



Chemisches und Veterinär-
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Stuttgart | Sigmaringen

29th Mycotoxin-Workshop

Fellbach, Germany

14th – 16th May, 2007



Gesellschaft
für
Mykotoxinforschung e. V.

Society
for
Mycotoxin Research

29th Mycotoxin-Workshop

14th – 16th May 2007, Fellbach, Germany

Program
Abstracts
Participants

Organizers



Chemisches und Veterinär-
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für
Mycotoxinforschung e. V.
Society
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Mycotoxin Research

Organizing Committee:

Dr. Uwe Lauber

Chemisches und Veterinäruntersuchungsamt Stuttgart

Brigitte Gutmacher

Chemisches und Veterinäruntersuchungsamt Sigmaringen

Dr. Renate Schnauffer

Chemisches und Veterinäruntersuchungsamt Stuttgart

Prof. Dr. Dr. Manfred Gareis

Bundesforschungsanstalt für Ernährung und Lebensmittel Kulmbach

Organization Assistance:

Stefan Böttcher (CVUA Stuttgart): Workshop Website

Werner Gluitz (CVUA Sigmaringen): Database of Participants

Dr. Pat Schreiter (CVUA Stuttgart): Abstract Book

Anke Trebstein (CVUA Stuttgart): Abstract Book

This Mycotoxin-Workshop would not have been possible without the support of **Ms. Maria Roth**, the director of our institution, and our dedicated staff **Sonja Fochler**, **Margit Kettl-Grömminger**, **Yvonne Kiesswetter**, **Christina Lenz**, **Susanne Maier**, **Benedikta Schubert**, **Gabriele Schwab-Bohnert** as well as many other helping hands at the Chemisches und Veterinäruntersuchungsamt Stuttgart.

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Program

Monday, 14th May 2007

- 07:45** **Registration / Exhibition**
- 09:00** **Opening of the 29th Mycotoxin-Workshop**
U. Lauber, Organizing Committee, CVUA Stuttgart
P. Hauk, Minister of the Ministry of Nutrition and Rural Affairs of Baden-Württemberg
M. Gareis, President of the Society for Mycotoxin Research
- Regulation / Introduction**
- 09:30** **Current status of mycotoxin regulations and future trends within the European Union**
L-01
Usleber, E., Giessen/D, Lauber, U., Märtlbauer, E., Gareis, M.
- 1. Session: Analytical developments (OTA and Ergot)**
- 09:45** **Development and Application of a Molecular Detection and Quantification System for the Ochratoxin A producing Species *P. verrucosum***
L-06
Geisen, R., Karlsruhe/D, Schmidt-Heydt, M., Richter, W., Michulec, M., Buttinger, G.
- 10:00** **A new validated HPLC-FLD method for detecting ochratoxin A in dry-cured meat and in blue cheese.**
L-05
Dall'Asta, C., Parma/I, Galaverna, G., De Dea Lindner, J., Virgili, R., Dossena, A., Marchelli, R.
- 10:15** **Development and application of an enzyme immunoassay for ergot alkaloids**
L-04
Curtui, V., Giessen/D, Dewenter, L., Seidler, C., Schneider, E., Usleber, E.
- 10:30** **Coffee break / Exhibition**
- 11:00** **Analysis and occurrence of ergot alkaloids in food**
L-08
Müller, C., Berlin/D, Klaffke, H.S., Krauthause, W., Wittkowski, R.
- 2. Session: Analytical developments (Fusarium toxins)**
- 11:15** **Development of an isotope dilution mass spectrometry (IDMS) method for the analysis of zearalenone (ZEA)**
L-03
Cramer, B., Münster/D, Bretz, M., Humpf, H.-U.

11:30 **Analysis of T2 and HT2 toxins via HPLC-FLD – drawbacks and opportunities**
L-11
Trebstein, A., Fellbach/D, Maier, S., Humpf, H.-U., Lauber, U.

11:45 **Development of a multi-stable isotope dilution assay (SIDA) for the determination of trichothecenes in food commodities**
L-02
Asam, S., Garching/D, Rychlik, M.

12:00 *Lunch / Exhibition*

13:00 **Poster Session I**

3. Session: Analytical developments (Multimethods)

14:30 **Multi-methods for the determination of *Fusarium* toxins in crops**
L-07
Hettwer, U., Göttingen/D, Karlovsky, P.

14:45 **An LC-MS/MS-based multitarget method for the detection of 89 mycotoxins: Development and application**
L-10
Schuhmacher, R., Tulln/A, Sulyok, M., Krska, R.

15:00 **Metabolites profiling by LC-HRMS – a powerful tool for developing trace analytical methods for the right mycotoxins in the right places**
L-09
Nielsen, K.F., Lyngby/DK

15:15 *Coffee break / Exhibition*

4. Session: Occurrence of mycotoxins I

15:45 ***Alternaria* mycotoxins in Swedish feed grain**
L-15
Hägglom, P., Uppsala/S, Stepinska, A., Solyakov, A.

16:00 **Occurrence of mycotoxins in falcon tissues infected with *Aspergillus fumigatus***
L-16
Meyer, K., Freising/D, Lipovsky, J., Schulz, J., Bauer, J.

16:15 **Defining background levels – First results for mycotoxins in house dust**
L-19
Portner, C., Duisburg/D, Plegge, V., Türk, J., Butte, W.

17:15 *Visit to the Daimler-Benz-Museum, afterwards dinner in the „Weinstädle“*
meeting point for departure: Entrance of the conference venue „Neue Schwabenlandhalle“

Tuesday, 15th May 2007**5. Session: Toxicology I**

- 09:00**
L-28 **Mechanisms of the genotoxic effect of *Alternaria* toxins**
Marko, D., Karlsruhe/D
- 09:15**
L-24 **Effects of the level of feed intake and ergot contaminated concentrate on ruminal fermentation, ergot alkaloid metabolism and carry over into milk, and on physiological parameters in cows**
Schumann, B., Dänicke, S., Braunschweig/D, Lebzien, P., Ueberschär, K.-H., Spilke, J.
- 09:30**
L-30 **Ochratoxin A: 13-Week Oral Toxicity and Cell proliferation in Male Fischer F344/N Rats**
Rached, E., Würzburg/D, Hard, G.C., Blumbach, K., Weber, K., Draheim, R., Özden, S., Steger, U., Dekant, W., Mally, A.
- 09:45**
L-27 **Biotransformation of the mycoestrogen zearalenone in horses**
Malekinejad, H., Fink-Gremmels, J., Utrecht/NL
- 10:00**
Coffee break / Exhibition

6. Session: Toxicology II

- 10:30**
L-25 **Characterization of the cytotoxicity of mycotoxins: Information gained from variations of *in vitro* assay conditions**
Degen, G.H., Dortmund/D, Aggarwal, M., Behm, C., Föllmann, W.
- 10:45**
L-26 **Cytotoxicity, cellular uptake and metabolism of deoxynivalenol by human kidney cells and human lung fibroblasts in primary culture**
Königs, M., Münster/D, Schwerdt, G., Gekle, M., Humpf, H.-U.
- 11:00**
L-23 **Hydrolysis of deoxynivalenol-3-O-glucoside**
Berthiller, F., Tulln/A, Domig, K.J., Kneifel, W., Juge, N., Knasmüller, S., Adam, G., Krska, R., Schuhmacher, R.
- 11:15**
L-29 **Effects of deoxynivalenol (DON) on human cell lines**
Nielsen, C., Oberschleißheim/D, Dietrich, R., Didier, A., Lippke, H., Märtlbauer, E.
- 11:30**
Lunch / Exhibition
- 12:30**
Poster Session II

7. Session: Prevention I (plants and animals)

- 14:00**
L-34 **Detoxification of zearalenone by *Gliocladium roseum*: biological function and regulation of zearalenone lactonase synthesis**
Utermark, J., Goettingen/D, Karlovsky, P.
- 14:15**
L-32 **Effects of live yeast cell supplementation to high concentrate diets on the toxicokinetics of ochratoxin A in sheep**
Blank, R., Kiel/D, Wolfram, S.
- 14:30**
L-31 **The positive effects of a probiotic microorganism (*Eubacterium sp.* DSM 11798) on the duodenal and jejunal histological alterations caused by the trichothecene deoxynivalenol of broilers**
Awad, W., Vienna/A, Böhm, J., Razzazi-Fazeli, E., Ghareeb, K., Zentek, J.

14:45 *Coffee break / Exhibition*

8. Session: Occurrence and production of mycotoxins II

- 15:15**
L-33 **Effect of Thiophanate – methyl on mycotoxin production in cereals under field conditions and *in vitro***
Buschhaus, H., Düsseldorf/D, Ellner, F.M.
- 15:30**
L-18 **Infection and development of *Fusarium spp.* in maize**
Oldenburg, E., Braunschweig/D, Höppner, F., Weinert, J.
- 16:00** **Meeting of the members of the Society for Mycotoxin Research**
- 18:45** *Departure to the conference banquet in the „Alte Kelter“*
meeting point: at the rear of the the conference venue „Neue Schwabenlandhalle“

Wednesday, 16th May 2007**9. Session: Occurrence and production of mycotoxins III**

- 09:15**
L-13 **Fusarium mycotoxins in UK oats – Impact of agronomy and processing**
Edwards, S., Shropshire/UK, Scudamore, K.
- 09:30**
L-21 **Elucidating the mechanisms by which wheat tolerates the Fusarium mycotoxin deoxynivalenol.**
Walter, S., Dublin/IE, Brennan, J.M., Ansari, K.I., Arunachalam, C., Khan, M.R., Egan, D., Doohan, F.M.
- 09:45**
L-20 **Toxigenic potential and pathogenicity of *Fusarium poae* and *Fusarium avenaceum* on cereals**
Vogelgsang, S., Zurich/CH, Sulyok, M., Schuhmacher, R., Forrer, H.-R.
- 10:00**
L-12 **Field Studies of FHB in wheat development and Mycotoxin production using quantitative PCR and DON-ELISA**
Bauer, A., Freising/D, Seigner, L., Büttner, P., Tischner, H.
- 10:15** *Coffee break / Exhibition*

10. Session: Prevention II (plants)

- 10:45**
L-14 **Results of a 4-year survey on *Fusarium* head blight (FHB) in wheat and their use to predict and prevent mycotoxin contamination in wheat**
Forrer, H.-R., Zurich/CH, Schachermayr, G., Müller, M., Jenny, E., Vogelgsang, S.
- 11:00**
L-22 **Reduction of mycotoxins in cereal grain using the resistance of wheat and triticale varieties against *Fusarium***
Rodemann, B., Braunschweig/D, Bartels, G.
- 11:15**
L-17 **Auswirkungen von kleinräumigen Feldheterogenitäten im Boden und im Bestand auf den Mykotoxin Gehalt von Winterweizen**
Müller, M., Müncheberg/D, Koszinski, S., Verch, G., Sommer, M.
- 11:30** **Closing Session**
- 12:00** **End of Workshop**
- 13:00** *Visit to the CVUA Stuttgart for participants interested*

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Abstracts of Lectures

Current status of mycotoxin regulations and future trends within the European Union

E. Usleber¹, U. Lauber², E. Märtlbauer³, M. Gareis⁴

¹Justus-Liebig-Universität Giessen, Fachbereich Veterinärmedizin,
Professur für Milchwissenschaften, Ludwigstr. 21, D-35390 Giessen, Germany

²Chemisches und Veterinäruntersuchungsamt Stuttgart, Schaflandstr. 3/2, D-70736 Fellbach, Germany

³Ludwig-Maximilians-Universität München, Tiermedizinische Fakultät,
Lehrstuhl für Hygiene und Technologie der Milch, Schönleutner Str. 8, D-85764 Oberschleißheim, Germany

⁴Bundforschungsanstalt für Ernährung und Lebensmittel, Institut für Mikrobiologie und Toxikologie,
E.-C.-Baumann-Str. 20, D-95326 Kulmbach, Germany

Until recently, harmonized regulations within the European Union concerning mycotoxins were focussed on maximum levels for aflatoxins, ochratoxin A, and patulin, respectively. Aflatoxins and ochratoxin A were also used as model substances, to establish regulations dealing with aspects of sampling, sample size, and quality criteria of analytical methods for mycotoxins.

In the last few years, *Fusarium* toxins (deoxynivalenol, zearalenone, fumonisins, T-2 toxin/HT-2 toxin) dominated the agenda of authorities involved in regulatory activities. Toxicological evaluations, for example those published as „Opinions of the Scientific Committee on Food“ of the European Commission, are available for several *Fusarium* toxins for years now.

In response to the demands arising from these scientific assessments, the discussion concerning harmonized maximum values within the European Union intensified in recent years. Present (and future) maximum values have to be seen in the light of a controversial debate, in which aspects related to (i) consumers protection, (ii) potential economic consequences of regulations on the food and feed industry, (iii) regional differences in aspects of relevance of certain *Fusarium* toxins (and attitude towards these toxins) on agricultural production, and (iv) international trade may influence the individual point of view. Regulations for deoxynivalenol, zearalenone, and fumonisins are now either fully established or in an advanced stage of completion within the EU. In general, the values of these three toxins tolerated through the harmonized EU regulations exceed those of an earlier, corresponding German regulation, and are also not in agreement with risk assessment data. In parallel to food legislation, the European Commission recently published „Guidance Values“ for several mycotoxins in products intended for animal feed. Interestingly, some of these values are virtually identical with the maximum levels set for foodstuff. The German guidance values (Orientierungswerte) for deoxynivalenol and zearalenone in feed are in some cases even lower than the EU-levels for food.

Presently, maximum values are discussed for T-2 toxin and HT-2 toxin in foods, but these will not be finalized in 2007, against earlier expectations. The discussion on tolerance values for ergot alkaloids is a difficult one, because of the large number of individual compounds forming within this group. However, the frequent occurrence of these toxins in rye, and products made thereof, may require the establishment of maximum levels within the near future. At present, the European Commission recommends further work on improved analytical methods, and consequently more data on the occurrence of T-2/HT-2 toxin and of ergot alkaloids in foods and feeds. The next candidates in the discussion on maximum levels for mycotoxins could be the *Alternaria* toxins, although current knowledge on the occurrence of these toxins in foods and feed is relatively poor.

This short contribution will primarily summarize present regulations on maximum levels for mycotoxins, and give an outlook on the possible level for some mycotoxins for which regulations can be expected soon. Furthermore, the status of regulations concerning sampling and analytical methods for mycotoxins will be presented briefly. In addition, a short information will be given about the EU rapid alert system for food and feed (RASFF), with special emphasis on notifications with regard to mycotoxins.

Development of a multi-stable isotope dilution assay (SIDA) for the determination of trichothecenes in food commodities

Stefan Asam, Michael Rychlik

Lehrstuhl für Lebensmittelchemie der Technischen Universität München,
Lichtenbergstrasse 4, D-85748 Garching, Germany

Trichothecenes are regularly occurring in cereals and cereal products. This contamination may include both the very polar type B trichothecenes and the more nonpolar type A trichothecenes such as deoxynivalenol and T2-toxin, respectively. Thus the development of methods for multicomponent analysis of substances with decisively unequal physical-chemical properties require certain compromise in terms of extraction solvent, clean-up and chromatographic conditions. In consequence, differences in the efficiency of the extraction procedure, in analyte losses during clean-up and ion suppression in the ESI-interface during LC-MS/MS-analysis may occur, which altogether account for the recovery of the analyte. To avoid laborious and time-consuming method optimization and validation, the application of stable isotope labelled internal standards is generally recognised as the method of choice.

Therefore, we synthesized several labelled type A and type B trichothecenes and developed respective stable isotope dilution assays. Synthesis of [¹³C₂]-15-acetyldeoxynivalenol, [¹³C₂]-3-acetyldeoxynivalenol, [¹³C₂]-4-acetyldeoxynivalenol, [¹³C₄]-T2-toxin, [¹³C₂]-HT2-toxin, [¹³C₄]-diacetoxyscirpenol and [¹³C₂]-15-monoacetoxyscirpenol was accomplished by [¹³C₂]-acetylation of the corresponding parent alcohol, i.e. deoxynivalenol, nivalenol, T2-triol and scirpentriol. The resulting peracetylated compounds were selectively hydrolyzed to obtain the desired mono- and diacetates. All synthesized products were characterized by NMR and MS experiments. [¹³C₁₅]-deoxynivalenol was purchased commercially.

By using the synthesized labelled trichothecenes and [¹³C₁₅]-deoxynivalenol stable isotope dilution assays were developed. Food samples were spiked with internal standards, extracted with common extraction mixtures and purified by non retentative SPE-columns of different manufacturers. All trichothecenes under study were quantified simultaneously within one LC-MS run using single and tandem MS detection. The method revealed good sensitivity with low detection and quantification limits along with excellent recovery data and good precision in inter-assay studies. A series of food samples was analysed showing a frequent contamination of cereals with deoxynivalenol at a medium concentration level mostly lower than the regulatory limit in the EU. Besides deoxynivalenol larger amounts of 15-acetyldeoxynivalenol were detected in maize and processed maize snacks, which is critical due to the increased toxicity of the acetylated trichothecenes. T2-toxin and HT2-toxin were regularly found in oat and oat products with the sum of both contents generally exceeding 50 µg/kg.

Whereas the clean-up procedure using non retentative SPE-columns had no major effect on the recovery of the trichothecenes, the polar deoxynivalenol especially was incompletely extracted by the common extraction solvents, which are composed of at least 60 % organic solvent. Though T2-toxin was completely extracted quantitation using external calibration resulted in lower values than the SIDA method, which points to a certain ion suppressing effect of coeluting matrix components. As a consequence the use of stable isotope labelled internal standards is an essential tool to obtain accurate results for evaluating trichothecene contamination of food commodities.

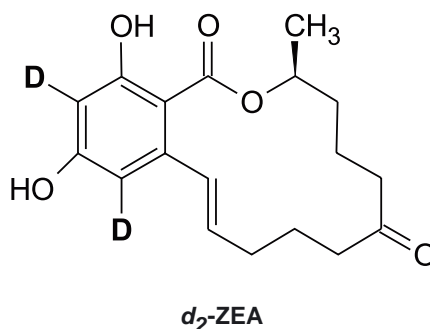
Development of an isotope dilution mass spectrometry (IDMS) method for the analysis of zearalenone (ZEA)

Benedikt Cramer, Michael Bretz, Hans-Ulrich Humpf

Institut für Lebensmittelchemie, Westfälische Wilhelms-Universität Münster,
Corrensstr. 45, D-48149 Münster, Germany

Zearalenone (ZEA) is a nonsteroidal mycotoxin with estrogenic activity that can disrupt the function of the endocrine hormone estrogen in animals and possibly in humans. It is produced by *Fusarium* species and frequently found in all major cereal grains as well as in processed food [1].

Several methods for the determination of ZEA in cereals and cereal products, such as HPLC coupled with fluorescence or UV-detection as well as GC-MS are described. In the last years, new multi methods using HPLC-MS/MS for the analysis of ZEA together with other *Fusarium* toxins were published. These methods have been shown to be fast, efficient and require only little clean-up. However, for reliable quantitation, suitable internal standards are necessary. Due to almost similar physical and chemical properties, stable isotope labelled analogues of the analytes have been shown to be the ideal internal standards for quantitation. While for trichothecenes such as DON or T-2 toxin the respective standards are available, an isotope labelled standard for the analysis of ZEA in cereals has not been reported.



We report the synthesis of stable isotope labelled 3,5- d_2 -ZEA as standard for HPLC-MS/MS analysis in cereal products. Starting from unlabelled ZEA we protected the carbonyl moiety of ZEA as ZEA-1,3-dioxolane. After hydrogen/deuterium-exchange under alkaline conditions, the dioxolane was cleaved and d_2 -ZEA yielded with an isotopic purity of 95%. The synthesized standard was successfully applied for quantitative determination of ZEA in cereal products by HPLC-MS/MS using a modified method of Klötzel *et al.* [2]. The recovery rates determined for ZEA in cereal samples ranged for 98 to 106%.

Reference

- [1] Opinion of the Scientific Committee on Food on *Fusarium* toxins, Part 2, 2000.
- [2] Klötzel, M.; Lauber, U.; Humpf, H. U. (2006): A new solid phase extraction clean-up method for the determination of 12 type A and B trichothecenes in cereals and cereal-based food by LC-MS/MS. *Molecular Nutrition & Food Research*, **50**, 261 – 269.

Development and application of an enzyme immunoassay for ergot alkaloids

V. Curtui, L. Dewenter, C. Seidler, E. Schneider, E. Usleber

Justus-Liebig-Universität Giessen, Fachbereich Veterinärmedizin,
Professur für Milchwissenschaften, Ludwigstr. 21, 35390 Giessen, Germany

Polyclonal antisera against ergonovine (ergometrine) were obtained after rabbits were immunized with an ergonovine-keyhole limpet hemocyanin conjugate. The specificity and sensitivity of these antisera was tested by using ergonovine coupled to horseradish peroxidase as enzyme linked toxin in a direct competitive enzyme-linked immunosorbent assay (EIA). Cross reactivities with ergonovine, ergotamine, ergocristine, ergosine, ergocornine, α -ergocryptine, β -ergocryptine and their corresponding epimers („inine-forms“) were tested.

The assay was characterized as group specific for ergot alkaloids. Considering the specific cross reactivity pattern of the individual alkaloids, a standard mixture containing defined amounts of ergonovine, ergotamine, and ergocristine was used for quantitative determination of the total amount of ergot alkaloids¹ in cereal samples.

The detection limit of the EIA was in a range of 14–20 $\mu\text{g}/\text{kg}$ total ergot alkaloids, recoveries from artificially contaminated samples ranged between 73 and 110 %. Results were in excellent agreement with those obtained by a HPLC-FLD method (detecting ergonovine, ergotamine, ergocristine, ergosine, ergocornine, α -ergocryptine, β -ergocryptine and their corresponding epimers) over a wide concentration range ($r^2 = 0.99$).

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¹total amount of ergot alkaloids was defined as the sum of ergonovine, ergotamine, ergocristine, ergosine, ergocornine, α -ergocryptine, β -ergocryptine, their corresponding epimers, and possible other ergot alkaloids which were not available as standards.

A new validated HPLC-FLD method for detecting ochratoxin A in dry-cured meat and in blue cheese.

C. Dall'Asta¹, G. Galaverna¹, J. De Dea Lindner², R. Virgili³, A. Dossena¹, R. Marchelli¹

¹Dipartimento di Chimica Organica e Industriale, Università di Parma, Viale G.P. Usberti 17/A, I-43100 Parma, Italy

²Dipartimento di Genetica Biologia dei Microrganismi Antropologia Evoluzione, Università degli Studi di Parma, Parco Area Scienze 11/A, I-43100 Parma, Italy

³Stazione Sperimentale per l'Industria delle Conserve Alimentari, V.le F. Tanara 31/A, I-43100 Parma, Italy

Moulds are usually considered as undesirable in food since they may produce mycotoxins, which are toxic secondary metabolites affecting a large variety of food commodities. Some of the most common mycotoxins are carcinogenic, genotoxic or may affect the kidneys, the liver and the immune system, thus causing severe health hazard to the human population. Nevertheless, moulds are intentionally added to several food products to induce the formation of specific flavours.

The growth of moulds on the surface of dry-cured meat products during the ripening phases is generally appreciated because their enzymatic activities contribute to the development of the characteristic flavour of these products. *Penicillium spp.* and *Aspergillus spp.* are common contaminants of dry-cured meat products; within these species, some strains are able to produce mycotoxins in suitable environment and substrate conditions.

The occurrence of ochratoxin A in meat and meat products can be ascribed to an indirect transmission via the ingestion of OTA-contaminated feed by pigs or to direct contamination due to mould growth in the outer layers of meat products. Processing procedures such as ripening have been proved to be ineffective for OTA reduction in meat products.

Another example about the technological use of moulds in food commodities is represented by blue cheeses, which are cow's milk or goat's milk cheeses with an extensively spotted or veined structure due to blue or blue-green moulds. In particular, *Penicillium roqueforti* is used as a secondary starter culture for the ripening of blue-cheese. Unfortunately, superficial contamination of the cheese with OTA-producing strain may occur during ripening and these moulds may enter the cheese after piercing.

Whereas several validated official analytical methods for OTA detection have been published for food matrices other than meat and cheese, in case of meat products and blue-cheeses a limited number of analytical procedures are available.

In this study, we developed a fast and sensitive method for the quantification of the mycotoxin ochratoxin A (OTA) in dry-cured meat products, which does not require a clean-up step, by HPLC with an alkaline mobile phase (pH = 9.8). Validation procedures for specificity, trueness, ruggedness, stability, recovery and repeatability were performed. The procedure was applied to representative dehydration levels of dry-cured meat samples.

Moreover, after the development of a suitable sample preparation procedure, the HPLC-FLD method was applied to several blue cheeses from the market. Evidence for the occurrence of ochratoxin A in blue cheeses is reported for the first time. The development of an accurate and reliable procedure for the extraction of OTA from cheese as well as the availability of a new sensitive HPLC-FLD detection method has allowed to determine ochratoxin A in complex matrices such as cheese even at very low levels (LOD in cheese: 0.02 µg/Kg).

Although the OTA contamination levels found in blue cheeses were very low, so that no risk for the consumer is to be foreseen, nevertheless the occurrence of ochratoxin A in such products opens a new issue for risk assessment and quality control, as far as the origin of OTA contamination and how to prevent it.

Development and Application of a Molecular Detection and Quantification System for the Ochratoxin A producing Species *P. verrucosum*

Rolf Geisen¹, Markus Schmidt-Heydt¹, Wolfgang Richter², Magdalena Michulec³,
Gerhard Buttinger³

¹Federal Centre for Nutrition and Food, Location Karlsruhe, Haid-und-Neu-Str. 9, 76131 Karlsruhe, Germany

²Bavarian State Research Center for Agriculture, Institut of Animal Nutrition and Forage,
Prof. Dürrwächter-Platz 3, 85586 Poing, Germany

³European Commission, Directorate General Joint Research Centre,
Institute for Reference Materials and Measurements, Retieseweg 111, 2440 Geel, Belgium

P. verrucosum is the species which is ultimately responsible for the presence of ochratoxin A in cereals and cereal products. The other known ochratoxin A producing *Penicillium* species is *P. nordicum*, which however usually does not occur in cereals.

It has been shown, that wheat with a certain degree of contamination by *P. verrucosum* contains ochratoxin A. That means that the detection of *P. verrucosum* at a certain contamination level is a quality criterion for cereals. The detection of *P. verrucosum* by conventional plating techniques however is very time consuming, because it is a slow growing species. In addition for the identification taxonomical skills are needed. A specific PCR method for *P. verrucosum* would speed up this process very much and would simplify the quality control of grain samples.

A requirement for a specific PCR reaction is the availability of unique DNA sequences. For this reason a part of the ochratoxin A polyketide synthase gene (*otapksPV*) has been cloned and sequenced. It has been confirmed by gene inactivation, that the identified gene is indeed involved in ochratoxin A biosynthesis. A primer pair was obtained from the *otapksPV* sequence. This primer pair is specific for the two ochratoxin A producing *Penicillium* species. Because *P. nordicum* does not occur in wheat, the cross reactivity between both species does not pose a problem. It could be shown, that the PCR system gave a positive reaction with wheat samples infected with *P. verrucosum*. A Real Time PCR system was also applied to be able to quantify *P. verrucosum* in wheat. If the contamination level was above $10^3 - 10^4$ cfu/ml the Real Time system could reliably detect and quantify *P. verrucosum* in the sample.

Interestingly all tested samples which gave negative results with the Real Time PCR reaction, did not contain measurable amounts of ochratoxin A, whereas all samples with positive Real Time PCR results proved to contain ochratoxin A. A Real Time PCR analysis of inoculated wheat samples stored under different conditions, revealed a high increase of the *P. verrucosum* genome copy numbers with samples stored at 24% moisture. Samples stored at 14% moisture showed no increase of the *otapksPV* copy numbers, suggesting the *P. verrucosum* was not able to grow under these conditions.

Multi-methods for the determination of *Fusarium* toxins in crops

Ursula Hettwer, Petr Karlovsky

Molekulare Phytopathologie und Mykotoxinforschung, Department für Nutzpflanzenwissenschaften,
Universität Göttingen, Grisebachstr. 6, 37077 Göttingen, Germany

The genus *Fusarium* consists of a large number of species which are abundant in soil, growing saprophytically or colonizing living plants. Certain *Fusarium* species are important pathogens on crop plants, especially cereals, but also on sugar beet and vegetables. *Fusarium* species often produce mycotoxins, which affect human and animal health. The most investigated mycotoxins of *Fusarium* spp. produced on plants are trichothecenes, fumonisins and zearalenone. For these toxins maximum limits are statutory in the European community whereas for other *Fusarium* mycotoxins, such as the enniatins, moniliformin or fusaric acid, tolerable levels are not yet defined.

