40th MYCOTOXIN WORKSHOP

Conference Abstracts

Munich
June 11-13, 2018

The 40th Mycotoxin Workshop is organized by the

Chair of Food Safety, Veterinary Faculty,
Ludwig-Maximilians-University Munich (LMU)

in cooperation with the

Central Institute of the Bundeswehr Medical Service Munich

Gesellschaft
für
Mykotoxinforschung e. V.
Society
for
Mycotoxin Research

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in cooperation with the Central Institute of the Bundeswehr Medical Service Munich

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with assistance of the team of the Chair of Food Safety
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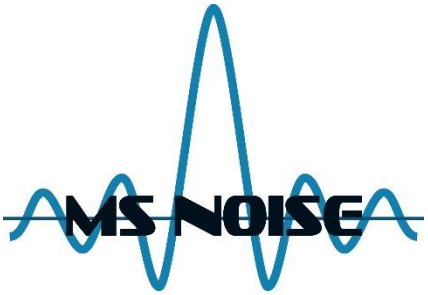

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Mandlstr. 23, 80802 München
(http://kwh.kath-akademie-bayern.de/how-to-find-us.html)

Sunday, June 10th 2018

18:00 Get together, Augustiner am Dom (Marienplatz)
Frauenplatz 8, 80331 München (www.augustineramdom.de)

Monday, June 11th 2018

08:00 Registration

09:00 Welcome

Session 1: **History**

09:10 **20th anniversary of the Society for Mycotoxin Research**
L1 Erwin Märtlbauer

09:30 **10th anniversary of cooperation with Springer**
L2 Stefanie Dether, Ewald Usleber

09:45 **Fumonisins: 30 years of research**
L3 Hans-Ulrich Humpf

10:00 **Cyclopiazonic acid: 50 years of research**
L4 Vladimír Ostry, Jakub Toman, Frantisek Malir

10:15 **Two forms of historic ergotism – two groups of toxins?**
L5 Ewald Usleber, Julia Irina Bauer, Madeleine Gross

10:30 – 11:15 **Coffee break/Exhibition (Foyer/Suresnes castle)**

Session 2: **Mycology/Toxigenic fungi – genetics and ecological function**

11:15 **Molecular biomarkers and phenotypic characterization as a means of determining genetic diversity in *Aspergillus flavus* isolates**
L6 Alfred Mitema, Sheila Okoth, Suhail M. Rafudeen

11:30 **Characterization of TcsC, the only type III histidine kinase in the pathogenic mold *Aspergillus fumigatus*, which is a new and promising therapeutic agent**
L7 Anja Spadinger, Anna Vincek, Annegret Wiedemann, Frank Ebel

11:45 **L8**	**Trichothecene-conjugating UDP-glucosyltransferases: Substrate specificities, kinetics and inhibition by culmorin** Herbert Michlmayr, Alexandra Malachová, Elisabeth Varga, Marta Piątkowska, Katrin Rehak, Julian Welsch, Gerlinde Wiesenberger, Julia Weber, Philipp Fruhmann, Christian Hametner, Franz Berthiller, Gerhard Adam
12:00 **L9**	**The biosynthesis of citrinin by *P. expansum* supports colonization of apples** Najim Touhami, Sebastian T. Soukup, Markus Schmidt-Heydt, Sabine E. Kulling, Rolf Geisen
12:15 **L10**	**Evidence that *Fusarium* toxins protect fungi against a wide range of predators** Yang Xu, Maria Vinas, Albatol Alsarrag, Ling Su, Katharina Pfohl, Wei Chen, Petr Karlovsky
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13:00 – 14:00	**Poster session/Exhibition (Suresnes castle)**
Session 3:	**Analytics**
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14:15 **L12**	**The face of *Stachybotrys* – novel cytotoxic meroterpenoids, chemically inspired isolation approaches and development of an LC-MS/MS multi-method** Annika Jagels, Viktoria Lindemann, Yannick Hövelmann, Alexa Zielinski, Melanie Esselen, Jens Köhler, Florian Hübner, Hans-Ulrich Humpf
14:30 **L13**	**Metabolic profiling and differentiation of *Alternaria* strains by Fourier Transform Ion Cyclotron Resonance mass spectrometry** Marina Gotthardt, Basem Kanawati, Philippe Schmitt-Kopplin, Michael Rychlik
14:45 **L14**	**Comprehensive proteomic analysis of *Penicillium verrucosum*: Strategies on using different database searches** Katharina Nöbauer, Markus Schmidt-Heydt, Ebrahim Razzazi-Fazeli

15:00 **L15**	**Using the model organism *Caenorhabditis elegans* for the toxicity testing of citrinin, zearalenone and zearalenone-14-sulfate** Julia Keller, Antje Borzekowski, Hajo Haase, Liliane Rueß, Ralph Menzel, Matthias Koch
15:15 **L16**	**Animal feed matrices: How to get control of a complex analytical problem?** David Steiner, Michael Sulyok, Lidija Kenjeric, Rudolf Krska
15:30 – 16:30	**Coffee break/Exhibition (Foyer/Suresnes castle)**
16:00 – 17:00	**Poster session/Exhibition (Suresnes castle)**
16:00 – 17:00	**Meeting of the members of the Society for Mycotoxin Research**
17:00	**Draught beer, wine and soft drinks (Foyer/Garden)**
18:00 – 23:00	**Dinner (Foyer/Garden)**

Tuesday, June 12th 2018

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09:15 **L18**	**Fish feeds – how much mycotoxin contamination can be assumed?** Constanze Pietsch
09:30 **L19**	**Relevance of mycotoxins in insect based food and feed** Jan Woyzichovski, Kelly Niermans, Rainer Benning, Ronald Maul
09:45 **L20**	**Forecasting model for the control of *Fusarium* diseases and the mycotoxin content of wheat and maize** Joseph Verreet, Tim Birr, Holger Klink

10:00
L21
Influence of agricultural practices and weather conditions on the occurrence of *Fusarium* toxins in Spanish local crops
Marta Herrera, Patricia Alfonso, Susana Lorán, Noemí Bervis, Raquel Anadón, Raquel Ciércoles, Antonio Herrera, Agustín Ariño

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Leaching of the mycotoxins deoxynivalenol and zearalenone from contaminated maize residues – a potential risk for agricultural soils
Friederike Meyer-Wolfarth, Elisabeth Oldenburg, Torsten Meiners, Katherine Muñoz, Stefan Schrader

10:30 – 11:15 **Coffee break/Exhibition (Foyer/Suresnes castle)**

Session 5: **Degradation and biotransformation**

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High physical properties of biochar issued from cashew nut shell to adsorb mycotoxins (aflatoxins and ochratoxin A) and its effects on toxigenic molds
Abderahim Achmadou, Alfredo Napoli, Noel Durand, Didier Montet

11:30
L24
The effects of feeding diets with *Fusarium* toxin contaminated maize treated with graded concentrations of sodium sulfite (SoS) on porcine health and adaptive immunity
Lisa Bahrenthien, Jeannette Kluess, Andreas Berk, Susanne Kersten, Jana Frahm, Janine Winkler, Martin Beer, Kerstin Wernike, Timm Harder, Dian Schatzmayr, Heidi Elisabeth Schwartz-Zimmermann, Annette Zeyner, Sven Dänicke

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L25
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Christelle El Hajj Assaf, Nikki de Clercq, Geertrui Vlaemynck, Els van Coillie, Els van Pamel

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L26
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Laura Righetti, Tania Körber, Enrico Rolli, Michael Rychlik, Michele Suman, Gianni Galaverna, Chiara Dall'Asta

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Herbert Michlmayr, Marta Piatkowska, Kristina Funtak, Florian Kastner, Christian Hametner, Gerlinde Wiesenberger, Lydia Wölflingseder, Doris Marko, Franz Berthiller, Gerhard Adam

12:30 – 13:30	**Lunch (Foyer)**
13:00 – 14:00	**Poster session/Exhibition (Suresnes castle)**
Session 6:	**Animal health**
14:00 **L28**	**Evaluation of concentrations of deoxynivalenol (DON), zearalenone (ZEN) and their metabolites in physiological samples of piglets as diagnostic markers** Susanne Kersten, Alma Nuhanovic, Janine Winkler, Jana Frahm, Jeanette Kluess, Sven Dänicke
14:15 **L29**	**Identification and characterization of novel DON metabolites in different animal species** Heidi Elisabeth Schwartz-Zimmermann, Christian Hametner, Veronika Nagl, Iris Fiby, Lukas Macheiner, Janine Winkler, Sven Dänicke, Erica Clark, James J. Pestka, Franz Berthiller
14:30 **L30**	**Impact of T2-toxin and zearalenone on the pharmacokinetics of midazolam as CYP3A probe drug in an in vivo porcine animal model** Wim Schelstraete, Mathias Devreese, Siska Croubels
14:45 **L31**	***Fusarium* toxin contaminated diet modified lipopolysaccharide (LPS)-induced acute cellular immune response in pigs** Jana Frahm, Jeannette Kluess, Susanne Kersten, Erik Bannert, Tanja Tesch, Susanne Bühler, Sven Dänicke
15:00 – 16:00	**Coffee break/Poster session/Exhibition (Suresnes castle)**
16:15 – 16:30	**Departure of the sightseeing busses**
18:00	**Arrival and welcome aperitif at Löwenbräukeller** (Stiglmaierplatz) Nymphenburger Str. 2, 80335 München (http://www.loewenbraeukeller.com)
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Wednesday, June 13th 2018

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09:30 **L32**	**Combinatory effects of glutathione-modulation on the toxicity of deoxynivalenol (DON) and NX-3 and its co-regulated metabolite butenolide (BUT)** Lydia Woelflingseder, Giorgia Del Favero, Elke Heiss, Gerhard Adam, Doris Marko

09:45
L33
Tubulo-interstitial cross talk modulates the impact of ochratoxin A (OTA) on renal epithelial cells and renal fibroplasts
Marie-Christin Schulz, Michael Gekle, Gerald Schwerdt

10:00
L34
Analysis of the interactions between the mycotoxin deoxynivalenol and cadmium, an environmental contaminant in different target organs
Thanh-Huong Le, Imourana Alassane-Kpembi, Isabelle P. Oswald, Philippe Pinton

10:15
L35
A target fishing approach to explore the mechanism of action of emerging mycotoxins – a case study on alternariol
Luca Dellafiora, Georg Aichinger, Doris Marko, Chiara Dall'Asta

10:30
L36
Estrogenicity of *Alternaria* toxins in complex mixtures
Georg Aichinger, Hannes Puntscher, Benedikt Warth, Doris Marko

10:45 – 11:30 **Coffee break/Poster session/Exhibition (Foyer/Suresnes castle)**

Session 8: **Human health/Exposure/Toxicology II**

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Fungal and mycotoxin burden in Portuguese bakeries
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Unraveling the prevalence of zearalenone biomarkers of exposure in the Portuguese population: An elaborate biomonitoring study
Carla Martins, Arnau Vidal, Marthe De Boevre, Carla Nunes, Duarte Torres, Ana Goios, Carla Lopes, Ricardo Assunção, Paula Alvito, Sarah De Saeger

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L39
Investigations on zearalenone exposure by urinary biomarkers in German adults
Gisela H. Degen, Nurshad Ali

12:15
L40
Human study on the pharmacokinetics of 2'R-ochratoxin A
Franziska Sueck, Benedikt Cramer, Vanessa Hemp, Hans-Ulrich Humpf

12:30 **Closing Session**

SERVICE PORTFOLIO FOR MYCOTOXIN ANALYSIS

Mould can infest food and feed of vegetable origin in the field or during storage. This is how moulds can especially impair grains, nuts, dried fruit, spices, hay, straw and silage. During their metabolism, they produce dangerous toxins, so-called mycotoxins. These belong to one of the strongest toxins occurring in nature. Humans and animals ingest these toxins primarily through food.

The most common mycotoxins are those belonging to aflatoxins. Aflatoxins may be contained in dried fruit, pistachios, nuts, peanuts, oil seeds, coffee and spices. Mycotoxins in the fusarium category may be contained in grains such as wheat, maize, oats and barley. Other mycotoxins can be found in rye, fruits or vegetables. They also enter the human food chain through mouldy feed, such as through milk. In addition, mycotoxins are heat-resistant. Generally, they are not destro yed during the processing or preparation of food.

Not only customers and consumers expect flawless products from the producers, processors and retailers, but industry standards and law makers also prescribe maximum values. Regulation (EC) No. 1881/ 2006 regulates the maximum levels of aflatoxin, patulin, fusarium toxins and other mycotoxins.

You can reduce your risk of processing contaminated products through regular sampling and analysis by SGS. Have your raw materials regularly inspected by our experts in order to prevent mycotoxins from entering the processing chain.

WE CAN PROVIDE YOU WITH

- sampling on site, also in the country of origin
- combination packages for different mycotoxins
- low limits of determination for dietetic foods
- rush analyses with 24-hour service

At the same time you can make use of the long-standing expertise of SGS in analysis and assessment as well as of our global network of laboratories and regional offices.

WE ANALYSE

- aflatoxins (B1, B2, G1, G2, aflatoxin M1)
- patulin
- ochratoxin A
- fumonisins B1, B2
- fusarium such as deoxynivalenol, nivalenol, T2 toxin, HT2 toxin and zearalenone.
- alkaloids of rye ergot (ergot alkaloids)

Professional sample preparation and the most modern measurement and analysis equipment guarantee reliable results.

Ask about our services in residue analysis (different plant protection products, dioxins, MCPD, MOSH/ MOAH, heavy metals) and micro-biological analysis.

In collaborating with SGS, show that you take the dangers involved with mycotoxins in food and feed just as serious as do authorities and consumers.

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WHEN YOU NEED TO BE SURE

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Abstracts of lectures

L1

20th anniversary of the Society for Mycotoxin Research

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The Society for Mycotoxin Research (Gesellschaft für Mykotoxinforschung e.V.) was founded in Munich, Germany, in 1997. Presently, the Society has more than 200 members from 20 countries and organizes an annual scientific meeting, the Mycotoxin Workshop. In 2005, Mycotoxin Research became the official journal of the Society, which is co-published by Springer and the Society since 2009. Proudly, the Society also awards the Brigitte Gedek Award for Mycotoxin Research.

This short review will present an overview on the background and the vision of the Society as well as on the major achievements of the last twenty years. Basic information on the Society can be found on the homepage (http://www.mykotoxin.de).

L2

10th anniversary of cooperation with Springer

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Mycotoxin Research, the official journal of the *Society for Mycotoxin Research*, was founded in 1985 by Rainer Schmidt, to provide a publication platform for this – then still relatively new – field of research. Many prominent scientists in the area of mycotoxin research supported the journal, either by submitting manuscripts or by acting in the advisory board. The year 2000 saw the beginning of the cooperation between the Society and Rainer Schmidt, by publishing the Proceedings of the Mycotoxin Workshops in *Mycotoxin Research*.

In 2005, *Mycotoxin Research* became the official journal of the Society and was co-published by Rainer Schmidt and the Society, with Manfred Gareis acting as the editor-in-chief. Soon this successful enterprise brought a rapidly growing demand for greater visibility, including online access to articles, and the workload of publishing a journal as a 100% volunteer product began to exceed available capacity in 2007. The Society looked for a professional publisher, and found a competent partner with Springer in Heidelberg, Germany. Following some productive talks, a publishing agreement was signed by Springer (Dieter Czeschlik) and the Society (Manfred Gareis) in May 2008.
Since volume 25, 2009, *Mycotoxin Research* has been co-published by Springer and the Society, with four issues per year. Furthermore, all backfile articles of volumes 1-24 have been digitalized, and more than 1100 articles are now easily accessible for the scientific community. Membership in the Society includes a print copy and free access to online articles of *Mycotoxin Research*. Every article published in *Mycotoxin Research* is "Society-branded", displaying the logo of the *Society for Mycotoxin Research* on the title page. Authors of articles can opt either for classical publication, without publication fee, or for open access publication. Major milestones in aspects of visibility and database coverage were the inclusion in PubMed/Medline in 2012, and inclusion in Clarivate Analytics database products (Science Citation Index Expanded, Current Contents) in 2015.

The journal received its first impact factor in 2016. With an IF of 2.7 in 2007, *Mycotoxin Research* is now fully established in the upper field of journals in its field of research. This successful story has been made possible by authors providing excellent manuscripts, by a competent and highly motivated editorial board, by a panel of expert reviewers, and of course by the constructive and harmonic cooperation between the Society and Springer. Everyone is cordially invited to visit the result of this cooperation at https://www.springer.com/life+sciences/microbiology/journal/12550.

L3

Fumonisins: 30 years of research

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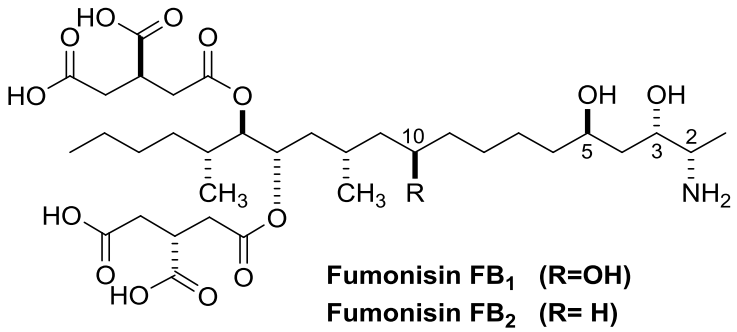
Fumonisins are a group of mycotoxins produced by *Fusarium verticillioides* (formerly *Fusarium moniliforme*) and the chemical structures of the main compounds, fumonisin FB_1 and FB_2, have been described for the first time in 1988 by South African researchers [1].



However the story of fumonisins started much earlier and already in the seventies leukoencephalomalacia (LEM), the characteristic disease of fumonisins in horses, has been described and linked to *F. verticillioides* cultures. It took several years to identify fumonisins as cause of this disease. The isolation and structure elucidation was rather challenging as fumonisins are rather polar compounds and do not show any UV-activity.

Besides the structure elucidation in 1988 Gelderblom et al. [2] already demonstrated in the same year the cancer promoting activity of pure cultures of *F. verticillioides* in rat liver in a short-term cancer initiation/promotion assay.

In the following years research activities have been intensified and research groups around the world were working on the toxicity, mode of action, structure elucidation of further isomers, stereochemistry, analytical methods, thermal stability, biosynthesis, etc.. In this presentation the research over the last 30 years will be summarized.

[1] Bezuidenhout SC, Gelderblom WCA, Gorst-Allman CP, Horak RM, Marasas WFO, Spiteller G, Vleggaar R. Structure elucidation of the fumonisins, mycotoxins from *Fusarium moniliforme*. J Chem Soc Chem Commun, **1988**, 743-745.

[2] Gelderblom WCA, Jaskiewicz K, Marasas WFO, Thiel PG, Horak RM, Vleggaar R, Kriek NP. Fumonisins-novel mycotoxins with cancer promoting activity produced by *Fusarium moniliforme*. Appl Environ Microbiol, **1988**, 54, 1806-1811.

L4

Cyclopiazonic acid: 50 years of research

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Mycotoxin cyclopiazonic acid (CPA) an indole tetramic and a lipophilic monobasic acid was first discovered and characterized in year 1968. For the remainder of this abstract, the term CPA refers to α-CPA. CPA was isolated from culture extracts as a main toxic metabolite of *Penicillium cyclopium* Westling (correct name: *Penicillium griseofulvum* Dierckx) during routine toxicity screening of microfungi isolated from groundnuts that caused acute toxicosis in ducklings and rats. CPA is produced by several *Penicillium* species (*P. griseofulvum, P. camemberti, P. commune, P. dipodomyicola*) and *Aspergillus* species (*A. flavus, A. oryzae,* and *A. tamarii*).

A variety of analytical methods have been used to detect and quantify CPA in fungal cultures and in food, including chromatographic methods (TLC, HPLC and LC–MS/MS), immunochemical methods (ELISA, IAC) and capillary electrophoresis. CPA has been reported to occur in food commodities (e.g. oilseeds, nuts, cereals, dried figs, milk, cheese and meat products). CPA is frequently detected in e.g. peanuts and maize. The potential toxic risk of CPA to humans and animals was initially considered as low because it was less potent compared with other mycotoxins (e.g. aflatoxins). Consequently, CPA did not attract the attention of the scientific community. Scientific interest in CPA increased in 1977. It was demonstrated that *Aspergillus flavus* could produce CPA in food and feed. CPA is toxic to several animal species, such as rats, pigs, guinea pigs, poultry and dogs. After ingesting CPA-contaminated feeds, test animals display severe gastrointestinal upsets and neurological disorders. Organs affected include the liver, kidney, heart, and digestive tract, which show degenerative changes and necrosis. Biologically, CPA is a specific inhibitor of sarco(endo)plasmic reticulum Ca^{2+}-ATPase.

Since 1970, approximately 27 CPA-type mycotoxins have been reported in different fungal extracts of *Aspergillus* and *Penicillium* species e.g. CPA-type mycotoxins: indole derivatives (β-CPA, α-CPA-imine, iso-α-CPA, cyclo-acetoacetyl-L-tryptophan, pseuboydone E) and CPA-type mycotoxins: oxindole derivatives (2-oxo CPA, speradines, aspergillines and cyclopiamides).

Conclusion: Selected recent research topics such as a research on the diversity and genetic variability of CPA production in *Aspergillus flavus* and on the problem of biocontrol strategy for use of the non-aflatoxigenic strains of *A. flavus* and CPA as a pathogenicity factor for *A. flavus* were researched.

Supported by Ministry of Health, Czech Republic - conceptual development of research organization („National Institute of Public Health – NIPH, IN 75010330“) and the specific research project (no. 2105/2017) of the Faculty of Science, University Hradec Kralove, Czech Republic.

L5

Two forms of historic ergotism - two groups of toxins?

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Ergotism has been one of the most devastating diseases in European history. It is commonly assumed that the two manifestations of ergotism, "Ergotismus gangraenosus" and "Ergotismus convulsivus", are both caused by ergot of rye (*Claviceps purpurea* sclerotia). As the toxic compounds in ergot are the ergot alkaloids, both forms of ergotism are by implication associated with this group of toxins.

However, there are some inconsistencies in the ergot theory with regard to the convulsive form, some of which have been discussed over centuries. Proposed alternative agents of the convulsive form, such as *Lolium temulentum*, *Bromus* spp., or *Raphanus raphanistrum*, have been rejected by the opinion-forming works of J. Taube (1782) and C.J. Lorinser (1824). The convulsive form predominantly affected the poor, rural population and was more severe in children, while the gangrenous form was present in all social and age groups.

Furthermore, reported cases of gangrenous ergotism peaked west of the River Rhine around AD 1000-1200, while outbreaks of convulsive ergotismus mainly occurred east of the River Rhine and peaked around AD 1600-1800, nearly 500 years later. Therefore, the gangrenous form happened in the medieval warm period, while the convulsive form occurred during a period which is called "the little ice age". The average temperatures differed by more than 1 °C between both periods, which most likely had an impact on agricultural production, and on growth activity of weeds and fungi. Finally, the symptoms of convulsive ergotism as described in historic reports do not fit well into current knowledge about pharmacological properties and toxic effects of ergot alkaloids.

Recently we found tremorgenic indole-diterpene alkaloids, co-occurring with ergot alkaloids, in ergot of rye and barley. Furthermore, Lolium sp., Bromus sp. or other Poaceae weeds infesting rye fields may also contain both groups of toxins in varying proportions. Indole diterpene alkaloids such as lolitrem B and paxilline are able to cross the blood-brain barrier and have shown to cause ryegrass staggers in hervibore animals. The main symptom of staggers, spasms, would fit well into the description of convulsive ergotism. We therefore conclude that neurotoxic indole diterpene alkaloids could have contributed to historic convulsive ergotism, either alone or in combination with ergot alkaloids.

This work was financially supported by the Brigitte and Wolfram Gedek foundation.

L6

Molecular biomarkers and phenotypic characterization as a means of determining genetic diversity in *Aspergillus flavus* isolates

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Toxigenic *Aspergillus* species produce mycotoxins that are carcinogenic, hepatotoxic and teratogenic immunosuppressing agents in both human and animals. Kenya frequently experiences outbreaks of aflatoxicosis with the worst occurring in 2004, which resulted in 215 deaths. We examined the possible reasons for these frequent aflatoxicosis outbreaks in Kenya by studying *A. flavus* diversity, phenotypes and mycotoxin profiles across various agricultural regions. Using diagonal transect random sampling, maize kernels were collected from Makueni, Homa Bay, Nandi, and Kisumu counties. Out of 37 isolates, nitrate non-utilizing auxotrophs complementation test revealed 20 vegetative compatibility groups. We designated these groups by the prefix ''KVCG'', where ''K'' represented Kenya and consequently assigned numbers 1 to 20 based on our findings. KVCG14 and KVCG15 had highest distribution frequency (n = 13; 10.8 %). The distribution of the L, S and S/L-morphotypes across the regions were 57 % (n = 21); 7 % (n = 3) and 36 % (n = 13) respectively. Furthermore, a unique isolate (KSM015) was identified that had characteristics of S-morphotype, but produced both aflatoxins B and G. Coconut agar medium (CAM) assay, TLC and HPLC analyses confirmed the presence or absence of aflatoxins in selected toxigenic and atoxigenic isolates. Diversity Index (H) analyses ranged from 0.11 (Nandi samples) to 0.32 (Kisumu samples). Heterokaryon compatibility ranged from 33 % (for the Makueni samples, n = 3) to 67 % (Nandi samples, n = 6). To our knowledge, this is the first reported findings for *A. flavus* diversity and distribution in Nandi, Homa Bay and Kisumu counties and may assist current and future researchers in the selection of biocontrol strategies to mitigate aflatoxin contamination as has been researched in Makueni and neighbouring counties.

Keywords: aflatoxins, morphotypes, genetic diversity, heterokaryon compatibility, TLC, HPLC, fluorescence.

USHEPiA, University of Cape Town and University of Nairobi for funding and offered laboratory for research

L7

Characterization of TcsC, the only type III histidine kinase in the pathogenic mold *Aspergillus fumigatus*, which is a new and promising therapeutic target

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Type III histidine kinases are signaling molecules that enable fungi to adapt to hyperosmotic environments. Some compounds, like fludioxonil, can artificially activate this process leading to a dramatic and unbalanced increase in the internal osmotic pressure. We have analyzed the resulting anti-fungal effect in detail for the pathogenic mold *Aspergillus fumigatus*. Fludioxonil results in an immediate halt of the hyphal growth, a dramatic swelling that is accompanied by a reorganization of the cell wall and finally culminates in lysis. Remarkably, the whole process completely depends on the type III histidine kinase TcsC. We found similar effects for several other clinically relevant molds, some of which are very difficult to treat. This and the fact that type III histidine kinases do not have homologs in animals and humans make these agents an interesting new option to expand the therapeutic arsenal of anti-mycotica.

To improve this anti-fungal activity on the molecular level, we have started to analyze the functional importance of the different TcsC domains (compare Figure 1). Our data show that the C-terminal kinase module is per se permanently active. In the full length protein, however, it is negatively regulated by the N-terminal sensing module, which consists of 6 so-called HAMP domains. A construct containing the kinase module and only the proximal HAMP domain is constitutively inactive indicating that this domain can turn off the activity of the kinase module. This phenotype is partially lifted in a construct containing the kinase module and two proximal HAMP domains. All 6 HAMP domains are required to gain full activity of TcsC. A GFP-TcsC protein was generated to study the localization of TcsC. Expression of this fusion protein in a Δ*tcs*C mutant restored the wild type phenotype indicating that the GFP portion has no negative impact on the function of TcsC. GFP-TcsC shows a distinct intracellular location pattern, which changes after activation. In conclusion, we have identified TcsC as a promising new therapeutic target and have started to characterize its structure-function relationship and its subcellular localization.

Caption: Figure 1: Domain organisation of the type III histidine kinase TcsC.

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L8

Trichothecene-conjugating UDP-glucosyltransferases: substrate specificities, kinetics and inhibition by culmorin

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Fusarium species infest cereal crops and produce several toxic metabolites, the most problematic being trichothecene-toxins such as deoxynivalenol (DON), which is considered to be a relevant virulence factor promoting disease spread. Plants are able to detoxify *Fusarium* toxins by glucosylation, a reaction catalyzed by members of the large and functionally versatile family of UDP-glycosyltransferases (UGTs).

Transcriptome analysis of barley upon *Fusarium* infection and DON application led to identification of the first crop plant UGT (HvUGT13248) able to glucosylate DON.

We have recombinantly expressed, affinity-purified and characterized HvUGT13248 and homologous UGTs from rice (OsUGT79) and Brachypodium (Bradi5g03300, Bradi5g02780) β-glucosylation of several trichothecenes (DON, nivalenol, fusarenon X, T-2 toxin, HT-2 toxin, T2 triol, neosolaniol, 4,15-diacetoxyscirpenol) was observed only at position C3-OH of the toxins as shown by NMR analysis of glucosides synthesized by OsUGT79 and HvUGT13248. All enzymes kinetically prefer type A trichothecenes like HT-2 toxin and T-2 triol over DON. C-4 acetylated toxins (e.g. T-2 and fusarenon X) were only glucosylated by HvUGT13248 to a low degree. Furthermore, hydroxylation of C-4 influences substrate affinity. For example, Bradi5g03300 clearly prefers DON, while Bradi5g02780 is only active with NIV. Through generation of hybrid proteins and site directed mutagenesis we could identify an amino-acid residue that seems to be crucial for discrimination between DON and NIV.

Culmorin is another *Fusarium* metabolite reported to co-occur with DON, yet its function is still unknown. We observed increased toxicity of DON in the presence of culmorin in a root growth assay with Apogee wheat. We present evidence that culmorin inhibits trichothecene-conjugating UGTs in a cooperative binding mode in vitro; with about equal, 10-fold or even 2000-fold higher binding affinity compared to DON as estimated in the cases of OsUGT79, HvUGT13248 and Bradi5g03300, respectively. We therefore hypothesize that culmorin may interfere with plant defense by targeting DON-detoxification.

L9

The biosynthesis of citrinin by *P. expansum* supports colonization of apples

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Penicillium expansum is the most important post-harvest pathogen of apples. After infection, it leads to green rot of the fruits. In addition to spoil the product, the fungus can produce two important mycotoxins, in particular patulin and citrinin. Usually much higher amounts of patulin are produced on apples, compared to citrinin. This is in contrast to the fact that many strains of *P. expansum* can produce high amounts of citrinin on laboratory media, which demonstrates their general capacity to produce high amounts of that toxin. It has been shown by various authors that patulin acts as a pathogenicity factor. Mutants in the biosynthesis of patulin were less able to infect the fruits, depending on the apple variety analysed. However, citrinin also plays a role during the establishment of the colonization. Mutants in the biosynthesis of citrinin, also showed a reduced colonization capacity. The addition of external citrinin re-established this capacity. A detailed analysis showed that citrinin plays a role as a protective metabolite against the ROS activity of apples. Reactive oxygene species (ROS) can degrade citrinin, which is one reason for the low concentrations of citrinin in apples.

L10

Evidence that *Fusarium* toxins protect fungi against a wide range of predators

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The most fundamental question of research on mycotoxins is why mycotoxins exist. What kind of selection pressure maintains the integrity of biosynthetic pathways for mycotoxins, how do mycotoxins contribute to the fitness of their producers and what drives their diversification in closely related species? According to ecological metabolite hypothesis, the biological function of fungal secondary metabolites in general and mycotoxins in particular is to modulate interactions of their producers with other organisms. The role of mycotoxins in two kinds of biotic interactions has been addressed experimentally: colonization of host organisms by fungal pathogens, in which fungal toxins act as virulence factors, and mycoparasitism, in which mycotoxins fulfill the role of agents of chemical defense. Fungal virulence factors have attracted continuous attention of many plant pathologists and researchers studying fungal diseases of humans. As a result, conclusive evidence for the role of trichothecenes, fusaric acid, gliotoxin and further toxins in pathogenesis has accumulated. Chemical defense against mycoparasitism have been studied sparingly but conclusive evidence for the role of zearalenone and few further metabolites in defense have been obtained.

The biological function of the large majority of mycotoxins, however, remains unknown. Only a small fraction of fungal species can colonize living hosts and mycoparasitism does not occur frequently. In search for biological function of mycotoxins, defense role of these metabolites in protection of their producers against predators have been postulated in 1980th. In following three decades a wealth of data on the toxicity of mycotoxins to insects have been generated but because the defense hypothesis have seldom been tested in ecologically relevant settings, evidence for the role of any *Fusarium* mycotoxin in defense against predation has been lacking. We investigated the role of mycotoxins of *Fusarium* spp. in interaction with predators systematically. The results provide conclusive evidence for the role of fungal toxins in defense against a wide range of predators including nematodes, crustaceans, primitive arthropods and insects.

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L11

Application of loop-mediated isothermal amplification (LAMP) for identification and detection of mycotoxin producing molds

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Detection and identification of mycotoxigenic molds in food and feed as well as in the commodities used for their production is an alternative approach in quality control and hazard analysis. The polymerase chain reaction (PCR) has been the gold standard over the past two decades. However, in recent years, LAMP was introduced as a novel technique for isothermal DNA amplification. Assays are based on the binding of 4 primers to 6 binding sites in the target DNA and are thus highly specific. Moreover, no thermal cycling is necessary thus making elaborate and costly lab equipment superfluous and signals can be detected visually without any further manipulations. Tests are performed within 45-60 min, including sample preparation. These features make LAMP assays well suited for applications in the field or at the point of analysis. The application of the technology is exemplified by an assay that was recently developed for the detection of typical aflatoxin producing mold species. This LAMP reaction detects the presence of the nor1 (=AflD) gene in 15 different aflatoxigenic species within the genus *Aspergillus* in a group specific manner. Presence of the gene is indicated by a color change from yellow to red in positive reactions that can be detected by the naked eye. The assay was used to detect potential aflatoxin producers in crude preparations of surface washings made from samples of rice, maize, nuts, powdered spices and dried fruits. Results where available within 60 min and showed a positive correlation to contamination of rice samples with aflatoxins and aflatoxin producing *Aspergillus* spp.

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L12

The face of *Stachybotrys* – novel cytotoxic meroterpenoids, chemically inspired isolation approaches and development of an LC-MS/MS multi-method

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The genus *Stachybotrys* is commonly associated with indoor water damage and has become increasingly popular since the 1990s within cases of idiopathic pulmonary hemosiderosis in infants and building-related illnesses. *S. chartarum* was found in the homes of the infected infants and in this regard, there are still controversial discussions, whether the fungal growth was correlated with the diseases. *Stachybotrys* produces a broad variety of secondary metabolites including macrocyclic trichothecenes, atranones and phenylspirodrimanes. In the past, most attention was put to the highly toxic macrocyclic trichothecenes and investigations concerning the phenylspirodrimanes have been neglected, although they occur in even higher levels and in a remarkable multitude of different compounds. Moreover, they are known to be cytotoxic as well as immunosuppressant and additionally, combinatory effects among them have never been considered.

For this purpose, a series of phenylspirodrimanes occurring in all common *Stachybotrys* species were isolated in order to be used as reference standards for the development of an LC-MS/MS quantitation multi-method. The general objective of this multi-method is to enable the determination of the secondary metabolites in fungal extracts and various matrices, such as building materials, dust, feedstuff, and physiological samples. A noteworthy representative of the phenylspirodrimane family is stachybotrydial. Its structural properties led to the development of chemically inspired isolation approaches, either based on supplementation of the culture media or semisynthesis. Both strategies could potentially be utilized to simplify access to certain reference standards from *Stachybotrys*.

Ultimately, in the course of gaining new insights into the secondary metabolite profile, three novel cytotoxic meroterpenoids, namely stachybotrychromenes A-C, were discovered. Their structures were elucidated by extensive spectroscopic analysis (NMR, HRMS, CD). First cytotoxic effects have been determined and the new compounds clearly contribute to the overall toxicity profile of this fungus.

Consequently, the genus *Stachybotrys* offers an enormous potential for future investigations to conclusively determine the role of this fungus within specific building-related case studies as described above and to clarify the relationship between human health and fungal contamination in indoor environments in general.

