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Institute of Animal Hygiene, TU München, Freising

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Chair of Food Safety, Ludwig-Maximilians-Universität, München

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Miroslav Ferianc
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Martina Chvílová

Web support

Hartmut Wöhrle

Under the auspices of

Faculty of Chemical and Food Technology, Slovak University of Technology
Ministry of Agriculture and Rural Development of the Slovakia
National Focal point for scientific and technical matters for EFSA

The Organizing Committee would like to thank to the Faculty of Chemical and Food Technology, Slovak University of Technology and Ministry of Agriculture and Rural Development of the Slovakia

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# Program

## Sunday – May 31st 2015

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<tr>
<td>17:00 – 19:00</td>
<td>Early bird registration</td>
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<tr>
<td>18:00</td>
<td>Get together – Flag ship restaurant</td>
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<td>Address: Namestie SNP 8, Bratislava</td>
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## Monday – June 1st 2015

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<tr>
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<tbody>
<tr>
<td>8:00</td>
<td>Registration</td>
</tr>
<tr>
<td>9:00</td>
<td>Welcome</td>
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<tr>
<td></td>
<td>Manfred Gareis – President of the Society for Mycotoxin Research</td>
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<td></td>
<td>Jana Feriancova – Chair of the Organizing Committee</td>
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## New metabolites and biosynthesis

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<td>Coffee break / Exhibition / Poster Session</td>
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## Analytical methods

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11:30 Detection of novel metabolites of HT-2 and T-2 toxin in barley by stable isotope labelling and LC-HRMS
Jacqueline Reiterer, Elisabeth Varga, Alexis Nathanail, Christoph Bueschl, Justyna Rechthaler, Niklas Kampleitner, Imer Maloku, Franz Berthiller, Marc Lemmens, Rainer Schuhmacher

11:45 Hydrophilization of quantum dots for application in immunochromatography of mycotoxins
Astrid Foubert, Natalia Beloglazova, Anna Gaynbugh, Mickael Tessier, Tangi Aubert, Zeger Hens, Sarah De Saeger

12:00 Capacitive biosensor based evaluation of antigen-antibody interactions for the screening of mycotoxin’s conjugates
Natalia Beloglazova, Astrid Foubert, Pieterjan Lenain, Sarah De Saeger

12:15 Lunch

13:00 Poster session / Exhibition

Toxicology and miscellaneous topics

13:30 Orally administered T-2 and Fumonisin B1 affects cation exchange of rabbit erythrocytes
András Szabó, Judit Szabó-Fodor, Hedvig Fébel, Róbert Romvári, Melinda Kovács

13:45 Intestinal toxicity of the masked mycotoxin deoxynivalenol-3-β-D-glucoside
Sabria Mimoun, Alix Pierron, Leticia S. Murate, Yannick Lippi, Nicolas Loiseau, Ana-Paula Bracarense, Laurence Liaubet, Gerd Schatzmayr, Franz Berthiller, Wulf-Dieter Moll, Isabelle Oswald

14:00 Genotoxicity of the food contaminant deoxynivalenol is modulated by the host microbiota acquired at birth
Delphine Payros, Eric Oswald, Isabelle Oswald

14:15 Multimycotoxin analysis of Aspergillus clavatus-infected feed samples implicated in two outbreaks of neuromycotoxicosis in cattle in South Africa
Christo Botha, Matthew Legg, Mariëtte Truter, Michael Sulyok

14:30 Mycotoxins in beetles Tenebrio molitor grazing on Fusarium species
Zhiqing Guo, Katharina Pfohl, Petr Karlovsy, Heinz-Wilhelm Dehne, Boran Altincicek

14:45 Coffee break / Exhibition / Poster Session

15:00 – 16:00 Meeting of the Society for Mycotoxin Research
17:00  Official audience in Primate’s Palace  
Meeting point: Primacialne namestie 1, Bratislava  

17:45  Guided City train  
Meeting point: Primate’s Palace, Primacialne namestie 1, Bratislava  

19:00 – 22:00  Welcome reception and dinner in the Moyzes hall  
Address: Vajanskeho nabrezie 12, Bratislava  

Tuesday – June 2nd 2015  
8:00  Registration  

Ochratoxin A – anniversary session I  
9:00  Ochratoxin A: History  
L14  František Malíř, Vladimír Ostrý  

9:15  Ochratoxin A: Occurrence in food and feed  
L15  Vladimír Ostrý, František Malíř  

9:30  Ochratoxin A: Toxigenic fungi  
L16  Rolf Geisen, Markus Schmidt-Heydt, Dominic Stoll, Najim Touhami  

10:00  Ochratoxin A: Chemistry  
L17  Hans-Ulrich Humpf  

10:30  Coffee break / Exhibition / Poster session  

Ochratoxin A – anniversary session II  
11:15  Ochratoxin A: Toxicology  
L18  Gisela H. Degen  

11:45  Overview on molecular mechanism involved in ochratoxin A nephrotoxicity and carcinogenity  
L19  Annie Pfohl-Leszkowicz  

12:00  Mycotoxins at the blood-brain barrier: Metabolism, toxicity, barrier integrity and transfer to the brain  
L20  Matthias Behrens, Sabine Hüwel, Hans-Joachim Galla, Hans-Ulrich Humpf  

12:15  Lunch  

13:00  Poster session / Exhibition
Occurrence and degradation

13:30  Effect of plastic mulches on mycobiome diversity and mycotoxin occurrence in soil
Katherine Muñoz Sepulveda, Markus Schmidt-Heydt, Dominic Stoll, Dörte Diehl, Rolf Geisen, Gabriele E. Schaumann

13:45  (Modified) Alternaria mycotoxins in foodstuffs: Occurrence and influence of food processing
Jeroen Walravens, Hannes Mikula, Michael Rychlik, Stefan Asam, José Diana Di Mavungu, Liesbeth Jacxsens, Carl Lachat, Anita Van Landschoot, Lynn Vanhaecke, Sarah De Saeger

14:00  Occurrence of ergot alkaloids and their epimers in feed in the Czech Republic
Martina Bolechová-Čumová, Markéta Pospíchalová

14:15  Safety of mycotoxin binders used in broiler feed: interactions with the oral absorption of veterinary drugs and coccidiostats
Thomas De Mil, Mathias Devreese, Nathan Broekaert, Sophie Fraeyman, Siegrid De Baere, Patrick De Backer, Siska Croubels

14:30  Plasma based mycotoxin degradation
Lars ten Bosch, Georg Avramidis, Katharina Pfohl, Stephan Wieneke, Wolfgang Viöl, Petr Karlovsky

16:45  Cruise by sightseeing boats
Welcome drink and coffee time
Meeting point: Fajnorovo nabrezie 2, Bratislava

19:00  Dinner – Brewery on the Danube
Address: Tyrsovo nabrezie 1, Bratislava

Wednesday – June 3rd 2015
8:00  Registration

Metabolism and toxicokinetics

9:00  Impact of phase I metabolism on cellular uptake, oxidative stress and genotoxicity of alternariol and its monomethyl ether
Christine Tiessen, Gudrun Pahlke, Hannes Mikula, Johannes Fröhlich, Doris Marko
9:15  Toxicokinetics of T-2 toxin and T-2-glucoside and *in vivo* hydrolysis of T-2-glucoside in broiler chickens
Nathan Broekaert, Mathias Devreese, Thomas De Mil, Sophie Fraeyman, Marthe De Boevre, Sarah De Saeger, Patrick De Backer, Siska Croubels

9:30  Unravelling the role of ruminal development in the biotransformation of deoxynivalenol and its acetylated derivatives: a comparative toxicokinetic approach
Gunther Antonissen, Bonnie Valgaeren, Mathias Devreese, Siegrid De Baere, Ellen Heyndrickx, Sarah De Saeger, Philipp Fruhmann, Gerhard Adam, Piet Deprez, Siska Croubels

9:45  (Deepoxy)-deoxynivalenol sulfonates as major deoxynivalenol metabolites in rats: The missing link in ADME studies
Heidi Elisabeth Schwartz-Zimmermann, Christian Hametner, Veronika Nagl, Wulf-Dieter Moll, Franz Berthiller

10:00  Coffee break / Exhibition / Poster session

### Inhalative exposure

10:45  Occupational exposure to airborne mycotoxins during handling of contaminated corn
Stefan Mayer, Mareike Reichel, Scarlett Biselli

11:00  Occupational exposure to aflatoxin B1 in one waste sorting plant
Susana Viegas, Luisa Veiga, Ana Almeida, Anita Quintal Gomes, Elisabete Carolino, Carla Viegas

11:15  Mycotoxins and indoor environments: Mycotoxin production during the development of toxigenic fungal species on materials used in indoor furnishings
Brankica Aleksić, Sylviane Bailly, Marjorie Draghi, Marlène Lacroix, Isabelle Oswald, Enric Robine, Jean-Denis Bailly

11:30  Mycotoxins-producing ability of indoor airborne fungi isolated from librarian environment affected by mould contamination
Anna Micheluz, Michael Sulyok, Sabrina Manente, Rudolf Kraska, Giovanna Cristina Varese, Giampietro Ravagnan

11:45  Closing session
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- Fumonisins
- Ochratoxin A
- Aflatoxin G1/G2
- Aflatoxin B1
- Diacetoxyscirpenol
- Deoxynivalnol
- T2 Toxin
- Zearalenone
- Ergot Alkaloids

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## New metabolites and biosynthesis

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**L10** Intestinal toxicity of the masked mycotoxin deoxynivalenol-3-β-D-glucoside  
Sabria Mimoun, Alix Pierron, Leticia S. Murate, Yannick Lippi, Nicolas Loiseau, Ana-Paula Bracarense, Laurence Liaubet, Gerd Schatzmayr, Franz Berthiller, Wulf-Dieter Moll, Isabelle Oswald

**L11** Genotoxicity of the food contaminant deoxynivalenol is modulated by the host microbiota acquired at birth  
Delphine Payros, Eric Oswald, Isabelle Oswald

**L12** Multimycotoxin analysis of *Aspergillus clavatus*-infected feed samples implicated in two outbreaks of neuromycotoxicosis in cattle in South Africa  
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Zhiqing Guo, Katharina Pfohl, Petr Karlovsky, Heinz-Wilhelm Dehne, Boran Altincicek

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**L15** Ochratoxin A: Occurrence in food and feed  
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Anna Micheluz, Michael Sulyok, Sabrina Manente, Rudolf Kraska, Giovanna Cristina Varese, Giampietro Ravagnan
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| P2 | Development of an enzyme immunoassay for the tremorgenic mycotoxin paxilline  
Julia I. Bauer, Madeleine Gross, Sandra Wegner, Ewald Usleber |
| P3 | Mycotoxin cocktail in early maturing cotton oilseed cake varieties associated with cattle feeding problems  
Agha Waqar Yunus, Michael Sulyok, Josef Böhm |
| P4 | The role of the National Focal Point and EFSA in the area of food contaminants  
Milo Bystrický, Zuzana Bírošová |
| P5 | Current situation of ochratoxin A in Chile  
Gisela Ríos, Mario A. Vega, Victor H. Campos-Requena, Katherine Muñoz Sepulveda |
| P6 | Development of an LC-MS/MS method for the quantitative determination of deoxynivalenol, 3- and 15-acetyl-deoxynivalenol and their in-vivo metabolites in calf plasma  
Siegried De Baere, Mathias Devreese, Gunther Antonissen, Bonnie Valgaeren, Philipp Fruhmann, Gerhard Adam, Siska Croubels |
| P7 | Carryover of fumonisins in feed materials using HPLC-MS  
Edit Deák, Gábor Rónaszéki, Dániel Hüse |
| P8 | Development of an LC-MS/MS method for the simultaneous determination of beauvericin, enniatins (A, A1, B, B1) and cereulide  
Marlies Decler, Andreja Rajkovic, Benedikt Sas, Annemieke Madder, Sarah De Saeger |
| P9 | Quantitative determination of tenuazonic acid in broiler chicken plasma using a validated liquid chromatography-tandem mass spectrometry method  
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| P10 | The contamination of German food with *Alternaria* mycotoxins – a fast multi analyte LC-MS/MS approach  
Sebastian Hickert, Hans-Ulrich Humpf |
| P11 | LC-MS/MS method for determination of mycotoxins in animal feed  
Piotr Jedziniak, Katarzyna Pietruszka, Olga Burek, Henryka Wiśniewska-Dmytrow |
| P12 | Ergot alkaloids in compound feed – results of an interlaboratory validation study and possible analytical stumbling blocks  
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Grzegorz Koczyk, Delfina Popiel, Adam Dawidziuk

P14 Determination of aflatoxin M1 in milk and powdered milk
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P15 Study of ochratoxin A and citrinin content in Czech lager beers by fast method using direct sample injection combined with fused core column on-line SPE-HPLC with fluorescence detection
Ivona Lhotská, Dalibor Šatínský, Lucie Havlíková, Petr Solich

P16 Design of a novel microfluidics platform for mycotoxin food contaminant determination
Jonathan H. Loftus, Christine E. Loscher, Richard J. O’Kennedy

P17 Development of cost-effective and rapid mycotoxin multiresidue method for baby food by SPE and DSPE technology as alternative to affinity chromatography
Jens Luetjohann, Fabian Mueller, Stefan Neubauer, Juergen Kuballa, Eckard Jantzen

P18 Development of a sensitive UPLC-MS/MS-based multiresidue method for the simultaneous determination of Alternaria toxins in baby food
Jens Luetjohann, Katharina Feist, Stefan Neubauer, Michael Rychlik, Juergen Kuballa, Eckard Jantzen

P19 Is the narrow focus on aflatoxins in sub-Saharan Africa plausible?
Limbikani Matumba, Christof Van Poucke, Michael Sulyok, Clare Narrod, Sarah De Saeger

P20 Validation of a method for the analysis of sterigmatocystin in cereals using immunoaffinity columns
Claire Milligan, Elaine Marley, Phyllis Brown

P21 Comparison of various commercially available multi-mycotoxin immunoaffinity columns to direct injection
Dave Leeman, Elaine Marley, Joyce Wilcox, Claire Milligan

P22 Spatial distribution of phytopathogenic Fusarium fungi and its mycotoxins within heterogeneous wheat fields
Marina Müller, Sylvia Koszinski, Donovan E. Bangs, Marc Wehrhan, Andreas Ulrich, Gernot Verch, Alexander Brenning

P23 Analysis of patulin in multifruit thick juice and concentrates using a molecularly imprinted polymers based SPE
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P24 Influence of reduced water supply on the Fusarium toxin contamination of maize ears
Elisabeth Oldenburg, Frank Ellner, Siegfried Schittenhelm

P25 Rapid diagnostic testing of toxigenic microfungi isolated from foodstuffs – an application of chromogenic media
Marie Jefremova, Vladimír Ostrý, František Malíř, Jiří Ruprich
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● patulin
● alkaloids of rye ergot (ergot alkaloids)

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Unusual metabolites in the ETP-like gene Cluster of *Claviceps purpurea*

**Julian Dopstadt**¹*, Lisa Neubauer², Paul Tudzynski², Hans-Ulrich Humpf¹

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The phytopathogenic ascomycete *Claviceps purpurea* is an important food contaminant as it infects a broad range of grasses, among them economically important cereal crop plants. The secondary metabolites best characterized so far are the toxic ergot alkaloids. They show different toxic effects ranging from gangrene of extremities to hallucinations and spasm.

The biosynthetic gene cluster which we characterized in *Claviceps purpurea* probably encodes a class of secondary metabolites originating from diketopiperazines: the Epipolythiodioxopiperazines (ETP). Their distinct structural feature is the presence of unique di- or polysulfide bridges. Various of them are immunosuppressive and cause apoptotic and necrotic cell death *in-vitro*. Their toxicity and their efficient suppression of cell proliferation have made them attractive as potential therapeutic agents for diseases such as cancer.

The genes in the identified cluster show high similarity to those of the Gliotoxin cluster in *Aspergillus fumigatus*. After the activation of the cluster genes by overexpressing the cluster-specific transcription factor, we unexpectedly could identify a wide range of new unusual metabolites, which differ from the major structure of the known ETP backbone. Whereas any predictable metabolites or pathway intermediates of the ETPs were not formed.

Detailed HRMS and NMR studies revealed that a number of them occur as a 2,3-oxopiperazinethiones and 2,3-dioxopiperazines and show significant similarities to Leptomaculins A and E, which are described as stress-driven products from the fungus *Leptosphaeria maculans*. Furthermore, we tentatively characterized a unique N-methylthio-diketopiperazine structure.

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Production of novel NX-type Trichothecenes in Liquid Media by Genetic Engineering of *F. graminearum* PH-1

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*Fusarium graminearum* is a filamentous fungus that infects cereals, mainly wheat and maize, thereby causing significant economic losses worldwide. Its main virulence factor is the trichothecene mycotoxin deoxynivalenol (DON), which acts as a eukaryotic translational inhibitor, as well its acetylated precursor ADON. The acetyl group of ADON can be located either in C-3 or in C-15 position, depending on Tri8 genotype. Recently, trichothecenes bearing high structural and functional similarity to DON were found in a North American wild isolate of *F. graminearum* (WG-9). These novel trichothecenes, named ‘NX-toxins’, resemble DON and its acetylated derivates, however without the C-8 keto group. Cultivation of WG-9 on rice yielded the 3-acetylated NX-2 toxin. Yet, it is impossible to produce 13C-labelled standards when using rice-based or complex media. In order to enable toxin production in repressing glucose-based media, WG-9 was transformed with a construct enabling constitutive overexpression of the Tri6 transcription factor. The same construct was transformed in a *F. graminearum* PH-1 based strain capable of producing the 15-acetylated NX-4 toxin. In both cases, NX production in liquid media could be significantly increased, but the PH-1 derived strain produced up to eight times more NX-toxins than its WG-9 counterpart. Several media will be tested in order to further optimize toxin production and increase possible yields.
Overexpression of the *Fusarium fujikuroi* global regulator gene *FfSGE1* revealed an extended fusaric acid gene cluster

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It has been known for decades that the rice pathogen *Fusarium fujikuroi* and closely related *Fusarium* spp. produce the mycotoxin fusaric acid (FSA) as well as the two derived compounds 9,10-dehydrofusaric acid (DH-FSA) and 10-hydroxyfusaric acid (HO-FSA). Recently, we characterized the five co-regulated FSA cluster genes *FUB1-FUB5*, out of which two are essential for FSA biosynthesis. Among those, *FUB1* encodes the key enzyme, a polyketide synthase (PKS).

Through overexpression of the global regulator gene *FfSGE1*, we were now able to identify seven additional FSA cluster genes, *FUB6-FUB12*, that are separated from *FUB1-FUB5* by three non-cluster genes. While the previously published FSA gene cluster did not harbor a pathway-specific transcription factor (TF) gene, to our great interest, even two genes of the extended cluster encode TFs of the fungal-specific Zn²Cys⁶-type. Cluster gene expression and product formation was shown to be completely dependent on one of the TFs, Fub10. In contrast, evidence suggests that the second TF, Fub12, regulates the conversion of FSA into its two derivatives, DH-FSA and HO-FSA, which seems to serve as FSA detoxification mechanism for the fungus. Our experiments indicate that, next to Fub12, the cluster-encoded major facilitator superfamily transporter Fub11 is also important for fungal self-protection.

Finally, through characterization of the extended FSA gene cluster, we were able to further elucidate FSA biosynthesis and shed light on the intensely discussed question of how nitrogen is incorporated into FSA. In addition to the PKS Fub1, we now identified a second biosynthetic key enzyme, the non-canonical non-ribosomal peptide synthetase (NRPS) Fub8. We suggest that Fub8 is necessary for the activation of an amino acid precursor which can be further modified by Fub1, underlining a unique way to gain a fungal PKS-NRPS hybrid compound.
Selective Biosynthesis of Zearalenone Conjugates by Fungi

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Zearalenone (ZEN) conjugates such as glucosides and sulfates have already been detected in different food and feed commodity also containing the free toxin. It is known that these conjugates can be hydrolyzed by human intestinal microbiota. Taking into account this liberation of additional ZEN, the true toxin load available for absorption is underestimated. In order to include ZEN conjugates in routine analysis, reference substances are needed.

The objective of this study was to develop a strategy for a facilitated selective biosynthesis of ZEN conjugates. First experiments indicated a mixed metabolite formation of several tested fungal strains. Therefore, *Rhizopus* and *Aspergillus* strains were screened for their potential to selectively synthesize the ZEN derivatives ZEN-14-sulfate (Z14S), ZEN-14-glucoside (Z14G) and ZEN-16-glucoside (Z16G) by varying the incubation conditions. The metabolite screening was conducted by adding ZEN to liquid cultures of the fungal species in the vegetative or generative growth phase. Various time points after ZEN addition were selected for sampling. All media samples were analyzed for metabolite formation by HPLC-MS/MS. The ZEN conjugate formation differs significantly depending on the fungal strain, growth phase and time of harvest. For Z14S formation *A. oryzae* is most suitable and ZEN-glucosides are formed by several *Rhizopus* species. High conversion rates of up to 90% of the initial ZEN could be achieved showing selective biosyntheses of Z14S and ZEN-glucosides with >95% exclusive formation.

A selective biosynthesis strategy for Z14S, Z14G and Z16G was successfully developed suited to provide required ZEN conjugate standards. Further studies will focus on a large-scale production and the development of a clean-up method. Additionally, a consecutive in situ biosynthesis of ZEN conjugates is conceivable by using *Fusarium* sp. for ZEN biosynthesis with subsequent conjugation of the formed toxin utilizing *Rhizopus* or *Aspergillus* sp.
Development of Stable Isotope Dilution LC-MS/MS Assays for Selected *Fusarium* Toxins Using Synthesized Labeled Internal Standards to Follow the Fate of Mycotoxins During the Malting and Brewing Process

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*Fusarium* head blight is assigned to a devastating fungal disease that has become of increasing importance in recent years. Heavy infestation of brewing barley with *Fusarium* species can induce a severe mycotoxin contamination and can impair the solubility as well as the malting and brewing properties. This disease is caused by various *Fusarium* species producing a wide range of toxins.

Greenhouse and field trials were conducted to compare the epidemiology of *F. culmorum*, *F. graminearum*, *F. avenaceum*, *F. tricinctum*, *F. langsethiae* and *F. sporotrichioides*. Besides malting quality factors (e.g. protein solubility, germination capacity, sorting) the metabolite spectra and the *Fusarium* DNA of pure inoculated material was investigated.

For the determination of potentially occurring mycotoxins including modified metabolites (e.g. deoxynivalenol-3-glucoside, deoxynivalenol (DON), acetylated DON derivatives, HT2-toxin, T2-toxin, enniatins, beauvericin, zearalenone) two different multi-mycotoxin methods were developed and either applied for solid samples (e.g. barley grains, malt, rootlets) or liquids like wort and beer. The quantification of mycotoxins in barley, malt and beer was carried out by stable isotope dilution assays using liquid chromatography-tandem mass spectrometry. As internal standards the previously synthesized 13C- and 15N-labeled analogs (e.g. 13C2-acetylated DON, 13C4-HT2-toxin, 13C2-T2-toxin, 15N3-enniatins, 15N3-beauvericin) were used.

The fate of *Fusarium* mycotoxins during the malting and brewing process was followed by the established LC-MS/MS-multi-methods.

During malting and brewing, a difference in the amount of *Fusarium* DNA and *Fusarium* toxins was observed. Mostly fungal growth was accompanied by the production of mycotoxins during the germination stage resulting in heavily loaded malt. The significant increases of some (modified) metabolites were noticeable and might result from the detoxification process in plants.
Detection of novel metabolites of HT-2 and T-2 toxin in barley by stable isotope labelling and LC-HRMS

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HT-2 toxin (HT2) and T-2 toxin (T2) are secondary metabolites of fungi of the genus Fusarium (especially F. sporotrichioides, F. langsethiae and F. poae) and are classified as type-A trichothecene mycotoxins. Contaminated small grain cereals, as barley, constitute a health risk for humans and animals since the native mycotoxins as well as its plant biotransformation products may contribute to toxicological effects [1].

The aim of this study was to get insights into the metabolism of HT2 and T2 in barley. Therefore, we employed a recently developed metabolomics workflow [2] based on stable isotope labelling and high resolution mass spectrometry (HRMS) measurements in fast polarity switching mode. Raw full scan data were processed with MetExtract software [3,4] to exclusively extract all HT2 or T2-derived metabolites. Structure annotation was done by calculation of possible elemental formulas and further HRMS/MS measurements. As a result, glucosylated forms of the toxins, malonylglucoside and feruloyl conjugates were elucidated for the first time. To determine time courses of metabolite formation and to establish mass balances, a second experiment was performed. Relative and absolute quantification of the biotransformation products at different time points showed that HT2 glucoside is a main metabolite not only of HT2 but also of T2 toxin which in both cases reaches its maximum already 1 day after toxin treatment.

References:


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Hydrophilization of quantum dots for application in immunochemical detection of mycotoxins

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Nowadays different labels are developed for application in immunochemical assays in order to reach the highest possible sensitivity. Quantum dots (QDs), small semiconductor nanoparticles, are one of the most promising labels due to their unique spectral properties. They are characterized by a high fluorescence quantum yield, stability against photobleaching, and size-tunable absorption and emission bands. The stable photoluminescence makes QDs ideal nanoprobes for chemical, biomedical and therapeutic labeling and imaging.

The synthesis of QDs results in very hydrophobic nanoparticles that are only soluble in non-polar solvents. However, almost all immunochemical detection methods require the use of aqueous buffered solutions, so QDs need to be solubilized while keeping their optical properties. Additionally, the solubilization of the QDs makes a future conjugation to biomolecules, such as proteins, possible. Hydrophilization can be achieved by exchanging surface ligands with water-soluble bifunctional molecules such as amphiphilic polymers, peptides, acidic ligands or by encapsulation in different carrier vehicles e.g. polymeric microbeads, liposomes or silica nanoparticles.

In this work different hydrophilization strategies of CdSe/CdS QDs were compared, such as encapsulation with jeff amine-based polymer, silica and liposomes. Silica with different functionalities (e.g. epoxide, mercapto and amine groups) were incorporated which allowed to use different bio-conjugation techniques to couple them to antibodies. A multi-mycotoxin LFIA system based on the use of green, red and orange-emitted QDs with different covering was developed and is able to detect four mycotoxins (deoxynivalenol (DON), zearalenone (ZEN) and ochratoxin A (OTA) and aflatoxin B1 (AFB1)) in cereals. There is no need for any mathematical or statistical processing of the obtained results what results in a user-friendly and sensitive detection method with cut-offs according to EU legislation.

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Capacitive biosensor based evaluation of antigen-antibody interactions for the screening of mycotoxin’s conjugates

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Antibody recognition of exogenous molecules is a key feature of the immune response. The high selectivity and affinity of antibody–antigen interactions is one of the most exquisitely tuned examples of non-covalent bonds. Several experimental techniques have been developed to study the antibody affinity. ELISA-based techniques are the most widely used because of their sensitivity, simplicity, rapidity and low reagents’ consumption. The performance of ELISA tests depends on many factors, i.e. used buffers, percentage of organic solvents, pipetting errors, time controls etc. This hampers a correct interpretation of the obtained results and might hinder conclusive answers.

Capacitive based label-free biosensors offer an unique platform which does not require complicated sample pretreatment or concerns about using solvents. A typical capacitive sensor generates a signal upon target analyte binding due to changes in dielectric properties and charge displacement. Capacitance values depend on the nature of the introduced molecules and their interaction with the capacitor interface. The observed change in the capacitive signal can thus be used to quantify interactions between ligands immobilized on the metal surface and the target compound.

Different mycotoxin-protein conjugates were synthesized in-house using various linkers and conjugation strategies. The aim of the presented work was to screen these conjugates in order to identify the best performing conjugate for use in a rapid lateral-flow test. The screening was performed by immobilizing monoclonal antibodies on the sensors’ electrode surface and monitoring the binding behaviour of the various conjugates.