Since it is not possible to prevent *Fusarium* infection in the field and mycotoxin levels can increase during storage, contamination of crops by mycotoxins must be monitored. As outlined above, *Fusarium* species are capable of producing a broad range of mycotoxins. Therefore screening methods suitable for the detection of as many toxins as possible at the same time are desirable. HPLC coupled with mass spectrometry allows the identification and quantification of many targets at the same time with high reliability. Because detection limits are affected by sample purity, cleanup procedures are required. Many protocols are published on clean-up by solid phase extraction (SPE) with different matrices, but most of them focus on the reduction of UV-interfering substances. For HPLC-Mass spectrometric methods the reduction of the quenching effects during ionization is more important. The high operation costs of mass spectrometers as compared to UV spectroscopy further increases the need to maximize data output from a single run. We present three protocols for the simultaneous determination of several *Fusarium* toxins, focusing on the reduction of costs and simplification of sample handling:

- A method for combined cleanup and determination of both type A and type B trichothecenes
- An easy method for combined clean-up and detection of trichothecenes together with zearalenone

Furthermore the suitability of polymer-phase-SPE for Fumonisin and enniatin cleanup will be discussed.

Analysis and occurrence of ergot alkaloids in food

Carola Müller, Horst Klaffke, Werner Krauthause, Reiner Wittkowski

Bundesinstitut für Risikobewertung, Thielallee 88-92, D-14195 Berlin, Germany

Als Mutterkörner werden die von dem parasitären Pilz *Claviceps spp.* gebildeten Sklerotien (*Secale cornutum* – Überwintungsform des Pilzes) bezeichnet, die verstärkt in feuchten Jahren auf Getreideähren und Gräsern vorkommen können. Die lila-braun gefärbten Sklerotien können bis zu 4 cm lang werden und eine Vielzahl an Alkaloiden enthalten [1]. Der Gehalt und die Verteilung der Alkaloide sind abhängig vom produzierenden Pilzstamm, der Wirtspflanze, der geographischen Herkunft und den Witterungseinflüssen.

Im Mittelalter starben Hunderttausende von Menschen nach dem Verzehr von mit Mutterkorn verunreinigtem Getreide. Als Symptome der akuten Vergiftungen mit Mutterkorn – bereits 5 bis 10 g können tödlich wirken – werden Übelkeit, Kopfschmerzen, Parästhesien, Krämpfe, Tod durch Atemlähmung und Kreislaufversagen genannt. Bei Schwangeren kommt es zudem zu Uteruskontraktionen verbunden mit Blutungen, Tetanus uteri, gefolgt von Erstickung des Embryos, Aborten und Uterusrupturen [2]. In einer toxikologischen Bewertung von Mutterkornalkaloiden in Roggenmehl [3] kommt das Bundesinstitut für Risikobewertung (BfR) zu dem Schluss, dass die Datenlage bezüglich der Mutterkornalkaloid-Belastung derzeit nicht ausreicht, um eine fundierte Risikobewertung durchzuführen. Ziel des Forschungsvorhabens ist es daher, eine effektive und schnelle Methode zur Bestimmung von Mutterkornalkaloiden in Getreiden und Getreideprodukten zu entwickeln und zu validieren, um damit weitere Daten zur Exposition des Verbrauchers zu erheben. Darüber hinaus wird vom BfR empfohlen, EU-einheitliche Höchstmengen für Mutterkornalkaloide in Lebensmitteln festzulegen.

Die vorgestellte Analysen-Methode basiert auf einer basischen Extraktion mit einem Gemisch aus Ethylacetat, Methanol und Ammoniumhydroxid, einer einstufigen Aufreinigung mittels Festphasenextraktion an basischem Aluminiumoxid und anschließender HPLC-FLD- bzw. LC-MS/MS-Detektion. Bisher wurden diverse Getreide und Getreideprodukte untersucht, wobei in einer Vielzahl von Proben Mutterkornalkaloide nachgewiesen werden konnten. Die weiteren Untersuchungsergebnisse sowie die ausführlichen Validierungsdaten werden vorgestellt.

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Metabolites profiling by LC-HRMS – a powerful tool for developing trace analytical methods for the right mycotoxins in the right places

Kristian Fog Nielsen

Center for Microbial Biotechnology, BioCentrum-DTU, Technical University of Denmark, Denmark,
Building 221, DK-2800 Kgs. Lyngby, Denmark, Tel: + 45 45252602; Fax: + 45 45884922;
kfn@biocentrum.dtu.dk, www.cmb.dtu.dk

Analytical chemists are highly depended on input from mycologists on what to look for in the feed/food of interest, and history has shown that it is impossible to retrieve the (species specific) metabolite potential from the literature, due to misidentifications, contamination of cultures, and poor analytical chemistry. The key point is to establish the metabolic potential on the right substrates (varying nutrition, a_w , O_2 etc.) of the fungi isolated from the food/feed to be investigated. Luckily metabolite profiling techniques have improved tremendously during the last years and HPLC with Time-of-Flight, Orbi-Trap MS detection provides accurate-mass data on all ionisable compounds in the sample. Combining with database like Anitbase many compounds can be identified/dereplicated in the sub micro gram scale and if available in microgram scale even structurally elucidated by LC-NMR. However one could go directly from the metabolic profiles (without compound identification) and use the newest high sensitive triple-quadrupole MS/MS instruments to look for the same compounds in crude extracts from infected samples. These metabolites can then be selected for structure elucidation if found in the habitat studied, a methods refereed to as reverse selection (Figure 1). Thus focusing on the ecological important metabolites and not metabolite which the fungus is only producing in other habitats. Moreover one can predict precursor and daughter ions of mycotoxins transformed by the plant, e.g. glucosylated metabolites. This combined with more focus on highly hydrophilic compounds which are underrepresented, as they are difficult to separate and purify, should let us find many more mycotoxins, their masked analogues, as well as fungal metabolites modulating their effects.

Reverse selection strategy

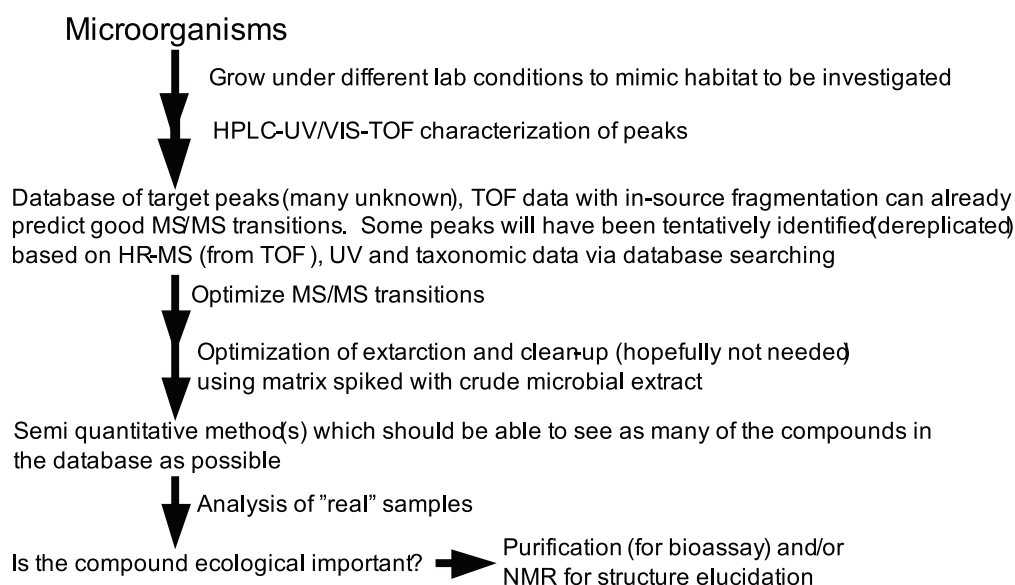


Figure 1. The reverse selection strategy for identification of novel mycotoxins and other ecological important metabolites.

An LC-MS/MS-based multitarget method for the detection of 89 mycotoxins: Development and application

R. Schuhmacher^{1,2}, M. Sulyok¹, R. Krska^{1,2}

¹Center for Analytical Chemistry, Dept. for Agrobiotechnology (IFA-Tulln),
University of Natural Resources and Appl. Life Sciences Vienna, Konrad Lorenz Str. 20, A-3430 Tulln, Austria

²Christian Doppler Laboratory for Mycotoxin Research, Dept. for Agrobiotechnology (IFA-Tulln),
University of Natural Resources and Appl. Life Sciences Vienna, Konrad Lorenz Str. 20, A-3430 Tulln, Austria

Numerous genera of fungi, such as *Fusarium*, *Penicillium* and *Aspergillus* produce a great number of different toxic metabolites. The types of toxins can be grouped into different compound classes, are frequently species specific and show a great variety of chemical structures. This has led to the development of many analytical methods dealing with single classes of compounds including a limited number of target analytes. Lately, additive and synergistic effects have been observed concerning the health hazard posed by mycotoxins: This has resulted in the search for multiresidue methods for the simultaneous determination of different classes of mycotoxins.

The development of such a multitoxin method is, however, a difficult task, as the compounds of interest show a broad range of physical and chemical properties. Ionic, very polar toxins such as moniliformin can be found as well as apolar cyclic peptides like beauvericin. As a consequence, the development of a multitoxin method makes it necessary to find the „best compromise“ for sample extraction, sample preparation and chromatographic separation, which may be far from optimum concerning certain target toxins. With respect to end determination, HPLC coupled to a tandem mass spectrometer can be regarded to be the best choice since most of the toxins are non-volatile and show low UV-absorptivities. On the other hand, LC-MS/MS, which is the only technique capable of the selective and simultaneous detection of a large number of analytes in complex matrices, is prone to suffer from unpredictable matrix-induced signal suppression. This can lead to low accuracies in toxin quantitation.

Recently, we have developed a method for the extraction, separation and the detection of 39 mycotoxins including trichothecenes, zearalenone and its derivatives, fumonisins, aflatoxins, ochratoxins, enniatins and ergot alkaloids at $\mu\text{g}/\text{kg}$ -levels [1]. The method does not use any sample clean-up; it consists of an extraction step, followed by filtration and dilution prior to LC-MS/MS measurement.

This work describes the extension of this method by some other 50 analytes. Currently, the potential of the extended method for the semiquantitative screening of food and food products is under investigation. We have applied the multi-target method to various molded food samples with the aim to evaluate the toxin spectrum of these samples. This contribution will summarize the characteristics of the method as well as the results we obtained for the real-world samples.

Acknowledgement

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Analysis of T2 and HT2 toxins via HPLC-FLD – drawbacks and opportunities

Anke Trebstein^{1,2}, Susanne Maier¹, Hans-Ulrich Humpf², Uwe Lauber¹

¹Chemisches und Veterinäruntersuchungsamt Stuttgart,
Schaflandstr. 3/2, D-70736 Fellbach, Germany

²Institut für Lebensmittelchemie, Westfälische Wilhelms-Universität Münster
Corrensstr. 45, D-48149 Münster, Germany

In the group of *Fusarium*-toxins the type-A trichothecenes T2 and HT2 are currently the most important ones. Mainly occurring in cereals – especially in oats and oat based foodstuff – they are objects for EU limit values in the near future. In practice, GC-MS or LC-MS/MS is the method of choice to determine T2 and HT2 toxins. The main advantage of the latter technique is getting robust, reliable and sensitive data upon short and simple clean-up, since matrix depending effects can be compensated, e.g. by isotopically labeled standards. However, due to high costs GC-MS or LC-MS/MS are not available in every lab in the routine analysis. Hence, an alternative, comparably sensitive determination method, e.g. HPLC with corresponding detection, is absolutely necessary.

In 2003, Pascale *et al.* [1] demonstrated one of the first HPLC-methods for determination of T2-toxin using immunoaffinity columns (IAC) for clean-up and fluorescence detection after precolumn derivatisation with dimethylaminopyridine (DMAP) and 1-anthroylnitrile (1-AN). In 2005, Visconti *et al.* [2] completed this method using almost the same parameters for quantification of both trichothecenes: T2 and HT2. Visconti's method works properly for matrices like wheat, barley and corn. However, it was also stated that this protocol can not be applied to oats and oat-containing products due to interfering peaks in the chromatography at the retention time of the HT2-derivative [2]. This is especially unsatisfying, because oats is one of the matrices most frequently contaminated with T2 and HT2 toxins. As the toxins, different compounds present in oats are converted in fluorescent molecules via derivatisation and result in interfering peaks in the chromatography, mainly with the HT2-derivative. In addition to matrix depending interference, interfering signals arising from solvents or materials were also detected.

In general, following IAC clean-up and precolumn derivatisation with DMAP and 1-AN the HPLC-FLD method is an effective and comparably sensitive alternative to the determination method using LC-MS/MS. During establishing of this method and improvement for determination of HT2 toxin as well, the following items were investigated:

- Possibilities for elimination of oats-specific interfering compounds which occur despite using highly-specific immunoaffinity columns for T2 and HT2.
- Potential interfering compounds originating from solvents and materials in general.
- Improvement of chromatographic separation using different gradient systems and HPLC-columns.

The lecture deals with difficulties which occurred during establishing this determination method in laboratory for routine analysis.

Acknowledgement

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Field Studies of FHB in wheat development and Mycotoxin production using quantitative PCR and DON-ELISA

Astrid Bauer, Luitgardis Seigner, Peter Büttner, Helmut Tischner

Bayerische Landesanstalt für Landwirtschaft, Institut für Pflanzenschutz IPS 2c,
Lange Point 10, 85354 Freising, Germany

Fusarium head blight (FHB) and the resulting mycotoxin (mainly DON) contamination of crops are a great problem in winter wheat since the 1990ies.

The main object of this project is to establish a solid base of epidemiological data for developing a *Fusarium* -forecasting model. Therefore two different varieties of winter wheat (susceptible and less susceptible) are grown at two different locations. For inoculation with *Fusarium* maize stubbles from the last harvest (1 stubble/m²) are applied on the field. Meteorological data are collected by weather stations nearby experimental plots.

Sampling takes place twice a week from BBCH 37 up to harvest from two different leaf levels (F and F-1) and ears. Samples are checked by quantitative Real-time PCR for *F. graminearum* and Tri5-DNA. Tri5 gene expression was studied by relative quantifications using the housekeeping gene beta-tubulin2 for normalization. The Tri5-gene is the key gene in Trichothecene production in *Fusarium* species. Additionally the DON concentration in the samples was determined by DON-ELISA.

Parts of the extensive results of 2004 and 2005 will be presented.

Fusarium mycotoxins in UK oats – Impact of agronomy and processing

Simon Edwards¹, Keith Scudamore²

¹Harper Adams University College, Newport, Shropshire TF10 8NB, UK

²KAS Mycotoxins 6 Fern Drive, Taplow, Maidenhead, Berkshire, SL6 0JS, UK

An investigation was conducted from 2002 to 2005 to determine the impact of agronomy on the fusarium mycotoxin content of UK barley and oats. Samples of oats and barley were collected from fields at harvest with associated agronomy data.

Mycotoxin levels were usually low or absent in UK barley samples with only one sample exceeding 1 000 ppb deoxynivalenol and five samples exceeded 100 ppb HT2+T2 ($n=446$). Mycotoxin levels in UK oats were generally low except for HT2 and T2. These mycotoxins were detected above the limit of quantification (10 ppb) in 92 percent of samples ($n=458$). Thirty percent of oat samples exceeded 500 ppb HT2+T2 and the maximum was 9 990 ppb HT2+T2.

Statistical modelling of the agronomic data identified several agronomic factors that influenced the HT2+T2 content of oats. There was a significant interaction between year and region, indicating that weather probably had an influence. Previous crop and cultivation had a significant interaction indicating that crop debris may be a source of inoculum. And there were significant differences between varieties with the most popular winter variety, Gerald, having the highest HT2+T2 content. Organic samples were significantly lower than conventional samples, this may, in part be due to differences in previous crop, cultivation and variety choice but the modelling indicated that some other difference, not accounted for within the model, was also having an impact.

Mycotoxin analysis of samples from replicated winter oat variety trials in 2004 and 2005 also identified significant differences between varieties with a similar pattern to the observational study. Samples from spring oat trials have been consistently lower than winter oat trials and no differences have been detected between spring varieties.

A laboratory scale de-hulling experiment ($n=4$) was conducted to identify the distribution of HT2 and T2 within the hulls and groats (de-hulled oats), as only groats are used for human consumption. The experiment determined that the majority of these mycotoxins were present in the hulls with at least a 90 % reduction during processing from oats to groats.

The effect of industrial processing of oats for human consumption on mycotoxin content has been studied as part of a large UK project to determine the fate of fusarium mycotoxins in the cereal food chain. Results to date from this study have identified that a similar reduction (90–99 %) occurs during industrial processing. Nearly all this reduction occurs during de-hulling.

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Results of a 4-year survey on *Fusarium* head blight (FHB) in wheat and their use to predict and prevent mycotoxin contamination in wheat

Hans-Rudolf Forrer¹, Gabriele Schachermayr², Matthias Müller³, Eveline Jenny¹,
Susanne Vogelgsang¹

¹ Research Station Agroscope Reckenholz-Tänikon ART, Reckenholzstrasse 191, CH-8046 Zurich, Switzerland

² Indo-Swiss Collaboration in Biotechnology, EPFL, CH-1015 Lausanne, Switzerland

³ Center for Plant Protection in Arable Farming, Education in Agriculture, Liebegg, CH-5722 Gränichen, Switzerland

Traditional agriculture, based on intensive soil tillage techniques with heavy machinery, is a real threat for maintaining soil fertility. The soil structure becomes increasingly unstable, the soil loses its load capacity and eventually degrades.

To improve the situation and to avoid increasing problems with soil compaction, soil erosion, and nitrate leaching, several Swiss cantons started between 2000 and 2003 to subsidise conservation tillage and especially no-tillage. No-tillage, a plant production system without any tillage from previous harvest to direct seeding offers the best condition to conserve and improve the soil structure. The soil cover with straw and plant residues is an essential factor to protect the soil and maintain a high biological activity; however, it could also be an important source for infections of cereals with FHB causing fungi. Therefore and to examine the impact of no-tillage on the prevalence of FHB fungi and mycotoxin contamination in wheat, the canton of Aargau sponsored an FHB survey from 2001 to 2004.

Besides elucidating and quantifying effects of soil tillage, we also wanted to investigate and separate rotational and varietal effects on the incidence of FHB fungi and mycotoxin contamination. Hence, we collected wheat samples from ploughed or no-tillage fields, each with or without maize in the previous crop, and with the less susceptible varieties Arina and Titlis or any other variety. All samples were collected at harvest by the farmers according to a standardised sampling protocol and sent to ART Zürich-Reckenholz. With a seed health test, we screened for the incidence of FHB fungi including all toxigenic *Fusarium* sp. as well as *Microdochium nivale*. In addition we quantified the deoxynivalenol (DON) content of ground grain samples with a Ridascreen[®] DON-kit, an enzyme immunoassay.

In all four years, *M. nivale* (MN), *F. graminearum* (FG), *F. poae* (FP), and *F. avenaceum* (FA) were the most important FHB fungi in 340 samples, with a mean of 8.7 %, 7.5 %, 2.3 % or 0.6 % infected grains, respectively. Highest amount of infected grains were observed with MN in 2001 (15 %). This shows the high significance of MN, since 10

Since we found clear and quantifiable effects of pre-crop, tillage, and variety from our survey, and FG proved to be the actual key problem with respect to toxigenicity, we developed the DON forecast system FusaProg [1]. To further promote no-tillage or other soil conserving tillage systems despite their risk for FG infection and DON contamination, we also started to develop and evaluate cultivation methods and systems to lower the risk of FHB and mycotoxin contamination [1, 2].

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Alternaria mycotoxins in Swedish feed grain

Per Häggblom, Anna Stepinska, Alexey Solyakov

Department of Animal Feed, National Veterinary Institute, SE-751 89 Uppsala, Sweden

Moulds of the genus *Alternaria* are common saprophytes ubiquitous in soil and on decaying feed and food products or plant pathogens causing leaf spots or blights on many plants.

Alternaria alternata produces several mycotoxins such as alternariol (AOH), alternariol monomethyl ether (AME), tenuazonic acid (TeA), altenuene (ALT) and altertoxin I (ATX-I). *Alternaria* mycotoxins are reported from fruits, tomatoes, olives, melons, pepper, apples, grains, sunflower seeds and oilseed rape meal etc.

Heavy and frequent rainfalls in the southern part of Sweden resulted in high moisture levels in the grain crops during the harvest season in 2006. Field observations indicated heavy infections of dematiaceous fungi in several agricultural regions due to elevated moisture levels. In a study carried out in the autumn of 2006 cereal samples of barley, wheat and oats intended for animal feed as well as straw was collected at the farm level.

In the laboratory investigations carried out *Alternaria spp.* were frequently isolated from all grain and straw samples. The presence of *Alternaria* mycotoxins in the whole grain and straw samples were analysed and AOH, AME or TeA were isolated using reversed phase HPLC-with UV or fluorescence detection after solid-phase extraction using C18 or silica columns.

The results up till now showed levels of AOH (in 16 out of 18 samples in the range 9–335 ppb) and AME (in 7 out of 18 samples in the range 1–184 ppb). TeA was detected in all 18 analysed samples, 4 out of 18 samples contained TeA, in the range 0.98–4.31 ppm. Interestingly, 2 of the 4 samples where the highest levels of TeA were detected were from oats. Preliminary results do not indicate any negative effect on the feeding of pigs.

This is the first investigation of *Alternaria* mycotoxins in Swedish feed grain.

Occurrence of mycotoxins in falcon tissues infected with *Aspergillus fumigatus*

K. Meyer¹, J. Lipovsky¹, J. Schulz², J. Bauer¹

¹TU München, Lehrstuhl für Tierhygiene, Weihenstephaner Berg 3, D-85354 Freising, Germany

²TiHo Hannover, Institut für Tierhygiene, Tierschutz und Nutztierethologie, Bünteweg 17p, D-30559 Hannover, Germany

The imperfect fungi *Aspergillus fumigatus* commonly existing autochthonal as a saprophyte is also known as an opportunistic pathogen causing severe diseases such as pulmonary invasive aspergillosis, leading to rising mortality rates in livestock and high economic losses in animal production. Especially birds are susceptible to infections with the fungus due to their anatomic particularities in the respiratory tract. For the small conidiospores of *A. fumigatus* (2–3 μm in diameter) the deeper airways are easy to reach and body temperature of birds enables a fast fungal growth. Once colonised the lung tissue *A. fumigatus* dispose of a wide range of virulence factors and toxic or immunosuppressive secondary metabolites. Beside the antibiotic and angiogenesis inhibiting Fumagillin this are especially the toxins of the fumitremorgin-verruculogen-group or the highly cytotoxic gliotoxin.

Tissue samples of ten falcons perished in a falcon breeding centre in northern Germany were taken for pathological, microbiological and mycotoxicological examinations. The samples showed marked necrotic nodular in lung tissues and in some respiratory airways pronounced hyphae formation and even sporulations were found. The air sacs were lined with a fungal sward and some airways were clogged with fungal mycelium. For mycotoxin analysis, kidney, liver, and lung of the birds were examined. The investigations have been performed by LC-MS-MS. Beside Gliotoxin other neuro- and cytotoxic secondary metabolites of *Aspergillus fumigatus* like Verruculogen, TR 2-toxin, Fumitremorgin B and C as well as Fumagillin have been detected. Concentrations were highest for Gliotoxin (up to 3 $\mu\text{g/g}$), but those of Verruculogen and Fumitremorgin C also exceeded the concentrations known to produce neurotoxic effects. To our knowledge this is the first time that toxins of the Fumitremorgin-Verruculogen-group were isolated from naturally infected avian tissues.

Auswirkungen von kleinräumigen Feldheterogenitäten im Boden und im Bestand auf den Mykotoxin Gehalt von Winterweizen

Marina Müller¹, Sylvia Koszinski², Gernot Verch³, Michael Sommer²

¹Leibniz-Zentrum für Agrarlandschaftsforschung Müncheberg (ZALF), Institut für Landschaftsstoffdynamik, Eberswalder Strasse 84, D-15374 Müncheberg, Germany

²Leibniz-Zentrum für Agrarlandschaftsforschung Müncheberg (ZALF), Institut für Bodenlandschaftsforschung, Eberswalder Strasse 84, D-15374 Müncheberg, Germany

³Leibniz-Zentrum für Agrarlandschaftsforschung Müncheberg (ZALF), Forschungsstation Landwirtschaft, Eberswalder Strasse 84, D-15374 Müncheberg, Germany

In Endmoränen-Gebieten des Landes Brandenburg sind die Winterweizen-Anbauflächen durch ihre Größe und durch ihre Relieferung gekennzeichnet. Wie Untersuchungen zeigen, sind innerhalb solcher großräumigen Getreidefelder pflanzliche Parameter wie der Ertrag oder der Proteingehalt des Getreides unterschiedlich. Kuppen und Senken als extreme Reliefpositionen zeigen durch ihre unterschiedlichen Bodeneigenschaften und Feuchtebedingungen ein optisch unterschiedliches Pflanzenwachstum. Die Pflanzendichte, die Pflanzenhöhe, der Zeitpunkt der Blüte und die Dauer der Abreife des Korns differieren auf diesen Standorten. Die vorgestellten Untersuchungen gehen deshalb der Frage nach, ob es durch diese Feldheterogenitäten auch zu unterschiedlichen Belastungen des Winterweizens mit Mykotoxinen innerhalb eines pflanzenbaulich gleich behandelten Feldes kommen kann.

Um die Komplexität der Einflussfaktoren auf die Konzentration des Deoxynivalenols in den geernteten Weizenkörnern zu erfassen, wurden entlang eines Niederschlagsgradienten in der Uckermark (langjährige Niederschlagsmittelwerte zwischen 400 und 550 mm) hügelige Winterweizenflächen mit unterschiedlicher Bodenbearbeitung (gepflügt und pfluglos) und Vorfrucht (Raps, Mais) ausgewählt, und auf diesen Flächen die Probenahmeorte auf den Kuppen und in den Senken festgelegt. Gleichzeitig wurden Bodenproben entnommen, die Bodenprofile bestimmt sowie direkt im Bestand eine Feuchtemessung von Anfang Juni bis Ende Juli vorgenommen.

Die Auswertung der Deoxynivalenol-Analysen in den Winterweizenkörnern der Jahre 2004 – 2006 zeigt den hauptsächlichlichen Einfluss der Bodenbearbeitung und der Vorfrucht sowie der Senkenposition auf die Konzentration dieses Mykotoxins. Die lehmig-anmoorigen Böden einiger Senken, die erhöhte Bestandesfeuchte an diesen Standorten und die verzögerte Abreife des Weizens scheinen dabei die Ursache für erhöhte DON Konzentrationen in diesen Senken-Bereichen zu sein. Kommen andere, *Fusarium*-fördernde Faktoren wie pfluglose Bodenbearbeitung, Mais als Vorfrucht und empfindliche Winterweizensorten hinzu, sind die DON-Gehalte auf allen Feldpositionen höher, die Maximalwerte werden auch hier meist in den Senken gemessen. Niedrige Weizen-Erträge auf den Kuppen sind mit geringen DON Werten korreliert, Regionen höherer Niederschläge führen vor allem auf den Kuppen zu höheren DON Gehalten. Feuchtere Böden in den Senken erhöhen auch die Feuchte direkt im Pflanzenbestand und bieten daher dem Pilz bessere und längere Wachstumsperioden, in denen Mykotoxine gebildet werden können. Höhere durchschnittliche Niederschlagswerte haben dabei weniger Einfluss auf die Mykotoxin-konzentration in diesen feuchten Senken-Habitaten.

Infection and development of *Fusarium* spp. in maize

Elisabeth Oldenburg¹, Frank Höppner¹, Joachim Weinert²

¹Institut für Pflanzenbau und Grünlandwirtschaft, Bundesforschungsanstalt für Landwirtschaft, Bundesallee 50, D-38116 Braunschweig, Germany

²Department für Nutzpflanzenwissenschaften, Fachgebiet Pflanzenpathologie und Pflanzenschutz, Universität Göttingen, Grisebachstrasse 6, D-37077 Göttingen, Germany

Unter mitteleuropäischen Klimabedingungen wird der Mais häufig von DON-bildenden *Fusarium*-Arten wie *F. graminearum* infiziert. Dieser Befall erfolgt bodenbürtig und wird vom Infektionsdruck und den Klimabedingungen am Wuchsstandort der Pflanze beeinflusst. Über witterungsspezifische Einflüsse, die eine *Fusarium*-Infektion des Maises in Mitteleuropa begünstigen, gibt es bisher wenige Kenntnisse. In diesem Beitrag werden dazu eigene Untersuchungsergebnisse und ein Befallsschema für den Mais vorgestellt.

Die Infektion einzelner Organe hängt vom pflanzlichen Entwicklungszustand ab und tritt in der Regel zeitlich und in der Ausprägung unterschiedlich in Erscheinung. Die Gefahr eines Befalls der Wurzeln und des unteren Stängelbereichs mit dem Krankheitsbild der Wurzel- bzw. Stängelfäule besteht potentiell während der gesamten Vegetationszeit. Trockenperioden, insbesondere auf sandigen, leichten Böden, und Verletzungen des Pflanzengewebes begünstigen diese Infektionen.