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L13

Metabolic profiling and differentiation of *Alternaria* strains by Fourier Transform Ion Cyclotron Resonance mass spectrometry

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As widely distributed plant pathogens, *Alternaria* fungi infect crops, herbs, and vegetables growing in the fields and cause losses during postharvest storage. Besides the immediate harvest failures, the formation of secondary metabolites is a severe problem for consumers. Some secondary metabolites are considered as mycotoxins, but evaluation of toxicological potential and estimation of occurrence data are still missing or are not finalized yet [1]. Besides the already investigated secondary metabolites, *Alternaria* fungi might produce further metabolites with unknown structure and toxicological potential. To cover the *Alternaria* mycobolome (fungal metabolome) consisting of hundreds of metabolites, a non-targeted metabolomics approach by means of ultra-high resolution mass spectrometry was performed.

Various *Alternaria* strains were cultivated in liquid media and extracts were analysed by Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). Ultra-high resolution and high sensitivity of FT-ICR-MS allowed unequivocal sum formulae annotation of measured masses. By applying various software tools, variations in the mycobolome of *A. alternata* and *A. solani* isolates were visualized and were mainly based on various perylene quinone derivatives. Moreover, FT-ICR-MS allows not only distinction between species but it also indicates variations within one species. Targeted LC-MS/MS analysis verified sum formulae annotation of FT-ICR measurements by analyzing six mycotoxins and confirmed metabolic differentiation of *Alternaria* strains.

Our results demonstrate that FT-ICR-MS is a promising instrument in non-targeted metabolomics to get deeper insights into the mycobolome of *Alternaria* discovering similarities and differences among strains.

[1]: EFSA on Contaminants in the Food Chain (CONTAM); Scientific Opinion on the risks for animal and public health related to the presence of *Alternaria* toxins in feed and food. *EFSA Journal* 2011;9(10):2407.

L14

Comprehensive proteomic analysis of *Penicillium verrucosum:* Strategies on using different database searches

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Mass spectrometry based identification of proteins in species lacking validated protein sequence information is a major problem. In the present study ochratoxin A (OTA) producing *Penicillium verrucosum* was used to identify and quantitatively analyse proteins of an organism with yet no protein information available. It is the first shotgun proteomics approach to identify proteins of this fungus. The use of three different databases, two genetically closely related organisms (*Penicillium chrysogenum* and *Penicillium nordicum*) and an "ab initio" translated one from DNA data of P. verrucosum, for subsequent database search was evaluated. In addition, two different search methods differing in their stringency were compared. The results clearly show that the amino acid exchange in one of the search methods (thorough search) increased the number of identified proteins, especially in the databases of homologous species. The new approach, using an "ab initio" translated database to identify proteins, resulted in the highest number of identifications even with the stringent search method. In this "ab initio" translated database from DNA sequences of P. verrucosum 3631 proteins could be identified. Additionally, a Sequential Window Acquisition of all Theoretical fragment-ion spectra analysis (SWATH) was done to find differentially regulated proteins at two different time points of the growth curve. We compared the proteins at the beginning (day 3) and at the end of the log phase (day 12). In total 251 regulated proteins could be found, four of them with a p-value<0.05. Downregulated proteins are involved in protein biosynthesis and ribosome biogenesis whereas upregulated proteins are part of proteolysis, and gluconeogenesis.

L15

Using the model organism *Caenorhabditis elegans* for the toxicity testing of citrinin, zearalenone and zearalenone-14-sulfate

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To keep up with emerging mycotoxins and their transformation products fast and reliable toxicity tests are needed. Toxicity testing of mycotoxins is carried out usually by performing in vitro assays or is evaluated by using laboratory animals like mice, rats or chicken in in vivo studies.

Settled between classical *in vitro* approaches and *in vivo* studies with higher animals are tests with the nematode *Caenorhabditis elegans* (Fig 1). Since Sydney Brenner described 1974 the cultivation and handling of *C. elegans*, this worm is widely used as model organism in developmental biology and neurology. Due to many benefits like easy and cheap cultivation, a completely sequenced genome and short generation time, it also plays an important role in toxicological research. Finally, the high number of conserved genes between human and *C. elegans* make the worm an ideal candidate for toxicological investigations.

In this study we used *C. elegans* to access the toxic effects of the relevant food mycotoxin citrinin (CIT), the mycoestrogen zearalenone (ZEN) and the modified mycotoxin ZEN-14-sulfate (ZEN-14-S) on different lifetable parameters including reproduction, thermal and oxidative stress tolerance and lifespan. All tested mycotoxins significantly decreased the amount of offspring. In case of ZEN and CIT also significant negative effects on stress tolerance and lifespan were observed compared to the control group.

Moreover, metabolization of mycotoxins in the worms was investigated by using LC-MS/MS. Extraction of the worms treated 5 days with mycotoxin-containing and UVC-killed bacteria showed metabolization of ZEN to α-ZEL and β-ZEL (ZEL = zearalenol, ratio about 3:2). ZEN-14-S was reduced to ZEL-14-S and CIT was metabolized to mono-hydroxylated CIT.

Caption: Figure 1: *Caenorhabditis elegans* wild type in larval stage L4

L16

Animal feed matrices: How to get control of a complex analytical problem?

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1
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Feedstuffs are at the beginning of the human food chain and thus represent an important basis for the production of our food. Influencing factors such as climatic parameters or improper storage conditions enable the occurrence of a variety of contaminants like secondary fungal metabolites or plant toxins in the food chain. Animal livestock is particularly endangered by the consumption of contaminated feed and thus show disease symptoms like estrogen syndrome or feed refusal. Therefore, there has been an increasing trend towards multi-targeted analysis especially based on LC-ESI-MS/MS to cover most of the relevant feed contaminants by using a single method. Due to the very diverse composition of starchy, oily and protein-containing ingredients, this sample type poses a special analytical challenge in terms of absolute and relative matrix effects. Previous publications have been exclusively focusing on cereal-based single feed ingredients and thus show no comprehensive illustration of the complex matrix type. In this work, a liquid chromatography-electrospray ionization tandem mass spectrometric method was developed, to allow a simultaneous quantification of several hundred natural contaminants and anthropogenic residues. On the basis of spike experiments, a comparison of self-designed feed matrices with real samples (cattle, pig, chicken, fish) was carried out. The focus is on the assumption that significant differences in signal suppression and enhancement (SSE) as well as in the extraction efficiency (RE) within different lots of a sample type is substantially influencing the method performance. In order to perform a complete characterization and assignment of corresponding matrix effects, analytical identification data of the single feed ingredients were evaluated. The aim of this work is to demonstrate a comprehensive overview and analytical characterization of both, compound feed formulas and single feed ingredients.

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L17

Fungal and mycotoxigenic burden from feed applied in Portuguese aquaculture

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Aquaculture is one of the most important animal husbandry systems, allowing a regular growth of 1% per year in the last three decades worldwide. As in other animal production, feed safety is an essential factor to assure the productivity of those aquatic activities.

The aim of this study was to assess fungal and mycotoxigenic burden in 7 different feeds, 4 filters from aquaculture recirculation tanks and 7 water samples from the same tanks. Each sample suspension was inoculated on to 2% malt extract agar (MEA) with 0.05 g.L-1 chloramphenicol media and dichloran glycerol (DG18) agar-based media. After incubation of the MEA and DG18 plates at 27 ◦C for 5 to 7 days, fungal densities (colony-forming units, CFU/m2/g) were determined and morphological identification was achieved through macro and microscopic characteristics. Molecular detection of the toxigenic *Aspergillus* sections Flavi, Fumigati, Circumdati and Versicolores will be performed shortly. Thirty seven mycotoxins were analyzed using LC-MS/MS system and detection was carried out using high performance liquid chromatograph (HPLC) Nexera (Shimadzu) with a mass detector API 4000 (Sciex). *Fusarium verticilloides* was the most prevalent in all samples analyzed, being detected on 3 out of 7 (42.6%) feed samples. *Fusarium culmorum* was also identified in one of the 3 feed samples. Besides *Fusarium* genus also *Aureobasidium* and *Penicillium* were found in one and in two water samples, respectively. The mycotoxins detected were deoxynivalenol, zearalenone, fumonisin B1, fumonisin B2, T2, ochratoxin A and mycophenolic acid. Most of the mycotoxins were detected only in the dry feed samples and only mycophenolic acid was found in one sample of semi-moist feed. Thus, the two samples of dry feed presented contamination by 6 to 7 mycotoxins.

In light of the results obtained from this first screening about fungal and mycotoxigenic burden in Portuguese Aquaculture, further studies should be performed focusing on raw materials and dry feed samples. Molecular detection of fungal species should target, besides *Aspergillus* genera, also *Fusarium* specific species.

L18

Fish feeds - how much mycotoxin contamination can be assumed?

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The occurrence of mycotoxins in feedstuffs for animals is indisputable. However, their presence in fish feed and their subsequent effects on fish have not sufficiently been investigated due to the fact that the analyses are laborious and in most cases also very expensive. For a comprehensive analysis of the risks for fish in aquaculture, the exact composition of compound feed has to be known in order to calculate their potential contamination with different mycotoxins. Unfortunately, fish feed producers rarely publish the exact composition of their feeds. Therefore, a feed composition calculator was established based on the average nutrient content of different feed ingredients according to the experience of a local feed producer. This allowed estimation of the proximate percentage of feed ingredients in commercial feed based on a list of gross nutrients in this feed and the ingredients that had been used for feed production. Based on this, the percentage of ingredients in more than 70 commercially available fish feeds was estimated resulting in an accuracy of more than 99% for the protein content and fat content, and more variation in the carbohydrate fraction. For further calculations, a literature search yielded information on the average, median, minimum and maximum contamination of feed ingredients. Based on this the average, minimum and maximum contamination of more than 70 commercially available fish feeds with the 10 most commonly present mycotoxins (according to the current knowledge). The calculations revealed that especially for the mycotoxins deoxynivalenol, zearalenone, fumonisin B1, moniliformin, and enniatin B higher values in different fish feeds can be expected. The obtained data were compared to already published contamination values in commercial fish feeds and known critical effect concentrations in fish. For most mycotoxins, no critical concentration in the feeds was estimated with respect to the currently recommended maximum allowable contamination levels in fish feeds, but problems for some fish species may be assumed. Since the contamination of feed ingredients is variable from year to year and insufficient storage of feedstuffs also contributes to contamination of feeds before they are actually used for feeding of fish, this risk assessment remains incomplete due to a lack of exact data. Nevertheless, the assessment can result in recommendations of certain feed ingredients that show low mycotoxin contaminations in general, and thus, appear to be less problematic for fish than others.

L19

Relevance of mycotoxins in insect based food and feed

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Along with the increase in the global human population there is a rising demand for protein rich refined food of animal origin. Conventional livestock will not be able to cover this growing demand. Thus, there is an increasing need for protein from alternative sources in the future. Additionally, efficient sources for feeding carnivorous livestock are required. Insects could be such a source of animal protein incorporated in the food and/or feed chain. The fat fraction and processed proteins of yellow mealworm larvae (*Tenebrio molitor*) are, together with six other insect species, legally allowed in animal feed within the EU and increasing numbers are registered as novel food in the EU following Regulation (EU) 2015/2283. However, data on hazardous chemicals in reared insects are scarce [1]. Before considering *T. molitor* larvae as suitable for consumption, the possible presence of contaminants must be evaluated. The presentation focusses on mycotoxins as one of the contaminants of high concern in food and feed. In a feeding study *T. molitor* larvae were nourished on grain flour containing different levels of natural mycotoxin contamination as well as spiked zearalenone (ZEN) levels. Biological parameters such as survival, feed conversion and weight gain were evaluated and parent mycotoxins along with their known metabolites were measured using LC-MS/MS technique.

For all diets survival was high and an average survival of 99.8% was found which is in accordance with a similar study reporting a high tolerance of two further insect species for e.g. Aflatoxin B1 and ZEN [2]. However, a significant increase in larval weight gain was found in our study between the diets containing naturally contaminated wheat and the other diets after eight weeks of exposure. No such effects were found for artificially contaminated feed. No ZEN or ZEN metabolites were detected in the mealworm larvae after harvest. However, ZEN, α- and β-zearalenol (ZEL) were found in the residue samples consisting of feces and small amounts of non-consumed feed. α- and β-ZEL were formed in the insects in equal amounts and accounted for up to 60% of the total mycotoxin recovery in the groups fed with naturally contaminated wheat flour. Overall recovery of ZEN and its metabolites was high in the residue, indicating that for unknown metabolites only small amounts have to be expected. For ZEN the study indicates a low risk for toxin accumulation in the edible parts of the insect. However, the intense formation of the more potent metabolite α-ZEL should be addressed in further studies.

[1] S. Belluco, C. Losasso, M. Maggioletti, C. C. Alonzi, M. G. Paoletti, A. Ricci, Comprehensive Reviews in Food Science and Food Safety 2013, 12, 296-313. [2] L. Camenzuli, R. Van Dam, T. de Rijk, R. Andriessen, J. Van Schelt, H. Van der Fels-Klerx, Toxins 2018, 10, 91.

L20

Forecasting model for the control of *Fusarium* diseases and the mycotoxin content of wheat and maize

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Winter wheat grain (3 susceptible cultivars from 2008 to 2017) and maize silage (4 susceptible cultivars from 2011 to 2015) from ten locations in Schleswig-Holstein were analyzed for the occurrence of *Fusarium* species and for DON and ZEA content and the effect of weather, cultivar susceptibility, crop rotation and soil cultivation was determined.

The same seven *Fusarium* species were detected in both wheat and maize. The DON and ZEA producers *F. graminearum* and *F. culmorum* dominated in all years, followed by *F. avenaceum* and *F. poae*.

The DNA amounts of *F. graminearum* and *F. culmorum* and DON and ZEA contents of wheat grain differed significantly between individual years and within each year between locations due to weather at flowering. Significant correlations were found between precipitation and temperature at flowering and DON and ZEA contents in wheat grain at harvest. Three multiple regression models were developed for forecasting DON and ZEA contamination of wheat grain at harvest. Their predictive performance was evaluated with new data on weather at flowering and DON and ZEA contents in wheat grain at the same locations. Regressing the observed versus predicted DON and ZEA values showed that 82 % and 83 % of the variation in the observed DON and ZEA values is explained by the variation in the predicted values. The models predicted correctly in 96.9 % of the cases whether the DON content was either lower or higher than the maximum threshold of 1250 µg/kg. For ZEA, the models provided 93.8 % correct predictions. Cultivar-specific reductions of DON and ZEA contents were observed in all years. In the low to moderately susceptible cultivar, DON and ZEA contents were reduced by 53 % and 52 % and in the moderately to high susceptible cultivar by 18 % and 22 % compared to the high susceptible cultivar. Triazole fungicides applied at flowering reduced DON and ZEA contents by 47 % and 50 %, respectively.

Ploughing reduced DON contents in maize silage by 78 % and ZEA contents by 61 % compared to pre-crop maize and conservation tillage. The cultivation of maize with crop rotation and ploughing reduced mycotoxin contents by 87 % (DON) and 65 % (ZEA). More tolerant cultivars had lower mycotoxin contents than more susceptible ones.

Weather conditions at flowering and DON contents of maize grains at harvest significantly correlated at ten Bavarian locations, each with six cultivars, from 2010 to 2014. To exactly determine the period of flowering, the temperature sum model of Rath et al. (2005) was used. Precipitation and temperature during that period correlated with DON content of maize grains (e.g. R2 = 0.91 for cultivar "Susann"). Based on this correlation, a multiple regression model was developed for the weather-based prediction of DON contents in maize grains. The model predicted DON contents in Austria in 2014 86 % and in 2015 89 % correctly.

Rath, J.; Herrmann, A.; Höppner, F. (2005): MaisProg - Abreife und Ernte von Silomais schätzen. Mais 3, 94 – 96.

L21

Influence of agricultural practices and weather conditions on the occurrence of *Fusarium* toxins in Spanish local crops

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Fusarium fungi are important in the cereal food chain because can reduce crop yields and contaminate maize grains with mycotoxins. A tool for the prevention and reduction of *Fusarium* mycotoxins in maize crops is the application of Good Agricultural Practices (GAP) during production and harvest, taking into account the local crops, climate, and agronomic practices.

The objective of the study was to monitor *Fusarium* toxins contamination in locally grown maize to identify risk factors for mycotoxin contamination. Five maize growing areas located in Aragón (NE Spain) were selected in 2016 and 2017 crop seasons. Detailed information on agricultural practices were obtained from farmers, including date of harvest, previous crop, pests, crop residues management, fertilization, irrigation and water stress between flowering and harvest. Maize samples were analyzed for deoxynivalenol (DON), fumonisins (FUM), zearalenone (ZEA) and T-2/HT-2 toxins by quantitative lateral flow devices and confirmation by liquid chromatography with appropriate detectors.

DON and FUM were detected in 4 out of 5 locations on both crop seasons ranging from 580-3,500 µg/Kg and 360-4,600 µg/Kg, respectively, in different harvesting dates. Other mycotoxin detected was ZEA (70-320 µg/Kg), while none of the samples was contaminated with T-2/HT-2 toxins. The highest levels of DON and ZEA in maize may be related with high temperatures during flowering coupled with sprinkler irrigation as well as second-crop maize under direct sowing. FUM occurrence was relevant in maize crops affected by corn-borers and over-fertilized with nitrogen (710 to 4,600 µg/Kg). Regarding European legislation, some maize samples surpassed the maximum levels in 2 areas for at least one mycotoxin. These samples belonged to a delayed harvesting of grain (after 15th of November).

These preliminary results confirm that local environmental conditions and agricultural practices can play a relevant role in mycotoxin contamination in maize. The study will be extended during at least one more crop season to obtain results that will assist producers regarding the environmental and agronomic risk factors that promote mycotoxin contamination in local maize crops.

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L22

Leaching of the mycotoxins deoxynivalenol and zearalenone from contaminated maize residues – a potential risk for agricultural soils?

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Unusual patterns and distributions of toxigenic phytopathogens may lead to an increased plant disease incidence and higher mycotoxin levels in crop products and crop residues due to climate change. By leaching from host tissue of infected plants, mycotoxins may pose an environmental contamination risk of soil and water. In agroecosystems, fungivorous soil organisms can be antagonistic to *Fusarium* infection and mycotoxin contamination and act, therefore, as biological and chemical regulators. Against this background, a microcosm-study was conducted to assess the leaching potential of the mycotoxins deoxynivalenol (DON) and zearalenone (ZEN). Furthermore, the biocontrol-impact of earthworms (*Aporrectodea caliginosa*) and collembolans (*Proisotoma minuta*) on the fungal plant pathogen *Fusarium graminearum* and its mycotoxins in finely chopped maize stubbles, soil, and leaching water should be quantified. Soil fauna was exposed for a time span of 6 weeks to artificially-infected maize stubbles highly contaminated with DON (10,462 µg/kg) and ZEN (2,780 µg/kg) considering two different temperatures (17°C, 25°C). At the end of the experiment, each microcosm was divided into two sections. One section was sampled immediately to gain residual maize stubbles and soil. The other section was irrigated with 200 ml of tab water before sampled accordingly. Residual maize stubbles (irrigated/non-irrigated), soil and leaching water samples were analysed for mycotoxins via liquid chromatography coupled to high resolution mass spectrometry.

Considering DON, 67-82% of the mycotoxin content in the residual maize stubbles leached from the plant material by irrigation and was recovered in soil (1.9-3.4 µg/kg) and leaching water (12-295 µg/l). In the case of ZEN, 27-50% of the mycotoxin leached from the residual maize stubbles due to watering, but was only occasionally detected in traces in the soil and not found in the leaching water.

A faunal effect was observed concerning the reduction of DON and ZEN in residual maize stubbles, but this effect is obviously influenced by the temperature regime.

In conclusion, the results clearly reveal a leaching potential of both DON and ZEN, respectively. The soil fauna may be involved in the natural self-regulation of the soil system. Still little is known about the ecotoxicological consequences or possible combinatory effects of different mycotoxins.

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L23

High physical properties of biochar issued from cashew nut shell to adsorb mycotoxins (aflatoxins and ochratoxin A) and its effects on toxigenic molds

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Biochar is a microporous and adsorbent solid carbon product obtained from the pyrolysis of various organic materials (biomass, agricultural waste). Biochar is distinguished from vegetable charcoal by its manufacture methods. Biochar is used as the amendment in soils to give them favorable characteristics under certain conditions, i.e absorption of water and its release at low speed.

Cashew nuts shell from Mali is usually discarded on land by local processors or burnt as a mean for waste management. The burning of this biomass poses serious socio-environmental problems including greenhouse gas emission and accumulation of tars and soot on houses closed to factories, leading to neighbor complaints.

Some mycotoxins as Aflatoxins are carcinogenic compounds resulting from the secondary metabolism of molds that develop on plants in the field and during their conservation. They are found at high level on some seeds and nuts in Africa. Ochratoxin A , member of mycotoxins, is produced by various species of *Aspergillus* and *Penicillium*. Human exposure to Ochratoxin A can occur through consumption of contaminated food products, particularly contaminated grain, as well as coffee, wine grapes.

We showed that cashew shell biochars produced at 400, 600 and 800°C adsorbed aflatoxins (B1, B2, G1, G2) at 100% by filtration (rapid contact) as well as by stirring (long contact). The average percentage of adsorption of Ochratoxin A was 35% by filtration and 80% by stirring. The duration of the biochar-mycotoxin contact was a significant parameter.

The effect of biochar was also tested on two strains of toxigenic molds: *Aspergillus parasiticus* (producers of Aflatoxins) and *Aspergillus carbonarius* (producers of Ochratoxins). The growth of the strain *Aspergillus carbonarius* was inhibited at up to 60% by the biochar at 600°C. An opposite effect to the inhibition was observed on *Aspergillus parasiticus* using the same biochar.

In conclusion, we observed that biochar adsorbs mycotoxins: Aflatoxins and Ochratoxin A to different degrees; 100% adsorption of aflatoxins under all conditions (filtration and stirring) and adsorption of Ochratoxin A varied depending on the type of biochar and the experiment conditions (35% by filtration and 85% by stirring).

The effects of biochar at 600 °C on the toxigenic molds: *Aspergillus parasiticus* and *Aspergillus carbonarius*, varied according to the experimental conditions and the strains. We observed an opposite effect on the growth with an inhibition of *Aspergillus carbonarius* up to 60% and a stimulated growth of *Aspergillus parasiticus*.

L24

The effects of feeding diets with *Fusarium* toxin contaminated maize treated with graded concentrations of sodium sulfite (SoS) on porcine health and adaptive immunity

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The effects of *Fusarium* toxins and especially deoxynivalenol (DON) as one of the most relevant toxins under central European production conditions, on the porcine immune system is of particular interest. However, controlled in vivo trials addressing the adaptive immunity of pigs are scarce. In addition, decontamination strategies may also affect pig health by themselves or in interaction with *Fusarium* toxin contamination. Therefore, the aim of the present study was to address these questions in fattening pigs using an influenza vaccination to depict the functionality of the adaptive immune system.

Material and Methods: Ninety-six barrows (BHZP, n=16/diet) with an initial body weight (BW) of 32.5 ± 3.4 kg were housed individually in floor pens and fed a diet containing either 10 % uncontaminated or DON contaminated maize (44.4 mg DON/kg maize) that was previously wet preserved with one of three sodium sulfite (SoS) levels (0, 2.5, 5.0 g/kg maize) and 15 g propionic acid per kg maize at 20% moisture content for 63-70 days. Each of the resulting six feeding groups (CON-, CON2.5, CON5.0, DON-, DON2.5 and DON5.0) were further subdivided: half of the animals were vaccinated against influenza (H3N2, H1NA, H1N2; Respiporc FLU3, IDT Biologika GmbH, Dessau-Roßlau, Germany) or injected with physiological saline as placebo (all intramuscular, 1st vaccination experimental week 4, booster 3 weeks later). Blood samples were taken at the first day of trial and in week 4, 7, 8 and 10 and vaccine titers were determined by ELISA. Parameters of clinical chemistry were analyzed on an automated analyzer (total protein, albumin, urea, glucose, triglycerides, cholesterol, total bilirubin, aspartate aminotransferase, alanine aminotransferase, γ-glutamyltransferase, alkaline phosphatase) for the first day of trial, week 4 and week 10.

Results: Pigs receiving influenza vaccination established protective titers already one week after the booster whereas all pigs injected with the placebo were negative at all sampling times. The titer formation was neither affected by the contaminated maize nor by the detoxification treatment of the maize with SoS. Most parameters of clinical chemistry showed an impact of time but were hardly influenced by the treatments.

Conclusion: The results of the present investigation demonstrated that neither dietary DON concentrations of up to 4.3 mg DON/kg diet nor SoS treatment of the feedstuff resulted in an alteration of the response of the adaptive immune system of the animals with respect to antibody titers.

L25

Influence of ascorbic acid on patulin concentration in cloudy apple juice

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Moulds have the ability to produce toxic secondary metabolites, mycotoxins, which, unlike their producing organisms, cannot be eliminated by cooking or by the various treatments of food/feed. According to the Food and Agriculture Organization (FAO) of the United Nations about 25% of food is contaminated with mycotoxins. This contamination affects foodstuffs at all stages of the food chain, causing considerable economic damage and having severe health consequences.

Patulin (PAT), is most commonly encountered in products derived from fruit processing, especially apples. The contamination is mainly caused by *Penicillium expansum*. Many studies report the natural presence of PAT in various fruit processing products such as juices, apple compotes or jams. Its presence in food is regulated and maximum levels of 50 µg/kg for fruit juices, 25 µg/kg for apple purees and compotes and 10 µg/kg for food intended for babies and young children have been set by the European Union. Despite these regulations, PAT is still being found in commercial food and/or beverage products, sometimes exceeding the maximum limits. Along the food processing chain, patulin content can be affected by factors like storage, handling, transport, pasteurization, filtration, etc. Other non-thermal processing techniques can affect the PAT content. In our study, we were interested in the effect of ascorbic acid (AA) on PAT degradation.

AA, often used in the food industry, is a powerful antioxidant resulting in an inhibition of browning reactions caused by oxidation of polyphenols. It can also be used to improve product quality (e.g. colour, palatability) and restore possible nutritional losses that occur during processing. In addition, AA has been described to act as a nucleophile, increasing PAT degradation.

To study its impact on PAT in cloudy apple juice, the addition of different concentrations of AA was evaluated, in presence or absence of oxygen, under different storage temperatures. The same experiment was performed on pure PAT standard in aqueous solution. The analysis showed that the highest PAT reduction rate of 60% was achieved after 6 days of incubation, with initial PAT concentration of 100 ppb and 0.25% (w/v) AA, respectively. It was also found that the treatment by AA resulted in generation of degradation products less toxic than PAT: Z/E-ascladiol and desoxypatulinic acid (DPA). In conclusion, these data suggest that AA, approved by FDA and described as a food additive improving numerous products qualities, also has an effect on toxins such as patulin thereby generating less toxic compounds.

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L26

Uptake and biotransformation of T2 and HT2 in micropropagated *Triticum durum* Desf.

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Plants employ different detoxification mechanisms to cope with the adverse effect of xenobiotics, such as mycotoxins. Multiple enzymatic pathways may lead in the formation of modified mycotoxins that, once entered the food and feed production chain, may significantly contribute to the overall toxic load related to mycotoxin exposure. Next to other mycotoxins, type A trichothecenes T2 and HT2 toxin inhibit protein synthesis and cell proliferation, thus they represent a high-risk potential for chronic intoxication of humans and animals. Because of their toxic potential, EFSA CONTAM Panel set a group TDI of 0.02 µg/kg b.w. for the sum of T2 and HT2 toxin (EFSA, 2017). Understanding the plant metabolism of mycotoxins and thus the toxicological role of resulting modified forms is becoming increasingly important for risk assessment. Up to now only few data of plant metabolites of T2 and HT2 toxin is available.

In vitro techniques represent a consolidated approach to investigate the metabolic fate of mycotoxins enabling the characterization of phase I and II biotransformation products. For this purpose, an in vitro model was set up to elucidate the uptake and metabolic fate of T2 and HT2 in durum wheat. Five wheat varieties with different levels of resistance, namely Cysco, Normanno, Iride, Kofa and Svevo were chosen. Leaves and roots experiments were independently set up to study the organ- and tissue-biotransformation dependency. To monitor the extent of mycotoxin absorption, its concentration was determined in the growing media (t=0, t=24h, t=7d and t=14d) by targeted LC-MS/MS analysis. The varieties, considering to the different plant cultures, were compared to illustrate the differences in mycotoxin uptake from the media. An almost complete and quick absorption of T2 toxin and contemporaneous formation of HT2 was noticed, whereas HT2 showed a slower uptake by uninfected plant organs.

After treatment with T2 and HT2, roots and shoots were profiled with ultra-high performance liquid chromatograph-high resolution mass spectrometry (LC-HRMS). A large spectrum of phase I and phase II biotransformation metabolites were depicted.

L27

Enzymatic synthesis of 3-lactyl-deoxynivalenol and 3-propionyl-deoxynivalenol

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Treatment of contaminated grain with lactic acid bacteria instead of drying is an increasingly suggested treatment method to reduce the DON content of low quality grain. We wondered whether the enigmatic compound 3-lactyl-DON might be generated under these conditions. This mycotoxin "responsible for vomiting in humans and swine" was isolated in China from naturally contaminated barley already in 1982. Structural characterization of the substance initially called CDB2 indicated that this compound was 3-lactyloxy-7,15-dihydroxy-12,13-epoxy-trichothec-9-en-8-one or 3-lactyl-deoxynivalenol. Theoretically 3-lactyl-DON could be a metabolite produced by (DON challenged) lactic acid bacteria, by plant cells (in the presence of DON and lactic acid), or by (lactic acid stressed) *Fusarium*. We tested the hypothesis, that the Tri101 acetyltransferase of *Fusarium graminearum* can also utilize lactyl-CoA instead of acetyl-CoA to acylate the C3-OH of DON. Lactate is normally utilized via lactate dehydrogenase and pyruvate-dehydrogenase and no lactyl-CoA is formed. Yet, *Escherichia coli* grown on propionic acid as sole carbon source induces a propionyl-CoA synthase (prpE) that can also act as lactyl-CoA synthase (ATP + CoA + lactate = lactyl-CoA + AMP + PPi). Combining recombinant prpE from E. coli and FgTri101 protein in vitro with the substrates allowed enzymatic synthesis of both 3-lactyl-DON and 3-propionyl-DON in good yield. The substances were purified by preparative HPLC and the structure confirmed by NMR. We could furthermore show by expression in *E. coli* that also *F. graminearum* possesses a functional propionyl-CoA synthase gene. Knockout strains have been prepared to test whether this gene is necessary for formation of the acyl-DON-derivatives. The available 3-lactyl- and 3-propionyl-DON substances are used to investigate the toxicological properties of these novel (or "re-emerging") modified mycotoxins, and to characterize conditions leading to lactyl/propionyl-DON formation.

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L28

Evaluation of concentrations of deoxynivalenol (DON), zearalenone (ZEN) and their metabolites in physiological samples of piglets as diagnostic markers

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The usefulness of concentrations of DON, ZEN and their metabolites in physiological samples of pigs for diagnostic purposes is still not well established. The experimental conditions in previous studies, as e.g. special feeding regimes before sampling or limited variation in the exposure limit the validity of the data under practical conditions. Therefore, the main aim of this experiment was to determine the relationship between DON, ZEN and their metabolites in diets and physiological samples of pigs over a wide exposure range and under ad libitum feeding conditions. Additionally, effects on peripheral circulation, as a possible cause of peripheral necrosis should be assessed.

To obtain diets containing increasing concentrations of DON and ZEN, a control diet and a *Fusarium* toxin contaminated diet, including 12.5% contaminated maize, were produced. Those two diets were gradually blended to get 10 separate diets containing increasing concentrations of DON (0.2 – 7.4mg/kg) and ZEN (0.01 – 0.36 mg/kg). Those 10 diets were fed to 80 female piglets, which were housed in 20 slatted floor pens with 4 piglets per pen (2 pens/diet) for three weeks. Individual life weight and feed consumed per pen were recorded weekly. Rectal and peripheral surface temperatures (at tail and ears) were also recorded once a week. At the end of the feeding period piglets were slaughtered and blood, urine, bile and liquor were sampled for the analysis of DON, ZEN and their metabolites by LC-MS/MS. In addition, organ weights were recorded and samples of tails and ears were taken for histological analysis.

Linear regressions between diet concentrations and serum concentrations of DON (r2=0.767) and ZEN and its metabolites (r2=0.574) could be established. The peripheral surface temperatures do not hint at a link to the concentrations of *Fusarium* toxins in the diet. Further results will be presented.

L29

Identification and characterization of novel DON metabolites in different animal species

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The mycotoxin deoxynivalenol (DON) is a frequent contaminant of food and feed. Mammals detoxify DON by glucuronidation, the extent and regioselectivity of which is species-dependent. So far, only DON-3-glucuronide (DON-3-GlcAc) and DON-15-GlcAc have been unequivocally identified as mammalian DON glucuronides, and DON-7-GlcAc has been proposed as further DON-metabolite. In the present work, qualitative analysis of urine samples of pigs, rats, mice and cows treated with DON revealed additional DON and deepoxy-DON (DOM) glucuronides. To elucidate their structures, DON and DOM were incubated with human (HLM) and rat liver microsomes (RLM). In addition to the expected DON/DOM-3- and 15-GlcAc, minor amounts of 4 DON- and 4 DOM-glucuronides were formed. Isolation and enzymatic hydrolysis of four of these compounds yielded iso-DON and iso-DOM, the identity of which was eventually confirmed by NMR. Incubation of iso-DON and iso-DOM with RLM and HLM yielded two main glucuronides for each parent compound, which were isolated and identified as iso-DON/DOM-3-glucuronide and iso-DON/DOM-8-glucuronide by NMR measurements. Iso-DON-3-GlcAc, most likely misidentified as DON-7-GlcAc in the literature, proved to be a major DON metabolite in rats and a minor metabolite in pigs. In addition, iso-DON-8-GlcAc turned out to be one of the major DON-metabolites in mice. DOM-3-glucuronide was the major DON-metabolite in urine of cows and an important DON metabolite in rat urine. Iso-DOM-3-GlcAc was detected in urine of rats and cows. Finally, DON-8,15-hemiketal-8-glucuronide, a previously described by-product of DON-3-GlcAc production by RLM, was identified in urine of mice and rats.

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L30

Impact of T-2 toxin and zearalenone on the pharmacokinetics of midazolam as CYP3A probe drug in an in vivo porcine animal model

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Cytochrome P450 enzymes (CYP450) are important catalyzing proteins, involved in the biotransformation and elimination of endogenous and xenobiotic compounds. However, ingestion of xenobiotics like drugs, food/feed, or food/feed-contaminants, can alter the CYP450 activity. Consequently, co-ingestion of drug and/or food/feed can lead to altered disposition of a victim drug, leading to either less efficacy or toxic responses. Nonetheless, regarding drug-food contaminant interactions, literature reports are scarce. Mycotoxins are an example of food and feed contaminants which are highly prevalent secondary metabolite produced by several fungal species (1). Pigs are a very sensitive species towards the effect of mycotoxins, such as T-2 toxin (T-2) and zearalenone (ZEA). In addition, the pig has been increasingly proposed as a valuable animal model in pharmacological and toxicological research, due to its resemblance in anatomy and physiology with humans (2,3). Previous in vitro work at our laboratory has shown that T-2 and ZEA can potently inhibit CYP3A activities in porcine microsomes (4).

The present study aimed to evaluate the potential in vivo impact of T-2 and ZEA on the drug metabolizing CYP3A enzymes. Eight pigs (7 weeks of age, 4 males and 4 females) were fed two times daily with control feed or feed contaminated with T-2 (500 µg/kg feed) or ZEA (500 µg/kg feed) for a two week period. Subsequently midazolam, a CYP3A probe substrate, was intravenously administered (dose, 0.035 mg/kg BW) and blood samples were collected at regular time intervals. After a two day rest period, an oral dose (0.15 mg/kg BW) was given and blood samples were collected as before. Afterwards, pigs were euthanized and liver and intestinal mucosa samples (duodenum, jejunum, and ileum) were collected for the preparation of microsomes. Plasma concentration-time data of midazolam and in vitro assessment of the CYP450 activity will be used to model the impact on the pharmacokinetics of midazolam. The results of the analysis and model development will be presented at the congress during an oral presentation.