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Orally administered T-2 and Fumonisin B1 affects cation exchange of rabbit erythrocytes

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Pannon White weaned rabbits were fed on diets artificially complemented with 2 mg/kg diet T-2 toxin, or 10 mg/kg diet fumonisin B1 (FB1), and both toxins in a combination (2+10 mg/kg, resp.). The control was fed on a toxin free, fully identical diet. Blood was sampled after 2 and 4 weeks of administration. Body and liver weight of the T-2 group was lower after 4 weeks. After full red cell lysis in a hypotonic buffer cellular membranes were isolated with centrifugation (30000 g/10 min) and the cation transport was implemented as the breakdown of ATP in the absence and in the presence of a selective sodium pump inhibitor, ouabain. Results were interpreted as the difference between the inhibited and non-inhibited treatments, and were given as liberated inorganic phosphate (nmol Pi/mg protein/ h.). The red blood cell (RBC) total, ouabain sensitive Na+/K+ ATPase activity decreased after 4 weeks in the T-2 group, increased in the FB1 group and antagonistic effect was revealed by the T-2+FB1 group (enzyme activity identical with the control). The RBC membrane fatty acid composition was altered by both mycotoxins similarly during the entire feeding. Considering hematology, after 4 weeks T-2 alone and in combination with FB1 increased mean cell volume (MCV). Time-dependent alterations in the T-2 group were significant for MCV and the mean cell hemoglobin, both parameters increasing. The active monovalent cation transport was significantly influenced by both T-2 and FB1 as well. Most probably FB1 exerts its sodium pump activity modification via an altered ceramide metabolism (behenic acid (C22:0) proportional decrease in the RBC membrane composition), while for T-2 toxin a moderate membrane disruption and enzyme (protein) synthesis inhibition was supposed (ca. 75% decrease of the ouabain sensitive sodium pump activity).

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Intestinal toxicity of the masked mycotoxin deoxynivalenol-3-β-D-glucoside

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BACKGROUND: Deoxynivalenol (DON), the most prevalent mycotoxin in Europe, frequently co-occurs with deoxynivalenol-3-β-D-glucoside (D3G). Although considered as a detoxification product in plants, the toxicity of this metabolite in mammals is largely unknown.

OBJECTIVES: The aim of this study was to assess the intestinal toxicity of D3G in comparison to that of DON.

METHODS AND RESULTS: The toxicity of D3G and DON (0-10 μM) was studied in vitro, on Caco-2 cell line, and ex vivo on porcine jejunal explants. First, an in silico analysis revealed that D3G, contrary to DON, was unable to bind to the peptidyl transferase center of ribosomes, the main target for DON toxicity. As a consequence, D3G did not activate JNK and P38 Mitogen-activated protein kinases. Conversely to DON, D3G had no effect on cell viability and on the barrier function as measured by the trans epithelial electrical resistance (TEER). Treatment of intestinal explants for 4 hours with 10 μM DON, induced intestinal lesions and up-regulated the expression of pro-inflammatory cytokines. In contrast, D3G did not alter histomorphology and did not trigger intestinal inflammation. Microarray analysis of DON-treated explants demonstrated an effect of the toxin on various genes involved principally in the immune responses. By contrast, the expression pattern of D3G-treated explants was similar to the control explants.

CONCLUSION: Our data indicate that D3G has no toxicological relevance compared to DON at least on the gut. However the possible reconversion of D3G to the molecule could contribute to the overall toxicity of this “masked” mycotoxin.
Genotoxicity of the food contaminant deoxynivalenol is modulated by the host microbiota acquired at birth

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Deoxynivalenol is the most prevalent mycotoxin in Europe and contaminates food chain. Whether the toxic effects of DON on morphological and immune alterations in intestine were known, DON effects on DNA damage are less characterized. Among microbiota, essential component in intestinal physiology, an increased number of newborns and adults carried Escherichia coli strains producing colibactin. In animal colonized at birth with colibactin producing E. coli, transient DNA damages are observed in the intestine.

The aim of this study was to evaluate the genotoxicity of the food contaminant DON, in animals colonized at birth with or without commensal E. coli producing colibactin.

**In vitro**, cells were infected with E. coli producing Colibactin and co-exposed with increasing doses of DON. Genotoxicity was measured using in cell western method. **In vivo**, newborns’ rats were colonized at birth by commensal E. coli strain and feed with DON-contaminated diet. DNA damages were quantified in jejunal epithelial cells.

Mammalian cells exposed in vitro to E. coli strains producing Colibactin and co-exposed 24 hours with increasing doses of DON exhibit more DNA damages than cells solely exposed to genotoxic E. coli strains or DON. **In vivo**, a significant increase in DNA damages was observed in animals colonized by genotoxic E. coli strains and co-exposed to DON in a dose- and time-dependent manner.

DON is classified by IARC as “not classifiable as to its carcinogenicity to humans”. Our results indicate that the genotoxicity of DON depend on the presence of commensal genotoxic E. coli strain acquired at birth. This raises questions about the potential role of DON in carcinogenesis.
Multimycotoxin analysis of *Aspergillus clavatus*-infected feed samples implicated in two outbreaks of neuromycotoxicosis in cattle in South Africa

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*Aspergillus clavatus* poisoning is a highly fatal neuromycotoxicosis of ruminants, especially cattle, caused by the ingestion of infected sprouting grain and sorghum beer residue. Locomotory disturbances, tremors and paralysis are observed. Histologically degeneration and necrosis of larger neurons in the medulla oblongata, the midbrain, the thalamus and the ventral horns of the spinal cord are observed. Although a range of mycotoxins such as patulin, cytochalasin E and pseurotin A have been isolated there is limited information on which specific mycotoxin or group of mycotoxins are involved during outbreaks of intoxication in livestock. Two outbreaks of *A. clavatus* intoxication in cattle were investigated. Feed samples were collected for fungal identification and culture and multimycotoxin analysis. A range of fungal metabolites were detected and estimated concentrations (μg/kg) will be presented. Both the sprouting barley and brewer’s grain were predominantly infected with *A. clavatus* and to a lesser extent *Rhizopus arrhizus*. The only common *Aspergillus* secondary metabolite present in all the samples was pseurotin A. Patulin and cytochalasin E were present in the sprouting barley samples as well as the *A. clavatus* isolates cultured on malt extract agar for 2 weeks. However, both these mycotoxins could not be detected in the brewer’s grain sample.
Mycotoxins in beetles *Tenebrio molitor* grazing on *Fusarium* species

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*Fusarium* species infect a wide array of crop plants and produce numerous human-health threatening toxins. Recently, we found that the common pest of stored products, the meal beetle *Tenebrio molitor*, was preferably feeding on wheat grains colonized with *F. proliferatum*. This was surprising since *F. proliferatum* was capable of increasing beetle's mortality under some tested conditions. We draw the hypothesis that *F. proliferatum* attracts meal beetles to gain fitness benefits by getting dispersed using the beetles as vehicles.

In this study we tested the *Fusarium* species, *F. avenacum*, *F. culmorum*, *F. graminearum*, *F. poae*, *F. proliferatum* and *F. equiseti*. After feeding on *Fusarium* mycelium, live conidia were traceable in beetles' excreta and beetles were capable of contaminating a high proportion of wheat kernels. The duration of fungal dissemination by those beetles and fungal colony forming unit density in beetles excreta were recorded for up to 20 days. Fungal DNA in live beetle was quantified by qPCR at different time points after feeding on *Fusarium* spp. mycelia and conidia to monitor whether *Fusarium* species proliferate in beetles. Conidia were traceable for at least up to 20 days in beetles' excreta and also *F. proliferatum* DNA were positively detectable in live beetles collected at 1 day, 5 days, 10 days and 15 day time points. HPLC-MS was performed to detect beauvericin, fumonisins, enniatins in live beetles. Beauvericin was found in beetles fed on *F. poae*, *F. proliferatum*, *F. equiseti* or *Beauveria bassiana* mycelium. Both fumonisins and enniatins content in live beetles were under the detection limits.

Our study indicates that 1) there is no association between the fungal DNA biomass and mycotoxins production in beetles and 2) *F. proliferatum* attract meal beetles to gain fitness benefits by getting dispersed by beetles. A deeper understanding of the impact of insects on toxin-producing fungi dissemination within storage grains is essential for food security.
Ochratoxin A (OTA) was discovered in South Africa, as a toxic metabolite of *Aspergillus ochraceus* from corn meal intentionally inoculated with said microfungus (Van der Merwe, 1965). Shortly thereafter, naturally occurring OTA was isolated for the first time from a commercial corn sample in the United States (Shotwell et al., 1969). In the same year of 1969 isolated the same mycotoxin from *Penicillium verrucosum* (van Walbeek et al., 1969).

Further milestones in OTA research in years 1965 – 2015:
1. OTA toxicology – nephrotoxicity and carcinogenicity in human and animal
2. OTA and ADME (“Absorption, Distribution, Metabolism, and Excretion”)
3. The role of OTA in mycotoxicosis (e.g. Balkan Endemic Nephropathy /BEN/ and Chronic Interstitial Nephropathy /CIN/ in human and Porcine Nephropathy)
4. Biological monitoring of OTA in human blood, urine and milk
5. OTA dietary exposure and health risk assessment
6. Important dietary exposure sources of OTA (e.g. foodstuffs of plant and animal origin)
7. Important dietary exposure sources of OTA (feedstuffs)
8. Trends in OTA analytical determination
9. OTA regulations in foodstuffs


*Dedicated to the memory of all researchers, who importantly contributed to the field of OTA research into the development of those knowledges.*

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Ochratoxin A (OTA) belongs to nephrotoxic mycotoxins with carcinogenic effects. OTA is produced by \textit{Aspergillus} and \textit{Penicillium} species. Naturally occurring OTA was isolated for the first time from a commercial maize sample in USA (Shotwell et al., 1969). OTA has been found worldwide in various foodstuffs and feedstuffs of plant and animal origin. In foodstuffs of plant origin, OTA has been found, in particular, in cereal products, beer, coffee, cacao, chocolate, spices (e.g., dried red pepper, chili powder, black pepper, cayenne pepper, nutmeg, coriander, ginger, curcuma, caraway), vegetables, green, fruit and black tea, pistachios, figs, raisins, grape juice, wine, liquorice and chestnuts. Foodstuffs of animal origin, such as raw pork meat, pork blood products, kidney, poultry liver or milk, are indirectly contaminated by OTA when animals are fed with contaminated feedstuffs. However, meat products, such as raw ham muscle, cured meats, salami or dry-cured ham, may also be contaminated by OTA in a direct way. In particular, OTA is produced by the ochratoxigenic microfungi, \textit{Penicillium nordicum}, growing on products made of pork meat during their ripening. Similar situation is in hard and blue cheeses (Ostry et al., 2013). An occurrence of OTA in foodstuffs of plant origin was determined from tenths to hundreds ng/g. An occurrence of OTA in foodstuffs of animal origin was determined from tenths to units ng/g.


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Ochratoxin A: Toxigenic fungi

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Ochratoxin A was first described 50 years ago by van der Merwe, Steyn and Fourie (Nature, 1965) as a toxic secondary metabolite from Aspergillus ochraceus. In the meantime several other important ochratoxin A production species within the genus Aspergillus or Penicillium have been identified and described. Some of them are potent producers. However ochratoxin A, as a secondary metabolite, is not constitutively produced also by these producers, but its production is tightly regulated. Growth parameters like substrate composition, temperature or water activity, especially, play an important role in the regulation of ochratoxin A biosynthesis. Changes in these parameters are sensed and transmitted to the transcriptional level by signal transduction cascades which results in the regulation of the activity of ochratoxin A biosynthesis genes. This regulation is part of an adaptation to certain environments. The gene clusters responsible for ochratoxin A biosynthesis are partly elucidated in Penicillia and Aspergilli. The available data show that the ochratoxin A biosynthesis genes are completely different in both genera. This situation rather argues for an independent evolution than for a horizontal gene transfer mechanism. Because of these facts, the ecological reason for ochratoxin A biosynthesis maybe different for the two genera. An overview of the current knowledge about biological aspects of ochratoxin A biosynthesis will be given.
Ochratoxin A (OTA) is a mycotoxin produced by *Aspergillus* and *Penicillium* species. Chemically OTA consists of a dihydroisocoumarin moiety linked through its 7-carboxyl group by an amide bond to L-ß-phenylalanine. Its chemical name according to IUPAC is: N-\{(3R)-5-chloro-8-hydroxy-3-methyl-1-oxo-3,4-dihydro-1H-isochroman-7-yl\}carbonyl\}-L-phenylalanine:

Ochratoxin A is one of the most-abundant food-contaminating mycotoxin and its chemical structure was described for the first time in 1965 in a Nature-Paper by van der Merwe et al. [1].

During the last fifty years OTA has intensively been studied and the PubMed database lists almost 3000 publication with the keyword "Ochratoxin A". Most important topics are the analysis, occurrence, formation and toxicity.

This presentation will focus on the chemical and molecular properties of OTA and discuss the most important aspects including stability, isomerization, thermal degradation and analysis of OTA.

Ochratoxin A (OTA) presence in many food commodities necessitates a toxicological risk assessment as basis for risk management measures taken to protect humans and animals against adverse health effects from OTA exposure. Risks from chemicals are assessed based on hazardous properties, dose-response and exposure data. In this regard, OTA is one of the most thoroughly studied mycotoxins: Key studies on critical toxicities in animals, i.e. nephrotoxicity and carcinogenicity, provide data on dose-response relationships needed for a hazard assessment of OTA. Studies conducted in cell culture or more complex *in vivo* models have led to a better understanding of mechanisms resulting in cell dysfunction and toxicity. OTA has genotoxic activity, and cause aneugenic and clastogenic effects at sufficiently high concentrations. Yet, there is some controversy on the role of DNA-adducts and the question whether OTA genotoxicity is 'thresholded' or not, a relevant aspect in a low-dose extrapolation of carcinogenic risk for human exposure scenarios. Both, rodent tumour data showing a clear sublinear dose-response, and a recent mode-of-action analysis support the view that 'safe' levels for OTA exposure can be derived, such as tolerable intake values set by scientific committees.

There is a rich database on human OTA exposure with estimates based on food contaminant analysis and consumption patterns. Biomonitoring, as complementary approach to assess intake from all sources and routes, has been used to investigate OTA exposure in several populations and subgroups. Analysis in human blood, breast milk and urine documents OTA exposure in most parts of the world. The biomarker data often indicate exposure at low levels which do not raise concern. But, high OTA exposure was reported in regions with a high incidence of nephropathy, and implicates OTA as etiologic factor in human disease. Yet, also other nephrotoxic agents, e.g. aristolochic acid and co-exposure to citrinin, have to be considered in this context. Therefore, further experimental studies on combination effects for mixtures (at human-relevant doses) are indicated. New developments in biomarker analysis, including multi-mycotoxin methods, will improve our knowledge on human exposure patterns, and can help to identify high risk cohorts.
Overview on molecular mechanism involved in ochratoxin A nephrotoxicity and carcinogenity

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Ochratoxin A (OTA), a para-chlorophenolic mycotoxin, is widely found as a contaminant of improperly stored food. OTA is mainly noted for its nephrotoxicity and to date is the most potent renal carcinogen in rats (and in poultry), ever studied by the National Institute of cancer. OTA is implicated in the etiology of Balkan endemic nephropathy (BEN) and its associated urinary tract tumours. This presentation gives an overview on the metabolic pathways involved in the toxicity of OTA. OTA promotes oxidative DNA damage through production of reactive oxygen species (ROS) but also generates covalent DNA adducts. It has been difficult to separate the biological effects caused by DNA adduction from that of ROS generation. OTA is genotoxic following oxidative metabolism by certain cytochrome P450 or peroxidase enzymes. This activity is thought to play a central role in OTA-mediated carcinogenesis and may be divided into direct (covalent DNA adduction) and indirect (oxidative DNA damage) mechanisms of action. An indirect mechanism is supported by: OTA-dependent lipid peroxidation and free radical formation in mammalian cells; decreased of vitamin E in plasma of rats; depletion of glutathione (GSH) in liver of mice; oxidative DNA damage in vitro; and oxidative DNA damage in rodents. Evidence for a direct mode of genotoxicity has been derived from ms/ms analyses of OTA DNA-adducts. Altogether, the data show that the C5−Cl atom of OTA is critical for direct genotoxicity but plays a lesser role in OTA-mediated cytotoxicity. Like other chlorinated phenols, OTA undergoes an oxidative dechlorination process generating a quinone (OTQ)/hydroquinone (OTHQ) redox couple. The SAR studies point different mechanisms of action (MOA) for OTA genotoxicity and nephrotoxicity, and are consistent with recent findings showing OTA mutagenicity to stem from direct genotoxicity, while cytotoxicity is derived from oxidative DNA damage. OTHQ, OTB-GSH and C-C8dGOTA are relevant biomarkers.
Mycotoxins at the blood-brain barrier: Metabolism, toxicity, barrier integrity and transfer to the brain

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Fungi of the different genera produce toxic secondary metabolites. To induce adverse effects in the brain, these mycotoxins would require to penetrate or weaken the blood-brain barrier. Although neurotoxic effects have been described, a systematic study investigating the effects of mycotoxins on the blood-brain barrier is still missing.

In the present study results of an in vitro cell culture model are presented. Primary porcine brain capillary endothelial cells (PBCEC) were harvested, purified and seeded on Transwell® inserts. PBCEC form a monolayer with strong intercellular tight junctions, which inhibit the paracellular diffusion of xenobiotics into the brain tissue. Before applying the test compounds in transport studies, they were tested for their effects on cellular viability using the Cell Counting Kit-8 (CCK-8). The tightness of the barrier in vitro was ensured by transendothelial electrical resistances (TEER) of >600 Ω•cm² using a CellZcope® impedance spectrooscope. The obtained values are much higher and closer to the barrier integrity in vivo compared to commercial mammalian brain capillary endothelial cell lines. Samples from the blood and the brain compartment were withdrawn at time points up to 48 h and analyzed via LC-MS/MS and LC-HRMS.

Fumonisins (FB₁, HFB₁), moniliformin (MON), trichothecenes (DON, 3-Ac-DON), zearalenon (ZEN, α-zearalenol (α-ZEL), ochratoxin A (OTA), ochratoxin alpha (OTα), citrinin (CIT) and dihydrocitrinone (DHCIT) were chosen as test compounds to cover a wide range of polarity and molecular size.

The data show that DON, 3-Ac-DON, FB₁ and OTA disturb the blood-brain barrier integrity by effecting cellular viability and TEER. Furthermore phase I and II metabolites of ZEN, α-ZEL were detected and quantified. The results indicate that HFB₁, ZEN metabolites, OTA, OTα, CIT and DHCIT might be substrates of efflux transporters at the blood-brain barrier.
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Effect of plastic mulches on mycobiome diversity and mycotoxin occurrence in soil

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Plastic mulching (PM) is a widely used agricultural management, enhancing soil temperature and improving water conservation, factors that influence the microbiological compartment with unknown effects on mycobiome and mycotoxin biosynthesis. The aim of this study was to assess the effect of PM on mycobiome diversity and mycotoxin occurrence in soil in example of asparagus crops.

Soil samples from white (PM) and green asparagus (bare soil), cultivated in a ridge-furrow system, were obtained from three different depths. The occurrence of five mycotoxins (Deoxynivalenol [DON], T-2, Zearalenone [ZEN], Ochratoxin A and Fumonisin B1) was investigated using LC-HRMS. Total colony-forming unit was used as indicator of mycobiome biomass. Identification of soil-borne filamentous fungi was done by DNA sequencing and characterization of mycotoxigenic capacity via HPTLC-Fl. Soil samples were characterized based on elemental analyses, water content, pH and cation exchange capacity. Soil temperatures and environmental conditions were also recorded.

A significant effect of plastic covers on the investigated soil physicochemical properties was not observed. Differences were observed in relation to soil temperature and water content lengthwise, with values <10% at the topsoil. A higher fungal compartment was observed in covered soil, which can be explained by the effect of PM on soil temperature. The mycotoxins DON and ZEN were found in soil samples with a higher prevalence of DON in covered soils (especially at the topsoil) compared to non-covered soils. Moreover, a high dominance of pathogenic and mycotoxin producing fungal species in the topsoil was observed in both crops, accompanied by the production of the mycotoxin as a possible adaptation response of the producing fungi against environmental conditions at the top soil. Results of this study confirm the effect of plastic covers on soil temperature with clear effects on mycobiome diversity and mycotoxin biosynthesis in soil.

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The fungal genus *Alternaria* contains numerous species that can contaminate a wide variety of crops in the field and cause post-harvest decay, leading to economic losses. Also, these species are able to produce mycotoxins with toxic properties. Based on the recommendations listed in EFSA’s scientific opinion on *Alternaria* toxins (2011), this research’s objective was to a) develop analytical methods for the simultaneous determination of free and modified *Alternaria* toxins in various matrices, b) gather quantitative occurrence data in foodstuffs available on the Belgian market, and c) investigate the effect of food processing on the *Alternaria* toxin levels. Multiple fast and sensitive UPLC-MS/MS methods for the determination of free (AOH, AME, ALT, TeA, TEN, ATX I) and modified (AOH3S, AME3S, AOH3G, AME3G) *Alternaria* toxins in cereal products, various types of beers, fruit and vegetable juices, tomato products, lentils, vegetable oils and oilseeds were developed and validated (in agreement with Regulation N° 401/2006/EC; Commission Decision N° 2002/657/EC). Subsequently, 300 samples of a variety of commercially available foodstuffs were analyzed between Feb/13 and Feb/15. High prevalence of AOH (<LOQ–41.6 μg/kg), AME (<LOQ–6.1 μg/kg) and TeA (7.7–330.6 μg/kg) was observed in tomato products. Moreover, higher concentrations of AOH were found in sesame seeds (<LOQ–98.6 μg/kg), whereas AME was frequently detected in both sesame seeds (<LOQ–155.7 μg/kg) and sesame oil (10.2–64.4 μg/kg). For the first time, the occurrence of modified *Alternaria* toxins was demonstrated in tomato products and sesame seeds. Additionally, dietary exposure within a specific population group (vegetarians) will be assessed through food frequency questionnaires, and combined with the occurrence data to carry out a probabilistic exposure assessment. Finally, stability of (modified) *Alternaria* toxins was confirmed during the beer production chain and the production of tomato based foodstuffs.

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Occurrence of ergot alkaloids and their epimers in feed in the Czech Republic

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Ergot alkaloids are mycotoxins produced predominantly by fungi of the *Claviceps* genus, which mainly affect cereals and wild grasses. Ingestion of ergot alkaloids can cause severe health problems in humans and animals. Thus, there is a need for methods that allow determination of these dangerous toxins.

A method based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been developed for the simultaneous determination ergot alkaloids which are included in Commission recommendation 2012/154/EU. Mean recovery, precision, matrix effects and limits of quantification (LOQ) were assessed for cereal and grass matrices within the method validation. Sample preparation based on modified QuEChERS approach was employed.

The presented method was used to inspect various feed samples. Ergot alkaloids were found mainly in cereal based products.
Safety of mycotoxin binders used in broiler feed: interactions with the oral absorption of veterinary drugs and coccidiostats

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Mixing mycotoxin binders in animal feed is nowadays the most commonly used method to prevent damage caused by mycotoxins. Mycotoxin binders aim to adsorb the toxin to their surface in the gastro-intestinal tract of the animal, thereby preventing the systemic uptake of the mycotoxin. The adsorption mechanisms are deemed to be non-specific and in 2010 EFSA stated that their safety, in terms of non-specific binding, should be investigated. The aim of this project is to investigate possible interactions of mycotoxin binders with the oral absorption of frequently used veterinary drugs and coccidiostats in broiler chickens.

First, the in vitro adsorption of sulfadiazine (SDZ), trimethoprim (TRIM), tylosin (TYL), doxycycline (DOX)\textsuperscript{1}, diclazuril (DICL), lasalocid (LAS) and salinomycin (SAL) to 6 different mycotoxin binders and active carbon was assessed in a single-concentration adsorption screening assay. Next, two in vivo experiments were executed in broiler chickens with 2 selected clay-based binders and active carbon. Pharmacokinetic profiles were constructed for SDZ+TRIM and DOX\textsuperscript{1} in broilers that received a single oral bolus containing the daily dose of either drug, or the combination of binder and drug. A third experiment assessed the oral absorption of TYL, DOX, SAL and DICL after a 2-week exposure period to the binder.

Several binder-drug combinations showed over 90% in vitro adsorption for TRIM, DOX, DICL, LAS or SAL. Results of the first two in vivo experiments demonstrated a significant interaction of 2 clay-based binders and DOX\textsuperscript{1}, since these binders were able to lower the oral bioavailability of DOX to less than 40% compared to broilers receiving only DOX. No interactions of these binders with SDZ + TRIM were seen. Results of the third in vivo experiment will be presented on the workshop.

\textsuperscript{1} De Mil T. et al., In vitro adsorption and in vivo pharmacokinetic interaction between doxycycline and frequently used mycotoxin binders in broiler chickens. 2015, J. Agr. Food Chem.

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Plasma based mycotoxin degradation

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The control of mycotoxins is a challenging task. Lots of different chemical, biological and physical approaches have been made throughout the years. The main problems occurring when treating plants, food and feedstuff are not only diminishing nutritional values and negative effects on their palatability. Chemical methods often require suitable facilities and relatively long treatment durations of the products due to e.g. additional drying and washing steps. Biological methods are frequently working with systemic effects, such as the depression of incorporation of the unwanted toxins in living tissue due to enzymatic binding of the toxins in the gastrointestinal tract. A novel and gentle treatment method based on physical principles is the use of a gas discharge in order to decontaminate foods and feedstuff. We present the effectiveness of a cold atmospheric pressure air plasma (APAP) for the general degradation of mycotoxins. We treated surfaces contaminated with different individual Fusaria and Aspergilli mycotoxins, such as Fumonisin B1, Enniatin A, Sterigmatocystin etc. The results indicated a significant degradation of most toxins. Subsequently, the degradative effects of the plasma were also tested on mixtures of extracts containing these mycotoxins which were produced by fungal species in liquid media. Beside the dependency of the process on the electrical power dissipated into the discharge we present the dose dependent decay of the mycotoxins.

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Impact of phase I metabolism on cellular uptake, oxidative stress and genotoxicity of alternariol and its monomethyl ether

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Alternariol (AOH) and its methyl ether (AME), mycotoxins formed by \textit{Alternaria alternata}, have been shown previously to act as topoisomerase poisons in mammalian cells, thus mediating DNA damaging properties. Recently, both mycotoxins were found to represent substrates for cytochrome P450 monooxygenases, forming catecholic metabolites. So far the impact of these metabolites on the DNA damaging properties has not fully been elucidated yet. In the present study we investigated the toxicity of the potential phase-I metabolites 4-hydroxy alternariol (4-OH-AOH) and 4-hydroxy alternariol monomethyl ether (4-OH-AME) in comparison to the parent toxins. Under cell-free conditions, both metabolites were found to exceed the inhibitory properties of the parent compounds with respect to the inhibition of topoisomerase II activity. However, in cell culture (KYSE510, human oesophageal cells) both metabolites exhibited a reduced potency to stabilize the covalent topoisomerase-DNA-intermediate compared to AOH and AME. Thus, hydroxylation resulted in a decrease of cellular topoisomerase poisoning. In accordance with these results, 4-OH-AOH and 4-OH-AME exhibited lower DNA-damaging properties in the comet assay in comparison to the parent mycotoxins. Furthermore it has to be pointed out that the formation of catecholic structures did not result in an increase of oxidative DNA-damage, analysed by the use of formamidopyrimidine-DNA-glycosylase in the comet assay. Analysis of cellular uptake by LC-MS demonstrated substantially lower cellular concentrations of the hydroxylated metabolites compared to AOH and AME, together with the formation of further methylated metabolites at least in the case of 4-OH-AOH. Thus, despite enhanced inhibitory properties of the metabolites under cell-free conditions, substantially decreased cellular levels support the conclusion that hydroxylation in position C4 of AOH or AME is associated with detoxification at least with respect to genotoxicity.
Toxicokinetics of T-2 toxin and T-2-glucoside and in vivo hydrolysis of T-2-glucoside in broiler chickens

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T-2 toxin (T-2) is regarded as the most acute cytotoxic mycotoxin within the Fusarium trichothecene mycotoxin family. As it is mainly detected in cereals, animals on a cereal based diet, such as broiler chickens, are highly exposed to this toxin. Additionally to the free mycotoxin, modified glycosylated forms of the mycotoxin may contribute to the degree of contamination as they might be hydrolyzed to the native mycotoxin upon oral ingestion. For T-2, the main modified form is T2-glucoside (T2-G). Although only limited occurrence data are available for T2-G, incidences of over 70% have been reported in cereals.