Primären Befall durch *Fusarium* spp. findet man in Kolbenanlagen, die nicht befruchtet und von der Pflanze nicht weiterentwickelt wurden, ab dem Zeitpunkt der weiblichen Blüte. Wird die Pflanze als Silomais für die Wiederkäuerfütterung genutzt, sind diese infizierten Kolbenanlagen zum Zeitpunkt der Siloreife die am höchsten mit DON belasteten Pflanzenorgane. Da sich die Pathogene von den primär infizierten Kolbenanlagen in die angrenzenden Pflanzenteile ausbreiten, können auch Blattscheiden, Blattspreiten und Stängel erhebliche Mengen des Mykotoxins DON enthalten.

Als Ergebnis einer mehrjährigen bundesweiten Erhebungsstudie zum Vorkommen von DON in Silomais, bei der Wetterdaten an den Standorten dokumentiert wurden, führten erhöhte Niederschlagsmengen in der frühen Wuchsphase Auflauf bis Blüte zu signifikant höheren DON-Gehalten im Ernteprodukt. Im Verlauf der nachfolgenden Reifephase bis zur Ernte war dagegen kein Einfluss durch die Niederschlagsmenge erkennbar. Der Temperaturverlauf zeigte ebenfalls keinen eindeutigen Zusammenhang mit den DON-Gehalten im Silomais. Es wird daher vermutet, dass die höheren Niederschlagsmengen vor der Blüte der Pflanze die Entwicklung des Pilzinokulums am Boden begünstigten und dadurch ein stärkerer Befall der anfälligen Pflanzenorgane resultierte. Nach erfolgter Infektion konnte sich der Pilzbefall dann innerhalb der Pflanze unabhängig von den Witterungsbedingungen weiterentwickeln. Die Kolbenfusariose entwickelt sich häufig erst, wenn die Siloreife der Pflanze überschritten ist und im Kolben TM-Gehalte über 50 % erreicht werden. Die Primärinfektion erfolgt hier an der Kolbenspitze unter Beteiligung der Narbenfäden. Im weiteren Verlauf seines Wachstums breitet sich der Pilz über die Spindel in den Körnern aus. Ein schlechter Lieschenschluss wirkt sich fördernd auf die Infektion aus, da dies mechanische Verletzungen der Körner durch Insektenbefall, Vogelfraß oder Hagelschlag nach sich ziehen kann. Anhaltend feuchte Witterung im Herbst begünstigt einen massiven Kolbenbefall, da dadurch die Abreife der Körner verzögert wird. Inwieweit sortenbedingte Unterschiede im Hinblick auf eine Anfälligkeit gegenüber *Fusarium*-Infektionen von Kolbenanlagen und Kolben bestehen, muss noch im Detail erforscht werden. Es sollten zudem erweiterte, den spezifischen Schadbildern abgepasste Boniturverfahren zur Bewertung des jeweiligen Krankheitsverlaufs entwickelt werden, um eine bessere Risikoabschätzung für die Maisproduktion und eine züchterische Sorten-Optimierung in Bezug auf die jeweilige Nutzungsform zu ermöglichen.

Defining background levels – First results for mycotoxins in house dust

Christoph Portner^{1, 2}, Volker Plegge¹, Jochen Türk¹, Werner Butte²

¹Institut für Energie- und Umwelttechnik e. V. (IUTA), Umweltmedizin/Analytik,
Bliersheimer Str. 60, D- 47229 Duisburg, Germany, e-mail: Plegge@iuta.de

²Carl von Ossietzky Universität Oldenburg, Fakultät V, Institut für reine und angewandte Chemie,
Carl-von-Ossietzky-Str. 9–11, D-26111 Oldenburg, Germany

Schimmelpilze sind in Wohnungen ubiquitär. Sie können beim Menschen schwerwiegende gesundheitliche Probleme bewirken. Neben der allergenen und infektiösen Wirkung von Schimmelpilzen ist die Bildung von Mykotoxinen von besonderer Bedeutung.

Innenraumkontaminationen stellen grundsätzlich ein hohes Umweltrisiko dar, weil sich die Bevölkerung in Ländern moderaten Klimas, wie in Deutschland, zu mehr als 80 % ihrer Zeit in Innenräumen aufhält. Mittel- und schwerflüchtige Mykotoxine reichern sich im Hausstaub an, weshalb Hausstaub ein idealer Indikator für eine Innenraumkontamination mit Mykotoxinen ist. Gleichzeitig ist Hausstaub auch ein wichtiger Pfad der Exposition mit diesen Toxinen. Bislang wurden Mykotoxine in Hausstaub nur an wenigen, häufig schimmelpilzindizierten Proben untersucht [1]. Typisch sind Gehalte im unteren $\mu\text{g}/\text{kg}$ -Bereich. Untersuchungen über das Vorkommen von Mykotoxinen in „normalen“, nicht schimmelbelasteten Wohnungen, d. h. über ihre Prävalenz, liegen bislang nicht vor.

In dieser Studie werden bevölkerungsrepräsentative Hausstaubproben untersucht, die im Rahmen von Bioziduntersuchungen gesammelt wurden. In diesen nicht induzierten Proben werden sieben Mykotoxine quantifiziert (Aflatoxin B1, Citrinin, Deoxynivalenol, Diacetoxyscirpenol, Gliotoxin, Ochratoxin A, Sterigmatocystin). Zudem wird per MS-Screening nach Schimmelpilz-Markern wie Ergosterin und weiteren Mykotoxinen gesucht [2]. Für über 150 Proben liegen bereits Ergebnisse vor. Von den untersuchten Toxinen kommen unter anderem Sterigmatocystin und Gliotoxin sowie insbesondere Deoxynivalenol im Hausstaub vor. Darüber hinaus werden aktuelle Proben von Schimmelschadensfällen und Kontrollproben gesammelt. Hierzu liegen ebenfalls erste Ergebnisse vor.

Der Hausstaub wird mittels Methanol/Wasser (84:16) und Ultraschall extrahiert. Aufgrund der unterschiedlichen Polarität der Analyten wurde auf eine Matrixabtrennung mittels Festphasenextraktion verzichtet. Stattdessen wurden die Extrakte direkt analysiert. Die Analyse erfolgte mittels HPLC-MS/MS (Shimadzu Prominence HPLC, Säule: Varian Pursuit XRs $5\ \mu\text{m}\ 150\times 2,0\ \text{mm}$, Applied Biosystems Q-Trap 3200) mit positiver und negativer Elektrosprayionisierung. Das MSⁿ-Screening erfolgte parallel, d.h. im gleichen Lauf im Trap-Modus (Enhanced MS).

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Toxigenic potential and pathogenicity of *Fusarium poae* and *Fusarium avenaceum* on cereals

Susanne Vogelgsang¹, Michael Sulyok², Rainer Schuhmacher², Hans-Rudolf Forrer¹

¹Research Station Agroscope Reckenholz-Tänikon ART, Reckenholzstrasse 191, CH-8046 Zurich, Switzerland

²Center for Analytical Chemistry, Department of Agrobiotechnology (IFA-Tulln), University of Natural Resources and Applied Life Sciences, Konrad Lorenz Strasse 20, A-3430 Tulln, Austria

Fusarium head blight (FHB) is one of the most noxious cereal diseases, causing substantial yield losses in small-grain cereals, a decline in grain quality, and the contamination of grain and straw by mycotoxins. In many cases, FHB is caused by a complex of *Fusarium* species producing different toxins. In Europe, the predominant toxigenic FHB pathogens are *F. graminearum*, *F. culmorum*, *F. avenaceum*, and *F. poae*. The species composition varies in time and space and there is evidence that *F. poae* and *F. avenaceum* are more prevalent and that their impact might be greater than the high number of publications on *F. graminearum* suggests.

Compared with toxins produced by *F. graminearum* (e.g. deoxynivalenol, zearalenone), some toxins of *F. poae* (nivalenol, diacetoxyscirpenol, monoacetoxyscirpenol, T-2, HT-2, beauvericin) and of *F. avenaceum* (enniatiins, moniliformin) display a far greater toxicity. This could have significant implications on human and animal health since plants and harvested grains infected by *F. poae* and *F. avenaceum* lack symptoms as distinct as those observed from *F. graminearum*. Therefore, and due to the lack of systematic monitoring of the associated toxins, contaminated cereal lots could be overseen and used for consumption or fed to animals.

Besides our work on epidemiology and prevention of FHB caused by *F. graminearum* (e.g. [1]), we have been examining the pathogenicity and toxigenicity of *F. poae* and *F. avenaceum* *in vivo* and *in vitro*. In a 3-year field experiment, the susceptibility of 14 Swiss winter wheat varieties was investigated by artificial inoculation with suspensions of a mixture of 3 poly-conidia isolates of either *F. poae* or of *F. avenaceum*. In climate chamber trials, individual single-conidia isolates were used to inoculate the wheat cultivar Apogee. Collected data included visual assessment of FHB incidence, yield, the incidence of infected grains by *Fusarium* species with a seed health test (field experiment only), as well as the analysis for selected mycotoxins in finely ground grains. In an *in vitro* study, the toxigenic potential of these isolates was assessed through incubation on 4 different cereal substrates. For both, *in vivo* and *in vitro* experiments, sample preparation and HPLC-MS/MS analysis was conducted according to [2]. Half a gram of ground sample was extracted using an acidified acetonitrile/water mixture. The raw extract was diluted 1:2, 1:100, and 1:7 500, respectively, and all three dilutions were subsequently analysed. Quantification was performed via external calibration. Blank samples were spiked, extracted, and analysed for control purposes.

We observed significant differences in the susceptibility of wheat varieties towards the mixture of *F. poae* and *F. avenaceum* isolates as well as substantial differences in pathogenicity between individual isolates on Apogee. Furthermore, the results from the *in vitro* experiment demonstrated strong substrate effects as well as isolate-specific substrate effects on the amount and type of toxins detected. Possible implications from these field and laboratory results for food and feed safety will be discussed.

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Elucidating the mechanisms by which wheat tolerates the *Fusarium* mycotoxin deoxynivalenol.

Stephanie Walter*, Josephine M. Brennan, Khairul I. Ansari,
Chanemougasoundharam Arunachalam, Mojibur R. Khan, Damian Egan, Fiona M. Doohan

Molecular Plant Microbe Interactions Group, School of Biology and Environmental Sciences,
Agricultural and Food Science Centre, University College Dublin, Belfield, Dublin 4, Ireland

* Author for correspondence: Phone: +353-1-7167110; e-mail: stephanie.walter@ucd.ie

Deoxynivalenol (DON) is a mycotoxigenic compound produced by *Fusarium* fungi. This toxin acts as an aggressive factor for these fungi when they attack wheat heads and cause *Fusarium* head blight (FHB) disease. Wheat cultivars differ in their susceptibility to FHB disease and their tolerance of DON. We used functional genomics techniques (differential display RT-PCR, spotted microarray analyses and gene-specific RT-PCR analysis) to identify a) wheat genes that respond to DON and b) wheat genes that are associated with the DON tolerance of wheat cultivar CM 82036. Based on our results, we propose a model whereby the early oxidative stress response and detoxification pathway contribute to DON tolerance. In wheat spikelets, DON-responsive wheat transcripts included retrotransposons and transcripts that code for proteins involved in defence [1], detoxification, oxidative stress response, protein synthesis, DNA replication and repair. Analysis of gene expression in double haploid progeny from a 'CM 82036' (DON tolerant) × 'Remus' (DON intolerant) cross showed that the effect of DON on the accumulation of several transcripts (including those coding for a bZIP transcription factor, an MRP ABC transporter, a glucosyltransferase and a cytochrome P450) was both genotype-specific and generally higher in progeny that inherited the FHB resistance QTL *Fhb1* from 'CM 82036'. Both the MRP ABC transporter gene and *Fhb1* are located on the short arm of chromosome 3B, but not in the same position.

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Reduction of mycotoxins in cereal grain using the resistance of wheat and triticale varieties against *Fusarium*

Bernd Rodemann, Gerhard Bartels

Biologische Bundesanstalt für Land- und Forstwirtschaft, Institut für Pflanzenschutz in Ackerbau und Grünland, Messeweg 11-12, D-38104 Braunschweig, Germany

Durch das Inkrafttreten der EU-VO 856/2005 zum 01.07.2006, gefolgt von der EU-VO 1881/2006 vom 19.12.2006 werden unter anderem die Höchstgehalte für die Mykotoxine Deoxynivalenol und Zearalenon in getreidebasierenden Lebensmitteln geregelt. Die deutsche Verordnung, die nur Höchstmengen für Speisegetreide und Brot und Backwaren beinhaltete, wird durch die EU-VO abgelöst und damit werden erstmals Grenzwerte für unverarbeitetes Getreide („unprocessed cereals“) festgelegt. Um diese Vorgaben einzuhalten, gilt es das Risiko des Fusariumbefalls vorbeugend durch Einhaltung pflanzenbaulicher Parameter wie Vorfrucht, Bodenbearbeitung, Pflanzenschutz, Düngung und insbesondere durch den Anbau einer resistenten Sorte bei der Aussaat zu minimieren.

In Deutschland werden zur Zulassung angemeldete Weizen- und Triticale Sorten dreijährig an bis zu sechs Standorten unter erhöhtem natürlichem Befallsdruck getestet und eine visuelle Befallseinschätzung gegenüber *Fusarium spp.* für das Bundessortenamt vorgenommen. Um reproduzierbare Ergebnisse zu erarbeiten, die eine Sicherheit in der Resistenzbewertung der Sorten ermöglichen, wird auf den Versuchsstandorten zusätzlich Inokulum mit erregerspezifischen Methoden ausgebracht.

Ergänzend wird die Kontamination des Ernteguts der Testsorten auf die Mykotoxine Deoxynivalenol und Zearalenon untersucht. Bei einigen Fragestellungen werden die Feldprüfungen ergänzt durch biochemische Analysen zur Bestimmung des Pilzbesatzes. Auf der Basis der erhobenen Daten werden die Sorten durch das Bundessortenamt (BSA) in Ausprägungsstufen (Skala 1 – 9) bewertet und die Ergebnisse in der jährlich erscheinenden Beschreibenden Sortenliste veröffentlicht.

Ergebnisse der Resistenzuntersuchungen des Jahres 2006 im Weizen an sechs Standorten wiesen einen mittleren Ährenbefall von 9 % auf, der bei den getesteten Genotypen zwischen 0,3 % und 25,2 % variierte. Die Neuzulassungen des Jahres 2006 gruppieren sich mit Werten zwischen 2,3 % und 10 % in den gering- bis mittelanfälligen Bereich ein.

Unter Berücksichtigung des Ährenbefalls und der Deoxynivalenol-Belastung erfolgte eine Einstufung über einen sog. *Fusarium*-Toxin-Index. Für die sehr gering bis gering anfälligen Sorten wie Solitär, Toras, Petrus oder Romanus wird die offizielle BSA-Einstufung nach APS-Noten durch Einbeziehung beider Parameter bestätigt. Wird die Sortenleistung nach Wirkungsgraden bewertet kann durch den Anbau o.g. sehr gering anfälliger Sorten sowohl der visuelle Ährenbefall als auch der DON-Gehalt im Vergleich zu hochanfälligen Sorten wie Ritmo und Quebon um 80 – 85 % reduziert werden.

Unter Berücksichtigung aller Ergebnisse der Resistenzprüfungen mit naturidentischer Inokulation, ergibt sich ein enger Zusammenhang mit Korrelationskoeffizienten von $r = 0,86 - 0,95$ zwischen dem sichtbaren Ährenbefall und der DON-Belastung im Erntegut.

In der Triticale - *Fusarium*resistenzbewertung war im Mittel der Jahre 2005 und 2006 ein Ährenbefall von 6 % festzustellen, der innerhalb der Genotypen zwischen 0,5 % und 18 % variierte. Gegenüber der hochanfälligen Sorte Versus mit 14 % Befall konnte durch die Sorten Benetto und Vitalis sowohl der Ährenbefall als auch die DON-Belastung im Erntegut um 70 – 78 % reduziert werden.

Hydrolysis of deoxynivalenol-3-O-glucoside

F. Berthiller¹, K.J. Domig², W. Kneifel², N. Juge³, S. Knasmüller⁴, G. Adam⁵, R. Krska¹,
R. Schuhmacher¹

¹Christian Doppler Laboratory for Mycotoxin Research, Dept. for Agrobiotechnology (IFA-Tulln), University of Natural Resources and Appl. Life Sciences Vienna, Konrad Lorenz Str. 20, A-3430 Tulln, Austria

²Division of Food Microbiology and Hygiene, Department of Food Sciences and Technology, University of Natural Resources and Appl. Life Sciences Vienna, Muthgasse 18, A-1190 Vienna, Austria

³Institute of Food Research, Norwich Research Park, Colney Lane, Norwich NR4 7UA, United Kingdom

⁴Institute of Cancer Research, Department of Medicine I, Medical University of Vienna, Borschkegasse 8a, A-1090 Vienna, Austria

⁵Inst. of Appl. Genetics and Cell Biology, Dept. of Appl. Plant Sciences and Plant Biotechnology, University of Natural Resources and Appl. Life Sciences Vienna, Muthgasse 18, A-1190 Vienna, Austria

Mycotoxin glucosides, formed by plants infected with toxigenic fungi, escape routine detection and have the potential to be reactivated in the digestive tract of mammals, as has been shown for zearalenone-4-O-glucoside (Z4G) in swine [1].

The aim of our studies was to find out whether deoxynivalenol-3-O-glucoside (D3G) [2] is a „masked mycotoxin“ by determination of its hydrolysis susceptibility to acidic pH, hydrolytic enzymes and intestinal bacteria. So far, D3G was found in naturally contaminated wheat up to 12 % of the DON value and in naturally contaminated maize even up to 50 % of the DON value. Therefore, the possible additional mycotoxin load should not be considered negligible.

D3G, as also observed for Z4G, showed high stability to acidic conditions down to a pH value of 0.7. The hydrolysis of those substances in the stomach of mammals is therefore extremely unlikely. Although the human and plant β -glucosidases had no hydrolytic effect, cellulase could partially hydrolyse D3G. In addition, several bacterial strains such as *Enterococcus durans*, *E. mundtii* and *Lactobacillus plantarum* showed the capacity to hydrolyse D3G to deoxynivalenol (DON). Feeding studies with rats are currently undertaken to determine the physiological relevance of D3G. We conclude that D3G should be regarded as a potential masked mycotoxin.

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Effects of the level of feed intake and ergot contaminated concentrate on ruminal fermentation, ergot alkaloid metabolism and carry over into milk, and on physiological parameters in cows

Barbara Schumann¹, Sven Dänicke¹, Peter Lebzien¹, Karl-Heinz Ueberschär¹, Joachim Spilke²

¹Institute of Animal Nutrition, Federal Agriculture Research Centre (FAL),
Bundesallee 50, 38116 Braunschweig, Germany

²Biometrics and Informatics in Agriculture Group, Martin-Luther University, Halle/Saale, Germany

The aim of the present study was to examine the effects of ergot contaminated concentrate at differing levels of feed intake on ruminal fermentation, ergot alkaloid metabolism and carry over into milk, as well as on various physiological parameters in dairy cows. Twelve double fistulated (in the rumen and the proximal duodenum) Holstein Friesian cows were fed either the control diet (on a dry matter (DM) base: 60 % maize silage, 40 % concentrate) or the contaminated diet (concentrate contained 2.25 % ergot, which caused an alkaloid concentration of the daily ration between 504.9 and 619.5 $\mu\text{g}/\text{kg DM}$) over a period of four weeks. Daily feed amounts were adjusted to the current performance which resulted in a dry matter intake (DMI) variation between 6.0 and 18.5 kg/day. The actual alkaloid exposure varied between 4.1 and 16.3 $\mu\text{g}/\text{kg}$ body weight when the ergot contaminated concentrate was fed. Isovalerate, propionate and $\text{NH}_3\text{-N}$ concentrations in the rumen fluid were significantly influenced by ergot feeding, and the amount of ruminally undegraded protein, as well as the fermentation of NDF, tended to increase with the ergot supplementation at higher levels of feed intake, which might indicate a shift in the microbial population. Other parameters of ruminal fermentation such as ruminal pH, fermented organic matter as a percentage of intake, or the amount of non-ammonia N measured at the duodenum were not significantly influenced by ergot feeding. Approximately 67 % of the alkaloids fed were recovered in the duodenal digesta, and approximately 24 % were excreted with the faeces. No alkaloid residues could be detected in the blood or milk samples, and the activities of liver enzymes in the serum were independent of ergot feeding. The anally measured body temperature of the cows significantly increased after ergot administration ($p=0.019$). Thus, body temperature can be regarded as a sensitive parameter to indicate ergot exposure of dairy cows.

Characterization of the cytotoxicity of mycotoxins: Information gained from variations of *in vitro* assay conditions

Gisela H. Degen, Manoj Aggarwal, Claudia Behm, Wolfram Föllmann

Institut für Arbeitsphysiologie an der Universität Dortmund (IfADo),
Ardeystrasse 67, D-44139 Dortmund, Germany

Cytotoxicity assays with mycotoxins are carried out for various reasons, either to characterize suitable concentration ranges to be used in other short term tests on genotoxicity, to compare the potency of several toxins, and/or to study combination effects of mixtures. Concentration-dependent effects of mycotoxins on cultured cells can be evaluated by a number of assays which differ in demand for execution, performance and throughput, but also with regard to endpoints and types of damage detected (cell death or impairment of cell proliferation and function).

From recent studies with ochratoxin A, citrinin, patulin and zearalenone, examples will be given to illustrate how detection methods for metabolic activity of mitochondria or lysosomes (Cell Titer Blue and Neutral Red uptake assays) were applied to assess the cytotoxicity of individual compounds and of relevant mixtures. Also data are given to which extent variations of assay conditions (cell density and treatment time, as well as medium composition and cell type) can affect concentration-effect-curves, *i.e.* the results of cytotoxicity assays, expressed as IC₅₀ values. On the one hand, this data indicate the need for standardization of treatment conditions when comparing potencies of various mycotoxins and caution against cross-comparisons between measures of cytotoxicity gained in different assays, even with the same assay and cell type. On the other hand, differences in the outcome of assays, conducted with a low or high fraction of growing cells during treatment, provide already some insight whether a compound acts predominantly cytotoxic or cytostatic. For an in-depth characterisation of „cytotoxicity“ induced by mycotoxins, additional assays with parameters that distinguish between cell death by necrosis or by apoptosis, and or flow cytometric analysis (for cell cycle arrest) are recommended.

Cytotoxicity, cellular uptake and metabolism of deoxynivalenol by human kidney cells and human lung fibroblasts in primary culture

Maika Königs¹, Gerald Schwerdt², Michael Gekle², Hans-Ulrich Humpf^{1*}

¹Institut für Lebensmittelchemie, Westfälische Wilhelms-Universität Münster, Germany

*e-mail: humpf@uni-muenster.de

²Physiologisches Institut, Universität Würzburg, Würzburg, Germany

Concerning its rate of occurrence deoxynivalenol (DON) is one of the most important mycotoxin in cereal commodities. At the level of the whole animal DON causes diverse effects. The acute toxicity varies upon the severity and duration of the exposure. The toxic effects range from causing diarrhoea, vomiting, gastro-intestinal inflammation to necrosis of several tissues. It also affects the immune system and leads to kidney lesions [1]. At the cellular level, the main toxic effect of DON is the inhibition of protein synthesis via binding to the ribosomes. Apart from this primary toxic effect it is assumed that there are several other secondary cellular effects [2].

Although DON has been tested in different human and animal cells for its cytotoxicity, these tests are of limited significance and do not necessarily reflect the situation in humans. The great disadvantage of cell lines consists in their modifications, their tumour derivation or their alteration during long-time cultivation.

In order to come closer to the human situation, we studied the effect of DON in human cells from primary culture. In our investigations we compared effects on renal proximal tubule epithelial cells (RPTEC) and lung fibroblasts (NHLF) measuring cell viability, apoptotic and necrotic cell death, collagen and fibronectin secretion.

We could demonstrate that DON has a distinct cytotoxic effect on human primary cells. A reduction in viability has been observed in both cell types, with fibroblasts reacting more sensitive. Furthermore we showed that DON is able to cause apoptosis as well as necrosis in the human primary cells. Depending on the analysed cell types DON induces cell death through different pathways. While necrotic cell death occurs in proximal tubule cells, DON leads to caspase-3-mediated apoptosis in lung fibroblasts.

We also studied the metabolism and the cellular uptake of DON using LC-MS/MS. The results indicate that DON is neither metabolized by proximal tubule cells nor by fibroblasts. No accumulation in the cells occurs, but cellular uptake of DON has been observed. The results shows that the uptake is higher in kidney cells than in fibroblasts. For the first time the amount of DON that is incorporated into cells was quantified.

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Biotransformation of the mycoestrogen zearalenone in horses

Hassan Malekinejad¹, Johanna Fink-Gremmels²

¹Faculty of Veterinary Medicine, Urmia University, Urmia, Iran

²Utrecht University, Faculty of Veterinary medicine, VFVT, Yalelaan 104, 3508 TD Utrecht, The Netherlands

Zearalenone (ZEA) is an estrogenic mycotoxin that despite its non-steroidal structure interacts with both types of the oestrogen receptors (ER α and ER β). This receptor interaction seems to account for the clinical signs of hyperoestrogenism observed in pigs and other animal species (for review see [1]). In previous studies we showed that ZEA undergoes two major biotransformation pathways including hydroxylation by 3 α - and 3 β -hydroxy steroid dehydrogenase, followed by conjugation. We also reported that there are significant differences between various animal species in terms of metabolite production. For example, α -zearalenol (α -ZOL) with a high binding affinity to oestrogen receptors was found to be the major hepatic and extra-hepatic metabolite of ZEA in porcine tissues, whereas β -zearalenol (β -ZOL), with a significantly lower oestrogen-like activity, is formed by the poultry livers, and bovine liver and granulosa cells [2,3].

Recently, maize (corn), known to be regularly contaminated with ZEA, is used with increasing frequencies in diets for horses. However, only very few reports are available about the adverse effects of ZEA on equine species. These previous reports showed that feed intake was decreased in horses fed grains contaminated by *Fusarium* toxins. Moreover, in a recent study it was demonstrated that ZEA and its metabolites could cause apoptosis in granulosa cells isolated from equine ovaries.

With the present study we aimed to describe the hepatic biotransformation of ZEA by primary equine hepatocytes and subcellular liver fractions including microsomes and post-mitochondrial fractions obtained from horse liver tissue. Results show that comparable to pigs, horses seem to convert ZEA predominantly to the α -ZOL isomer, which is known to have a higher estrogenic activity than the parent ZEA. Both, ZEA as well as its metabolites, are successfully conjugated in the liver samples and hepatocytes at low toxin concentrations. However, in the presence of ZEA concentrations exceeding 50 μ Mol, the glucuronidation capacity was saturated, and only a small fraction of the toxins (ZEA and its metabolites) were conjugated.

These data are of clinical relevance, as they indicate that horses toxify ZEA into the more potent α -ZOL. The latter might be pre-systemically cleared by glucuronidation, but this conjugation step is easily saturable. Forthcoming studies should therefore address the analysis of systemic toxin levels following oral exposure of horses, and measure the effects of these compounds on the reproductive performance of both, male and female animals.

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Mechanisms of the genotoxic effect of *Alternaria* toxins

Doris Marko

Institute of Applied Bioscience, Section of Food Toxicology, University of Karlsruhe (TH),
Adenauerring 20a, 76131 Karlsruhe, Germany

Contamination of food and feed with toxin-producing fungi represent a major risk for diseases in humans and animals. Exposure to *Alternaria spp.*, especially *Alternaria alternata* has been associated with enhanced incidence of oesophageal cancer. Extracts of *A. alternata* have been described as genotoxic and mutagenic *in vitro*. However, the underlying mechanism of action has not been elucidated so far.

We investigated the genotoxic effect of the *Alternaria* toxins alternariol (AOH), alternariol monomethyl ether (AME), altenuene and isoaltenuene with emphasis on potential mechanisms involved. AOH and AME significantly increased the rate of DNA strand breaks in human colon carcinoma cells at concentrations $\geq 1 \mu\text{M}$ and $25 \mu\text{M}$, respectively, measured as DNA strand breaks by single cell gel electrophoresis (comet assay). In contrast, altenuene and isoaltenuene did not affect DNA integrity up to $100 \mu\text{M}$. The rate of DNA strand breaks induced by AOH and AME were not modulated by formamidopyrimidine-DNA-glycosylase (fpg), thus excluding enhanced oxidative DNA damage.