1. Lee HJ, Ryu D. Worldwide Occurrence of Mycotoxins in Cereals and Cereal-Derived Food Products: Public Health Perspectives of Their Co-occurrence. J Agric Food Chem. 2017;65(33):7034–51.
2. Puccinelli E, Gervasi PG, Longo V. Xenobiotic metabolizing cytochrome P450 in pig, a promising animal model. Curr Drug Metab. 2011;12(6):507–25.
3. Swindle MM, Makin A, Herron AJ, Clubb FJ, Frazier KS. Swine as Models in Biomedical Research and Toxicology Testing. Vet Pathol. 2012;49(2):344–56.
4. Schelstraete W, Devreese M, and Croubels S. 2017. "Impact of *Fusarium* Mycotoxins on in Vitro Activity of Major Hepatic Cytochrome P450 Biotransformation Enzymes in Pigs." Presented at the 1st MycoKey International Conference, "Global Mycotoxin Reduction in the Food and Feed chain", 11th -14th September 2017.

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L31

Fusarium toxin contaminated diet modified lipopolysaccharide (LPS)-induced acute cellular immune response in pigs

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It was recently shown that chronic oral exposure to *Fusarium* toxin contaminated feed modulates the metabolizing and detoxifying properties of the liver in pigs suffering from a systemic inflammation as induced by systemically administrated lipopolysaccharides (LPS) (Bannert et al. 2015, Tesch 2015, Renner et al. 2017). Little is known about the immune modulating effect of *Fusarium* toxins on the cellular immune response in pigs *in vivo*. Thus, the aim of the present study was to determine the role of chronic oral *Fusarium* toxin exposure on the blood leukocyte function and the interaction between *Fusarium* contaminated feeding and endotoxin-induced innate immune response in young pigs. For this purpose, barrows (n=44) were fed either a mycotoxin-contaminated (4.59 mg deoxynivalenol (DON)/kg feed) or a control (CON) diet for four weeks. At the end of the feeding trial, pigs were equipped with indwelling catheters (~48h recovery) to facilitate a 60 min pre- and post-hepatic (portal *versus* jugular catheter) infusion of either physiological saline (CON_CONjugular-CONportal, DON_CONjugular-CONportal) or LPS (7.5µg/kg body weight, CON_CONjugular-LPSportal, CON_LPSjugular-CONportal, DON_CONjugular-LPSportal, DON_LPSjugular-CONportal). Blood samples (*Vena jugularis interna* and *Vena portae hepatis*) were collected frequently until 180 min *post infusionem* and were used for flow cytometric analysis. In order to characterize and determine lymphocyte subsets we used a panel of monoclonal antibodies against porcine CD3 (T-cells), CD4 (T-helper cells), CD8 (cytotoxic T-cells), CD21 (B-cells) and CD14 (monocytes) cell surface molecules. Furthermore, we characterized the cellular function of neutrophils by quantifying the intracellular free radical production, oxidative burst capacity and phagocytosis of fluorescence-labeled bacteria. Although chronic oral *Fusarium* toxin exposure had no impact on the proportion of T- or B-cell populations in blood our results show a changed pattern of LPS-induced acute cellular immune response in pigs which were fed with a *Fusarium* contaminated diet compared to their CON-fed counterparts. In conclusion, our results suggest immune modulating potential of *Fusarium* contaminated diet in endotoxemic pigs.

References:

Bannert E, Tesch T, Kluess J, Frahm J, Kersten S, Kahlert S, Renner L, Rothkötter HJ, Dänicke S. (2015) Metabolic and hematological consequences of dietary deoxynivalenol interacting with systemic *Escherichia coli* lipopolysaccharide. Toxins (Basel). 2015 Nov 16;7(11):4773-96.

Tesch T, Bannert E, Kluess J, Frahm J, Kersten S, Breves G, Renner L, Kahlert S, Rothkötter HJ, Dänicke S. (2015) Does Dietary Deoxynivalenol Modulate the Acute Phase Reaction in Endotoxaemic Pigs?--Lessons from Clinical Signs, White Blood Cell Counts, and TNF-Alpha. Toxins (Basel). 2015 Dec 23;8(1).

Renner L, Kahlert S, Tesch T, Bannert E, Frahm J, Barta-Böszörményi A, Kluess J, Kersten S, Schönfeld P, Rothkötter HJ, Dänicke S. (2017) Chronic DON exposure and acute LPS challenge: effects on porcine liver morphology and function. Mycotoxin Res. 2017 Aug;33(3):207-218.

L32

Combinatory effect of glutathione-modulation on the toxicity of deoxynivalenol (DON) and NX-3 and its co-regulated metabolite butenolide (BUT)

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Various secondary metabolites of *Fusarium*, such as the trichothecenes DON and NX-3 or BUT, have been reported to trigger ROS production and oxidative damage in mammalian cells[1,2]. Glutathione (GSH) plays on the one hand a central role in the maintenance of the intracellular redox homeostasis and represents on the other hand a crucial substrate to detoxify thiol-reactive xenobiotics. Previous studies argue for an involvement of GSH in the detoxification of BUT and DON[2,3].

In the present study, we addressed the question whether the intracellular GSH level affects the toxicity of DON and NX-3 and therefore plays a crucial role in the respective detoxification processes. Furthermore we investigated whether the co-occurrence of BUT, reported to decrease the intracellular GSH-level, might enhance the toxicity of DON and NX-3. Pre-treatment of human liver cells (HepG2) with L-buthionine-[S,R]-sulfoximine, a well-known inhibitor of GSH synthesis, aggravated substantially BUT-induced cytotoxicity but only marginally affected the cytotoxicity of DON and NX-3. In line with literature, BUT reduced intracellular GSH after short term incubation. However, after 24 h incubation a substantial increase of the intracellular GSH levels was observed for DON, NX-3 and, even more pronounced, for BUT. Thus, for combinatory cytotoxicity experiments, the experimental design with respect to time and concentration ratios of pre- or co-incubation was found to be critical, but in most conditions the combination with BUT resulted in stronger toxic effects than DON would have induced as single compound.

These combinatory effects underline the potential contribution of non-regulated secondary metabolites like BUT, considered as *per se* non-toxic, to the toxicity of co-occurring mycotoxins such as DON or structurally related trichothecenes, arguing for further studies on the toxicological relevance of these metabolites in naturally occurring mixtures.

References: [1]Mishra et al., 2014, Toxicol Appl Pharmacol; [2]Wang et al., 2006, Toxicol Lett; [3]Stanic et al., 2016, J Agric Food Chem.

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L33

Tubulo-interstitial cross talk modulates the impact of ochratoxin A (OTA) on renal epithelial cells and renal fibroblasts

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Introduction: Ochratoxin A (OTA) is a potential nephropathic substance. The process of epithelial-to-mesenchymal-transition (EMT) is a crucial event in the development of interstitial nephropathies. During EMT epithelial cells dedifferentiate to mesenchymal cells with loss of epithelial junctions, enhanced expression of mesenchymal proteins like fibronectin. Furthermore, it is suggested that EMT contributes to the development of interstitial fibrosis due to an epithelial-fibroblast cross talk via soluble mediators (e.g. cytokines, prostaglandins and microRNAs (miRs)).

Based on this knowledge, our hypothesis is that tubule-fibroblast cross talk modulates the effects of OTA.

Material/Methods: Our experiments were performed in epithelial cells derived from proximal tubule of the rat (NRK-52E) and renal fibroblasts of the rat (NRK-49F) under mono- and co-culture conditions. Cells were incubated with 10-100nM OTA for 48h. We investigated the impact of OTA on two EMT-relevant proteins (E-cadherin and fibronectin) and two proteins, which can contribute to the cross talk (WISP1 and COX-2), via western blot. Alterations of their mRNA-contents were analyzed by RT-qPCR. Moreover the abundance of miR-21, -29b and -200a were measured by RT-qPCR.

Results: NRKE: Under monoculture conditions, OTA affected only the following targets: E-cadherin protein content was decreased and the abundance of the measured miRs were decreased. By contrast, under co-culture conditions OTA induced an increase of fibronectin protein amount and WISP-1 mRNA abundance in addition to a decrease of E-cadherin and COX-2 protein content. These protein changes are not reflected by changes in mRNA abundance. The amount of miR-21 and -200a was increased, whereas miR-29b was unchanged.

NRKF: In monoculture, OTA caused an increase of collagen I and fibronectin protein amount whereas COX-2 and WISP1 protein amount was unchanged. Moreover the abundance of miR-200a was increased. By contrast, in co-culture OTA had no impact on the collagen I or fibronectin protein amount whereas COX-2 and WISP1 protein content were increased. OTA led to an increase of the abundance of COX-2 mRNA and miR-29b.

Conclusion: Our data show that OTA supports the development of EMT. Moreover, our results suggest that the effects of OTA are significantly affected by an epithelial-fibroblast crosstalk, making it worthwhile to study its impact in more complex cell systems in order to evaluate its pathogenetic potential.

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L34

Analysis of the interactions between the mycotoxin deoxynivalenol and cadmium, an environmental contaminant in different target organs

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Most of the toxicological data concern the effects of food contaminants when present alone; however, food and feedstuffs are usually contaminated by more than one toxic compound. Mycotoxins such as deoxynivalenol (DON) and Cadmium (Cd), a common and widespread toxic heavy metal, are frequent contaminants of agricultural commodities worldwide. This raises concern of about consumers' exposure to the cocktail of DON and Cd. However, the co-contamination of DON and Cd has never been addressed.

To characterize their toxicity in combination, their effects alone or in mixture were investigated in different human cell lines from intestine (Caco-2), kidney (HEK-293), blood (HL-60) and liver (HepG2). Cytotoxicity, chosen as readout, was assessed through ATP measurement. The nature of the interactions between Cd and DON was determined by the Isobologram-Combination index method.

Caco-2 and HEK-293 cells, models of first barrier against food contaminants and target organ of both Cd and DON respectively, were exposed to increasing doses of DON, Cd and their mixture at different ratios corresponding to plausible scenarios of exposure (DON/Cd of 2/1; 1/1; 1/2 and 1/8). Whatever the ratio, for increasing cytotoxicity, the interactions observed ranged from additivity to antagonism in Caco-2 cells and from moderate antagonism to additivity in HEK-293 cells. At ratio DON/Cd of 1/1, for increasing cytotoxicity, the interactions ranged from synergy to antagonism in HL-60 cells and from antagonism to synergy in HepG2 cells.

Our results show that the consequences of a combined exposure to food and environmental contaminants differ depending on the cell line. This suggests that the effects could vary in different target organs. The interactions between DON and Cd should be studied in whole animals to confirm these data before they can be used for risk assessments.

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L35

A target fishing approach to explore the mechanism of action of emerging mycotoxins - a case study on alternariol

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Food may be contaminated by fungal secondary metabolites, which are referred to as “mycotoxin” when evoking toxicity. Most of them lack regulations due to the shortage of toxicity and occurrence data. In addition, the presence of still uncharacterized toxic metabolites cannot be excluded. To fill these knowledge gaps, first-line workflows are needed to assign putative activity, hierarchize the endpoints to test and guide experiments. In this framework, the activity of compounds can be assessed identifying unexpected biological targets via “target fishing”. Here, we present a proof of concept dealing with alternariol (AOH), an unregulated emerging mycotoxin produced by Alternaria spp with wide occurrence and multi-target toxicity. Currently, the understanding of its mechanism of action (MOA) is still fragmentary and cannot properly support risk assessment. Therefore, a target fishing study was used to explore the molecular basis of AOH toxicity. An *in silico/in vitro* workflow based on ligand-based virtual screening and structure-based modeling coupled to experimental trials for validation is presented.

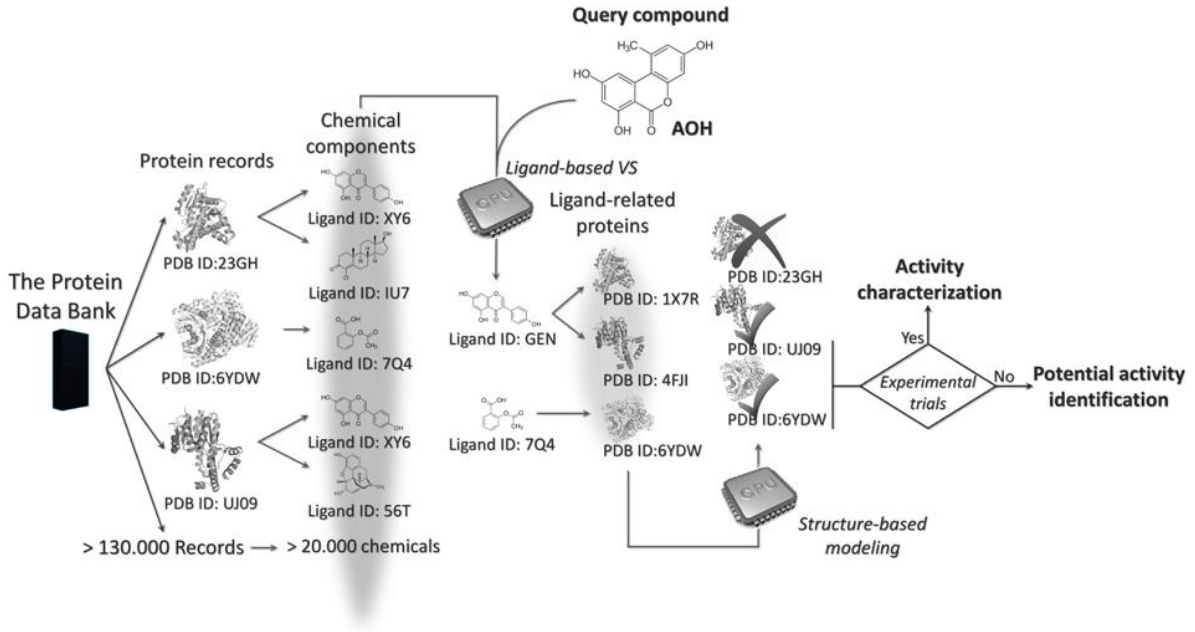

Figure: Target fishing. Known compounds underwent ligand-based screening to identify chemical similarity to AOH. Once identified the most similar ones, the interaction of AOH with the respective targets was assessed at molecular level to check activity.

The procedure was validated using atromentin, a fungal secondary metabolite produced by mushrooms and microfungi (including *Aspergillus* spp). For the first time, the procedure identified the estrogen receptors (ERs) among the possible targets predicting a mild estrogenicity. The activity was confirmed *in vitro* in Ishikawa cells using the estrogenic impact on alkaline phosphatase expression as read-out, proving the procedure reliability. After validation, the procedure was applied to AOH. Among the possible targets, we found ERs – in agreement with the current knowledge – as well as 17-β-hydroxysteroid dehydrogenase (17βHSD), an enzyme involved in estrogens production. The analysis of the binding pose in comparison to those reported by crystallographic studies of polyphenolic inhibitors revealed a strong fitting into the binding pocket. The inhibition 17βHSD was identified as an endpoint to test with priority to better understand the MOA of AOH. In addition, the effect of phase-I and –II metabolism on the ability to fit the enzyme pocket was assessed to identify which metabolites may mediate the effect *in vivo*. Overall, this work proved the efficacy of target fishing to identify molecular targets of molecules of food interest. This may succeed in gaining insights on the MOA of known mycotoxins, as in the case of AOH, or to early identify potentially adverse activity of poorly characterized fungal metabolites, as in the case of atromentin.

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L36

Estrogenicity of *Alternaria* toxins in complex mixtures

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Black molds of the genus *Alternaria* grow on a wide variety of food and feed items and are known to produce more than 120 secondary metabolites. Early studies indicated that alternariol (AOH), one of the most studied *Alternaria* toxins, might possess estrogenic activity. However, due to the apparent low estrogenic potency, this observation was not followed with high priority at that time. Recently we demonstrated that not only AOH but also its monomethyl ether (AME) and certain phase I metabolites possess estrogenic properties. The advances in mycotoxin analysis in the last decade demonstrated that contaminated food and feed might contain not only single mycotoxins but a spectrum of secondary fungal metabolites. In the present study we addressed the question whether estrogenicity might also be of relevance in complex mixtures of *Alternaria* metabolites.

The estrogenic activity was studied in Ishikawa cells, an endometrial carcinoma cell line, expressing both isoforms of the estrogen receptor. An estrogenic stimulus activates the expression of alkaline phosphatase (AlP). A complex extract of *Alternaria* alternata (DSM62010) infested rice failed to induce AlP expression up to 4 µg/mL, a concentration already inducing substantial DNA-damage (comet assay) and cytotoxicity. Characterisation of the extract by a LC-MS/MS-based multimethod revealed a cumulative concentration of about 0.4 µM of genotoxic members of the perylene quinone family (altertoxin II and stemphyltoxin III). In contrast, AOH and AME only reached levels in the lower nanomolar range, thus clearly below the concentrations estrogenicity could be expected. Therefore, due to the respective toxin pattern, genotoxicity of the perylene chinones dominated the cellular effect, impeding to reach estrogenic concentrations of AOH and AME in the complex mixture. These results indicate that estrogenicity of complex *Alternaria* metabolite mixtures not only depend on the presence of the estrogenic constituents but also on the formation, stability and degradation of genotoxic perylene chinones such as ATX-II. However it has to be pointed out that, so far, genotoxic perylene chinones are hardly found in processed food, raising the question whether these compounds might be degraded during food production. Such a loss of these dominant genotoxicants might open the stage for the remaining estrogenic metabolites to act.

L37

Fungal and mycotoxins burden in Portuguese bakeries

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Flour is a complex organic dust consisting of one or a mixture of several cereal grains (wheat, rye, millet, barley, oats or corn cereal) that have been processed or ground by milling. In addition, flour may contain a diverse number of contaminants, such as fungi and mycotoxins.

The aim of this study was to assess the exposure to fungal burden (fungi and mycotoxins) on 13 Portuguese artisanal bakeries applying active and passive methods as sampling strategy. Air samples of 100 liters (impaction method) and 600 liters (impinger method), surface samples, settled dust samples, and electrostatic dust cloths (EDC) were collected in each bakery. Quantification and morphological identification by culture-based methods as well as molecular detection of the toxigenic *Aspergillus* sections Flavi, Fumigati, Circumdati and Versicolores were performed in all collected samples. Mycotoxins in the air and settled dust samples were analyzed by LC-MS/MS system. Separation and detection was carried out using high performance liquid chromatograph (HPLC) Nexera (Shimadzu) with a mass spectrometry detector API 4000 (Sciex).

Fungal load in indoor air ranged from 0 to 2590 CFU·m−3 on MEA, with 30 out of the 49 (61.2%) air samples collected presenting higher fungal load when compared to the outdoor sampling. *Cladosporium* sp. was the most prevalent species in indoor air samples in both media (29.7% MEA; 48.7% DG18), followed by *Penicillium* sp. (22.3% MEA; 30.5% DG18). *Aspergillus* spp. was observed on air samples on MEA and DG18 (0.3 and 1.2%, respectively). Among *Aspergillus* genera, section Candidi was the most prevalent (62.5%) on MEA followed by Nigri (25%), whereas sections Candidi and Circumdati (37.9%) were more prevalent on DG18. *Aspergillus* section Fumigati was possible to detect in 22.4% on air, 27.8% on surface swabs and in 7.4% on EDC samples; section Versicolores was detected in one air sample through molecular tools. Only settled dust samples contained mycotoxins: ochratoxin A was detected in the settled dust from 10 bakeries, deoxynivalenol-3-glucoside in 9 bakeries, and deoxynivalenol and zearalenon in all the settled dust samples.

Considering the fungal and mycotoxins burden found is proposed that fungal and mycotoxins assessment should be always performed in order to guarantee an accurate risk characterization in this occupational environment.

L38

Unraveling the prevalence of zearalenone biomarkers of exposure in the Portuguese population: An elaborate biomonitoring study

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Zearalenone (ZEN) is a mycotoxin that occurs widely in food commodities with particular incidence in cereals. Due to chemical structures similar to the endogenous estrogen 17-β-estradiol, ZEN and its metabolites exert estrogenic toxicity. Therefore, it is crucial to assess ZEN exposure among the population. Biomarker-driven research appeared as a promising method to assess the mycotoxin exposure in humans. For this reason, ZEN metabolites such as α-zearalenol (α-ZEL), β-zearalenol (β-ZEL), α-zearalenal (α-ZAL), β-zearalenal (β-ZAL), zearalanone (ZAN) (phase I) and the glucuronides ZEN14GlcA, α-ZEL14GlcA and β-ZEL14Glc (phase II) were identified in biological fluids. With a potency factor of 60 relative to ZEN, α-ZEL is the most relevant metabolite in terms of human health. ZEN is characterized by a fast metabolism and excretion, therefore urine is the matrix commonly used to assess the exposure to this mycotoxin and its metabolites. To date, in Portugal, there is a lack of human studies to assess biomarkers of exposure to ZEN.

In the Scope of National Food, Nutrition, and Physical Activity Survey of the Portuguese General Population (2015-2016), 94 participants collected 24h-urine samples, and performed non-consecutive dietary assessments (two 24-hour recalls, 8-15 days apart). Following a salt-assisted matrix extraction, urine samples were analyzed using LC-MS/MS for the simultaneous determination of ZEN, α-ZEL, β-ZEL, α-ZAL, β-ZAL, ZAN and ZEN14GlcA.

ZEN and ZEN-14-GlcA were detected in 52% (36/69) and 14% (10/69) of the analyzed samples, with a mean concentration of 1.2 and 6.9 µg/L, respectively. The metabolites α-ZEL, β-ZEL, α-ZAL, β-ZAL, ZAN were not detected in the urine samples. Considering the 24h-urinary volume, the mean dietary excretion of ZEN and ZEN-14-GlcA was 1.5 and 7.8 µg/day, respectively.

The present biomonitoring study generates reliable data regarding the exposure of the Portuguese population to ZEN. These data are crucial to perform a more realistic risk assessment, contributing to the knowledge of factors that may be associated with this exposure.

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L39

Investigations on zearalenone exposure by urinary biomarkers in German adults

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The *Fusarium* mycotoxin zearalenone (ZEN) is known to exert estrogenic effects in rodents and domestic animals, with pigs as the most sensitive species. EFSA derived a tolerable daily intake (TDI) of 0.25 µg/kg b.w. for humans in 2011; it is now set as group health-based guidance value for ZEN and all its modified forms in light of the following [1]: ZEN is often found in various cereal crops and grain-based foods along with more polar metabolites formed by plants and fungi such as ZEN-O-β-glucosides and ZEN-14-sulfate. These modified forms contribute to ZEN exposure, as they are hydrolyzed in the gastrointestinal tract of animals and humans, and then metabolized to α- and β-zearalenol (α-ZEL and β-ZEL).

ZEN as well as α-ZEL and β-ZEL are mainly excreted as conjugates in urine which can enable a biomarker-based exposure assessment in human cohorts [2]. Here we report on a new study in a cohort of German adults (n=60) where ZEN and its metabolites were determined by a sensitive LC-MS/MS method after enzymatic hydrolysis of spot urines and immunoaffinity column cleanup of the analytes. Biomarkers were detected in all urine samples of the cohort (see table).

Analyte	Range (ng/mL)	Mean ± SD (ng/mL)	Median (ng/mL)
ZEN	0.04-0.28	0.10 ± 0.05	0.07
α-ZEL	0.06-0.45	0.16 ± 0.07	0.13
β-ZEL	0.01-0.20	0.05 ± 0.04	0.03

Interestingly, average urinary levels of α-ZEL, the more potent estrogenic metabolite, were higher than those of ZEN whilst β-ZEL (less estrogenic than ZEN) was found at lower levels than the parent mycotoxin. Similar results were found in 10 persons who collected multiple urine samples to provide more insight into intra-individual temporal fluctuations and inter-individual differences in ZEN biomarker levels. Despite some uncertainties in a back-calculation of urinary biomarker levels to ingested mycotoxin amounts, the exposure of most individuals in our cohort appears to be below the group TDI. But, in one person's urines the sum of all analytes was higher on two days (1.6 and 1.01 ng/mL) indicative of an exposure about twice the group TDI.

In conclusion, human biomonitoring can reduce existing uncertainties in consumer exposure to the estrogenic mycotoxin ZEN and its modified forms and aid in identifying persons at higher risk.

References

[1] EFSA Panel on Contaminants in the Food Chain (2016) Scientific opinion on the appropriateness to set a group health-based guidance value for zearalenone and its modified forms. EFSA Journal 14(4):4425 (46 pp); doi: 10.2903/j.efsa.2016.4425
[2] Mally A, Solfrizzo M, Degen GH (2016) Biomonitoring of the mycotoxin zearalenone: current state-of-the art and application to human exposure assessment. Arch. Toxicol. 90:1281–1292

L40

Human study on the pharmacokinetics of 2'R-ochratoxin A

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Ochratoxin A (OTA) is a toxic secondary metabolite produced by several fungal species of the genus *Penicillium* and *Aspergillus* that can contaminate food and feed. During food processing of contaminated raw materials, OTA can isomerize up to 26% to 2'*R*-ochratoxin A (2'*R*-OTA) at high temperatures as applied during roasting (Figure 1) [1]. So far, the only commodity where 2'*R*-OTA has been detected is roasted coffee. In human blood from coffee drinkers both ochratoxins can be found in concentrations between 0.02-0.41 ng/ml with the 2'*R*-OTA concentration being in average half of that of OTA but in certain cases even exceeds that of the parent compound [2]. The discrepancy between exposure and occurrence of 2'*R*-OTA in the human body, especially in comparison to OTA is an interesting yet unanswered phenomenon.

Possible explanations for these relatively high 2'*R*-OTA concentrations in human blood could be further unidentified sources of exposure, but also higher binding affinities to proteins especially to human serum albumin compared to OTA resulting in a longer biological half-life.
To identify further food sources for 2'*R*-OTA, over 30 thermal processed foodstuffs were analysed for OTA and 2'*R*-OTA by HPLC-MS/MS. Besides coffee samples 2'*R*-OTA was only detectable in trace levels in malt coffee powder whereby the 2'*R*-OTA/OTA ratio was comparable to coffee.
To evaluate a possible accumulation in human blood a human study was conducted. Seventeen healthy coffee drinkers had to refrain from coffee for eight weeks to have no exposure to 2'*R*-OTA during that time. To pursue the decrease of 2'*R*-OTA a venous blood sample from each participant was taken every second week and analysed for OTA and 2'*R*-OTA by HPLC-MS/MS using isotope labelled standards for quantification. The results of this human study will be presented and discussed.

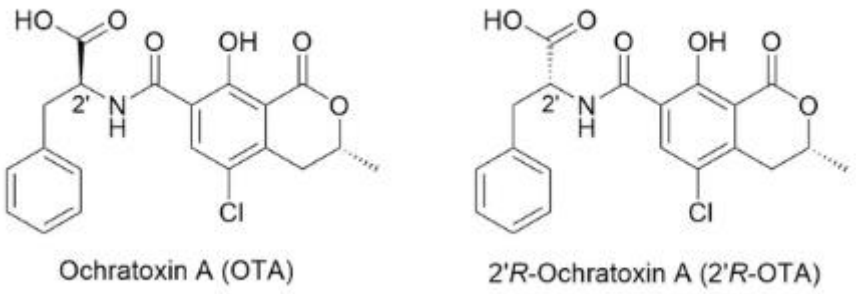

Figure 1: Molecular structures of OTA and its thermal isomer 2'*R*-OTA.

[1] Cramer, Königs, Humpf, J. Agric.Food Chem. 56 (2008) 5673–5681.
[2] Cramer, Osteresch, Muñoz, Hillmann, Sibrowski, Humpf, Mol. Nutr. Food Res. 59 (2015) 1837-1843.

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Abstracts of posters

P1

New derivative of fusaric acid in plants: Can *N*-methylamide reported half a century ago be confirmed?

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Fusaric acid (FA) is a well-known mycotoxin produced by numerous *Fusarium* species and the causative agent of wilting diseases of different crops. The involvement of FA in plant diseases was demonstrated by E. Gäumann for tomato already in 1952. After establishing that FA was detoxified by resistant tomato varieties, the structure of major detoxification product was determined by Klüpfel in 1957 as *N*-methylated amide of fusaric acid, based on the behavior of the radioactively labeled compound in paper chromatography and its chemical degradation.

The aim of our study was to verify the structure elucidation after more than half a century using current techniques. For this purpose, we selected tomato variety Money Maker (*S. lycopersicum*), which is susceptible to Fusarium wilt, and Red Currant (*L. pimpinellifolium*), which is resistant to Fusarium wilt. Tomato shootings were placed in FA solution until they had taken up a dose of 150 mg FA/kg fresh weight. After 72 h incubation, plants were harvested and their extracts were analyzed for the presence of FA and its derivatives by HPLC. 31% and 67% of unmodified FA were found in Red Currant and Money Maker varieties, respectively. Besides, several metabolites of FA were detected in acid and alkaline methanolic extracts of both varieties. The dominant metabolite, which was found in alkaline extracts and showed the same UV absorption spectrum as FA with a maximum at 271 nm, was purified for further reanalysis. The relative mass of positively ionized molecule of the metabolite was 194.1165. *N*-methylated amide of FA has a predicted m/z of 193.1335, which shows that the metabolite did not possess the structure published by Klüpfel. Considering that no metabolites with m/z close to 193.1335 were found in acidic and alkaline extracts, transformation of FA into the postulated compound in tomato appears questionable. Structural elucidation of the purified metabolite is in progress.

P2

Mycotoxins meet phytoestrogens: Combinatory effects of endocrine active compounds in beer

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Some mycotoxins produced by *Fusarium* spp. like zearalenone (ZEN) and α-zearalenol (α-ZEL) are known to possess endocrine disruptive potential due to their structural similarity to the human 17β-estradiol (E2).

As humans are typically not exposed to isolated, but to co-occurring compounds, complex mixtures have to be taken into account. For example, ZEN and α-ZEL - which are mainly found in cereals and cereal-based foods – might be ingested together with polyphenols present in beer. Those are predominantly originating from the use of hops during the brewing process, like the phytoestrogen 8-prenylnaringenin (8-PN) and the anti-oxidative and chemopreventive agent xanthohumol (XAN). Though XAN does not show estrogenic effects per se, previous studies indicate a role as modulator of estrogenicity.

In the present study, we addressed the question whether hop polyphenols have the potential to interact with mycoestrogens regarding their estrogenic potential. Thus, ZEN, α-ZEL, 8-PN and XAN have been tested alone as well as in combinations in the estrogen-sensitive Ishikawa cell line. The reporter-enzyme alkaline phosphatase was used to assess the estrogenic stimulus of the substances alone, in combination and in comparison to E2.

By means of mathematical models, in particular the combination index, interactions have been identified. XAN and 8-PN were found to induce antagonistic effects on the estrogenicity of the selected *Fusarium* toxins. Especially XAN seems to ameliorate the estrogenic properties of ZEN and α-ZEL. Thus, hop polyphenols might have a protective impact on the adverse endocrine properties of *Fusarium* contaminations.

P3

Intestinal toxicity of the type B trichothecene mycotoxin fusarenon-X: Whole-transcriptome profiling reveals new signaling pathways

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The few data available on fusarenon-X (FX) do no support derivation of health-based guidance values for this mycotoxin, although preliminary results suggest higher toxicity compared to other regulated trichothecenes. Using histo-morphological analysis and whole-transcriptome profiling, the present study was designed to get a global view of intestinal alterations induced by FX. The well-described trichothecene deoxynivalenol (DON) served as a benchmark. FX exposure induced more severe intestinal histological alterations compared to DON. Intestinal inflammation was the hallmark of the molecular toxicity of DON, but also of FX. The dose-response analysis for FX revealed that benchmark doses for up-regulation of key-inflammatory genes expression were 4 to 45-fold higher than the previously reported ones for DON. Transcriptome analysis revealed that both mycotoxins down-regulated PPAR and LXR-RXR signaling pathways controlling lipid metabolism. Interestingly, several pathways including VDR/RXR activation, ephrin receptor signaling, and GNRH signaling were specific to FX and thus, discriminate the intestinal transcriptomic fingerprint of the two mycotoxins. Altogether, these results demonstrate that FX induces a more potent intestinal inflammation than DON. This study also reveals specific FX-targeted pathways, indicating that the toxicity of DON cannot serve as a benchmark for FX, and that toxicity evaluation of each trichothecene should be conducted separately.

The authors are grateful to the Bioinformatics platform from Toulouse Midi-Pyrenees génopole (GenoToul) for providing resources for the microarray analysis. This work was supported by the ANR-CESA project DON&Co and PHC Stefanik 35813XM.

P4

Ozone technology for the reduction of zearalenone contamination in whole maize flour and its impact on technological quality

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The study of alternatives to reduce mycotoxin contamination in grains and their products are necessary. The use of Ozone gas is a promising technology, being considered a green chemical process and Generally Recognized as Safe (GRAS). The aim of this study was to evaluate the effectiveness of ozone technology on the reduction of zearalenone (ZEN) concentration in whole maize flour (WMF), as well as also evaluating the impact of this technology on the product rheological properties. The study was performed on WHF sample naturally contaminated with ZEN (958 ± 293 µg/kg), exceeding the maximum limits of both Brazilian and European regulations. Ozone treatment was applied at a flow rate of 0.8 L/min and concentration of ~51±3.4 mg/L in the gas stream, under different exposure time. The initial sample moisture content was 10.8%. ZEN analysis were performed using methanol:water as extraction solvent; Immunoaffinity columns as cleaned up step and HPLC/fluorescence as the mycotoxin detection/quantification. The ZEN concentration was significantly reduced ($p<0.05$) by the ozone processing: the mean reduction after 5 min up to 60 min ranged from 40 to 62.3%. The rheological properties of the ozonated WMF was evaluated through the Rapid Viscometer Analyser (RVA) and changes were observed in the visco-amylographic profile of the ozonated flour. Although the ozone technology was effective in the degradation of ZEN in whole maize flour, more studies are needed to better understand the effects on the product constituents and properties, as well as to define the most effective use for this process.

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P5

Mycotoxins and moulds in *Silybum marianum* L. Gaertn plants

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The botanical name for milk thistle is *Silybum marianum* (L.) Gaertn., a member of the plant family *Asteraceae*. It comes from the Mediterranean area: southern and south-eastern Europe, North Africa, West Asia and some regions of Central Asia (India, Pakistan). As other botanicals it can be contaminated with a variety of fungi and their secondary metabolites. Supplements based on milk thistle are widely consumed due to their possible beneficial effect on liver health.

The aim of this study was to evaluate mycotoxins and moulds contamination of *Silybum marianum* L. Gaertn. plant. The material included samples, which were divided into 2 groups: flowering plant and fruiting plant. Moulds and yeasts were determined using YGC medium (yeast extract, glucose and chloramphenicol). The results are expressed as the number of colony forming units (cfu) per gram of a sample. The identification of moulds was done to the genus. Mycotoxins determination was performed using HPLC-FLD method (immunoaffinity column AflaTest from Vicam-AF; immunoaffinity column OchraPrep from R-Biophram Rhône Ltd–OTA) and HPLC-MS/MS method (Bond Elut®Mycotoxin column from Agilent - DON, NIV, ZEN, T-2 and HT-2).

The mycological analysis of the plants showed significant levels of moulds content (an average of 2,6 x 10^5 cfu/g) and it was genus *Cladosporium* that prevailed (51%), followed by *Alternaria* (20%) and *Fusarium* (15%). In case of fruiting plant, the average mould contamination was 6,7 x 10^5 cfu/g with the prevalence of *Cladosporium* (52%) and *Alternaria* (21%) genera. The lowest level of moulds was found in the flowering plant 3,2 x 10^4 cfu/g with the prevalence of *Cladosporium* (25%) and *Fusarium* (17%).

The most frequently detected mycotoxin was ZEN, occurring in 47% of the samples. The highest level of this mycotoxin was ZEN_{max} = 823,45 ppb. Up to 27% of the samples contained DON, T-2, HT-2 and the highest concentration reached the value of 80,00 ppb, 451,67 ppb and 226,14 ppb, respectively. NIV was found only in one sample (NIV_{max} = 3,00 ppb). There was no AF or OTA found in the material.

On the basis of the results it can be concluded that the incidence of mycotoxins and fungal profile of *Silybum marianum* L. Gaertn plant is high. The manufacturers should take into account and monitor the level of mycotoxins contamination in raw material, which is intended for the production of supplements.