In order to assess whether T2-G adds to the toxicity of T-2, a toxicokinetic study of T-2 and T2-G was performed in broiler chickens. A two-way cross-over trial was set up with an orally (PO) and intravenously (IV) administered bolus of T-2, at a dose of 0.74 mg/kg BW, in 6 broilers. Afterwards, three of those birds were administered an equimolar dose of T2-G at 1 mg/kg BW, both PO and IV. Blood was collected up to two hours post administration and analysed by a validated LC-MS/MS method.

After IV or PO administration of T2-G, only trace amounts of T-2 could be detected (< 1 ng/mL). These results demonstrate that the in vivo hydrolysis of T2-G to T-2 is negligible.

Plasma concentration-time profiles of T-2 and T2-G after IV administration were analysed by means of a two-compartmental model. The absolute oral bioavailability of T-2 and T2-G, a measure of systemic exposure to the toxin, was 2% and 9%, respectively. Following toxicokinetic parameters were calculated and will be presented at the conference: volumes of distribution, total body clearance, distribution and elimination rate constants and half-lifes.

The compared to T-2 high oral bioavailability and negligible in vivo hydrolysis of T2-G to T-2 highlights the need to investigate the (cyto)toxicity of this modified mycotoxin in order to perform an appropriate risk assessment.

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Unravelling the role of ruminal development in the biotransformation of deoxynivalenol and its acetylated derivatives: a comparative toxicokinetic approach

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Recently, a survey by Antonissen et al. (2014) demonstrated the presence of several mycotoxins in veal feed samples, especially in roughage and concentrate feed. Deoxynivalenol (DON) was most prevalent, contaminating 80% of the roughage samples and all of the 15 concentrate samples. Fibrous feed compounds such as roughage and cereal-based low-iron concentrates have been added to the diet to ensure a better ruminal development and improved animal welfare (Brscic et al., 2010). It is suggested that an optimal rumen functioning is responsible for detoxification of several Fusarium mycotoxins, including DON, making ruminants less sensitive to these toxins (Fink-Gremmels, 2008). However, in veal calves on a classic milk replacer diet, ruminal development is absent, potentially making them more sensitive to mycotoxins.

The aim of this study was to investigate the impact of the ruminal development on the biotransformation of DON and its acetylated derivatives (3- and 15-acetyl-DON, 3- and 15-ADON) in calves. A comparative toxicokinetic study was performed in two ruminating and two non-ruminating male calves. Each animal received respectively a bolus of DON (120 μg/kg bodyweight (BW)), 3-ADON (25 μg/kg BW), and 15-ADON (50 μg/kg BW) by intravenous (IV) injection and per os (PO), for each toxin in a cross-over design respecting a wash-out period of 96h. Concentrations were based on average feed intake and maximum contamination levels of the feed as described in the survey. Following mycotoxin bolus administration, blood and urine was collected at different time points post administration. An LC-MS/MS method was developed to quantitate DON, 3-ADON, 15-ADON as well their metabolites, namely de-epoxy-DON, DON-3-glucuronide, DON-3-sulfate, DON-15-sulfate, 3-ADON-15-sulfate, 15-ADON-3-sulfate and DON-3,15-di-sulfate, in plasma and urine samples. Results of the toxicokinetic analyses will be presented at the conference.

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(Deepoxy)-deoxynivalenol sulfonates as major deoxynivalenol metabolites in rats: The missing link in ADME studies

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The *Fusarium* mycotoxin deoxynivalenol (DON) is a major fungal contaminant of food and feed. Its ADME has frequently been investigated in rats, whereupon widely different biological recoveries were obtained. In studies with the native toxin, only about 30% of the administered dose were typically recovered. However, biological recoveries were close to 90% in studies using radio-labelled DON.

Only in 2013, one DON sulfonate and one de-epoxy-DON (DOM) sulfonate were reported to be major natural DON metabolites in excrements of DON treated rats and tentative structures were proposed. Yet, in the same year, three DON sulfonates of different structure and stability were discovered as post-harvest treatment products of DON with inorganic sulfur reagents. Therefore, we assumed formation of several different DON- and DOM sulfonates as DON metabolites in rats. In order to verify this hypothesis, we analyzed urine and faeces samples of a previous rat trial where DON and DON-3-glucoside (D3G) had been administered by gavage and less than 30% of the parent toxins had been recovered.

By chemical synthesis, preparative isolation and NMR analysis of the expected compounds, several DON-, DOM- and D3G sulfonates could be identified and structures proposed in the literature could be disproved. LC-MS/MS based methods for determination of the novel DON and DOM (conjugate) sulfonates and their parent compounds DON, DOM and D3G in faeces and urine of rats were developed and validated. Application of the biomarker methods to excrements of 6 rats administered 2.0 mg/kg bw DON or 3.1 mg/kg bw D3G yielded a biological recovery of 75 ± 9% for the DON group and 68 ± 8% for the D3G group. DON-, DOM- and D3G sulfonates excreted in faeces accounted for 48% and 47% of the total amount of administered DON and D3G, whereas urinary excretion of sulfonates was < 1%. Hence, DON-, DOM- and D3G sulfonates were the missing link to reasonable biological recoveries in ADME studies with DON or D3G treated rats.

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In 2013 in Germany aflatoxin M1 concentrations above the maximum level have been detected in milk. After identification of contaminated corn from Serbia as source the further use of this maize in Germany has been interdicted. About 100,000 tons of contaminated corn had to be stored out. Such tasks are associated with high airborne dust concentrations. In the present case the exposure to airborne dust may go along with an inhalative intake of airborne mycotoxins and thus may represent an additional health risk for the workers.

During the outstoring of the corn, the exposure to airborne dust and mycotoxins has been measured using a high volume airsampler. Samples have been taken in one of the storage halls during the transshipment with a wheel loader on two days. The samples have been analysed with a multimycotoxin screening method using HPLC-MS/MS and with a standardised method using HPLC-FLD with post-column derivatisation and a modified immunofinity column cleanup for aflatoxins. Additional samples for airborne dust have been taken at further workplaces along the transshipment.

The airborne dust concentrations in the storage hall exceeded the occupational limit concentration by a factor of 12 to 15. However, dust concentrations in the cabin of the wheel loader and at the other work places have been well below the occupational limit. In the airborne dust samples 22 mycotoxins have been detected in concentrations of up to 18 mg/kg for fumonisin B1.

Based on the airborne mycotoxin concentrations the inhalative intake has been calculated. The risk assessment is hampered by a lack of safety limit values for this route of exposure. Due to this limitation an alternative approach for risk assessment of airborne mycotoxin exposure will be presented.
Occupational exposure to aflatoxin B1 in one waste sorting plant

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In the management of solid waste fungi and their metabolites are the risks more reported in the literature, however there still a lack of information regarding occupational exposure to fungi and mycotoxins.

This study intends to assess occupational exposure to aflatoxin B1 and know if there is a relation with the contamination by the A. flavus complex.

Occupational exposure assessment to AFB1 was done with a biomarker of internal dose that measures AFB1 in the serum. The quantification was done with RIDASCREEN AFB1 30/15 enzyme-linked immunosorbent assay (ELISA; R Biopharm) and it was calibrated with aflatoxin standards from 1 to 50 ng.ml\(^{-1}\). Values below 1 ng.ml\(^{-1}\) were considered non detectable since these are below the detection limit. Twenty six workers from the waste company were enrolled in this study. A control group (n = 30) was also considered in order to know the AFB1 background levels for the Portuguese population.

Fungal assessment was achieved by conventional and molecular methods. Air samples (50 L) from different working locations through impaction method onto malt extract agar (MEA) supplemented with chloramphenicol (0.05%), with a flow rate of 140 L/min. Surfaces' samples were collected by swabbing the surfaces of the same indoor sites. To obtain molecular identification by qPCR of toxigenic strains from A. flavus complex, air samples (250L) were collected using the Coriolis \(\mu\) air sampler at 300 L/min airflow rate.

All the workers showed detectable levels of AFB1 with values ranging from 4.7 to 25.9 ng. ml\(^{-1}\) and with a median value of 10.1 ± 5.7 ng.ml\(^{-1}\). In the control group, the AFB1 values were all below 1 ng.ml\(^{-1}\). Significantly higher concentrations of mycotoxin were found in waste workers compared to controls (U = 0.000, P < 0.0001).

Eight different species of filamentous fungi were identified being the species from A. flavus complex one from the three most found in air (8.7%) and surfaces (13.8%). However, toxigenic strains from this complex were not amplified by qPCR.

The results obtained suggest that exposure to AFB1 occurs and may be related with the continuous fungal contamination that occurs during all the waste management chain resulting in a continuous waste mycotoxin contamination.

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Mycotoxins and indoor environments: Mycotoxin production during the development of toxigenic fungal species on materials used in indoor furnishings

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In industrialized countries, people spend 80-95% of their time inside buildings. Many physical, chemical or microbiological agents can induce disorders in occupants such as allergies, infections, poisoning. Among the most frequent microbiological pollutants, some moulds are capable of growing on construction and decoration materials when appropriate environmental conditions are present (mainly humidity).

Some of the species commonly observed in indoor environments are also able to produce mycotoxins.

If the health effects caused by the ingestion of the main mycotoxins are well described and allowed establishment of regulations in foods, the possible effects related to inhalation or contact with contaminated materials are very poorly documented.

We characterized the production of mycotoxins by 3 fungal contaminants of indoor environments (Aspergillus versicolor, Penicillium brevicompactum and Stachybotrys chartarum), during their development on 5 materials: wallpaper, fiberglass wallpaper, vinyl wallpaper, fir, fiberglass and fiberglass.

The wallpaper appeared to be a very good support for both development and sporulation of all species; as well as for toxinogenesis of Aspergillus versicolor and Stachybotrys chartarum. By contrast, Penicillium brevicompactum preferred fiberglass. Conversely, the vinyl wallpaper was an unfavorable support for development and subsequently for toxin production of all tested strains.

Contamination of the supports with the different mycotoxins could reach several mg of toxin per m² of contaminated material.

As an example, one square meter of wallpaper contaminated by Stachybotrys chartarum contained an average of 29 mg of macrocyclic trichothecenes and around 112 mg of sterigmatocystin after development of Aspergillus versicolor.

Although there is no clear data on the dose-effect relationship for these toxins, it is likely that such a level of contamination could have an impact on health if toxins are aerosolized and inhaled.

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Mycotoxins-producing ability of indoor airborne fungi isolated from librarian environment affected by mould contamination

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In the last years, the manage of increasing fungal contaminations inside libraries and archives due to inadequate conservations, such as malfunction of air conditioning systems or lack of cleanliness, has become a complex and expensive problem. Although indoor environments are climate controlled (18–20 °C, 50–60% relative humidity), some fungal species are still able to grow on materials, preferentially in air-stagnation microenvironments.

Regardless the biodeterioration phenomena, these fungi can exert potential impact on human health of workers and students. In indoor environment, the high presence of microorganisms in the air, in the dust or at least as mould contamination on building materials as well as on Cultural Heritage, can be the cause of the well known problem called Sick Building Syndrome (SBS).

The contaminated environment of a Ca’ Foscari University of Venice library (Italy) was used as a case study. Stored in Compactus® shelves, more than 27,000 books were affected by a widespread spotted fungal contamination. The major fungal species isolated from both air and contaminated book samples were: Aspergillus creber, A. protuberus, Penicillium chrysogenum, P. brevicompactum, Cladosporium cladosporioides, A. penicillioides, Eurotium chevalieri, E. halophilicum and A. jensenii. These were selected for the multi-mycotoxin analyses with LC-MS/MS in order to determine their mycotoxin-production ability. Several metabolites partly of toxicological relevance were identified, especially for fungi belonging to the revised group Aspergillus section Versicolores, i.e. sterigmatocystin, methoxysterigmatocystin and related precursors and side metabolites from the biosynthetic pathway. Moreover, specific investigation has concerned the not well-known fungus E. halophilicum as emerging contaminant implicated in book contaminations in specific indoor niches.
Abstract of posters
Immunochemical screening for several mycotoxins in beer

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Beer is the most consumed alcoholic beverage in the EU. In 2013, the average per capita consumption accounted for 71 litres; in some countries it was over 100 litres. Therefore, the presence of mycotoxins in beer potentially is a significant source of intake. Research in this field has mainly been focused on the Fusarium mycotoxins deoxynivalenol (DON) and zearalenone (ZEA), which are known to frequently contaminate brewing grains. Relatively few information is available about the occurrence of other groups of mycotoxins in beer such as ergot alkaloids or Alternaria toxins. Although no regulatory levels in food have been set for these toxins so far, they are frequently detected in grains and products thereof. We therefore performed a preliminary survey of these toxins in beer (n=44) using enzyme immunoassays for ergonovine (syn. ergometrine) and alternariol (AOH) as rapid screening methods. DON and ZEA analysis by EIA was also included in this survey.

Beer samples were degassed and either analyzed directly after dilution in EIA buffer solution (ergonovine, AOH, ZEA) or extracted by liquid-liquid partitioning with ethyl acetate (DON). Cloudy beers were additionally filtered before analysis. EIA detection limits in beer were in the range of pg/ml to low ng/ml. Positive results were obtained in all test systems, in most cases at very low concentration levels close to the detection limit. The highest contamination levels were detected for DON with a maximum concentration of 20 ng/ml in wheat beer. Although beer was not found to be a major source of intake of ergonovine or AOH, the presence of these toxins in raw materials used for brewing should be further studied.
Development of an enzyme immunoassay for the tremorgenic mycotoxin paxilline

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The indole-diterpenoid paxilline (PAX) is a tremorgenic mycotoxin produced by fungal species from several genera, for example *Penicillium paxilli*, *Claviceps paspali*, and *Neotyphodium lolii*. PAX and its reduced form paxitriol serve as intermediates in the biosynthesis of many other tremorgenic mycotoxins (e.g. penitrems, lolitrems). These compounds cause neurological disorders in mammals, for example the "staggers syndrome" in ruminants. Although tremorgenic mycotoxins have been found in pasture and in foodstuffs in Europe, data on the occurrence in food and feed are scarce.

The present study describes a new immunochemical method for the detection of PAX in food and feed in order to establish a fast, simple and sensitive analytical device. PAX was converted to its carboxymethyl oxime (PAX-CMO) and then conjugated to keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA) using an activated ester method. Rabbits were immunized with the PAX-CMO-KLH conjugate. Antibody titers were screened in an indirect test format by using the PAX-CMO-BSA conjugate as a solid phase antigen. First results indicate a strong immune response and the successful production of specific antibodies against PAX. Further studies on test sensitivity and specificity will be presented in this contribution.

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**Mycotoxin cocktail in early maturing cotton oilseed cake varieties associated with cattle feeding problems**

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Cottonseed cake in Asia has been associated with health issues in ruminants in the recent years. Five cases of toxicity in cattle were alleged to be due to the use of early maturing cottonseed cake. Four to five months after post-production storage feeding problems arose. All cotton cake samples showed high bacterial and fungal contamination with more than a dozen different fungus genera. Screening for mycotoxins revealed toxic levels of mycotoxins including aflatoxins, ochratoxins, rubrofusarin, cyclopiazonic acid, equisetin, tenuazonic acid, nitropropionic acid, and citrinin. In a diagnostic feed trial comparing high versus low contaminated cotton seed cakes two buffalo calves of the control group showed signs of complex toxicity. All calves lost body weight during the three weeks of feeding the contaminated cotton cake concentrates and recovered the next ten months.

The results indicate that low agricultural practice may result in low quality of processed and stored cotton seed cake and such concentrates could be of negative health impact on ruminants in Pakistani regions.

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The role of the National Focal Point and EFSA in the area of food contaminants

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Under the Regulation (EC) No 178/2002 of the European Parliament and of the Council laying down the general principles and requirements of food law and procedures in matters of food safety, the European Food Safety Authority was established. The European Food Safety Authority (EFSA) is the keystone of the European Union (EU) in risk assessment pertaining food and feed safety. In close collaboration with national authorities and in open consultation with its stakeholders, EFSA provides independent scientific advice and clear communication on existing and emerging risks at the Community level. The role of EFSA’s Scientific Panels is to deliver scientific advice for Europe’s decision-makers in the areas of food and feed safety, nutrition, animal health and welfare, plant protection and plant health. The Panel on Contaminants in the Food Chain (CONTAM) provides an independent scientific advice on contaminants in the food chain and undesirable substances for instance natural toxicants, mycotoxins and residues of unauthorised substances. In case of mycotoxins, Panel CONTAM provides scientific advice and risk assessments on mycotoxins for EU risk managers to help them assess the need for regulatory measures as regards the safety of mycotoxin-contaminated food and feed. It performs its work either in response to requests for scientific advice from risk managers or on its own initiative. At the national level in the Slovak Republic the role of Focal Point is represented by the National Focal Point for Scientific and Technical matters for EFSA (NFP) which is situated at the Department of Food Safety and Nutrition at the Ministry of Agriculture and Rural Development of the Slovak Republic (MPRV SR). The main role of the NFP is to coordinate scientific risk assessment and risk communication at a national level as well as the exchange of information and the coordination of the cooperation of scientists in the area of risk assessment.
Current situation of ochratoxin A in Chile

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A very low incidence of mycotoxins has been reported in Chile. The first OTA alert was reported in 2004, when OTA in plasma from two agricultural zones showed a range of 0.07 – 2.75 μg kg⁻¹. During 2006, a median of 0.15 μg kg⁻¹ (n = 91) was found in flour collected from the local market. In addition, rice and starch from corn presented < LOQ levels with more than 50 % of the samples with negative OTA, and just one sample resulted higher than the European Community limit (EC 472/2002) with 12.5 μg kg⁻¹. Another study of liver, kidney and pork meat (n = 60) showed an average of < 1.0 μg kg⁻¹, with an upper level of 5.0 in liver, 1.5 in meat, and 1.2 μg kg⁻¹ in kidney.

In 2009, OTA was detected in all human milk samples from lactating women (n = 9) soon after delivery with an average of 106 ng L⁻¹, and levels of OTα were 40 ng L⁻¹, but increased considerably upon enzymatic hydrolysis with β-glucuronidase/sulfatase (up to 840 ng L⁻¹) in human milk, considered an indicative of an efficient lactational transfer of OTA. This showed that the exposure of infants to OTA exceeded the tolerable daily intake (TDI) of 5 ng kg⁻¹ body-weight/day proposed by the Nordic Expert Group (Denmark).

In 2010, a study of instant and roasted coffee (n = 63) from supermarkets found concentrations between 0.16 – 7.25 μg kg⁻¹, which were below the limit proposed by FAO/WHO (12 μg kg⁻¹ b-w/day) considering that the calculated daily intake was 0.22 ng kg⁻¹ b-w/day. Between 2007 and 2009, Chilean wines (73% red and 27% white) for importation and internal consumption were analysed by OIV method showing that only a 2.7 % (n = 1188) of the samples were positive being 0.35 μg kg⁻¹ the highest level. These results show that Chile has a privileged situation with the lowest incidence of OTA worldwide.

The results indicate that exist evidence of OTA in some sectors in contact with the Chilean population. Bigger number of data is urgently required, and other food commodities must be studied.
Development of an LC-MS/MS method for the quantitative determination of deoxynivalenol, 3- and 15-acetyl-deoxynivalenol and their in-vivo metabolites in calf plasma

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A selective and sensitive analytical method based on ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) has been developed for the quantitative determination of deoxynivalenol (DON), 3-acetyl-deoxynivalenol (3-ADON), 15-acetyl-deoxynivalenol (15-ADON), deepoxy-deoxynivalenol (DOM-1), DON-3-glucuronide (DON-3-Glc), DON-3-sulfate (DON-3-S), DON-15-sulfate (DON-15-S), 3-ADON-15-sulfate (3-ADON-15-S), 15-ADON-3-sulfate (15-ADON-3-S) and DON-3,15-di-sulfate (DON-3,15-DS) in calf plasma. 13C15-Deoxynivalenol was used as internal standard.

Sample preparation consisted of a deproteinization and phospholipid removal step using an OasisÒ OstroTM 96-well plate. The UPLC-MS/MS system consisted of an Acquity UPLC H-Class Quaternary Solvent Manager and Flow-Through-Needle Sample Manager in combination with a Xevo TQ-SÒ mass spectrometer, equipped with an electrospray ionization probe (Waters, Zellik, Belgium). To optimize the chromatographic separation between the isobaric compounds (3- and 15-ADON; DON-3-S and DON-15-S, 3-ADON-15S and 15-ADON-3-S), different types of stationary phases, mobile phase compositions and gradient elution programs were evaluated. The MS/MS instrument was operated in the selected reaction monitoring mode using for each analyte two precursor ion > product ion transitions.

The method was validated for all analytes of interest (linearity, accuracy and precision, limit of quantification, limit of detection, specificity, stability) and the results fell within the ranges specified.1

The applicability of the method was shown by the analysis of plasma samples that were obtained during a comparative toxicokinetic study with DON (120 μg/kg bodyweight (BW)), 3-ADON (25 μg/kg BW), and 15-ADON (50 μg/kg BW) after intravenous injection and oral administration to ruminating and non-ruminating calves. Results of the method optimization, validation and applicability will be presented.

Acknowledgments

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Mycotoxins are substances naturally produced by several *Fusarium* species normally present as some forms of defence for the organism. These mycotoxins are capable of causing short and long term disease even and death in humans and animals. Because of their pharmacological activity, some mycotoxins or mycotoxin derivatives have found to be used as antibiotics, growth promotants, and other kinds of drugs; still others have been intend to be developed and used implicated as chemical warfare agents.

The European Commission regulates limits of fumonisin in corn and corn-based foods for a total content of FB1 and FB2 in the range of 200–4000 μg/kg depending on the intended use. The U.S. Food and Drug Administration specifies significantly higher limits of all three total fumonisins (FB1+FB2+FB3): a limit of 2000–4000 μg/kg in human food and 5000–100000 μg/kg in animal feed. Fumonisins have been implicated as a possible cause of human esophageal cancer, equine leukoencephalomalacia, – a serious disease in horses, and porcine edema – a disease in swine. Poultry and cattle are not especially susceptible to fumonisins.

The fumonisins formed organometallic compound with trace metal ions on the sample's flow path. In order to eliminate carryover of fumonisins a wash solvent mixture were examined. The carboxyl groups of fumonisins may preferentially pair with hydrogen ions in the presence of low pH. To wash the fumonisins from the metal, the inner surface of the injection needle was rinsed with 1% formic acid in methanol/acetonitrile/isopropanol. To test the solvent mixture, standard was injected, followed by one blank injection. To check the carryover of fumonisin from complementary and complete feed was determined both in case of using and skipping the wash solvent method. As a result the carryover of fumonisin could be achieved on 100% by finding using the best wash solvent mixture.
Development of an LC-MS/MS method for the simultaneous determination of beauvericin, enniatins (A, A1, B, B1) and cereulide

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Beauvericin and the related enniatins are secondary metabolites produced by different Fusarium species. These fungal metabolites are known as mycotoxins. The emetic toxin cereulide is produced by the bacterium Bacillus cereus. Although these toxins have a different origin, the striking structural and functional similarities should allow a combined detection in food matrices. Since these fungal and bacterial toxins are cyclodepsipeptides with ionophoric properties, the toxicity is related to their ability to form membrane carriers for cations resulting in disturbances in the physiological cation level in the cell. In the area of food safety, the determination of the occurrence of these medium-sized cyclodepsipeptides in food and feed is imperative.

A method was developed and validated for the simultaneous determination of beauvericin, the related enniatins and cereulide in cereal and cereal-based food matrices such as wheat, maize, rice and pasta. The analytical method of choice for combined detection was ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS). A Waters Acquity UPLC system coupled to a Waters Quattro Premier XETM Mass Spectrometer operating in ESI+ mode was used. The mobile phase consisted of variable mixtures of water and methanol/acetonitrile (20/80, v/v), 0.3 % formic acid and 1 mM ammonium acetate. Gradient elution was chosen to allow separation of the different toxins within a time frame of 7 min. For the first time beauvericin (and the related enniatins) were analyzed together with cereulide. Sample treatment involved liquid extraction of analytes using organic solvents without any further clean-up step. The method validation included the determination of selectivity, repeatability, limit of detection, limit of quantification, recovery and linearity. For all compounds the mean recovery varied between 85% and 105 % in the different matrices. The developed method proved to be sensitive and repeatable.

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Quantitative determination of tenuazonic acid in broiler chicken plasma using a validated liquid chromatography-tandem mass spectrometry method

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Alternaria mycotoxins, such as tenuazonic acid (TeA), are frequently detected in feed and food samples. Notwithstanding its high prevalence, little is known about the possible effects of TeA on animal or human health. For a better understanding of these effects and thus for a better risk assessment, elucidating the toxicokinetic characteristics of TeA is crucial. Therefore, the aim was to develop a sensitive and specific liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the quantitative determination of TeA in broiler chicken plasma.

Plasma sample preparation consisted of a protein precipitation method using acetonitrile, followed by evaporation of the supernatant. The dry residue was dissolved in 5 mM ammonium formate in water (pH 9)/methanol (95/5, v/v). Chromatographic separation was achieved on a double end-capped Zorbax® Eclipse Plus column. Mobile phases A and B were run in a gradient elution program and consisted of 5 mM ammonium formate in water (pH 9) (A) and methanol (B). The LC effluent was interfaced to a TSQ® Quantum Ultra triple quadrupole mass spectrometer operating in the negative h-ESI mode. Acquisition was performed in the selected reaction monitoring mode.

The LC-MS/MS method was successfully in-house validated according to the protocol described by De Baere et al. (1). Linearity (correlation coefficient (r) and goodness-of-fit (g)), within- and between run accuracy and precision, limit of detection (LOD), limit of quantification (LOQ), specificity, carry over, extraction recovery (RE) and signal suppression or enhancement (SSE) were evaluated. The results for r, g, within- and between-run accuracy and precision fell within the acceptance ranges. The LOQ and LOD were 5.0 ng/mL and 0.22 ng/mL, respectively.

Finally, the LC-MS/MS method was successfully applied to a toxicokinetic study of TeA in broiler chickens, therefore demonstrating the suitability for this application.

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The contamination of German food with *Alternaria* mycotoxins – a fast multi analyte LC-MS/MS approach

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Fungi of the genus *Alternaria* occur worldwide and infect various agricultural crops including grain, fruit (apple), solanaceous herbs (tomato, potato) and sunflowers. Besides their phytotoxic - and thereby detrimental - activity, they produce a variety of about 70 mycotoxins such as tenuazonic acid (TeA), alternariol (AOH), alternariol mono methyl ether (AME), altenuene (ALT) and isoaltenuene (isoALT), altertoxins (ATX), Tentoxin (TEN) and AAL toxins (AAL).

These toxins show a broad range of adverse effects, including teratogenic, cytotoxic and mutagenic effects. Up to date, no legal maximum levels or guidance levels for *alternaria* toxins in food or feed have been set. Besides the insufficient knowledge about the toxic effects, this is due to the lack of occurrence data.

We developed a simple and fast LC-MS/MS method for the quantification of TeA, AOH, AME, ALT, isoALT, ATX I, TEN, and AAL TA\(_1\) and TA\(_2\), covering the mainly occurring toxins in foodstuff. The method relies on a single extraction step and a dilute-and-shoot approach without further sample preparation. The method was validated for a variety of food matrices and a broad range of food samples from retail in Germany (region of Muenster) was analyzed.