AOH effectively competed with the minor groove binding ligand Hoechst 33258 with an EC_{50} -value of $8 \pm 1 \mu\text{M}$ indicating substantial affinity to the minor groove of the DNA. A number of DNA minor groove binding ligands affect mammalian topoisomerases I and II. Therefore, we investigated the impact of *Alternaria* toxins on the different classes of topoisomerases. AOH was found to inhibit the catalytic activity of topoisomerase I at concentrations $\geq 50 \mu\text{M}$. In contrast AME, bearing a methoxy group at position 9, did not affect the catalytic activity of topoisomerase I up to $100 \mu\text{M}$. The catalytic activity of topoisomerase II α and II β was significantly suppressed by AOH at $\geq 25 \mu\text{M}$ and $\geq 150 \mu\text{M}$, respectively. AME did not affect the activity of topoisomerase II β up to $200 \mu\text{M}$, but was found to be equipotent to AOH with respect to the inhibition of topoisomerase II α . Thus, topoisomerase II α was identified as the most sensitive target so far for both, AOH and AME. In accordance with the results in the comet assay, altenuene and isoaltenuene did not affect the activity of topoisomerase I and II.

Furthermore, we investigated the mode of interaction of AOH with the potential target enzymes. AOH was found to stabilise the catalytically generated DNA-topoisomerase intermediate of topoisomerase I and II, thus acting as a so called topoisomerase poison. The stabilisation of the DNA-topoisomerase II α intermediate was observed in the concentration range leading to enhanced DNA strand breaks in the comet assay.

In summary, AOH and AME were identified as potent inhibitors of topoisomerase II α , which might at least contribute to the DNA strand breaking properties of these mycotoxins.

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Effects of deoxynivalenol (DON) on human cell lines

Carina Nielsen, Richard Dietrich, Andrea Didier, Harald Lippke, Erwin Märtlbauer

Chair of Hygiene and Technology of Milk, Faculty of Veterinary Medicine,
Ludwigs Maximilians-Universität Munich, Schönleutner Str. 8, D-85764 Oberschleißheim, Germany

Effects of trichothecenes in animals comprise toxic alterations of the immune system, growth depressions and reproduction disorders. To expand insights into molecular pathomechanisms underlying the trichothecene toxicity, in this study human cell lines were stressed with these mycotoxins. Altered pathways were identified by a microarray approach and results were confirmed on RNA and protein level.

To get an overview on *in vitro* cytotoxicity, the 50 % toxic dose of six trichothecenes (DON, nivalenol, T2, HT2, satratoxin G and H) were determined in six human cell lines (HepG2, HeP2, Jurkat, A204, CaCo2 and U937). With special regard to DON a time-kinetic experiment using non-cytotoxic equivalence-doses was performed in five of the above mentioned cell lines. Subsequently, mRNA expression profiles of marker-genes derived from different metabolic pathways and potentially influenced by trichothecenes were assayed by the means of real-time RT-PCR followed by relative quantification. As investigations at single gene can not provide a comprehensive picture of all factors involved, additionally an expression profile of HepG2 cells was generated in a microarray approach.

Present results clearly show that mycotoxin-treated HepG2 exhibit alterations of gene expression in different metabolic pathways. Especially, factors belonging to the MAP-kinase signalling pathway, transcription factors as well as the cytokine TNF-alpha were affected. It is known that p38 MAP-Kinase, extracellular signal regulated kinase (ERK) and c-Jun N terminal protein kinase (JNK) MAPKs family are activated by a variety of stressors, toxins and receptor agonists [1]. The relevance of DON concerning activation of the MAP-kinase pathway has recently been shown *in-vitro* and *in vivo*-studies [2,3]. However, most of these studies focus on MAP-kinase pathway in view of an immuno-modulatory effect. Little is known about other metabolic consequences of an DON-activated MAP-kinase pathway (e.g. proliferation, differentiation and growth modulation). In this study influences of DON on factors of the MAP-Kinase pathway including transcription factors and other target genes were identified.

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Ochratoxin A: 13-Week Oral Toxicity and Cell proliferation in Male Fischer F344/N Rats

Eva Rached¹, Gordon C. Hard², Kai Blumbach³, Klaus Weber³, Regina Draheim³, Sibel Özden², Ulrich Steger⁴, Wolfgang Dekant¹, Angela Mally¹

¹Department of Toxicology, University of Würzburg, Versbacher Strasse 9, 97078 Würzburg, Germany

²Private Consultant, Tairua, New Zealand

³RCC Ltd., CH-Itingen/Füllinsdorf, Switzerland

⁴Department of Surgery I, University of Würzburg, Oberdürrbacher Strasse 6, 97080 Würzburg, Germany

Ochratoxin A (OTA) is nephrotoxic and a potent renal carcinogen. Male rats are most susceptible to OTA toxicity and chronic administration of OTA (70 and 210 $\mu\text{g}/\text{kg}$ b.w.) for two years has been shown to induce high incidences of adenomas and carcinomas, arising from the straight segment of the proximal tubule epithelium. In contrast, treatment with a lower dose of 21 $\mu\text{g}/\text{kg}$ b.w. did not result in increased tumor rates, suggesting a non-linear dose-response for renal tumor formation by OTA.

Since the mechanism of OTA carcinogenicity is still largely unknown, this study was conducted to investigate early functional and pathological effects of OTA and to determine if sustained stimulation of renal cell proliferation might play a role. Male F344/N rats were treated with OTA for up to 13 weeks under conditions of the NTP bioassay. Cell proliferation in the renal cortex and outer stripe of the outer medulla (OSOM) was determined using bromodeoxyuridine (BrdU) incorporation and immunohistochemistry.

Histopathological examination showed renal alterations in mid and high dose animals involving single cell death and prominent nuclear enlargement within the straight proximal tubules. Treatment with OTA at doses of 70 and 210 $\mu\text{g}/\text{kg}$ b.w. led to a marked dose- and time-dependent increase in renal cell proliferation, extending from the medullary rays into the OSOM. No effects were evident in kidneys of low dose animals or in the liver, which is not a target for OTA carcinogenicity. A NOEL in this study was established at 21 $\mu\text{g}/\text{kg}$ b.w., correlating with the dose in the NTP 2-year bioassay that did not produce renal tumors. The apparent correlation between enhanced cell turnover and tumor formation induced by OTA indicates that stimulation of cell proliferation may play an important role in OTA carcinogenicity and provides further evidence for an epigenetic, thresholded mechanism.

The positive effects of a probiotic microorganism (*Eubacterium sp.* DSM 11798) on the duodenal and jejunal histological alterations caused by the trichothecene deoxynivalenol of broilers

Wageha Awad¹, Josef Böhm¹, Ebrahim Razzazi-Fazeli¹, Khaled Ghareeb², Jürgen Zentek¹

¹Institut für Ernährung, Veterinärmedizinische Universität Wien,
Veterinärplatz 1, A-1210 Vienna, Austria

²Institut für Tierhaltung und Tierschutz, Veterinärmedizinische Universität Wien,
Veterinärplatz 1, A-1210 Vienna, Austria

During the last few decades, the investigation of the biodegradability of mycotoxins has become an area of great interest. Specific rumen and intestinal micro flora detoxify DON by the removal of the epoxide oxygen. The de-epoxidation is an important step in the detoxification of trichothecenes. Therefore, the objective of the present study was to investigate the effect of 10 mg/kg DON in the presence or absence of a microbial feed additive (BBSH 798) on performance and intestinal histology.

Materials and Methods: Two hundred and seventy-seven, 1-d-old broiler chicks were distributed randomly into three groups, either in the presence or absence of the microbial feed additive BBSH 798 (*Eubacterium sp.* DSM 11798). Feed consumption, body weight gain and feed: gain ratio were measured weekly during the 6-wk experiment. Histological sections of duodenum and jejunum, stained by haematoxylin and eosin, and examined by light microscope. In the duodenum and jejunum, the villus length was measured from the villus tip to the bottom. The villus width measured at the bottom of villi. The measurement was done by the Scion Image Program. The mean villus heights and width from 15 birds were expressed as a mean villus height for one treatment group. ANOVA and subsequent Duncan's Multiple Range Test were used for comparisons between groups.

Results: The general performance of the birds was not influenced ($p > 0.05$) by feeding DON. The absolute or relative organ weights were not altered ($p > 0.05$), moreover, the relative liver weight was slightly decreased in broilers fed the diet containing DON. DON altered small intestinal morphology, especially in the duodenum and jejunum where the villi were shorter ($1237 \pm 10 \mu\text{m}$, $976 \pm 5 \mu\text{m}$, respectively) and thinner ($190 \pm 2 \mu\text{m}$, $158 \pm 1 \mu\text{m}$, respectively) compared to controls (length was $1667 \pm 4 \mu\text{m}$, $1064 \pm 5 \mu\text{m}$, and width was $249 \pm 3 \mu\text{m}$, $175 \pm 2 \mu\text{m}$, in duodenum and jejunum respectively). Probiotic supplementation to the contaminated diet significantly ($p < 0.05$) diminished the effects of toxin on the villus height of the duodenum ($1548 \pm 7 \mu\text{m}$), and the jejunum ($1050 \pm 6 \mu\text{m}$).

Conclusion: Feeding DON contaminated diets at 10 mg/kg did not depress the growth performance of broilers. However, DON altered the small intestinal morphology, especially in the duodenum and jejunum. Furthermore, the results indicate that supplementation of the probiotic BBSH 798 (*Eubacterium sp.* DSM 11798) is beneficial in counteracting the toxicity of DON. The *Eubacterium sp.* transforms DON into its metabolite DOM-1 and reduces the risk of undesirable and harmful effects of DON.

Effects of live yeast cell supplementation to high concentrate diets on the toxicokinetics of ochratoxin A in sheep

Ralf Blank, Siegfried Wolfram

Institute of Animal Nutrition and Physiology, Christian-Albrechts-University, 24098 Kiel, Germany

Introduction: Ruminants are relatively resistant against acutely toxic effects of the mycotoxin ochratoxin A (OA) due to an extensive hydrolysis of OA to its less toxic metabolite ochratoxin α ($O\alpha$) and phenylalanine by rumen microorganism. According to previous studies in sheep, however, systemic availability of OA is increased when animals are fed high concentrate diets. Under such feeding conditions the degradation of OA in the rumen seems to be limited due to a lower post feeding rumen pH compared to roughage diets and a concomitant reduction of the rumen protozoa which are substantially involved in ruminal OA degradation. Feeding strategies like supplementation of live yeast cells or sodium bicarbonate to high concentrate diets have been shown to stabilize or increase the post feeding ruminal pH. The present study was conducted to investigate whether supplementation of live yeast cells to high concentrate diets affects the toxicokinetic of OA in sheep.

Materials and Methods: Twelve castrated male sheep (b.w. 90 kg) housed in metabolism crates were divided into two feeding groups and fed a diet consisting of 70 % concentrate and 30 % grass silage without and with supplementation of 0.4 g live yeast cells (Levucell[®] SC 20, Lallemande S. A., France) for three weeks. The energy and protein supply was 2.0 times the requirements for maintenance. After 3 weeks all animals received a single dose of 150 g of OA-contaminated wheat containing 2.46 mg OA. Wheat was offered at the beginning of the feeding time to ensure a complete ingestion of the OA dose. Blood samples from the jugular vein were taken and faeces and urine were collected over a period of 6 days. Thereafter, sheep received an additional single dose of OA-contaminated wheat and rumen fluid samples were taken before and 1, 4, 7, 10, and 24 hours post feeding by means of an oro-ruminal probe. Ochratoxin A and its metabolite $O\alpha$ were analysed in faeces, urine, ruminal fluid and serum samples by HPLC.

Results: Supplementation of live yeast cells tended ($p < 0.10$) to increase post-feeding ruminal pH due to a faster pH recovery following the post feeding drop. Nevertheless, the higher concentration of OA in rumen fluid of sheep fed live yeast cells compared to the control group indicate a slower degradation in the first hours post feeding. However, this effect was counter-balanced in the later hours post feeding as indicated by a similar concentration of $O\alpha$ in rumen fluid in both feeding groups. The systemic availability of OA as well as the faecal and renal excretion of OA and its metabolite $O\alpha$ were not influenced by the live yeast cell supplementation.

Conclusions: The results of this study indicate that supplementation of live yeast cell to high concentrate diets may have a beneficial effect on rumen pH, but the changes in pH may be possibly too small to have a major impact on the toxicokinetics of OA.

Effect of Thiophanate – methyl on mycotoxin production in cereals under field conditions and *in vitro*

Herbert Buschhaus¹, Frank M. Ellner²

¹NISSO CHEMICAL EUROPE GmbH, Steinstrasse 27, 40210 Düsseldorf, Germany

²Federal Biological Research Centre for Agriculture and Forestry, Institute for Ecotoxicology and Ecochemistry in Plant Protection, Königin-Luise-Str. 19, 14195 Berlin, Germany

Thiophanate-methyl (TM) is a benzimidazole fungicide, which has been on the market for over 30 years. This compound has a broad spectrum of activity against many economically important plant pathogens belonging to the groups of Ascomycetes, Deuteromycetes, and Basidiomycetes. TM has been in the registration process as „DONstopp“ designed for control of *Fusarium spp.* in cereals and for reduction of the mycotoxin content as the name implies. Under field conditions TM was applied at a rate of 770 g/ha at BBCH growth stages varying from 61 to 75 and reduces Deoxynivalenol (DON) by 45 to 59 % compared to the untreated control, which had an average amount of 1.2 mg DON/kg. Zearalenon (ZEA) was reduced around 74 % by all treatments. The influence of the application at different growth stages was more pronounced concerning the reduction of DON in comparison to ZEA, where virtually no difference between the different applications could be observed. DON reduction, however, was highest after the application between BBCH stages 61 – 71 at a level of approximately 56 % with regard to the untreated control. Even at BBCH stage 75, an average reduction of 45 % could be achieved.

In a second trial series, TM was applied 1–3, 6–10, and 11–23 days after artificial inoculation of *F. graminearum* spores (505 spores/ml). Up to 10 days after the artificial inoculation TM could reduce the amount of DON between 65 and 67 %. Even the last application still reduced DON by 44 %.

In vitro studies were performed with different *Fusarium* species isolated from maize at various locations throughout the world. The isolates were cultivated on maize grits containing 0, 0.5, 1.0, 10, or 50 µg TM/mg medium, respectively, for at least 10 days under best conditions. Significant effects on growth could be observed with concentrations starting at 10 µg TM/mg medium. The influence on mycotoxin formation varied depending on the *Fusarium* strain investigated. Fumonisin (FB) concentration produced by *F. verticilloides* declined successively with increasing TM concentrations with a total reduction by 75 % at 50 µg TM/g medium, whereas it remained nearly unchanged up to TM concentrations of 10 µg/mg medium in the presence of *F. proliferatum*. FB formation was reduced by 90 % at 50 µg TM/g medium.

The pattern of two *F. graminearum* strains representing NIV and DON chemotypes became comparable with each other under conditions of decreasing mycotoxin formation from 10 µg TM/g medium upwards.

Detoxification of zearalenone by *Gliocladium roseum*: biological function and regulation of zearalenone lactonase synthesis

Jan Utermark, Petr Karlovsky

Molecular Phytopathology and Mycotoxin Research, Goettingen University,
Grisebachstrasse 6, D-37077 Goettingen, Germany, www.gwdg.de/instpht/karlovsky/

Zearalenone is a resorcylic lactone derivative produced by plant pathogenic *Fusarium* species, which exerts estrogenic effects on mammals. The biological role of zearalenone in the fungal biology has been unknown. We established that zearalenone inhibits the growth of most filamentous fungi. Only myco-parasitic ascomycetes *Gliocladium roseum*, which is known to hydrolyze zearalenone by the activity of zearalenone-specific lactonase, was not affected by zearalenone. This observation lead us to raise the hypothesis that zearalenone acts as agent of interference competition and zearalenone lactonase protects *G. roseum* from its effect. To test the hypothesis we inactivated *zes2* gene encoding zearalenone lactonase in *G. roseum* using *Agrobacterium tumefaciens*-mediated genetic transformation. The mutants were unable to hydrolyze the lactone bond of zearalenone and displayed an increased susceptibility to zearalenone, corroborating the hypothesis about the ecological function of this mycotoxin.

In order to facilitate the investigation of the regulation of zearalenone lactonase synthesis, we constructed promoter fusions of *zes2* with reporter genes encoding Green Fluorescent Protein (GFP) and luciferase from *Gaussia princeps*. *G. roseum* strains harboring the fusion constructs exhibit fluorescence (GFP) or emit light (luciferase) upon exposure to zearalenone. We used the strains to elucidate the inducibility of zearalenone lactonase genes by different zearalenone derivatives. Furthermore, these strains serve as bioassays for zearalenone. In contrast to yeast-based bioassays for zearalenone described so far, *G. roseum*-based bioassays possess a high specificity for zearalenone and its derivatives and do not respond to phytoestrogens and steroids.

Abstracts of Posters

Large Scale Production of T2- and HT2-toxin using the FCPC-System

Marita Beyer, Eva Groß, Hans-Ulrich Humpf*

Institut für Lebensmittelchemie, Westfälische Wilhelms-Universität Münster,
Corrensstr. 45, D-48149 Münster, Germany, *e-mail: humpf@uni-muenster.de

The type A trichothecenes T2- and HT2-toxin are toxic secondary metabolites from fungi of the *Fusarium* genus. Until now, there are no legal limits for these toxins, but the occurrence in cereals, especially in oats imply health risks for the consumer. Therefore, it is important to undertake further studies on the stability and toxicity, and to determine a selective and sensitive method for the analysis of T2- and HT2-toxin. Although, most toxins are commercially available, their high prices are the limiting factor on the realization of the experiments. Thus, we developed a method in order to obtain large amounts of T2- and HT2-toxin.

F. sporotrichioides (DSM 62423) was chosen for the biosynthetic production as this strain was described to produce high amounts of T2-toxin [1]. Maize was inoculated with cultures of *F. sporotrichioides* for 21 days at 15 °C. Under these conditions the fungus produced mainly T2-toxin, HT2-toxin and neosolaniol were formed as by-products. The cultures were extracted with 70% methanol and the extract was subsequently lyophilized. The anhydrous residue was extracted with ethyl acetate, and the following purification was carried out by fast centrifugal partition chromatography (FCPC). This technique is based on the separation of solutes, by their partition between two immiscible liquid phases. In the fast centrifugal partition chromatograph more than 1 000 small chambers (called „loculi“) are linked in series and arranged in a circle around a rotor. Setting the rotor in motion (800 – 1 000 rpm), a permanent gravitational field keeps the stationary phase in the loculi, while the mobile phase is pumped through the system. During this process, chromatographic separation is achieved through the different distribution of the sample constituents in the mobile and stationary phase. Using this system we were able to isolate gram quantities of T2-toxin and, lower quantities of HT2-toxin and neosolaniol. Starting from T2-toxin, higher amounts of HT2-toxin, and also T2-triol and T2-tetraol were obtained by alkaline hydrolysis.

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MSTFA/TMS-imidazole: faster and simpler trimethylsilylation of type A + B trichothecenes

Andreas Breidbach, Vitalija Povilaityte, Jörg Stroka

European Commission, Directorate General- Joint Research Center,
Institute of Reference Materials and Measurements, Retieseweg 111, B-2440 Geel, Belgium

To make type A + B trichothecenes amenable to separation by gas chromatography (GC) their hydroxyl functions need to be derivatized. Because of their favourable properties trimethylsilyl-(TMS) derivatives are the preferred choice. For the trimethylsilylation of these trichothecenes Trimethylsilyl-imidazole (TMSIM), with or without the addition of Trimethylchlorosilane (TMCS) and N,O-Bis(trimethylsilyl)acetamide (BSA), has commonly been used in the past. TMSIM, pure or as mix with TMCS and BSA, has a detrimental effect on the GC column and needs to be removed before injection. This is accomplished either by evaporation or by liquid-liquid extraction of the TMS-trichothecenes with an organic solvent (f.i. isooctane) against water or buffer.

We propose the use of N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) containing 5% TMSIM to convert type A + B trichothecenes to their respective TMS derivatives. MSTFA, pure or as mix with TMCS, TMS-iodine, or TMSIM, has been used as TMS reagent of choice for the GC separation of many different substance groups, foremost steroids, for many years in doping control analysis.

We will show that the silylating potential of this reagent is high enough to also convert the C₇-OH groups of deoxynivalenol and nivalenol at room temperature within 15 min. At the same time this mix is not harmful to the GC column and trichothecenes are quite stable in it, therefore there is no need for removal of excess reagent. This makes the derivatization step very fast and convenient: just add 50 μ L of the reagent to the dry extract, mix, incubate 15 min at room temperature, and inject into the GC. An additional positive effect is the smaller volume of the injection solution, which increases the concentration of the analytes significantly resulting in increased signal-to-noise ratios.

T-2 and HT-2 by GC/MS for official food control - a validated method

Andreas Breidbach, Vitalija Povilaityte, Carsten Mischke, Ivanka Doncheva, Jörg Stroka

European Commission, Directorate General- Joint Research Center,
Institute of Reference Materials and Measurements, Retieseweg 111, B-2440 Geel, Belgium

The two mycotoxins, T-2 toxin (12,13-Epoxytrichothec-9-ene-3- α ,4- β ,8- α ,15-tetrol-4,15-diacetate-8-isovalerate) and HT-2 toxin (12,13-Epoxytrichothec-9-ene-3- α ,4- β ,8- α ,15-tetrol-15-acetate-8-isovalerate) are produced by fungi of the *Fusarium* genus. One of the two, T-2 toxin, has been subject of a SCOOP exposure assessment in 2003. One major finding was the fact that about 80 % of all measured samples were below the limit of detection. The reason for this were insufficiently high limits of detection of the employed detection methods. Also, legislative limits for T-2 and HT-2 toxins will be introduced soon by European Commission Regulation 1881/2006. To our knowledge up-to-date there exists no fully validated or officially accepted method for the detection of T-2 and HT-2 toxin working at the desired target levels.

We present here a method for the detection of T-2 and HT-2 toxin in different matrices based on immunoaffinity (IA) clean-up and GC/MS detection. In brief, a finely ground sample is suspended in methanol/water (80/20, v/v) for 30 min. After filtration the filtrate is diluted to methanol/water (16/84, v/v) and filtered again to remove any precipitation. This second filtrate is then, after addition of stable-isotope labelled T-2 toxin (¹³C₂₄-T-2), applied to an IA column specific to T-2 and HT-2. After washing and elution with pure acetonitrile the cleaned-up extract is dried down and derivatized by addition of N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA)/Trimethylchlorosilane (TMCS) (99/1, v/v). This solution was then injected into a GC/MS system without removal of the excess reagent.

The above method was then validated in a collaborative trial involving 26 laboratories from 16 European countries. Three different matrices, one straight cereal mix, one resembling a commercial baby food and one a compound animal feed, were tested at blank and naturally contaminated levels. The consensus values for the total concentrations of the sum of T-2 and HT-2 ranged from 11 $\mu\text{g}/\text{kg}$ in the lowest contaminated baby food matrix to 122 $\mu\text{g}/\text{kg}$ in the highest contaminated animal feed matrix. The relative standard deviation under reproducibility conditions (RSD_R) for the determination of T-2 ranged from 12 to 28 %. For the determination of HT-2 RSD_R ranged from 17 to 32 %. These RSD_R s are all well within the permissible range given in European Commission Regulation 401/2006, namely that the RSD_R should be smaller than 60 % for T-2 below 250 $\mu\text{g}/\text{kg}$ and for HT-2 below 200 $\mu\text{g}/\text{kg}$.

We therefore consider this method suitable for official food control and will submit it to CEN for standardization.

A strategy to assess measurement uncertainty using certified reference materials for method validation

Gerhard Buttinger, Magdalena Michulec

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements,
Reference Materials Unit, Retieseweg 111, 2440 Geel, Belgium

The awareness for the necessity of comparable analysis results is constantly growing in times of globalization and international trade. Comparable analysis results are not only necessary to minimize risks for the consumers but also economical risks for trading partners. Comparable analysis results can only be achieved through traceability to a common standard. This standard might be a SI unit or a reference method where the determination of the measureand is method dependent. Traceability can only be guaranteed via an unbroken chain of unbiased comparisons.

Therefore the analysis results will only become comparable if the performance of the methods used is known and all sources of bias are identified. Method validation is the only stage where the performance of a method for a particular scope is evaluated. All other quality control measures (like proficiency test and quality control charts) are only in place to monitor eventual changes of method performance after completion of the validation. During method validation among other parameters: measurement uncertainty, accuracy (trueness and precision), linearity and limits of quantification and detection, respectively are evaluated.

Method performance criteria always have to be agreed on with the customer, but as a guideline in the mycotoxin area the legal performance criteria laid down in Commission Regulation 882/2004 [1] and 401/2006 [2] apply. In the latter, the performance of the method is characterised by repeatability, reproducibility and recovery rate, but it is also stated that results have to be reported with the measurement uncertainty. The only guidance given on how to assess measurement uncertainty is the reference to the „Report on the relationship between analytical results, measurement uncertainty, recovery factors and the provisions of EU food and feed legislation“ [3], where eight different procedures are described.

The use of certified reference materials (CRM) (certified calibrators/calibrants and certified matrix materials) can facilitate the performance assessment and method validation. Certified reference materials provide due to their certified property value, not only an easy way to assess trueness but also due to their well characterised stability and homogeneity, an interpretation of repeatability and precision data.

A strategy how to use those additional assets of a CRM to evaluate measurement uncertainty and to derive other characteristics of the method during validation is presented.

Reference

[1] Official Journal L 165, 30.4.2004, pp. 1 – 141

[2] Official Journal L 70, 9.3.2006, pp. 12 – 34

[3] http://ec.europa.eu/food/food/chemicalsafety/contaminants/report-sampling_analysis_2004_en.pdf

Tailoring β -cyclodextrins for mycotoxin recognition

C. Dall'Asta¹, P. Cozzini², A. Amadasi², R. Pela¹, A. Dossena¹, R. Marchelli¹

¹Department of Organic and Industrial Chemistry, University of Parma
Viale G.P. Usberti 17/A, I-43100 Parma, Italy

²Molecular Modeling Laboratory, Department of General and Inorganic Chemistry
Viale G.P. Usberti 17/A, I-43100 Parma, Italy

Aflatoxins are natural substituted coumarins produced by *Aspergillus flavus*, *A. nomius* and *A. parasiticus*, which can be found on foodstuffs supporting fungal growth. These toxins are hepatocarcinogens and mutagen agents and in 1993 they were declared as Group 1 carcinogens by the International Agency for Research on Cancer (IARC).

Ochratoxin A is a mycotoxin produced by *Penicillium verrucosum*, *Aspergillus ochraceus* and other related species, which is nephrotoxic, hepatotoxic, immunosuppressive, teratogenic and carcinogenic in several animal species and has been classified as a possible carcinogen for humans (Group 2B) by IARC.

There has been a recent increase in mycotoxin contamination of foods, causing concern among producers and consumers. Therefore, the availability of reproducible and sensitive methods for screening foodstuffs is essential.

In the present work we have investigated the mechanism of OTA inclusion into cyclodextrins by molecular modeling techniques, and have tried to better understand the nature of the interactions between aflatoxin B1 and cyclodextrins, previously studied by Dall'Asta *et al.* using spectroscopic techniques [1].

Docking techniques and the HINT (Hydropatic INTeraction) program were used to explain interactions of Aflatoxin B1 and Ochratoxin A with b- and g-cyclodextrins. The work is aimed at designing a chemosensor to identify very low concentrations of these mycotoxins by exploiting the affinity of the cyclodextrin cavity for many small organic molecules. These include the fluorescent side of the toxin, which is protected from the solvent, thus enhancing the luminescence.

HINT is a „natural“ forcefield, based on experimentally determined LogP octanol/water values, that is able to consider both enthalpic and entropic contributions to binding free energy with an unified approach [2, 3]. HINT is normally applied to predict the DG of binding for protein-ligand, protein-protein and protein-DNA interactions. The leading forces in biomolecular processes are the same as those involved in organic host-guest inclusion phenomena, therefore we applied this methodology for the first time to cyclodextrin complexes. The results allowed us to explain spectroscopic data in absence of available crystallographic or NMR structural data.

Exhaustive comprehension of the AFB1-CD inclusion mechanism, as well as the study of OTA-CD interaction, may lead to the design of chemosensor devices based on fluorescence. The well-known specificity of fluorescent probes would allow for more sensitive and accurate detection of mycotoxins, even at very low concentrations, minimizing the risk of false negative/positive results usually associated with ELISA tests. Moreover, chemosensors based on a luminescence response may be integrated into microarray systems, which could be applied for early detection of post-harvest contamination, providing for an easy-to-use control tool for mycotoxin analysis.

Reference

- [1] Dall'Asta, C.; Corradini, R.; Ingletto, G.; Galaverna, G.; Marchelli, R. (2003): *J. Incl. Phenom. Macr. Chem.*, **45**, 257–263.
- [2] Cozzini, P.; Fornabaio, M.; Marabotti, A.; Abraham, D. J.; Kellogg, G. E.; Mozzarelli, A. (2002): *J. Med. Chem.*, **45**, 2469–2483.
- [3] Kellogg, G. E.; Abraham, D. (2000): *J. Eur. J. Med. Chem.*, **35**, 651–661.