The study was supported from statutory funds related to the project entitled ‘Research on the occurrence and toxicity of secondary metabolites of moulds and identification of organisms by molecular biology methods’ (Ministry of Science and Higher Education, Republic of Poland)

P6

Fungal profile and mycotoxins in milk thistle herbal supplements

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Milk thistle (*Silybum marianum* (L.) Gaertn) is a flowering plant, growing in Mediterranean countries, also known as Mary thistle or holy thistle, and is widely used to make natural remedies. Milk thistle dietary supplements are used to treat liver and biliary disorders, some people claim it can lower cholesterol and help to manage type 2 diabetes. Among other potentially beneficial properties of the thistle are anti-hypercholesterolemic and anti-oxidant, chemoprotective effects against lung and prostate cancers.The active ingredient in milk thistle is called silymarin.

The aim of this study was to evaluate mycotoxins and moulds contamination of milk thistle dietary supplements from Polish market. The material consisted of 37 samples, which were divided into 3 groups: milk thistle powder (n=10), seeds (n=15), fruits (n=12). Moulds and yeasts were determined using YGC medium (yeast extract, glucose and chloramphenicol). The results are expressed as the number of colony forming units (cfu) per gram of a sample. The identification of moulds was done to the genus. Mycotoxins determination was performed using HPLC-FLD method (immunoaffinity column AflaTest from Vicam-AF; immunoaffinity column OchraPrep from R-Biophram Rhône Ltd–OTA) and HPLC-MS/MS method (Bond Elut®Mycotoxin column from Agilent - DON, NIV, ZEN, T-2 and HT-2).

The mycological analysis of powder supplements showed significant levels of moulds content (an average of 3,2 $x10^4$ cfu/g) and it was genus *Eurotium* that prevailed (33%), followed by *Aspergillus* (31%) and *Penicillium* (25%). In case of seeds, the average mould contamination was 3,3 x 10^4 cfu/g with the prevalence of *Absidia* (54%) and *Eurotium* (14%) genera. The lowest level of moulds was found in the fruits (an average 2,7 x 10^3 cfu/g) with the prevalence of *Eurotium* (55%).

The most frequently detected mycotoxins were HT-2 and T-2, occurring in 100% of the samples. The highest level of those mycotoxins was ($HT\text{-}2_{med}$ = 73,17ppb, $HT\text{-}2_{max}$ = 985 ppb; $T\text{-}2_{med}$ = 76,82 ppb, $T\text{-}2_{max}$ = 1789,77 ppb). Up to 97% of the samples contained ZEN with highest concentration 201,33 ppb. DON was present in 78% of the samples (DON_{max} = 3919,35). Moreover, 60% of the samples contained NIV (NIV_{max} = 56,93 ppb). There was no AF or OTA found in the material.

The results confirm high contamination of mycotoxins and moulds in milk thistle herbal supplements. Based on our results, it is recommended that producers of dietary supplements should include mycotoxin and moulds analysis in product quality control.

The study was supported from statutory funds related to the project entitled ‘Research on the occurrence and toxicity of secondary metabolites of moulds and identification of organisms by molecular biology methods’ (Ministry of Science and Higher Education, Republic of Poland).

P7

Effective mycotoxin detoxification strategies to ensure safer feed

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

We can distinguish two main categories within the wide group of mycotoxin detoxifying agents: adsorbing agents, which the aim is to decrease mycotoxin bioavailability by including them in the compound feed, leading to a reduction of mycotoxin uptake; and biotransforming agents, which the aim is to degrade mycotoxins into less toxic metabolites by using microorganisms or enzymes. Within the adsorbing agents the most commonly used are activated carbons, aluminosilicates (HSCAS), modified aluminosilicates and yeast cell walls. Within the biotransforming agents, there are a wide array of bacteria, fungi and different enzymes, although few of them are used in commercial products.

The efficacy of mycotoxin detoxifying agents is very often assessed in static in vitro models measuring the percentage of adsorption or the degree of detoxification achieved by the agent. However, Vekiru et al. (2007) and Versantvoort et al. (2005) showed that detoxifying agents generally become less efficient when gastro-intestinal conditions are simulated. Their efficacy may be overestimated in static models making more reliable the use of dynamic gastro intestinal models.

The efficacy of adsorbing agents depends on the intermolecular interactions between the adsorbing agent and the mycotoxin, such as electrostatic, hydrophobic or shape effects and the characteristics of the adsorbing agents, including atom composition, total charge and charge distribution, size of the pores or accessible surface. The efficacy of biotransforming agents depends on the activity and survival of the microorganism in the digestive tract, the specificity of the enzyme and its substrate, the time for the enzymatic reaction to occur, the toxicity of the produced metabolites and their intestinal absorption compared to the parent mycotoxin.

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

P8

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

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The aim of this study was to measure the efficacy of an algo-clay complex on T2-HT2 toxins, fumonisins and aflatoxins individual liver toxicity. Three trials were conducted by the Samitec Institute of Analytical (Brazil). 1080 broilers chickens (Cobb 500) were used in total for the 3 trials. 360 animals were allocated to 5 treatments with 6 replicates within each of the 5 tests treatments or 12 replicates within the control treatment. Each group contained 10 animals. The study was run from day 1 to day 21. 3 trials were set up allowing to test each mycotoxin individually at a contamination level of 2.8 ppm for aflatoxins, 100 ppm for fumonisins and 2 ppm for T-2/HT-2 toxins. Treatments differed by the presence each individual mycotoxin object of study, alone or with an inclusion of the algo-clay complex at 2.5 kg/ton or 5 kg/ton. Performance and liver parameters were measured: feed intake (FI), body weight (BW), individual relative liver weight (RWL), Sphinganine-to-Sphingosine ratio (Sa/So) for fumonisins, Lamic/Samitec Index (LSI) for aflatoxins and total plasma proteins (TPP). The inclusion of 0.50% of algo-clay complex in the diets containing mycotoxins significantly improved FI and BW compared to diets containing mycotoxins only ($P \leq 0.05$). In each study, the RWL of the birds receiving 0.50% of the algo-clay complex, was significantly improved when compared to those receiving mycotoxins only. The inclusion of 0.50% of algo-clay complex in the diets containing 2.8 ppm of aflatoxins improved significantly the LSI compared with those diets containing aflatoxins only ($P \leq 0.05$). The inclusion of 0.25% and 0.50% of algo-clay complex in the diets containing 100 ppm of fumonisins diminished significantly the Sa/So compared with those from the birds fed with fumonisins only ($P \leq 0.05$). The inclusion of 0.25% and 0.50% of algo-clay complex improved TPP ($P \leq 0.05$). The algo-clay complex decreased significantly ($P \leq 0.05$) the deleterious hepatic effects and performance losses caused by very high level of 3 types of mycotoxins on broiler.

P9

Risks & benefits of foods: RiskBenefit4EU project and the case study involving mycotoxins and cereal-based foods

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Human diet may present both risks and benefits to consumers. Risk-benefit assessment (RBA) intends to estimate the benefits and risks for humans following exposure (or lack of exposure) to a particular food or food component and to integrate them in comparable measures. For instance, any policy action directed at the adverse effects also affects the degree of beneficial effects and vice versa. In the last years, a few number of European projects within the RBA of foods have been conducted and some challenges remained and new became apparent, however the RBA methodologies are far from being well established. In contrast, risk assessment (RA) within food safety is a well-established discipline across the EU. In Portugal, a recent example is the RA study performed under MYCOMIX, a national funded project that studied the toxic effects of children exposure to multiple mycotoxins. MYCOMIX revealed a potential health concern associated to aflatoxins intake through the consumption of cereal-based foods with different contents of whole-grain. The output of this project also highlighted the knowledge gaps on the contra-balance beneficial health effect of these foods, and the need to determine the risk-benefit, since they are vehicles of food components that could be assumed as beneficial.

"RiskBenefit4EU – Partnering to strengthen the risk-benefit assessment within EU using a holistic approach" is a recent European project funded by EFSA integrating a multidisciplinary team from Portugal, Denmark and France. This project aims to strengthen the EU capacity to assess and integrate food risks and benefits in the areas of microbiological, nutritional and chemical components through the development of a harmonized framework. To validate all the developed tools, a Portuguese case study on cereal-based foods, gathering data as mycotoxins and *Bacillus cereus* intake (risks) and the consumption of fibres (soluble/insoluble) (benefits) will be considered. RiskBenefit4EU will contribute for the development and the establishment of RBA as a tool to provide scientific evidence to inform risk management decisions in the area of food safety and nutrition.

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P10

Climate change and aflatoxins exposure in Portugal – could we expect a significant health risk?

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In recent decades, changes in climate have caused impacts on natural and human systems on all continents and across the oceans. Climate change (CC) has become one of the most critical issues for the sustainable development of human societies and the functioning of ecosystems on earth. Portugal is highly vulnerable to CC impacts due to its Europe south-western geographical situation, mainly through decreasing annual precipitation, more intense extreme weather and climate events. In fact, CC is considered as one of the biggest global threats to human health of the 21st century and its effects will undoubtedly influence agricultural systems and food safety. Due to the potential impact on the occurrence of food hazards, increased concern has being aroused. Mycotoxins, natural contaminants produced by fungi, are among the most important of such hazards, especially due to their potential to establish deleterious health conditions. From all known mycotoxins, aflatoxins stand to the most toxic for humans. Dietary aflatoxins exposure is considered a significant risk factor for hepatocellular carcinoma (HCC). Considering the impact of CC in mycotoxin contamination of food products available in Portugal, the present study aims to discuss the potential influence of CC in the health risk associated to aflatoxins dietary exposure of Portuguese population. To tackle this objective, the estimated number of hepatocellular carcinoma (HCC) and the associated burden of disease in terms of Disability Adjusted Life Years (DALYs) were derived. Results showed that the number of extra cases of HCC associated to Portuguese aflatoxin dietary exposure ranged from 0.52 and 2.00. The derived DALYs ranged between 8.0 and 30.9. It is expected that in the future the number of DALYs and the associated HCC due to aflatoxin exposure should increase due to CC. These results should represent an alert for the potential consequences of an incompletely explored perspective of CC – the health impact of the human exposure to aflatoxins. Politics and decision-makers should be involved and committed to implement effective measures to deal with CC issues and to reduce its possible consequences.

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P11

Effects of Nzu (calabash clay) on mineral and aflatoxin contents in cows' milk

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Expensive and non-availability of adsorption materials for the reduction of Aflatoxin M1 (AFM1) in Nigeria drive the demand for a local readily available adsorbent. Adsorption studies of AFM1 was performed using Nzu (calabash clay) (0.5, 1 and 2%) at aflatoxin contamination rates of 9, 231 and 435ng/L for 5hrs at 4, 16, 28 and 32 °C. The aflatoxin adsorbing capability of the adsorbent depend on the adsorbent concentration, contact time and treatment temperature. Nzu exhibited very poor adsorbent capacity at 3 treatment temperatures (4, 16, 28 °C) also released lead (pb) into the milk, it decreases the calcium and iron by 25 and 30% respectively, however at 32 °C Nzu demonstrated significant reductions ($P < 0.05$) at all contamination rates , also released lead (pb) into the milk and reduced the calcium and iron contents by 40 and 60% respectively. Results from this study indicates, the potential of Nzu reducing aflatoxin AFM1 in developing tropical countries but cant still be used because of its release of lead (Pb) into products and reduction of calcium (Ca) and iron (Fe) from products.

Keywords: aflatoxin M1, calabash clay, local adsorbent, lead, calcium, iron

P12

Sodium sulfite detoxification of deoxynivalenol (DON)-contaminated maize and its impact on performance and plasma mycotoxin patterns in fattening pigs

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A wide range of mycotoxin detoxification in feedstuff are under investigation, whereby the chemical detoxification of DON contaminated cereal grains with sodium sulfite – Na_2SO_3 - appears rather promising. The aim of this study was to examine the detoxification of DON-contaminated maize with sodium sulfite (SoS) and its impact on the performance of fattening pigs.

Material and Methods: For wet preservation, uncontaminated (CON) and mainly DON-contaminated (DON) maize kernels (44.4 mg DON/kg maize) were treated with one of three SoS-levels (0, 2.5, 5.0 g/kg maize) with addition of 15 g/kg propionic acid at 20% moisture content for 63-70 d. The different maize batches were included at 10% in the diet (barley-wheat-soybean meal based) resulting in the following six feeding groups: CON- (0 g SoS/kg maize), CON2.5 (2.5 g SoS/kg maize), CON5.0 (5.0 g SoS/kg maize), DON- (no SoS treatment), DON2.5 (2.5 g SoS/kg maize) and DON5.0 (5.0 g SoS/kg maize). Barrows (BHZP, n=16/diet) with an initial body weight (BW) of 32.5 ± 3.4 kg were housed individually in floor pens and equally assigned to the six diets. Performance parameters such as BW, live weight gain, average daily feed intake (ADFI) and feed:gain ratio (F:G ratio) were determined during the 10 week-fattening phase. Furthermore feed (continuous) and blood samples (week 10) were taken for DON and DONS (DON sulfonates) analysis. Data were statistically analyzed with proc mixed (SAS 9.4) applying mycotoxin presence (DON vs. CON), SoS-level (0, 2.5, 5.0 g/kg maize) and their interaction as main factors.

Results: DON concentration in diet DON- amounted to 4.3±0.34 mg/kg feed and was reduced to 3.0±0.12 mg/kg feed in diet DON2.5 and to 1.5±0.14 mg/kg feed in DON5.0. This was reflected in plasma concentrations: in DON- pigs, mean plasma levels were determined at 21.2±6.4 ng DON/mL and decreased to 77% (DON2.5) and 52% (DON5.0) of the DON- group. In the two latter groups this coincided with a concurrent increase in DONS-levels, which are detoxification products of DON. Pigs receiving diet DON- demonstrated the lowest performance, which was recovered to control levels by both SoS-treatments, irrespective of SoS dose.

Conclusion: Our results indicate that SoS wet-preservation of DON-contaminated maize successfully reduced DON concentration in the feedstuff and this was also reflected in pigs' plasma concentrations upon feedstuff inclusion in the diet. The DON related decrease in performance in group DON- was completely recovered to control levels in group DON5.0.

P13

Ethanol extracts of *Thymi herba* and *Origani herba* effect for cytotoxicity of DON

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Cereals, such as wheat, maize and oats are often infected by *Fusarium* species. These fungi may produce trichothecenes, a class of mycotoxins. Deoxynivalenol (DON) is the most prevalent trichothecene in cereals and feed in Europe and North America [1]. At the level of the whole animal, the toxic effects of the mycotoxin deoxynivalenol (DON) range from causing diarrhoea, vomiting, gastro-intestinal inflammation to necrosis of several tissues. It also affects the immune system and leads to kidney lesions [2]. Thyme (*Thymus vulgaris* L.) and oregano (*Origanum vulgare* L.) are aromatic plants of the Mediterranean flora commonly used as spices and for medicinal purposes. Oregano, similar to thyme, evinces antioxidant and antimicrobial activities and some reports deal with its antimutagenic and anticarcinogenic effects [3].
The aim of the current research was to assess possible protective effect of *Thymi herba* and *Origani herba* ethanol extracts against cytotoxic effect by DON.

Methods: Two cell lines BHK–21 (baby hamster kidney fibroblasts) and MH–22A (mouse hepatocarcinoma) were incubated with DON (10 µg/mL) for 24 h and 48 h with or without of Thymi herba (30, 20, 10 µg/mL) and Origani herba (30, 20, 10 µg/mL) ethanol extracts. To determine the viability, based on metabolic activity of cells, a colorimetric assay was performed using the MTT [4].
Results: DON decreased BHK-21 cell viability 50%, but not effected MH–22A cell viability after 24 h. BHK-21 cells treated with DON and Origani herba ethanol extract (30 and 20 µg/mL) increased cell viability 30% ($P>0.05$) compared with cells treated only with DON and Thymi herba ethanol extract (30, 20, 10 µg/mL) increased cell viability 60% ($P<0.05$), 44% ($P<0.05$), 52% ($P<0.05$) respectively. MH–22A cell viability, treated with DON, decreased 34% after 48 h, both plants extracts in all concentrations decreased cell viability approximately 20%.
Conclusion: Ethanol extracts of *Thymi herba* and *Origani herba* protected cells against cytotoxicity of DON.

References:
1. GAREIS, M. Collection of occurrence data of *Fusarium* toxins in food and assessment of dietary intake by the population of EU member states. Report of Experts Participating in SCOOP Task 3.2. 10-Part A: Trichothecene, 2003, 13-235.
2. KÖNIGS, Maika, et al. Cytotoxicity, metabolism and cellular uptake of the mycotoxin deoxynivalenol in human proximal tubule cells and lung fibroblasts in primary culture. Toxicology, 2007, 240.1-2: 48-59.
3. BUKOVSKÁ, Alexandra, et al. Effects of a combination of thyme and oregano essential oils on TNBS-induced colitis in mice. Mediators of inflammation, 2007.
4. VISTICA, David T., et al. Tetrazolium-based assays for cellular viability: a critical examination of selected parameters affecting formazan production. Cancer research, 1991, 51.10: 2515-2520.

P14

Synthesis and characterization of ochratoxin A metabolites to develop a LC-MS/MS based biomarker method

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Ochratoxin A (OTA) is one of the most important and wide-spread mycotoxins worldwide. It has been shown that OTA has numerous toxic effects to humans and animals where the most prominent one is nephrotoxicity. Due to the different toxicity of OTA and its degradation products such as ochratoxin alpha (OTα), it is essential to elucidate the biotransformation in humans and animals. So far, knowledge of the metabolism of OTA and OTα is limited.

The aim of this work is the analysis of ochratoxin metabolites in humans as well as in rats. The metabolism was verified *in vitro* with the usage of liver microsomes. Therefore, liver microsomes of rats and humans were incubated with the toxins and NADPH to collect possible phase I-metabolites. In a second approach, the phase II-metabolism concerning the formation of glucuronide conjugates was investigated. The formed metabolites were subsequently analyzed on a Sciex 6500+ LC-MS/MS system using electrospray ionisation.

The functionalization catalyzed by cytochrome P450 containing monooxygenases results in the introduction of hydroxyl groups to the toxins. Similar to Yang *et al.* (Anal. Bioanal. Chem. 407, 2015) hydroxylation at the positions 4, 5', 7' and 9' was observed at low intensities. The microsome assays with UDP-glucuronic acid have identified three different OTA-glucuronides, which were formed in a higher quantity. For structural elucidation, the OTA glucuronides formed were isolated by solid-phase extraction and preparative HPLC and characterized.

Hereafter, a LC-MS/MS based method to determine OTA, OTα, ochratoxin B and their metabolites was developed and validated for mammalian urine.

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P15

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

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Citrinin is produced by a number of *Aspergillus* and *Penicillium* fungi and has been found in a variety of foods such as grains, cheese and red yeast rice, the latter of which is regulated in the EU (Commission Regulation (EU) No 212/2014). Although legislation is currently in place for only red yeast rice there is considerable interest in Europe with regards to levels in foods including cereals as this toxin is considered as a potential issue during storage often occurring simultaneously with ochratoxin A with both toxins considered as potential agents of Balkan endemic nephropathy.

R-Biopharm Rhône have developed a new immunoaffinity column that selectively isolates and concentrates citrinin from a wide range of commodities including cereals and red yeast rice. Red yeast rice is available as a loose powder and also as a food supplement prepared in tablet or capsule form. Samples were analysed using immunoaffinity clean up with HPLC and the results compared with an LC-MS/MS method.

P16

Determination of aflatoxin M1 in milk products using an automated immunoaffinity cartridge system

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Aflatoxin M1 is a major metabolite of aflatoxin B1 in humans and animals and may be present in milk from animals fed with aflatoxin B1 contaminated feed. Current European Legislation is in place for aflatoxin B1 in animal feed in order to reduce the risk of aflatoxin M1 in the food chain. For aflatoxin M1 legislative levels are often very low therefore suitable methods are required.

R-Biopharm Rhône has developed a patented, online immunoaffinity cartridge which can be used prior to HPLC for analysis of aflatoxin M1. The cartridges are used together with the RIDA®CREST handling system to combine automated online sample clean-up with quantitative analysis. Each cartridge is calibrated and can be used for up to 12 samples before the cartridge is automatically removed and replaced with a new one.

The cartridges contain monoclonal antibodies attached to a polymer making them highly specific and sensitive. A key advantage of this process is that during the LC run of one sample, the next sample is simultaneously passed through the affinity cartridge, reducing the time taken for subsequent sample clean-up to almost zero.

Here, methods and results will be presented for the automated, rapid determination of aflatoxin M1 in various commodities, including milk, infant milk formula, cream, yoghurt and cheese.

P17

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

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EU regulations for mycotoxins are highly complex with varying limits applied to specific commodities. There are limits in place for a number of mycotoxins for cereals and cereal products therefore a multi-toxin method targeting a wide range of toxins is of particular interest. In addition, baby foods and animal feeds are composite in nature and again are regulated for a number of toxins therefore, a multi-toxin immunoaffinity column can offer many advantages.

R-Biopharm's new immunoaffinity column, 11+Myco MS-PREP® utilises a single extraction method for the analysis of 11 mycotoxins (aflatoxins B1, B2, G1, G2, ochratoxin A, fumoninisins B1, B2, deoxynivalenol, zearalenone, T-2 and HT-2) prior to detection by LC-MS/MS. In the study, a number of cereals and cereal samples were analysed in order to determine recoveries and results demonstrate that the use of an immunoaffinity column enables the concentration of the mycotoxins prior to detection, improving sensitivity and eliminates the use of isotopic labelled standards.

P18

The assessment of the occurrence of nivalenol, deoxynivalenol and deoxynivalenol-3-glucoside in Polish winter wheat

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The occurrence of mycotoxins in cereal grain is a very important factor affecting food safety. Also a very important is the presence of modified mycotoxins in cereal grains. Occurrence of NIV, DON, and DON-3G in 92 samples of various winter wheat cultivars cultivated in the 2015/2016 vegetation season in various regions of Poland was evaluated using the LC chromatography technique with an UV detector. Cross reactivity of antibodies contained within DON-NIV WB immunoassay columns made possible to determine DON-3G (i.e., DON masked form) simultaneously with NIV and DON. IAC effectiveness was checked in a validation experiment. NIV/DON/DON-3G was identified above the method LOD in 70%/83%/27% of the samples, respectively, but at safely low levels. Mean concentrations of the analytes were (all data in μg/kg): 35.0 (range 5.1–372.5)/140.2 (range 10.5–1265.4)/41.9 (range 15.8–137.5), respectively. The DON-3G/DON molar ratio ranged from 4.3 to 37.0%. The results obtained in this work are important for food safety, especially since literature data on the contamination of food with DON-3G are quite scarce. Although the frequency of occurrence of the discussed mycotoxins was high, the quality of the wheat was generally high. Nevertheless, the presence of a glycosidic derivative of deoxynivalenol could increase the risk to food safety, as it could be hydrolyzed by intestinal microflora.

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P19

Evaluation of the mushroom *Pleurotus eryngii* mycelium as biosorbent for aflatoxin B1

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Aflatoxin B1 (AfB1) is a major threat to human and animal health, due to its potent hepatotoxic, carcinogenic and mutagenic effects. Removal or inactivation of aflatoxins in food and feedstuffs by chemical and physical methods is limited, due to safety issues and possible loss of nutritional value of the treated commodities.

Dietary supplementation of animals with aluminosilicates, bentonites and activated carbons are the preferred technologies for the mycotoxins detoxification of feedstuffs, but could have negative impact on the nutritional quality of decontaminated feed. For this reason, interest in the use of natural microbial adsorbents has increased.

In this study we investigated the capability of ground not-viable mycelium (*Pleurotus eryngii* ITEM 13681) edible fungi to absorb AfB1.

For the preparation of biosorbent, the mycelium was autoclaved, lyophilized and finely ground. High performance liquid chromatography (HPLC) with florescence detector was used to define the efficiency of adsorption of this biomaterial.

Preliminary studies were based on the evaluation of the influence of different parameters: pH (5, 7), AfB1 concentrations (from 50 to 1000 ng/mL), time (from 30 to 120 min), temperature (25, 37 °C), fungal mass (from 50 to 1000 mg), on the absorption capability of the mycelium of *P. eryngii*. This biomaterial seems able to absorb AfB1 at 37 °C, irrespective of the pH, to values that depend on mycotoxin concentration and biosorbent mass. AfB1 mass balance demonstrated that mycelium extraction with methanol and ethyl acetate was respected, in fact mycotoxin recovery has been 108±6% and 91±6%, respectively.

Optimal absorption was obtained at the lowest AfB1 concentration and the highest fungal mass. Stability of AfB1-biosorbent binding and desorption studies were carried out varying, respectively, the pH at 7 and 3, for 24 hours of incubation at room temperature in the dark. Results showed that the percentage of the released mycotoxin were 11±2% at pH 7 and 18±4% at pH 3. These assays revealed that AfB1 was stably and spontaneously absorbed by *P. eryngii* mycelium, over time.

Finally, Langmuir model, better described the absorption mechanism at isothermal conditions.
Our results suggest that biosorbent obtained from the mycelium of *P. eryngii* could represent a cheap and effective feed additive for AfB1 detoxification.

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P20

First *in vitro* screening of laccase mediator systems to reduce the fusaric acid levels

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Fusaric acid (FA) is produced by several *Fusarium* species, in particular *Fusarium oxysporum*, *F. proliferatum* and *F. fujikuroi.* This picolinic acid derivative is known for its high phytotoxicity, it causes diseases in great variety of plants (tomato and banana), directly related to the severity of damping off, vascular wilt and root rot diseases of numerous vegetable crops. In addition, it exhibits toxicity towards animals, including notochord malformation in zebrafish and neurochemical effect in mammals. This mycotoxin is commonly widespread on corn, wheat and derived products.

Many plant species can metabolize fusaric acid into less phytotoxic products. In this study, the action of laccase mediator systems (LMSs) toward fusaric acid is investigated for the first time. For this purpose, several *in vitro* degradation assays were performed, screening the effect of different redox mediators on the activity of laccase (EC 1.10.3.2) from *Trametes versicolor* towards FA. As redox mediators, two natural products syringaldehyde (SA), acetosyringone (AS) and two synthetic molecules, 2-azino-di-[3-ethylbenzo-thiazolin-sulphonate] (ABTS), 2,2,6,6-tetramethylpiperidinyl-oxyl (TEMPO), were investigated. The FA levels were evaluated by high performance liquid chromatography (HPLC) with diode array (DAD) detector at wavelength of 271 nm. FA presence was monitored at 72 and 144 hours.

No degradation was observed in the presence of three redox mediators (AS, SA, TEMPO). Adding ABTS seems to facilitate the complete degradation of FA within 3 days.

Our preliminary data showed encouraging results, although the mechanism of the degradation remains to be investigated. In order to identify putative biodegradation products of FA by LMS activity, we intend to purify the products and elucidate their structure using standard methods of natural product chemistry. Further study will be needed to evaluate the toxicity of these products.

P21

Detection and quantification of aflatoxin-producing *Aspergillus* spp. in maize grain from the Philippines

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Aflatoxins, considered to be the most toxigenic mycotoxins, are mainly produced by *Aspergillus flavus* and *A. parasiticus*. Data on aflatoxigenic fungi and aflatoxins in Philippine maize are limited. Hence, the objectives of the study were to isolate mycotoxigenic fungi by culture plate method, to test for the presence of aflatoxin biosynthetic pathway gene, *afl*D (= *nor*1) and to quantify DNA of aflatoxigenic *Aspergillus* spp. in 208 farm maize samples collected from different regions of the Philippines by real-time PCR. *Aspergillus* section Flavi was the most frequently isolated from maize seeds (52%) followed by *Fusarium* (34%) and *Aspergillus* section Nigri (22%). All samples except three were positive for *afl*D. The range of aflatoxigenic *Aspergillus* DNA was from 1 to 166 µg kg-1 grain sample. About 90% of the samples contained more than the 20 µg kg-1 aflatoxins, which is the permissible level for aflatoxins in the Philippines. The analysis of other mycotoxins is underway. This work provides a current status of aflatoxin levels in maize grains in the Philippines as a basis for the assessment of exposure of consumers in the Philippines, showing that there is an urgent need to increase awareness and implement management strategies to reduce aflatoxin levels in food and feed products.

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P22

Reliable quantification of mycotoxins with Lateral Flow Tests

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Although lateral flow tests (strip tests) are commercially available since a decade, most people still consider them as qualitative or semi-quantitative only. Nevertheless, this technology made significant progress during the last five years and now the strip tests are able to produce reliable quantitative results for mycotoxin concentrations.

This poster will describe the procedure of the US Department of Agriculture used for the performance evaluation of rapid test for mycotoxins. Furthermore, the results obtained by the USDA operators will be shown and clearly demonstrate the capabilities of modern lateral flow test.

P23

Project Aflatox® III - how to use the database to study mycotoxigenic fungi metabolism: A glimpse to the interconnections between molecular structure modifications and biological activity

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The Aflatox® Project (www.aflatox.it), funded by the Cariplo Foundation, is aimed at the development of a biotechnological polyphasic approach to identify and design new bioinorganic compounds possessing biological activities against phytopathogenic fungi contaminating cereals and food/feed derivatives, with a particular focus on aflatoxigenic species. Controlling fungal contamination on crops is considered a priority by sanitary authorities of an increasing number of countries, due also to the fact that the geographic areas interested in mycotoxin outbreaks are widening. Among the different pre- and post-harvest strategies that may be applied to prevent fungal and/or mycotoxin contamination, fungicides still play a prominent role. The previous Aflatox® I and II abstracts illustrate, respectively, the experimental flow-chart and the database produced from the evaluation of compounds under analysis. The present contribution shows some examples of how the search in such a database could be conducted for specific research topics in the study of mycotoxigenic fungi. What we report is how, starting from the collected data, we selected groups of molecules, modified in both structure and chemical-physical characteristics, to analyze different aspects of fungal development and secondary metabolism in *Aspergillus*, *Fusarium* and *Penicillium* species; in particular:

1) the species-specific fungistatic effect of cuminaldehyde-derived molecules on *Penicillium nordicum*, *P. commune*, *Fusarium proliferatum*, *F. verticilloides*, *F. graminearum*, *Aspergillus parasiticus* and *A. carbonarius*;
2) the relationship between predicted antioxidant scavenging ability of jasmone-derived molecules and their effect on sclerotia biogenesis in *A. flavus* aflatoxigenic strains;
3) the differential proteome-changes induced by cinnamaldehyde-derived ligand and relative zinc complex;
4) the importance of lipophilicity in determining thiosemicarbazones anti-aflatoxigenic activity;
5) the molecular docking to predict the interaction between selected molecules and aflatoxins biosynthetic pathway enzymes.

This study was supported by a grant from “Fondazione Cariplo” (Project N. 2014-0555).

P24

Novel insights on the toxicity of deoxynivalenol in A431 cells: Influence on cellular mechanotransduction

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Cells in the human body are continuously subject to chemical, mechanical and physical stimulation. From the toxicological perspective, chemical-driven pathways have paramount importance, and mycotoxins belong to the wide group of contaminants that can disrupt cell homeostasis, as well as interaction with the extracellular environment. In the present study, the classical toxicological approach was combined with biophysical methods, to describe the effects of deoxynivalenol (DON, vomitoxin) on complex cellular responses, i.e. reaction to biomechanical stimulation (cyclic uniaxial stretching 0.5 Hz, 24 h). The experiments were performed in the epidermoid carcinoma cell line A431 and a complex multi-parametric workflow for image analysis was developed in order to analyze the effects of living cells directly on the deformable membranes. In control conditions, cells responded to mechanical stimulation with morphological adaptation together with the remodeling of tubulin cytoskeleton, as well as redistribution of lysosomes. Pre-incubation with DON altered these responses in concentration-dependent manner (0.1, 1, 10 µM) with direct effect on the capability of the cell to react to the cyclic uniaxial stretching. Moreover, biomechanical pre-conditioning of A431 appeared to reduce the sensitivity of the cells to the effect of the mycotoxin. In conclusion, DON appeared to modulate the capability of A431 cells to respond to complex challenges also in a concentration range that is not directly cytotoxic, thus offering new perspectives for the evaluation of the effects of the mycotoxin in vitro.

P25

Determination and occurrence of tenuazonic acid in food

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Alternaria toxins are mycotoxins produced by *Alternaria* species. *Alternaria alternata* is the most common mold in harvested foods and the most important mycotoxin-producing species. Due to their growth at low temperatures, these species are also responsible for spoilage of foods during refrigerated transport and storage. [1]

Alternaria species produces more than 70 phytotoxins. Tenuazonic acid (TEA) is one of these mycotoxins with a high acute oral toxicity in several animal species. An increased level of this toxin indicates that mold-infested ingredients have been processed.[1]

According to a scientific opinion by EFSA on the risk of *Alternaria* toxins in food from 2011, a threshold of toxicological concern (TTC) of 1.5 µg/kg b.w. per day was estimated for TEA. The TTC concept is a scientific tool used for orientation not for legal opinion.

Several techniques, based on chromatography are suitable for the quantification of *Alternaria* toxins. LC-MS/MS has become the method of choice [1]. Tenuazonic acid was determined after derivatisation with 2,4-Dinitrophenylhydrazin [2].

The present work shows the sample preparation and the results of the analysis of several food products. Tenuazonic acid was found most frequently and in high levels.

[1] EFSA Journal 2011; 9(10): 2407
[2] Siegel, D. et al. J. Chromatogr. A (2009): 4582

P26

Some aquaculture species are extremly sensitive to mycotoxins

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Mycotoxins are highly toxic secondary metabolites produced by certain fungi that contaminate a wide variety of grains and other commodities used in animal and human production. The awareness of mycotoxin-related issues in aquaculture has grown as feed manufactures and producers realize the importance of mycotoxins and their potential to negatively impact production.

Aflatoxin (AfB1) is highly carcinogenic and the negative effects of AfB1 on liver health and liver histology were reported in several economically relevant aquatic species such as sea bream, tilapia, catfish, shrimps and pangasius. Histopathological changes such as necrosis, presence of inflammatory cells and biliary enlargement were observed as well as the accumulation of AfB1 in the muscles and in the liver.

Trichothecenes such as deoxynivalenol (DON) are inhibitors of protein synthesis. These mycotoxins are highly immunosuppressive and cause effects such as reduction of growth performance, feed efficiency and histopathological changes in aquatic species like Atlantic Salmon, carp and rainbow trout.

Zearalenone (ZEN) is an estrogenic mycotoxin and it is the major cause of hyperesterogenism in animals such as pigs and poultry. Shrimps in particular are quite sensitive to zearalenone (ZEN) and effects on growth and reproduction have been observed as well.

Fumonisins (FUM) are toxic and carcinogenic for several aquatic species, such as catfish, trout, white leg shrimp and tilapa. Some trials in channel catfish and rainbow trout revealed several mutagenic and toxic effects, like degeneration of several organs such as kidneys and liver, or developmental abnormalities. Fumonisins undergo synergistic interactions with aflatoxin B1, increasing the severity of this mycotoxin.

Aquatic species can be quite sensitive to mycotoxins as well, especially due to the increasing tendency to include plant-derived meals in aquatic diets. Over a periode of 4 years (2014-2017), 53 samples of finished aquaculture feeds were analyzed within the scope of BIOMIN Mycotoxin Survey Program. All samples contained 10 or more different mycotoxins and metabolites. ZEN was the most common mycotoxin and was detected in 79% of all samples at a mean concentration of 40 ppb for positive samples. In order of prevalence for the other mycotoxins, DON was in 53% (mean of 59 ppb) and FUM was in 47% (mean of 213 ppb). AfB1 and ochratoxin A occurrence are often related to storage conditions but also grain damage in the field; Both were present in 11% of all samples.

P27

Monitoring of deoxynivalenol (DON) levels in winter wheat fields in Bavaria before harvest

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To give a first assessment of annual levels of DON and to support production and commercialisation of winter wheat according to legal limits established with EU Regulation No. 1881/2006 a monitoring of winter wheat fields prior to harvest is conducted in Bavaria. From 2012 to 2017 overall 892 samples of wheat ears were taken 10 to 14 days before harvest from voluntarily participating farmers who were selected by millers and from agricultural consultant officers.

The mean value of DON ranged from 92 µg/kg in the year 2015 to 524 µg/kg in the year 2012 for samples of wheat ears. The correspondending values of an independent monitoring of harvested winter wheat showed a mean DON value of 40 µ/kg in 2015 and a mean DON value of 623 µg/kg in 2012.