In the first set of samples analyzed, TeA, AOH, AME and TEN were found in almost all samples, mostly occurring in low levels ranging from 100 to 500 μg/kg for TeA. Some samples showed TeA contamination of up to 1120 μg/kg (sunflower seeds). TEN concentrations ranged from 40 to 50 μg/kg with maximum concentrations of 400 μg/kg (sunflower seed). AOH was only found in low levels of up to 8 μg/kg. AME was found in levels from 2–4 μg/kg with maximum concentrations of 20 μg/kg (sunflower seed).

The authors thank ABSciex (Darmstadt) for supplying us with a QTRAP 5500 mass spectrometer.
An LC-MS/MS based method for determination of several mycotoxins (nivalenol, deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, HT-2 toxin, T-2 toxin, ochratoxin A, fumonisins B1 and B2, aflatoxin B1, B2, G1 and G2, sterigmatocystin and zearalenone) in animal feed has been developed and validated.

After extraction of the mycotoxins with acetonitrile/water/acetic acid (79/20/1) extract was divided into two parts. One portion of extract, used for aflatoxins analysis, was cleaned-up with immunoaffinity columns. Second portion, used for analysis of other mycotoxins, was cleaned-up with C18 sorbent in the presence of magnesium sulphate. Extracts were mixed with internal standards solution and determined with UHPLC-MS/MS technique. Mycotoxins were separated with Luna Phenyl column (150×2.0mm, 3 μm, Phenomenex) with 15 min – gradient elution of methanol-and 0.01 M ammonium acetate pH 6.8. Mass spectrometer worked with multiple reaction monitoring, for all analytes 2 transitions were monitored.

The method was validated: linearity, precision (repeatability and within-laboratory reproducibility), recovery, limit of detection and limit of quantitation were calculated. Within-laboratory reproducibility was in the range of 10–25%, with recovery above 70%.

The effectiveness of the presented method was proven in the analysis over 100 animal feed samples as well as matrix reference materials.
Ergot alkaloids (EA) are mycotoxins that are produced by all species of the *Claviceps genua*, most notably by *Claviceps purpurea*. Given the current state of knowledge more than 600 plant species are susceptible to infections with *Claviceps purpurea*. In particular, this includes the economically important cereals like wheat, rye, triticale, barley, spelt, millet and oat. Up to date more than 50 different EAs have been identified. In risk assessment, the focus is particularly aimed at ergometrine, ergotamine, ergocornine, ergosine, ergocristine, and ergocryptine and the corresponding isomers ergometrinine, ergotaminine, ergocorninine, ergosinine, ergocristinine and ergocryptinine.

In Europe current regulation only sets maximum levels for ergot sclerotia in certain feed and food ([Regulation (EU) No. 1272/2009; Directive (EU) 2002/32/EC](#)), however maximum levels for EA are not yet established. In the year 2012, the European Food Safety Authority (EFSA) for the first time inferred health-based guidance values for tolerable intake quantities of ergot alkaloids for acute and chronic exposure.

In this context a method for the determination of ergot alkaloids in cereal and cereal flour by HPLC-FLD was fully validated and published in the official compilation of the German Food and Feed Code. Based upon this method an interlaboratory validation study considering mixed feeding stuff analysis was carried out in 2012/2013. Since analyses with HPLC-FLD often produce false positive findings for samples with a complex composition, the more specific LC-MS/MS was applied. Various mixed feeding stuffs with different levels of ergot alkaloids were prepared and used as reference material. The results of the interlaboratory validation study and challenges in ergot alkaloid analyses will be presented in detail.
Detecting mycotoxin producers in environmental samples via phylogeny-based preamplification and sequencing protocol

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The advances in next-generation sequencing presently allow for efficient sampling of fungal taxa present in the environment, via high throughput sequencing of conserved, taxonomic marker sequences. A similar conceptual approach can be used to sample underlying chemotypes, biosynthesised by core genes of common descent. On basis of analysis of over 150 model fungal genomes, we have previously demonstrated the “distant siblings” property of extant non-reducing polyketide synthases. The present day repertoire of aromatic compounds is synthesised by distantly related PKSs (“siblings” due to duplication, “distant” due to selective losses). The separation of major groups of homologous genes associated with synthesis of toxic compounds (e.g. aflatoxin, aurofusarin, zearalenone) permits specific characterisation of toxigenic potential in environmental samples.

Based on the phylogenetic results, we developed a preamplification protocol targetted to conserved ketoacyl synthase fragment in major clades of non-reducing polyketide synthases. The result of our pilot sequencing experiments (454 and Illumina MiSeq) supports use of preamplification and pooled environmental sample sequencing in detection and annotation of biosynthetic potential associated with mycotoxin biosynthesis. Preliminary assays validated our approach on both samples directly obtained from the environment (collected dust samples), as well as on the pooled samples of cultured fungi.

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Determination of aflatoxin M1 in milk and powdered milk

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Aflatoxins are one of the most important groups of mycotoxins. They are produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus* strains. The most toxic and prevalently produced compound is aflatoxin B1, which can be transformed into its hydrolyzed form – aflatoxin M1 (AFM1). Aflatoxin M1 can be found in milk or milk products obtained from livestock that have ingested contaminated feed. It is less harmful than aflatoxin B1, but it is known that juveniles are more sensitive to aflatoxins than adults. The International Agency for Research on Cancer classified AFM1 as a class 2B human carcinogen.

The aim of the study was to gather information about the occurrence of AFM1 in food products with the established values of the maximum levels.

A total of 36 liquid milk and powdered milk samples were purchased in local shops in Bydgoszcz, North Poland. Powdered milk was dissolved in preheated water. Afterwards liquid milk or dissolved powdered milk were centrifuged to remove fat. Skimmed milk sample was passed through Afla M1 HPLC (Vicam) immunoaffinity column. After the washing step AFM1 was eluted from the column by the mixture of ACN:MeOH (3:2). Finally, the sample was evaporated to dryness and dissolved in 1 ml of mobile phase.

The Merck-Hitachi HPLC-FLD system was used to separate and detect AFM1. The separation was carried out on the Luna C18 column (250x4.6 mm, 5 μm, Phenomenex). The mobile phase consisted of H2O:ACN:MeOH (6:2:2). The flow rate was kept at a level of 1 mL/min.

The validation study showed that the method met the performance criteria in accordance with the EU Regulation 401/2006. The retention time of AFM1 was 10.7 min. The limits of detection (LOD) and quantitation (LOQ) of AFM1 were 0.003 μg/kg and 0.01 μg/kg, respectively. The recoveries of AFM1 from liquid milk and milk powder were between 83.9-89.2 % and 84.3-86.0 %, respectively. None of the milk samples contained AFM1 at a concentration level higher than LOD value.

Ochratoxin A (OTA) and citrinin (CIT) are produced by some species *Aspergillus* and *Penicillium*, which can contaminate cereals. Due to their relatively high chemical and thermal resistance, cereal products such as beer are also affected and the mycotoxins are not eliminated totally during food-processing. Some mycotoxins have already been tested in beer, including OTA, but CIT is considered more heat-labile and isn’t examined.

The aim of this work was simultaneous determination of OTA and CIT in beer with on-line extraction and preconcentration using switching columns in HPLC with sensitive and selective fluorescence detection reaching trace amount of mycotoxins.

The analytes were on-line preconcentrated and separated in time less than 6 min, after direct injection of 100 μL of filtrated beer. Both preconcentration and separation were performed on fused-core columns, Ascentis Express RP-C-18 guard column and Ascentis Express Phenyl-Hexyl, respectively. For better chromatography separation of the analytes, the mobile phases in both dimensions were acidified, separation was achieved with gradient elution.

The optimized and validated method showed high sensitivity with limit of detection 10 and 20 ng L\(^{-1}\) for OTA and CIT, respectively. Accuracy as the mean recoveries of OTA and CIT both in light and dark beer samples were found in the range 98.3–102.1%.

The mycotoxins were analyzed in 49 Czech beer samples, the content in light, dark and wheat lagers was studied. Low concentration levels of OTA and CIT below the maximum tolerable limit were found. OTA was detected in all beer samples (mostly about hundred time lower amount than EU limit for OTA in alcoholic beverages 2 μg L\(^{-1}\)), the presence of CIT was observed in 4 samples only. Although that confirms its low stability, neither citrinin is removed by brewing beer process totally.

The study was supported by the Charles University in Prague – project 17/2012/UNCE and project of specific research, no. SVV 260 184.
Design of a novel microfluidics platform for mycotoxin food contaminant determination

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Mycotoxins are major food contaminants with multiple toxic effects. Current methods for analysis of mycotoxins lack sensitivity and require skilled personnel using laborious analytical methods in laboratory facilities. Thus, there is a pressing need for the development of a portable, cost-effective, highly sensitive method of mycotoxin analysis to protect consumers. We envisage that achieving this will encompass production, isolation and purification of specific anti-mycotoxin recombinant antibodies and their subsequent optimisation and validation on a microfluidic platform. The device will be capable of detecting the primary mycotoxins and their metabolites. Furthermore, there is a lack of detailed understanding of how mycotoxins affect the immune system. This project aims to address this issue by examining the effects of mycotoxin exposure, alone and in combinations, on some of the key cells involved in the immune response. Such an approach will provide important insights into the mechanisms by which mycotoxins modulate the immune response to exert their immunosuppressive activity. To date, there is no highly successful incorporation of such measurements, in combination with total mycotoxin determination, on any easy-to-use detection devices. The project will design a test capable of measuring the potential synergistic effects of multiple mycotoxins for generating toxicity in food samples. This work ultimately aims to develop a multiplex microfluidics device for rapid ‘on-site’ quantification of mycotoxins whilst incorporating a novel method for elucidation of the effects these toxins in food samples exert on immune cells. The inclusion of an analytical step demonstrating how differing combinations of mycotoxins affect immune cells is potentially a highly novel and important advance in food safety. This strategy should move mycotoxin analysis from the lab to the field and allow early intervention, where necessary, improving food safety and providing cost savings.

This research is supported by the Irish Research Council.
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Development of cost-effective and rapid mycotoxin multiresidue method for baby food by SPE and DSPE technology as alternative to affinity chromatography

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Every year approximately 20 % to 25 % of grain crops worldwide are contaminated by fungi. The most occurring species are of *Penicillium*, *Aspergillus* and *Fusarium* genus, which produce mycotoxins in certain stage of growth on grain under specific climate conditions. Because of global warming and climate changes the contamination of grain crops by fungi is expected to increase in the coming years. Mycotoxins have an acute and also chronical toxic impact on human beings and livestock. To look ahead analytical procedures for the determination of mycotoxins in staple foods and feeds will become more and more important, since the demand of countries governments for more stringent controls of their imported foodstuff is still rising.

In this paper a sensitive and highly selective multiresidue method (MRM) for the analysis of 16 mycotoxins in cereal products applying DSPE and SPE cleanup technology is presented. Following a QuEChERS-like extraction step using buffered conditions the extract is cleaned in DSPE mode followed by an SPE step. For both cleanup modules sorbents of normal phase and reverse phase type were applied. A change in the solvent composition of the mobile phase during the subsequent cleanup steps was crucial for the efficiency in the removal of unwanted co-extractives while retaining the mycotoxins of interest. The optimization of selectivity and sensitivity of DSPE and SPE steps are shown in details.

The validation was performed according to DIN 32645. The LOQs determined for the method for example for aflatoxins were between 0.01 and 0.1 ppb. This selective MRM protocol represents a valid alternative to the cleanup with affinity chromatography.
Development of a sensitive UPLC-MS/MS-based multiresidue method for the simultaneous determination of *Alternaria* toxins in baby food

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*Alternaria* toxins are secondary metabolites of the fungi *Alternaria alternata* mainly occurring in cereals, vegetables, fruits and oil seeds. These mycotoxins act fetotoxic, mutagenic and teratogenic in mice. Furthermore tenuazonic acid can inhibit protein biosynthesis and comprises a cancerogenic potential. Although there are no maximum levels required by the European Union yet, it is of high interest to determine these compounds in food to ensure safety especially for sensitive consumers like infants because of their adverse side effects.

Thus we present a new sensitive UPLC-MS/MS MRM for the determination of *Alternaria* toxins in baby food. The method includes the simultaneous detection of alternariol (AOH), alternariol monomethylether (AME), altenuene (ALT) and tentoxin (TEN) as well as tenuazonic acid (TeA) after derivatization with 2,4-dinitrophenylhydrazone (2,4-DNPH).

The method was validated according to DIN 32645 for various matrices including cereal products, purees of fruits and vegetables as well as beverages. Possible matrix effects were determined for each commodity group. Recoveries were between 80 and 90 %. Limits of detection (LOQs) were in the range of 1 to 4 ppb without cleanup procedure. We estimate to reach LOQs ≤ 1 ppb by optimizing the extraction method with a SPE cleanup step.

In the present study the applicability of this new methodology to various commodities for the sensitive and selective determination of *Alternaria* toxins in baby food to delimitate hazards for infants was demonstrated.
Is the narrow focus on aflatoxins in sub-Saharan Africa plausible?

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Aflatoxins are the most commonly known and thoroughly studied mycotoxins. They are considered to be among the most toxic mycotoxins and have been associated to epidemics of liver diseases. In spite of substantive linkage between aflatoxins B1 and human hepatic/extra-hepatic carcinogenesis, in some cases the etiology of the disease could not be unequivocally established [Tandon et al., 1978, Arch Pathol Lab Med 102(7):372-376]. Despite evidence of synergistic interaction between mycotoxins, in instances where the relationship seem fairly tenable, there is no record of assays of other mycotoxins which might have co-occurred with the aflatoxins and augmented the epidemics [Lewis et al., 2005, Environ Health Persp 1763-1767; Probst et al., 2007, Appl. Environ. Microbiol. 73(8), 2762-2764]. Likewise, aflatoxin is virtually the sole focus in developing countries including those in sub-Saharan Africa. In contrast, recent multi-mycotoxins surveys collaboratively done with oversea laboratories indicate widespread co-occurrence of a number of different mycotoxins in staple foods in Africa [Adetunji et al., 2014; Mycotoxin Res, 30(2), 89-102; Ediage et al., 2014, J Agric Food Chem 62: 4789-4797; Matumba et al., WMJ(in press) http://dx.doi.org/10.3920/WMJ2014.1773; Mohale et al., 2013, Mycotoxin Res, 29(4), 209-219; Warth et al., 2012, J Agric Food Chem 60(36), 9352-9363]. Moreover, mycotoxins other than aflatoxins have been repeatedly found to be more prevalent and in much higher concentrations. While recognizing the potential toxicity of the aflatoxins, this article critically examines the rationale of the current narrow focus on aflatoxins in Africa.
Validation of a method for the analysis of sterigmatocystin in cereals using immunoaffinity columns

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Sterigmatocystin is a precursor in the metabolic pathway for aflatoxin formation and like aflatoxin can be produced by several Aspergillus species. The main producer is A. versicolor which is ubiquitous in nature and has been found to grow on corn, bread, dried fruit, cheese, rice and fermented meat products. Although there are many reports on the occurrence of sterigmatocystin in various commodities, many of the thin layer chromatography methods that were traditionally used lack adequate specificity.

In March 2013, the European Food Safety Authority (EFSA) issued a tender for surveillance work on sterigmatocystin in a variety of grains including wheat, barley, rye, oats and rice intended for human consumption from 3 different European countries. R-Biopharm Rhone has developed an immunoaffinity column which selectively isolates and concentrates the mycotoxin from a range of cereal samples. The result is better clean up leading to improved chromatography and ultimately lower limits of detection.
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Comparison of various commercially available multi-mycotoxin immunoaffinity columns to direct injection

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R-Biopharm Rhone’s immunoaffinity columns offer a versatile solution for multi mycotoxin analysis whereby the immunoaffinity columns can be used in tandem with one another to cover the regulated mycotoxins applicable to a particular food matrix.

AOF MS-PREP® and DZT MS-PREP® immunoaffinity columns were tested in tandem to determine 11 legislated mycotoxins (total aflatoxin, ochratoxin A, fumonisin, deoxynivalenol, zearalenone, T-2 and HT-2) in maize. The samples were analysed using a single extraction followed by immunoaffinity clean-up with the columns connected in tandem. The eluted solution was analysed by LC-MS/MS. In addition, the samples were analysed by two other commercially available immunoaffinity columns and were also compared to analysis by direct injection.

When using the R-Biopharm Rhone immunoaffinity columns, normal HPLC standards were used for calibration and no issues were observed with ion suppression or enhancement which can often adversely impact identification and quantification.
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Spatial distribution of phytopathogenic *Fusarium* fungi and its mycotoxins within heterogeneous wheat fields

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FHB and mycotoxin distributions can be highly heterogeneous at the field and landscape scale. Environmental and agricultural management factors can determine patches or patterns of phytopathogenic fungal populations. Müller et al. (2010, 2011) described the up to two-fold higher DON and six-fold higher ZEA concentrations in depression positions within a uniformly managed wheat field compared to the hilltops. The authors explained these patches by the more pronounced relative humidity in depressions deriving from air humidity as well as from the soil moisture conditions at these positions. For this reason, these sites are regarded as the hot spots of inoculum for epidemiological behaviour.

This study (Müller et al. submitted) explores variable within-field and landscape humidity conditions and their potential influence on fungal infection and mycotoxin production in a susceptible winter wheat cropping system. Occurrence of fungi, mycotoxins, and environmental data were collected on four farm fields, two each in 2009 and 2011, under winter wheat following maize. Environmental data hypothesized to describe humidity were topography (TWI), soil electrical conductivity (ECa), and a proxy for canopy density derived from remote sensing imagery (NDVI).

In the wet year of 2011, a high *Fusarium* infection rate resulted in a high abundance of trichothecene-producing fungi as well as high concentrations of mycotoxins. Simultaneously, fusaria inhibited the development of other filamentous fungi. Overall, a very heterogeneous distribution of pathogen infections and mycotoxin concentrations were displayed in each field in each landscape. The NDVI serves as an important predictor of the occurrence of phytopathogenic *Fusarium* fungi and their mycotoxins in a field and landscape scale. In addition, the ECa reflects the distribution of the most frequently occurring mycotoxin deoxynivalenol within the fields and landscapes. In all cases, TWI was not found to be a significant predictor.

Analysis of patulin in multifruit thick juice and concentrates using a molecularly imprinted polymers based SPE

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Patulin is a mycotoxin produced as a secondary metabolite of particularly Aspergillus and Penicillium fungi present in agricultural commodities. Contamination of agricultural commodities is a real problem due to their toxic effects such as genotoxicity. Several countries have instituted Patulin restrictions mainly on apple based products. For instance, in European Union, the limit is set to 50 micrograms per kilogram (ug/kg) in both apple juice and cider, 25ug/kg in solid apple products and 10ug/kg in products for infants and young children. However, some countries such as Sweden, Russia, China also extend this regulation to products which do not contain apple such as berries, hawthorn, tomato or sea-buckthorn.

On other part, thick juices are obtained by mixing apple juice with some fruits such as mango. The viscosity of the solution makes the analysis more complex. A similar problem is encountered with juice concentrates, a very important intermediate product in agrofood industries.

This poster shows solid phase extraction (SPE) clean-up methods developed for these two kinds of products by using sorbents based on Molecularly Imprinted Polymers (MIP). MIPs are affinity columns made with very stable polymers to aqueous or organic solvents as well as temperature. These cost-effective products are widely used for clean-up and preconcentration applications.

Several matrices based on mixture with apple or pure fruit not containing apple. The method developed has been evaluated with several matrices containing or not apples and shows high recovery yield and excellent clean-up.

Keywords:
Solid phase extraction, Molecularly Imprinted Polymers, Patulin, clean-up method, Thick Juice, concentration
P24

Influence of reduced water supply on the *Fusarium* toxin contamination of maize ears

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For large parts of Central Europe more frequent summer droughts are predicted as a consequence of global warming. In a 2-year field experiment it was examined whether limited watering either before or after flowering does affect the risk for DON contamination in maize ears.

The trial was conducted near Braunschweig during the main growing season of 2012 and 2013. The total experimental area (162 m²) was divided into eight equally sized subplots. The water supply was controlled by means of a sprinkler irrigation system installed in a mobile rain-out shelter. The cultivar ‘Oldham’ was inoculated with a mixture of each 1 million conidia of *F. graminearum* and *F. culmorum* sprayed on the maize silks at full flowering. At harvest twenty ears were manually removed from each subplot. After separating the husks, the ears were crushed, dried and ground to a fine powder. The samples were analysed for *Fusarium* mycotoxins by LC-MS (5500 QTrap).

In both years of investigation deoxynivalenol (DON) was the dominating *Fusarium* toxin followed by 3-acetyldeoxynivalenol (3-acDON). While these two toxins occurred in all ear samples, zearalenone and nivalenol were only occasionally found in low concentrations (<0.2 mg kg⁻¹). When the plants were cultivated with only the half amount of water until the end of flowering followed by the full amount thereafter, the mean DON concentrations were slightly higher (2012: 1.85 mg kg⁻¹, 2013: 3.56 mg kg⁻¹) compared with the opposite water regime (2012: 1.34 mg kg⁻¹, 2013: 3.04 mg kg⁻¹). The mean concentrations of 3-acDON ranged from 0.71 to 1.35 mg kg⁻¹ with no significant difference between the years and the water regimes. The present study revealed that early and late drought did not differently affect the *Fusarium* toxin contamination of maize ears.
P25

Rapid diagnostic testing of toxigenic microfungi isolated from foodstuffs – an application of chromogenic media

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Toxigenic microfungi of *Aspergillus* and *Penicillium* species are considered to be of major relevance for human health. The presence of toxigenic microfungi (e.g. *Aspergillus flavus*, *A. carbonarius*, *A. ochraceus*, *Penicillium verrucosum* and *P. citrinum*) in foodstuffs represents a potential risk of mycotoxins (e.g. aflatoxins, cyclopiazonic acid, ochratoxin A and citrinin). It is necessary setting rapid methods for toxigenic moulds isolated from foodstuffs in order to reduce the risks for consumers' health. An application of chromogenic media presents one of the possibilities. Chromogenic media serve as nutrient systems for the cultivation of toxigenic microfungi. ADMB agar (Aspergillus Differentiation Medium Base), AFPA agar (Aspergillus flavus and parasiticus Agar), CA (Coconut Agar), AHT (Ammonium Hydroxide Test) and EAT (Ehrlich’s Aldehyde Test) for rapid testing of *Aspergillus flavus* (producers of aflatoxins and cyclopiazonic acid) were used. The rapid testing of ochratoxigenic microfungi *A. carbonarius*, *A. ochraceus* and *Penicillium verrucosum* can be proved by using diagnostic nutrient media CGYE agar (Chloramphenicol Glucose Yeast Extract), 6 MFA agar (Medium for cultivation of *Aspergillus ochraceus*) and DRYES agar (Dichloran Rose Bengal Yeast Extract Sucrose agar). CA (Coconut Agar) was used for testing of *Penicillium citrinum*, citrinin producer. The chromogenic media are suitable for a laboratory with basic equipment for microbiological and mycological analysis. One of the cost/benefits of chromogenic media is the rapid diagnosis which takes place, within 48 – 72 hours only, but it presents a screening/survey test only. The results of microfungi toxigenity must be confirmed by chromatographic methods (e.g. HPLC, LC-MS/MS) or molecular biological methods (e.g. PCR, RT-PCR).

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An occurrence of toxigenic microfungi in grapes in the Czech Republic: A role of climate change and global warming?

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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 in temperate climates (e.g. *Aspergillus flavus*, *A. carbonarius*, *Fusarium verticillioides* and *Penicillium spp.*). In 2004 was carried out a pilot study in the Czech Republic that addressed the colonization of grapes by toxigenic microfungi from the Znojmo wine region. During the harvest were taken 22 grape samples representing 12 varieties of grape wine. The most important mycotoxin – producing microfungi were *Alternaria alternata* contributing for about 16 (73 %), while *Cladosporium spp.* (*Cladosporium herbarum, C. cladosporioides*) constituted up to the 12 (55 %) and *Rhizopus nigricans* 6 (27 %) of all the toxigenic microfungi. Other potential toxigenic species, e.g. *Penicillium aurantiogriseum*, *P. spinulosum* *P. citrinum*, *P. expansum* and *Aspergillus clavatus* were present, but with a minor contribution (from units to 10 %) (Varga et al., 2007).

A recent similar study was prepared in the Znojmo wine region in the year 2014. The aim of this study was a comparison of mycological profile of grapes from the year 2004 and year 2014. Twenty five grape samples representing 15 varieties of grape wine were gathered. *Penicillium expansum* was isolated in 23 (92 %) grape samples. *P. expansum* isolates are potential patulin and citrinin producers. *Alternaria alternata* group was isolated in 8 (32 %) grape samples. Other potential toxigenic species, e.g. *Penicillium aurantiogriseum*, *P. roqueforti*, *P. crustosum* and *Aspergillus clavatus* were present, but with a minor contribution (up to 4 %).

The mycological profile obtained in year 2014 was significantly different from the mycological profile in year 2004. We can only speculate if this reason are the climatic changes and/or other ecological and environmental factors. Further studies are needed to clarify the results of this examination. We can recommend a regular monitoring of the colonization of grapes by toxigenic microfungi before and during the harvest.


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Colonization with *Fusarium* spp. and mycotoxin content of cultivated and wild *Amaranthus* spp.

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*Amaranthus* spp. is a niche product, but due to rising health consciousness, products made of amaranth are becoming more popular. *Amaranthus* spp. are grown as ancient crops, mainly in Central and South America, but also in Europe the cultivation areas are increasing, exclusively in ecological farming systems. Besides, *Amaranthus* spp. occur as wild weeds in. Infection by fungal species and the contamination of mycotoxins in *Amaranthus* spp. is rarely described.

A total of 37 amaranth seeds and popcorn from retail in Germany were collected and analyzed for fungal biomass using qPCR and mycotoxins using HPLC-MS/MS. The DNA of *F. culmorum*, *F. graminearum*, *F. proliferatum* and *F. tricinctum* were found, while *F. equiseti*, *F. poae*, *F. subglutinans* and *F. verticillioides* were not detected. Analyses revealed the contamination of 13 samples with enniatins (A, A1, B, B1) with amounts up to 54 µg/g (sum of enniatins) as well as two samples with zearalenone. Fumonisins, beauvericin, trichothecene A and B were not found.

Seven weed samples (*A. retroflexus*) were collected from maize fields in Germany. The DNA of *F. graminearum*, *F. culmorum* and *F. avenaceum* were detected, while *F. verticillioides*, *F. proliferatum*, *F. poae* and *F. subglutinans* were not found. Samples infected with *F. avenaceum* were contaminated with enniatins in amounts up to 410 ng/g enniatin B and low amounts of beauvericin.

The results show the *Fusarium* spp. infection and the contamination with mycotoxins in wild and cultivated *Amaranthus* spp. In order to ensure food safety the investigation of amaranth, a product with increasing interest, is necessary. It is unknown if the infection of wild amaranth plants contributes to the infection pressure of *Fusarium* spp. in maize fields.
Results of mycotoxin survey in wheat and barley harvested in last 5 (wheat) or 8 (barley) years in the Czech Republic have been analysed. The attention has been paid to *Fusarium* mycotoxins limited in foodstuffs on the basis of Commission Regulation No. 1881/2006, i.e. to deoxynivalenole (DON) and zearalenone (ZEA). A representative set of cereal samples intended for food production was collected each year immediately after harvest in a close cooperation with farmers. In total, 500 samples of wheat was analysed for DON and ZEA content in the period of 2010-2014. The proportion of wheat samples above legislation limit for DON (1250 μg/kg) varied between 0 % in 2014 to 4 % in 2012 and 2013. The maximum content of DON in particular year varied from 498 μg/kg (2014) to 5183 μg/kg (2012). For ZEA content, only in one harvest year (2011) a wheat sample exceeding the legislation limit (100 μg/kg) has been found, having 192 μg/kg. In total, 480 samples of barley was analysed during 2007–2014. The proportion of barley samples above legislation limit for DON varied between 0 % in 2010, 2012, 2013 and 2014 to 26 % in 2009. The maximum DON content in particular year varied from 219 μg/kg (2014) to 7050 μg/kg (2009). For ZEA content, none of the analysed barley samples exceeded the legislation limit. The maximum ZEA values varied between <2 μg/kg (2014) to 99 μg/kg (2012). The most influencing factor proved to be harvest year. Mycotoxin content was also negatively influenced by maize as preceding crop: 8 of 10 DON above-limit wheat samples and 16 of 19 DON above-limit barley samples were grown after maize.