Rapid Kinetic Assays – the new generation of Fluorescence Polarization Immunoassays for the detection of mycotoxins

Ursula Dahmen-Levison, Frank Mallwitz

Aokin AG, Robert-Rössle Str. 10, D-13125 Berlin, Germany, www.aokin.com

Rapid kinetic assay have been evaluated for the detection of zearalenon (ZON) in wheat. The **Rapid kinetic assay** is a practical and easy to use technique for rapid and reliable mycotoxin detection in food and feed. The method allows a five-minute quantitative mycotoxin determination in the ppb range after a 10 minute sample preparation.

Competitive Immunoassay:

A fluorescently labelled ZON forms a complex with an Anti-ZON antibody, resulting in a slowing down of the rotation of the labelled ZON. The observed changes in fluorescence polarization between free and bound labelled ZON can be rapidly and accurately measured. These values are related to the concentration of unlabeled ZON present in a sample.

Sample preparation and handling:

A sample is ground and then extracted with a water/solvent/additive mixture in a blender for 3.5 minutes. The ZON recovery is greater than 95 %. The mixture is then filtered and processed for three minutes by centrifugation through a column to minimize deleterious matrix effects. The sample preparation has been tested for raw materials like wheat, rye, triticale and maize and also food and feed products such as bread, cookies, pasta and feed pellets. The sample preparation uses a novel spin filtration step. This sample preparation will be compared to Immuno Affinity clean up.

Instrumentation:

Plane polarized light is used for excitation. Fluorescence emission intensities are determined for the parallel and vertical polarization directions. The FP470 has a stabilized light source, a temperature-stable sample compartment, and a robust design and construction. The standard deviation for P in the FP470 is 0.1 mP at 1 nMol Fluorescein. Calibration curves have been determined for a dynamic range of 1 ppb to 100 ppb. The calibration is stable over time and utilized for all measurements.

Summary:

The aokin method provides a new fast and quantitative and analytical tool for mycotoxin detection. It has been developed for single sample analysis and stands out by its high reproducibility and linearity.

Use of Gas Chromatography Isotope Dilution Mass Spectrometry in Deoxynivalenol Analysis

Mari Eskola, Gerhard Buttinger

European Commission, DG Joint Research Centre,
Institute for Reference Materials and Measurements B-2440 Geel, Belgium

Gas chromatography-mass spectrometry (GC-MS) is one of the most widely applied analytical techniques for the determination of type A and B trichothecenes in foods and feeds. One of the biggest problems in trichothecene analysis has been the lack of suitable internal standards, and it has been suggested that members of the trichothecene group, not normally occurring in cereals, would be the best choice. Isotopically labelled deoxynivalenol, T-2 toxin and HT-2 toxin, which are on the market now, clearly improve this situation. Use of an isotopically labelled analogue of the analyte and determination of the isotope amount ratio by mass spectrometry (IDMS) is a principle having a high potential to obtain accurate results.

In this study gas chromatography-mass spectrometry operating in electron impact mode was applied for analysis of deoxynivalenol (DON). ¹³C-labelled deoxynivalenol (¹³C-DON) was used as an internal standard. Since similar mass fragments are seen in the spectra for trimethylsilylated DON and trimethylsilylated ¹³C-DON, fluoroacyl derivatisation technique was used for derivatising DON and ¹³C-DON instead of silylation. DON and ¹³C-DON have a 15 amu difference in molecular mass which is the same mass as is the mass of a methyl group. During the electron impact ionisation the molecule bonds between the silicon atom and the methyl groups of the trimethylsilylated DON as well as trimethylsilylated ¹³C-DON break, and hence mass fragments with a 15 amu difference are formed. This phenomenon does not allow to use the ratio of the response of the two isotope mass fragments with the 15 amu mass difference (one fragment resulting from DON and the other from ¹³C-DON) in quantification of the results.

When fluoroacyl derivatisation was used clearly separate ions for both DON and ¹³C-DON were detected in the spectra, and hence the IDMS technique can be applied for deoxynivalenol analysis. The method will be further improved and used for developing reference materials for trichothecenes.

Determination of Ochratoxin A in liver and kidney of pigs – Validation of a test method by means of InterVal

Rudolf Hackenberg, Carolin Stachel

Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL)
Mauerstraße 39-42, 10117 Berlin, Germany, e-mail: crlvdrug@bvl.bund.de

Ochratoxin A (OTA) is a mycotoxin which is produced by various *Aspergillus* and *Penicillium* species, e.g. *aspergillus ochraceus*. Intake of contaminated feed by pigs may result in residues in kidney, serum and liver due to the long biological half-life time in this species. Depending on the dose, OTA may show nephrotoxic effects and is also suspected of having a carcinogenic potential in humans [1].

The validated method for the determination of OTA in liver and kidney of pigs comprises several steps. 5 g of homogenized matrix are enzymatically hydrolyzed for about 12 hours. After adjusting the pH-value to 3, the analyte is extracted from the aqueous phase with three 8 ml portions of ethyl acetate. The combined organic solvent is evaporated to dryness, then the residue is dissolved in 1 ml of methanol. 8 ml of water and 1 ml of NaCl-solution are added, the pH value is adjusted to 8, followed by defatting with 6 ml of n-hexane which is discarded. After adding phosphate buffer, the analyte is extracted from the aqueous phase and enriched on an immunoaffinity column. The concentration of Ochratoxin A is determined by HPLC and fluorescence detection.

Specific factors which may be of importance for the performance of the method were varied in the validation study as it is required by Commission Decision 2002/657 [2] when applying the approach of in-house validation.

These factors were the type of matrix (liver-kidney), the time of sample storage as well as storage of extracts, the state of the matrix (fresh or lyophilised), the lot of the immunoaffinity columns, the standard solution used for spiking and finally the operator. In total, 48 analyses including blank samples were performed in 8 series. The experimental plan – which means the combination of the factor variations and randomization of the sequence of the analytical runs – was set up by means of the statistical software InterVal [3] which was also used for evaluating the study. The results for each concentration level are given in the table below. After correcting for the mean rate of recovery a decision limit CC_{α} of 0.20 $\mu\text{g}/\text{kg}$ and a detection capability CC_{β} of 0.26 $\mu\text{g}/\text{kg}$ are obtained.

When testing the matrices for the validation study, we noticed a natural contamination with Ochratoxin A in all of the kidney samples at low levels. This underlines that the method is suitable for the analysis of real samples.

concentration [$\mu\text{g}/\text{kg}$]	within laboratory reproducibility s_{wIR} [$\mu\text{g}/\text{kg}$]	reproducibility s_{wIR} [%]	recovery [%]
0.10	0.018	18.5	85.3
0.20	0.022	11.0	79.4
0.40	0.034	8.6	76.4
0.60	0.048	8.0	75.4
1.00	0.077	7.7	74.6

Reference

- [1] Opinion of the Scientific Committee on Food on Ochratoxin A
(http://ec.europa.eu/food/fs/sc/scf/out14_en.html and references given herein)
- [2] Official Journal of the European Communities, 17.08.2002, L 221/8–36
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Purity and Stability of Ergot Alkaloid Calibrants

Martin Hafner¹, Franz Berthiller¹, Michael Sulyok¹, Rainer Schuhmacher¹, Colin Crews²,
Kristian Fog Nielsen³, Rudolf Krska¹

¹Christian Doppler Laboratory for Mycotoxin Research, IFA-Tulln
University of Natural Resources and Applied Life Sciences Vienna,
Konrad Lorenz Straße 20, A-3430 Tulln, Austria

²Central Science Laboratory, Food Science Group Sand Hutton, York YO41 1LZ, UK

³Technical University of Denmark, BioCentrum-DTU, DK-2800 Kgs. Lyngby, Denmark

Ergot alkaloids are mycotoxins based on a common ergoline tetracyclic structure. Members of the three subclasses (clavines, simple lysergic acid derivatives and ergopeptides) can be produced by a variety of fungal species. In Europe, ergopeptides are mostly encountered in *Claviceps*-infected cereals, especially rye. Consumption of contaminated food causes symptoms such as tremor and hallucinations up to limb loss due to suppressed blood supply. Analytical methods for the determination of ergot alkaloids are constantly being developed and refined. As for any reliable quantitative analytical system, stable standards are necessary. Being lysergic acid derivatives, ergopeptides have asymmetric carbon atoms at positions 5 and 8. While the conformation of C-5 is fixed, the C-8 atom can form two interchangeable epimeres. One form (R-form, denoted by the suffix -ine) is biologically active, the other form (S-form, suffix -inine) is biologically inactive.

The aim of this study was to determine the purity of seven ergot alkaloid standards and to evaluate storage conditions for standard solutions (solvent and temperature) in order to reduce or avoid epimerization. The purity of the solid standards was determined via HPLC-UV-TOF/MS after dissolution in acetonitrile. The purity of each component was calculated based on its UV-absorption signal. Its identity and that of impurities were determined via their exact masses measured by subsequent TOF-MS. For storage, the stability of standard solutions of ergot alkaloids in aprotic solvents (chloroform and acetonitrile) and aqueous acidic and alkaline conditions at -20 °C, +4 °C and +20 °C was tested. Samples were stored at -80 °C after one, three and six weeks at the test temperature for isochronous measurements by HPLC-MS/MS. Ergine, ergocornine, ergocristine, ergocryptine, ergometrine, ergosine and ergotamine were evaluated. Only the R-form (-ine) was added to the solvents before storage. Generally, protic solvents and higher temperatures promote epimerization of ergot alkaloid toxins while an aprotic environment and low temperatures inhibit the process.

Determination of citrinin in cereals by ethyl acetate extraction, aminopropyl clean-up and HPLC-FLD (Polaris C18-A column)

Anja Hartl, W.-R. Stenzel

Fachbereich Veterinärmedizin, Wissenschaftliche Einrichtungen Veterinary Public Health,
Institut für Lebensmittelhygiene, Freie Universität Berlin, Königsweg 69, D-14163 Berlin, Germany

Citrinin wird überwiegend von Lagerpilzen, Spezies der Gattungen *Penicillium* und *Aspergillus*, auf Lebens- und Futtermitteln gebildet. Hauptproduzent von Citrinin auf Getreide ist in gemäßigten Klimazonen *Penicillium verrucosum*, weitere wichtige Citrininproduzenten sind *Penicillium citrinum*, *Penicillium expansum*, *Penicillium radicola*, *Monascus ruber* und *Monascus purpureus*. Bisher gestaltete sich die Analytik von Citrinin im ppb-Bereich aufgrund der Instabilität und der Polarität des Toxins als schwierig. In Anlehnung an die Methode von Meister [1] wurde eine simultane Bestimmung von Ochratoxin A und Citrinin in Zerealien entwickelt [2], deren Nachteil die Verwendung von Dichlormethan als Extraktionsmittel ist.

Darauf basierend wird eine alternative Methode zur Bestimmung von Citrinin in Weizen, Gerste und Roggen vorgeschlagen. Es werden verschiedene zu Dichlormethan alternative Lösungsmittel für die Extraktion von Citrinin erprobt. Von den getesteten Lösungsmitteln erzielt Essigsäureethylester die höchsten Wiederfindungsraten. Nach der Filtration mittels Phasentrennpapier erfolgt das Clean-up an Aminopropylphasen [2]. Da mit Essigsäureethylester ein Großteil polarer und mittelpolarer Substanzen aus der Matrix mit extrahiert werden und Störpeaks im HPLC-Chromatogramm zu verzeichnen sind, muss auf eine alternative HPLC-Methode zurückgegriffen werden. An dieser Stelle liefert die Polaris C18-A Trennsäule in Kombination mit einem Acetonitril/Phosphorsäure-Gradienten die gewünschte Trennung. Mit dieser HPLC-Methode erhält man scharfe Citrinin-Peaks ohne Peaktailing. Für Roggen, Gerste und Weizen ergeben sich Wiederfindungsraten von 77–92% (*rsd* 4,8–5,5%). Die nach DIN 32 645 ermittelten Nachweis- und Bestimmungsgrenzen betragen 0,6–0,9 µg/kg und 1,7–3,3 µg/kg. Die Methode wird zusätzlich mit natürlich kontaminiertem Material getestet, einer homogenen Mischung aus kontaminiertem Angkak und Weizenmehl.

Reference

- [1] Meister, Ute (2004): New method of citrinin determination by HPLC after polyamide column clean-up. Eur. Food Res. Technol. **218**: 394–399.
- [2] Hartl, Stenzel (2006): Solid phase extraction on aminopropyl columns for determination of citrinin and ochratoxin A in cereals. 28. Mykotoxinworkshop 29–31.05.2006, Bydgoszcz, Polen, Tagungsband S. 101

New fast alternative Clean-up Method for Deoxynivalenol from Feed

Ayman Hashem¹, Elisabeth Korte²

¹LAVES Futtermittelinstitut Stade, Heckenweg 4, 21680 Stade, Germany

²Varian Deutschland GmbH, Alsfelder Strasse 6, 64289 Darmstadt, Germany,
e-mail: elisabeth.korte@varianinc.com

Mycotoxins intake from contaminated food and feed is recognized worldwide as a growing health and safety concern. Of special interest in temperate climate regions are the widely distributed *Fusarium* fungi. *Fusarium* toxins can produce a wide range of mycotoxins including several Trichothecenes, with Deoxynivalenole (DON) as the most frequently occurring mycotoxin. Suitable fast, sensitive and reliable methods for the clean-up and determination of *Fusarium* toxins are therefore required.

The poster presents a new alternative method to the commonly used extraction and clean-up step of DON from different feed matrices. This new clean-up method is performed on a Bond Elut Mycotoxin cartridge, which has previously been used for the effective clean-up of 12 Trichothecenes and Zearalenone from cereal-based foods contaminated with *Fusarium* toxin [1]. In this poster, our results indicate that the new Bond Elut Mycotoxin method is much faster than the previously used clean-up procedure with Immunoaffinity sorbents (IAC). Using the Bond Elut method we could shorten the clean-up time to almost half of the time required by IAC clean-up, thus increasing our sample throughput.

High precision and linearity was achieved with the Bond Elut Mycotoxin method by using an internal standard. With the IAC method, the use of an internal standard is difficult, as it requires a selective antibody, which is not easily available.

As the performance of the Bond Elut Mycotoxin cartridges is similar or even better and the columns are more cost-effective, the new clean-up procedure is a very good alternative to the previously used IAC method.

Reference

[1] M. Klötzel, U. Lauber, H.-U. Humpf, (2006): *Mol. Nutr. Food Res.*, **50**, 261 – 269

Entwicklung und Validierung von Enzymimmuntests zum Nachweis von T-2 und HT-2 Toxin

Katja Hocke¹, Richard Dietrich¹, Valeriu Curtui², Georg Langenkämper³, Uwe Lauber⁴, Paul Majerus⁵, Anke Trebstein⁴, Ewald Usleber², Erwin Märtlbauer¹

¹Lehrstuhl für Hygiene und Technologie der Milch, Ludwig-Maximilians-Universität München, Schönleutner Str. 8, 85764 Oberschleißheim, Germany

²Professur für Milchwissenschaften, Justus-Liebig-Universität, Ludwigstr. 21, 35390 Gießen, Germany

³Bundesforschungsanstalt für Ernährung und Lebensmittel, Standort Detmold, Schützenberg 12, 32756 Detmold, Germany

⁴Chemisches und Veterinäruntersuchungsamt Stuttgart, Schaflandstr. 3/2, 70736 Fellbach, Germany

⁵Landesuntersuchungsamt – Institut für Lebensmittelchemie, Maximineracht 11a, 54295 Trier, Germany

Im Rahmen eines vom Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz (BMELV) geförderten Forschungsvorhabens wird derzeit die T-2 und HT-2 Belastung in Lebensmitteln des deutschen Marktes untersucht. Neben verschiedenen chromatographischen Analyseverfahren sollten hierbei, auch im Hinblick auf die Anzahl der zu untersuchenden Proben, Enzymimmuntests eingesetzt werden.

Zur Erstellung empfindlicher Enzymimmuntests wurde die Eignung verschiedener polyklonaler Kaninchenserum- bzw. monoklonaler Antikörper überprüft. Zum spezifischen Nachweis von T-2 Toxin wurde ein konsekutiver, direkt-kompetitiver EIA, zum Summennachweis von T-2 und HT-2 Toxin ein simultaner, direkt-kompetitiver EIA entwickelt. Die entsprechenden Nachweisgrenzen lagen bei 25 pg/ml bzw. bei 70 pg/ml. Zum Toxinnachweis in Lebensmitteln wurde ein einfaches und robustes Extraktionsverfahren entwickelt. Die Extraktion erfolgt mittels Methanol/Wasser (70:30), der Rohextrakt wird über Flüssig-Flüssig-Verteilungschromatographie (Ethylacetat) gereinigt und schließlich mit n-Heptan entfettet. Die Nachweisgrenzen liegen bei Verwendung des spezifischen T-2 Toxintests bei 0,15 ppb bzw. bei Einsatz des Gruppennachweises bei 0,25 ppb. Die Wiederfindungsraten und die Reproduzierbarkeit des Analyseverfahrens wurden anhand von künstlich kontaminierten Lebensmittelproben (Weizenmehl, Hartweizennudeln und Kleinkindernahrung) überprüft. Die durchschnittliche Wiederfindungsrate für T-2 lag bei 81,7%.

Das EIA-Verfahren zum Nachweis von T-2 und HT-2 Toxin wurde des Weiteren im Rahmen des Verbundprojektes in vier verschiedenen Labors etabliert und anhand von Laborvergleichsuntersuchungen validiert. Neben der oben skizzierten Methodik wurden in den anderen Labors zum Teil LC-MS/MS und GC-ECD als alternative Analyseverfahren eingesetzt. Bislang wurden vier verschiedene Lebensmittel (z.B. Hafer, Haferflocken, Säuglings- und Kleinkindernahrung) vergleichend untersucht. Die nachgewiesenen T-2/HT-2-Konzentrationen lagen hierbei im Bereich von < 0,3 bis 160 ppb. Trotz der Untersuchung von teilweise sehr niedrig kontaminierten Proben (2–24 µg/kg) und der unterschiedlichen Analysemethoden wurden ausgezeichnete Laborvergleichsdaten erhalten.

Acknowledgement

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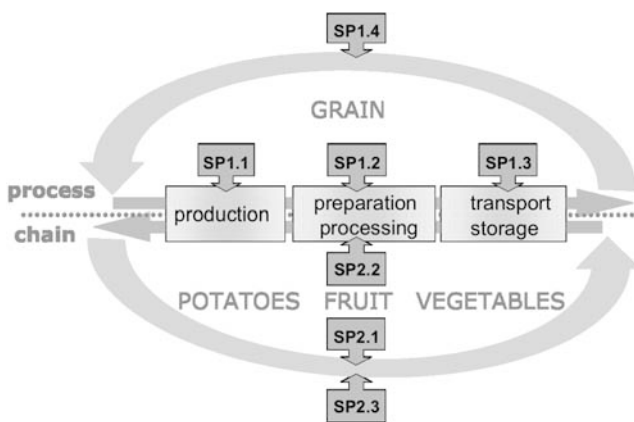
Indicators and sensor technology for the identification of mycotoxine producing fungi in the processing of grain

Christine Idler¹, Andrea Jonitz¹, Michael Kumke², Claudia Rasch²

¹Leibniz-Institut für Agrartechnik Bornim e.V., Max-Eyth-Allee 100 D-14469 Potsdam, Germany

²Universität Potsdam, Institut für Chemie, Karl-Liebknecht-Str. 24-25 D-14476 Potsdam, Germany

The German Ministry of Education and Research (BMBF) will provide funding of more than 1.9 millions Euro for a large joint project on the development of innovative sensor-based techniques and processes in the field of food quality and safety. In this research-project „Exploration of sustainability potentials by use of sensor-based technologies and integrated assessment models in the production chain of plant related food“ 13 partners from universities, non-university institutions and industry will cooperate within the projects. The expected results shall contribute to maintain freshness and improve safety of produce.



In one of the seven subproject (SP 1.2) – *Indicators and sensor technology for the identification of myco-toxine producing fungi in the processing of grain* – will be tested spectroscopic methods for the detection on moulds and/or mycotoxins.

Therefore laser-induced fluorescence (LIF), diffuse reflection (DR) spectroscopy as well as ion mobility (IM)spectrometry will be estimated and applied for the detection of mycotoxin contaminations on grain, e.g., in storage facilities, during loading, or before processing. In order to evaluate the capabilities of each method basic parameters are determined first for model systems and in a subsequent step for real samples.

Topics of the project are:

- Spectral range for DR spectroscopy (UV – NIR range) and characterization of suitable absorption and reflection bands for the recognition of mycotoxin contaminants on grain.
- Excitation and emission settings in LIF for the detection of mycotoxin contamination, either of the mycotoxins or fungi, respectively. The application of time-resolved detection schemes is tested in order to minimize the possible influence of signals originating from the complex background matrix.
- Characterization of potential application of IM spectroscopy for the detection of mycotoxin contamination in dust/air of grain storage and processing facilities.
- Possibilities of locally resolved detection schemes e.g., for storage facilities are evaluated e.g., in combination with fibre optics.
- Determination of the limit of detection (LOD) for DR spectroscopy, LIF, and IM spectrometry.

Selectivity, sensitivity, and cross sensitivity are characterized for the different spectroscopic methods. For data evaluation advanced algorithms are developed. The ultimate goal is to build a mobile spectrometer system for the detection of mycotoxin contamination that can be used *in-situ* directly at the storage and processing facilities.

Nationales Referenzlabor für Mykotoxine - Eine neue Einrichtung im Bundesinstitut für Risikobewertung

Sabine Kemmlein, Horst Klaffke

Bundesinstitut für Risikobewertung, Thielallee 88-92, D-14195 Berlin, Germany

Referenzlaboratorien sind für den gesundheitlichen Verbraucherschutz von zentraler Bedeutung. In Übereinstimmung mit den Ausführungen des Artikel 33 der Verordnung (EG) Nr. 882/2004 des Europäischen Parlaments und des Rates vom 29. April 2004 über amtliche Kontrollen zur Überprüfung der Einhaltung des Lebensmittel- und Futtermittelrechts sowie der Bestimmungen über Tiergesundheit und Tierschutz sind die Mitgliedstaaten der Europäischen Union aufgefordert, zur Unterstützung der Gemeinschaftslaboratorien (CRL) der EU entsprechende nationale Referenzlaboratorien (NRL) u. a. für Kontaminanten wie Mykotoxine zu benennen. Im Oktober 2006 erfolgte, neben weiteren nationalen Referenzlaboratorien, die Zuweisung der Aufgabe des NRL Mykotoxine an das Bundesinstitut für Risikobewertung durch das Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz.

Das Nationale Referenzlabor für Mykotoxine in Lebens- und Futtermitteln bildet primär die Kommunikationsstelle zwischen den Länderlaboratorien und den Europäischen Gemeinschaftslaboratorien und unterstützt die zuständigen Lebensmittelüberwachungsbehörden bei der Gestaltung der Kontrollsysteme. In enger Abstimmung und mit der Unterstützung der Behörden der Länder sollen durch das NRL Mykotoxine analytische Methoden erarbeitet bzw. validiert und Programme zur Schulung und Fortbildung der Überwachungslabore angeboten werden. Eine weitere Aufgabe ist die Durchführung regelmäßiger Vergleichstests zwischen den verschiedenen Laboratorien, die mit den genannten Analysen beauftragt sind, um eine hohe Qualität bei der Überwachung zu gewährleisten bzw. zu fördern. Die für solche Untersuchungen notwendigen Referenzmaterialien (Probenmaterialien, Standardsubstanzen) und Fachinformationen werden durch das NRL Mykotoxine zur Verfügung gestellt, soweit gleichwertige kommerziell angebotene Referenzmaterialien nicht zur Verfügung stehen

Um den intensiven Informationsaustausch zwischen allen mit der Überwachung betrauten Einrichtungen zu fördern sollen durch das NRL Mykotoxine Workshops organisiert werden. Weiterhin kann das Nationale Referenzlabor für Mykotoxine auch als Schiedsstelle im Falle unklarer oder strittiger Analysergebnisse fungieren.

Quick and cheap determination of trichothecenes in cereals and cereal products by LC-MS-MS after clean-up with dispersive solid-phase extraction

Ulrike Kocher

Chemisches und Veterinäruntersuchungsamt Sigmaringen,
Hedinger Str. 2/1, 72488 Sigmaringen, Germany

Trichothecene gehören zu einer Gruppe der von *Fusarien* gebildeten Toxine. Aufgrund ihrer sehr unterschiedlichen Struktur hat man diese Gruppe in weitere Untergruppen wie Typ A und Typ B Trichothecene eingeteilt. Lebensmittelrechtlich ist aufgrund einer bestehenden Höchstmenge Deoxynivalenol als wichtigster Vertreter der Typ B Trichothecene zu nennen. Künftig ist auch für die Typ A Trichothecene T2 und HT2 eine Höchstmengenregelung beabsichtigt.

Zur Probenvorbereitung werden aufgrund ihrer einfachen Anwendung häufig spezielle SPE-Kartuschen eingesetzt: Zum einen die bereits seit über 10 Jahren auf dem Markt befindlichen MycoSep[®] und MultiSep[®]-Kartuschen von Romer Labs [1] sowie seit 2006 die BondElut[®] Mycotoxin-Kartuschen von Varian [2]. Bei beiden Verfahren werden die Trichothecene zunächst mit einem Acetonitril/Wasser-Gemisch aus dem Lebensmittel extrahiert. Der nächste Schritt ist jeweils ein sogenanntes einstufiges Clean-up, wobei nur ein Aliquot des filtrierten Rohextrakts durch die Hersteller-Kartuschen gedrückt wird. Ein Aliquot des Eluats wird dann zur Trockene eingeeengt und in einem für die Messung geeigneten Fließmittel gelöst. Die Nachteile dieser Verfahren liegen darin, dass es sich um Hersteller-spezifische Methoden handelt und dass die eingesetzten Kartuschen teuer sind.

Basierend auf ein für die Pestizidanalytik entwickeltes Verfahren, das auch unter dem Namen QuE-ChERS (Quick, easy, Cheap, effective, Rugged, Safe [3], [4], [5]) bekannt ist, wird ein neuartiges Clean-up zur Bestimmung von 12 Typ A und Typ B Trichothecenen vorgestellt.

Wie bei den o. g. Herstellermethoden wird der Rohextrakt durch Extraktion der Probe mit einem Acetonitril/Wasser-Gemisch hergestellt. Das Clean-up erfolgt in einem Zentrifugenröhrchen mit Schraubverschluss, das im Labor mit einer definierten Menge Aminopropyl-Anionenaustauscher, Aktivkohle und wasserfreiem Magnesiumsulfat befüllt wird. Ein Aliquot des zentrifugierten Rohextraktes wird auf dieses Gemisch pipettiert. Nach kurzem, intensivem Schütteln erfolgt eine Zentrifugation. Ein Aliquot des klaren Überstandes wird in einem Mikroal zur Trockene eingeeengt und der Rückstand in Acetonitril/Wasser gelöst. Zur Quantifizierung dient ein LC-MS-MS (API 3000) mit einem CTC-PAL Probengeber.

Die dispersive Festphasenextraktion (dispersive SPE, DSPE) mit einem Aminopropyl-Anionenaustauscher, Aktivkohle und wasserfreiem Magnesiumsulfat stellt eine preiswerte, qualitativ gleichwertige Alternative zu den bereits vorhandenen Hersteller-spezifischen Clean-up-Verfahren zur Bestimmung von 12 Typ A und Typ B Trichothecenen dar.

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Determination of 7 *Alternaria*-Toxins in edible oil and oilseeds by LC-MS-MS

Ulrike Kocher

Chemisches und Veterinäruntersuchungsamt Sigmaringen,
Hedinger Str. 2/1, 72488 Sigmaringen, Germany

Die Gattung *Alternaria* (Schwärzepilze innerhalb der *Deuteromycetes*) besteht aus mehr als 40 Arten, die in unterschiedlichem Maße Toxine und sekundäre Metaboliten bilden. In einer toxikologischen Bewertung von *Alternaria* kommt das Bundesinstitut für Risikobewertung (BfR) zum Schluss, dass die Datenlage bezüglich der *Alternaria*-Toxinbelastung derzeit nicht ausreicht, um eine Risikoabschätzung für den Verbraucher vorzunehmen. Das BfR hält daher unter anderem weitere Untersuchungen zur Exposition für erforderlich. Vor diesem Hintergrund wurde beim Mykotoxin-Workshop 2006 in Bydgoszcz eine Methode zur Bestimmung von *Alternaria*-Toxinen mittels LC-MS-MS vorgestellt. Die Methode wurde an Säften, Kartoffel- und Tomatenerzeugnissen validiert. Mit der Methode konnten die *Alternaria*-Toxine Alternariol (AOH), Alternariol-monomethylether (AME), Altenuen (ALT), Tentoxin (TEN) sowie Tenuazonensäure (TEA) bestimmt werden. Ebenfalls wurde beim Mykotoxin-Workshop 2006 eine Multimethode zur Bestimmung von Mykotoxinen (ohne *Alternaria*-Toxine) in Speiseölen mittels LC-MS-MS präsentiert.