Providing basic information to farmers and millers about methods of prevention of DON producing *Fusarium* species and responsible practices for handling the mycotoxin problem were intensified to support quality of winter wheat in Bavaria. In 2015 a first attempt to assess the possibilities of remote sensing using Sentinel 1 satellite data was done. It is not possible to directly observe the flowering of wheat or infections by *Fusarium* funghi. In an approach combining crop growth modelling with earth observation data covering optical and microwave spectral ranges the best results for prediction of onset of flowering was achieved pointing to the potential of remote sensing in plant observation.

Under the prevailing agronomic conditions DON is a manageable yearly challenge in Bavarian winter wheat. Agronomic risk factors for DON like maize as pre-crop and no tillage are not decreasing. Strategies for prevention, timely information about DON levels and options how to handle contaminated batches play a major role in handling this ongoing challenge. An integrated approach taking into account all risk factors for prevention of *Fusarium* infection is best on farm level to prevent high DON values.

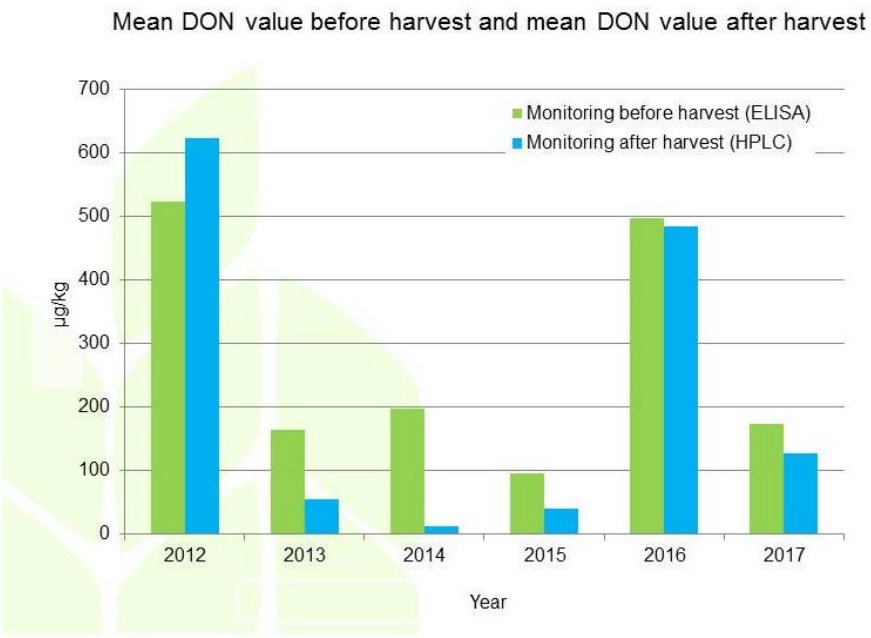

Figure: Mean DON values before harvest and after harvest

The technical assistance of I. Birken and J. Rieder in DON analysis is highly appreciated. The research was supported by the Cluster Ernährung of the Free State of Bavaria, BAYWA AG, Bayerischer Müllerbund e.V., Landhandelsverband Bayern e.V. and Verband der Getreide-Mühlen- und Stärkewirtschaft VGMS e.V..

P28

Optimization of the methodology for the detection/quantification of patulin and breakdown products

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Patulin (PAT) is a mycotoxin produced by several *Penicillium*, *Aspergillus* and *Byssochlamys* mould species commonly found on apples. Damaged and bruised apples are the most susceptible to contamination by PAT-producing moulds. Considering its cytotoxic, genotoxic and immunosuppressive properties, the European Union (EU) has established specific regulations to protect the consumer's health. A maximum level of 50 µg/kg of PAT is set for fruit juices and derived products, 25 µg/kg for apple purees and compotes and 10 µg/kg for food intended for babies and young children (EC Commission Regulation, 2006). Yet, some apple juices can be contaminated by PAT at levels exceeding these regulatory limits. Different studies focused on the degradation of this toxin and generation of breakdown products, that are less toxic, such as (E/Z)-ascladiol and desoxypatulinic acid (DPA). Chemical, physical and biological treatments have been described in literature. We focused on the chemical degradation of PAT by ascorbic acid (AA) and assessed the level of contamination by PAT and other metabolites in apple juice.

In a lab experiment, the influence of AA on the concentration of PAT in cloudy apple juice and pure PAT standard in aqueous solution was studied. However, during high-performance liquid chromatography (HPLC) analysis coupled to UV detector, different compounds appeared to interfere with each other not allowing a correct interpretation for each of the compounds considered: 5-hydroxymethylfurfural (HMF), appearing in some apple juices, interfered with PAT causing an overestimation of the concentration of the latter, and dehydroascorbic acid (DHA) resulting from the oxidation of AA interfered with (E)-ascladiol causing an overestimation of this latter. Because these interferences could cause a misidentification and/or overestimation of PAT and E-ascladiol, we needed to optimize our original in-house methodology (De Clercq et al., 2016) to assess the different compounds cited above. Pure standards of DHA, (E/Z)-ascladiol, HMF, PAT and DPA were used. Based on their chemical properties and different polarities, the HPLC conditions were optimized improving the separation of these compounds. Gradient elution using HPLC-grade water acidified with 0.1 % acetic acid and acetonitrile as mobile phase A and B, respectively, allowed separation of all compounds on an ACQUITY UPLC® H-Class System equipped with a Waters® ACQUITY™ UPLC™ Tunable UV (TUV) detector set at 276 nm system equipped with an Acquity HPLC® BEH C18 1.7 µm (2.1x150mm) column.

Our improved methodology allowed a good separation of all the compounds cited above, and could be used to study the influence of ascorbic acid on the degradation of PAT.

We would like to kindly thank Prof. Raffaello Castoria (Department of Agricultural, Environmental and Food Sciences, University of Molise, Italy) and Prof. Rosa María Durán Patrón (Department of Organic Chemistry, University of Cádiz, Spain) for providing the DPA standard as well as Dr. Olivier Puel (Toxalim laboratory, INRA Toulouse, France) for providing E/Z-ascladiol standards.

P29

Interaction of 2'R-ochratoxin A with serum albumins

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Ochratoxin A (OTA) is a nephrotoxic mycotoxin produced by *Aspergillus* and *Penicillium* species. OTA appears in different meals and drinks, including bakery and dairy products, wine, beer, and coffee. During the roasting process of OTA-contaminated coffee, its R-enantiomer, 2'R-ochratoxin A (2'R-OTA) is formed. 2'R-OTA seems less toxic than the parent compound OTA. Human serum albumin (HSA) is the most abundant plasma protein in the human blood. Albumin forms stable complexes with several compounds, which can strongly affect the tissue distribution and elimination of these molecules.

In this study, the interaction of 2'R-OTA with serum albumins was examined employing fluorescence spectroscopy, molecular modeling, and in vitro cell experiments. Binding constant of 2'R-OTA – HSA complex was approximately 10-fold lower compared to OTA-HSA. Modeling studies and investigations with site markers suggest that, similarly to OTA, 2'R-OTA occupies Sudlow's Site I as its high-affinity binding site on HSA. To characterize species-dependent alternations, bovine (BSA), porcine (PSA), and rat (RSA) serum albumins were tested as well. 2'R-OTA forms more stable complexes with BSA and RSA compared to HSA. Viability of MDCK kidney cells was determined after 24-hour incubation, during which 2'R-OTA showed significantly lower toxicity than OTA. Furthermore, the presence of 10% fetal bovine serum (FBS) or 40 g/L HSA in the cell media resulted in the considerable decrease of the toxic action of both mycotoxins. Co-treatment of cells with OTA and 2'R-OTA was examined in the absence and presence of 10% FBS: In both cases, even subtoxic concentrations of 2'R-OTA led to the significant increase of the toxic action of OTA.

In conclusion, 2'R-OTA forms stable complex with albumins. Its lower affinity for HSA (compared to OTA) is likely due to its different position on Site I. 2'R-OTA showed much lower species-dependent alternations than OTA, and formed the most stable complex with BSA among the tested albumins. Cell experiments suggest the significant biological relevance of 2'R-OTA – albumin interaction, and showed that its co-exposition with OTA can be problematic, despite the relatively low toxicity of 2'R-OTA.

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P30

Comparative genome analyses uncover a cryptic ochratoxin biosynthesis gene cluster in the genus *Penicillium*

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Species of the genus *Penicillium* belong mainly to the saprophytes which cause substantial food loss if plants are infected on the field or during postharvest storage under suboptimal conditions. Most Penicillia are able to produce harmful substances such as mycotoxins whose ingestion lead to health issues such as mycotoxicoses in humans and animals. Moreover, spores of filamentous fungi in general act as potent antigen and may trigger mycogenic allergies in exposed individuals. Genome sequencing and subsequent mining for secondary metabolite gene clusters is an important prerequisite for an adequate risk assessment. By sequencing the genomes of different species of the genus *Penicillium* and comparative analyses with existing genome data in online data bases, a high diversity of putative secondary metabolite gene clusters were revealed. An important outcome of analyses focused on the genomes of Penicillia occurring on salt-rich habitats, such as *P. verrucosum, P. nordium* and *P. thymicola*, was the identification of cryptic ochratoxin biosynthesis genes which show an exceptional high homology to the OTA-cluster described in *Aspergillus steynii* by Gil-Serna et al. (2014). If this genes are actively involved in ochratoxin biosynthesis in the analyzed Penicillia is now under investigation and will help to complete the picture about OTA-biosynthesis in Penicillia.

P31

Deoxynivalenol in diets of chickens does not impact some serum clinical biochemical markers

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The feed contaminant deoxynivalenol (DON) may produce significant health problems when fed to chickens even at low levels. Therefore, this study was conducted to investigate some impacts of a DON feeding (5 mg DON/kg feed) to broiler chickens for short and long duration (3 and 5 weeks respectively) on common serum clinical biomarkers. A total of 19 serum samples (controls for 3 and 5 weeks versus DON-fed groups of same age) were analyzed for aspartate transaminase, glutamate dehydrogenase, and bile acids as biomarkers for liver function, urea and uric acid as indicators for kidney function, fructosamine as independent biomarker for diabetes and lipase as a marker for pancreas function.

The results showed that there were no statistical significant differences ($p > 0.05$) between DON-fed groups and control groups in the terms of clinical serum biomarkers suggesting that feeding DON at a level of 5 mg/kg feed does not affect the overall clinical health of broiler chickens.

In conclusion, hepatotoxic effects did not occur during the course of experiment. Furthermore, pancreas and kidney functions of broilers were altered, urea significantly and lipase in tendency under diets contaminated with DON at level of 5 mg/kg feed.

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P32

Ochratoxin A formation in rye – a sampling study

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OTA detection in grain lots still remains a huge challenge for mills. Even if the number of samples taken per lot is increased or dynamic sampling is used, OTA hot spots can still remain undetected.

In Northern or Central Europe OTA contamination is often caused by *Penicillium* species during grain storage. Environmental factors like grain humidity or changes in storage temperature can greatly influence OTA formation. For example, a shift in temperature caused by day and night variation leads to air moisture condensation in a silo cell. Dropping water may dampen the grain. At these points free water becomes available for *Penicillium* growth. Depending on the species water activity levels of 0.8 are sufficient for mould formation and toxin production. As a consequence, OTA hot spots are formed.

New sampling strategies for hot spot detection are needed to avoid cost-intensive recalls. Dust sampling was to be evaluated for its potential to identify OTA hot spots in rye lots. For this purpose, different storage scenarios were tested in small batches to simulate hot spot formation in a silo cell. To 24 uncontaminated rye lots (10 kg each) different amounts of rye dust were added. Moreover, water was evenly spread across the rye to raise grain humidity in several lots. Kernels visibly infected with *Penicillium verrucosum* were added to some of these lots. Another approach for evoking OTA hot spots was to add 200 g of rye kernels soaked with a *P. verrucosum* spore suspension. The infected, wet kernels were directly placed in the middle of a 10 kg rye lot. Infected kernels were as well added to half of the control lots, in which rye humidity was not increased. After a sufficient incubation period, dust was separated from the lots and kernels were milled. Dust as well as rye flour was analysed by LC-MS/MS. First results showed that the simulation of OTA production in a silo cell was successful. Tremendous OTA contaminations up to 1-2 mg/kg were found in kernels and even more in dust after adding moist, infected kernels to the middle of a lot. Grain dust analysis was shown to identify OTA contamination in rye lots and can be used as a proper tool for an in-process early warning system in mills. Aiming at quantitative determination, follow-up studies are currently performed to reveal reliable correlation models between dust and kernel contamination.

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P33

Deoxynivalenol induces oxidative stress in prostate cancer cells

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It is estimated that global contamination with mycotoxin is approximately 25% of all cereal crops. One of the mycotoxins produced by *Fusarium* species is deoxynivalenol (DON) known as vomitoxin. It is usually detected in wheat, maize, barley, rice and rye. Prolonged exposure to DON can potentially result in health consequences on endocrine and nervous system.

The present study evaluated the effect of DON on the oxidative activity of mitochondria, mitochondrial potential and induction of oxidative stress (ROS) in prostate cancer cells (PC3, DU-145, LNCaP, 22Rv1). Our study showed that treatment with DON (5 μM; 3 μM; 2 μM) in all investigated human prostate cancer cell lines caused significant increase in reactive oxygen species (ROS) production with simultaneous increase in mitochondrial potential as well as decrease in oxidative activity of mitochondria.

To conclude, DON might trigger oxidative stress in human prostate cancer cells and may result in DON-induced damage of DNA.

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P34

Ultrahigh liquid chromatography Orbitrap mass spectrometry method for the quantitative analysis of *Fusarium* mycotoxins in forage maize and maize silage

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Forage maize is frequently infected by a variety of mycotoxin producing *Fusarium* species. Nevertheless, the occurrence of mycotoxins in products intended for animal feed has predominately studied in cereals. As staple feed constitutes a considerable part in the diet of cattle, there is also the need to determine the presence of mycotoxins in this kind of feed.

Therefore, the objective of the present study was to develop and validate a sensitive and reliable method based on ultrahigh performance liquid chromatography (UHPLC) coupled to high resolution mass spectrometry (HRMS) for the simultaneous determination of deoxynivalenol (DON), 3+15-acetyldeoxynivalenol (3+15-AcDON), deoxynivalenol-3-glucoside (DON3G), de-epoxy-deoxynivalenol (DOM-1), zearalenone (ZEN), alpha-zearalenol (α-ZEL) and beta-zearalenol (β-ZEL) in forage maize and maize silage. The sample preparation consisted of a simple liquid/solid extraction followed by a clean-up step using Bond Elut Mycotoxin® columns.

The full MS/data dependent MS/MS acquisition mode enabled the monitoring of the precursor ion as well as of two fragment ions per mycotoxin which were used for the correct identification and conformation. A high mass accuracy (< 3 ppm) was obtained for both matrices. The method was successfully validated for forage maize and maize silage according to the Commission Decision 2002/657/EC guidelines (1). The recovery, repeatability and reproducibility were in the range of 92 - 108 %, 2 - 16 % and 3 - 12 %, whereas the decision limit (CCα) and the detection capability (CCβ) varied from 11 - 107 µg/kg and 20 - 125 µg/kg, respectively. Finally, the method was applied to forage maize as well as maize silage samples collected in Northern Germany. All samples analysed contained at least one mycotoxin. DON and ZEA were the most abundant mycotoxins occurring in concentrations up to 10972 µg/kg and 1644 µg/kg, respectively.

(1) Commission decision (2002/ 657/EC) of 12 August 2002, Implementing council directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. In: Official Journal of the European Union (L221), p. 8–36.

P35

Genotoxicity assessment of mycotoxins ZEA, DON, AFB1 to bovine peripheral blood lymphocytes

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Zearalenone (ZEA) and deoxinivalenol (DON) are fusariotoxins produced by genera *Fusarium* of the northern temperete regions. Aflatoxin B1 (AFB1), mainly produced by *Aspergillus flavus* and *A. parasiticus* [1]. AFB1 due to climate change become a food and feed safety issue in Europe. Mycotoxins are genotoxic, carcinogenic and immunosuppressive substances, and cause acute and chronic toxicity. Related health problems are difficult to diagnose, mainly due to cryptic, long-term and chronic exposures [2].

In the current work were investigated genotoxic effects of mycotoxins DON, ZEA, AFB1 and DON/ZEA combinations (as naturally estimate the highest concentrations in real practice in Lithuanian feed) in vitro cultures of bovine lymphocytes.

A possibly genotoxic effect of mycotoxins AFB1, ZEA, DON, and ZEA/DON on bovine lymphocytes, after exposition to the highest concentrations, were determined in vitro. Analysis of chromosomal aberration spectrum showed the highest total number of cells with lesions after exposition of lymphocyte cells to ZEA (1.560 µl/L) and the lowest after exposition to DON (1.250 µl/L). The highest percentage of lesions after exposition of lymphocytes to mycotoxins was accounted for by breaks and gaps of chromatids indicative of instability of genome. The highest percent of gaps was determined after exposition of lymphocyte cells to DON and the highest percent of breaks after exposition to combination ZEA/DON (1.560 µl/L 1.250 µl/L). It was also determined that ZEA affected the changes in the number of chromosomes (aneuploidy). Evaluation of the spectrum of chromosome aberrations after exposition of bovine lymphocyte cells to AFB1 (8.5 µl/L) revealed the strongest abnormal changes of chromatids: breaks and gaps.

In conclusion: the frequencies of chromosomal aberrations of control and mycotoxin-affected lymphocyte cells differed. The mycotoxin-affected bovine lymphocyte cells had breaks and gaps, which represent the initial lesions of DNA.

References:

1. Alberts J, Engelbrecht Y, Steyn P, Holzapfel W, Van Zyl W. Biological degradation of aflatoxin B1 by *Rhodococcus erythropolis* cultures. Int. J. Food Microbiol. 2006; 109: 121–126.
2. Battilani P., Toscano P., Van der Fels-Klerx H.J., Moretti A., Camardo Leggieri M., Brera C., Rortais A., Goumperis T., Robinson T. Aflatoxin B1 contamination in maize in Europe increases due to climate change. Sci Rep. 2016; 6.

P36

Approaches to investigate the effectiveness of aflatoxin binders in chickens

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Aflatoxins, as the most potent natural carcinogens, can cause severe public health issues. As humans are exposed to aflatoxins through food of plant and animal origin, managing aflatoxins in contaminated feed is of extreme importance. Today there is a vast number of mineral clays on the market which are sold as aflatoxin binders. However, not all subtypes of mineral clays are toxin binders. Furthermore, only few products on the market were tested *in vitro* and even fewer products were investigated in *in vivo* studies to verify the effectiveness of the product.

To assess an effectiveness of an aflatoxin binder mixed into chicken feed we need analytical method(s) for determination of aflatoxins and their metabolites in serum and chicken excreta. Comparison of the concentrations of aflatoxin biomarkers in serum of the aflatoxin groups with and without binder indicates whether aflatoxin absorption was reduced by the binder. However, taking of serum samples is more involved than excreta sampling and aflatoxin-lysine, the main serum metabolite after Pronase digestion, is not commercially available. Therefore, it is worth to also investigate the suitability of excreta analysis for assessing the effectiveness of aflatoxin binders in chickens. However, the avian peculiarity that urine is combined with feces in the cloaca before being excreted complicates the testing of aflatoxin binders in chickens. Unless truly urinary biomarkers like the aflatoxin B_1-N^7-guanine adduct are formed to sufficient extent, the concentrations of general aflatoxin biomarkers in excreta have to be compared. Theoretically, bound aflatoxins should remain attached to the binder during extraction of excreta, so that aflatoxin concentrations in excreta should be higher in the aflatoxin group without binder. However, bound aflatoxins might be desorbed from the binder if organic extraction solvents are used. This poster will provide an overview of the possibilities to investigate the effectiveness of aflatoxin binders in chickens and summarize the pre-tests required for reliable determination.

P37

The occurrence of fungi and mycotoxins in acorns

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Oak fruit (acorn) are unstable and particularly suceptible to temperature and humidity changes. They fall off the tree with high moisture content and, unlike most fruit and seeds, get dry very quickly and thus get destroyed. They are also frequently infected with fungi which thrive in moist conditions acorns need to avoid dehydration damage. The aim of the study was the determination of the level of fungi and mycotoxins in acorns of pedunculate oak. Harvesting acorns was made in 2016 Czerniejewo (Poland). The laboratory sample was prepared, then the acorns were shelled and, based on the visual examination, divided into two groups: moulds free and contaminated ones. Mycological examination was carried out on YGC agar medium. The results are expressed as the number of colony forming units (cfu) per gram of a sample. Mycotoxins were analyzed with HPLC-FLD and LC-MS/MS methods. Total number of fungi (cfu/g) amounted at -1,0 x 10^5 and - 2,6 x 10^6 fungi in free vs contaminated ones, respectively. The acorns samples were dominated by moulds from genus *Penicillium* – 97% and *Mucor* – 3%. Cytotoxicity evaluation of the most frequently isolated moulds was performed using MTT test with swine kidney (SK) cells. MTT test showed that the most cytotoxic species was *Penicillium expansum* (IC50 0,061 cm^2/ml). Among the determined mycotoxins, mycophenolic acid (MPA) and patulin (PAT) were present at the highest concentrations. In a sample contaminated by moulds (2,6 x 10^6 cfu/g), MPA was detected at 14580 ppb and patulin at 50 ppb. In this sample ZEN, DON, NIV, DOM, MAS, DAS, T2 toxin and OTA were detected at low levels. In the sample uncontaminated by moulds, MPA was detected at 85,4 ppb. The following toxins were not found in any of the above acorn samples: AFM1, AFB1, AFB2, AFG1, AFG2, 3ADON, HT-2, D3G, FUSX, α-ZAL, β-ZAL, β-ZOL, α-ZOL, T2 tetraol, T2 triol, NEO, 15-ADON, FB1, FB2, FB3, ROQ C, GF, OTB, MEV. The study confirmed presence of mycotoxins in acorns. This raises a question, of whether mycotoxins present in acorns can hazardous to animals living in the wild, as they constitute a substantial part of their diet.

P38

Monitoring of trichothecenes A and B and zearalenone in food in the Czech Republic

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Fusarium toxigenic microfungi have the ability to produce *Fusarium* mycotoxins in foodstuffs of plant origin. Many of the cereal (wheat, rye, corn, barley and oats) can produce trichothecene mycotoxins type A (HT-2 toxin (HT-2), T-2 toxin (T-2), and diacetoxyscirpenol (DAS) and type B (deoxynivalenol (DON), and its derivatives of 3-acetyl-DON (3-AcDON), 15-acetyl-DON (15-AcDON), and deoxynivalenol-3-glucoside (DON-3-Glc), nivalenol (NIV), fusarenon X (FUS X) and zearalenone (ZEA).
According to recent knowledge about trichothecenes and ZEA toxicity, and information about their dietary exposure sources a new research project: „*Monitoring of trichothecenes type A and B and zearalenone in food in the Czech Republic*“ was realised.
The determination of trichothecenes and ZEA in food represents the first part of the new project. Foods of plant origin (28 kinds) were collected in four sampling terms in 12 regions of the Czech Republic during the year 2016. So they were mainly cereal products e.g. flour, bread, rolls, cookies, pastries but also rice or beer. In total, 336 food samples were gathered.
Validated multi-toxin LC-MS/MS method for determination of trichothecenes and ZEA in food was developed. Raw extracts (acetonitrile/water 50/50) are cleaned-up with unbuffered QuEChERS. Limit of quantification of the method (LOQ) varied between 0.04 – 1.16 ng/g depending on the type of *Fusarium* mycotoxins and sample matrix. Recoveries were determined in the range 80 – 120 %. The results of the determination of trichothecenes and ZEA in food samples are presented in the table.

Mycotoxin	n+% [a]	Range (ng/g)		Mean[b]	Median[b]	90th perc.[b]	LOQ
		min	max	(ng/g)	(ng/g)	(ng/g)	(ng/g)
DON	53.6	0.76	274	13.6	2.99	36.0	0.76
3-AcDON	1.49	6.91	57.7	0.49	0.21	0.21	0.41
15-AcDON	1.49	4.06	24.1	0.70	0.58	0.58	1.16
DON-3-Glu	6.55	0.69	36.7	0.52	033	0.33	0.65
FUS X	4.17	2.38	11.4	0.57	0.30	0.30	0.59
NIV	2.68	1.69	7.64	0.27	0.17	0.17	0.33
T-2	11.6	0.29	13.9	0.29	0.09	0.41	0.18
HT-2	10.7	0.71	17.4	0.72	0.23	0.95	0.45
DAS	1.79	0.33	0.66	0.07	0.06	0.06	0.11
ZEA	38.4	0.13	11.6	0.42	0.02	1.23	0.04

[a]n+%= percent of positive samples; [b]middle bound approach for left-censored data < LOQ, trichothecenes and ZEA concentrations are considered as being 1/2 LOQ

Conclusion: The results of DON, NIV, T-2/HT-2 toxins and ZEA in food obtained in study will be used for dietary exposure assessment and health risk characterization for Czech population.

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P39

Growth inhibition of *Fusarium graminearum* by biologically active compounds in buckwheat grain

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Fusarium graminearum is a pathogen of cereals and a producer of toxic mycotoxins. Buckwheat grains are rich in biologically active compounds which can penetrate the cell wall of microscopic fungi and affect their development. The study was aimed to determine inhibitory effects of buckwheat grain and hull extracts against *F. graminearum* and to identify phenolic compounds of buckwheat grain with a potential application in the prevention of fungus occurrence.

F. graminearum monoculture was grown on a potato dextrose agar medium (20 ml) supplemented with raw extracts of buckwheat grain and hulls, prepared in dimethyl sulphoxide (DMSO) solvent (extraction ratio 1:10) and solutions of pure phenolic compounds: rutin (150.0 µg/ml), quercetin (20 µg/ml) and their mixture, prepared by diluting one-component solutions at a ratio of 1:1, 3,4-dihydroxybenzoic acid (3,4-DHBA, 50 µg/ml), p-hydroxybenzoic acid (p-HBA, 20 µg/ml) and their mixture (1:1), *p*-coumaric (20 µg/ml), ferulic (20 µg/ml) and their mixture (1:1), vanillic (20 µg/ml) and sinapic acid (20 µg/ml). DMSO solvent was used as control. The extension diameter (cm) of hyphae was measured at 24 hours' intervals for 7 days.

The first significant ($P<0.05$) symptoms of mycelial growth inhibition were recorded after 96 hours of incubation. Buckwheat hull extract showed from 10 % to 15 % stronger antifungal activity than grain extract. A solution of rutin and quercetin mixture gave ~10 % better *F. graminearum* growth inhibition than buckwheat hull extract. This mixture, as well as that of 3,4-PHBA with p-HBA, provided 20–30 % significantly stronger inhibition of the fungus than single-component solutions. The solutions of p-coumaric, sinapic acids inhibited *F. graminearum* growth significantly 2.0–4.6 % better compared with 3,4-DHBA. The single-component solutions of p-HBA, vanillic and ferulic acids did not statistically significantly differ in the effect on *F. graminearum* growth. Buckwheat hulls contain higher concentrations of phenolic compounds than grain. Therefore phenolic compounds extracted from hulls could be used as a natural preventive product against grain infection with microscopic fungi and mycotoxins.

P40

A regional mycotoxin survey of food cereals from Central Ukraine

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Ukraine is one of the world's largest producer of cereals, but very few data have been published on the contamination of domestic cereal products with mycotoxins. This study therefore aimed at performing a small survey (n=42) on a variety of local products from retail stores in the area of Bila Tserkva (situated in the Kiev district) and adjacent regions of Central Ukraine. Samples included wheat (n=10) and products thereof (flour (6), pasta(8)), barley (5), oats (4), maize (4), millet (3), and buckwheat (2). Samples were purchased in 2017 as offered to the consumer, and analysed using enzyme immunoassays for deoxynivalenol (DON; LOD 20 ng/g), zearalenone (ZEN; 1.7 ng/g), T-2/HT-2 toxin (T-2/HT-2; 0.8 ng/g), and ochratoxin A (OTA; 0.1 ng/g). Overall, the toxin levels were low in all products, well below the European Union maximum levels (EC 1881/2006). Most samples were positive for DON (83%, 20-830 ng/g) and T-2/HT-2 (88%, 0.9-76 ng/g), few samples contained ZEN (21%, 14-40 ng/g) or OTA (17%, 0.1-0.8 ng/g). Most notably, wheat and products thereof (flour, pasta) contained low (DON, T-2/HT-2) or no detectable (ZEN, OTA) toxin contamination. The median toxin levels in these products were 50 ng/g (DON), 3 ng/g (T-2/HT-2), <LOD (ZEN), and <LOD (OTA). Even the highest levels of DON (barley), T-2/HT-2 (maize), ZEN (millet) and OTA (buckwheat) were low when compared with data from other European countries. Although this study was limited with regard to sample numbers and sampling period, the results indicate that the contamination situation of cereals in Central Ukraine with mycotoxins may differ from that of other European regions.

P41

Mycotoxins contamination in winter varieties of cereal species on the light soils

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Winter cereals are a group of annual plants that need a period of low temperatures in winter to produce flower buds and produce fruit. The group of these plants includes: wheat, barley, triticale and rye, oilseed rape, turnip rape and vetch. Winter cereals are used both in the food and feed industry. The scale and wide range of grain utilization oblige to produce cereals of the best quality, free from contamination, one of which may be mycotoxins, secondary mould metabolites. The aim of this research was to assess the mycotoxicological contamination of winter cereal varieties. The research was carried out as part of the BIOSTRATEG project in 6 locations throughout Poland. The test material consisted of 7 varieties of winter cereals (42 samples): wheat (Artist), barley (Kosmos), triticale (Meloman) and 4 varieties of rye (population - Dańkowskie Rubin, hybrid - KWS Binntto and KWS Serafino, population from own reproduction - NN). *Fusarium* mycotoxins (NIV, DON, 3ADON, MAS, DAS, T-2, HT-2, as well as ZEN) were determined using HPLC-MS/MS method. The samples clean-up was performed using Bond Elut® Mycotoxin (Agilent) columns. Ochratoxin A was determinated using HPLC-FLD method. The samples were cleaned using OchraPrep immunoaffinity column (R-Biopharm Rhône Ltd).

The most frequently occurring mycotoxin was DON. It was detected in 40 of the 42 cereal samples (95%). Its mean concentration was 70 µg/kg. The maximum value was recorded in the NN rye sample (354 µg/kg). The highest mean concentrations were obtained for the triticale samples (170 µg/kg), and the lowest for barley (20 µg/kg). T-2 toxin was detected in 88% of the samples (mean concentration was 2 µg/kg). The maximum value of this toxin was found in a triticale sample (15.6 µg/kg). The highest mean concentrations were recorded in wheat samples (3.9 µg/kg), and the lowest in samples of Dańkowskie Rubin rye (0.7 µg/kg). Similarly, a high overall percentage of contamination (83%, 35 samples) was obtained for the HT-2 toxin. Its mean concentration was 7.7 µg/kg. A wheat sample contained HT-2 toxin at the highest concentration of 53.7 µg/kg. The lowest mean concentrations of HT-2 toxin were found in Dańkowskie Rubin rye samples (3.0 µg/kg), while the highest in wheat samples (18.9 µg/kg). 76% of the analyzed samples contained ZEN, with mean concentration of 2.5 µg/kg. The maximum concentration of this mycotoxin was recorded in a wheat sample (41.6 µg/kg). Wheat samples contained ZEN at the highest average concentration (8.4 µg/kg), while the lowest concentration was found in barley samples (0.6 µg/kg). NIV, 3ADON and MAS occurred in 36%, 24% and 14% of the samples, respectively. The highest concentration of NIV and MAS was obtained in the barley sample (18.9 µg/kg and 2.34 µg/kg), and 3ADON in a wheat sample (3.0 µg/kg). The highest mean NIV concentration was recorded in wheat samples (5.2 µg/kg). DAS occurred only in one sample (barley, 1 µg/kg). Ochratoxin A was detected in 3 of the 42 cereal samples (7%), and its highest concentration was 2.8 µg/kg (KWS Serafino rye sample).

It was concluded that winter cereals contained the majority of *Fusarium* metabolites, but no tested sample exceeded the maximum acceptable levels of mycotoxins (Commission Regulation 1881/2006 EC and Commission Recommendation 2013/165/EU).

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P42

Zearalenone induces oxidative stress in prostate normal cells

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Zearalenone (ZEA) is a fungal mycotoxin present in cereals, which is one of the endocrine disrupting chemicals (EDC). ZEA through its structural similarity to 17β-estradiol might induce estrogen associated cell signaling and modulates hormonal balance in animals. ZEA is also reported to induce reactive oxygen species (ROS), DNA damage, apoptosis and mitochondrial depolarization.

This study was undertaken to verify if ZEA might induce oxidative stress in normal prostate tissue and potentially modulates its proper functioning in humans. PNT1A cells were treated with ZEA (30-10µM), 17β-estradiol (100nM) as positive control, and non-treated as control cells for 48h. We observed that ZEA induces oxidative stress in normal prostate cells associated with decrease in cell viability. Moreover, we observed concentration-dependent decrease in the expression of mitochondrial superoxide dismutase 2 (SOD2).

Our results indicates that ZEA induces oxidative stress in normal prostate cells what might alter its functioning. Together with previously reported oxidative stress induced by ZEA in Sertoli cells, our results might suggest that ZEA influences males' physiology.

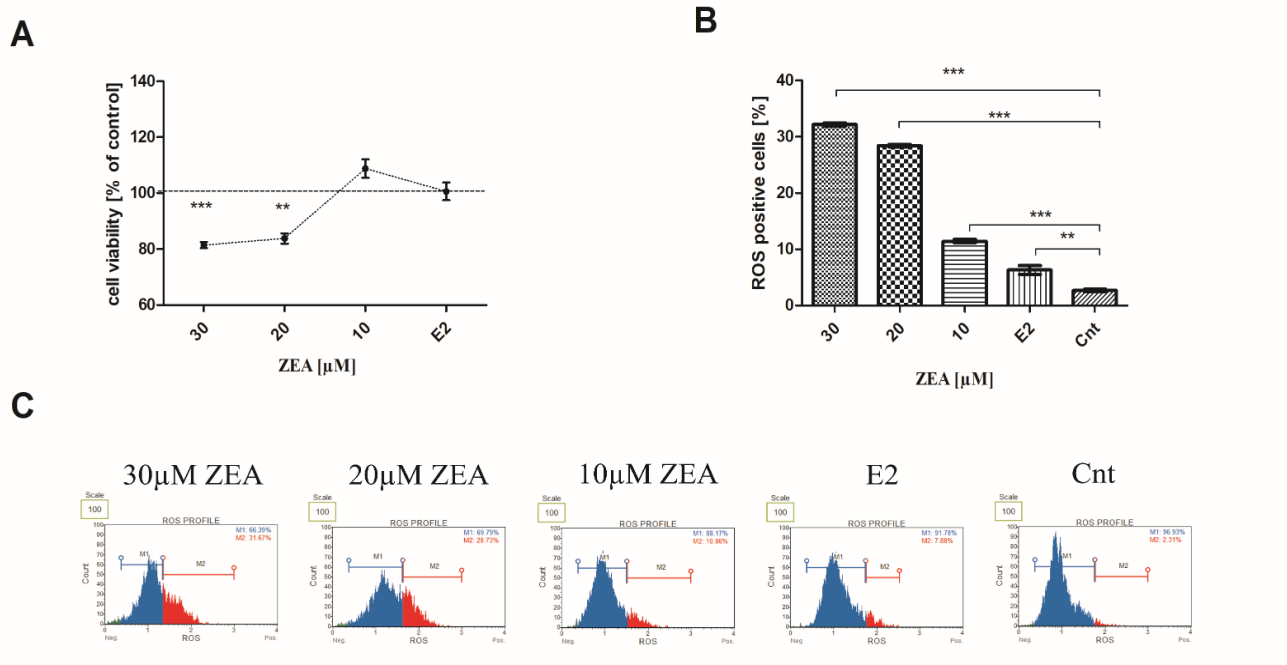

Figure: A- cell viability assay (MTT) results, B- results from Oxidative Stress Muse® kit expressed as ROS positive cells, C- representative results, **p<0.01, ***p<0.001 as compared to Cnt. ZEA-zearalenone, Cnt-control, E2-17β-estradiol (100nM).

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P43

Contamination of Polish winter rye by ergot and its alkaloids

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Ergot (*Claviceps purpurea*) is a fungal pathogen that infects various grass and small grain cereal species; open-pollinated grasses such as rye and triticale are the most vulnerable for the infection. The ergot can decrease crop yield by 5-10 %, although the more significant problem is connected with accumulation of toxins, known as ergot alkaloids (EAs), in sclerotia. EAs may induce numerous health problems in humans and animals. The specific symptoms of poisoning include convulsive disorder, fever, hallucinations, swelling, and gangrene.