For the reduction of mycotoxin content in a food chain, monitoring at its entry is very important. Information about situation together with possibility of identification of risk factors and quick feedback to farmers poses such knowledge-based monitoring a powerful tool for further increasing of food safety.

This work was financially supported by the Ministry of Agriculture of the Czech Republic by the institutional support for long-term development (decision of the Ministry of Agriculture No. RO0211 dated 28.2.2011) and projects QG60047 and QG50041.
In 2014 the organic farming, which is continuously growing, reached almost 12% of the farmland acreage in the Czech Republic. The crops of great importance are cereals followed by fodder crops. The organic wine market is increasing and it is becoming popular as well. Organic products have been grown in compliance with principles of organic farming that typically excludes the use of chemicals as fertilizers, fungicides, herbicides and other types of pesticides. The study deals with analysis of mycotoxins in organic and conventional farm products such as raw materials for feed production. The multiresidue mycotoxin method for feed analysis by ultra performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) which has been recently developed represents useful tool for feed analysis. The method for determination of 17 mycotoxins (deoxynivalenol, nivalenol, HT-2 toxin, T-2 toxin, ochratoxin A, zearalenone, aflatoxins, fumonisins, beauvericin and enniatins) based on unbuffered QuEChERS method has been validated.

The study presents results of mycotoxin screening performed on raw feed material samples of conventional and organic origin produced in 2013 and 2014.
The occurrence of mycotoxins in different types of flours

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The aim of this research was to determine the occurrence of storage mycotoxins produced by fungi of the genus *Aspergillus* and *Penicillium* in different types of flour widely available in shops and mills in Poland. It was checked whether the type of packaging and species of cereals have influence on the contamination of storage products of flour by mycotoxins. It was also investigated what the correlation is between the two dependent variables. To determine the content of mycotoxins, RidaScreen's enzyme-linked immunosorbent assay (ELISA) was used.

It was noted that there were significant differences in the occurrence of mycotoxins in flour depending on the plant species from which it was made. It was found that wheat flour and spelt flour are the least contaminated of all tested flour, and the accepted limits of aflatoxins and ochratoxin A are not exceeded in them. The maximum allowable limit for aflatoxins was exceeded only in rice flour packaged in paper, and the limit for ochratoxin A was exceeded in rye flour, buckwheat flour and amaranth flour, stored both in plastic and paper packaging. There was no significant effect of packaging (plastic, paper) on the mycotoxin content in the tested flours. The highest content of ochratoxin A was found in buckwheat flour stored in paper packaging and buckwheat flour stored in plastic packaging, in which the allowed limit for ochratoxin A was exceeded 16 times.
Survey of aflatoxins in selected dried fruits and nuts on the markets in Vienna

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Aflatoxins are secondary metabolites of storage fungi. They are mainly produced by Aspergillus flavus and Aspergillus parasiticus. The most important aflatoxins are B1, B2, G1, G2, and the so-called milk toxins M1 and M2. Aflatoxin B1 (AFB1), which acts primarily hepatotoxic, is the most toxic and most dominant aflatoxin. In general, high concentrations of aflatoxins have been reported in some fatty plant foods (peanuts, pistachios, almonds, hazelnuts, etc.) dried fruits (figs, dates) as well as certain types of grains (maize, millet, wheat, rice).

In the present work, dried fruits (figs, baby figs, raisins, apricots, dates, sultanas, mulberries) and nuts (pistachios, almonds, and peanuts) were purchased from the markets in Vienna and analysed for their aflatoxin content. The sample preparation was performed by immunoaffinity chromatography prior to HPLC-FLD determination. Sample preparation was optimized for the commodities. While with dried fruits and distilled with water slurries were produced, nuts were ground fine. Purification and extraction of the sample was optimized for each commodity. An aliquot was taken (usually 0.5 g sample), diluted and applied on the immunoaffinity columns. The validation of the whole method was performed on certified reference material from FAPAS, where recoveries within one day and on three consecutive days were determined. A total of 98 food samples were evaluated for their aflatoxin content. In the present work only pistachios were identified as AF-positive commodities. The results indicated that 16 samples (16%) contained detectable concentrations of aflatoxins. In detail in 12 pistachios samples concentrations above the quantification limit were measured. However, in 4 pistachios samples (4%) the maximum levels of AFs in pistachios were exceeded dramatically.
Representative and fast analysis of ochratoxin A in green coffee via dust sampling

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The EU maximum level for ochratoxin A (OTA) in roasted coffee beans is set to 5 μg/kg. Depending on regional climate conditions, raw coffee beans can be heavily contaminated. Thanks to improvements within the producing process, nowadays contents are much lower compared to the past. However, industrial self-control remains the only possibility for further improvement and to protect consumer’s health. Therefore, a suitable OTA detection procedure including sampling is needed for reception and import control. Due to a heterogenous distribution in the lots, sampling and sample preparation are the most important but at the same time most laborious steps in the control of OTA.

To cope with this problem, an innovative sampling regimen based on dust was developed and tested. Particles from the surface of beans are abraded during transport or routine handling procedures and are ubiquitously found in lots of green coffee. In coffee warehouses, every incoming lot is cleaned separately before storage. Samples of dust were taken from the cleaning process. In parallel, beans from the respective lots were taken dynamically out of a gravity spout. Dust samples were fractionated according to particle size. As no grinding or homogenisation step was needed, sample preparation was reduced to a short extraction step of the dust. In contrast, each coffee bean sample had to be pre-grinded and wet-milled to obtain a homogenous sample before analysis by LC-FD.

Concentrations measured in coffee dust were up to 100 times higher than in green beans. A dust fraction was identified, whose contamination was highly correlated to the contamination in the beans (R = 0.92). Based on this fact, data models can be set up that allow calculation of OTA contents in beans from concentrations determined in respective dust. Hence, dust sampling is a promising way to obtain representative samples in a very easy way and to speed up the measurement of OTA in green coffee in future.
Sampling of grain lots for mycotoxins – uncertainty, risk and approach

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Risk management has to deal with a lot of different uncertainties contributed via different steps of mycotoxin control. According to the FAO Sampling Tool (http://www.fstools.org/mycotoxins/), the variance of measuring 20 μg/kg of aflatoxin B1 in corn (1 kg sample, 10 g test portion) adds up to 530 (μg/kg)^2 with sampling and sample preparation contributing 46% and 53%, respectively. The variance ascribed to sampling can be reduced by 90% when taking 10 kg instead of 1 kg of sample. However, to grind 10 kg of corn is often too laborious to be performed on-site.

Different lot sizes from lorry to barge (25 t, 350 t, 1000 t) of corn and wheat were controled using both conventional grain and innovative dust sampling methods. Samples were analysed by LC-MS/MS multitoxin methods or on-site using lateral flow devices. Results of dust samples were recalculated to contaminations in grain using respective data models (rapidust procedure). Effort to perform the measurements was recorded in terms of time and resources needed. Variability in concentration of different mycotoxins was illustrated as well as the portion of controlled and not-controlled parts of a lot. When comparing conventional kernel to indirect dust sampling, the standard deviation for aflatoxin B1 in corn was reduced from 40% (10 kg sample, grinded ≤ 1 mm) to 14% by using the latter. Furthermore, dust procedures speeded up the sample preparation after comprehensive static sampling (10 kg per 25 t) from > 2 h to < 10 min, making it generally applicable to intake control. Considering FAO data and data obtained in the studies risks were assessed for the different sampling approaches taking three scenarios of mycotoxin occurrence (high, low, and random incidence) as a basis. Finally, based on the probabilities of false negative results, the estimated consequential costs of a wrong decision, and the measurement costs including labour, total costs for the grain industry were estimated for the control options.
Innovative approaches for controlling mycotoxins in stored grains for animal and poultry feed

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We designed an innovative technology for mycotoxins control in a large scale in Alexandria port, Egypt. We succeed to control mycotoxins from high levels to lower levels. We achieved an effective suppression for the Aflatoxins with our new technology for 9000 Tons of yellow Corn in less than 72 days. It is a great worthy to mention that this is the first time to achieve this huge amounts of stored grains with a simple and Eco-friendly technology. Finally, we can recommend the application of our technology to both developing and developed countries.
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Aggressiveness of *Fusarium graminearum* chemotypes producing nivalenol or deoxynivalenol in maize

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*Fusarium graminearum* Schwabe [teleomorph = *Gibberella zeae* (Schwein) Petch] is a mycotoxigenic pathogen which attacks maize plants and is one of the causative agents of the Gibberella ear rot disease. *F. graminearum* typically produces the mycotoxines trichothecene B such as nivalenol (NIV) and deoxynivalenol (DON). Thus *F. graminearum* can be defined as either NIV or DON chemotype depending on the toxin produced.

Unlike in small grain cereals, the pathogenicity and aggressiveness of NIV and DON chemotypes have rarely been investigated in maize. The aim of this study was to address the aggressiveness of *F. graminearum* NIV and DON chemotypes on artificially inoculated maize (var. Gaspe Flint) under greenhouse conditions. Disease symptoms, fungal biomass and mycotoxins content were monitored in infected ears.

The results show that *F. graminearum* DON chemotype caused intense ear rotting, extensive kernel colonization and produced a considerable amount of the toxin deoxynivalenol. In the contrary, the NIV chemotype developed less severe symptoms, colonized kernels to a lesser extent and secreted less toxin nivalenol. Therefore, *F. graminearum* DON chemotype was significantly more aggressive than NIV chemotype on maize ears.
Analysis of 814 feed samples sourced worldwide in 2014 for more than 380 mycotoxins and secondary metabolites

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A total of 814 feed samples such as corn, wheat, barley, silage, as well as finished feed and others were screened for more than 380 mycotoxins and other secondary metabolites. The feed samples were collected worldwide in 2014 and analyzed with a multi-mycotoxin LC-MS/MS method at IFA-Tulln according to Vishwanath et al (2009). The analytical method was transferred to a more sensitive mass spectrometer (QTrap® 5500) and extended to cover more than 380 metabolites (Malachova et al, 2014; Streit et al, 2013). The accuracy of the method is monitored by regular participation in proficiency tests, which includes a separate testing scheme on “animal feed” (BIPEA, Gennevilliers, France).

On average 30 different metabolites were detected per sample. 78% of the samples tested positive for zearalenone, 67% for deoxynivalenol, 55% for DON-3-glucoside and 49% for nivalenol (average of positives 186, 1003, 114, 128 μg/kg; max. 11192, 28864, 3204, 11232 μg/kg). 48% of the samples were contaminated with fumonisins and 43% with A-trichothecenes (average of positives 900, 56 μg/kg; max. 52438, 3768 μg/kg).

The “emerging mycotoxins” beauvericin, emodin and enniatin B and B1 were the most frequent mycotoxins found in over 85% of the samples analyzed (average of positives 26, 59, 43, 52 μg/kg; max. 1610, 4155, 2668, 5507 μg/kg).

As the sensitivity of LC-MS/MS increased by 200-fold in the last 10 years, more mycotoxins and other secondary metabolites are detected per sample nowadays. In consequence, further data on the metabolic fate, mode of action, toxicity and interactions of mycotoxins are required to interpret the health risk. Nevertheless, Fusarium mycotoxins like deoxynivalenol, zearalenone and fumonisins are still among the most frequently occurring agriculturally relevant mycotoxins.

References:
Fusarium Head Blight (FHB) is a fungal plant disease evoked by the fungus *Fusarium*. One of the most important species *Fusarium graminearum* (Fg) is responsible for mycotoxin contamination in wheat plants causing severe yield and quality losses.

In a recent metabolomics experiment two near-isogenic lines (CM-NIL-38, CM-NIL-51) differing in QTLs with and without resistance genes Fhb1 and Qfhs.ifa-5A were studied. Samples were analysed by GC-MS and LC-HRMS [1, 2] to study the Fg-wheat interaction in primary and secondary metabolism. Different bioinformatics methods were applied to the processed data sets in order to spot important metabolites or identify relationships between metabolites that could play a major role in the development of disease or resistance.

A Bayesian two-sample test [3] was applied to decipher whether and at which time points a metabolite is differentially expressed e.g. due to different treatments. Time points where the metabolic response starts to differ were elucidated. Moreover, focus was laid on database-derived Fg metabolites to distinguish between metabolites solely derived from Fg. Metabolic correlation networks were established to visualize interactions within and between primary and secondary metabolism. Networks composed of metabolites of wheat plants exposed to different treatments or originating from different genotypes are opposed and investigated for differences in their structure. Connections of the known GC-metabolites with the largely unknown LC-metabolites provided interesting insight into the global metabolic response of wheat upon infection with Fg. Both time series and network correlation analysis further helped to interpret the mostly unknown LC-HRMS derived wheat secondary metabolites.

The authors thank the Austrian Science Fund (project SFB Fusarium F3706-B11) for the financial support.

References
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3  Stegle et al. 2010. DOI:10.1089/cmb.2009.0175.
Determination of *Alternaria* toxins in tomato juice, wheat and sunflower seeds by liquid chromatography tandem mass spectrometry

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Alternaria species (e.g. *Alternaria alternata*) produce more than seventy secondary metabolites, but only few of them have been structurally identified and reported as hazardous mycotoxins to animals and humans (Devari et al. 2014; European Food Safety Authority 2011). Alternaria species can occur in vegetables, cereals, fruits and oilseeds and the continuous consumption of food contaminated with Alternaria toxins can cause fetotoxic and teratogenic effects. Among these Alternaria mycotoxins altenuene (ALT), alternariol (AOH), alternariol monomethyl ether (AME), tentoxin (TEN) and tenuazonic acid (TEA) are the main toxins of concern; therefore they are the focus of the present study.

We developed a liquid chromatography tandem mass spectrometric (LC-MS/MS) method for these toxins in tomato juice, wheat and sunflower seed samples. The proposed method is a modification of an earlier in-house developed method in which an addition derivatization step for TEA with 2,4-dinitrophenylhydrazine was used (Tölgyesi et al. 2015). The previous version of the method was validated for tomato samples and successfully proved its performance in a proficiency test in 2014. In the present method the pre-column derivatization has been excluded to further simplify the method, while it could be shown that a modified chromatographic separation allows to compensate need for derivatisation to a large degree, thus improving the ease to implement the method.

The method will be subject to an inter-laboratory validation study with the aim to identify a good candidate for a European standard by the European Committee for Standardization (CEN). Therefore, mycotoxin laboratories have been invited to participate in the validation of the method.
An occurrence of ochratoxin A in white and parboiled rice

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Rice (Oryza sativa L.) is a staple food for over half of the world’s population, especially in Asia. Rice is the agricultural commodity with the third-highest worldwide production, after sugarcane and maize (FAOSTAT, 2015).

Ochratoxin A (OTA) is a very important mycotoxin which is produced by Aspergillus and Penicillium species. An occurrence of OTA in rice has been reported in Canada, Czech Republic, Chile, China, Egypt, Ivory Coast, Japan, Jordan, Korea, Morocco, Nigeria, Pakistan, Portugal, Spain, Tunisia, Turkey and Vietnam (Bansal et al., 2011; Majeeda et al., 2013; Lai et al., 2015). The study objective was the determination of OTA in samples of white and parboiled rice as one of OTA dietary exposure sources.

Sixty samples of rice were collected from retail in 12 regions of the Czech Republic in the year 2014. It represents 30 samples of white rice and 30 samples of parboiled rice. Validated and accredited ultra-trace HPLC-FD method was employed for OTA determination. Rice samples were cleaned by means of immunoaffinity chromatography (OCHRAREP® columns, R-Biopharm, Germany). Limit of quantification of the method (LOQ) was 0.2 ng/g.

Occurrence of OTA in white and parboiled rice was not proved. All results were under limit of quantification of the method.


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Occurrence of ochratoxin A in green coffee and in diet supplements based on green coffee extract

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Ochratoxin A (OTA) is produced by Penicillium verrucosum and some species of Aspergillus. It is detected in a number of raw materials and plant products, among others: cereals, spices, dried fruits, cocoa, coffee or animal offal. OTA impact on the health of humans and animals is varied. Green coffee has not been considered as hazardous to humans, due to its exposure, as it has not been consumed in this form so far. Green coffee and green coffee extract belong to a group of functional foods and thus are treated as dietary supplements. The maximum acceptable levels of OTA have been established for coffee (roasted, ground and instant). There is no legislation, however, for green coffee and dietary supplements based on green coffee.

The aim of the study was to evaluate OTA and moulds contamination of green coffee and food supplements containing green coffee extract, available in Poland. The material consisted of 34 green coffee samples and 22 samples of food supplements.

The mycological analysis of green coffee showed significant levels of moulds content an average of 6.6 x 103 cfu/g (nd – 5.7 x 104 cfu/g) with the prevalence of Aspergillus (63%) and Penicillium (25%) genera. In the case of dietary supplements the average contamination with moulds was 2.3 x 103 cfu/g (nd – 4.2 x 104 cfu/g) and it was the genus Penicillium that mostly occurred (72%).

OTA determination was performed by HPLC with FLD. The sample was purified on the immunoaffinity columns OchraPrep by R-Biopharm Rhône Ltd, according to the procedure recommended by the manufacturer. The analysis showed the presence of OTA in 26% of coffee and 50% of the supplements samples, respectively. The average contamination of green coffee with OTA was 3.1 ppb (0.4 ppb – 7.44 ppb) and the average content of OTA in food supplements was 3.2 ppb (0.46 ppb – 31.4 ppb). The studies confirmed that ochratoxin A can contaminate yet another raw material as well as food commodity, being part of human diet.

Prevention and treatment trends of civilization diseases have currently been focusing on the use of the active compounds of natural origin. The group of such compounds include natural lovastatin called monacolin K. At the beginning of 2014, EFSA confirmed the cause – effect relationship between the consumption of monacolin K (from fermented red rice) and maintaining proper levels of LDL cholesterol in blood. Monacolin K is produced, among others, by Monascus purpureus, but some strains of the mould also produce citrinin (CIT). On the basis of the data on the presence of citrinin in some supplements based on fermented rice (i.e. 15.2mg/kg, Taiwan and 64.7μg/caps., the USA), the European Commission Regulation No 212/2014 amended the Regulation No 1881/2006 with regard to the maximum contamination levels of “citrinin” in food supplements, based on fermented rice with Monascus purpureus. Due to the nephrotoxicity and uncertainty regarding carcinogenicity and genotoxicity of citrinin the maximum permissible content of this mycotoxin in the supplements based on fermented rice was determined at 2mg/kg.

The aim of this study was to verify the literature data of the citrinin presence in supplements with therapeutic content of monacolin K, used in prevention and treatment of lipid metabolism disorders.

Material for the study consisted of 15 dietary supplements containing an extract of fermented red rice, available in Poland. The products were: tablets, capsules with powdered and oily content and sachets. The content of monacolin K in the supplements ranged from 1.5 to 4%.

The extraction of CIT was done on the immunoaffinity column CitriTest HPLC (Vicam). Citrinin was analyzed with HPLC-FLD (Lachrom Elite, Merck-Hitachi). The limit of detection (LOD) and quantification (LOQ) was determined at 16ppb and 26ppb respectively.

None of the analyzed samples contained citrinin exceeding the LOD level.
Improved methodology for the simultaneous screening of mycotoxins and pesticide residues in spices using the ultra-performance liquid chromatography – Orbitrap high resolution mass spectrometry

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In this study an ultra-high performance liquid chromatography coupled to high resolution Orbitrap mass spectrometry (UHPLC-Orbitrap-MS) was demonstrated as a promising detection technique for the routine analysis of mycotoxins and pesticides contamination in spices. An optimisation of analytical parameters was performed in order to assess the application of a rapid one-stage extraction technique and a multi-component detection method based on the Orbitrap technology. The elaborated procedure was compared with the conventional triple quadrupole tandem mass spectrometric detection (UHPLC-QqQ-MS/MS). The values of recovery (75-120%) and repeatability (10-15%) for both methods showed a good conformity to relevant EU guidelines. In general, the proposed UHPLC-Orbitrap-MS method indicated a sufficient correlation with the UHPLC-QqQ-MS/MS method. However, the results at levels close to the detection limit (3 μg kg⁻¹) of contaminants were significantly influenced by the mass resolution depending on the detection techniques. The developed HRMS method was applied for the analysis of fifty ground paprika samples containing blends of harvested in India and China sweet and hot paprika. The results of analysis demonstrated a ubiquitous presence of three mycotoxins (fumonisin B1, ochratoxin A, and sterigmatocystin), and a wide occurrence of twelve pesticide residues. In addition, a notable difference in concentration of fumonisin B1 was determined depending on the harvest period (2009 and 2013) reaching the maximum concentrations of 33 μg kg⁻¹ in sweet paprika. No significant correlation was found between the presence of mycotoxins and levels of pesticides with only exception of decreased fumonisin B1 content in samples with an elevated concentration of metalaxyl fungicide. Results of the current study demonstrated the proposed HRMS method as a promising high-throughput technique suitable for the assessment and control of mycotoxins in spices.

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Colonization of maize and rice grains in Costa Rica with *Fusarium* spp.

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A total of 17 and 22 maize and rice grain samples respectively meant for human consumption from Costa Rica were screened for seven *Fusarium* species and thirteen mycotoxins. Using a PCR-based method, it was possible to determine that *F. graminearum* (55 %) and *F. proliferatum* (91 %) were predominant in rice; while in maize *F. culmorum* (82 %), *F. verticillioides* (71 %) and *F. proliferatum* (82 %) were the most commonly detected fungi. *F. avenaceum* was not found in maize or rice samples and *F. poae* was found only in maize samples (41 %).

Mycotoxin quantification was performed by high performance liquid chromatography coupled with mass spectrometry (HPLC-MS/MS). The contamination level for each mycotoxin was highly variable in both rice and maize samples. In rice, zearalenone and beauvericin were found in 86 % and 64 % of the samples respectively, while only 27 % of samples contained fumonisin B1. Enniatins A, A1, B and B1 and apicidin were only detected in rice (5 % to 9 % of samples). In maize, most of the samples were contaminated with fumonisin B1 (94 %) and half of the samples with zearalenone (53 %) while only 18 % of the samples had nivalenol, deoxynivalenol and beauvericin. Although, in 41 % of the maize samples *F. poae* was present, none of the mycotoxins related with this fungus (trichotheccene A like t2-toxin and diacetoxyscirpenol) were found.

It is important to note that 24 % and 41 % of maize and rice samples respectively were contaminated with more than 0.1 mg/kg of zearalenone and 47 % of maize samples presented more than 1 mg/kg of fumonisin B1. One maize sample was contaminated with more than 9 mg/kg of fumonisin B1, results that should be considered for health safety reasons since maize is a staple food in Costa Rica.
Contamination of food and feed by Deoxynivalenol in the years 2010-2014 in Slovak Republic depending on weather conditions

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Mycotoxins are generally considered as unavoidable contaminants in food and feed and they are a serious problem worldwide. There is a great interest in these naturally occurring chemical substances because of their toxic, detrimental and sometimes carcinogenic effects on human health as well as on animal health. Presentation is aimed at pointing out the occurrence of Deoxynivalenol in food of plant origin and feed. Comparison of analytical data is evaluated for period 2010-2014.

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A highly specific enzyme immunoassay for the mycotoxin sterigmatocystin

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Sterigmatocystin (STC) is a mycotoxin, produced by several species within the genus Aspergillus and structurally related to the aflatoxins. The International Agency for Research on Cancer (IARC) has classified STC as a group 2B carcinogen. Compared to aflatoxins, data on STC occurrence in food, feed, and indoor environment are scarce. Although several analytical procedures have been developed for the determination of STC, rapid and inexpensive screening tests are not yet available. We therefore developed a simple and straightforward method for the detection of STC.

STC was derivatized with glycolic acid to its STC-glycolic acid-ether (STC-GE). The STC-GE was conjugated to keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA), and to horseradish peroxidase (HRP) via an activated ester method. Rabbits were immunized either with the STC-GE-KLH or the STC-GE-BSA. Specific antibody titer was screened in a direct EIA using the STC-GE-HRP and microtiter plates coated with anti-rabbit IgG in a double antibody solid phase system. The antibody specificity was determined by testing competitive binding inhibition by O-methylsterigmatocystin (OMSTC) and the aflatoxins (B, G, M). In a first application study, the toxin production profiles of five STC- or aflatoxin-producing Aspergillus spp. were tested.

A specific immune response could be detected in all rabbits. All antisera enabled a highly sensitive detection of STC, the 50% inhibition concentration (IC50) values of the standard curves were 0.5-2 ng/ml. The detection limit of the most sensitive test was in the range of 100 pg/ml. All sera were highly specific, and only minimal cross-reactivity was observed for OMSTC (approx. 0.1%) and the aflatoxins (approx. 0.01%). It is concluded that the EIA system described here is well suited as a rapid screening method to determine the STC-production ability of fungal species. Further application studies on the detection of STC in food and feed are in progress.
Comparison of two analytical methods for monitoring zearalenone in edible oils – results of in-house validation and investigation of commercially available samples

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Zearalenone (ZEA) is a nonsteroidal mycotoxin produced by several Fusarium species, which are commonly found on nearly every type of grain in Europe.

Because of its lipophilic properties ZEA concentrations in edible oils, particularly corn oils are often several times higher than in other cereal products. Notably, levels of ZEA have also been reported in wheat germ and soy bean oils. In the European Union a maximum level for ZEA in refined corn oil of 400 μg/kg is currently in place.

The fact that edible oils could form an important contribution to human ZEA exposure accentuates the need of validated analytical methods. Two analytical approaches are currently in consideration:

Method 1 is based on the dilution of the oil sample and subsequent liquid-liquid partitioning with a mixture of methanol and ammonium bicarbonate.

Method 2, more selective for ZEA, is based on solid phase extraction using hydrazine-functionalized particles in commercial SPE cartridges. The latter method was recently developed by the Federal Institute for Materials Research and Testing (BAM). Sample extracts obtained by both methods were measured using HPLC-FLD.

In this study we present the results of in-house validation of both methods according to CEN TR 16059. In addition to corn oils, soy bean, sunflower, wheat germ, sesame and pumpkin seed oils were analysed with the two methods.

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Detection of aflatoxin from some *Aspergillus* sp. isolated from wheat seeds

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Ten seed borne fungi (*Alternaria* sp., *Aspergillus* sp., *Aureobasidium* sp., *Cladosporium* sp., *Dreschslera* sp., *Penicillium* sp., *Rhizoctonia* sp., *Stemphylium* sp., *Mucor* sp. and *Rhizopus* sp.) were isolated and identified from two wheat varieties, the highest frequency of seed borne fungi was observed on wheat cultivar site Mol14 *Alternaria* sp. Their mean and standard deviation was (5.5± 1.69) while the lowest frequency fungal isolated was *Dreschslera* sp. and *Rhizopus* sp. Their mean and standard deviation was (0.1± 0.64). The aflatoxin-producing isolates appeared as gray or black colonies in the UV hotographs, whereas nonproducing isolates appeared as white colonies, the plate five colony four (P5CO4) showed the positive results which means the presence of aflatoxin as compared to the control which showed the negative results. Ammonium Hydroxide Vapor-Induced Color Change method used which the dish was inverted and 1 or 2 drops of concentrated ammonium hydroxide solution are placed on the inside of the lid. The undersides of aflatoxin-producing colonies quickly turn plum-red after the bottom of the Petri dish has been inverted over the lid containing the ammonium hydroxide as positive result in (P5CO4) and (P7CO4) observed. Essentially no color change occurs on the undersides of colonies that are not producing aflatoxin this indicate to the negative results (control). The main objective of this study is to isolation, identification and rapid detection of aflatoxin from wheat seed borne fungi.
Application of biochip array technology to the simultaneous screening of mycotoxins in animal feed

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Introduction
The determination of mycotoxins is important for food and feed safety as they are undesirable contaminants of cereal products and grass seeds and can cause adverse health effects in humans and animals. Biochip array technology allows the simultaneous screening of multiple analytes from a single sample, which consolidates testing and reduces the quantity of samples to be assessed by confirmatory analysis. This study reports the applicability of biochip array technology to the simultaneous screening of multiple mycotoxins from a single feed sample at or below regulatory limits.