Mit der Multimethode zur Bestimmung von Mykotoxinen in Speiseöl können neben den 5 o.g. *Alternaria*-Toxinen auch Altertoxin I (ATX-I) und die AAL-Toxine TA1 und TA2 erfasst werden. Zur Extraktion dieser 7 Toxine sind zwei unterschiedliche Extraktionsverfahren erforderlich. Mittels sogenannter „staggered Injection“ mit 2 binären Pumpen (Agilent 1100) und einem CTC-PAL-Probengeber mit 2 Injektionsventilen, einem Säulenofen für 2 Trennsäulen (Agilent 1100) erfolgt die Messung der beiden Extrakte an einem Triple-Quad-Massenspektrometer (API 3000). Diese Messtechnik erlaubt eine zeitsparende Analyse der Extrakte, da während der Säulenconditionierung der ersten Säule bereits die Messung auf der zweiten Säule erfolgen kann.

Es wurden bislang 38 überwiegend kaltgepresste Speiseöle untersucht. Toxine waren nachweisbar in Distelöl, Olivenöl, Rapsöl, Sesamöl und in Sonnenblumenöl. Mit Ausnahme einer Probe enthielten alle Sonnenblumenöle *Alternaria*-Toxine. TEN war in 10 Proben (bis 44 µg/kg), TEA in 9 Proben (bis 390 µg/kg) nachweisbar. Ein Sesamöl enthielt auffällig viel AME (85 µg/kg). Ebenfalls auffällig waren die Toxin-Rückstände im Distelöl, allerdings wurden bislang erst zwei Proben untersucht. In Distelöl konnten, ebenso wie bei Sonnenblumenöl, bis zu vier Toxine in einer Probe festgestellt werden.

Aufgrund der Befunde in Speiseölen wurden unter anderem auch Sonnenblumenkerne und Sesam auf *Alternariatoxine* untersucht. In 12 von 14 Proben Sonnenblumenkerne wurden messbare Rückstände an *Alternaria*-Toxinen festgestellt. Auffällig war eine Probe, die 4 Toxine enthielt: AOH (13 µg/kg), AME (4 µg/kg), TEA 1000 µg/kg sowie TEN 880 µg/kg. Insgesamt enthielten 11 Proben TEA, 10 Proben TEN, 1 Probe AOH und 1 Probe AME. Zwei der drei untersuchten Sesamproben enthielten TEA, eine Probe enthielt zusätzlich AOH und AME.

Determination of T-2 and HT-2 toxins in oats and other cereal varieties by HPLC-FLD

Ute Meister

Institut für Lebensmittel- und Umweltforschung e.V.,
Arthur-Scheunert-Allee 40-41, D-14558 Nuthetal OT Bergholz-Rehbrücke, Germany

The *Fusarium* toxins T-2 and HT-2 toxin are occurring in cereals, oats – regarded as especially healthy variety – mainly being affected. The toxicity of T-2 toxin and the main metabolite HT-2 toxin was evaluated by the Scientific Committee for Food (SCF), in 2001. The general toxicity, the hematotoxicity and the immunotoxicity of T-2 toxin are assessed especially critically. The TDI-value for the two toxins amounts to 0.06 $\mu\text{g}/\text{kg}$ body weight per day.

The EU plans to define a limit value for these mycotoxins. However, the data situation is insufficient due to missing suitable analytical methods. For the definition of such limit values an exact knowledge of the occurrence of mycotoxins in raw materials and in processed products is necessary. For this purpose, reliable and sensitive measuring methods are required.

As food industry is requested to analyze the raw materials and products for the T-2 and HT-2 toxins a method was developed which is feasible with the usual HPLC equipment. According to this method T-2 and HT-2 toxins in different grain varieties and milling products can be determined by routine with moderate instrumental expenditure. The method is based on HPLC and fluorescence detection. After extraction with methanol/water the samples are purified on immunoaffinity columns. The sample residue is concentrated to dryness and derivatized with 1-anthrolylnitrile in the presence of 4-dimethylaminopyridine (DMAP). The T-2 and HT-2 toxins are separated from the peaks of the grain matrix and the derivatization agent according to the relatively extensive HPLC-gradient method.

The method was validated for oats, wheat, rye, barley, and maize. The method has a low detection limit (1–2 $\mu\text{g}/\text{kg}$), a good recovery rate (70–99%), and a good precision (relative standard deviation 3–8%).

The analysis of 55 oat samples from food industry and of various selected oat products has shown that positive results of oats especially from the northern regions in Europe have to be taken into consideration. The two toxins also were detected frequently in maize, however in lower concentrations. T-2 and HT-2 toxins were detected occasionally in randomly selected wheat and barley samples, and not at all in rye samples. The results are demonstrated on the poster.

Application of planar chromatography to determination of mycotoxins in foodstuffs and biological materials

Vladimir Ostry, Jarmila Skarkova, Jiri Ruprich

National Institute of Public Health in Prague, Centre for the Hygiene of Food Chains,
Brno, CZ-612 42, The Czech Republic

Planar chromatography or instrumental high performance thin-layer chromatography (HPTLC) is a modern separation technique, distinguished by flexibility, reliability and cost efficiency. Together with HPLC and GC it belongs to the microanalytical methods, which play an important role in research and routine laboratories. In many cases instrumental thin-layer chromatography offers a more suitable solution and often it is used as confirmatory or alternative technique. Due to the off-line principle, thin-layer chromatography offers enormous flexibility, parallel separation of many samples with minimal time requirement, simplified sample preparation due to single use of the stationary phase and possibility of multiple evaluations of the plate with different parameters because all fractions of the sample are stored on the plate.

One of important fields of application planar chromatography is analyzing of contaminants in food, feed, and biological material. Among them mycotoxins, secondary metabolites produced by certain fungi in agricultural products, are usually determined with various chromatographic methods (HPLC, HPTLC, TLC, GC) and with immuno-chemical methods (ELISA). Here HPTLC procedures for quantitation of various mycotoxins (aflatoxins, ochratoxin A, deoxynivalenol, zearalenone, patulin, sterigmatocystin, fumonisins, alternaria toxins, cyclopiazonic acid, tenuazonic acid) in food, feed, and other biological materials are described.

The ground samples are blended with suitable extraction solvents (e.g. methanol, ethyl acetate, chloroform) to extract toxins from matrices. The extracted solutions are further cleaned up either by liquid-liquid extraction, solid-phase extraction or immuno-affinity chromatography. The purified extracts are analyzed by instrumental high-performance thin-layer chromatography (HPTLC) with the Camag equipment (Automatic TLC Sampler III and TLC Scanner II). The silica gel 60 HPTLC plates (Merck) are used for separation with various mobil phases in twin trough vertical or horizontal developing chamber. One dimensional TLC is sufficient for the separation and quantitation of all determined mycotoxins. In some cases a pre- respectively post-chromatographic derivatization is necessary. Densitometric evaluation is carried out with Camag TLC Scanner II and CATS software. The developed methods were evaluated by analysis of various samples of food, feed, human urine and animal kidney. The certified reference materials of Community Bureau of Reference and spiked samples were used for validation of the methods. The limit of quantification (LOQ) was calculated by means of equations given in International Conference of Harmonization (ICH) guideline. The recovery was tested by running recovery experiments with spiked samples.

In summary, the selective, sensitive and precise HPTLC methods for quantitation of aflatoxins (B1, B2, G1, G2, M1), ochratoxin A, patulin, deoxynivalenol, zearalenone, sterigmatocystin, fumonisin B1, alternene, cyclopiazonic acid, and tenuazonic acid in food, feed and biological materials were developed.

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Sensible Method for Determination of DON in Cocoa by means of HPLC-Techniques

Marion Raters, Reinhard Matissek

Food Chemistry Institute (LCI) of the Association of the German Confectionery Industry (BDSI),
Cologne, Germany, www.lci-koeln.de

Deoxynivalenol (DON) is one of a group of mycotoxins known as type B trichothecenes. These form a very extensive collection of over 170 mycotoxins whose key structural feature is a cyclic sesquiterpene framework with an epoxy ring. The frequency with which DON occurs in certain raw materials and the concentrations found make it one of the world's most significant mycotoxin contaminants [1].

The primary objective of this study was to establish a current situation assessment of the possible occurrence of deoxynivalenol in cocoa and cocoa products. Since there was no analytic method for determining DON in cocoa and cocoa products, a special method was developed. The applicability and consistency of the method was confirmed by performing recovery assays on various cocoa products. A special post-column derivatisation procedure to increase selectivity and raise sensitivity by a factor of 80. The method's limit of detection (LOD) was thereby reduced to 7 $\mu\text{g}/\text{kg}$; the limit of quantification (LOQ) was 14 $\mu\text{g}/\text{kg}$.

The method was used to test 230 samples for possible DON content, ranging from cocoa beans to cocoa bean shells, nibs, cocoa liquor and cocoa powders through to finished cocoa-based products. In the case of cocoa beans and cocoa bean shells, DON content close to the detection limit was only determined in isolated cases. No DON content was detected in nibs, cocoa liquor, cocoa powders and finished cocoa-based products [2]. The findings are presented.

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Ergot alkaloids: Quantitation and recognition challenges

Hans Reinhard, Heinz Rupp, Otmar Zoller

Swiss Federal Office of Public Health, Food Safety Division, CH-3003 Bern, Switzerland

Detection and legislation of ergot alkaloids (EAs) is hampered by their large number of substances, their incomplete toxicological assessment and the industrial feasibility regarding their removal. Many attempts concerning the analytical challenge have been fronted [1, 2], while wide spread application of LC-MS/MS has nowadays opened new possibilities concerning especially the characterization of the detected analytes.

In Switzerland limiting values for ergot (sklerotia, not EAs) are set to 500 mg/kg in cereals intended for flour production and to 200 mg/kg in grains foreseen for consumer delivery, respectively [3]. Additionally, legislation tends to introduce a convention method where only a few known but available ergot alkaloids are analyzed to interpret food quality in ready-to-eat food. This work intends to pave the way to this revision.

From the 109 bread, flour and baby food samples taken in 2001, 2003 and 2005, 40 contained or were made of rye. Samples were analyzed with LC-FLD and LC-MS/MS and compared to EA values found in 1985 [1]. Sample treatment consisted basically of acidic extraction, automated clean up over Oasis-MCX tubes and subsequent chromatographic separation and analysis. The EA content of a sample was defined as sum of the 16 alkaloids ergometrin(in)e, ergosin(in)e, ergotamin(in)e, ergostin(in)e, ergocornin(in)e, α -ergokryptin(in)e, β -ergokryptin(in)e and ergocristin(in)e. It turned out that comparability between FLD and MS/MS was satisfactory but depended on the analyte under investigation and that the use of an internal standard was crucial. We abandoned methysergide as internal standard due to instability problems and got reliable results applying dihydro-ergocristine (DHET) as internal standard for MS/MS. Unfortunately, DHET is naturally occurring and detectable in percent levels (referred to total EA content) depending on sample matrix and was found especially in samples exceeding EA contents of 100 ng/g. Here, the availability of an isotopically marked ergot alkaloid standard is demanding.

Compared to ergot values found in rye flour and bread in 1985 (140 / 21.3 ng/g, resp. [1]), the expected variability for every year is lower than the variability among the data of a certain year. The median values found in rye flour and bread (fresh weight) was 172/87 ng/g (2001, $n = 13/14$), n.a./120 ng/g (2003, $n = n.a./7$) and 160/156 ng/g (2005, $n = 2/2$), respectively.

To allow for unexpected EA recognition, additional LC-MS/MS experiments were run. Since quadrupole (but not trap) instruments lack of sensitivity in scan mode, we operated the first quadrupole of our triple quadrupole instrument as bandpass (resolution drop by 2 V) during ordinary SRM, allowing ions of widened m/z ranges ($m/z -15$ to $m/z +40$) to enter the collision quadrupole. The analyzing quadrupole was set to the common daughter ion $m/z = 223$ of EAs (9,10-unsaturated ergoline cation). The interesting mass region was then scanned by running multiple SRM experiments with the first quadrupole set from $m/z = 530$ to 630 in 20 amu steps. In a few samples ergovalin(in)e was tentatively identified by these means.

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Microarray and Real Time analysis of the expression of trichothecene biosynthesis genes in cereals

Markus Schmidt-Heydt, Rolf Geisen

Federal Research Centre for Nutrition and Food, Haid-und-Neu-Str. 9; 76131 Karlsruhe, Germany

Different species of the genus *Fusarium* are known to produce trichothecenes of type B, like deoxynivalenol or nivalenol. Among other *Fusaria*, *F. culmorum* is responsible for the occurrence of deoxynivalenol in cereals. The biosynthesis is thereby greatly dependent on environmental conditions, like substrate, pH, water activity and temperature. This regulation in dependence of growth parameters is mediated at the level of transcription of the trichothecene biosynthesis genes. Because of the fact, that the activation of transcription can be detected earlier before the toxin is produced, transcription activation can be regarded as an early indication for mycotoxin biosynthesis.

In this work we have analyzed the correlation between activation of trichothecene biosynthesis genes and deoxynivalenol production in wheat as natural habitat at different water activities. Expression analysis of the trichothecene biosynthesis cluster genes was performed with microarray. For validation, a Real Time PCR mediated expression analysis has been carried out with the *tri5* gene, which encodes for a trichodiene synthase. Deoxynivalenol biosynthesis has been determined by ELISA.

According to the data shown, there is a correlation between expression of the trichothecene biosynthesis genes and deoxynivalenol production. High expression levels could be found at conditions conducive for trichothecene biosynthesis and vice versa. Hence gene expression could be taken as an early indication for trichothecene biosynthesis.

Evaluation of the commercial ELISAs for detection of total aflatoxins in pig feeds

Yun-Kyung Song¹, Byeong-Yeal Jung¹, Jeong-Soo Choi¹, Ki-Beom Kweon¹, Duck-Hwa Chung²,
Son-Il Pak³, Chang-Hee Kweon¹, Choi-Kyu Park¹

¹Animal Disease Diagnostic Division, National Veterinary Research and Quarantine service,
480 Anyang 6-dong, Manan-gu, Anyang City, Gyeonggi-do, 430-824, Republic of Korea

²Division of Applied Life Science, Gyeongsang National University,
900 Gajwa-dong, Chinju City, Gyeongsangnam-do, 660-701, Republic of Korea

³Department of Veterinary Medicine, Kangwon National University,
192-1 Hyoja-dong, Chunchon City, Gangwon-do, 200-701, Republic of Korea

Aflatoxins produced by *Aspergillus spp.* are recognized as a major concern in animal and human health. In pigs, ingestion of aflatoxin-contaminated feeds causes immunosuppression, hepatotoxicosis, and poor feed efficiency. This study was conducted to evaluate the performance of commercial ELISA kits for the screening of aflatoxins in pig feeds.

Twenty-nine pig feed samples were examined for total aflatoxins using three ELISA kits, simultaneously. From three repetitions of each assay, the average intra-assay precisions expressed as coefficient of variation (*CV* %) were 6.90, 5.64 and 5.78 for Veratox Quantitative Aflatoxin test, AgraQuant Total Aflatoxin assay 4/40 and RIDASCREEN FAST aflatoxin, respectively. On the other hand, the average inter-assay precisions were 12.29, 21.5 and 17.06 % within range of quantitation.

Twenty of the above 29 samples were further examined for total aflatoxins using a high performance liquid chromatography (HPLC) and the results of HPLC were compared with those of three ELISA kits. A statistical comparison of the results between HPLC and ELISAs showed that the correlation coefficient values for Veratox, AgraQuant and RIDASCREEN were 0.96, 0.95 and 0.96, respectively ($p < 0.0001$).

From our results, the ELISA kits exhibited high correlation with HPLC in the detection of aflatoxins in pig feeds. Consequently, it is considered that the ELISA kits are simple and useful methods for the screening of aflatoxins in pig feeds.

Rapid methods for the analysis of mycotoxins – the fast alternative to classic analysis?

Rolf Steinmüller

Neogen Europe Ltd., Cunningham Building, Auchincruive, KA6 5HW, Ayr, United Kingdom

Mycotoxins are toxic natural secondary metabolites formed by fungi growing on agricultural commodities in the field or during storage. Contamination of cereals and related products with mycotoxins causes food- and feed-borne intoxication in man and farm animals. Although the non-fastidious *Fusarium* moulds can be found world-wide, they are prevalent in zones with a moderate climate. In Europe the commodities affected most frequently by *Fusarium spp.* are barley, maize, and wheat. These are also the most important commodities in agricultural production.

Numerous surveys and monitoring studies on the presence of fungal metabolites indicate that deoxynivalenol (DON), a member of the type B trichothecenes, belongs to the most prevalent *Fusarium* mycotoxins in agricultural commodities in Europe. Since mycotoxins are potential health risks for humans and animals, several countries, including the EU regulates DON in food and feed at maximum tolerable levels. Therefore, accurate and precise analytical methodologies are required.

This overview presents the analysis of DON by rapid methods such as: enzyme linked immunosorbent assay (ELISA) and flow through membrane based immunoassay in comparison to physicochemical methods like HPLC. The need for rapid yes/no decisions has led to a number of new screening methods. In particular, rapid and easy-to-use tests based on immunoanalytical principles have made good progress. These methods (NEOGEN Veratox DON 5/5 and NEOGEN DON Reveal) are currently commercially available and are, as the presented data shows, both reliable and rapid methods. This review focuses on the basic principle of each rapid method as well as advantages and limitations of each method. In the case of rapid methods, the clean-up procedures are generally not as intensive as the other analytical techniques. A sample homogenate containing toxin is either directly detected using a standard microtiter strip or a membrane-base format (dip strip) is used for the purpose of screening for the presence of the DON. These tests, especially the single-step lateral flow immunochromatographic assay (NEOGEN DON Reveal) can be performed without special technical knowledge and can be easily integrated into production processes. Thereby reduce both the time and expenses required for analysis and are therefore more economical than conventional methodologies (HPLC).

First steps in developing an ELISA for sterigmatocystin

Ilka Toepfer², Karin Petersen², Rolf Mentlein³, Werner Butte¹

¹Carl von Ossietzky University, Institute for Pure and Applied Chemistry, PB 2503, D 26111 Oldenburg, Germany

²HAWK, Institute for Conservation and Restoration, Bismarckplatz 10/11, D 31135 Hildesheim, Germany

³Institute of Anatomy, Christian Albrechts University of Kiel, Otto-Hahn-Platz 8, 24118 Kiel, Germany

Introduction: *Aspergillus versicolor* is one of the fungi most often detected in mouldy indoor environments [1]. It is capable of sterigmatocystin (ST) production. Engelhart *et al.* [2] detected ST in a few samples of carpet dust from damp indoor environments. To investigate the association between the amount of *A. versicolor* conidiospores and the ST concentration in house dust as well as a possible background concentration of ST a high number of samples has to be analysed. Therefore we are looking for a fast, simple and rather inexpensive method for the detection of ST in house dust. Common methods for the detection of mycotoxins in food are high performance liquid chromatography (HPLC) and enzyme linked immunosorbent assay (ELISA). ELISA is a simple method which allows investigating several samples within a short time. The production of ST and first steps in the development of an ELISA for ST are described here.

Selection of a toxinogenic strain and culture conditions: Nine strains of *Aspergillus versicolor* and two strains of *A. flavus* were isolated from building materials and air samples. These isolates were cultivated in the dark on maltextract-, oatmeal-, potato-dextrose-, cornmeal- and ricemeal-agar for 14 days at 29 °C. The production of ST was examined by the agar-plug method using thin-layer chromatography (TLC) [3]. The *A. versicolor* strain most productive was selected. The highest production of ST occurred on potato-dextrose-agar. On the contrary growth in liquid potato-dextrose medium yielded only low concentrations of ST or no ST at all. For the production of ST in a higher scale mashed potatoes powder (Pfanni, Germany) was dissolved in tap water containing 2% (w/v) dextrose and spread in sterilised baking trays (30×50 cm). A dense conidial suspension was applied and the trays were incubated for 14 days at 29 °C in the dark (covered with aluminium foil).

Extraction of Sterigmatocystin: Production of ST was investigated after 7 and 14 days by TLC. After 14 days the mycelium was removed from the medium, dried at 40 °C and milled in a kitchen blender. The mycelium was extracted with chloroform in a Soxhlet apparatus for 20 hours and the solvent removed in a rotary evaporator.

Purification of Sterigmatocystin: Column chromatography was used for ST purification [4] [column: 4×60 cm, filling: deactivated silica gel (10% water), eluent: dichloromethane]. Fractions of 10 ml were collected and its ST content analysed by TLC. Fractions containing ST were combined. ST was recrystallized using n-hexane/chloroform and acetone. The purity of ST was determined by NMR spectroscopy (¹H, ¹³C). Its purity exceeded 95%.

Konjugation of Sterigmatocystin: ST was conjugated to keyhole limpet hemocyanin and bovine serum albumine, respectively, using the hemiacetal method originally published by Li and Chu [5].

Planned steps: The immunisation of rabbits with the conjugated ST still has to be accomplished. Experimental details for a competitive ELISA to detect ST in house dust will then be developed and validated.

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Using of Cliquid™ software to quickly build custom LC/MS/MS methods for mycotoxin analysis

Kristin von Czapiewski, André Schreiber, Mark J. Kuracina, Alek Dooley

Applied Biosystems, Frankfurter Strasse 129 B, 64293 Darmstadt, Germany

Increasing demand by regulatory authorities to monitor a wider range of food contaminants at increasingly lower levels has led the technique of LC/MS/MS to be adopted by many labs. More reliable, specific and sensitive LC/MS/MS has become the analysis technique of choice for food contaminants as mycotoxins, pesticides, azo-dyes, and veterinary drug residues.

Here the new Cliquid™ software provides an easy way of developing such screening methods for a multitude of potential pollutants. Built with an intuitive point-and-click interface, Cliquid™ Software provides preconfigured methods and reporting tools that simplify the complex process of performing food contaminant analysis. Cliquid™ Software also provides the flexibility to create and add new methods specific to a particular laboratory's needs.

Built into the software is an MRM catalogue, which can be used to quickly build LC/MS/MS method, based on MRM transitions, compound dependant parameters and retention time information saved into the catalogue. The MRM catalogue can be adjusted and extended easily with new compounds or more parameters.

The poster shows an example of rapid method development of a mycotoxin screening method.

***Fusarium* mycotoxins in Nigerian maize**

Timothy O. Adejumo¹, Ursula Hettwer², Petr Karlovsky²

¹Dept. of Microbiology, Adekunle Ajasin University, P.M.B. 01, Akungba-Akoko, Nigeria

²Molecular Phytopathology and Mycotoxin Unit, Dept. of Crop Sciences, University of Göttingen, 37077 Göttingen, Germany, e-mail: toadejumo@yahoo.com

One hundred and eighty two maize samples for human consumption from four maize-producing states of south western Nigeria were screened for contamination of *Fusarium* species and 19 mycotoxins using the High Performance Liquid Chromatography coupled with Mass Spectroscopy (HPLC/MS). Mycological examination showed that *Fusarium verticillioides* was the most commonly isolated fungi (71%), followed by *F. sporotrichioides* (64%), *F. graminearum* (32%), *F. pallidoroseum* (15%), *F. compactum* (12%), *F. equiseti* (9%), *F. acuminatum* (8%), *F. subglutinans* (4%) and *F. oxysporum* (1%). Sixty six samples (36.3%) were contaminated with trichothecenes, deoxynivalenol (DON) (mean: 226.2 $\mu\text{g}\cdot\text{kg}^{-1}$ range: 10–745 $\mu\text{g}\cdot\text{kg}^{-1}$), 3-mono-acetyldeoxynivalenol (3-AcDON) (mean: 17.3 $\mu\text{g}\cdot\text{kg}^{-1}$; range: 1–72 $\mu\text{g}\cdot\text{kg}^{-1}$) and diacetoxyscirpenol (DAS) (mean: 16.0 $\mu\text{g}\cdot\text{kg}^{-1}$; range: 1–51 $\mu\text{g}\cdot\text{kg}^{-1}$) were detected in 22%, 17% and 9% of total samples respectively. 93.4% of the total samples were contaminated by zearalenone (ZON), α - and β -zearalenols (α - and β -ZOL), fumonisin B₁ (FB₁) or enniatins (ENN). The fraction of contaminated samples were 73% for FB₁ (mean: 117 $\mu\text{g}\cdot\text{kg}^{-1}$, range: 10–760 $\mu\text{g}\cdot\text{kg}^{-1}$); 57% for ZON (mean: 49 $\mu\text{g}\cdot\text{kg}^{-1}$, range: 115–779 $\mu\text{g}\cdot\text{kg}^{-1}$) and 13% for α -ZOL (mean: 63.6 $\mu\text{g}\cdot\text{kg}^{-1}$, range: 32–181 $\mu\text{g}\cdot\text{kg}^{-1}$), while ENN A₁, B and B₁ were present in 3%, 7% and 3% of the samples. Only the FB₁ content was significantly different at 95% confidence level among the four states. There were no 15-mono-acetyldeoxynivalenol, nivalenol, HT-2 toxin, neosolaniol, T-2 toxin, T-2 tetraol, T-2 triol, monoacetoxyscirpenol, fusarenone-X and β -ZOL detected. This is the first comprehensive report about the natural occurrence of DON, 3-AcDON, DAS, α -ZOL and ENN in Nigerian maize for direct human consumption.

DON-Gehalte im deutschen Weizen und Roggen

Sandra Masloff, Georg Langenkämper, Thomas Betsche

Bundesforschungsanstalt für Ernährung und Lebensmittel (BfEL),
Institut für Biochemie von Getreide und Kartoffeln, Schützenberg 12, D-32756 Detmold, Germany

The annual monitoring study „Besondere Ernte- und Qualitätsermittlung“ is delivering representative results of the crop and the quality of cereals from the German Federal States. The part „quality“ of this monitoring study is a mandatory research field of the BfEL (according to the German „bill for agricultural statistics“). Contents of Undesired compounds such as mycotoxins, heavy metals and pesticides are determined in the Biochemistry Institute. In the year 2006, deoxynivalenol (DON) was determined in a total of 471 wheat and 263 rye samples. These samples were drawn by institutions commissioned by the Federal States.

DON-contents were lower in 2006 than in the preceding years. The results of the monitoring study 2006 will be presented.

Contamination with aflatoxins and ochratoxin A in grains come from Poland and their biological detoxification

Joanna Biernasiak, Katarzyna Śliżewska, Małgorzata Piotrowska, Zdzisława Libudzisz

Institute of Fermentation Technology & Microbiology, Technical University of Lodz,
Wólczanska 171/173, 90-924 Lodz, Poland

The aim of the work was to determine microbiological contaminated and level of contamination with aflatoxins and ochratoxin A in grains used in production of probiotic. In research was determined the influence of the fermentation process of probiotic bacteria and yeast (*Lactobacillus paracasei/casei* ŁOCK 0920, *Lactobacillus brevis* ŁOCK 0944, *Lactobacillus plantarum* ŁOCK 0945, *Saccharomyces cerevisiae* ŁOCK 0142) on reduction of pathogenic microflora as well as reduction of mycotoxin concentration. Three kinds of grains (barley, wheat and corn) come from different regions of Poland were used in experiment.

The range of research work included identification of selected micro-organisms (total number of bacteria, *coli* bacteria, *Escherichia coli*, *Salmonella*, *Pseudomonas*, *Staphylococcus*, lactic acid bacteria, aerobe and anaerobe spore forming bacteria, as well as yeast and moulds) occurring in barley, wheat and corn grain. Also the level of contamination of the grains mentioned above was determined for aflatoxins and ochratoxin A with the use of ELISA test. The fermentation was conducted in previously optimized conditions (37 °C during 24 h) for probiotic preparations production. The fermentatum medium applied was fodder composite from the most polluted barley (50 %), wheat (45 %) and corn (5 %) grain mixed with water in 1:1.5 proportions.

All grains of barley, wheat and corn coming from different regions of Poland that were subject to microbiological analysis full filled microbiological requirements contained in the norm PN–R–64791:1994. The requirements and tests contained in the norm refer only to the total number of bacteria, anaerobic spore forming, *Salmonella*, *Staphylococcus* and total number of moulds. The tests conducted additionally related to marking the total number of aerobic spore forming, *coli* bacteria and *Pseudomonas* showed that the grains subject to examination are not free from microbiological threats, which was confirmed by the presence of *coli* bacteria (10^3 and 10^5 cfu/g) and *Pseudomonas* (10^2 and 10^3 cfu/g). The highest level of microbiological contamination was found in case of barley grains, and the smallest was found in corn grains. However, it should be emphasized that none of the kinds of grain examined was found to contain typical morbid bacteria *Escherichia coli*.