The contmination of 122 samples of rye grain harvested in Poland with ergot and 12 of its alkaloids (EAs) was evaluated. The sample preparation included: milling of grain with sclerotia, extraction with acetonitrile - ammonium carbonate (84:16 v/v) mixture, and purification on glass chromatographic column filled with neutral alumina. The samples were analyzed using an H-class ultra-high-performance liquid chromatograph coupled with a LCQ Premiere XE time-of-flight high-resolution mass spectrometer.

Ergot sclerotia were found in all samples. The mean content of ergot sclerotia in grain ranged between 0.74 and 1.06 g/kg, and the mean concentration of ergot alkaloids in grain ranged between 270.1 and 580.4 µg/kg, depending on the region of cultivation. Thirty-seven percent of the samples were infected with ergot below the 0.5 g/kg level set by the European Commission as the maximum permissible level for ergot, and in those samples the mean ergot alkaloids concentration was 155.8 µg/kg (range 4.7–667.9 µg/kg). A statistically significant correlation (R^2 = 0.6941) between ergot content and concentration of ergot alkaloids was found. Ergot alkaloids concentration in grain was re-calculated into ergot alkaloids concentration in sclerotia, and was found to vary widely from 114 to 3167 mg/kg. Ergot alkaloids profiles were most frequently dominated by R-configured ergopeptides, such as ergocryptine, ergocornine and ergocristine (31%, 29%, and 16% of the samples, respectively).

P44

Enzymatic degradation of fumonisin B1 and zearalenone in grain maize silage

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Mycotoxin contamination occurs widely in feedstuffs of plant origin. Contamination of animal feed with mycotoxins compromises animal health and may lead to carry-over into animal products (eggs, meat, or milk). Moreover, mycotoxins lead to huge economic losses. Thus, strategies to eliminate or inactivate mycotoxins in food and feed are urgently needed.

Enzymatic detoxification of mycotoxins in situ has been discussed since the 1970s as a strategy for the elimination of mycotoxins from grain, feed and food. *Fusarium* mycotoxins, e.g. fumonisin B1 (FB1) and zearalenone (ZEN), are among the most common and toxicologically most important mycotoxins in feed and food. Our group developed enzymes that catalyze the degradation of *Fusarium* mycotoxins in the gastrointestinal tract, i.e. the fumonisin esterase FumD (FUMzyme®, registered in the European Union as a feed additive) and the zearalenone hydrolase ZenA (ZENzyme®). FUMzyme® hydrolyzes the two tricarballylic acid side chains of fumonisins, resulting in the formation of non-toxic hydrolyzed fumonisins. ZENzyme® exhibits lactonase activity against ZEN, forming the non-estrogenic hydrolyzed zearalenone.

Application of enzymes in silage is a new approach for the detoxification of mycotoxins in feed. In this study, we tested the FB1- and ZEN- degrading capability of FUMzyme® and ZENzyme® in grain maize silage. We also evaluated, if these enzymes have an influence on certain indicators of silage quality (pH, volatile fatty acid levels and water soluble sugar levels). Two 90-day experiments were conducted in microsilos (~700 g ensiled material) with naturally contaminated corn meal, FUMzyme® (1st silage trial) or ZENzyme® (2nd silage trial) in the presence or absence of further additives (silage stimulant and/or an acid-based feed preservative).

Results showed that FUMzyme® was highly active and FB1 was completely degraded during ensiling, without any negative impact on the fermentation process. Acidity of silage and production of lactic acid in FUMzyme® treated samples were comparable to those of a FUMzyme® free control treatment. Addition of a silage inoculant stimulated the fermentation and induced a rapid pH drop in presence or absence of FUMzyme®.

Similarly, ZEN concentrations were significantly reduced by ZENzyme® during ensiling. Moreover, we did not find any negative effect of ZENzyme® on indicators of silage quality, such as utilization of water soluble carbohydrates and production of desired volatile acids.

P45

Patulin in the interaction between *Penicillium expansum* and *Rhodosporidium paludigenum*

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Rhodosporidium paludigenum is a biocontrol yeast for the control of blue mold disease on several fruits and fruit-derived products, as well as detoxifying patulin, which is mycotoxin produced by *Penicilliium expansum*. In our study we found that at low cell density, *R. paludigenum* can stimulate patulin synthesis, while the growth of *P. expansum* was inhibited. Potential increase of patulin levels is an undesirable side effect of the application of *R. paludigenum* to fruit preservation. After culturing 10^3 spores/mL *P. expansum* with 10^5 cells/mL *R. paludigenum* for 4 days, patulin concentration was 1.66 times higher than that of controls without *R. paludigenum*, while the expression of three genes involved in patulin synthesis PatK, PatL and PatN were 3.18, 1.45 and 2.46 times up-regulated, respectively. Our results imply that *R. paludigenum* cultures for the control *P. expansum* on fruits have to be adjusted to an adequate cell density in order to prevent increase of patulin accumulation.

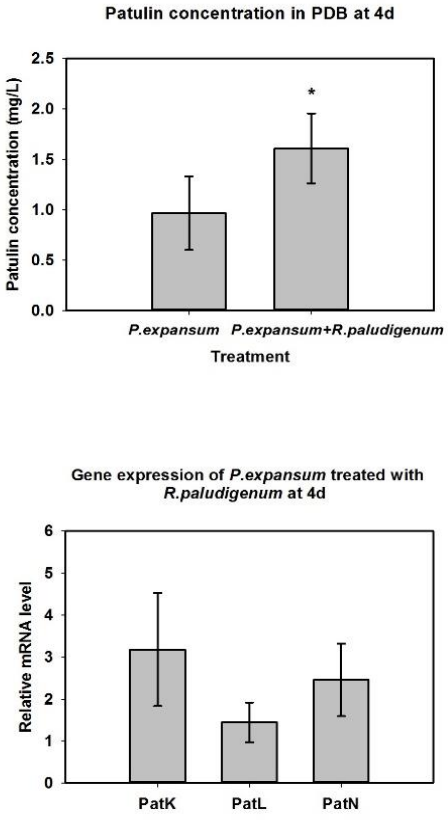

Figure: Patulin concentration & relative mRNA level

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P46

Comparative analysis of mycotoxin occurrence in different types of forage

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Feed raw materials show different profiles of mycotoxin contamination depending on the type of culture and storage conditions. In previous studies, we highlighted the difference of mycotoxin occurrence in different types of cereals and corn materials. Ruminant diets include high levels of various forages, the aim of the present study was to compare the occurrence of mycotoxin in the most frequent types of forage: corn silage, grass silage, hay and straw. This study used the LABOCEA database composed of chromatography analyses run with LC MS/MS from 2013 to 2016 and including 931 forage samples. This database has the unique characteristic of quantifying 45 mycotoxins per sample, which is very rare in the available data on forages. Data show that corn silage is the most poly-contaminated forage as 78% of the samples contain more than 5 mycotoxins on the contrary to straw, hay and grass silage, with 46%, 15% and 11% respectively. As shown on other feed materials, fusariotoxins are also the most frequent mycotoxins in forages, but on the contrary to grains, deoxynivalenol (DON) is not always the most frequent. Mycotoxins like zearalenone (ZEA) can be more often positive in grass silage and hay than DON. Nevertheless, the median level of DON is always the highest in all types of forage samples, corn silages having higher DON median level of contamination (1090 ppb) than straw (345 ppb), grass silage (70 ppb) and hay (25ppb). Apart from fusariotoxins, our data also illustrate the specificity of grass to have endophytic toxins like ergot alkaloids (6 alkaloid toxins are quantified) on the contrary to corn silage and straw. In this study Alternaria mycotoxins are not as rare as described in other studies as tenuazonic acid contaminates more than 20% of grass silage and hay samples. Regarding storage mycotoxins, as demonstrated in other studies, hay and straw are a good substrate for the development of *Aspergillus versicolor*. Consequently, the contamination in sterygmatocystin is the highest in these types of forage whereas aflatoxins and ochratoxins were almost not detected in the four types of forage tested. This unique wide study on mycotoxin contamination in forages is a first step in the identification and understanding of the different profiles of mycotoxin contamination in the main forages.

P47

Non-invasive GC-MS analysis of trichodiene as volatile mycotoxin biomarker in cereals

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Due to the increasing consumption of cereals worldwide, monitoring of growth, storage and processing is becoming more crucial. Particularly when stored, infested grains breed fungal clusters (“hot spots”) in which mycotoxins greatly exceed allowed maximum levels. Because of their unpredictable presence, current sampling and procedures for mycotoxin analysis are complex and contain invasive and cost intensive steps.

A possible approach to avoid sampling inaccuracies is the non-invasive analysis of the homogeneous gas phase above the crops, instead of analyzing random samples. However, this procedure requires microbial volatile organic compounds (MVOC´s) being released by the samples. Previous investigations revealed trichodiene to be a precursor in trichothecenes biosynthesis – one of the largest mycotoxin groups with more than 180 compounds. Due to its non-functionalized sesquiterpene structure, quantification of trichodiene using Headspace GC-MS methods is possible (e.g. [1]). Thereby, it could be used as biomarker for trichothecene contamination in foodstuff.

However, further studies are necessary. The correlation between trichodiene concentration in the gas phase and trichothecenes mass fraction in the sample must be examined closely to draw conclusions about the trichothecene content in samples. Realizing this idea, would widely extend the applicability of trichodiene and enormously simplify trichothecene quantification. Hence, this first step of an ongoing study aims to develop a laboratory reference method using trichodiene as volatile biomarker to quantify trichothecenes in cereals. Static headspace and SPME-enrichment coupled to gas chromatography with mass spectrometry (GC-MS) were employed. In a second step, this reference method is intended to validate new approaches for fast on-site screening of trichodiene in cereals.

References:

[1] Becker, E.; Herrfurth, C.; Irmisch, S.; Köllner, T. G.; Feussner, I.; Karlovsky, P.; Splivallo, R. (2014): Infection of Corn Ears by *Fusarium* spp. Induces the Emission of Volatile Sesquiterpenes. Journal of Agricultural and Food Chemistry, 62, pp. 5226 - 5236.

P48

Individual and combined toxic effects of zearalenone and its metabolites (alpha zearalenol and beta zearalenol) on human liver cancer cell line HepG2

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Zearalenone (ZEA), a mycotoxin produced by several *Fusarium* spp., is most commonly found as a contaminant in stored grain and has chronic estrogenic effects on mammals. The reproductive system is the major target of the mycotoxin toxicity and ZEA intoxication was associated with decreased fertility, reduced litter size and change in serum levels of progesterone and oestradiol. In addition, ZEA has been shown to be immunotoxic, hepatonephrotoxic and enhancer of lipid peroxidation. ZEA derivatives (a-zearalenol (a-ZOL), b-zearalenol (b-ZOL)) can also be produced by *Fusarium* spp. in corn stems infected by fungi of this genus in the field and in rice cultures. Also, following oral exposure, zearalenone is metabolized in various tissues, particularly in the liver, the major metabolites being a-ZOL and b-ZOL. The co-exposure of cells to mixture of a combination of mycotoxins may cause an increase of toxicity produced by these mycotoxins. In this in vitro study, we compared the effects of zearalenone (ZEA), two of its derivatives: a-zearalenol (a-ZOL), b-zearalenol (b-ZOL) on the viability and oxidative stress response of human liver cancer cell line (HepG2). HepG2 cells were cultivated at a density of 1*105 cells/mL until confluence in 96 or 24 wells plates and treated for 72h at 37oC with different concentrations (0-100mM) of toxins (ZEA, a-ZOL and b-ZOL) and their binary combinations in a 1:1 ratio. Cell viability was assessed after 72h using a neutral red assay. Effect of the toxins and their binary combinations on oxidative stress were assessed through the measurement of the activity of enzymes involved in the antioxidative response (superoxide dismutase - SOD, glutathione peroxidase - GPx, catalase-CAT) and on total antioxidant capacity of the Hep2 cells. Assessment of the enzyme activity was performed using Cayman kits according to the producer recommendation from the supernatant issued from ultrasonicated cells. Total antioxidant capacity was also analysed in the cells and was based on the absorption of ABTS+ cation. T test were performed in order to assess the significant statistic differences between treatments. Chou-Talalay method was used for the study of the combined toxicity of mycotoxins in order to determine the type of interaction between ZEA and its metabolites (synergic, additive or antagonistic). As resulted from the neutral red assay, the amounts of toxins necessary to inhibit viability by 50% were: 54.61 mM for ZEA; 119.9 mM for a-ZOL; 15.23 mM for b-ZOL and for their combinations 46.64 mM for ZEA+a-ZOL; 19.92 mM for ZEA+b-ZOL and respectively 17.99 mM for a-ZOL+b-ZOL combination. The investigations concerning the effect of toxins on oxidative stress and on the type of interactions between the toxins are under analysis.

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P49

Neutralization of aflatoxin B1 (AFB1) induced lethality in Zebrafish treated with an AFB1 binding monoclonal antibody and a DNA aptamer

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There is a need to develop new methods to neutralize the effects of mycotoxins when they are fed to domestic animals as many of the mycotoxin binders in current use are only partially effective and often have undesirable side effects. Mycotox Solutions Inc. has hypothesized that antibodies and aptamers (chemical antibodies) designed to specifically bind AFB1 can be used not only for the quantitation of mycotoxins but also as agents to bind and neutralize mycotoxins when they are incorporated into animal diets. The objective of the current study was to determine the lethality of AFB1 alone, a monoclonal antibody (mAb) alone and AFB1 in the presence of either the mAb or aptamer in Zebrafish embryos (ZFE). The ZFE mortalities (lethality) in the antibody study after a 96 h incubation period were zero for ZFE administered mAb (0.5 µM) alone, 80% ± 3.3 SD for ZFE administered AFB1 (0.25 µM) alone and zero for ZFE administered the AFB1 (0.25 µM) + mAb (0.5 µM) mixture. In the aptamer study, the mortalities after a 96 h incubation period were zero for ZFE administered the aptamer (0.25 µM) alone, 82.2%, ± 1.9 SD for ZFE administered AFB1 (0.25 µM) alone, and zero for ZFE administered the AFB1 (0.25 µM) + aptamer (0.25 µM) mixture. The number of replicates for each treatment was 30. These data demonstrate that mAbs and aptamers that are designed to specifically bind AFB1 are capable of completely neutralizing the effects of AFB1 in a ZFE model. Thus, it is evident from the results of this study that both specific aptamers and antibodies should be highly effective in neutralizing the toxic effects of not only AFB1 but also other mycotoxins in animals that are fed diets containing mycotoxins. Additional research is required to confirm the results of current study using model animals such as rats or ducklings. The current data suggest that antibodies and aptamers may provide a new and highly effective method for safely controlling mycotoxicosis in animals. Both mycotoxin binder technologies are patented (Pending).

The ZFE lethality studies were carried out by Phylonix Pharmaceuticals Inc,Cambridge, MA (USA) under the excellent technical guidance of Wen Lin Seng, Ph.D.

P50

Prevalence of citrinin in the Belgian feed chain and its toxicokinetic profile in broiler chickens

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According to the scientific opinion on the mycotoxin citrinin (CIT), published by the European Food Safety Authority (EFSA, 2012), occurrence and toxicity data to perform a risk assessment are lacking, showing the need to collect quantitative data by occurrence and toxicity studies. The main aim of this project is therefore to gather information on the prevalence of CIT in feed and different foodstuffs available on the market in Belgium, to identify all relevant sources of intake and their importance. Combining these occurrence data with toxicokinetic data obtained from animal studies, a risk assessment in Belgium (exposure assessment and risk characterization for both the Belgian population and pig and poultry sector) can be performed. In this study, we report our first results regarding the presence of CIT in broiler chicken feed and preliminary data concerning its toxicokinetic profile in broiler chickens, including data on absolute oral bioavailability. First, sensitive and specific methods for the quantitative determination of CIT in chicken plasma and feed using liquid chromatography combined with electrospray ionization (ESI) tandem mass spectrometry (LC–MS/MS) were developed. For plasma clean-up, a straightforward deproteinization step using acetonitrile was performed, and phospholipids were removed using a Waters Ostro® 96-well plate. For feed, a simple QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe)-based extraction protocol was developed. The analytical methods were in-house validated. Second, CIT concentrations were measured in 38 Belgian broiler chicken feed samples using the validated LC-MS/MS method, revealing the presence of CIT (<LOQ – 3.90 µg/kg) in 45% of the samples. Next, in order to investigate the absolute oral bioavailability and toxicokinetics, CIT was administered both orally and intravenously (cross-over) to broiler chickens, blood was collected and absorption, distribution, metabolism, and excretion (ADME) parameters were determined. An additional pilot study was carried out to investigate possible phase I and II metabolites of CIT in plasma using high resolution-mass spectrometry (HRMS).

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A survey of citrinin and ochratoxin A in food and feed in Belgium

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According to the scientific opinion of the European Food Safety Authority (EFSA) on the nephrotoxic mycotoxin citrinin (CIT), occurrence and toxicity data to perform a risk assessment regarding this toxin are lacking. Hence, a survey of CIT was performed in several food and feedstuffs available on the markets in Belgium, with the prospect of identifying all relevant sources of intake and their importance. Cereals and cereal-based products are the major contributors of dietary exposure to CIT, as well as food supplements based on red yeast rice (RYR) powder for which a legal maximum limit of 2 mg/kg was set. Ochratoxin A (OTA), another nephrotoxic mycotoxin, was also included in this study, since both mycotoxins often co-occur in food and feedstuffs. In a first stage, UPLC-ESI+/--MS/MS methods for the simultaneous analysis of CIT and OTA in feed and food products were developed for cereal-based products, fruit and vegetable juices, herbs and spices, nuts and seeds, alcoholic beverages, baby food, soy and vegetarian products, food supplements and meat products. A QuEChERS-based extraction method was used without any further clean-up step to extract the mycotoxins from the abovementioned matrices. Final extracts were analyzed using a Waters Acquity UPLC system coupled to a Xevo TQ-S mass spectrometer equipped with an electrospray interface operated in both positive and negative ionization mode. Chromatographic separation was achieved using an Acquity UPLC HSS T3 column by applying gradient elution and the total run time was 10 min. The methods were successfully validated for several parameters with regards to specificity, linearity, apparent recovery, limit of detection, limit of quantification, repeatability, reproducibility, matrix effects and measurement uncertainty to meet the criteria mentioned in Commission Regulation No. 401/2006/EC and Commission Decision No. 2002/657/EC. This analytical method was applied for the detection and quantification of CIT and OTA in 100 broiler chicken and pig feed samples and 200 food samples. Analysis of the feed samples revealed the presence of CIT (<LOQ – 3.90 µg/kg) and OTA (<LOQ – 5.60 µg/kg) in more than 50% of these products. For food samples, CIT and OTA were most frequently detected in cereal-based products (<LOQ – 0.25 µg/kg). For the food supplements, the RYR supplement was highly contaminated with CIT (1224.6 µg/kg). Consequently, this study shows the need for further toxicokinetic and toxicity studies to set legal limits for CIT in the interests of public health.

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P52

Mycotoxin occurrence under short-term plastic mulching in strawberry cultivation

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Plastic mulching (PM) is widely used for the production of off-season commodities, because of its positive effects on soil temperature and soil moisture. Further, these modifications may lead to a restructuration of soil microbial communities, with unexpected effects on the occurrence of mycotoxins in agricultural soils. In this study, the occurrence of the mycotoxins DON, NIV, OTA, CIT, ZEN and, T-2 was investigated in relation to modifications of soil physicochemical parameters under short-term PM. Ergosterol was used as indicator of fungal biomass. No-mulch (NM) was used as control. Samples were collected at the time of planting (T0) and successively at two (T1) and four months (T2) after planting, in three soil depths (0-10, 10-30 and 30-60 cm). In total, 90 soil samples were analyzed. Mycotoxins were analyzed via LC-HRMS after soil extraction with methanol-water mixture. Soil samples were additionally characterized for soil physicochemical and microbial parameters. Approximately 50% of the samples resulted positive at least to one mycotoxin. DON and NIV were the mycotoxins with the highest detection frequencies. The levels of DON and NIV observed in soil samples ranged from 1.0-12.0 and 8.1-12.4 µg/kg, respectively. Mycotoxin concentrations could not be attributed to fungal biomass. Under PM, DON and NIV concentrations increased from T0-T2, pointing to either a higher mycotoxin formation or to less efficient mycotoxin degradation. The highest concentrations were observed at the soil depth 10-30 cm, which coincides with the highest root density. Under NM, the highest concentrations were observed at T1 for DON and T2 for NIV. No clear pattern of mycotoxin occurrence was observed under NM. In general, no significant differences in mycotoxin concentrations were found between the managements. To conclude, no significant differences on soil physicochemical and microbial properties were observed between PM and NM. But, two different patterns of mycotoxin occurrence were observed. These differences can be partly attributed to modification of soil temperature together with the impervious matter of PM which hampers water penetration and colonization by other species. The experiment is ongoing for the investigation of long-term effects of PM on soil microbiology and the fate of mycotoxins.

P53

Mycotoxin levels in human milk samples from Lower Saxony and Bavaria (LUPE 8)

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In Biomonitoring studies, contrasting scenarios regarding exposure to mycotoxins with human milk have been described [1]. As part of the LUPE (Länderuntersuchungsprogramme) projects, the concentration of 15 mycotoxins in human milk samples (DON, OTA, CIT, OH-CIT, ZEN, AFTXs, FB1, T-2, AME, AOH and ENNB1) from two cohorts in Germany was investigated: Lower Saxony (n=78) and Bavaria (n=79). The QuEChERS method [2] with small modifications was used for the extraction. Sample analysis was done via LC-HRMS by interpolation of data in matrix matched calibration curves, range 0.1-50 µg/L. For DON, the working range was set at 10-100 µg/L. The recovery rates ranged between 41-104%. Additionally, the occurrence of mycotoxin conjugates in human milk was investigated via enzymatic hydrolysis (EH) with β-Gluc/ArylS. More than 70% of the samples were positive for at least one mycotoxin. The most frequently detected mycotoxins are presented in table 1. For these mycotoxins, the infant daily dose (IDD) was calculated and compared with TDI values [1, 3, 4]. The proposed TDI values were exceeded, particularly with DON, AFB_1 and AFM_1. OTA could not be detected, differing from a previous German study [5], attributed to the low recovery rate of the method (41%). Higher mycotoxin levels after EH were sporadically observed, which does not clearly evidence an excretion of mycotoxins also as conjugates. Results of this study confirm the occurrence of mycotoxins in German human milk samples at levels that may exceed in some cases recommendations.

Table 1: Mycotoxin occurrence in human milk samples from Lower Saxony (LS) and Bavarian (B), infant daily dose (IDD) and comparison with TDI-values

Mycotoxin	*Cohort*	*Positive (%)*	*Average ± SD (µg/L)*	*IDD[a] (µg/kg b.w.)*	*TDI-values[2], [b] (µg/kg b.w.)*	*Hazard Index*
DON	LS	24.4	11.92 ± 5.74	2.21	1	**2.2**
	B	3.8	32.91 ± 41.13	6.09		**6.1**
AFB_1	LS	38	0.20 ± 0.00	0.03	$6.8x10^{-4}$[b]	**43.9**
AFM_1	B	38	0.08 ± 0.03	0.01	$6.8x10^{-4}$[b]	**14.6**
CIT	LS	15.4	0.40 ± 0.30	0.07	0.2[c]	0.4
	B	13.9	0.19 ± 0.12	0.03		0.2
FB_1	LS	19.2	5.81 ± 8.23	1.07	2	0.5
	B	15.2	5.01 ± 11.89	0.93		0.5
ZEN	LS	15.4	0.32 ± 0.21	0.06	0.25	0.2
	B	3.8	0.23 ± 0.17	0.04		0.2

[a] Calculated as average concentration in milk × 0.185 L per kg bw [3, 4]; [b] TDI values are in this case not adjusted [1], a TDI-adjustment on factor 3 for infants below 16 weeks is under discussion [4].
[c] [6]; [d] LNC: level of no concern for nephrotoxicity.

References: [1] Fromme et al. 2016. nt J Hyg Environ Health 219(2), 143-165. **[2]** Rubert et al. 2014. Anal Chim Acta, 820, 39-46. **[3]** Degen et al. 2017. Toxicol Lett, 277, 69-75. **[4]** EFSA 2017. EFSA Journal 15 (5): 4849, 1-58. **[5]** Muñoz et al. 2013. Gesundheitswesen 75, 194-197. **[6]** Kuiper-Goodman 1990. Can J Physiol Pharmacol. 68(7):1017-24

P54

Ochratoxin A in black pepper, white pepper and clove sold in Yaoundé (Cameroon) markets: Contamination levels and consumers' practices increasing health risk

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Background: Spices are widely consumed in Cameroon, but there is no data available concerning their mycotoxin contamination. A cross-sectional study was conducted from February to June 2017 to assess the occurrence of Ochratoxin A (OTA) in black pepper, white pepper and clove sold in three main markets of Yaoundé (the capital of Cameroon), and the health risk that can be associated taking into account consumers' practices during and after purchase.

Results: Data obtained revealed that 10% of black pepper samples and 40% of the white pepper samples contained OTA at levels ranging between 1.15–1.91 µg/Kg and 1.81–4.89 µg/Kg, respectively. This is below the established European Commission regulated limit of 15 µg/Kg in spices. This toxin was not detected in clove samples. Considering the consumption data collected from our survey, the maximum OTA daily intake was estimated at 0.182 and 0.699 ng/kg bw/day for black pepper and white pepper, respectively. This is lower than the EFSA tolerable value of 17 ng/kg bw/ day. However, it also comes out that 88% of respondents did not have an idea of mycotoxins. Furthermore, 5–20% of them do not pay attention to some general quality indicators such as odour, colour and presence of moulds reported by the others when purchasing or storing the three spices, the latter being the most pertinent indicator of the possible presence of mycotoxins. These spices are generally stored at room temperature and for prolonged periods, conditions which are suitable for fungal growth and mycotoxin production.

Conclusion: Although the present study shows limited or no health risk associated to OTA contamination in black pepper, white pepper and clove, it is necessary to enhance consumers' awareness on mycotoxins and their associated impact on health, and to sensitise them on preventive measures to be taken.

P55

Assessment of aflatoxin levels in processed and unprocessed vegetable oils in Nigeria

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Oils play vital role in a well-balanced diet. However, aflatoxin is a major mycotoxin known to frequently contaminate crop produce and processed form which consequently affect the health of human and animal in developing countries especially Africa. The survey was carried out to determine the rate at which aflatoxin travel from unprocessed to processed vegetable oils and molecular characterization of identified fungi was done as well. Twenty samples of processed and unprocessed vegetable oils from factories were collected and assessed for aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2) using High Performance Liquid Chromatography (HPLC). Unprocessed (Corn oil, Coconut oil, Soya oil, Palm kernel oil, Palm oil, Groundnut oil, Cotton oil, Sun-flower oil and Canola oil) had the following aflatoxin concentrations respectively; 157 ng/kg, 148 ng/kg, 128 ng/kg, 141 ng/kg, 102 ng/kg, 96 ng/kg, 102 ng/kg, 28 ng/kg, 11 ng/kg and 19 ng/kg while the processed form of the same oil batches had the following aflatoxin concentrations; 33 ng/kg, 17 ng/kg, 21 ng/kg, 9 ng/kg, 5 ng/kg, 2 ng/kg, 4 ng/kg and Cotton, Sun flower and Canola oils had no detectable aflatoxin. Comparing the rate at which the toxin (aflatoxin) travel from unprocessed to processed oil, unprocessed vegetable oils had more aflatoxin load than processed oil and this indicates a serious hazard for human health. The study also implies refining process reduces aflatoxin to a tolerable limit of 5 ng/kg recommended by European Union even though there is no threshold at which no harmful effect is observed. It is therefore recommended that National control authorities should place a national surveillance plan to monitor and help ensure compliance with the requirements of the legislation as regards aflatoxin.

P56

Project Aflatox® I – a biotechnological approach for the development of new antifungal compounds to protect the environment and the human health

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With a steadily increasing world population, a more efficient system of food production is of paramount importance. One of the major causes of food spoilage is the presence of fungal pathogens and the production and accumulation of mycotoxins: due to their implication in crop production, food quality and human and animal health, aflatoxins represent a major concern for both developed and developing Countries. However, despite of countless efforts, to date the problem of food and feed contamination remains unsolved, since the essential factors that affect aflatoxin production are various and hard to handle together. In this scenario, the exploitation of bioactive natural sources to obtain new agents with novel mechanisms of action may represent a successful strategy to minimize at the same time mycotoxin contamination and the use of harmful pesticides.

In 2015 the Aflatox® Project was funded by Cariplo Foundation: purpose of our project was the development of new-generation inhibitors of aflatoxigenic *Aspergillus* spp. proliferation and of toxin production through the modification of naturally occurring molecules; a panel of at least 180 compounds, based on a class of molecules named thiosemicarbazones, have been analyzed for their antifungal and antiaflatoxigenic ability. Accordingly, with the experimental design, the activities of the different research groups joining the project were organized as follows:

1) synthesis and characterization of new bioactives obtained by condensation of thiosemicarbazide with aldehydes or ketones of natural origin;
2) evaluation of effect on *A. flavus* growth, development and aflatoxin biosynthesis;
3) improvement of compounds performance by modifying the chemical scaffold and/or via complexation with biocompatible metal ions (i.e. copper and zinc);
4) evaluation of cytotoxicity, genotoxicity and epi-genotoxicity of new-synthesized compounds through the use of validated assays on human cell lines (colon, epidermis and lung tissues), *Salmonella*/microsome test and *Allium cepa* micronucleus test;
5) in vitro evaluation of the best performing compounds effect on maize kernels infection by *A. flavus*;
6) creation of a Quantitative Structure-Activity Relationship (QSAR) database correlating chemical structures with biological/toxicological activities.

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P57

A web-portal for meta-analysis of mycotoxin occurrence in agricultural commodities and food products

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Mycotoxins are toxic products of fungal metabolism and the most prevalent contaminant in various agricultural commodities either before harvest or in postharvest conditions (e.g. during storage).

Mycotoxins pose a serious threat to human health and cause economic losses in livestock.

Numerous studies assessing the presence, levels and the effects of mycotoxins in various crops are being carried out globally.

As the data collected from fields and laboratories expands, statistics become a real need, to gain human-friendly and direct information about the experiences indifferent geographical locations which will aid the researchers in developing and planning their future experiments as well as help in monitoring the effects.

To accomplish this, a relational database with data logic layer and statistics in a user-friendly web interface needs to be established.

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Investigation of patulin and citrinin in grape must from grapes naturally contaminated by toxigenic strains of *Penicillium expansum*

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The change in climate is a widely acknowledged fact. Recently is intensively monitored the effects of climate change and global warming on the occurrence and distribution of toxigenic microfungi - producers of important mycotoxins patulin (PAT) and citrinin (CIT) in foodstuffs in temperate climates.

The mycobiota of grape berries from selected Czech vineyards was examined. Altogether five Czech vineyards with 25 grape samples representing 15 grape varieties were involved in the study. Grape samples were collected in autumn of 2014.

Strains of *Penicillium expansum* were isolated and confirmed by PCR methods in 23 (92%) grape samples. A toxigenic potential (PAT and CIT production) of the selected *P. expansum* isolates was investigated in vitro on semisynthetic liquid culture - YES medium (25 °C, 7 days) and in vivo on freshly harvested white table grapes (25 °C, 7 days).

Twenty three samples of grape must were prepared from grape samples naturally contaminated by toxigenic strains of *P. expansum*.

Extraction method of grape must samples was based on unbuffered QuEChERS. UPLC-MS/MS method for the simultaneous determination of PAT and CIT was applied. Limit of quantification for PAT and CIT was 100 ng/g and 2 ng/g respectively.

The results of determination of PAT and CIT in 23 grape must samples demonstrated the occurrence of PAT in 10 (43%) grape must samples (middle bound approach; mean: 171 ng/g; median: 50 ng/g; range: 143–644 ng/g) and the occurrence CIT in 2 (9%) grape must samples (middle bound approach; mean: 1 ng/g; median: 1 ng/g; range: 2.5–3.5 ng/g). This is the first report on the natural occurrence of CIT in grape musts.

Conclusion: Although further large-scale trials are needed, these results support the consideration of the possible occurrence of mycotoxins in grape musts and wines including CIT, PAT, ochratoxin A, fumonisin B2 and *Alternaria* mycotoxins. We can also recommend a regular monitoring of the colonization of grapes by toxigenic microfungi before and during the harvest and determining respective mycotoxins.

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P59

Comparison of different sample preparation procedures for LC-MS/MS determination of B-trichothecenes in feedstuffs

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B-trichothecenes are a large group of mycotoxins produced by moulds belonging to the genus *Fusarium*. In central Europe the most important are deoxynivalenol (DON), nivalenol (NIV) and fusarenon-X (FUS-X) in grains and feedstuffs. These toxins are responsible for a wide range of toxicity in animals including vomiting, feed refusal, weight losses. Moreover, in recently years researcher paid more attention to modified mycotoxins e.g. deoxynivalenol 3-glucoside (DON-3G), 3- and 15-acetyldeoxinivalenol (3- and 15-AcDON), because they are undetected by conventional analytical methods targeted for mycotoxins. Occurrence of modified forms may contribute to the total dietary exposure of parent toxins DON. The aim of this study was comparison of sample preparation procedures for determination of B-trichothecenes (DON, 3Ac-DON, 15Ac-DON, DON-3G, NIV, FUS-X) in feedstuffs using LC-MS/MS technique.

In this study we compare few techniques of sample preparation: immunoaffinity columns (IAC) DONTestTM, OASISTM solid-phase extraction (SPE) cartridge, Mycosep Trich 225 column, modified QuEChERS, and 'dilute and shoot' approach. The analytes were determined with LC-MS/MS technique (LC-MS 8050, Shimadzu), with chromatographic separation performed on Luna Polar C18 1.6 µm column (Phenomenex). The mobile phase was composed of 0.2% acetic acid and acetonitrile. During 12 min of analysis all compounds were fully separated. Sample preparations were performed according to manufactures recommendation and with ours experience. During this study few validation parameters were established: limit of quantification (LOD), limit of qualification (LOQ), recovery (RE) and matrix effect (ME). Most efficient technique was Mycosep Trich 225 column. The RE for all compounds were above 84%, except DON-3G (30%), however this compound feature with high signal to noise ratio (S/N), what enable to achieving low LOQ (2 µg/kg). In turn, application of IAC or SPE clean-up with OASIS cartridge have some limitations. IAC did not show cross-reactivity feature to 15-AcDON, OASIS column show low RE for FUS-X (below 5%) and high matrix suppression for DON-3G (32%).

Alternatively, QuEChERS technique and 'dilute and shoot' approach can be used as a cheaper option prior to LC-MS/MS detection, however with lower sensitivity for the methods ranging from 50 to 100 µg/kg. Overall, the result demonstrate that the Mycosep Trich 225 column are the best opinion for sensitive determination of all B-trichothecenes. Future studies require fully validation of this sample preparation. Described method could be adopted as a confirmatory method for evaluation of DON, its metabolites, NIV and FUS-X in feedstuffs.

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P60

Fungal infection of brown trout (*Salmo trutta* morpha *trutta*) – case study

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In the last few years, during migration of brown trout (*Salmo trutta* m. *trutta*) to Polish rivers for spawning, fish health disorders have been noticed. The observed clinical signs concerned mainly males and manifested skin depigmentation with circular or oval lesions. Those symptoms developed rapidly, causing destruction of the epidermis and mortality of the fish.

Samples from the skin and internal organs were collected for microbiology, toxicology and histopathology examinations. For fungal growth, homogenates of tissue samples were inoculated on Sabourauda medium and cultured at 27°C for 5 days. Total DNA from fungal hyphae were isolate and conventional PCR targeting ITS region of rDNA were performed. Amplification products were sequenced. For histopathology, the collected tissue samples were fixed in 10% neutral-buffered formalin, routinely processed and embedded in paraffin blocks which were then cut at 4 µm. The cut sections were stained with hematoxylin and eosin and examined using light microscopy for a presence of histopathological lesions. For toxicological analysis LC-MS/MS technique was adopted for determination of 25 mycotoxins (including ZEN, a-zal, sterigmatocystin and Enniatin B) in gastric contents, kidney and liver. Sample preparation based on extraction with ACN:H2O:HCOOH (79:20:1 v:v:v) and clean-up with OASIS HLB cartridges. Characterized method performance for all analytes were satisfactory, with limit of quantification (LOQ) most of analytes at level 2 µg/kg.