Methodology
Simultaneous competitive chemiluminescent immunoassays, defining discrete test sites on the biochip surface and applied to the Evidence Investigator analyser, were employed. The system incorporates dedicated software to process and archive the multiple data generated. Mycotoxins were extracted from feed by a generic liquid/liquid extraction prior to application to the biochips.

Results
Aflatoxins, ochratoxin A, fumonisins, trichothecenes zearalenone, ergot alkaloids and paxilline were detected. The detection limits were at or below the regulatory limits in feed. Initial authentic feed sample comparisons (n=8) with LC-MS/MS showed 100% agreements for all analytes.

Conclusions
The results indicate applicability of biochip array technology to the simultaneous screening of multiple mycotoxins from a single feed sample. This represents a useful multi-analytical tool to increase the screening capacity in test settings.
Genome shuffling for zygomycetous fungi from tempeh against mycotoxins

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Feed and food contaminated with mycotoxins not only endanger the human health but also affect economical situation in terms of international trade for several countries. Based on losses and harm of mycotoxin, thus, controlling the mycotoxin level is urgently needed especially in developing countries. One promising biological strategy for reducing mycotoxin contamination is by enzymatic or microbial degradation, since it is efficient, specific, and environmentally friendly way of detoxification.

Genome shuffling is intensively applied for increasing production of metabolites by microbe strains, enhancing strains tolerance as well as improving substrate uptake. Genome shuffling may also be used to improve the capability of microbe on mycotoxins detoxification. This technique is initiated by constructing parent strains library. The use of natural genetic diversity as a starting point has been done by collecting forty five traditional inoculums (usar) or tempeh product (tempeh kedelee, dage, oncom, gembus, dablongan and other various tempeh type) from different traditional tempeh producers and traditional market areas in Java, Indonesia. Fungal purification has been conducted by modification techniques of single spore isolation, and mostly around two to four different strains have been identified by microscopic identification. Each traditional inoculum or tempeh product mainly consisted of Rhizopus and Mucor genera. Random amplified polymorphic DNA (RAPD) method has been established to distinguish identical and non-identical strains. Internal transcribed spacer (ITS) sequenced analysis will be used for further strains identification. Next, these wild strains will also be involved in the parental collection to generate the growth character of the complex progeny for mycotoxins detoxification purpose.
The combination of metabolic engineering and high resolution mass spectrometry sheds light on bikaverin biosynthesis in Fusarium fujikuroi

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The search for new, yet unknown secondary metabolites of occurring molds is an important step in peril prevention as these metabolites can be highly toxic and bioactive. However, exactly this bioactivity can also be advantageous for mankind, serving as new therapeutic agents and expanding the known structure pool for pharmaceuticals.

In this study, we have identified such an unknown metabolite in Fusarium fujikuroi, an ascomycetous fungus normally infecting rice plants. This fungus gained attention due to produced phytohormones, gibberellines, which constitute a virulence factor of the fungus but are nowadays also used as plant growth regulators in agriculture.

Since the genome of this fungus is fully sequenced, the identified metabolite could be assigned to the corresponding gene cluster through genomic engineering, showing that the substance is dependent on the bikaverin gene cluster. Although all genes for the biosynthesis of the PKS-derived pigment are known, only two bikaverin precursors, nor-bikaverin and pre-bikaverin, are established so far. We classified the new metabolite as a bikaverin precursor designated as oxo-pre-bikaverin and elucidated its structure by NMR. To decipher the whole bikaverin biosynthetic pathway and to overcome negative regulation circuits, the structural cluster genes BIK2 and BIK3 were overexpressed independently in the ΔΔbik2/bik3+OE::BIK1 mutant background (1) by using strong constitutive promoters. With the help of the software MZmine 2 (2), the metabolite spectra of the created mutants were compared, revealing further possible intermediates.

(1) Wiemann et al., Molecular Microbiology 2009, 72, 931–946.

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Diversity of ABC and MFS transporters in phytopathogenic fungi involved in the export of toxic substances

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Survival of microorganisms can depend on the development of various resistance mechanisms against different synthetic and natural antifungal agents e.g. mycotoxins, fungicides and drugs. Mechanisms of resistance to toxic compounds relate to qualitative factors such as the absence or presence of a sensitive target site or to quantitative factors such as uptake, transport, storage and metabolism. The broad-specificity, adaptable drug transporters, of ABC and MFS classes, represent quantitative factors that can provide protection of organisms against natural mycotoxins and fungicides. In plant pathogens, their action modulates the baseline sensitivity to fungicides, contributing to multidrug resistance (MDR). The ABC (ATP-binding cassette) proteins constitute some of the key determinants of azole sensitivity of laboratory strains of model species (e.g. C. albicans, A. nidulans, M. graminicola). The MFS proteins are the second group of transporters involved in the transport of various compounds. The role of MFS transporters in sensitivity and resistance to toxic compounds is also supported by experimental evidence (e.g. FLR1 resistance factor of baker's yeast S. cerevisiae).

Our research highlights polymorphism and expression patterns of ABC and MFS transporters in the diverse strains of phytopathogenic species of the Fusarium genus. As part of the research we conducted bioassays, PCR and gene expression experiments on sensitive and resistant fungal strains. Our experiments aim to correlate morphological changes in mycelia with polymorphism of candidate resistance genes and their expression in stress conditions. To confirm the participation of selected genes in the development of resistance through overexpression of existing transporter variants – the susceptible strains were subjected to selection pressure due to application of toxic compounds and the resulting changes in expression of potential resistance genes were examined.

Research funded under the project: “Molecular diagnostics of fungicide resistance in phytopathogenic fungi” LIDER/27/204/L-3/11/NCBR/2012.
Penicillium expansum growth and patulin (PAT) production mainly occur during long-term storage of apples. Isoepoxydon dehydrogenase, encoded by the *idh* gene, is a key enzyme in the patulin biosynthesis pathway. In this study, an RT-qPCR technique was developed to measure *idh* gene expression. *Idh* expression was compared to PAT production and growth of *P. expansum* on apple puree agar medium (APAM), under different storage conditions.

Three *P. expansum* strains were one-point inoculated onto APAM plates. The plates were stored under three conditions: 20°C–air, 4°C–air and 4°C–controlled atmosphere (CA; 3%O₂, 1%CO₂). Samples were taken when colony diameters reached 0.5cm and 2cm. An RT-qPCR was developed to quantify the *idh* gene relative to three housekeeping genes (*18S, β-tubulin* and *calmodulin*). RNA was extracted from all samples and, after DNase treatment, converted to cDNA which, in turn, was used as a template for qPCR. In parallel, the samples were analyzed for their PAT content by means of HPLC-UV (LOD = 3μg/kg).

At 4°C–CA, APAM plates of *P. expansum* colonies at a growth stage of 0.5cm contained less than 50μg/kg PAT. At 4°C and 20°C–air, PAT concentrations were in the range of <LOD–500μg/kg and 800–2,500μg/kg, respectively. PAT of 2cm large colonies at 4°C–CA accumulated to a range of 10,000–30,000μg/kg. Storage at 4°C and 20°C–air led to an increase of PAT to ranges of 20,000–60,000μg/kg and 80,000–300,000μg/kg, respectively. In order for PAT to be produced, *idh* needs to be expressed. Colonies grown at 4°C–CA, producing less than 50μg/kg on APAM, showed very low *idh* expression levels (<0.05A.U.). All others grown at cold storage showed low *idh* expression (0.70–2.55A.U.), while at ambient storage higher expression profiles (1.80–7.34A.U.) were observed. The results may be explained by a delayed metabolism under stress conditions (low temperature and/or oxygen). A good correlation was found between *idh* expression and patulin production. Our data confirm storage to have an effect on patulin production, by acting at the transcriptional level of the tested gene.

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Comparison of the patulin production of *Penicillium expansum* isolates from apples, under different steps of long-term storage

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*Penicillium expansum* growth and patulin (PAT) production mainly occur during long-term storage of apples. Long-term storage involves a long period (≥3 months) of controlled atmosphere storage (CA; 0.5–3.5°C, 1–3%O2, <1–3%CO2), followed by a short period under cold air. After delivery at the apple juice producer, apples are kept for 1 day maximum at ambient temperature before processing. The aim of our study was to investigate the extent by which each successive step during long-term storage contributes to the PAT production by *P. expansum* strains.

Fungal isolates from Belgian apples of different orchards/industry were collected and identified to species level by means of random amplification of polymorphic DNA (RAPD) and β-tubulin gene sequencing. All 27 *P. expansum* field isolates and 7 reference strains were one-point inoculated onto apple puree agar medium (APAM) plates. After 5 days at 25°C, PAT content was determined by HPLC-UV. Subsequently, 6 isolates were selected and one-point inoculated onto APAM. The plates were first stored for 2 months at 1°C–CA (3%O2, 1%CO2) (step 1), after which some plates were transferred for 3 days to 1°C–air (steps 1+2), and finally for 1 day to 20°C–air (steps 1+2+3). After step 1, 1+2 and 1+2+3, plates were analyzed for their PAT content.

*P. expansum* and *P. solitum* were identified as the most prevalent *Penicillium* species associated with Belgian apples. Although rather small differences were found in the genetic profiles of the RAPD dendrogram of the *P. expansum* group, a high diversity in PAT production could be observed. Two high, 2 average and 2 low PAT producers were selected to investigate the effect of each successive step of long-term storage on the *in vitro* PAT production. No significant differences between the storage steps were observed but PAT production seemed highly strain dependent. A high spore inoculum may lead to a strong PAT accumulation already during the first step of long-term storage at 1°C–CA.

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Blocking of some stages of melaninogenesis can enhance aflatoxin B1 production in *Aspergillus flavus*

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Both aflatoxin B1 (AFB1) and melanin produced by *Aspergillus flavus* are products of a polyketide biosynthesis. Inhibition of AFB1 biosynthesis reduces the harmfulness of *A. flavus*, whereas blocking of melanin biosynthesis is able to reduce the viability of this fungus. Thus, the development of a complex preparation blocking both AFB1 and melanin production in *A. flavus* is a point of practical interest.

As we have shown earlier, some phosphoanalogues of amino acids and peptides are able to regulate polyketide biosynthesis in phytopathogenic fungi. The purpose of this study was to screen such compounds for potential inhibitors of the AFB1 and melanin production in *A. flavus*. As a result, two different groups of inhibitors have been revealed:

**I. Inhibitors of AFB1 biosynthesis**
1. \( \text{CH3(NH2)-P(S)(OH)2} \)
2. \( \text{CH3-CH(NH2)-P(S)(OH)2} \)
3. \( \text{CH3-CH(NH2)-P(O)(OC2H5)OH} \)
4. \( \text{CH3-CH(NH2)-CO-NH -CH(CH3)-P(O)(OCH3)OH} \)

**II. Inhibitors of melaninogenesis**
5. \( \text{CH3-CH(NH2)-P(O)(OH)2} \)
6. \( \text{NH2-CH2-CH2-CH2-ONH2} \)
7. \( \text{D,L-Ala-P(O)(CH3)OH} \)
8. \( \text{NH2-CH2-CH2-CH2-NHOH} \)

Compounds 1-4 reduced AFB1 production by *A. flavus* up to 6 times as against the control, but did not influence on a colony pigmentation. Compounds 5-8 caused colony discoloration and, at the same time, significantly enhanced the AFB1 production. It is known that AFB1 and melanin biosynthetic pathways have common initial stages and then diverge. Colony discoloration could be caused by blocking of melaninogenesis after the divergence point (see figure); due to such blocking, toxin production is increased. The further testing of different combinations of compounds from the groups I and II will probably allow us to develop a complex preparation able to block both toxin and melanin production.

**Caption:** Simplified scheme of the aflatoxin B1 and melanin biosynthetic pathways in *Aspergillus flavus*.

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Activation of the Beauvericin Cluster through deletion of the Histone Deacetylase Ffhda1 in *Fusarium fujikuroi*

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Gene regulation by chromatin modifications is a promising mechanism to express silent and almost silent fungal secondary metabolite gene clusters. Recent genome sequencing of the rice pathogen *Fusarium fujikuroi* revealed the presence of 45 putative secondary metabolite gene clusters of which most are cryptic and not expressed under laboratory conditions. The production of several well-known secondary metabolites of this fungus was recently shown to be controlled by the histone deacetylases Hda1 and Hda2. In this work we detected a strong peak in the $\Delta$HDA1 deletion mutant using high performance liquid chromatography coupled to a diode array detector (HPLC-DAD). Further analyses of this unknown compound revealed that it is the cyclic peptide beauvericin. The use of the $\Delta$HDA1 mutant led to significant up-regulation of the respective non-ribosomal peptide synthetase (NRPS) gene and adjacent genes as well as a 1000-fold increased beauvericin yield compared to the wild type. Mutation of a conspicuous DNA-binding motif in the promoter region of the NRPS gene resulted in significant elevation of product yields suggesting that a yet unknown repressor controls the expression of cluster genes.
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Pathogenicity and mycotoxin profiles of *Fusarium* isolated from tomato

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Tomato is one of the important economic vegetable crops which is attacked by several diseases among which are caused by *Fusarium* species. With respect to Nigeria, consistent field observations cum speculations reveals that, apart from *F. oxysporum*, there are other suspected and unidentified *Fusarium* species responsible for tomato diseases. We therefore hypothesized that other soil-borne *Fusarium* species other than *F. oxysporum* may have or not overtime established relationship with commercial tomato cultivars in Nigeria, knowing fully well that host infection can be either asymptomatic or symptomatic which may directly or indirectly influence the amount of mycotoxins produced. Based on this, we prioritise pathogenicity potentials of isolated *Fusarium* species and their mycotoxin status with or without tomato plants. *Fusarium* species were isolated from the soil samples collected from tomato growing fields and rotten tomato fruits in Southwestern Nigeria. Following standard protocols, greenhouse inoculation shows that suspected isolates significantly (P<0.05) retarded seed germination and causes seedling diseases of both tomato cultivars tested, though more on UC82B cultivar. Symptoms of diseases begin in seedling as leaf curl, slight vein clearing on outer leaflets with green colour gradually turning yellow and eventually affected the seedling before maturity. Taxonomical analysis and mycotoxin profiles indicate that *F. oxysporum* and strains of a yet undescribed clade of *G. fujikuroi* species complex is responsible for the disease.

The investigation was supported by TWAS-DFG programme – Nigeria cooperation visits to Germany.
Fusarium graminearum, *F. cerealis* and *F. culmorum* are major producers of trichothecenes B, the presence of which in human diet and feedstuff is of concern because these fungal metabolites can harm the health of farm animals and humans when infected grain is ingested. Acetylation of DON on carbon 3 is regarded as a self-protection mechanism of DON producers. It is hypothesized that in the so-called DON chemotype of *F. graminearum*, the acetyl group on C3 is removed during active export of the metabolite from fungal mycelium. Other chemotypes of trichothecene B producers are 3-ADON chemotype, which produce predominantly 3-ADON, 15-ADON chemotype, which produce predominantly 15-ADON, and NIV chemotype, which produce NIV and fusarenon X (Fus X). NIV chemotypes also produce low amounts of DON. 3-ADON and 15-ADON chemotypes also produce DON, the amount of which exceeds the amount of 3-ADON and 15-ADON in some strains.

The cause of the existence of chemotypes in *F. graminearum* and other DON/ADON producers are differences in the specificity of acetyltrichothecene esterase (Tri8), which has 3,15-diADON as a substrate. Esterase produced by 3-ADON chemotype preferentially removes acetyl from carbon 15, while esterase produced by 15-ADON chemotype preferentially removes the acetyl group on carbon 15. The region of the protein responsible for the specificity towards carbon 3 or 15 has been identified in the work of Alexander et al. (2011).

To study Tri8 biochemically, we expressed the Tri8 gene from different *Fusarium graminearum* strains and in *E. coli* and enriched the protein both in its native and His-tagged form. In spite of testing many constructs and diverse conditions, we were never able to detect enzymatic activity. We therefore switched to a yeast expression system used by Alexander at al. (Fung. Genet. Biol. 2011, 48:485-495). Preliminary results on the yield and activity of Tri8 in different expression systems will be presented.
The role of fungicides, their influence on expression of key efflux and mycotoxin biosynthetic genes in the control of resistant Fusarium strains

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Development of increased resistance to fungicides has become an important factor, not only because of limiting their efficiency, but also due to the concern about the influence of chemical treatments on the mycotoxin production. It is known that external parameters such as temperature, water activity, pH, light and variety of stresses can have a significant impact on the secondary metabolism. As a rule, the mycotoxin biosynthetic genes are not constitutively expressed, instead being induced in certain growth phases (e.g. sporulation) or under specific culture conditions (such as oxidative stress resulting from hydrogen peroxide addition or presence of particular sugars in the medium).

We demonstrate the expression patterns of mycotoxin biosynthetic genes (\textit{Tri5} – trichothecene synthase, \textit{Tri6} – trichothecene transcription factor, \textit{Tri12} – trichothecene efflux pump, \textit{Zea2} – zearalenone synthase, \textit{Zeb2} – zearalenone transcription factor and \textit{Zra1} - zearalenone efflux pump) in the fungal strains treated with the different fungicide substances (flusilazole, carbendazime).

The past results pointed to non-lethal amounts of fungicides, leading to a possible increase in mycotoxin biosynthesis (e.g. suboptimal doses of triazole fungicides transcriptionally activate the trichodiene synthase gene in \textit{F. graminearum}). Our accumulated results show, that even a very effective fungicide (carbendazim) applied to a full-grown pathogen, can result in enhanced mycotoxin production by surviving mycelium. Thus, the expression of mycotoxin biosynthetic genes is strongly influenced not only by the amount or the type of antifungal compound, but also the timing of fungicide exposition relative to mycelium growth.

Research funded under the project: “Molecular diagnostics of fungicide resistance in phytopathogenic fungi” LIDER/27/204/L.3/11/NCBR/2012.
Molecular and biochemical characterization of patulin producing and non-producing Penicillium species in apples fruits from Morocco

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In recent years, there has been a growing interest in the study of the basic biology and genetics of toxigenic Penicillia because of their natural occurrence in food products and of the toxic effects of their secondary metabolites on humans.

Among Penicillium species, Penicillium expansum is a well-known postharvest pathogen causing decay of apple fruits during storage and it is also responsible for patulin biosynthesis, a mycotoxin that exhibits a number of toxic effects in mammals.

In this study, authors report the isolation of patulin-producing Penicillia in apples collected in different markets in four localities in Morocco.

Fungi were identified by ITS-sequencing and further characterized using a specific PCR-based method targeting the isoeoxydon dehydrogenase (IDH) gene to discriminate among patulin-producing and non-producing strains. Patulin production was also determined using a reversed-phase, diode-array-detection, high-performance liquid chromatography (RP-DAD-HPLC).

Sequencing data showed that 79.5% of contaminant fungi belonged to Penicillium genus and P. expansum was the most isolated species followed by P. chrysogenum (~9.7%), P. crustosum (~6.4%) and P. polonicum (~3.3%).

Molecular analysis revealed that 67.5% of the Penicillium species produced the expected IDH-amplicon denoting patulin production in these strains. However, patulin production was not chemically confirmed in all P. expansum and the isolation of IDH-/patulin+ strains poses the hypothesis that the gentisylaldehyde is not a direct patulin precursor supporting previous observations that highlighted the importance of the gentisyl alcohol in the production of this mycotoxin.

Total agreement between IDH-gene detection and chemical method employed was observed in 54.8% of the P. expansum and for 100% of the other species.

Overall the data reported here showed considerable epidemiological differences and a substantial genetic variability within P. expansum population from Morocco.

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*Fusarium graminearum* secondary metabolism and lysine biosynthesis: Role of *PPT1*

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The phytopathogen *Fusarium graminearum* produces numerous secondary metabolites such as zearalenone and aurofusarin via polyketide synthases (PKS) and siderophores by nonribosomal peptide synthetases (NRPS). Former research showed that the inactivation of the biosynthesis of single secondary metabolites does not decrease virulence significantly. Yet, all PKSs and NRPSs are inactivated simultaneously by knocking out *PPT1*, a phosphopantetheinyl transferase which is responsible not only for posttranslational modification of PKSs and NRPSs, but also for modification of the lysine biosynthesis enzyme alpha-aminoadipate reductase (AAR) encoded by *LYS2*, thus leading to a lys auxotrophic phenotype. In this project we plan to impair the fungus’ secondary metabolism by PKS and NRPS while maintaining lysine biosynthesis. The re-establishment of lysine production is of great importance as lysine auxotrophy significantly reduces virulence of *Fusarium graminearum*, which infects mainly grains and cereals that contain only low amounts of lysine. To uncouple lysine biosynthesis from modification of PKSs and NRPSs we are replacing the gene pair *Fg-PPT1* and *Fg-LYS2* by its homologs from *Saccharomyces cerevisiae* (*Sc-LYS5* and *Sc-LYS2*). *Sc-Lys5* modifies Lys2 (inactive AAR), specifically at conserved residues, with the phosphopantetheinyl moiety, but presumably does not act on PKSs and NRPSs. Hence the integration of the *Saccharomyces cerevisiae* genes is believed to restore lysine biosynthesis but to impair the fungus in activation of NRPSs and PKSs. The resulting strain will be used for virulence experiments to test the hypothesis that secondary metabolites are collectively necessary for pathogenicity.
Whole genome shotgun sequence analysis of the ascomycete *Penicillium verrucosum*

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Recently we have performed a whole Genome Shotgun sequencing of the wheat contaminating ascomycete *Penicillium verrucosum* BFE808 using the NGS Illumina Technology (60 x coverage).

Subsequent analyses of the genome sequence, which has about 32 MB, using e.g. Augustus, SeqManNGen and the antismash algorithm, we could identify 41 secondary metabolite biosynthesis gene clusters. Of this gene clusters 14 belong to the PKS type, 9 to the NRPS and 7 to the fatty acid or terpene type and 11 could not yet be further specified. Moreover we could identify the gene clusters for ochratoxin and citrinin biosynthesis. Future analyses of the draft genome sequence will give a deeper insight in secondary metabolite biosynthesis of *P. verrucosum* in comparison to other ochratoxin or citrinin producing fungi.

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**Fusarium graminearum** has been reported to be able to manipulate ethylene signalling to increase virulence. Ethylene, a plant hormone, is produced from the immediate precursor 1-aminocyclopropane carboxylic acid. ACC is formed from methionine, catalysed by ACC-synthase. ACC-synthase and ACC-deaminase candidate genes were identified in the genome of *Fusarium graminearum*. As an important stress response, ethylene is formed after wounding, infection and high auxin levels. It has been reported that ACC-synthases are very instable proteins and not easy to handle *in vitro*. Therefore an *in vivo* assay was set up in a modified *Escherichia coli* T7-express strain. Since *Fusarium graminearum* contains an active ACC-deaminase, which degrades ACC to α-ketobutyric acid (KBA), we generated knock-out strains to test the virulence of the mutant. One of the ACC-deaminase candidate genes turned out to be a cysteine desulphydrase, while the other had the expected activity. ACC synthase candidate genes are already knocked out as well, virulence testing is in progress.
Synthesis of Deoxynivalenol-15-β,D-glucoside

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Mold-infested plants are able to modify Deoxynivalenol (DON) as part of their defense against xenobiotics. The phase II detoxification process of plants includes conjugation reactions such as glycosylations. These altered forms of DON belong to the broader group of so called “masked mycotoxins” that are neither recognized by routine screenings nor regulated by legislation. Nevertheless they are harmful for mammals as hydrolysis during digestion can convert them back to their toxic precursors. Due to the lack of reference materials, detection as well as quantification is still a problem or even impossible for most masked mycotoxins. Therefore we decided to develop a reliable strategy for the synthesis of Deoxynivalenol-15-β,D-glucoside.

Starting from glucose pentaacetate (1) an appropriate acetimidoyl donor was prepared by deprotection of the anomeric center with benzylamine, followed by a reaction with N-phenyltrifluoroacetimidoyl chloride. This glucosyldonor 2 was then used in a Lewis acid mediated glycosylation of 3-Acetyldeoxynivalenol (4) to obtain acetyl protected 3-ADON-15-β,D-glucoside 5 in good yields. Final acetyl-deprotection of compound 5 afforded DON-15-β,D-glucoside.

3-Acetyldeoxynivalenol (4) could be synthesized in a one pot reaction from DON by selective protection of position 7 and 15 with phenylboronic acid, followed by acetylation of position 3. Cleavage of the phenylboronic protecting group by transesterification with methanol afforded 3-ADON (4).

Caption: Synthetic route to DON-15-glucoside
Analysis of *Alternaria* mycotoxin production influenced by temperature, incubation time and substrate

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The genus *Alternaria* is known to form a broad spectrum of possibly human or animal health endangering mycotoxins, occurring as natural contaminants throughout the food chain. The production of these Alternaria toxins is dependent on the species and a complex interaction of various environmental factors e.g., temperature or moisture content, that are not fully understood\(^1\).

The aim of this study was to determine the influence of temperature (7°C, 25°C), substrate (rice, wheat kernels) and incubation time (4, 7, 14 days) on the production of the mycotoxins alternariol (AOH), alternariol monomethyl ether (AME), tentoxin (TEN), altenuene (ALT), altenuisol (ATL), tenuazonic acid (TeA), altenuic acid III (AA III), altertoxin I (ATX I), altertoxin II (ATX II), stemphyllotoxin III (STTX III) and AAL toxin by three different Alternaria isolates from the species groups *Alternaria infectoria* and *A. tenuissima*.

Experiments were performed in triplicate and samples were extracted with an acetonitrile-water-acetic acid mixture. ESI-HPLC-MS/MS was used for the quantification of the Alternaria toxins.

The results showed that TeA is the most extensively produced toxin under nearly all conditions. AAL toxin was not detected at all.

At 7 °C only TeA, AOH, AA III and STTX III could be detected. In comparison to this all examined toxins were found at 25 °C.

The incubation time had different effects on the production of some toxins. For example, the TEN production increased constantly from day 4 to day 14 and in contrast to this the TeA production increased first from day 4 to day 7 and decreased from day 7 to day 14.

The substrate only marginally influenced the mycotoxin patterns of the three *Alternaria* strains. The two *A. tenuissima* strains produced high amounts of toxins at 25 °C (e.g. approx. 9000 mg/kg AOH) and in contrast to this the *A. infectoria* strain could only produce low amounts (e.g. 0.4 mg/kg AOH) except for STTX III which was produced up to 500 mg/kg.

Characteristics of selected strains of *Stagonospora nodorum* detected in Poland in terms of necrotrophic activity and production of proteinaceous toxins.

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*Stagonospora nodorum* is one of the major pathogens of wheat and triticale, and causes major losses. Following infection causes necrotic areas in which they are growing and from which spreads to the same plant, as well as to neighboring plants. The appearance of this pathogen during high humidity and temperature can lead to a serious reduction in the yield by reducing the photosynthetically active surface of the leaf and damage to the chaff. In the last few years there have been reports of production by *S. nodorum* protein 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. The positive 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. There were no negative effects of the elimination of the dominant allele of susceptibility to the toxin, while the increase in resistance was found, which positively corresponds with the research Oliver and others (2014). Until now seven different proteinaceous toxins have been described. For genes encoding three of them the nucleotide sequence have been published and the population studies on the global sample of isolates of *S. nodorum* are being performed. Poster shows data on isolates from the Polish and complementary vision of Europe's population of *S. nodorum*. Among the tested isolates the most numerous group consists of isolates producing Tox1 and Tox3. The two toxins are produced by approximately 80% of the isolates, and ToxA by 9.5%. The results confirm literature data on European populations of *S. nodorum*.

Selected isolates producing different toxins were grown in liquid medium and the resulting culture filtrates were tested for necrotic activity and fractionated on ion exchange columns to obtain partially purify the toxins. Toxicity of separated fractions was tested on the leaves of selected wheat varieties to examine the selective susceptibility / resistance. The HPLC chromatograms of selected culture filtrates and partially purified fraction were registered and selected toxins were identified.