It was stated that after 6-hour fermentation with addition of probiotic cultures the amount of aflatoxines decreased of 64 % in relation to initial concentration in the fermentation medium, and 50 % in case of ochratoxine A. After the fermentation finished the decrease both of aflatoxines and ochratoxine A was comparable and equaled 77–83 %.

Comparing the microflora pattern during feed fermentation we stated that addition of probiotic cultures not only influences the effective detoxication of toxins examined, but it also allows for proper development of desired microflora during fermentation, such as lactic acid bacteria and yeast. At the same time, it hampers development of *coli* bacteria, aerobic spore forming, *Pseudomonas* and *Staphylococcus* bacteria and moulds.

Occurrence of T-2 toxin and HT-2 toxin in foods from the German market in 2006 – preliminary results from a joint research project

V. Curtui¹, R. Dietrich², K. Hocke², U. Lauber³, E. Märtlbauer², P. Majerus⁴,
A. Trebstein³, E. Usleber¹

¹Justus-Liebig-Universität Giessen, Fachbereich Veterinärmedizin,
Professur für Milchwissenschaften, Ludwigstr. 21, 35390 Giessen, Germany

²Ludwig-Maximilians-Universität München, Tiermedizinische Fakultät,
Lehrstuhl für Hygiene und Technologie der Milch,
Schönleutner Str. 8, 85764 Oberschleißheim, Germany

³Chemisches und Veterinäruntersuchungsamt Stuttgart, Abt. 3.5 – Mykotoxine,
Schaflandstr. 3/2, 70736 Fellbach, Germany

⁴Landesuntersuchungsamt Rheinland-Pfalz, Standort Trier, Institut für Lebensmittelchemie,
Maximineracht 11a, 54295 Trier, Germany

Within a joint research project entitled „Optimisation and validation of the analysis of type A trichothecenes (T-2 toxin and HT-2 toxin) and occurrence of these mycotoxins in food in Germany“, supported by the German Federal Ministry of Food, Agriculture and Consumer Protection (BMELV), analytical data were generated on the contamination levels of foods from the German market with T-2 and HT-2 toxins in the year 2006.

Nearly 1 500 food samples (cereals and cereal-containing foods) were predominantly purchased from retail shops in Germany and analysed for T-2 and HT-2 toxins by enzyme immunoassay, GC-ECD, or LC-MS/MS, respectively. Detection limits (sum of T-2 toxin and HT-2 toxin) were in the range of 0.5–2 µg/kg. All analytical methods were validated through intra- and interlaboratory studies. Low levels of T-2 toxin and/or HT-2 toxin were frequently detected in the majority of all samples, with HT-2 toxin being the predominant toxin in most – but not all – samples.

Relatively high levels of contamination (sum of T-2 and HT-2 toxins) were found for foods based on (or containing) oats (contamination rate: 92–100 %; median toxin level: 5–15 µg/kg). Other grains, in particular wheat, rye, barley, spelt, maize, and products thereof, respectively, were frequently contaminated (50–100 %), but with low or very low median levels, ranging between 0.5 and 2 µg/kg.

In general, the levels of T-2 toxin and HT-2 toxin were lower than those reported from other countries. This study will continue in the next two years, to enable a three-year overview concerning the average levels of T-2 and HT-2 toxins in foods, and to calculate the average intake of these toxins by the German consumer.

Acknowledgement

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The impact of anaerobic digestion on isolated spores of *Fusarium culmorum*

Bettina Frauz^{1,2}, Hans Oechsner², Ulrika Weinmann², Winfried Drochner¹

¹Institute of animal nutrition, University of Hohenheim, Emil-Wolff-Starße 10, 70599 Stuttgart, Germany

²Institute of Farm Machinery and Farm Structures, University of Hohenheim, Garbenstraße 9, 70599 Stuttgart, Germany

The Commission Regulation (EC) No 856/2005 sets maximum levels of pollutants in foodstuffs. Consequently *Fusarium* spores within cereals are problematic and to be inactivated in the given context. This inspires current research to create innovative routines of a safe disposal. One pioneering research approach at the State Institute of Farm Machinery and Farm structures in cooperation with the Institute of Animal Nutrition is to investigate the prerequisites of a potential deactivation of *Fusarium* spores in a fermenting process.

Anaerobic digestion as a biological process involves the conversion of complex organic feedstuffs into a range of smaller, simpler compounds under non attendance of oxygen. The use of digester effluent and residue as a fertiliser after the biogas process is, for the farmer, a very important factor in the calculation of the economic viability of this system [2].

However, the fertiliser value is not the only factor which needs to be considered before the effluent can be applied to arable lands. The presence or absence of any undesirable organisms must also be taken into account [2]. The fate of phytopathogenic organisms in anaerobic digestion has only been reported by these aforementioned authors although their great importance in digester systems fed with plant waste materials. The test agreement was accommodated follows:

Isolated *Fusarium culmorum* spores (FC 46) were derived from a beta testing received from the State Plant Breeding Institute were dissolved and added into the established Hohenheim Biogas yield test after VDI Guideline (VDI 4630, [1]). The results show that no vital spores could be detected after a retention time of 3.5 hours. This points out an encouraging sign for the use of anaerobic digestion for the treatment of infected plant material.

Reference

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Degradation of Deoxynivalenol during anaerobic fermentation

Bettina Frauz^{1,2}, Hans Oechsner², Margit Schollenberger¹, Winfried Drochner¹

¹Institute of animal nutrition, University of Hohenheim, Emil-Wolff-Starße 10, 70599 Stuttgart, Germany

²Institute of Farm Machinery and Farm Structures, University of Hohenheim, Garbenstraße 9, 70599 Stuttgart, Germany

A current research project at the State Institute of Farm machinery and Farm structures and the Institute of Animal Nutrition at the University of Hohenheim was launched to clarify the terms and conditions for a potential intermixture of Fusarium-contaminated substrate in agricultural biogas plants. The design of this research approach was set to modify fermentation in order to gain the aforementioned deactivation of the anti nutritives, such as Deoxynivalenol.

One option for evaluation of biogas potential is to determine appearing CH₄ yields of standard inoculated manure combined with the toxic substrate using a batch fermentation test, the so called Hohenheim biogas yield test [1] after VDI 4630 (directive for fermentation) [4]. The small dimension of the experimental facility, consisting of about 130 flask samplers with a volume of 100 ml allows an accomplishment out of a large number of repetitions or parallel tests with a reduced space requirement. Other than the mentioned methane production also the fate of Fusarium was recorded [3]. To evaluate the microbiological transactions deoxynivalenol standard solution in standard liquid manure und DON-free wheat flour were applied into the test system and incubated at 35 °C. Samples were taken at specific dates and analysed for the content of DON and its metabolite Deepoxydeoxynivalenol by HPLC/FLD or GC/MS after liquid/liquid extraction using kieselguhr material [2]. A complete degradation of DON was observed by a coevally detection of Deepoxydeoxynivalenol.

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The Effect of Temperature and Time Processing on Zearalenone Mycotoxin from Mainze Products

Valeria Gagiuc*¹, Mihaela Nicoleta Avram¹, Nastasia Belc¹, Enuta Iorga¹, Mariana Diaconu², Alecu Diaconu², Mihai Pricop³

¹Institutul de Bioresurse Alimentare, Str. Dinu Vintilă nr. 6, sect. 2, București, Romania

²Univ. Tehnică „Gh. Asachi” – Iași, Centrul de Cercetare și Transfer Tehnologic Polytech, Bd. Mangeron, nr. 59, Iași, Romania

³SCDA Podul Iloaiei, str. Națională, nr. 87, Iași, Romania

*Corresponding author. Phone: 040212109128; Fax: 040212113639, e-mail: bioresurse@adslexpress.ro

Mycotoxins contaminated food represent a risk for human and animal healthy. The best way to protect consumers against toxic effects of fungi and mycotoxins from cereals is to apply HACCP system during production and storage.

As processing is one of the methods for food decontamination, we tested the effect of processing temperature and time on zearalenone mycotoxin from contaminated maize. For this aim, 3 maize samples (naturally contaminated with ZON) were tested at 175 °C, 200 °C and 225 °C, respectively, and 15, 20, 25 and 30 min, respectively; there were obtained 36 samples that were tested for ZON concentration, using Ridascreen[®] Zearalenon test kit (ppt), according to protocol of R-Biopharm producer (Germany). Absorbance was measured at 450 nm, by microplate reader Sunrise (Tecan), fitted with RIDAWIN software (R-Biopharm). Results showed that ZON was stable at 175 °C for 15–25 min, but concentration was decreased at 200 °C after 20 min. It was thought that this situation is due to the fact ZON is a compound very stable at high temperature, as it is mentioned in literature.

Occurrence of type A and B trichothecenes in wheat, rye and oats from 2005 and 2006

Christoph Gottschalk¹, Jörg Barthel², Gabriele Engelhardt², Johann Bauer¹, Karsten Meyer¹

¹TU München, Chair of Animal Hygiene, Weißenstephaner Berg 3, D-85354 Freising, Germany

²Bavarian Health and Food Safety Authority, Veterinärstraße 2, D-85764 Oberschleißheim, Germany

Trichothecenes type A and B are known as cytotoxic and immunosuppressive mycotoxins produced by genera of *Fusarium*. Among cereals oats, maize, wheat, and rye are considered to be the most contaminated with type A trichothecenes like T-2 and HT-2 toxin. According to mean intake data provided by a study of the Scientific Cooperation (SCOOP 2003 [1]) the ingestion of these toxins could exceed the temporary TDI for the sum of T-2 and HT-2 especially in infants and young children. In order to set appropriate maximum levels till 1 July 2008 the European Commission claimed for more occurrence data of these toxins in cereals (Commissions Directive (EC) No 1881/2006 [2]).

Representative samples of wheat ($n = 130$), rye ($n = 61$) and oats ($n = 98$) have been drawn according to Commissions Directive (EC) 401/2006 [3] at mills, wholesale and retail stage in Bavaria between February and December of 2006. The samples were analysed for 14 type A and B trichothecenes by a sensitive LC-MS/MS method. For wheat, mean levels of T-2 and HT-2 (sum of the toxins) were low with $1.8 \mu\text{g}/\text{kg}$, but with an incidence of 94%. As expected, the contamination of deoxynivalenol (DON) was 100% (LOD: $0.04 \mu\text{g}/\text{kg}$) with a mean level of $57 \mu\text{g}/\text{kg}$ in the range of 0.06 to $1\,160 \mu\text{g}/\text{kg}$. The burden of rye with type A trichothecenes was comparable to that of wheat. DON was detected also in every sample with a mean level of $28 \mu\text{g}/\text{kg}$. Values ranged between 1.1 and $288 \mu\text{g}/\text{kg}$. In oats a mean level of $15 \mu\text{g}/\text{kg}$ and a maximum level of $85 \mu\text{g}/\text{kg}$ T-2 and HT-2 was determined, with a contamination rate of 100% (LOD: T-2 $0.03 \mu\text{g}/\text{kg}$, HT-2 $0.06 \mu\text{g}/\text{kg}$). For T-2 tetraol a mean level of $9.7 \mu\text{g}/\text{kg}$ and a maximum level of $85 \mu\text{g}/\text{kg}$ with an incidence of 90% was determined. 74% of the oat samples were contaminated with DON with a mean level of $2.8 \mu\text{g}/\text{kg}$.

The data will be discussed and evaluated with respect to possible health risk for certain consumer groups.

Reference

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Moulds and mycotoxins contamination of flours and brans in 2006 from retail trade

Jan Grajewski¹, Magdalena Twaruzek¹, Waldemar Korol², Beata Miklaszewska¹, Agnieszka Wozniak¹

¹Kazimierz Wielki University, Institute of Biology and Environmental Protection,
Chodkiewicza 30, 85064 Bydgoszcz, Poland

²National Research Institute of Animal Production, National Feed Laboratory,
Chmielna 2, 20079 Lublin, Poland

Cereal products due to the richness in nutritive substances and the storage conditions are prone to moulds and mycotoxins contamination. International project SCOOP Task 3.2.10 (and previous) confirmed a wide presence of mycotoxins in the food chains in a number of the EU members. The presented results are a part of the scientific project which is aimed at determining the possibilities of using the MTT test based on cells culture in the evaluation of the level of mycotoxins contamination of feeds and food commodities. Several dozen of flour samples: wheat, rye and corn, as well as wheat, rye and oats brans were analysed. To compare the quality of the foodstuff brans, a few samples of wheat and rye feedstuff brans were used. The samples were collected at random from various shops and supermarkets from Bydgoszcz and Torun. The aim of the study was the contamination level evaluation of the cereal products, with microbiological, biological (MTT cytotoxicity test) and toxicological methods. Quality and quantity identification of moulds and yeast was done in accordance with PN ISO 7954: 1999 (inoculation on YGC medium – yeast extract, glucose, 100 ppm chloramphenicol, incubation 5–7 days in 25 °C). The extraction of mycotoxins was done on the immunoaffinity columns: AlfaTest, ZearalaTest, FumoniTest and DONTest by Vicam for aflatoxins (AF), zearalenon (ZEA), fumonisins (FUM) and deoxynivalenol (DON), as well as Ochraprep by R-Biopharm Rhône Ltd for ochratoxin A (OTA). Mycotoxins were evaluated with the HPLC method (MERCK-Hitachi) with fluorescence and UV detection. Cobra Cell was used for aflatoxins derivatization.

Cytotoxicity test was carried out on the samples in which the pathogenic fungi and higher mycotoxins concentration had been detected earlier. Colometric toxicity test – MTT with swine kidney (SK) cells was used. Different levels of moulds and yeast contamination were detected in the analysed samples. The level of moulds in the foodstuff brans ranged from 1.0×10^1 to 1.0×10^4 cfu/g. Higher contamination was detected in feedstuff brans 8.0×10^2 – 4.5×10^6 cfu/g. Moreover, differences in the moulds profile were noted. In the foodstuff brans dominated *Aureobasidium*, *Eurotium*, *Mucor* and *Penicillium*. While in the feedstuff brans the highest percentage participation was of *Aspergillus candidus* and *A. penicillioides*. No AF or ZEA contamination was detected in the brans. OTA was determined in all the feedstuff brans samples and at a higher range (0.36–18.0 ppb) than for the foodstuff brans samples (< 0.20–3.32 ppb). DON was also found in all the brans samples, however, the level of the contamination's concentration varied (< 50–705 ppb). Flour analysis showed that the average amount of fungi in all the samples ranged from 1.0×10 to 8.0×10^4 cfu/g. The differences in the moulds profiles were of quantitative nature only, with the presence of field and storage moulds (*Aspergillus*, *Penicillium*, *Eurotium*, *Cladosporium*). No AF and ZEA contamination was found in the flours. But for two cases, the level of DON contamination was low. In all the corn flours FUM presence was detected. Very high concentration of OTA was found in rye flour. Four samples exceeded the acceptable limit and in one case the concentration was above 90 ppb. It was proven that MTT test enables toxicity evaluation, caused by moulds and their secondary metabolites. Especially in the case of flour samples there is a correlation between the determined cytotoxicity and the amount of moulds and level of mycotoxins.

Estrogenic mycotoxins released from *Fusarium* infested wheat and corn fields

Niccolo Hartmann¹, Thomas D. Bucheli¹, Marianne Erbs¹, Felix E. Wettstein¹,
Susanne Vogelgsang¹, Hans-Rudolf Forrer¹, René P. Schwarzenbach²

¹Agroscope Reckenholz-Tänikon Research Station ART, Reckenholzstrasse 191, CH-8046 Zürich, Switzerland

²Swiss Federal Institute of Technology Zürich, Universitätsstrasse 16, CH-8092 Zürich, Switzerland

Resorcyclic acid lactones (RALs) are produced by a variety of *Fusarium* species that grow on corn, wheat, and other cereals, similar to the very well known deoxynivalenol (DON). They have been known to be potent natural estrogens for almost half a century. Zearalenone (ZEA) is the most prominent compound of the RALs. Plenty of effort has been spent to understand its occurrence and fate in food, feed and domestic animals. As of yet, the environmental exposure to the RALs has not been investigated. Due to their very high estrogenic potential, it is possible that RALs contribute to the total estrogenicity of surface waters if they are released from *Fusarium* infected crops.

The emission of RALs via drainage water from wheat and corn crops was thus studied in a worst case scenario field study with maximised infestation by *Fusarium graminearum*. Complementary, a monitoring network was established to trace RALs in various Swiss rivers. In addition, the sorption behaviour of ZEA was investigated in laboratory studies in order to determine the soil components responsible for sorption.

Up to now, we were able to detect ZEA in the drainage water, top soil, and plant material of the experimental field. However, other RALs could not be detected in any of these samples. ZEA concentrations ranged from 1 to 30 ppt in drainage water, from 5 to 10 ppb in the soil and were at around 6 ppm in grain samples. Concentrations of DON as measured in a few drainage water samples were about 5 to 10 times higher than for ZEA. The total ZEA loads released via drainage water were 56 μg and 100 μg during the wheat and corn cultivation period, respectively.

A preliminary estimated ZEA mass balance of the *Fusarium* infested wheat field showed that about 23 % of the produced ZEA were removed by harvest, 6 % were retained in the topsoil and only negligible amount was released via drainage water into the local creek. We assume that the rest remained in the plant debris on the field and in lower soil horizons.

Despite our observation of low ZEA concentrations and loads in our particular field study, ZEA might contribute to the total estrogenicity of local surface waters if these are fed mainly by drainage water from *Fusarium* infested wheat and corn fields.

Mykotoxine in Maiserzeugnissen

Matthias Hennel

Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit
Lebensmittelinstitut Braunschweig, Dresdenstrasse 2, 38124 Braunschweig, Germany

In den zurückliegenden Jahren sind bei diversen Mykotoxinen in Maisprodukten immer wieder mal auffällige Gehalte festgestellt worden. Daher sollte ein umfangreiches Untersuchungsprogramm im Jahr 2006 näheren Aufschluss über die Kontamination dieser Lebensmittel liefern.

Folglich wurden 77 Proben an Maismehl, Maisgriess und Popcornmais auf ihren Gehalt an Aflatoxinen, Ochratoxin A, Zearalenon sowie Fumonisin untersucht. Weitere Fumonisin-Analysen sind in Cornflakes sowie Erdnussflips durchgeführt worden.

Bei den Aflatoxinen und Ochratoxin A handelt es sich um von Lagerpilzen der Gattungen *Aspergillus* und *Penicillium* gebildete Toxine. Dagegen entstehen Fumonisine und Zearalenon durch Befall mit Feldpilzen der *Fusarium*-Gattung.

Die Analysenmethoden beruhen auf dem Prinzip der Aufreinigung an Immunoaffinitätssäulen und Messung mit HPLC und Fluoreszenz-Detektion. Für die Aflatoxine erfolgte eine Nachsäulenderivatisierung mit Brom während die Fumonisine mit Phthaldialdehyd vor der Säule derivatisiert wurden.

Die Betrachtung der Ergebnisse bestätigt die Relevanz der Mykotoxine in den genannten Matrices. Bei Maismehl, Maisgriess und Popcornmais kam es bei Aflatoxinen, Zearalenon und Fumonisin zu diversen Höchstmengenüberschreitungen. Insbesondere Fumonisine und Zearalenon waren auch in Bezug auf die Kontaminationshäufigkeit kritisch zu sehen. Die Kontamination mit Ochratoxin A war dagegen vergleichsweise gering. Die Erdnussflips und Cornflakes waren zu etwa zwei Dritteln mit Fumonisin kontaminiert. Die Maximalwerte lagen jeweils knapp unter der Höchstmenge.

Die regelmässigen Höchstmengen-Überschreitungen sowie das parallele Vorkommen mehrerer Mykotoxine veranlassen dazu, diese Analysen auch künftig fortzusetzen. Neben weiteren Mykotoxinen wird hier auch das toxische Moniliformin in das Untersuchungsprogramm aufgenommen.

***Fusarium langsethiae* pathogenicity and aggressiveness towards oat and wheat in an *in vitro* detached leaf assay**

Samuel Imathiu, Rumiana Ray, Matthew Back, Martin Hare, Simon Edwards

Crop and Environment Research Centre, Harper Adams University College,
Shropshire TF10 8NB, United Kingdom

High concentrations of HT2 and T2 have been reported on oats from several European countries. *Fusarium langsethiae* has been identified as the likely species responsible for production of these type A trichothecenes in oats. However, little is known about the pathogenicity and epidemiology of this newly identified *Fusarium* species.

An *in vitro* detached leaf assay involving artificial inoculation of wounded oat and wheat leaves was used to compare the pathogenicity and aggressiveness of *F. langsethiae* to known fusarium head blight pathogens at 10 and 20 °C. Whereas all fungi caused lesions of varying sizes on detached leaves of oat and wheat by the sixth day post-inoculation, no lesions were observed on any of the control leaves. All fungal species were pathogenic to oat and wheat leaves although there were highly significant differences in aggressiveness as measured by lesion length ($p < 0.001$) at both temperatures. The fungus \times host interaction for lesion length was also highly significant ($p < 0.001$). *Fusarium langsethiae* was aggressive on oat and wheat at both 10 and 20 °C. *F. langsethiae* caused more necrosis on developed lesions, particularly on oats, than any other fungal species. The detached leaf assay results indicate that *F. langsethiae* may be a potent pathogen of oat and wheat.

Clearly defined symptoms observed in the detached leaf assay may correlate to varietal resistance to *F. langsethiae*. If so, this would allow a rapid screening of potential breeding material and progeny of oats and other cereals which would aid the reduction of HT2 and T2 in raw and processed cereals.

Moulds and mycotoxins contamination level of maize grains used for bioethanol production in Poland

Grzegorz Klosowski, Jan Grajewski, Wioletta Lukomska, Robert Kosicki

Kazimierz Wielki University, Institute of Biology and Environmental Protection,
Chodkiewicza 30, 85-064 Bydgoszcz, Poland

The aim of the study was qualitative evaluation of the maize grains used for bioethanol production in Poland, picked during harvest in 2006. The examined samples were collected in various geographical-climatic regions of Poland. The material included in the study was raw and divided into three categories: grains immediately after the harvester, grains collected from the heaps in front of the distilleries and after drying and a short storage in a silo or warehouse. The following parameters were examined: mycotoxins concentration, the level of moulds contamination and humidity, as well as the possible efficiency of bioethanol from 100 kg of grains.

In case of the grains collected immediately after the harvester no aflatoxins or ochratoxin A contamination was found. Furthermore, zearalenon contamination was low, with the maximum level at 13 ppb and deoxynivalenol contamination ranged from 160 to 685 ppb.

The grains with humidity about 30% from the samples collected after a short period of storage on the heaps in front of the distilleries contained small amounts of aflatoxins (<0.28 ppb) and ochratoxin A (<0.36 ppb). In particular samples the concentration of zearalenon reached the level of 149 ppb and deoxynivalenol ranged from 87 ppb to 6 059 ppb.

In the samples taken from the grains stored in silos or warehouses no aflatoxins contamination was detected. Moreover, the level of ochratoxin A did not exceed 0.2 ppb. Zearalenon was found in three out of six examined samples in the concentration of 156 to 231 ppb. While deoxynivalenol concentration ranged significantly from 791 ppb to 3 407 ppb.

The level of moulds contamination varied depending on the humidity and storage period. Even in the grains of similar humidity, particular samples were found to have significantly different levels of moulds contamination.

In the samples taken from the grains immediately after the harvester, no contamination of the species: *Penicillium*, *Endomyces* and *Aspergillus flavus* was detected. The most commonly present species were *Acremonium* (from 2.13×10^4 cfu/g DM to 1.56×10^5 cfu/g DM), *Verticillium* (from 3.79×10^4 to 5.15×10^4 cfu/g DM) and *Cladosporium* (from 8.40×10^3 to 3.87×10^4 cfu/g DM).

In the samples of the fresh grains after the harvest with humidity level of about 30% stored for a short period on the heaps in front of the distillery mainly *Penicillium* in the range from 5.55×10^3 to 1.84×10^5 cfu/g DM was detected. Among other fungi species found were: *Endomyces fibuliger* (9.01×10^4 cfu/g DM) and single colonies of *Aspergillus flavus*. The number of yeast detected in the samples taken from the heaps was four times higher than in the samples taken after the harvester and reached 1.77×10^7 cfu/g DM.

The general level of moulds contamination of the analysed dried grains samples with humidity ranging from 10.5 to 15.4% was much lower than of the samples with humidity of 30%. However, significant differences in this respect were noted probably due to different conditions of drying and storage. In particular samples a greater number of *Fusarium* 8.64×10^5 cfu/g DM, *Acremonium* 2.25×10^5 cfu/g DM and yeast 1.11×10^6 cfu/g DM was detected.

Yield of fermentation (alcohol productivity) from 100 kg of the raw material, indicated with the fermentation method, was $26.75 \text{ dm}^3 \text{ A}_{100}$ (fresh grains after the harvest); $25.75 \text{ dm}^3 \text{ A}_{100}$ (grains after short period of storage on the heaps); $34.17 \text{ dm}^3 \text{ A}_{100}$ (dried grains).

Feeding properties and mycotoxin contamination of dried maize slops obtained by PLS technology

Grzegorz Klosowski¹, Jan Grajewski¹, Josef Böhm², Beata Miklaszewska¹, Anna Blajet¹

¹Kazimierz Wielki University, Institute of Biology and Environmental Protection,
Chodkiewicza 30, 85-064 Bydgoszcz, Poland

²University of Veterinary Medicine, Institute of Nutrition,
Veterinärplatz 1, A-1210 Vienna, Austria

The aim of the study was the evaluation of the dried maize slops (from fermented maize grains used in the production of ethyl alcohol) for its feeding properties and the level of contamination with mycotoxins and fungal microflora. Preliminary assessment of the possibility of obtaining the effect of the mycotoxins biodegradation, present in the raw material, during the technological process of alcoholic fermentation run according to the Pressureless Liberation of Starch technology (PLS), with *Saccharomyces cerevisiae* yeast, was done.

The examined samples of raw material and maize slops were taken from the production environment of the agricultural distillery, using the PLS technology. The maize grains used for alcohol production had humidity level of 25.65%. Yield of fermentation (alcohol productivity) from 100 kg of the raw material, indicated with the fermentation method, was 29 dm³A₁₀₀. The concentrations of the isolated mycotoxins in 1 kg of the dried material were: aflatoxin B1-0.38 ppb, zearalenon 24 ppb, deoxynivalenol 608 ppb. Ochratoxin A was not detected.

Fungi were detected at the level of 8.52×10⁴ cfu/g DM and yeast at the level of 1.13×10⁷ cfu/g DM of the maize grains. Among the most dominating fungal species isolated were: *Penicillium cyclopi-um*, *P. crustosum*, *P. brevicompactum*, *P. aurantiogruseum* and *P. coprophilum* (total number around 1.84×10⁵ cfu/g DM); *Mucor racemosus*, *M. hiemalis* and *Rhizopus nigricans* (total number around 1.63×10⁵ cfu/g DM); *Acremonium strictum* (1.35×10⁵ cfu/g DM); *Endomyces fibuliger* (9.01×10⁴ cfu/g DM); *Fusarium moniliforme* (6.73×10⁴ cfu/g DM); *Cladosporium cladosporioides* (3.63×10³ cfu/g DM). The remaining, unidentified fungal species constituted 2.10×10⁵ cfu/g DM. The preliminary studies of the mycotoxins levels in the post-distilled residue indicated that the technological process itself (heat processing, enzymatic amylolytic preparations treatment and yeast microflora) made it possible to achieve the effect of mycotoxins concentration reduction only in the case of deoxynivalenol. The concentration of DON in the maize slop, prepared for drying and of humidity 69.88%, was 379 ppb (DM). Whereas after the drying process in the tumble dryer the humidity was 8.39%, and the concentration was at the similar level, namely 396 ppb. Therefore, the biodegradation result of DON at the level of approximately 40% was achieved (in relation to the raw material). The biodegradation of other, examined mycotoxins detected in the residue was not observed, as their concentrations were at the level similar to the one found in the raw material. The dried maize slops contained much lower numbers of fungi (1.37×10³ cfu/g DM) and yeast (1.75×10³ cfu/g DM), when compared to the base material. The content of the basic feeding components in 1 kg of the dried maize slops obtained by the PLS technology was: raw proteins 26.2%; raw fat 3.26%; raw fibre 15.7%; and raw ash 1.1%.