Sequencing analysis identified *Fusarium* sp. in skin and internal organs samples. Histopathological examination revealed disruption of epidermal layer which was penetrated by fungal hyphae. Partially, there was complete loss of epidermis visible, accompanied by dermal necrosis, oedema and, occasionally, myofibrillar necrosis. Penetrating fungal hyphae were observed in dermis and hypodermis. Occasionally mild inflammatory infiltrations were present, usually limited to eroded dermal layer. There were no prominent changes observed in the liver, kidney and spleen. Toxicological analysis reveal occurrence of ZEN in each matrix at level range 2-25 µg/kg. Moreover, α-ZEL were found in two livers, however in traces amounts (below LOQ of method). In few cases sterigmatocystin and enniatin B were detected (in two kidneys and two livers), but at low level below 9 µg/kg.

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Development and validation of a LC-MS/MS based method for the determination of deoxynivalenol and its modified forms in cereals

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A liquid chromatography - tandem mass spectrometry (LC-MS/MS) based method for the determination of deoxynivalenol (DON), deoxynivalenol-3-glucoside (DON-3-Glc), 3-acetyl-deoxynivalenol (3-ADON) and 15-acetyl-deoxynivalenol (15-ADON) in cereals was developed. Analytes were separated on a Waters Acquity UPLC HSS T3 C18, 1.8 µm, 2.1 × 100 mm column using gradient elution on an Agilent 1290 UHPLC system. Electrospray negative ionization was used to charge the analytes and detect them using a Sciex 6500+ QTrap MS/MS system. Selected reaction monitoring transitions of the analytes and their 13C-labelled internal standards (IS) were used to monitor the analytes.

13C-labelled D3G was enzymatically produced using 13C-DON and 13C-sucrose using the published procedure of Michlmayr et al. (Toxins, 7, 2015). Baseline separation was achieved for the critical peak pair DON/D3G while 3-ADON/15-ADON were not fully baseline separated using the optimized chromatographic conditions after testing various C18-RP, fluorinated phases and chiral HPLC columns. After grinding, weighing and extracting the cereal samples with 20% aqueous acetonitrile, the extract was centrifuged and a mixture of the four 13C-labelled IS was added.

The method was successfully validated for two matrices. Linear calibration lines were obtained from the LOQs up to 1 mg/L, while the concentration of the IS was set at 100 µg/L. For maize, apparent recoveries were 127% for DON, 96% for DON-3-Glc, 75% for 3-ADON and 74% for 15-ADON without IS. Applying the IS improved the total recoveries to 103 % DON, 96% DON-3-Glc, 93% 3-ADON and 98% 15-ADON. For wheat apparent recoveries were 82% DON, 84% DON-3-Glc, 54% 3-ADON and 32% 15-ADON without IS. Applying the IS improved recoveries to 116% DON, 111% DON-3-Glc, 110% 3-ADON and 101% 15-ADON. The use of the IS therefore corrected the occurring matrix effects efficiently.

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Combined toxic effects of indoor fungal toxicant mixtures

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Inhalatory mycotoxin dose min 100x lower than while ingested might cause the same adverse biological effect in exposed individuals. The study presented was aimed to characterize cyto- and genotoxicity of „sick building“ originated *Aspergillus versicolor* (AV) and *Stachybotrys chartarum* (ST) complex metabolite mixtures in the system of human lung adenocarcinoma cells A549. Such results enable to model interactions of toxic effects of particular toxicants comprised in the mixtures. Metabolic activity of the A549 cells was measured as MTT reduction after exo- and endometabolite exract AV (+ pure sterigmatocystin), ST (atranone chemotype) - 5, 10, 20, 40 a 80 µg/mL, and combination of AV+ST extrolite - 20+20, 10+40 a 40+10 µg/mL/24 h exposure. One per cent DMSO was applied as negative control with no effect on the cell viability. Genotoxicity of all the metabolites given was tested by alcalic comet assay (tail length, tail DNA, tail intensity and tail moment) and stopping micronuclear cytokinesis assay (micronucleus, nuclear germ tube and nucleus-plasma bridge frequentions in binuclear cells) as well. The values obtained underwent statistical analysis by ANOVA, Tukey, Kolmogorov-Smirnov and Pearson $\chi 2$ tests. The level of $P<0.05$ was taken as significant. Result interpretation: additive effect of toxicant mixture components – meaured toxic parameters were not significantly different from the ones expected; synergistic effect – cell viability measured was significantly lower or the comet tail moment was significantly higher then their expected values; antagonistic effect – experimental values of cell viability were significantly higher than the expected ones or significantly under the expected intensity of the comet tail. AV+ST endometabolites expressed concentration dependent antagonistic activity in comet and micronucleus assays applied. On the other hand, both test confirmed a certain degree of the tested fungal exoproduct genotoxic synergism, that was clear from previous studies in vivo. To objectivize causal relationship between mycotoxin mixtures present in the indoor environment and particular ill health of the affected building occupants after inhalatory exposition, it is necessary: to set the minimal effective concentrations of the toxicants able to cause (clinical) symptoms in comparison with models in vitro; to choose optimal biological models to study mycotoxin pathogenity and pathophysiology (pulmonary deposition); to characterize short- and longterm human health damages after known mycotoxin exposition via airways.

The publication resulted from the project realization.

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The presence of enniatins and beauvericin in eggs and milk from Poland

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Enniatins A, A1, B, B1 (ENNs) and beauvericin (BEA) belonged to „emerging mycotoxins", are structurally related compounds (cyclic hexadepsipeptides) produced by various *Fusarium* species which invade and grow on crops and may produce these toxins under a favourable weather conditions. They occur as contaminants mainly in cereals, such as wheat, barley, and maize, but also in oats, rye and rice. In recent years those emerging *Fusarium* mycotoxins are receiving more attention because of their not fully known toxic effects and the constant increase of the reports of their occurrence in food and feed. Moreover, information about it levels in food of animal origin are limited.

The aim of the study was to evaluate the occurrence of enniatins and beauvericin in milk and eggs. A number of 44 hen egg samples and 114 bovine milk samples (51 of raw milk and 63 UHT milk samples) were collected from entire Poland and analysed using liquid chromatography-tandem mass spectrometry method. The samples were extracted with a mixture of acetonitrile:water:formic acid (79:20:1, v/v/v) and cleaned using Oasis HLB columns. These method allow for the determination of ENNs and BEA in the range of 0.001 µg/kg to 100 µg/kg in milk and egg samples.

The results indicated that 77.3% of egg samples were contaminated with at least one enniatin, with the concentrations ranging from 0.002 µg/kg for enniatin A to 31.8 µg/kg for enniatin B. Beauvericin was present in 42 out of 44 egg samples (0.01 – 5.13 µg/kg).

Among the 51 raw milk samples collected in the monitoring control program, 74.5% of samples were contaminated with at least one enniatin. Enniatin B was present in 66% of samples, enniatin A in 44% of samples, enniatin A1 in 11.4% of samples and enniatin B1 in one sample. Regarding the UHT milk samples, all 63 were contaminated with enniatin B with the concentration range from 0.246 µg/kg to 4.15 µg/kg, and no enniatin A1 was found in any of the milk samples. Enniatin A was present in 12 of samples (0.024 – 3.34 µg/kg), whereas enniatin B1 in 27 of UHT milk samples (0.038 – 2.104 µg/kg). Beauvericin was detected in 39 milk samples (62%) with the concentration ranging from 0.0018 µg/kg to 2.06 µg/kg.

The results of these studies demonstrated a constant contamination of milk and eggs in Poland with enniatins and beauvericin. Because of the absence of international legislation limits for this emerging *Fusarium* mycotoxins in food, more research is needed concerning transfer of those metabolites from feed to food of animal origin.

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Project Aflatox® II - a full-experimental database to collect and handle chemical and biological data for the development of new antiaflatoxigenic agents

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During the three years of the Aflatox® Project (*www.aflatox.it*) we worked to synthesize and examine properties of a large number of chemical compounds. Our attention focused on the research of very specific aflatoxin production inhibitors. At the same time, these molecules must be safe for human cells, for the plants on which fungi grow and for the whole ecosystem.

To achieve our goal, the project has been divided into three different sections. The first was the design and synthesis of some parent compounds from natural molecules. The second was the study of their biological effect and cytotoxicity. The third was the chemical modification of the most active compounds in order to study the mechanism of action and to improve the biological activity. In this last stage of the project, the compounds which had shown good results were not only modified in their chemical scaffold, but also used as chelating agents for bio-metal ions like zinc, copper or iron.

What we obtained is a panel of more than 170 compounds fully characterised and tested for antifungal and antiaflatoxigenic properties. Toxicological and genotoxicological evaluations were conducted on three different human cell lines, and on *A. cepa* root apices.

This huge amount of data would be certainly impossible to be analysed as a whole, so we managed to create a database which allows to produce Q-SAR (Quantitative structure-activity relationship) evaluation profiles. To build this database we used the Instant JChem software by ChemAxon which can record and handle large volumes of data coming from different fields. It allows also to run queries and make graphs based on the recorded information. This full-experimental database is a powerful tool to identify important requirements to be taken into account to develop a new generation of pesticides, responding to “greener” and environmentally sustainable agricultural strategies.
This study was supported by a grant from “Fondazione Cariplo” (Project N. 2014-0555).

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Fusarium mycotoxins in organic and conventional wheat grown in the Czech Republic

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It has often been questioned, whether organically grown cereals are more contaminated by mycotoxins than conventionally grown, because of an absence of synthetic fungicides in organic agriculture. In general, these comparisons are regarded to be more reliable in experimental field trials, as there may be variations in farming practice and environmental conditions between organic and conventional systems as well as between individual farms within both organic and conventional systems. On the other hand, comparisons of mycotoxin contamination of cereals originated from the ordinary farm production give a real picture of situation in a given area and can be valuable information for consumers.

We compared the content of *Fusarium* mycotoxins, deoxynivalenol (DON) and zearalenone, in wheat harvested in 2015 - 2017 in Czech organic and conventional farms. Two different approaches were used:

A) Set of 153 samples of organic wheat (ORG) was compared to set of 330 samples of conventional wheat (CONV), analysed in the frame of regular mycotoxin monitoring programme of Czech Ministry of Agriculture.
B) The same set of 153 organic wheat samples (ORG) was compared to compatible set of 153 conventional wheat samples (CONV_select). Organic and conventional samples were pair-matched, with regard to preceding crop and location (district) of growing.

Mycotoxin analyses were performed in one single laboratory. Using approach A, mycotoxin contamination was significantly higher in conventional (CONV) than in organic wheat (ORG). Using approach B, mycotoxin contamination was comparable in both conventional and organic farming systems (Fig). We suppose, that the main reason may be different preceding crop composition. Whereas in the set of conventional samples (CONV) the preceding crops were as follows: rape and mustard 38%, maize 14%, forage crops (clover, alfalfa, leguminous) 14% and cereals 10%, in the set of organic samples (ORG) preceding crops were: forage crops 53 %, cereals 20%, rape and mustard 4% and maize 1%. If the difference in preceding crops (approach A) was eliminated by the means of creating pair-matched conventional set of samples with the same preceding crops (approach B), there was no difference in mycotoxin contamination between organically and conventionally grown wheat.

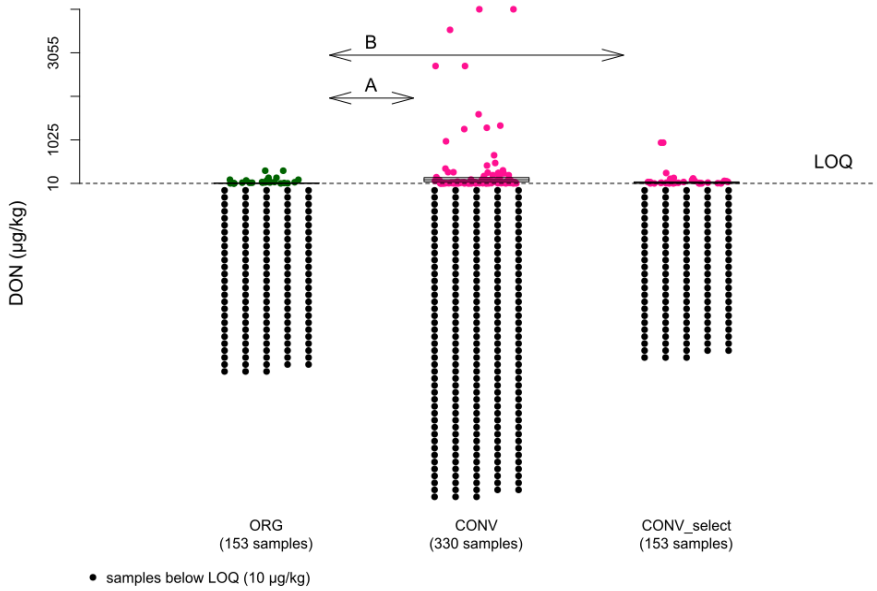

Figure: Comparison of deoxynivalenol (DON) in organic (ORG) and conventional (CONV) wheat using two approaches: A) randomly assembled, unequally large sample sets, B) paired sample sets, with regard to preceding crop and location

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Interaction of zearalenone and its reduced metabolites with serum albumins

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Zearalenone (ZEN) is a *Fusarium*-derived mycotoxin exerting xenoestrogenic effects in animals and humans. During the biotransformation of ZEN, mainly its reduced metabolites and glucuronides are produced. The dominant reduced metabolites are α-zearalenol (α-ZOL) and β-zearalenol (β-ZOL), while ZEN and ZOLs also form other reduced derivatives, including zearalanone (ZAN), α-zearalanol (α-ZAL), and β-zearalanol (β-ZAL). Glucuronide conjugates are less toxic than ZEN, while reduced metabolites can exert even considerably stronger toxic action than the parent compound ZEN. Serum albumin is the most abundant protein in the circulation. Formation of stable albumin complexes can significantly influence tissue distribution and elimination of several drugs and xenobiotics.

Our previous study demonstrated that ZEN forms stable complex with human albumin occupying a non-conventional binding site between subdomains IIA and IIIA. However, we had limited information regarding albumin-binding of reduced metabolites and species-dependent alternations of these interactions. Therefore, complex formations of ZEN, α-ZOL, β-ZOL, ZAN, α-ZAL, and β-ZAL with human (HSA), bovine (BSA), porcine (PSA), and rat (RSA) serum albumins were investigated by fluorescence spectroscopy. Among the tested mycotoxins, ZEN formed the most stable complex with HSA, followed by α-ZOL (2.5-fold lower affinity than ZEN) > ZAN and α-ZAL (3.5-fold lower affinity than ZEN) > β-ZOL and β-ZAL (more than 5-fold lower affinity compared to ZEN). Furthermore, very significant species-dependent alternations were observed, even more than one magnitude differences of binding constants occurred (e.g., β-ZOL-HSA vs. β-ZOL-RSA). Typically, mycotoxins formed the less stable complexes with PSA (and/or with BSA), while the most stable complexes were observed regarding RSA. Thermodynamic investigation of ZEN-albumin complexes also demonstrated relevant species-dependent alternations. Molecular modeling studies suggest that binding sites of ZEN, α-ZOL, and β-ZOL are located between subdomains IIA and IIIA on HSA. However, until the binding positions of ZEN and α-ZOL are similar, binding of β-ZOL shows differences, which may explain its significantly lower affinity towards HSA.

We can conclude that reduced metabolites bind typically lower affinity to albumins than ZEN. Furthermore, interactions of these mycotoxins with albumin show large species-dependent alternations, therefore, their biological importance may be various in different species.

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Aflatoxin B1 and ochratoxin A in medical herbs marketed in Latvia

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Cultivation of medical plants for the production of herbal teas is widely practiced In Latvia. Plant parts (flowers, fruits, stems, leaves, roots, etc.) are rich in alkaloids, glycosides, flavonoids, and other biochemically active substances. Undesired environmental and biochemical factors (e.g., temperature, moisture, air quality, insects, etc.) may influence fungal contamination of herbs with *Alternaria*, *Aspergillus*, *Fusarium*, and *Penicillium* strains. Aflatoxins (AFs: AFB1, AFB2, AFG1, AFG2) and ochratoxin A (OTA) are the most hazardous mycotoxins produced of *Aspergillus* or *Penicillium* strains during growth, pre- and pro-harvest periods.

The present study was dedicated for the assessment of AFB1 and OTA content in medicinal herbs. A total of sixty tea samples were purchased from the local Latvian drugstores. High-performance liquid chromatography coupled with time-of-flight mass spectrometry was used for the determination of mycotoxin contamination.

OTA was determined in 10% of the analyzed samples, reaching the maximum content of 30 ng/g in herbal blend used as bronchi-lung tea. AFB1 was relatively frequently presented in 20% of the samples. The concentrations of AFB1 ranged between 3 to 23 ng/g in the samples, which increase the concentration limit of 2 ng/g set for medical herbs by the European Pharmacopeia regulations. The maximum level of AFB1 was determined in herbal blend used as laxative.

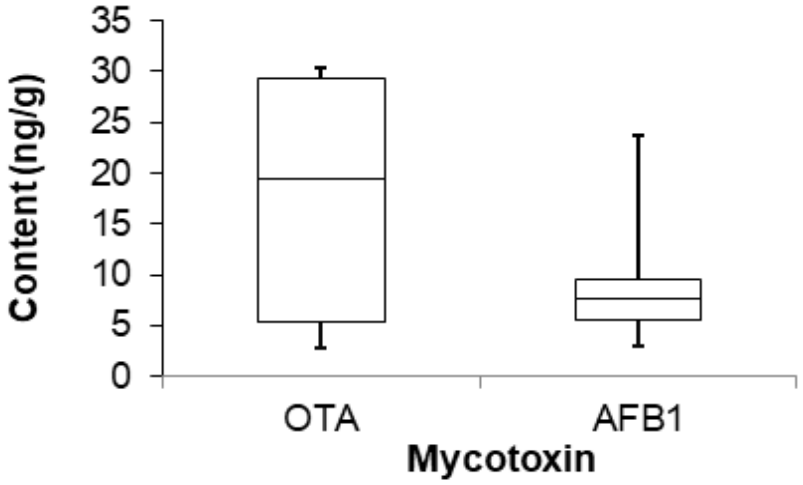

Figure 1. The results of OTA and AFB1 levels in dried medical herbs.

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P68

Heterologous expression of human UDP-glucuronosyltransferases in *Saccharomyces cerevisiae* for enzymatic synthesis of glucuronides of deoxynivalenol and zearalenone

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Humans are confronted with a vast array of xenobiotics in their diet, as medication or through drug abuse. Such substances are primarily metabolized by conjugation with glucuronic acid, a reaction catalyzed by UDP-glucuronosyltransferases (UGTs). The most important UGTs involved in mammalian phase II detoxification are members of the UGT subfamilies UGT1A and UGT2B. Glucuronides are excreted via urine and represent valuable biomarkers to investigate the metabolism of toxins and drugs. For example, monitoring glucuronides of the *Fusarium* metabolite deoxynivalenol (DON) in urine provides vital information about the exposure to this mycotoxin.

Access to suitable glucuronide-standards is crucial to develop analytical methods to monitor exposure and to determine the toxicokinetic properties of a given substance. Unfortunately, corresponding analytical standards are difficult to synthesize and only available for few substances. Enzymatic synthesis of glucuronide-standards using human UGT isoforms seems a reasonable approach that should deliver also metabolites relevant for the human metabolism. UGTs are membrane proteins and therefore difficult to isolate in active form. Presently, several (human) UGT isoforms are commercially available (e.g. recombinantly expressed in insect cells). However, these preparations are expensive, and in our experience, not suited for preparative synthesis of glucuronides. Therefore, we attempt to express human UGT isoforms known to glucuronidate the *Fusarium* toxins zearalenone (UGT1A1, UGT1A8, UGT1A9) and DON (UGT2B4, UGT2B7) recombinantly in yeast. Yeast microsomes containing active recombinant human UGTs will then be isolated and used to synthesize glucuronides of DON and zearalenone. Preliminary experiments showed that UGT1A isoforms can be functionally expressed in *Saccharomyces cerevisiae*, optimization of expression is in progress. As UGT activity is difficult to measure directly, sialidase fused to the C-terminus of the UGTs is used as reporter to monitor expression levels. Our goal is to establish a reliable method to synthesize glucuronides of mycotoxins and other compounds of interest in mg scale.

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Deoxynivalenol and ecological processes in agroecosystems: A preliminary study on soil activity

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Mycotoxigenic fungi represent an important fraction of soil fungal community, leading to the occurrence of mycotoxins in soils. Mycotoxins are toxic to humans and animals. However, the importance of mycotoxins as contaminants in agroecosystems and the potentially harmful effects on the soil microbial community have not yet been investigated. In this context, the aim of this study was to relate the deoxynivalenol (DON) content of naturally contaminated soil samples (range: 1.0-13.1 µg/kg) to soil activity. Soil microbial respiration (basal and substrate induced) and enzymatic activity (Chitinase, Aminopeptidase and Phosphatase) were at the focus of this study. Samples originated from fields with strawberry cultivation under either straw or plastic mulching (PM). Since PM increases soil temperature, the toxicity of DON was additionally evaluated in a substrate induced respiration experiment in a standard soil using the MicroResp™ system at two different temperatures 20 and 40 °C. The mycotoxin test concentrations ranged from 0.16 to 3,200 µg/kg. The results of this study indicate that the DON concentration measured in the samples did not affect the activity of the above mentioned enzymes. In the same way the basal soil respiration was not affected. However, a correlation with substrate induced soil respiration was observed (r = -0.644, p = 0.033). The substrate induced respiration experiment showed no effect at 20 °C, but at 40 °C a reduction of about 35 % was observed at the highest DON soil concentration compared to the control. The results of this study suggest an effect of DON on the microbial activity of soil. However, a toxic effect was only observed at the highest concentration which may not necessarily represent relevant environmental concentrations. The elevated temperature was crucial in the toxicity of DON in the studied standard soil. Further studies are needed to assess the toxicity of mycotoxins for soil microorganisms, especially in soils under intensive agricultural management practices.

P70

Colonization of maize in Germany by *Fusarium* spp., interdependencies of species on ears and stalks and mycotoxin contamination (project EarRot)

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Infection of maize by *Fusarium* spp. can lead to contamination with mycotoxins, which is detrimental to quality and relevant to both animal and human nutrition. As corn acreage has more than doubled in Germany over the past decade, the problem of infection increased. Additionally, the species spectrum of *Fusarium* on maize is continuously being expanded. Recent studies showed that in neighboring, warmer growing areas in the south of Germany, a shift in species composition on maize has recently occurred. Here, the abundance of 'new' species increases whereas the occurrence of the classical pathogen on maize, *F. culmorum*, is declining. To what extent this is also the case in Germany, regarding climate conditions, pre-crop, crop rotation, tillage, variety and usage type, is examined in this monitoring.

To determine the occurrence of 12 major species of *Fusarium* on maize, corn, rachis and stem material consisting of 189 cobs and 112 stem segments was analyzed for fungal colonization. *Fusarium* isolates were purified as single-spore-cultures and used for morphological identification as well as for extraction of genomic DNA and for molecular identification.

The results revealed the following 5 most frequently occuring species (with relative percentage): *F. graminearum* (73%), *F. poae* (17%), *F. verticillioides* (14%), *F. temperatum* (13%) and *F. cerealis* (13%). The results of the monitoring imply that besides the most frequently occuring species *F. graminearum*, species of the GFSC (*Gibberella fujikuroi* species complex), including the relatively new species *F. temperatum*, as well as *F. poae* and *F. cerealis* appear to be the major sources of infection. The goal of the EarRot project is to provide a comprehensive overview of the colonization of maize cobs and stems in Germany with *Fusarium* spp. and the associated mycotoxins.

P71

Evaluation of rapid tests for quantitative aflatoxin measurements in maize

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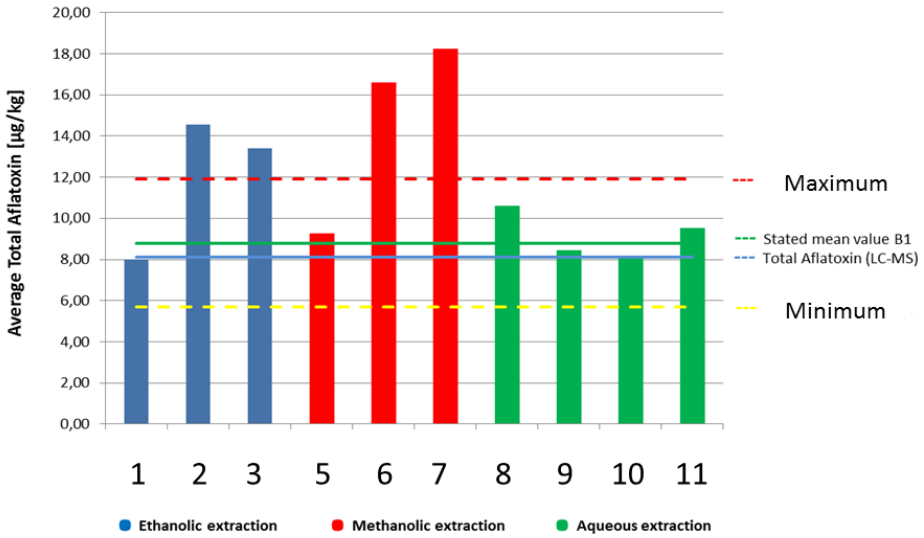
Several rapid test systems can be found on the market to determine the aflatoxin content in different matrices based on lateral flow devices. During the joint project “AflaNet – networking on aflatoxin reduction in the food value chain” founded by the German Ministry of Food and Agriculture, a number of different test kits were evaluated for their general applicability, procurement costs and comparative results. The aim of the investigation was to examine the performance in general and to issue recommendations for the applicability of rapid tests under sub-Sahara conditions. Keeping in mind that less trained persons (such as farmers) should be able to detect aflatoxins simply, properly and safely to ensure the daily food is safe to consume.

The average results of repeated extractions of the reference sample gave divergent content of the certified maize material (figure 1). Moreover, different extraction solvents seem to be more or less suitable for the determination of aflatoxin content when conducting rapid tests.

Due to the high fluctuation of the values of natural contaminated samples, several rapid test devices are less appropriate for a single determination. The risk is high of making a wrong decision regarding the destruction or use as food of the corn batch based on only one measurement. Moreover, the evaluated devices are all expensive and thus only affordable for agricultural communities but not for a single farmer with low income. Overall, we came to the conclusion, that one rapid test might be suitable for field use under harsh conditions. However, our examinations provide very differing values, so that a recommendation can hardly be expressed until now. Beside a reliable test strip a mobile analytical system is desirable which should be free of charge, universal applicable and can be loaded onto existing devices e.g. mobile phones which are present today everywhere in the world.



Caption: Comparison results of different rapid tests of reference material with certified content of 8.8 µg/kg±3.1 AFB1 (AFB2, AFG1 and AFG2 < 1 µg/kg) conducted with different extraction solvents. The average of min. 12 independent tests for each box is shown.

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Fulminant mucormycosis after a traffic accident: A case study report

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Mucor spp. are filamentous fungi of the Mucormycota phylum. This genus currently includes around seventy species. These fungi are ubiquitous, commonly found in soil, plants, and decaying matter being potential cytotoxins-producers. The species *M. circinelloides* was isolated from yoghurt and recognized as a probable cause of intoxication of above 200 consumers. *M. circinelloides* is the species rarely isolated, also from immunocompromised patients. Our clinical case report concerns lower limb infection caused by *M. circinelloides*, after a car accident.

A twenty-six-year-old man was diagnosed with an open, multiple level, and multiple fragment fracture of the left leg, with an open knee dislocation, crushing of the soft tissues of the shin and fracture of the right thigh anastomosed with intramedullary rod the year before. In addition, the right shoulder dislocation and the injury of the tibial artery and vein were found. A large postoperative wound appeared around the knee and shin. Antibiotic therapy was introduced. At the same time, the specimens for microbiological analysis were collected: wound swab, then rectal and skin swabs for VRE screenings. All cultures were negative. On the third day of the hospitalization, the next three wound swabs were collected and two turned out positive. *Enterococcus hirae* and *Pantoea agglomerans*, both multi-susceptible strains, aside with *Mucor* sp. were isolated from one positive sample, whereas *E. hirae* was identified in the second one, respectively. Amplification of fungal ITS region confirmed that it was *M. circinelloides* f. *circinelloides* strain. MTT test showed low cytotoxicity of the strain, yet no mycotoxins were detected in the analyzed sample.

On the fifth day of hospitalization, due to the deterioration of the wound, another swab was collected, and because fungal colonies were visible in the wound, amphotericin B was applied intravenously. Antifungal treatment was continued.

On the following days, due to the persistent purulent wound infection and the risk of sepsis, the left lower limb at one-third of the proximal thigh was amputated. After rehabilitation the patient in a good condition was discharged home.

This research was supported by the Nicolaus Copernicus University funds for the Department of Microbiology and from the funds allocated to the project ‘Implementation of molecular biology techniques in identifying of moulds to the species level’ implemented at the Kazimierz Wielki University.

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"DIFESAMAIS": An Italian project for the management of aflatoxin crops contamination

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Corn contamination by aflatoxins is one of the main issue that farmers of large areas of Northern Italy have to cope with. Different strategies have been implemented to try to reduce, if not eliminate, toxin contamination at the pre-harvest level; however, a careful analysis of the costs/efficacy of each of the various inputs must be performed in order to provide an acceptable income for the farmers. On the other hand, there is an increasing market demand for food and feed commodities obtained with environmentally low-impact practices (organic farming) possibly through the experimentation of innovative and organic defense techniques. Presently, it is largely accepted that minimization of mycotoxin risk requires a multifactorial approach since climatic, agronomic and phytoiatric factors are found to interact each other in complex, local ecological realities. The "DIFESAMAIS" project, funded by Emilia-Romagna Region (PSR 2014-2020), intends to design and validate an innovative combined approach addressed to the development and the valorization of organic farming/integrated production of corn, with particular attention to the maintenance of biodiversity. Both academic and industrial partners (PROGEO S.C.A. and AGRITES s.r.l.) were involved in the present project. The main stakeholders are the farmers belonging to PROGEO S.C.A. and, more generally, all the farmers which will have the opportunity to acquire the economic expertise and the technical tools to implement an ecologically sustainable maize cultivation by a reduction of chemical inputs. Among the various activities performed in the framework of the "DIFESAMAIS" project, we will report the validation of a sustainable procedure to reduce aflatoxin contamination by bio-competition approaches, based on: 1) the use of non-aflatoxigenic strains of *Aspergillus flavus*, and 2) the development and validation of technologies for European corn borer (*Ostrinia nubilalis*) control. Moreover we will report the interaction of the above reported treatments with different agronomic practices that were part of the experimentation scheduled for the "DIFESAMAIS" project.

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Characterization of *Fusarium graminearum* amidohydrolases

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Infection in cereals with *Fusarium* leads to production of antifungal compounds such as the hydroxycinnamic acid amid (HCAA) coumaroyl-tryptamin. To overcome the defense reaction of the plants, *Fusarium* can cleave HCAAs, which leads to release of tryptamine that is then converted into auxin (IAA, indole-3-acetic acid) at high yield. Massively elevated levels of IAA and the inactivation products IAA-glucoside and IAA-aspartic acid (IAA-Asp) were detected in infected 'Apogee' wheat.

We have started to characterize the large family of amidohydrolases of *Fusarium* by heterologous expression of 58 candidates in *yeast* and *E. coli*. While the amidohydrolase(s) responsible for cleavage of the tryptamine-derived HCAAs are still unknown, we could confirm that one member of a branch of six amidohydrolases with similarity to plant IAA-amino acid conjugate hydrolases indeed has activity with IAA-Asp. *Fusarium* can hydrolyse both IAA-Ala and IAA-Asp. However the recombinant enzyme in yeast is only able to hydrolyze IAA-Asp. Knock out of the candidate leads to a mutant that is no longer able to cleave IAA-Asp. However the knock out of the amidohydrolase gene did not lead to reduced virulence in wheat infection experiments, which may be due to redundancy. Alternatively, the concentrations of auxin might be sufficient for defense suppression even in the absence of the amidohydrolase, which is presumably converting back inactivated IAA-Asp to IAA inside the plant cell.

P75

Lot-to-lot variation and its impact on the accuracy of LC-MS based multi-mycotoxin analysis

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In the recent years, the LC-MS/MS based multi-analyte approach has been demonstrated to be a powerful technique for the simultaneous determination of mycotoxins in food and feed. Quantification of mycotoxins is increasingly based on the analysis of diluted crude extracts and external- or matrix-matched calibration. In essence, the response of the analyte is compared to a calibration curve of the analyte in neat solvent or matrix and, if necessary, corrected for the method bias. The method bias, expressed as apparent recovery (RA), can arise from incomplete recovery of the extraction (RE) or signal suppression/enhancement (SSE), also known as matrix effect. Due to the heterogeneous nature of the matrix, RE, SSE and/or RA may vary for different lots (quantity of material known to have uniform characteristics such as origin and variety) of the same matrix resulting in lot-to-lot variation. Lot-to-lot variation can lead to a matrix mismatch in the case that RA of the lot used for validation differs from the RA of the analyzed lot. Ignoring matrix mismatch leads to the introduction of an error of unknown magnitude. For mycotoxins, large differences in SSE have already been observed for different varieties of sorghum and rice. However, most method validation studies neglect the issue of lot-to-lot variation and validate the method based on a single lot of a matrix.

We hypothesized that neglecting the lot-to-lot variation during method validation can lead to an underestimation of the uncertainty of the measurement result. The objectives of this study were to estimate the contribution of the lot-to-lot variation to i) the uncertainty associated with RE, SSE and RA and ii) the expanded measurement uncertainty (U) of an LC-MS based multi-mycotoxin assay. This study presents the first evaluation of the accuracy of LC-MS based multi-mycotoxin determination in food and feed under consideration of the lot-to-lot variation, and differs significantly from assays which were validated based on a single lot of a matrix.

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P76

Detoxification of zearalenone by zearalenone hydrolase ZenA in an *in vitro* rumen model

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Zearalenone (ZEN) is one of the most frequently detected mycotoxins in feedstuffs. It shows estrogenic activity thereby compromising the fertility and reproduction of animals. In the rumen, ZEN is metabolized to α-zearalenol (α-ZEL), β-zearalenol (β-ZEL), α-zearalanol (α-ZAL), β-zearalanol (β-ZAL) and zearalanone (ZAN). As ZEN, these metabolites show estrogenicity, with α-ZEL being 60 times as potent as its parent compound. Previously, our group studied the bacterial enzyme zearalenone hydrolase ZenA (ZENzyme®) that converts ZEN to the non-estrogenic compound hydrolysed ZEN (HZEN). HZEN is subsequently converted to decarboxylated HZEN (DHZEN) by spontaneous decarboxylation. In this study we investigated the efficacy of ZenA to degrade ZEN in the rumen using an *in vitro* rumen fermentation model. To simulate conditions in the rumen, reactor bottles (n = 8) were inoculated with 1000 mL of a mixture containing 50% rumen fluid from slaughtered bulls, 30% tap water and 20% synthetic saliva (modified McDougall buffer) as well as 10 g of a common dairy total mixed ration. ZEN-containing culture material of *Fusarium graminearum* was added to the reactors to achieve an end concentration of 0.3 µM ZEN. ZenA was added to 4 of the reactors, while the remaining 4 reactors served as control. The reactors were incubated for 24 h at 39 °C. To investigate the metabolization of ZEN by the rumen microbiota and ZenA, samples of the fermentation broth were taken after 10 min, 20 min, 30 min, 1 h, 2 h, 3 h and 24 h of incubation and concentrations of ZEN, HZEN, DHZEN, α-ZEL, β-ZEL, α-ZAL, β-ZAL and ZAN were measured using liquid chromatography-mass spectrometry analysis. In the control treatment, ZEN was metabolized to α-ZEL, β-ZEL, β-ZAL and ZAN, with α-ZEL being the main metabolite. In the ZenA treated reactors, ZEN degradation to HZEN was observed already after 10 min of incubation and HZEN was the main metabolite at each sampling time point, whereas only minute concentrations of α-ZEL were detected. DHZEN appeared after 2 h of incubation. The concentration of β-ZAL was comparable to the control treatment. In conclusion, ZenA degraded ZEN to HZEN in a rumen fermentation model thereby preventing the formation of the highly estrogenic metabolite α-ZEL. These results indicate that application of ZenA as a ZEN-degrading feed additive in ruminants may be promising and should be investigated *in vivo* in future studies.