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Urinary biomarkers of citrinin and ochratoxin A exposure in two Bangladeshi cohorts: follow-up study on regional and seasonal influences

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Biomonitoring studies provide valuable insights into human mycotoxin exposure, especially when food contaminant data are scarce or unavailable. Our first analysis of citrinin (CIT) biomarkers in urines of two Bangladeshi cohorts indicated frequent exposure, and higher biomarker levels in the rural cohort than in samples of German adults [1]. This interesting result for CIT and a possible co-exposure to ochratoxin A (OTA) led us to conduct a follow-up study with analysis of biomarkers for both nephrotoxic mycotoxins. Urine samples were collected on two occasions (summer and winter season) from a rural and an urban cohort in the Raishahi district of Bangladesh, and analyzed by targeted methods.

Nearly all samples (90-97%) had detectable levels of CIT biomarkers (CIT and its metabolite HO-CIT), with clearly higher levels in urines collected in winter compared to summer season. In both sampling periods, total CIT biomarker concentrations were significantly higher in the rural cohort than in the urban cohort. OTA was also detected in most urine (80-100%), more frequently than OT-alpha (47-69%). The mean OTA level in winter urines of the rural cohort were significantly higher than in their summer urines; in the urban cohort no seasonal difference was found for low OTA levels. Statistical analysis of food frequency questionnaire data of the participants did not show significant association between urinary biomarkers levels and intake of certain types of food, except for a positive trend of correlation with high rice consumption.

Frequent detection of OTA and CIT biomarkers indicates widespread mycotoxin exposure in both cohorts. The season, region and food consumption pattern, are apparently major determinants for exposure to nephrotoxic mycotoxins in the population of Bangladesh.


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Investigations on biomarkers of citrinin and ochratoxin A exposure in humans

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Biomonitoring can serve to assess human exposure to citrinin (CIT) and ochratoxin A (OTA). OTA analysis in biological fluids is widely used, yet few data exist on the temporal variability of this biomarker in individuals. For CIT, biomarker-based analysis has to be developed further, as metabolism of CIT in humans has not been studied.

CIT, OTA and their metabolites dihydrocitrinone (HO-CIT) and ochratoxin alpha (OTα) were analysed by validated methods in blood and urine of two male volunteers (A, B) on their usual diet to obtain profiles over an extended time and to gain insight into individual biomarker variability. Urinary excretion kinetics of CIT and HO-CIT were studied in a female volunteer (C) who ingested 4 μg CIT, a non-toxic dose.

Analyte levels in plasma and urine of person A showed small fluctuations over the study period and mean concentrations of OTA, CIT and their metabolites were clearly lower than in person B. Biomarker levels in plasma of both males were considerably higher than those found in their urines, except for OTα which person B excreted at much higher levels than person A, possibly due to more efficient conversion of OTA to OTα. In person C, low pre-dosing levels of CIT and HO-CIT in urine increased soon (2h) after CIT ingestion, due to rapid mycotoxin absorption and subsequent metabolism. Urinary biomarker levels continued to increase during the next phase (up to 20h) and strongly declined thereafter. Within a day, about 36% of the ingested dose was excreted in urine, about 12% as CIT and the larger part (24%) as metabolite HO-CIT, therefore a useful second biomarker of exposure.

This first data on the kinetics of CIT in humans indicate efficient conversion of CIT to its less toxic metabolite HO-CIT and renal excretion of a considerable fraction of ingested mycotoxin. Moreover, it can be concluded that interindividual variability for the investigated biomarkers reflects both dietary exposure and internal disposition of ingested mycotoxins.

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The impact of deoxynivalenol on pigeon health: occurrence in feed, toxicokinetics and interaction with salmonellosis

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Deoxynivalenol (DON) is one of the most frequently detected mycotoxins in feed. This study is the first to assess the exposure and impact of DON on pigeon health (Columba livia). Therefore, the aims were 1) to determine the occurrence of mycotoxins in pigeon feed; 2) to assess the toxicokinetic characteristics of DON in pigeons; and 3) to evaluate the impact of DON on the susceptibility of pigeons for Salmonella infection.

A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was used to analyse the presence of 21 mycotoxins in 10 commercially available pigeon feed samples. The results demonstrated that DON was one of the most frequently detected mycotoxins, contaminating 5 out of 10 samples (range 176.8 - 1465.9 µg/kg). For the toxicokinetic study, 10 pigeons were administered DON (0.3 mg/kg body weight) both orally and intravenously in a two-way cross-over design. Blood was collected at different time points post administration. The concentration of DON in plasma was analysed by a validated LC-MS/MS method. Toxicokinetic analysis revealed that the absolute oral bioavailability (F) of DON in pigeons was low (30.4%), and comparable to other poultry species. DON showed a rapid clearance and a rather low volume of distribution, resulting in a short elimination half-life. The low F suggests that intestinal epithelial cells are exposed to significant DON concentrations that eventually may affect intestinal translocation and colonization of bacteria. Consequently, the effect of feeding a DON contaminated diet (3.5 mg DON/kg feed) on the susceptibility for infection with a pigeon-adapted Salmonella Typhimurium variant Copenhagen strain, was assessed in an experimental infection model based on Pasmans et al. (2003).

DON increased the severity of the macroscopic lesions related to salmonellosis in small intestine, liver, spleen and kidneys, however, without showing an effect on the Salmonella numbers in these organs.

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Effects of a lipopolysaccharide (LPS) stimulus in pigs chronically exposed to dietary deoxynivalenol (DON): Metabolic and hematological consequences.

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Investigating a potential modulatory effect of the Fusarium toxin deoxynivalenol (DON) to a subsequent immunological stimulus in pigs was one of the main objectives of the present project.

In our trial a total of 44 barrows were examined of which 20 were chronically exposed to a DON contaminated maize-based diet (approx. 4mg DON/kg feed) and 24 to an uncontaminated control feed. Pigs were equipped with different catheters (A. carotis comm., Vv. jugulares, V. portae hepatis) to facilitate pre- or post-hepatic treatments (LPS, 7.5 μg/kg BW; control, 0.9% NaCl) and blood sampling. This resulted in two feeding groups (CON or DON), which are each divided into three infusion groups (CONjug-CONpor, CONjug-LPSpor, LPSjug-CONpor). Frequent blood samples were taken from the different catheters before, during and after infusion (−30, +15, +30, +45, +60, +75, +90, +120, +150 and +180 min). Subsequently analyses for blood gases, electrolytes, pH, lactate (GEM4000, Werfen) and red hemogram (Celltac, Baumann Medical AG) were performed. Data were evaluated by PROC MIXED in SAS with group, catheter and time as main factors as well as their interaction.

Erythrocytes, hematocrit, hemoglobin and electrolytes were not affected by DON and LPS. In LPS infused groups pO₂ decreased overall (p<0.05) whereas pCO₂ was reduced only in arterial blood. DON had no effect on pO₂ and pCO₂. Irrespective of catheter localization pH was decreased (p<0.01) and lactate was increased (p<0.01) in LPS-groups, indicating an emerging lactic acidosis. All LPS infused groups reached maximum lactate levels at 180 min. The increase in lactate started earlier in DON-fed pigs than in the CON-fed, which became obvious at 75 and 150 min in groups DON_LPSjug-CONpor and DON_CONjug-LPSpor, respectively.

Chronic DON-feeding alters the porcine pathophysiological response to a subsequent LPS stimulus dependent on infusion site (pre- or post-hepatic).
Intraepithelial processes of dendritic cells in pigs exposed to the mycotoxin deoxynivalenol

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The mycotoxin deoxynivalenol (DON) is contaminating crops and has effects on the intestinal immune system. DON-exposure inhibits maturation and function of dendritic cells (DC), thus affecting the initiation or suppression of a mucosal immune response (Bimczok et al., Immunobiology, vol. 212 pp 655-666, 2007). There is evidence that DC extend processes into the gut epithelium to sample luminal antigens. We conducted an in vivo study with pigs (~40kg BW) fed a DON-containing diet (4 mg DON/kg feed) and infused with either NaCl or LPS (7.5 μg/kg BW) at the end of the experiments. Paraffin-embedded sections of jejunum were analysed microscopically with MHC II and laminin antibodies to estimate DON effects on intraepithelial DC-processes. Signals of intraepithelial MHC II immunoreactivity indicating DC-processes per 1000 μm basement membrane were counted in the epithelium. In DON-fed pigs the immunoreactivity was markedly reduced in comparison to control animals. After systemic intravenous LPS challenge the signal frequency was higher in control-fed pigs than in DON-fed. Further work is under progress to characterise the intraepithelial MHC II positive processes with respect to the interaction between DC and epithelial cells.
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Combined effects *in vitro* of mycotoxin binary mixtures

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Mycotoxins commonly contaminating food and raw materials occur simultaneously. Since a single fungus can produce an array of toxins and a plant can be infected by a few different fungi simultaneously, many mycotoxin interactions are possible. Several classes of fungal secondary metabolites are considered to be highly toxic. Although the toxic properties of single compounds are relatively well defined, the toxicity of mycotoxin mixtures still needs evaluation.

Thus the aim of this project was to examine the cytotoxicity of different food and feed relevant mycotoxins individually and in combinations.

The *in vitro* studies were performed on a swine kidney (SK) cell line as well as kidney epithelial cells from an African green monkey (Vero). Cytotoxicity of citrinin (CIT), ochratoxin A (OTA), deoxynivalenol (DON) and T-2-toxin was evaluated with MTT test.

The SK cells were more sensitive to T-2 toxin (IC$_{50}$ = 5.3 ng/ml) compared to Vero cells (IC$_{50}$ = 42.5 ng/ml), whereas the latter cell line was more sensitive to DON (IC$_{50}$ Vero = 1010 ng/ml, IC$_{50}$ SK = 2140 ng/ml, respectively). The cytotoxicity of OTA and CIT was similar in case of both cell lines. The level of cytotoxicity turned out to be as follows: T-2 > DON > OTA > CIT – irrespective of the cell line. Binary mixtures testing revealed possible synergistic effects of the examined mycotoxins.

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Impacts of deoxynivalenol contaminated feed on intestinal integrity and immune response in chickens

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Deoxynivalenol (DON) is a common feed cereal contaminant with a wide public health concern due to its high toxigenic potentials. Once ingested, the DON primarily targets and impairs the functioning of the epithelial cells lining of the gastrointestinal tract, which, as the first line of defense, is of paramount importance for host's health. Poultry are considered tolerant to the adverse impacts of DON. Therefore, it could be suggested that poorer quality grain is probably diverted to poultry feed which would probably result in a higher incidence and higher level of DON. The full effects of Fusarium mycotoxins are still not known and up to now the biological mode of action of DON is not clear. It may be related to the reduced protein synthesis by binding to the 60S subunit of eukaryotic ribosomes and impairment of the function of the peptidyl transferase or to the decreased of nutrient and energy availability at the cellular level. Therefore, the mode of action of Fusarium toxins in chickens need further research for being better understood.

In our recent studies, it was found that DON could modulate the gut health by altering the intestinal morphology, electrophysiology, immunology, oxidative stress and expression of nutrient transporters and immune relevant genes. Additionally, it is documented that oral exposure of DON reduced the height and width of intestinal villi which in turn resulted in a decrease in the absorptive surface area. Furthermore, oral DON exposure led to down-regulation of expression of the mRNA of SGLT1, GLUT1 and GLUT2.

Moreover, DON induced lipid peroxidation and oxidative stress of the intestinal cells as thiobarbituric acid reactive substances (TBRAS) was increased after DON exposure. These alternations may explain the reduction of glucose absorption and uptake by the intestines after oral DON exposure. These DON associated changes in the chicken gut could also affected the tight junctions of the intestinal epithelial cells and potentiate the entrance of pathogenic bacteria and viruses resulting in infectious diseases.

Taken together, oral exposure of chickens to DON mycotoxin adversely affects the gut health in the terms of intestinal morphology, expression of nutrients transport genes and expression of immune genes. DON also reduced the cytokines production and vaccinal immune response as indicated by its low level in the blood of chickens after oral exposure. It can be concluded that DON severely compromises several crucial intestinal functions. Consequently, it may increase the susceptibility to enteric infectious diseases and may have harmful sequelae for the health of both animals and humans.
Systemic effects of ergot alkaloids on piglets

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Members of the fungal genus Claviceps infest grass species, including cereal grains, and can produce ergot alkaloids (EA), which might exert adverse effects on humans and animals. In monogastric animals, such as pigs, EA can induce decreased feed intake and liver biochemical changes. Immunosuppressive effects have been reported in pigs fed mycotoxins contaminated diets; however data concerning systemic EA effects are scarce. The aim of the present study was to investigate the effects of EA on histology of intestine, liver and lymphoid organs of pigs submitted to chronic intoxication. A total of eighteen 4-week-old piglets were randomly assigned to three different groups, receiving separate diets for 28 days: a control diet, a diet contaminated with EA (2368μg/kg) or a diet contaminated with EA (4817μg/Kg). After the period of the experiment the animals were euthanized, and fragments of jejunum, liver, mesenteric lymph node and spleen were sampled. The samples were fixed in 10% buffered formalin solution and submitted to histological procedures. Slides were stained with HE for histological evaluation. The main histological changes induced by chronic ingestion of EA on intestine were mild villi atrophy, edema of lamina propria and enterocyte cytoplasmic vacuolation. A significant decrease on villi height and goblet cells was observed on pigs fed the contaminated diets compared to control diet. On liver the main findings were mild hepatocyte cord disorganization, moderate hepatocyte megalocytosis and mild inflammatory infiltrate. A significant increase in the lesional score was observed on the liver of pigs fed the contaminated diets (3.5 fold increase for lower dose and 4 fold increase for higher dose) compared to control group. No significant difference was observed on the lesional score of lymphoid organs among the groups. In conclusion, we observed that chronic ingestion of low and high doses of ergot alkaloids affect pigs’ intestinal and liver homeostasis. More studies are necessary to evaluate the possible interaction between EA and the immune system.

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Hepatic metabolism of the mycotoxin citrinin in vitro

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Citrinin (CIT) is a mycotoxin which causes nephrotoxic effects in several animal species, and has genotoxic properties. There is little knowledge on the metabolism of CIT, especially in humans and data on human exposure to CIT are scarce so far. First biomonitoring studies have demonstrated frequent occurrence of CIT and its metabolite dihydrocitrinone (HO-CIT) in urines from German and Bangladeshi adults [1, 2]. In this context, it is of interest that this metabolite (HO-CIT) has been recently characterized as detoxication product of CIT [3].

To gain further insight into the metabolism of CIT, we have now studied conversion of CIT to HO-CIT in an in vitro model, using primary cultures of freshly isolated mouse hepatocytes. The cells were incubated with CIT (at 10 and 100 μM) for up to 24 hours. During the incubation period aliquots of the culture medium were withdrawn and analyzed by established HPLC and LC-MS/MS methods [1, 2]. The analysis revealed a clear decline of CIT over time and the appearance of HO-CIT metabolite: After 3 hours about 4% of the parent compound was metabolized to OH-CIT. At the end of the incubation after 24 hours about 22 to 31 % of the initial CIT dose was metabolized to OH-CIT. From these results it is concluded that CIT metabolism can occur in the mammalian liver. Further studies are of interest aimed to identify the enzymes responsible for CIT conversion to HO-CIT, as well as a comparison between rodent and human hepatocytes, since the extent of CIT detoxication may differ between species.


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Immunotoxic effects of deoxynivalenol in broiler chickens

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The impacts of deoxynivalenol (DON) feeding either alone or in combination with a microbial feed additive (MFA) on the immune response to a viral vaccine and serum clinical chemical parameters were investigated.

Forty 1-day-old boiler chicks were weighed and randomly divided into four groups, 10 birds each group: control group fed with basal diet; DON group fed with basal diet artificially contaminated with 10 mg DON/kg feed; DON + MFA group fed with basal diet contaminated with 10 mg DON/kg feed and supplemented with 2.5 kg of MFA/ton feed; and MFA group fed with basal diet supplemented with 2.5 kg of MFA/ton feed. At 35 days of age, birds were slaughtered and blood was collected for investigating the antibody titre against infectious bronchitis virus (IBV) and clinical chemical parameters.

The results showed that DON reduced the titre against the viral bursitis (IBV), decreased the level of alanine transaminase (ALT) compared with control birds, increased the serum cholesterol compared with their control counterparts and increased the amount of circulating triglycerides compared with controls.

These results indicate that dietary DON altered the humoral immune response to viral vaccine and affected some of the serum clinical biochemistry parameters.

However, DON in combination with MFA did not affect serum IBV titre. In conclusion DON in the feed of broilers produced an impairment of the success of IBV vaccine and affected the health of birds.

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Mycotoxins in blood and urine of sporthorses

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Although mycotoxins are an important cause of diseases in horses, the overall exposure via feeds and straw is not very well studied. Blood and urine are suitable sample materials to monitor the level of exposure. In this long-term monitoring study we determine the levels of several mycotoxins in blood and urine samples of sporthorses in Germany. Sample material is kindly provided by the Deutsche Sporthochschule Köln. In the years 2013/2014, a total of 100 blood samples and 200 urine samples were obtained for analysis. The analytical parameters were deoxynivalenol (DON), zearalenone (ZEA), ergonovine, fumonisin (FB₁), and ochratoxin A (OTA). All mycotoxins were analyzed by enzyme immunoassays. The following detection limits were achieved both in blood and urine: DON, 12 ng/ml; ZEA, 1 ng/ml; FB₁, 0.5 ng/ml; ergonovine, 0.2 ng/ml; OTA, 0.05 ng/ml.

Blood serum was frequently positive for OTA (35%, mean value 0.077 ng/ml) and ergonovine (14%, mean value 0.020 ng/ml). Fusarium toxins were found less frequently.

Urine was frequently positive (70-95%) for all toxins except FB₁ (10% positives). The mean levels for DON (80 ng/ml), ZEA (10 ng/ml), FB₁ (0.6 ng/ml), ergonovine (0.6 ng/ml), and OTA (0.2 ng/ml) were much higher than the blood levels, which indicates that urine is the better sample material for mycotoxin monitoring in horses. In agreement with results from an earlier study on mycotoxins in horse feed [1], the data suggest that horses are continuously exposed to multiple mycotoxins. Present knowledge does not allow (except FB₁) a clear assessment of the relevance of the mycotoxin levels found here. It may be assumed that the mean levels do not present an immediate toxicological concern.


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LC-MS/MS based biomarker methods to investigate the toxicological relevance of fumonisin derivatives for rats

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Fumonisins are mycotoxins, produced by certain Fusarium spp. that frequently infect maize, and other fungi including Aspergillus species. Hydrolytic reactions during gastro-intestinal metabolisation, alkaline food processing or deliberate administration of a fumonisin degrading enzyme (FumD) lead to a conversion of fumonisin B1 (FB1) to partially and fully hydrolysed FB1 (pHFB1 and HFB1). Moreover, thermal treatment of contaminated food or feed may generate N-(1-deoxy-D-fructos-1-yl) fumonisin B1 (NDF) in the presence of glucose. The aim of the study was to assess the toxicological relevance of the fumonisin derivatives, in particular their effect on the sphingolipid metabolism compared to FB1, in rats. For this purpose, LC-MS/MS based methods for the determination of biomarkers of effect (elevation of the sphinganine (Sa) to sphingosine (So) ratio in urine and kidney samples) as well as biomarkers of exposure (excreted fumonisin derivatives in urine and faeces) were developed and validated on a 4000 QTrap subsequent to UHPLC separation.

Validation covered the determination of apparent recoveries, matrix effects, linearity, repeatability as well as limits of detection and quantification of Sa and So in urine and kidney samples and of fumonisin derivatives in urine and faeces of rats. The validated methods were applied to samples collected in the course of a feeding experiment in which male Sprague Dawley rats were exposed to diets containing 13.9 μmol/kg of high purity FB1, pHFB1, HFB1 and NDF, respectively (n = 4), for three weeks.

Sa/So ratios in urine and kidney samples were significantly elevated in samples of the positive control group fed with FB1-diet. In contrast, orally administered pHFB1, HFB1 and NDF did not affect sphingolipid metabolism in rats. Nevertheless, partial decomposition of NDF to FB1 was observed in faeces samples, suggesting partial reactivation during digestion in vivo.

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Impact of Alternaria toxin Altertoxin II on the Nrf2/ARE-pathway

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Recently we identified altertoxin II (ATX II) as one of the genotoxic compounds of complex toxin mixtures formed by Alternaria alternata [Schwarz et al. Arch. Toxicol. (2012)86:1911–1925]. However, so far, its mechanism of action has not been fully elucidated. Previous studies highlighted that ATX II may be able to form adducts with glutathione (GSH) in a cell free environment [Fleck et al., Chem. Res. Tox.(2014)27: 247-2532014]. In the present study we addressed the question whether also within cells GSH, the most abundant and crucial cellular thiol, is affected by ATX II. The incubation of HT29 colon carcinoma cells with ATX II induced a depletion of GSH levels already after short time incubation (1h). This initial decrease was then followed by an increase after longer incubation times (3h, 24 h). In order to examine whether these alterations of GSH levels could be attributed to the activation of the nuclear factor (erythroid-derived 2)-like 2/ antioxidant response element (Nrf2/ARE)-pathway additional immunocytochemical experiments were conducted. Confocal microscopy localization allowed monitoring the nuclear translocation of the transcription factor Nrf2 in response to incubation with ATX II. According to literature, after activation and translocation to the nucleus, Nrf2 binds to the ARE in the promotor region of the respective target genes triggering the expression of enzymes important for metabolism and the oxidative stress response. In agreement with this hypothesis, ATX II induced in HT29 cells an increase of transcription of γ-glutamate cysteine ligase (γ-GCL), the rate-determining enzyme in the GSH synthesis. In conclusion, the impact of ATXII on cellular GSH levels is mediated by the activation of the Nrf2/ARE signaling pathway in human colon carcinoma cells.
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Analysis of respiratory capacity of liver mitochondria *ex vivo* in DON and LPS treated pigs

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Deoxynivalenol (DON) is a trichothecene mycotoxin produced by *Fusarium graminearum* and is found as contamination in various cereals. In the present investigation we analysed the effect of DON contaminated feed on the liver physiology of pigs with and without endotoxic challenge by lipopolysaccharides (LPS). We focused on the mitochondrial respiration and Ca^{2+} accumulation capacity measured in isolated liver mitochondria. Pigs were fed for 4 weeks with DON contaminated (4 mg DON/kg) or control feed (CON). At the end of the experiment the animals received LPS (7.5 μg/kg BW, LPS) or saline (control,CON) systemically for 1 h via a jugular (ju) or portal (po) catheter. After 195 min pigs were slaughtered, the liver was removed and mitochondria were isolated by mechanical disruption of the liver tissue and sequential centrifugation steps. Oxygen consumption rate (OCR) of mitochondria was measured in a oxygraph with glutamate/malate (5 mM each) as substrates in presence (state 3) or absence (state 4) of ADP (2 μmol / 1.5 mL test solution). Respiratory control index (RCI) was calculated (OCR state 3 / OCR state 4) and mean values of 6-7 animals per group were calculated (Diagram). In animals receiving control feed, jugular LPS challenge and portal saline infusion (CON_LPSju-CONpo) showed a small, but statistical significant enhanced RCI in comparison to control group. This effect was absent in DON feed animals as well as in portal LPS challenge. The Ca^{2+} accumulation capacity measured in presence of 1 or 10 mM phosphate or 2 μM Cyclosporin A was not influenced by the different treatment regimes. We conclude that jugular LPS application triggers effects in periphery, which lead to fast (within 3 h) functional modifications in the liver mitochondria. In animals exposed to feed derived DON this effect was absent. Interestingly, portal applied LPS did not show an increase in RCI, indicating that LPS is unlikely directly modulating the mitochondrial activity in liver.

Caption: RCI of isolated liver mitochondria of pigs fed with DON contaminated or control (CON) feed and challenged acutely with LPS via jugular (ju) or portal (po) route (*p<0.05, ANOVA, Dunnett Test, ± SEM).

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Impact of Alternaria toxins on CYP1A1 expression in human esophageal tumor cells and relevance for genotoxicity

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The Alternaria toxins alternariol (AOH) and alternariol monomethyl ether (AME) have been reported previously to act as activators of the aryl hydrocarbon receptor (AhR) in murine hepatoma cells, thus enhancing the expression of cytochrome P450 (CYP)1A monooxygenases [1]. Concomitantly, both benzopyrones represent substrates of CYP1A, giving rise to catecholic metabolites indicating that the oxidative metabolism of these compounds may be of toxicological relevance. In the present study we investigated the impact of AOH and AME on AhR-mediated expression of CYP1A in human esophageal tumor cells KYSE510 and their impact on cell viability, generation of reactive oxygen species (ROS) and DNA integrity. Cytotoxic effects of AOH and AME were determined with the sulforhodamine B (SRB) assay after 24 h of incubation. KYSE510 cells were not significantly affected by both mycotoxins at concentrations ≤ 50 μM. ROS production was induced concentration-dependently by both mycotoxins with AOH exhibiting the highest potency concomitant with the most prominent CYP1A induction level. AhR-siRNA knockdown in human esophageal cells supported the hypothesis of AhR-mediated CYP1A1 induction by AOH in human tumor cells. However, the DNA-damaging potency of AOH was not compromised by suppression of the AhR in human esophageal cells. The results suggest that only minor DNA damage occurs at AOH concentrations relevant for CYP1A1 induction. Thus the expression of AhR seems of no relevance for genotoxic effects of AOH in esophageal cells although induction of CYP1A1 was AhR-dependent. Nevertheless, in combination with compounds or xenobiotics prone to metabolic activation by CYP1A the enzyme induction by Alternaria toxins might be of toxicological relevance. Of note, the perylene quinone altertoxin II (ATX-II), the more potent DNA-damaging mutagen formed by Alternaria alternata [2, 3] was found to induce CYP1A transcription even at 100-fold lower concentrations than AOH. As Alternaria mycotoxins in infested food have been linked to esophageal cancer [4], the discovery of AhR activation and CYP1A induction especially in human esophageal cells warrants further investigation.

References


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Real time analysis of gene expression in living yeast cells as a novel approach for mycotoxin cell damage detection

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Microorganisms respond to environmental stress by the activation of gene expression programs in order to adapt and survive. Fine tuned transcriptional activation in response to stress is the result of dynamic interactions of transcription factors with specific promoter binding sites. Mycotoxins are organic compounds that trigger a fast response within *Saccharomyces cerevisiae* cells. We have used a time resolved luciferase reporter assay in living yeast cells to gain insights into how Citrinin and Ochratoxin modulate gene expression in a dose sensitive manner. Specifically, the dose response behavior of different natural promoters reveals differences in their sensitivity and dynamics. Characteristic Citrinin dose response profiles are also obtained for artificial promoters driven by only one type of stress regulated consensus element, such as CRE, STRE or AP-1 sites. We aim to elucidate the signaling transduction pathways in response to Citrinin and Ochratoxin, and therefore isolate the key defense proteins. Real time analysis of gene expression in living yeast cells is extremely sensitive and requires low concentrations of the chemical species studied, thus this technology allows the study of cell damage at physiological levels.
Effects of short-term exposure of T-2 toxin and deoxynivalenol on gene expression and activity of the members of the glutathione redox system in broiler chicken

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The aim of this study was to evaluate the effects of T-2 toxin and deoxynivalenol exposure in young broiler chickens at a dose of 5.77 mg T-2 toxin/kg feed and 4.86 mg DON/kg feed. The changes of the glutathione redox system at enzymatic and molecular levels were followed in the first 24 hours of exposure in 1 and 3-week-old chickens. Liver samples were taken after 2, 4, 8, 12, 16, 20 and 24 hours of the beginning of the feeding trial. The activity of glutathione peroxidase (GPx), reduced glutathione content (GSH), some parameters of lipid peroxidation (CD, CT and MDA), and changes of the gene expression of phospholipid hydroperoxide glutathione peroxidase (GPX4), glutathione reductase (GR), and glutathione synthetase (GS) were determined in the liver of chickens.