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

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Most of the guidelines that are available on proper method validation have been designed for assays targeting only one or very few analytes. In case of multi-analyte analysis, following these guidelines without any modification is impractical in view of the workload resulting from the number of replicates, number of concentration levels and number of measurements for the within-laboratory reproducibility. In addition, it is unrealistic to expect that compliance to all performance criteria (e.g. a target range of 70-110% for recovery) might be obtained for all analytes in case of methods covering a diverse set of target substances. As a consequence, guidelines for multi-analyte determination (such as SANTE/11945/2015 for pesticides residues) allow for some flexibility as considers both performance criteria and experiments that are foreseen for method validation.

This presentation tries to identify the part of the experiments that might be skipped during validation in order to reduce the workload for LC-MS/MS based methods covering several hundreds of fungal metabolites. In particular, matrix effects as well as recoveries of the extraction step seem to be independent of the concentration, which would allow for spiking at a single high level, thus facilitating data evaluation. However, our results indicate that an additional effort to characterize relative matrix effects is an essential part of the validation process. This is not foreseen in current guidelines and might lead to an underestimation of the methods uncertainty.

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

P78

Strongly diminished auxin biosynthesis in *Fusarium graminearum* septuple knock out strains

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Fusarium graminearum is a plant pathogenic fungus with the ability to infect different small grain cereals, resulting in yield losses. Owing to its ability to produce many toxic secondary metabolites, the fungus is of concern for food and feed safety. Since the exact mode of infection has not been elucidated yet, we developed the hypothesis that the fungus may interfere with plant hormone signalling. *F. graminearum* is able to produce auxin from tryptamine (TAM) using seven Cu-amine oxidases (*AOX*). Due to the high redundancy, we performed seven consecutive knock outs (AoxΔ^7) using three different positive selection markers (*hph*, *nptII* and *nat1*) and one negative selection marker (HSVtk). Marker recycling was accomplished by taking advantage of the Cre-*loxP* system. In a TAM feeding assay, the resulting AoxΔ^7 strains produce less than 3% auxin compared to the wild type after 96 hours. Preliminary results of a virulence test on wheat cv. Apogee indicate a lower virulence.

P79

Beneficial effect of dietary bioactive compounds from grape seed meal, a by-product of wine processing on oxidative damage induced by AFB1 in liver of pig

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Aflatoxins are the most abundant mycotoxins produced by certain molds *Aspergillus* sp. which are widespread contaminants of foods and feeds. Their high toxicity to both animals and humans makes them one of the most dangerous known mycotoxins. The main target of aflatoxin toxicity is the liver, the main organ involved in toxin biotransformation. Aflatoxin B1 (AFB1) is the most potent aflatoxin that is activated in the liver to its reactive metabolites. It was demonstrated that both chronic and high-level aflatoxin exposure can induce acute hepatic necrosis, oxygen-free radical formation, liver cancer etc.

In the last decade there has been an increasing interest in the use of bio-adsorbing and bio-transforming agents such probiotics (ex. bacteria and yeast) and prebiotics (ex. carbohydrates) as mycotoxin mitigating agents. Recent studies showed that grape waste (grape pomace) is able to sequester rapidly AFB1 from liquid media due to their high content of fibers. These agents are also rich sources of bioactive compounds such as polyphenols, polyunsaturated fatty acids, vitamins and minerals with strong antioxidant activity which could increase the defense capacity of animal against the negative action of mycotoxins.

The present study aimed to investigate the protective effect of a dried milled grape seed meal (GS) on AFB1-induced oxidative stress and toxicity in liver of pigs after weaning. Antioxidant enzymes activity (SOD, CAT and GPx), lipid peroxidation and total antioxidant capacity was investigated.

Twenty four cross-bred weaning piglets (TOPIGS-40) were randomly assigned to one of the following diets: control (treatment1-T1), 300ppb AFB1 (treatment 2-T2), 8% GS (treatment 3-T3), and 300 ppb AFB1 plus 8% GS (treatment 4-T4), 6 animals per group, for 28 days. At the end of the experiment, liver samples were collected and stored at -80˚C until analyses.

The results showed that GS diet was able to reduce the high level of lipid peroxidation induced by AFB1 in liver (33.2 nmol TBARS/g, AF group vs 23.18 nmol TBARS/g control group). GS diet also increased the activity of hepatic antioxidant enzymes GPx, CAT and SOD and enhanced the total antioxidant capacity affected by AFB1 toxicity.

Supplementary studies are needed to investigate the molecular mechanism of grape seed and to optimize their level of dietary inclusion.

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P80

The LC-MS/MS analysis of pigs urine for mycotoxin biomarkers – comparison of two strategies in sample preparation

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Mycotoxins are commonly found in animal feed. Pigs are particularly vulnerable to the effects of mycotoxins - ingestion of mycotoxins can affect pig health and decreased productivity. Additionally, the presence of emerging and modified mycotoxins in feed, show a complex picture of animal exposure. The analysis of suitable biomarkers of exposure can help with assessment of mycotoxin exposure. Thus, sensitive method able to determine a wide range of mycotoxins and its metabolites is required. The aim of this study was developing and optimizing the LC-MS/MS method for the determination of mycotoxins and their major metabolites in pigs urine. Two sample preparation methods were tested: multi-mycotoxin immunoaffinity columns (IAC) and Oasis HLB (SPE) columns (both with and without beta-glucuronidase hydrolysis).

The LC-MS/MS conditions for analysis of about 30 analytes including eight regulated mycotoxins, their metabolites and additionally: citrinin, nivalenol, sterigmatocystin, beauvericin and enniatins were optimized. The analytical column used was a Luna C18 2 × 100 mm, 3 µm kept at 45 °C. The mobile phase consisted ammonium acetate acidified with acetic acid and methanol. Chromatographic run-time was 15 min.

Satisfactory recovery (70-100%) for EU regulated mycotoxins was obtained by using IAC columns without enzymatic hydrolysis (LOD 0.2-4 ng/mL). It is worth noting that citrinin, nivalenol, sterigmatocystin, beauvericin and enniatins and metabolites such as ochratoxin-alpha, T-2-triol, partially hydrolysed fumonisin B1 and also deoxynivalenol and deepoxy-deoxynivalenol glucuronides could be detected using IAC columns. Sample clean-up with OASIS columns was not sufficient: for most compounds, co-eluting and interfering matrix components were observed at this concentrations range. Satisfactory recovery (40-100%) for EU regulated mycotoxins was obtained by using SPE columns without enzymatic hydrolysis for samples spiked at higher concentrations (LOD 2-40 ng/mL). Sample pre-treatment with beta-glucuronidase resulted in about 10-20% lower recovery for both columns.

The IAC columns enabled sufficient clean-up, resulted in satisfactory recoveries and high sensitivity and were chosen for our future study. For indirect glucuronide determination, the second method with beta-glucuronidase pre-treatment was applied. The new LC-ESI-MS/MS methods for simultaneous determination of urinary biomarkers of mycotoxins have acceptable performance characteristics and will be applied to the analysis of pigs urine samples.

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P81

Determination of ochratoxin A in kidney and tumour tissue from patients with a renal cell carcinoma and urinary tract cancer

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The frequency of renal tumours and urinary tract tumours in the Czech Republic is high, but causality is not clearly known. As the mycotoxin ochratoxin A (OTA) is known for its carcinogenic and nephrotoxic effects in rodents, and a common food contaminant, it is a risk factor which may exert adverse effects on human health. To explore whether OTA may contribute to disease of the kidney in the Czech population we conducted a pilot study by using a suitable biomarker of exposure, i.e. OTA occurrence in kidney and tumour tissue from patients with a renal cell carcinoma and urinary tract cancer. Twenty five samples of human kidney and 25 samples tumour tissue were collected from 25 patients (18 men and 7 woman) with a renal cell carcinoma and urinary tract cancer in Czech Republic during the years 2015-2016. OTA analysis in kidney and tumour tissue has been carried out by a validated ultra-trace method, after sample extraction and clean-up on immunoaffinity columns (OCHRAPREP®, R-Biopharm) followed by HPLC with fluorescence detection. The limit of detection (LOD) and the limit of quantification (LOQ) for OTA in kidney and tumour tissue were 0.008 ng/g and 0.027 ng/g, respectively. The recovery of the method was 69.8 %. Relative standard deviation of repeatability (RSD_r) of the method was 4.06 %.

OTA occurrence in human kidney and tumour tissue are presented in the Table.

Sample	Incidence n+/n+%	Range min-max (ng/g)	Mean (ng/g)	Median (ng/g)	90th Percentile (ng/g)
Human kidney	25/100	0.072– 0.385	0.205	0.186	0.305
Tumour tissue	25/100	0.054– 0.431	0.192	0.191	0.334

n+= positive samples ≥ LOQ; n+%= per cent of positive samples

Conclusion: The results of this biomonitoring study in the Czech Republic indicate a variable but frequent exposure to OTA in the patients with a renal cell carcinoma and urinary tract cancer. Finally, additional epidemiology studies using human biomonitoring data are needed for a national and international comparison.

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P82

Effect of multi-mycotoxin exposure on the nutritional health status of children in Burkina Faso

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Mycotoxins are toxic metabolites with detrimental effects on humans upon contaminated food consumption. These fungal secondary metabolites affect various tissues and organ functions including lowering the immune system, hence allowing opportunistic ailments with undernutrition to set in. An emerging concern of children's health in Sub-Saharan Africa is their chronic exposure to multiple mycotoxins via foods, but also through breastmilk. Cases of multi-mycotoxin contamination and chronic malnutrition have been reported in the Haut-Bassin region in Burkina Faso. To date, exposure of humans to mycotoxins is verified through the analysis of biomarkers of exposure in biological fluids such as breastmilk, urine and plasma. A large cohort study of infants and their lactating mothers (n=153) was conducted in this region to correlate mycotoxins' exposure with the infant nutritional health status (October 2017 to December 2017). The occurrence of mycotoxin biomarkers in human breastmilk (n=150) and infant urine (n=153) was evaluated using LC-MS/MS. Anthropometric measurements and 24HRs data were collected from participants and were correlated with the means of mycotoxin concentrations using SPSS. The poster will give an overview of the research project, in combination with the obtained preliminary data.

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P83

Positive-negative selectable markers based on fusion genes allow efficient marker-free gene editing in *F. graminearum* as well as Cre/*loxP* aided recycling of transformation markers for consecutive knockouts

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Marker-free genetic engineering of filamentous fungi, such as *F. graminearum*, has proven to be a laborious task in the past, mainly due to the inability to efficiently recycle previously integrated markers. To amend this shortcoming, a series of bifunctional marker genes was generated by N-terminal fusion of the *Herpes simplex* virus thymidine kinase (HSVtk) to the resistance markers *hph* (hygromycin B), *nptII* (G418) and *nat1* (nourseothricin). These fusion genes can be selected for using the respective antibiotics in the fungus, and also in *E. coli*, while additionally allowing forced excision of the marker via 5-fluoro-2-deoxyuridine (FdU) counterselection. Here, we demonstrate the applicability of HSVtk fusion genes by constructing a *TRI8* allele-swapped *F. graminearum* PH-1 transformant. *TRI8* encodes an esterase that uses 3,15-diacetyl-deoxynivalenol as a substrate, and cleaves off an acetyl group from either position 3, or position 15, depending on the allele. PH-1 possesses the *TRI8* allele responsible for producing 15-acetyl-deoxynivalenol (15-ADON), however, the 3-acetyl-deoxynivalenol (3-ADON) chemotype is also frequently encountered in the wild.

An isogenic PH-1-derived strain, carrying the 3-acetyl *TRI8* allele, was generated in two steps. First, *TRI8* was deleted by integration of the HSVtk-*nat1* marker, after which the marker was replaced with the 3-acetyl *TRI8* allele via homologous recombination. The resulting strain no longer possessed the nourseothricin resistance of its precursor Δ*tri8*:HSVtk-*nat1* strain, and was PCR-verified on both flanks for correct integration. As expected, the chemotype was switched from 15-ADON to 3-ADON production. Furthermore, we constructed a *F. graminearum* PH-1-derived mutant that carries seven consecutive gene deletions using the Cre/*loxP* system for marker recycling. Treatment of protoplasts with recombinant Cre recombinase removes *loxP*-flanked cassettes very efficiently, reducing the screening effort required to obtain marker-free deletion strains.

P84

Database extension of filamentous fungi for MALDI-TOF MS – analytical challenges

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One of the most significant characteristics of filamentous fungi or moulds is their saprobiotic ability to grow on almost every material. Toxigenic strains are able to produce mycotoxins, which are occurring in the food chain. In addition, pathogenic fungi could cause infectious diseases and many molds can contribute to the development of asthma and allergies.

Classical mycological methods such as culturing and microscopic identification of characteristic phenotypic structures are often not sufficient to provide a valid identification on species level. Furthermore, such methods need a high level of expertise. The identification of filamentous fungi based on the creation of a protein fingerprint by MALDI-TOF is a fast method and does not require detailed knowledge of phenotypic structures.

Since many species are currently not included in the filamentous fungi database of Bruker Daltonik, the aim of this project is to improve and to extend the currently available database for routine identifications.

Fungal isolates were cultivated in SABOURAUD liquid medium and identified by classical methods and sequencing. Isolates were also processed according to the sample preparation protocol for filamentous fungi for MALDI-TOF MS identification. The resulting species identification was taken as benchmark for the MALDI-TOF MS database.

In order to identify filamentous fungi by MALDI-TOF MS a comprehensive database of well characterized isolates is necessary. Hence, more than 100 new isolates will be included in the database. This approach highlights important taxa which are still not included in the filamentous fungi library, e.g. *Stachybotrys* species. Moreover, analytical and technical challenges of the protein extraction method for filamentous fungi are presented and discussed. Some fungal species show problems in a proper protein extraction which are addressed in detail.

With this database extension it will be possible to identify filamentous fungi which are relevant in food, feed and in the clinical background down to species level. This will benefit the identification of filamentous fungi in the routine.

P85

Occupational exposure to mycotoxins in swineries

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Occupational exposure to aflatoxin B1 (AFB1) in swine farms was already reported (Viegas et al., 2013) and showed positive findings for AFB1 by ELISA in urine samples from workers that work in those farms. However, data regarding fungal contamination showed that exposure to other mycotoxins could be expected. A study was developed to analyze if exposure to further mycotoxins was occurring. Occupational exposure assessment to mycotoxins was done with a LC-MS/MS urinary multi-analyte approach. Besides urine samples, litter (n = 5), feed (10) and air samples (23) from swine farms were also analyzed by LC-MS/MS.

Deoxynivalenol (DON), zearalenone, 15-Acetyldeoxynivalenol, 3-Acetyldeoxynivalenol, fumonisins (FB1, FB2 and FB3), mycophenolic acid and sterigmatocystin (STE) were the most prevalent mycotoxins on litter and feed samples. All litter samples presented contamination by STE. Regarding air samples, only two samples have quantifiable values of STE. Twenty-five workers and nineteen control individuals participated in the study. Workers showed quantifiable results for deoxynivalenol-glucoside (DON-GlcA) (52%), ochratoxin (OTA) (4%), Aflatoxin M1 (AFM1) (16%) and citrinin (CT) (4%). For the control group, only CIT presented a quantifiable result.

Environmental samples analyzed presented multiple contamination and are probably related with workers exposure. Mycotoxins should be considered an occupational risk factor and risk management measures should be define and apply.

S. Viegas, L. Veiga, C. Verissimo, R. Sabino, P. Figueiredo, A. Almeida, E. Carolino, C. Viegas. (2013). Occupational Exposure to Aflatoxin B1 in Swine Production and Possible Contamination Sources, Journal of Toxicology and Environmental Health, Part A: Current Issues, 76:15, 944-951, DOI: 10.1080/15287394.2013.826569.

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P86

Exposure assessment to mycotoxins in a Portuguese fresh bread dough company by analysis of dust and urine samples

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Occupational exposure to mycotoxins can occur in many and different work environments. Critical workplaces for mycotoxins appearance have already been studied and nowadays it is possible to investigate that exposure to mycotoxins is likely through inhalation due to their presence in dust. This study aimed to assess occupational co-exposure to mycotoxins in a fresh bread dough company, an workplace not studied until now. Occupational exposure assessment to mycotoxins was done with a LC-MS/MS urinary multi-biomarker approach. Twenty-one workers and nineteen participants, which were used as controls, participated in the study. Additionally, a settled dust sample from the company was analyzed. As a result, workers showed quantifiable results for deoxynivalenol-glucoside (DON-GlcA) (43%) and Aflatoxin M1 (AFM1) (10%). For the control group, only CIT presented a quantifiable result. DON was the mycotoxin measured also in higher amounts in the settled dust sample (58.2 ng/g). Therefore, workers exposure is probably due to the raw materials contamination and their manual handling during the work routine in the company. We can conclude that workers and controls are exposed to several mycotoxins simultaneously and workers might have a higher exposure to airborne DON by flour dust. Risk management measures should be applied to prevent exposure and health surveillance programs should be defined.

Key-words: mycotoxins, occupational exposure, fresh bread dough company, multibiomarker approach

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P87

NX-toxins: Toxicity, stability and detoxification

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Deoxynivalenol (DON) produced by *Fusarium graminearum* is a well-known virulence factor on wheat and most likely on other hosts. North American isolates of *Fusarium graminearum* (NX strains) produce a new type A trichothecene mycotoxin, NX-2, with a similar structure as 3-ADON but without the keto group at C-8 (Varga et al., Environ. Microbiol. 2015 Aug; 17(8)). While the NX-strains produce mainly NX-2 on rice or synthetic media, NX-2 is de-acetylated to NX-3 in planta. Toxicity of NX-3 towards eukaryotic ribosomes is comparable to that of DON.

The NX strains possess a TRI1 allele, which differs from the one in PH-1 at 14 amino acids. Despite the fact that TRI1 is unlinked to the core TRI cluster, all natural isolates so far were genotyped as 3-ADON type. Introduction of the NX-allele of TRI1 into the 15-ADON producer PH-1 led to formation of NX-4, which is acetylated at C-15. Like other trichothecenes NX-4 inhibits eukaryotic translation, but has a lower inhibitory activity than DON or 15-ADON. Accordingly, in wheat infection assays with cultivar Apogee, NX-4 producing strains are slightly less virulent than the isogenic DON producer PH-1. Also, the plant response might be different. NX-3 glucoside (and also NX4-glucoside) can be formed by recombinant UDP-glucosyltransferases in vitro.

Recently up to 20% NX-producers were detected in an area of New York State, a much higher frequency than previously reported. A selective advantage of NX producing strains might be the fact that NX-type toxins can escape a second type of detoxification inactivation by Michael-adduct formation with glutathione.

Conversion of NX-3 into a non-toxic rearrangement product (NX3-M1) lacking the epoxide, has been observed during alkaline treatment, conditions mimicking food processing (baking), and even at temperature conditions that might occur in the field and during extended grain storage.

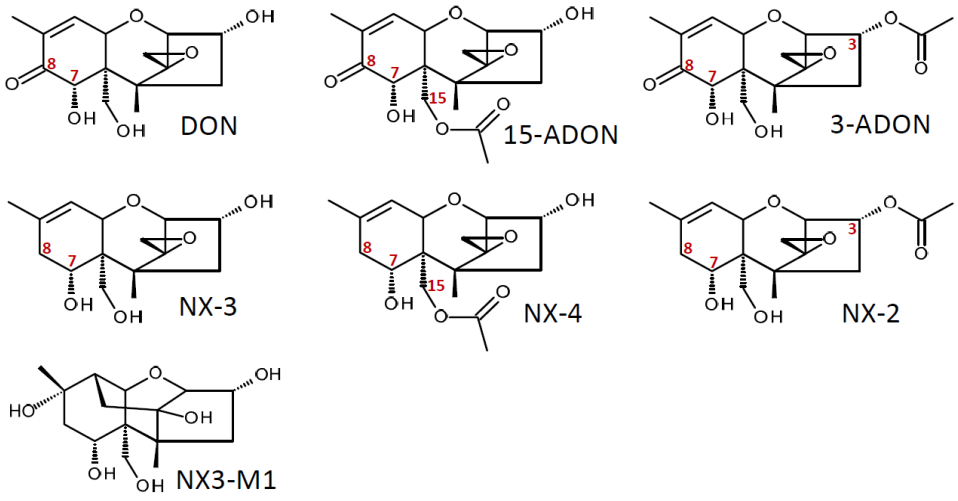

Figure 1: Chemical structures of DON, NX-2 and related metabolites

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Fum$_1$ gene-based analysis of potential fumonisin producers within the *Fusarium fujikuroi* species complex using a loop-mediated isothermal amplification (LAMP) assay with visual signal detection

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Members of the *Fusarium fujikuroi* species complex (FFSC, formerly known as the *Gibberella fujikuroi* species complex) are responsible for high economic losses due to destructive plant diseases and reduction in staple food crop quality. Many species of the group are potent producers of fumonisins in food/feed worldwide. Contamination with fumonisins can result in serious human and animal health problems. Among fumonisins, fumonisin B1 (FB1) is the most frequently found in naturally contaminated foods. It has been associated with nerve system disorders in horses, pulmonary edema in swine as well as neural tube defects and esophageal cancer in humans. FB1 induces disruption of the sphingolipid metabolism and has been considered as a category 2B carcinogen by the International Agency for Research on Cancer (IARC). The *fum$_1$* gene codes for a polyketide synthase involved in the biosynthesis of fumonisins. The gene is present in the genomes of all fumonisin producing *Fusarium* species. Reliable detection of that gene in pure cultures and in contaminated food may provide an estimate of the toxicological potential of a strain or a food sample. A LAMP assay based on the *fum$_1$* gene sequence was developed and tested with purified DNA of 91 FFSC strains representing 44 different species. Among them, the *fum$_1$* gene was detected in 21 species (37 strains) with *F. anthophilum, F. bulbicola, F. fujikuroi, F. globosum, F. nygamai, F. proliferatum, F. sacchari* and *F. verticillioides* being the most important fumonisin producers. Only one out of 69 other *Fusarium* spp. showed a cross reacted with the new assay. The LAMP assay had a limit of detection (LOD) of 5 pg of genomic DNA per assay and positive reactions were detected visually by color change from yellow to pink without any necessary manipulations. Usefulness of the method was validated by investigation of artificially contaminated samples. A detection limit of 10^4 *F. proliferatum* conidia per reaction was revealed for artificially contaminated rice after direct addition of sample lavage fluid. Furthermore, the LAMP assay was demonstrated to be also effective for the analysis of naturally contaminated maize. In conclusion, the new LAMP method has been demonstrated to be group specific, rapid, sensitive and reliable for the prognosis of a potential fumonisin risks during production and processing in the food and feed industry.

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Determination of aflatoxin-albumin adduct in bovine blood and comparison to aflatoxin M1 in milk

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Biomarkers are indicators of environmental pollution and illnesses and the analysis of physiological specimens can provide diagnostic information. The aflatoxin-albumin adduct (AAA) is a well-established biomarker in human blood for aflatoxin exposure. Aflatoxin B1 is the most toxic mycotoxin, which occurs in nuts, spices and maize. Due to the use of contaminated maize as a feeding stuff for dairy cows, there is a potential risk for humans through the consumption of the milk and for the animals themselves. The analysis of AAA in bovine blood is not established so far, but can provide detailed information for evaluation of the aflatoxin exposure of the animal. The aim of the present investigation was to investigate if AAA can also be detected in bovine blood and to compare this biomarker with the well-established biomarker aflatoxin M1 (AFM1) in milk.

Therefore, milk and blood samples of 22 dairy cows were collected at the same time from Kenyan farms which were recruited from an ongoing trial on aflatoxin mitigation. The milk was kept frozen at -20°C until analysis for AFM1 with a commercial enzyme-linked immunosorbent assay (ELISA) (Helica Biosystems Inc., Santa Ana, CA, USA) following the manufacturer's instructions. The blood samples were centrifuged and the serum aliquoted and frozen until the determination of AAA by ELISA according to Chapot and Wild (1991). AFM 1 (min-max; 2.2 – 487.9 ppt) as well as AAA (2.2 – 96.3 pg/ mg albumin) were detected in all samples. The correlation (r^2=0.67) between both biomarkers is highly significant. These results suggest that AAA is well suited as biomarker in bovine blood to measure the aflatoxin exposure and can also be consulted in non-lactating cows.

Reference

Chapot, B., Wild, C.P., 1991. ELISA for quantification of aflatoxin-albumin adducts and their application to human exposure assessment. In: Warhol, M., van Velzen, D., Bullock, G.R. (Eds.), Techniques in Diagnostic Pathology, Vol. 2. Academic Press, San Diego, pp. 135-155.

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

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Highly regulated mycotoxin analysis needs special clean-up or high-end analytical devices. Combine both to increase analytical speed, sample throughput, and to reduce costs by highly selective clean-up and full automatisation.

The FREESTYLE ThermELUTE™ system allows fully automated sample processing from the crude extract to the final chromatogram at low detection range. It combines highly selective immuoaffinity clean-up, a thermal denaturation of the antibody-toxin-complex and quantitative injection directly into HPLC, thus an ultimate sensitivity down to the ppt or even ppq level is achieved. A benefit of the technology is the high capacity and selectivity of the immunoaffinity columns, which allows matrix clearance and direct injection of up to 10 gram matrix equivalents (aflatoxin M1 in milk) into the HPLC. This leads to two aspects, a lower sample amount increasing the analytical speed and a higher sensitivity of HPLC-FLD or MS/MS, respectively. Not only standard matrices like hazelnut and corn but also spices or other difficult matrices can be analysed using the system down to baby food regulated levels for mycotoxins.

The technology reduces solvent amounts for extraction and clean-up by using miniaturized affinity columns with high loading capacity and excellent matrix clearance. High flow rates and low sample volumes allow a sample turnover time within less than 30 minutes including chromatography.

For aflatoxins B/G the lower ppt range quantification is possible and for aflatoxin M1 even in the ppq range reliable analytical results are found. Ochratoxin A and zearalenone analysis can be performed using the same technological approach. The LOD were determined for aflatoxins B1/B2/G1/G2 7 ppt / 2 ppt / 8 ppt / 2 ppt, the LOQ were determined ranging from 6 ppt to 24 ppt. For ochratoxin A analysis a LOD of 10 ppt and a LOQ of 31 ppt allow a sensitive and precise analysis. The analysis of aflatoxin M1 especially in milk, where up to 10 mL could be loaded, revealed a LOD of 0.7 ppt and a LOQ of 2.2 ppt. The quantification of zearalenone can be achieved with a LOD below 1 ppb and LOQ of 2.6 ppb.

Overall, the FREESTYLE ThermELUTE™ in combination with dedicated immunoaffinity columns increases sample throughput and saves time and money by overlapping processing of sample and analytical measurement.

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Molecular identification of *Penicillium* species

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Molecular biology techniques are the most sensitive and specific methods widely used in microorganism identification, especially pathogenic and environmental bacteria. The aim of the study was to identify toxinogenic mould strains to the genus and species level, using PCR and DNA sequencing.

A total of 28 mould strains initially identified to the *Penicillium* species based on standard phenotypic microbiological methods (microscopic techniques and selective media) were subjected to molecular analysis. Additionally, three strains from other fungal genera (*Mucor circinelloides*, *Fusarium oxysporum*, *Aspergillus fumigatus*) were included as controls. The mould strains were isolated from contaminated samples of feed, food, building materials and clinical material taken from a single patient diagnosed with mucormycosis. For DNA isolation, moulds were inoculated onto appropriate microbiological media. DNA isolation was performed using the method covered by the patent number WO / 2006/133701. The fungus-specific universal primers ITS1 and ITS4 were used to amplify the ITS region. PCR products were subjected to agarose gel electrophoresis and then visualized using the Gel Doc XR+ system and Image Lab software (Bio-Rad). Subsequently, the samples were purified using Exo/Sap mixture and subjected to bi-directional sequencing using BigDye Terminator v.3.1 chemistry (Applied Biosystems) and the primers ITS1 and ITS4. Sequencing reactions were done and analysed on ABI3730xl sequencer (Applied Biosystems) in oligo.pl DNA Sequencing Laboratory of the Institute of Biochemistry and Biophysics Polish Academy of Sciences (Warsaw, Poland). The sequences of the PCR products were compared with the data available in the CBS-KNAW and GenBank database.

In the case of all tested strains (100%, 32/32) previously identified based on phenotypic methods, ITS sequence analysis confirmed identification to the genus level. In addition, the majority (64.3%, 18/28) of the *Penicillium* strains were classified to the same species based on both methods. In the future, we aim to analyse the sequences of other fungal ITS regions.

The research was supported from the statutory funds allocated to the project entitled ‘Implementation of molecular biology techniques in identifying of moulds to the species level’ (Ministry of Science and Higher Education, Poland).

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Production, isolation and necrotrophic activity of selected *Parastagonospora nodorum* proteinaceous toxins

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Parastagonospora nodorum (*Stagonospora nodorum*) is one of the most important pathogens of grains grown in Poland - triticale and wheat. Infection causes necrotic spots on leafs in which they are growing, and can spread to the same plant, as well as to neighbouring plants. The favouring conditions of this pathogen are high humidity and temperature. Reduction of the photosynthetically active surface of the leaf and damage to the chaff leads to serious reduction in the yield. There have been reports of production by *S. nodorum* of proteinaceous toxins, which play a key role in the induction of necrotic changes in infected tissues of the host. These toxins affect the specific host genes. Interaction of the toxin with the products of dominant form of the gene leads to the induction of necrosis, and in the presence of only a recessive form is the insensitivity to a particular toxin. Experiments conducted under both laboratory and field conditions confirmed that the toxins are a major factor in the development of leaf and chaff septoria on wheat. Until now seven different proteinaceous toxins have been described.

The aim of the studies was isolation of selected necrotrotrophic effectors in amount which allowed to conduct further studies on susceptibility of wheat and triticale genotypes.

Selected isolates of *P. nodorum* were grown 4 weeks on liquid medium in darkness at room temperature on shaker. After filtration the resulting filtrates were separated on the strong cation exchanger with use of the gradient elution 50-300 mM NaCl. The obtained 5-ml fractions were tested for necrotoxicity on the leaves of selected wheat and triticale genotypes. Fractions with necrotic activity were combined and further purified on Superdex 75 chromatography column with acetate buffer. The collected fractions were analysed on HPLC system with BIOshell A400 Protein C4 column and tested for necrotic activity.

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Beauvericin and ennatins formation in maize kernels inoculated with new pathogen - *Fusarium temperatum*

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Fusarium temperatum is considered as a one of the most important causing factor of maize ear rot in Poland. The fungus produces two main secondary metabolites, like beauvericin (BEA) and the structurally related enniatins (ENN). They belong to group of N-methylated cyclic hexadepsipeptides. BEA is a specific cholesterol acyltransferase inhibitor, can be toxic to some several human cell lines and may induce apoptosis. The aim of studies conducted in 2017 was analysis of the profile of mycotoxin derivatives produced by *F. temperatum* in laboratory conditions.

Material and methods Biosynthesis of secondary metabolites was performed in vitro on rice and maize, according to methods described by Waśkiewicz et al. (2012). Beauvericin, enniatins, fumonisins and ergosterol (ERG) were extracted using methanol and analysed by HPLC.
Results The mycotoxin profile of 70 *F. temperatum* strains, originally collected from naturally infected ears has been studied. BEA was recognised as predominate hexadepsipeptide toxin. The mean level of BEA was 425.35 ppm on rice and 121.52 on maize kernels. Trace amounts of ENN on rice and maize kernels were noted for 21 and 24 isolates of fungus respectively. On rice the ERG level ranged from 12.24 ppm to 4110.64 ppm. On the second maize kernels used as substrate, the ability of producing ERG was significantly lover and ranged from 2.14 ppm to 716.39 ppm. Contamination of rice and maize kernels with fumonisins (FB_1 and FB_2) was not detected among all 70 *F. temperatum* isolates. Quite scant and contradictory reports in the literature about spectrum of *F. temperatum* mycotoxin production points to the need continuous in this aspect study.
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Contamination of wheat grains with *Fusarium* mycotoxins, depending on cereal crop rotation

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Agricultural production is often negatively influenced by the occurrence of microscopic filamentous fungi and their toxic secondary metabolites, mycotoxins. Cereals belong to the most frequently affected agricultural commodities, and in the moderate climatic zone *Fusarium* fungi/mycotoxins dominate. Numerous species of the genus *Fusarium* have phytopathogenic and toxigenic properties affecting yield, nutritive value and hygenic quality of harvest products from arable crops worldwide. These fungi can cause serious diseases in different vegetative and generative organs of the plant throughout the cultivation period. Trichothecenes and zearalenone are the most common *Fusarium* mycotoxins contaminating a wide range of crops in Poland.

Field experiment was conducted in the Institute of Soil Science and Plant Cultivation –State Research Institute in Puławy (Poland). Grain samples of two winter wheat cultivars (Bamberka -*Triticum aestivum spp*., Rokosz – *Triticum spelta L.)* collected in 2013 and 2014 were analyzed for contamination with deoxynivalenol (DON), zearalenone (ZEA), and T-2/HT-2 toxins by the enzyme-linked immunosorbent analysis (ELISA) method. The influence of cultivar and crop rotation on the contamination with mycotoxins was evaluated. Plants were naturally infected by fungal pathogens producing mycotoxins. The agrotechnical treatments differed with percentage of cereals in crop rotation (50, 75 and 100%). It was found that the wheat grains were contaminated with the tested mycotoxins (DON, ZEA, and T-2/HT-2).

The cultivar and percentage of cereals in crop rotation strongly modified the concentration of mycotoxins in grains. The highest level of each tested mycotoxin was found in Bamberka cv. compering to Rokosz cv. Moreover, the determined concentration of DON in Bamberka sample grow in crop rotation with 100 and 75% of cereals was close to safe level for human/animal health and was 1225 $\mu g \cdot kg^{-1}$. Samples from crop rotation 100 and 75% of cereals was the most abundant with all analyzed mycotoxins in both cultivars.

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Application of molecularly imprinted polymer solid-phase extraction (MISPE) to the determination of citrinin in cereals

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Throughout the last years, citrinin, produced by a variety of molds especially in cereals during storage, has moved into the focus of food safety [1]. In 2012, EFSA recommended to keep the level of citrinin ingestion below 0,2 µg/kg body weight, as nephrotoxic effects are expected negligible below this level. However, genotoxic and cancerogenic effects cannot be excluded and overall citrinin uptake through foodstuff should be as low as possible [2]. Determination of citrinin by chromatographic methods requires thorough sample preparation to avoid possible matrix interferences. To obtain suitable extracts, different clean-up techniques have been applied of which immunoaffinity chromatography (IAC) and, recently, solid-phase extraction using molecularly imprinted polymers (MISPE) have shown very promising [3,4].

Here we present the preparation and characterization of several molecularly imprinted polymers for the extraction of citrinin from different cereals. In comparison to citrinin-selective immunoaffinity columns, the developed polymers show superior physico-chemical stability facilitating easy and robust handling, while allowing for storage at room temperature and a remarkable reusability. Because of their feasible and inexpensive laboratory scale production, they appear as a valuable alternative to established immunoaffinity columns.

References:

[1] de Oliveira Filho, J.W.G. *et al.* A comprehensive review on biological properties of citrinin. *Food Chem. Tox.* **2017**, *110*, 130-141.

[2] López Sáncheza, P. *et al.* Generation of occurrence data on citrinin in food. *EFSA Supp. Publ.* **2017**, EN-1177.

[3] Marley, E., Brown, P., Leeman, D., Donnelly, C. Analysis of citrinin in cereals, red yeast rice dietary supplement, and animal feed by immunoaffinity column cleanup and LC with fluorescence detection. *J. AOAC Int.* **2016**, *99*, 1025-1031.

[4] Appell, M., Jackson, M. A., Wang, L. C., Bosma, W. B. Determination of citrinin using molecularly imprinted solid phase extraction purification, HPLC separation, and fluorescence detection. *J. Liq. Chromatogr. R. T.* **2015**, *38*, 1815-1819.

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