Activity of GPx and GSH content showed elevated levels in both ages in the T-2 toxin treated group compared to the control, in the first 8 hours of exposure, and in case of the 3-week-old group elevated levels were seen in GPx and GSH levels after 20 hours of exposure, which indicates a reactivation in the glutathione redox system. At molecular level we did observe only minor changes, which may be in association with a reserve of preformed or formed molecules in the liver, which was not depleted at protein level. In addition, the control group showed changes during the first 24 hour-period in gene expression and activity as well, which is similar to circadian changes of the antioxidant system. In the parameters of lipid peroxidation only minor changes were observed, which indicates that the potential oxidative stress inducing effect of the applied doses of T-2 toxin and deoxynivalenol was protected by the antioxidant defense of the liver in short term.

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Effects of chronic DON exposure and systemic LPS administration on liver histopathology in pigs

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Animal feed is frequently contaminated with the *fusarium* toxin Deoxynivalenol (DON) whereby pigs are the most susceptible species. Lipopolysaccharide (LPS) is a major component of the outer membrane of gram negative bacteria that mediates systemic inflammation. The aim of the present study was to investigate interactions of DON and LPS on liver morphology in pigs. In the trial the pigs were fed a control diet or a DON contaminated diet (4 mg/kg feed) for 4 weeks. At the end of the experiment the animals received LPS (7.5 μg/kg BW) or saline (control) systemically for 1 h via a jugular or portal catheter. After 195 min pigs were slaughtered and samples of the liver were taken for histopathological examination. The samples were fixed in 4% paraformaldehyde, dehydrated and embedded in paraffin. 5 μm sections were stained using haematoxylin and eosin.

The degree of liver damage was evaluated on basis of the modified histology activity index (HAI, 1). The HAI involves inflammation (portal, periportal and acinar infiltration of neutrophil and eosinophil granulocytes), necrosis (focal and confluent) and haemorrhages. The cumulative HAI was highest in the LPS treated groups, indication harmful effects of LPS on the liver. DON alone did not injure the liver (compared to control feed). However, the differences between LPS and saline infused pigs were greater in the DON fed animals, especially when LPS was injected into v. jugularis (Diagram). The increase of the HAI score after LPS administration is mainly ascribed to severe haemorrhage and inflammation. Necrotic lesions were found randomly in all experimental groups.

In conclusion, LPS mediated liver damage was significantly enhanced in DON fed pigs.

References

Caption: HAI score of pigs fed control (CON) or DON contaminated diet and systemically infused with LPS or saline (CON) via v. jugularis (jug) or v. portae (po); abc: columns with unlike superscripts differ significantly (Kruskal-Wallis, p < 0.05)

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Toxigenic potential of selected *Fusarium* spp. isolates

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Fusarium Head Blight (FHB) is one of the most common cereal diseases in Poland and worldwide. Wide range of pathogens from *Fusarium* genus is responsible for grain quality and quantity loss. It is a health risk for both humans and animals. The aim of the study was identification of toxigenic fungi and estimation of the genes occurrence responsible for mycotoxin production in the selected isolates of *Fusarium* spp. collected from organic farm in east-central Poland. The material were kernels of rye and triticale cultivars different in aspect of FHB resistance. In order to isolate the fungi present in the analyzed material Petri dish method was used. In result of mycological analysis *Fusarium culmorum*, *Fusarium graminearum* and *Fusarium avenaceum* isolates were chosen for PCR confirmation of identification correctness. Sequence of TEF1-α (Translation Elongation Factor 1-α) gene and species-specific primers were used. Futhermore 40 isolates of *Fusarium* species defined previously to the species by PCR technique were again analyzed by PCR but those assays were targeted toward toxin biosynthesis TRI13 and TRI7 genes responsible for trichotecenes, nivalenol and deoxynivalenol, production. The chemotype-specific primers are based on literature data. NIV and DON chemotypes of tested *Fusarium* species were determined also for grain species origin. Those assays occurred to be useful for assessing the risk of those trichotecenes contamination.
Individual and combined effect of *Fusarium* toxins *in vivo*

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Feed containing fumonisin (5 ppm; F), zearalenone (Z) and deoxynivalenol (D) (0.25 ppm+1 ppm; ZD) individually and these three toxins in combination (5 ppm+0.25 ppm+1 ppm; FZD) was fed to adult Pannon White (n =15/group) male rabbits (4±0.5 kg) for 65 days to determine the *Fusarium* toxin effect on breeding rabbit bucks’ sperm quality and endocrine function. The toxin levels were the lowest limit values for farm animals of the Commission Recommendation (2006/576/EC). On trial days 0, 30 and 65 blood and semen were sampled, and from semen pH, concentration, motility and morphology of the spermatozoa were investigated. The ratio of spermatozoa showing progressive forward motility decreased (P<0.05) from 80% to 67% in the FZD group. Differences were found between the data of the ZD (66.3%±23.7) and control animals (80.2%±11.2) concerning the normal morphology of spermatozoa. After gonadotropin-releasing hormone analogue treatment, the testosterone concentration was lowered in the FZD animals after 65 days. There was no difference among groups in feed consumption and BW.

Histophatology revealed lowered spermiogenesis activity occurred in lower percentage in the ZD group (30.77%), while in FZD it was much more progressed (64.28%), referring to a synergistic effect of the three toxins.

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Effects of a lipopolysaccharide (LPS) stimulus in pigs chronically exposed to dietary deoxynivalenol (DON): Clinical signs and leukocytes

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Information about the interaction between chronic deoxynivalenol (DON) exposure and a subsequent systemic lipopolysaccharide (LPS) challenge in pigs with respect to modulation of the inflammatory response is limited. The present study aimed to determine the effects on the acute phase reaction more detail.

A total of 44 barrows were either chronically exposed to a DON-contaminated (approx. 4mg DON/kg feed) or a control feed, surgically equipped with catheters at different locations (for pre- or posthepatic application and blood sampling) and an intraabdominal temperature logger. Animals were divided into six groups, whereby the first abbreviation refers to the diet and the other two indicate the infusion in V. jugularis interna and V. portae hepatis: CON_CONjug-CONpor, CON_CONjug-LPSpor, CON_LPSjug-CONpor, DON_CONjug-CONpor, DON_CONjug-LPSpor, DON_LPSjug-CONpor. Pigs were infused either with 0.9% NaCl or LPS (7.5 μg/kg BW) for 60min. Blood samples were taken every 15 to 30min for leukocyte count (Celltac, Baumann Medical AG), starting 30min before until 180min after start of infusion. Concurrently a range of clinical signs was scored (0 physiological, 1-5 pathological) and body temperature measured. Data were evaluated by PROC MIXED (SAS) with group, catheter and time as main factors and their interaction.

We found a severe leukopenia (p<0.001) and a significant increase in body temperature (p=0.003) in all LPS-infused pigs. Also we observed a uniform sequence of clinical signs caused by LPS, with tremor and cyanosis followed by hyperemic conjunctivae and injected episcleral vessels. Additionally there were differences due to DON. Group DON_LPSjug-CONpor showed consistently a 0.5°C lower elevation of temperature and clinical symptoms returned more uniformly to physiological levels at 180min compared to CON_LPSjug-CONpor. We concluded that dietary DON caused a modulation of the porcine acute phase reaction and altered thus the response to systemic LPS.
Mycotoxins are secondary metabolites of fungi, whereby fungi species belonging to different genera are known to generate mycotoxins, thus resulting in toxins with high structural diversity. Due to the inevitable entry of these toxic compounds into the food chain, they become a health issue. The development of sophisticated MS-based "multi"-methods has opened the possibility to assess concomitantly the occurrence of a broad spectrum of mycotoxins in one sample in reasonable time. Respective data published so far demonstrate that often food and feed is not contaminated with only one single but a spectrum of different mycotoxins [1, 2]. Clearly, this may result from the ability of several fungi species to produce more than one mycotoxin as it is described for several representatives of diverse fungi genera [3, 4]. Such co-contaminations highlight the need of studies on combinatory effects of mycotoxins in order to contribute to risk assessment, which is currently based on toxicity data of single compounds. So far, the knowledge on combinatory effects of different mycotoxins is still scarce and most studies focus on combinations of compounds produced by the same species. However, co-infestation of food commodities by species of different genera is suggested by several studies [5, 6].

This study, therefore, focuses on combinatory effects between a mycotoxin produced by *Alternaria* spp. and fusarotoxins. Preliminary tests on cytotoxicity of single compounds were conducted. Based on these data cytotoxic and non-cytotoxic concentrations were chosen for further tests on binary mixtures of mycotoxins in corresponding doses. Assessment of cytotoxicity was performed in the WST-1 in the colorectal adenocarcinoma cell line Caco-2.

Our results indicate that the tenuazonic acid, formed by *Alternaria* spp., modulates the toxicity of several fusariotoxins in an extenuating manner, especially those of deoxynivalenol, nivalenol and zearalenone.
Climate change effect in fungi and mycotoxins exposure – New tools for risk assessment

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The most relevant mycotoxins in the point of view of Human health and possible exposure are aflatoxin B1, ochratoxin A, fumonisins, zearalenone, deoxynivalenol and citrinin. These mycotoxins are toxics for Human and animals even when consumed in small amounts. Published articles reported several health effects such as carcinogenicity, mutagenicity, teratogenicity, cytotoxicity, neurotoxicity, nephrotoxicity and, also estrogenic and immunosuppressive proprieties. These chemicals do not disappear from the food even after cooking procedures. Several scientists reported that Portugal and other countries with temperate climate present higher risk of exposure to mycotoxins due to the tendency to the temperature increasing in future climate change scenarios. Recently, a scientific report submitted to the European Food Safety Authority stated that it is expected a higher risk of contamination by aflatoxin B1 related with the optimal conditions for the growth of A. flavus complex and its proliferation in several food and feed products. In Portugal, wheat and maize production have a significant economic relevance and are cultured for both Human consumption and feed production. To prevent a possible mycotoxin contamination of these products and, consequently, to avoid Human exposure, it is crucial the development of a support decision tool that allow the identification of the geographic areas with higher risk and to identify some of the preventive measures to apply. This tool can be used by risk managers and policy makers and can also be used by food and feed producers to anticipate the contamination and exposure risk and to adopt adequate preventive measures.

Only with a multidisciplinary approach is possible to interfere and reduce mycotoxins exposure in the future.

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Deoxynivalenol-sulfates: New conjugates occurring in planta and in human urine

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The trichothecene deoxynivalenol (DON) is a common food contaminant and plays a crucial role in the development and spread of the plant disease Fusarium head blight. DON is extensively metabolized in plants, animals, and humans resulting in metabolites of diverse chemical and toxicological properties.

The DON-sulfates are a so far little investigated group of DON conjugates. Recently, two DON-sulfate isomers, namely DON-3-sulfate and the DON-15-sulfate, were chemically synthesized to study their natural occurrence and toxicological potential [1]. The substances were used as reference standards to develop a quantitative LC-MS/MS method for their determination in wheat [2]. Both isomers were detected in DON treated wheat plants, demonstrating the plant’s potential to form these metabolites. Furthermore, it was shown that both isomers can be regarded as detoxification products of DON in wheat.

Besides its occurrence in planta, a DON-sulfate was also identified in human urine samples. The tested samples have been previously shown to contain very high concentrations of DON and its major urinary conjugates DON-3-glucuronide and DON-15-glucuronide [3]. Though the absolute concentration of the DON-sulfate was lower than those of DON-glucuronides, this novel human excretion mechanism warrants more detailed investigation. Future experiments will employ human cell lines to focus on the cellular uptake, a potential release of the parent toxin through intestinal cleavage and the potential impact on inflammatory processes of DON-sulfates.

References

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The assessment of zearalenone, deoxynivalenol and their metabolites in animal samples as indicator of dietary exposure

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Mycotoxin contamination on cereals poses a current problem, because their occurrence cannot completely be avoided. Of particular importance are the Fusarium mycotoxins zearalenone (ZEN) and deoxynivalenol (DON), which often co-occur in feed especially in maize and wheat. The analysis of European maize samples (n=223) of the harvest 2014 shows that 80 % of the samples were positively tested for ZEN and 92 % for DON, respectively (Naehrer, 2014). Because ZEN and DON might have negative effects on animal health and performance, the assessment of the dietary toxin exposure based on the analysis of different biological matrices represents an important aspect.

Therefore, a dose-response study with 30 dairy cows was performed with three different feeding groups, whereby the dietary ZEN and DON concentration of the highest Fusarium contaminated group was adapted to the current guidance values of the Commission of the European Communities (2006/576/EG, 2006). During the feeding trial, which covered a period of 13 weeks, starting at 7th day post-partum, plasma, urine and bile samples were collected on 3 or 4 different time points. Based on the analysis of ZEN, DON and their metabolites, which were examined for their usefulness as indicators of exposure, an evaluation was performed regarding the most suitable matrix for the assessment of toxin exposure through the diets. Thereby, the individual variation represents a particular challenge.

References


Fusaric acid (5-butyl-2-pyridinedicarboxylic acid) is a known wilting toxin of numerous formae specialis of *Fusarium oxysporum* (Sacc.), which is the causative agent of wilting diseases of different crops. It was first isolated by Yabuta, Kambe and Hayashi (1934) from culture filtrates of *Fusarium heterosporum* Nees, and later also found in other *Fusarium* species. Fusaric acid applied on plants causes necrosis on stems and leaves. The difficulty to verify the role of fusaric acid as a wilting substance *in vivo* can be explained based on the fact that the toxins are subjected to metabolic transformations. Sanwal (1956) showed that fusaric acid is converted to at least four other, as yet unidentified, compounds in living tomato plants, and one of the substances found was identified as N-methyl-fusaric acid amid which appears largely nontoxic for plants. Surprisingly, these results have not been verified using modern analytical techniques. Our plan is to verify and continue these studies after a gap of more than half a century. In this study, seven cultivars of tomatoes were tested for detoxification activity towards fusaric acid. The roots of tomato plants in early four-leaf stage were cleaned gently using sterilized water, placed in vials containing fusaric acid solution (150 mg/kg plant weight) and incubated at room temperature for 48 hours. After extraction of fusaric acid from plant material, the mycotoxin was quantified by HPLC-DAD. Ongoing experiments include treatment of plants with 13C-labeled fusaric acid, which will facilitate the search for further transformation products using mass spectrometry.

Enzymatic hydrolysis of zearalenone by ZenA of *Rhodococcus erythropolis* PFA D8-1

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Zearalenone (ZEN) is a mycotoxin produced by different *Fusarium* species, including *F. graminearum* and *F. culmorum* which infect major crop plants worldwide. Besides different physical and chemical treatments to reduce ZEN concentration in contaminated feed, biological decontamination is a favourable alternative. Many different microbes have been reported to degrade ZEN, but for most of them reaction products remained unknown. For 2 fungal strains (*Trichosporon mycotoxinivorans* and *Gliocladium roseum*), ZEN degradation has been studied in detail and loss of estrogenicity of reaction products has been confirmed. The lactonase ZHD101 of *G. roseum* was identified to be able to cleave the lactone ring of ZEN leading to hydrolysed ZEN, which is then further converted to decarboxylated hydrolysed ZEN.

We isolated new ZEN degrading microbial strains from soil samples. Our best ZEN degrading strain, PFA D8-1, was taxonomically assigned to the species *Rhodococcus erythropolis*. PFA D8-1 converted ZEN to the same reaction products as *G. roseum*. We prepared the primary reaction product hydrolysed zearalenone, which was previously only postulated but not isolated, found that it was non-estrogenic in assays with the breast cancer cell line MCF7 and the estrogen reporter yeast strain YZHB817, and abbreviated it HZEN. We made and screened a genomic library of PFA D8-1, found the gene encoding ZEN hydrolase, and named it *zenA*. We produced ZenA in recombinant *E. coli*, purified it by 6 x His-tag mediated nickel affinity chromatography, and characterised the enzyme by determining kinetic parameters and investigating effects of temperature and pH on enzyme activity. ZenA had higher substrate affinity and catalytic rate than the previously described ZHD101 of *G. roseum*. Together with the new information that the primary reaction product HZEN is non-estrogenic, the enzyme characteristics suggest that an application of ZenA for detoxification of ZEN contaminated animal feed might be feasible.
Comparison of porcine, chicken, and bovine derived peripheral blood mononuclear cells to study the cytotoxicity of DON and its metabolite after microbial transformation

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Deoxynivalenol (DON) is one of the most prevalent food- and feed-associated mycotoxins in cereals and cereal-derived products. Tissues with a high protein turnover, such as gut cells, or quickly proliferating cells, such as immune cells are highly sensitive to this mycotoxin. Genus novus species novus BBSH 797 of the Coriobacteriaceae family, isolated from bovine rumen fluid, transforms DON into the less toxic metabolite de-epoxy-deoxynivalenol (DOM-1). For that reason, BBSH 797 is used as feed additive to counteract the toxicity of DON. The effect of DON and DOM-1 was tested for porcine, chicken, and bovine peripheral blood mononuclear cells (PBMCs) via BrdU assay after stimulation with the T cell mitogen concanavalin A (ConA). DOM-1 did not affect the viability of the tested cell lines at the added concentration [1.4 – 365.7 μM], whereas DON alone showed already a cytotoxic effect at the concentrations starting at 0.02 μM. The calculated EC 50 values were 0.069 μM for porcine, 0.068 μM for chicken, and 0.03 μM for bovine PBMCs. This is interesting, as the in vivo sensitivity is known to vary from species to species, with pigs being the most sensitive.

The non-cytotoxic effect of DOM-1 cannot be explained by a decreased stability, as DOM-1 was retrieved in LC-MS/MS measurements, proving that DOM-1 was stable in the course of the experiment. Data therefore confirm the detoxifying abilities of Genus novus species novus BBSH 797 and support its use as a DON-detoxifying feed additive.
The interrelationship between the impact of aflatoxins and the role of a mycotoxin binder in the various growing phases of broiler chickens

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A study was conducted to investigate the relationship between the effect of aflatoxins fed either during the entire study or only in the first or second phase and the addition of a mycotoxin binder on broiler performance. A total of 800 day old AA male chicks were divided over 10 treatments with 8 replicates & 10 birds per replicate. Treatment characteristics are displayed in Table A. Corn naturally contaminated with aflatoxins was used, resulting in a final diet concentration of 120ppb. Average body weight (ABW) and feed intake (ADF) were measured on day 1, 21 & 41. On day 21 and 41 blood parameters and relative organ weights (ROW) were measured.

The results showed that feeding the contaminated diets during the entire study significantly reduced ABW, average daily gain (ADG) and ADF during the first & the second phase. Feed conversion ratio (FCR) as well as blood parameters or ROW were not affected. Binder inclusion significantly improved and restored ABW, ADG and ADF. Feeding aflatoxin contaminated diets during the first phase only led to a tendency for a reduction in ADF during the first 21 days. The binder partly restored this reduction. Other parameters were not influenced. Administering the aflatoxin contaminated diets only during the second phase led to a significant reduction in final ABW and ADG during the second phase. Binder inclusion had no effect. Other parameters were not influenced.

It can be concluded that feeding aflatoxin contaminated diets during the period from day 21-41 have a more severe impact on broiler growth performance compared to feeding the aflatoxin contaminated diets during the first 21 days. The effect of feeding contaminated diets only in a certain phase on growth performance was less severe compared to feeding them during the entire study. The inclusion of the binder was able to diminish the negative effects of aflatoxins on growth performance when the contaminated diets were fed during the entire period.
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Assessment and characterisation of yeast-based products intended to mitigate ochratoxin exposure using \textit{in vitro} and \textit{in vivo} models

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Ochratoxin A (OTA) a mycotoxin contaminating mainly cereals but also other crops, is accumulated in meat, and is not destroyed by food processing. It is nephrotoxic for pig and poultry, and implicated in human Balkan endemic nephropathy and associated urothelial cancer. One of the most promising and economical strategy for reducing animal exposure to mycotoxins is the utilization of binders in feed to reduce gastrointestinal absorption of mycotoxins. The aim of this paper was to evaluate the capacity of several yeasts to decrease genotoxicity of OTA and establish if the decrease is only due to binding of OTA on yeast. Ten poultries per group were fed two days with feed including yeast based-products, and then were fed seven days with feed including yeast and OTA. Genotoxicity and OTA derivatives formed in liver and kidney of poultry were analysed. In parallel to check more specifically the interest of yeast enriched either by glutathione (GSH) or selenomethionine (SE), human renal cells were exposed to OTA alone or in presence of GSH-yeast or with SE-Yeast. Addition of all yeast based products in feed of poultry reduced the amount of OTA in bile, plasma, liver and kidney except with GSH-yeast. DNA adducts are significantly decreased in liver and in kidney, except with SE-Yeast. In cell culture, OTA significantly decreases cell viability (60%; \textit{p}<0.01) and induces formation of two OTA-DNA-adducts. GSH-Yeast partially restores cells viability (70\% versus 60\%; \textit{p}<0.05) and avoid DNA adduct formation, explained by conversion of OTA into OTB and 4 OH OTA. SE-yeast has antagonistic effect (110\% versus 60\%; \textit{p}<0.01) and increased OTA-DNA adducts, correlated with the appearance of new OTA metabolites, notably quinone derivatives. The decrease of OTA toxicity observed with yeast was not only correlated to binding but also to biotransformation of OTA which is modulated by yeast. DNA adduct patterns were correlated with OTA derivatives formed in the kidney.
The effect of medium composition on \textit{in vitro} destruction of aflatoxin B1 by \textit{Phoma glomerata} PG41

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A number of bacteria and fungi, which destroy aflatoxins, are considered as sources of aflatoxin-catabolizing enzymes for potential application in decontamination of agricultural products. Aflatoxin B1-degrading \textit{Phoma glomerata} strain PG41 that we isolated from beans of \textit{Phaseolus vulgaris} colonized by an aflatoxigenic \textit{Aspergillus flavus} was used as one of such microbial sources. Culture liquid of PG41 grown on Czapek's medium supplemented with hydrolyzed casein (the standard medium) possessed the aflatoxin B1-destroying activity. This activity was determined by fungal metabolites, which were preliminary characterized as pH-dependent, freeze-thaw stable and relatively heat-labile high-molecular weight substances hydrolyzable by proteinase K. However, no studies have been carried out so far to determine the nutritional factors, which would promote the production of the B1-degrading metabolites.

The goal of the current research was to assay the influence of some nutrients on the growth of PG41 and extracellular destruction of B1 by this strain.

The fungus was grown at 27°C and 200 rpm for 7 days on the standard medium and on Czapek's media, in which casein hydrolyzate (CH, 3 g/L) was changed to yeast extract (YE, 1 g/L). The YE-containing medium was additionally supplemented with coconut oil (CO, 3 g/L). Fungal growth was estimated by a sedimentation method (PMV determination) or by the weight of dry biomass. To evaluate the B1 degradation level, the toxin was added in sterile samples of culture liquids to a final concentration 3 μg/ml. After a 72-h incubation, residual B1 was quantified by HPLC. The percent of destroyed B1 and specific activity of the destruction (ng of B1 destroyed by mg of dry biomass) were calculated.

It was found that medium composition greatly affected productivity of PG41 toward B1-destroying metabolites. If the nutrient medium contained YE instead of CH, the fungus grew as well as on the standard media (PMV ranged from 35 to 40%). The CH replacement with YE did not influence PG41 biomass production, but significantly \((p = 0.01)\) increased of the percent of the destroyed toxin. The enrichment of the YE-containing medium with CO greatly improved both the fungus growth and B1 degradation, however specific activity of B1 destruction by PG41 was higher on the medium with YE alone.

\begin{figure}
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\includegraphics[width=\textwidth]{influence_of_additives.png}
\caption{Influence of various additives to Czapeck media on the growth (grey columns) and specific aflatoxin B1-destroying activity (transparent columns) of \textit{Phoma glomerata} PG41. Bars represent standard deviation.}
\end{figure}

This research was financially supported by Russian Science Foundation (project No. 14-16-00150).
Trichothecene mycotoxins are naturally and worldwide occurring food contaminants, exposing adverse health effects in humans and animals. T-2 and HT-2 toxins are the most prominent members of Type A trichothecenes and are produced by several species of the Fusarium genus and contaminate mainly oats, which is an increasing problem in times of a growing interest on oat-derived products for a healthy and balanced diet. Concentrations of the sum of both toxins in oat products for human consumption may exceed the EU-guidance levels of 200 μg/kg (1), which is regarded critically, due to the high toxic potential of both secondary metabolites.

There are several methods reported to reduce the mycotoxin content in food, ranging from agricultural strategies to prevent the fungal infestation to food processing steps that either remove the toxins or destroy the mycotoxins’ molecular structure. Food extrusion is a high-temperature short-time (HTST) process and was shown to efficiently lower the mycotoxin content due to a combination of high temperatures and pressure and severe shear forces.

Knowledge about optimized food processing parameters to reduce the amount of mycotoxins in food products conduces to consumer protection and economic interests. We therefore investigated the stability of T-2 and HT-2 toxins in extrusion cooking under industrial conditions, regarding also the formation of degradation products.

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Protection of fungivorous Collembola against poisoning by mycotoxins

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Invertebrates are of great abundance in nature, and live in different habitats. Over time they adapted to their habitat and evolved the ability of specific mechanisms leading to an increase in their fitness. For example, Camponotus have symbionts Blochmannia to supplement for the nitrogen-deficient diet; mosquitos developed a special immune system to avoid the virus attack. Likely, fungivorous Collembola tend to expose to fungi which can produce mycotoxins. In this occasion, they may have evolved mechanisms for detoxification as self-protection.

In our study, we will investigate the way of detoxification by Collembola. On one aspect, bacteria associated with Collembola may help to accomplish this task; on the other, the insect itself could have developed detoxifying mechanisms, involving cytochrome P450, glutathione S-transferase and carboxylesterase. Furthermore, we will isolate the bacteria from Collembola which can detoxify the mycotoxins by culture-dependent and culture-independent techniques, and characterize the detoxification products.

The results showed that grazing of the Collembola (Folsomia candida) on mycelia of the fungus *Fusarium graminearum* lead to a decrease in the production of deoxynivalenol, 15-acetyl-deoxynivalenol and 3-acetyl-deoxynivalenol. However, the production of zearalenone was increased, which may be involved in the self-protection system of the fungus. The Collembola fed on mycelia containing mycotoxins showed a higher number of microorganisms but less species than Collembola fed on yeasts. We will extent the research on Collembola detoxifying the mycotoxins produced by *Aspergillus nidulans* and *F. verticillioides*, and elucidate detoxification processes. The results give an insight into the interaction between fungivores and mycotoxin producing fungi as well as processes of detoxification.


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**Alternaria** toxins in feed and food – method development and results from a survey of the German market

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**Alternaria** is a genus of worldwide occurring fungi, which can act as both saprophytes and plant pathogens. A fungal infestation of cultivated plants can lead to several plant diseases resulting in harvest losses and reduction in quality. Furthermore, the fungi are known to produce a variety of possibly human or animal health endangering mycotoxins, which can be present as natural contaminants within the entire food chain. Obtaining additional data relating to the contamination of food and feedstuffs with **Alternaria** toxins is a high priority in order to assess human and animal exposure and possible health risks. However, the analysis of **Alternaria** toxins is currently lacking some commercially available standard substances and standardized validated analytical methods¹. At the BfR a reliable method for the analysis of **Alternaria** toxins in food was developed. An extraction procedure based on QuEChERS² was applied. HPLC-MS/MS was used for identification and quantification of tenuazonic acid, alternariol, alternariol monomethyl ether, tentoxin and altenuene. The method was verified through an inter laboratory study and extended to additionally include other **Alternaria** toxins such as altertoxin I, alerotoxin II, stemphytoltoxin III, altenuisol, altenuic acid III and AAL toxin.

Various tomato-based products (e.g. tomato juice, sauce, ketchup) and also different kinds of flour (rye, wheat, spelt, buckwheat, oat flakes, sorghum, rice, maize) and further a selection of fruit and vegetable juices (e.g. currant, carrot, lemon) and some white and red wines from the German market were examined for the presence of **Alternaria** toxins. Data obtained from this market study will be presented and discussed.


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