Auswirkungen einer Inokulation von *Fusarium culmorum* auf die *Fusarium*-Population und den Mykotoxin-Gehalt in Winterweizen

Ulrike Korn¹, Marina Müller¹, Marion Tauschke¹, Dietmar Barkusky², Gernot Verch²

¹Leibniz-Zentrum für Agrarlandschaftsforschung Müncheberg (ZALF), Institut für Landschaftsstoffdynamik, Eberswalder Straße 84, 15374 Müncheberg, Germany

²Leibniz-Zentrum für Agrarlandschaftsforschung Müncheberg (ZALF), Forschungsstation Landwirtschaft, Eberswalder Straße 84, 15374 Müncheberg, Germany

Ackerbauliche Maßnahmen wie die Fruchtfolge, Bodenbearbeitung und Düngung beeinflussen die Befallsrate von Winterweizen mit *Fusarium*-Arten und eine Mykotoxin-Bildung ebenso stark wie die Niederschlagsmengen zum Blühzeitpunkt. Um die Wertigkeit dieser Einflussfaktoren auf den DON- und ZON-Gehalt zu bestimmen, wurden in einem Parzellenversuch wesentliche Faktoren, wie Standort- bzw. Bodeneigenschaften, Beregnung und Inokulation mit *F. culmorum* variiert.

Um in diesen Versuchen insgesamt einen hohen Belastungsdruck zu simulieren, wurde als Fruchtfolge Winterweizen nach Mais angebaut und eine *Fusarium* empfindliche Sorte (Tuareg, mittlere bis starke Anfälligkeit) gewählt.

Der Feldversuch wird auf zwei intensiv ackerbaulich genutzten Versuchsflächen (Müncheberg, Dedelow) in Nordostdeutschland durchgeführt. Die Versuchsstandorte unterscheiden sich in ihren Bodeneigenschaften. Der Grenzstandort für Weizen in Müncheberg steht einem für den Weizenanbau gut geeigneten Boden in Dedelow gegenüber. Der Versuch wurde im Herbst 2005 als Parzellenanlage mit je vier Wiederholungen pro Variante angelegt:

1. ohne Inokulation/ohne Zusatzberegnung (Müncheberg und Dedelow)
2. Inokulation mit *Fusarium culmorum*/ohne Zusatzberegnung (Müncheberg und Dedelow)
3. ohne Inokulation/mit Zusatzberegnung (nur Müncheberg)
4. Inokulation mit *Fusarium culmorum*/mit Zusatzberegnung (nur Müncheberg).

Für die Beimpfung im April 2006 wurden mit *F. culmorum* infizierte Weizenkörner (10 g/m²) auf den Boden ausgebracht. Die zusätzliche Beregnung von insgesamt 165 mm erfolgte in 10 Gaben von Anfang Juni bis Mitte Juli. Es wurden Bodenproben vor und nach der Inokulation genommen, und die Gesamtkeimzahlen der filamentösen Pilze sowie das *Fusarium*-Spektrum bestimmt. Weizenkörner wurden hinsichtlich ihrer *Fusarium*-Befallsrate untersucht und nach der Ernte der Ertrag sowie der DON- und ZON-Gehalt ermittelt.

Die Gesamtkeimzahlen in den Bodenproben aller Varianten zeigten keine Unterschiede. Die Boden- und die Körnerproben von Dedelow wiesen quantitativ und qualitativ mehr Fusarien auf als die Proben von Müncheberg.

Der Vergleich der Erträge zeigte einen Einfluss der Beregnung in Müncheberg. Die höheren Erträge der beregneten Parzellen in Müncheberg waren aufgrund der schlechteren Standort- und Bodenbedingungen insgesamt niedriger als die Erträge in Dedelow. Der DON-Gehalt in den Weizenkörnern in Dedelow (290–834 µg/kg) war höher als in Müncheberg (< 20–186 µg/kg). ZON konnte nicht nachgewiesen werden.

Insgesamt zeigt sich, dass die Unterschiede zwischen den Standorten am größten waren. Ein Einfluss der Beregnung konnte nur teilweise, eine Wirkung der Inokulation auf die *Fusarium*-Population und den Mykotoxin-Gehalt in Winterweizen gar nicht beobachtet werden. Trotz der *Fusarium* fördernden ackerbaulichen Maßnahmen und der Inokulation wurde am Standort Müncheberg im warmen und trockenen Sommer 2006 keine Mykotoxin-Belastung des Winterweizens nachgewiesen.

Production of the masked mycotoxins zearalenone-4-glucoside, α -zearalenol-4-glucoside and β -zearalenol-4-glucoside

Pamela Krenn¹, Franz Berthiller¹, Wolfgang Schweiger², Christian Hametner³, Roland Ludwig⁴, Gerhard Adam², Rudolf Krska¹, Rainer Schuhmacher¹

¹Christian Doppler Laboratory for Mycotoxin Research, Dept. for Agrobiotechnology (IFA-Tulln), University of Natural Resources and Appl. Life Sciences Vienna, Konrad Lorenz Str. 20, A-3430 Tulln, Austria

²Inst. of Appl. Genetics and Cell Biology, Dept. of Appl. Plant Sciences and Plant Biotechnology, University of Natural Resources and Appl. Life Sciences Vienna, Muthgasse 18, A-1190 Vienna, Austria

³Inst. for Appl. Synthetic Chemistry, Vienna University of Technology, Getreidemarkt 9, A-1060 Vienna, Austria

⁴Division of Food Biotechnology, Dept. of Food Sciences and Technology, University of Natural Resources and Appl. Life Sciences Vienna, Muthgasse 18, A-1190 Vienna, Austria

The aim of this study was to produce the zearalenone (ZON) derivatives zearalenone-4-glucoside (ZON-4G), α -zearalenol-4-glucoside (α -ZOL-4G) and β -zearalenol-4-glucoside (β -ZOL-4G). These substances can occur naturally as masked mycotoxins in cereals [1], but so far no analytical standards are commercially available.

We used a *Saccharomyces cerevisiae* strain expressing the *Arabidopsis thaliana* glucosyltransferase UGT73C6 [2] for the conversion of ZON to ZON-4G. For the production of the glucosides we developed a method with four main steps: 1) production of ZON-4G from ZON utilizing genetically modified yeast, 2) isolation and purification of ZON-4G on RP-C₁₈-SPE columns and preparative HPLC, 3) chemical reduction of ZON-4G with sodium borohydride to both α -ZOL-4G and β -ZOL-4G, and 4) isolation and purification of α -ZOL-4G and β -ZOL-4G with preparative HPLC.

The yeast was cultivated in SC-Leu medium until an OD₆₀₀ of about 1.0 was reached and concentrated to give an OD₆₀₀ of about 5.0. Afterwards, 50 mg ZON were added to the yeast in a 5 l bioreactor. After 48 h, the yeast produced 23 mg ZON-4G, 14 mg β -ZOL and small amounts of β -ZOL-4G. The remaining ZON and its metabolites ZON-4G and β -ZOL were isolated with RP-C₁₈-SPE columns from the medium. After subsequent preparative HPLC, 13 mg pure ZON-4G were gained. To produce both ZOL-glucosides, chemical reduction of ZON-4G with sodium borohydride in methanol was carried out. We obtained transformation rates of ZON-4G into α -ZOL-4G and β -ZOL-4G of about 50% each. After the reduction, the pH value of the solution has been carefully neutralised with diluted acetic acid to avoid degradation of the ZOL-glucosides, which were finally purified with preparative HPLC. A Phenomenex Luna semi preparative column (250 – 10 mm, 10 μ m) was used for purification applying an acetonitrile/water gradient. After drying of the ZOL-glucosides with nitrogen at room temperature, ¹H and ¹³C NMR spectra proved the identity of the produced substances.

Acknowledgement

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Reference Material for Ochratoxin A in Coffee

Rita Krüger, Matthias Koch, Irene Nehls

Federal Institute for Materials Research and Testing (BAM),
Division I.2 Organic Chemical Analysis; Reference Materials,
Richard-Willstätter-Str. 11, D-12489 Berlin, Germany, Phone: ++49 30 81041123, Fax: ++49 30 81041127

The aim of the project is to produce, to characterise and to certify a new reference material for the determination of ochratoxin A (OTA) in roasted coffee.

OTA, a toxic metabolite produced by *Aspergillus spp.* and *Penicillium spp.*, has a number of toxic effects in mammals. The most notable of which are nephrotoxicity and hepatotoxicity [1]. Above all it was classified as potential carcinogen of class 2 by the IARC (International Agency for Research on Cancer). OTA can occur in a large variety of food such as cereals, beans, groundnuts, spices, dried fruits, coffee, beer and wine [2]. Because of carry-over effects it can also be found in meat, especially in kidneys, from animals fed with contaminated feed [3]. Since wine, roasted coffee and instant coffee contribute significantly to the OTA exposition new limit values for these food have been established in Directive EC 123/2005 [4].

Certified reference materials are important instruments for the quality assurance in the analytical laboratory. Due to the new limit values for OTA in several food and the lack of commercially available reference materials on the other side, there is a great demand for the development and supply of respective matrix-reference materials [5].

In the frame of an ERM[®] initiative (European Reference Materials) a new reference material shall be prepared under responsibility of BAM. The targeted OTA content in roasted coffee of about 5 µg/kg corresponds to the new EC limit value. The material was prepared by spiking a sieved non-contaminated ground roasted coffee with OTA. Homogeneity and stability assessments of the confectioned material will be carried out as defined in the ISO Guide 35 [6] using HPLC-ESI-MS-MS and HPLC-FD. The OTA content will be determined by external certification studies.

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Occurrence of trichothecene toxins in wheat and foodstuffs of the Romanian market

Adrian Macri¹, Margit Schollenberger², Melanie Rühle², Constanta Modoran¹,
Maria Virginia Morar¹, Zoe Dancea¹, Winfried Drochner²

¹University of Agricultural Science and Veterinary Medicine, Cluj-Napoca,
Manastur 3-5, Cluj-Napoca, 400372, Romania

²Institute of Animal Nutrition, University of Hohenheim,
Emil-Wolff-Str 10, 70599 Stuttgart, Germany

Fusarium fungi play an important role as pathogens of cereals in Romania. The areas mostly affected are western, northern and central parts of the country as they are characterized by weather conditions favorable to the mycotoxin development. Furthermore, the wheat/maize rotation has become fairly common during the last years, thus contributing to the increasing risks of Fusarium infestation [1]. Investigations on wheat and maize contamination with trichothecene toxins were carried out in western Romania. Nothing is known about the situation in cereals from other parts of the country and about the trichothecene contents in bread and pasta [2].

A total of 88 samples was taken at random in August and September 2006 in central Transylvania and western Romania, consisting of wheat ($n = 42$), bread and rolls ($n = 32$) as well as pasta ($n = 14$). Wheat was obtained from farmers and a mill in the counties Alba, Cluj and Timis, other samples were collected in supermarkets and bakeries in Cluj-Napoca. 13 trichothecene toxins of the B-type as well as of the A-type were analysed by GC/MS [3] after solid-phase extraction. Detection limits varied between 2 and 20 $\mu\text{g}/\text{kg}$. In wheat the toxins nivalenol, deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-ADON), 4-,15-diacetoxyscirpenol, HT-2 and T2 toxin were detected in 1, 38, 15, 1, 21 and 1 sample, respectively. In bakery products DON, HT-2 and T-2 toxin and in noodles DON were found. The maximum concentrations of DON in wheat, bakery products and pasta were at 3 395, 352 and 35 $\mu\text{g}/\text{kg}$, respectively. These results prove contamination of trichothecenes in wheat and wheat-based foodstuffs in the central areas of Transylvania as well as in western Romania.

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Impact of variety and damage by *Ostrinia nubilalis* on *Fusarium* infection of maize cobs and mykotoxin contamination

Gunter Meyer

Biologische Bundesanstalt für Land- und Forstwirtschaft,
Messeweg 11 – 12, 38104 Braunschweig, Germany

Im Rahmen der Entwicklung einer Methode zur *Fusarium*-Resistenzprüfung von Maissorten wurden im Jahr 2006 an den Standorten Sickte (bei Braunschweig) und Münzesheim (Kraichtal, Baden-Württemberg) Versuche mit je 10 mittelfrühen bzw. mittelspäten Maissorten angelegt. Zur Ernte wurde die befallene Kolbenfläche bonitiert, *Fusarium*-Arten isoliert und bestimmt, sowie die Ernteproben mit ELISA auf Deoxynivalenol und Fumonisine untersucht.

In Sickte trat kein Maiszünsler auf und, mit Ausnahme einer Sorte mit 17 % befallener Kolbenfläche, kam es aufgrund der trockenen Witterung insgesamt nur zu einem sehr geringen Kolbenbefall mit *Fusarium* spp. von unter einem Prozent. An diesem Standort waren *F. avenaceum* und *F. poae* die am häufigsten isolierten *Fusarium*-Arten, während *F. graminearum*, *F. subglutinans* und *F. verticillioides* nur vereinzelt auftraten. Die Belastung der Proben mit DON war nur sehr gering (max. 0,2 ppm), während Fumonisine nicht nachgewiesen werden konnten.

Im Gegensatz dazu führten am Standort Münzesheim häufige Niederschläge im August und ein starkes Auftreten des Maiszünslers mit rund 26 % beschädigten Kolben zu einem hohen *Fusarium*-Befall der Maiskolben. Bei der befallenen Kolbenfläche gab es signifikante Unterschiede zwischen einzelnen Maissorten, nicht aber bei der Zusammensetzung des *Fusarium*-Spektrums. Ohne Maiszünsler-Schäden wiesen die mittelfrühen Sorten mit durchschnittlich 5,1 % befallener Kolbenfläche einen geringeren Befall auf als die mittelspäten Sorten mit 7,5 %. Mit Maiszünsler stieg die befallene Kolbenfläche auf rund 21 % bei den mittelfrühen Sorten bzw. rund 30 % bei den mittelspäten Sorten an. Gleichzeitig kam es zu einer Verschiebung im *Fusarium*-Spektrum: An nicht Maiszünsler-befallenen Kolben dominierte *F. graminearum* mit einem Anteil von rund 60 %, gefolgt von *F. avenaceum* bzw. *F. verticillioides* / *F. subglutinans* mit jeweils knapp 14 %, die an geschädigten Kolben mit einem Anteil von 36 % genauso häufig auftraten wie *F. graminearum*, während sich die Anteile der anderen Arten nur wenig veränderten.

Mit Ausnahme zweier Sorten lagen die DON-Gehalte der Proben aus Münzesheim über dem Grenzwert von 1,75 ppm der Mykotoxin-Höchstmengenverordnung und waren mit dem bonitierten Kolbenbefall der unbeschädigten Kolben stärker korreliert als mit dem Gesamtbefall. Insbesondere in stark befallenen Proben bzw. nach Maiszünslerbefall wurden neben hohen DON-Gehalten auch Fumonisin-Gehalte von bis zu 2,5 ppm nachgewiesen.

Influence of grain moisture on Ochratoxin A content in stored wheat

Magdalena Michulec¹, Gerhard Buttinger¹, Wolfgang Richter², Rolf Geisen³

¹European Commission, Directorate General, Joint Research Centre, Institute for Reference Materials and Measurements, Retieseweg 111, 2440 Geel, Belgium

²Bavarian State Research Center for Agriculture, Institut of Animal Nutrition and Forage, Prof. Dürrwächter-Platz 3, 85586 Poing, Germany

³Federal Centre for Nutrition and Food, Location Karlsruhe, Haid-und-Neu-Str. 9, 76131 Karlsruhe, Germany

Cereal storage from one harvest season to the other has been of concern from the earliest times. This problem has grown with increasing populations and developing commerce. Suboptimal storage conditions can cause e.g. a fungal infection of the cereals and as a result, loss of an entire stock.

Some moulds are not only source of economic loss of crop yields, but also cause a serious threat to human health and animal welfare, due to the production of associated mycotoxins. Due to that, quality control of storage practice, raw materials and final food products is exceedingly important.

The poster describes the outline of an experimental storage of wheat previously inoculated with *Penicillium verrucosum*. The storage trial was performed as described in details by Abramson et al [1].

Six 24 kg lots of wheat were inoculated and adjusted to moisture of 14 %, 19 % and 24 %. The lots were split into two portions and transferred into perforated plastic bags, which were stored in silos. During the trial the temperature was recorded every 4 hours and the CO₂ concentration was measured monthly. Each month samples were taken and analysed for Ochratoxin A (OTA) content, fungal growth and moisture.

After milling of the samples (< 1 mm) OTA was extracted from a 20 g aliquot by shaking with acetonitrile/water (60 + 40; v/v). The extract was filtered and then cleaned-up with immunoaffinity columns. The final determination was performed by HPLC with fluorescence detection. LOD of the method used is 0.09 µg/kg. The combined measurement uncertainty is 6.6 % (k = 2).

During the study, the impact of moisture on the OTA content in the stored material has been demonstrated. Considerable concentration of OTA (> 0.3 mg/kg) was found in samples containing 24 % of water. The quaint thing is that OTA was not detected in samples containing 19 % water even after 6 month of storage.

Reference

- [1] D. Abramson, W. Richter, J. Rintelen, R.N. Sinha and M. Schuster (1992): *Arch. Environ. Contam. Toxicol.*, **23**, 259 – 265.

An Approach upon the Mycotoxicological Charge Degree of Products of Vegetal Origin from the Chains of Cluj, Correlated with Their Fats Quality Contents

Maria V. Morar¹, Zoe Dancea¹, Constantin Bele¹, Ioana Z. Kleinhaus, Adrian Macri¹, Vlad Schlezak²

¹University of Agricultural Science and Veterinary Medicine, Cluj-Napoca, Calea Manastur Nr. 3-5, 400372 Cluj-Napoca, Romania

²University of Medicine and Pharmacy, Cluj-Napoca, Cluj-Napoca, Romania

In the actual period, of transition, on the commercial market of our country are often found alimentary vegetal product of indigenous or import provenience with questionable quality. The aim of the realized research was the appreciation of the quality of some aliments from different commercial units/ market from Cluj-Napoca.

Material and method: There were aleatorically sampled (by purchasing) a number of 17 samples from the products: pumpkin seed with hulls (5), pumpkin seed without hulls (2), decorticated pumpkin seed (2), roasted and salted groundnuts/peanuts (4), walnut kernel (3) and hazelnut (1). There was appreciated for all the samples the organoleptic characteristics, the raw chemical compositions (classical methodology), the fats quality (the free acidity expressed in mg KOH/g fat), the proteins quality (by determination of the easy hydrolysable bases) and the fungi charge (CFU/g by cultivation on solid Sabouraud medium). Simultaneously there were identified the most frequent fungi species, based on the cultural and morphological characters (stereo-microscopic, on preparations slides colored with bleu cotton).

Results and conclusions: At the organoleptic examination there were ascertained that 6 samples presented modified odor/smell and taste (rancid). The raw chemical composition data were comparable with data from the literature. Even if in all samples the water contents was low (under 7%), in 5 samples developed over 0.3 millions colonies/gram, dominant being those from the genus *Aspergillus* (especially *A. niger*), *Fusarium*, *Penicillium*. The value of the free acidity was variable, but increased (higher than 4 mg KOH/g fat) in the samples in which organoleptic modifications were distinguished. In four samples (walnut and peanuts) the free ammonia contents were between 97 and 135 mg %.

The correlation allowed the appreciation that circa a third of the alimentary products, aleatorically sampled from commercial units and from the market place, presented qualitative changes considered dangerous for the consumer health. Between these there were observed the occurrence of the mycets with toxigenic potential, which is superposing over the toxicity induced by the occurrence of the resulted compounds by the alteration of the proteins and fats. An important conclusion is related to the need for quality control intensifying and especially of the determination of the mycotoxins of those products, which are consumed in important quantity by human.

Ochratoxin A in pork meat at the Chilean retail market

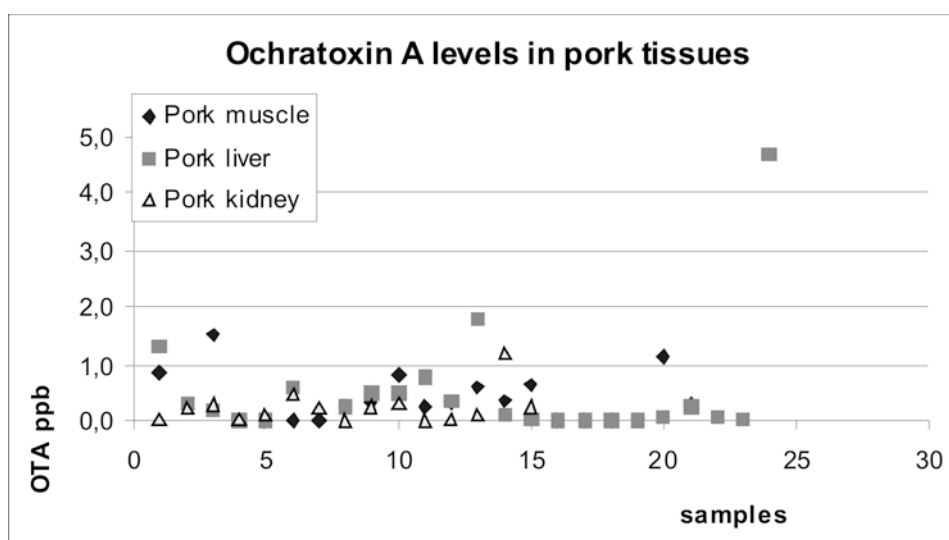
Katherine Muñoz, Mario Vega, Alejandra Opazo

Universidad de Concepción, Facultad de Farmacia Departamento de Bromatología, Nutrición y Dietética,
Casilla N° 237, Concepción, Chile

Ochratoxin A is a mycotoxin produced by a *Penicillium verrucosum* and by several *Aspergillus* species, such as *A. ochraceus*, *A. niger*, *A. carbonarius*. The presence of these moulds is related with good manufacturing practices, (GMP) especially at storage and transportation. These post harvest practices can be affected by a combination of environmental factors, being temperature and humidity the most important.

Normally, the best quality grains are used for human food, the lower quality grains used for animal feeding. In pork, OTA is distributed in blood, kidney, and liver and in less proportion in muscle. All these tissues are used frequently as commodities, contributing with the OTA intake.

In this study, the method of L. Monaci *et al.* (2004) that uses liquid-liquid extraction and HPLC, with some modification to improve detection was used for OTA in pig tissues. The method was previously validated and the samples were collected in the local market. A total of 60 samples were analyzed. The results are summarized in the next graphic.



More than fifty percent of the samples presented values below 1 ppb. The pork products (meat, kidney, blood and liver) are frequently contaminated with OTA, reason why these products could be an important dietary source for OTA.

Acknowledgement

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Experimental study for fumonisins and fungi screening in maize for silage and its relation with cut height and harvest date

Katherine Muñoz, Mario Vega, Ernesto Jahn, Ricardo Madariaga

Universidad de Concepción, Facultad de Farmacia Departamento de Bromatología a, Nutrición y Dietética, Casilla N° 237, Concepción, Chile

The maize crop has been highly related with fumonisin contamination, especially in tropical areas. One of most common uses of this crop is animal feeding. During this study four different cut heights and three harvest date for maize silage were considered: April 5, April 19, and May 10. Fungal flora was studied using a seriated dilution in a PDA media, followed by colony counting. The most representative fungal flora was *Mucor sp.*, *Cladosporium sp.*, *Alternaria sp.*, *Penicillium sp.* and *Fusarium sp.* mainly *Fusarium verticillioides*. For fumonisins analysis AOAC method was used. Forty-eight samples were analyzed. As results of this research, *Cladosporium sp.* and *Fusarium sp.* were the most predominant species. In relation to the mycotoxicological analysis, Fumonisin B1 and B2 were present just in some samples and at very low concentration, less than 2 ppm. The higher values for Fumonisin contamination were found at the harvest 2 (April 19).

Graphic 1: *Fusarium sp.* population in the three harvests, at the different heights of cut.

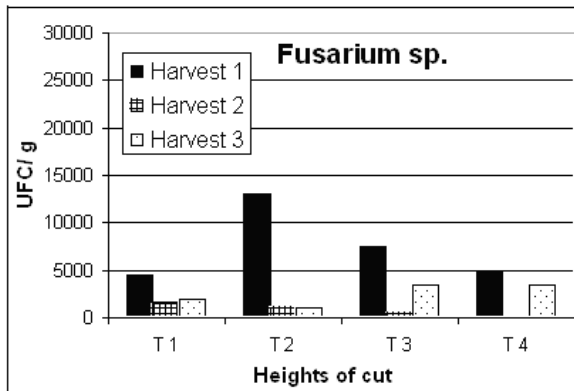


Table 1: Result average for FB1 and FB2 in the three harvests at four different heights of cut.

	Harvest 1		Harvest 2		Harvest 3	
	B1 ppm	B2 ppm	B1 ppm	B2 ppm	B1 ppm	B2 ppm
T ₁	0.057	0	0.029	0	0.013	0
T ₂	0	0	0.123	0.546	0	0
T ₃	0.011	0	0.105	0	0	0
T ₄	0.022	0	0	0	0	0

It was not established a relation between fungal contamination and FB1 and FB2, all the correlation values were below 0.01. This conclusion can be attributed to the matrix conditions; the low humidity and processing treatment can eliminate the mould contamination, but not the fumonisins presence. The information got from *Fusarium sp.* counting and fumonisins analysis shows that the corn residue (T₂) is the most critical area for microbiological and micotoxicological contamination.

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This study was done under the frame of the European Union Project INCO MYCOTOX ICA4-CT-2002-10043.

Comparison of mycotoxin contamination of ecologically and conventionally grown maize

Sabine Nutz, Ursula Hettwer, Petr Karlovsky

Department für Nutzpflanzenwissenschaften, Molekulare Phytopathologie und Mykotoxinforschung,
Georg-August-Universität Göttingen, Grisebachstr. 6, 37077 Göttingen, Germany

The contamination of maize and cereal crops with *Fusarium ssp* poses a serious problem in the production of food and feed. Apart from losses in yield, the decreasing of quality caused by the contamination with mycotoxins is the most serious problem. The infestation of maize and cereals with *Fusarium ssp* depends on several factors. Among the climate it depends also on the cultivation method like tilling practices and crop rotation. In this aspect, the strategy of organic farmers differs from the strategy of conventional farms.

In the years 2005 and 2006 18 different maize genotypes were grown under conventional and organic conditions. We measured the mycotoxin-content of the grain with HPLC/MS and for comparison the amount of fungal biomass with species-specific real-time PCR.

Searching of dietary exposure sources of ochratoxin A: The occurrence of ochratoxin A in red pepper

Vladimir Ostry¹, Jarmila Skarkova¹, Frantisek Malir², Pavlina Kreneckova¹, Jiri Ruprich¹

¹National Institute of Public Health in Prague, Centre for the Hygiene of Food Chains, Brno, CZ-612 42, The Czech Republic

²Institute of Public Health, CHL, CZ-500 02 Hradec Kralove, The Czech Republic

Ochratoxin A (OTA) is the best known potentially nephrotoxic and carcinogenic mycotoxin. The major dietary sources of OTA are cereals but significant levels of contamination may be found e.g. in grape juice, wine, coffee, cocoa, nuts, dried fruits and spices. OTA was determined in beer, pork and pig blood products too. The objective of the study was the current estimate of dietary exposure and risk characterization of OTA in red pepper.

Sample characterization:

Total 56 samples of red pepper were sampled in the Czech retail in year 2005–2006. A part of samples was sampled during the food inspection of Czech Agriculture and Food Inspection Authority (CAFIA).

OTA determination:

An HPLC and HPTLC methods for quantification of OTA in red pepper was used. The limit of quantification (LoQ) was 0.5 µg/kg (HPLC) and 1 µg/kg (HPTLC) respectively. Validation of the methods was performed according to the principles used for HPLC methods and HPTLC methods (ICH Guideline for planar chromatography).

Results on OTA in red pepper:

The average concentration of OTA in red pepper (own results and CAFIA results) was determined 16.3 µg/kg (median 3.6 µg/kg, max. conc. 153 µg/kg). The concentration of OTA < LOQ was considered as 1/2 LOQ for the statistical calculation.

Consumption data:

The consumption data on red pepper in the Czech Republic were from the National study on individuals (SISP 04). Study used 24 h recall, twice repeated on every individual (2003–2004), number of individuals was 2658 persons, represent the Czech population at age 4–90 years and both genders. The consumption of red pepper for average person 64 kg b.w. was determined 0.01 g/kg b.w./day.

Results and conclusions:

The „point“ estimation of dietary exposure dose of OTA for the Czech population (for average person 64 kg b.w.) was 0.16 ng/kg b.w./day (1.14 ng/kg b.w./week). This estimate was compared to known PT-DI/TWI to assess if dietary exposure represents a risk to consumer health (see table).

	Exposure limit	Scientific authority	Hazard index
PTDI	5 ng/kg b.w. / day	SCF (1998)	0.03
PTWI	100 ng/kg b.w. / week	JECFA (2001)	0.01
TWI	120 ng/kg b.w. / week	EFSA (2006)	0.01

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The occurrence of microfungi *Penicillium crustosum* in walnuts

Vladimir Ostry, Jarmila Skarkova, Pavlina Kreneckova, Jiri Ruprich

National Institute of Public Health in Prague, Centre for the Hygiene of Food Chains,
Brno, CZ-612 42, The Czech Republic

The study of the occurrence of toxigenic fungi in walnuts was a part of the project „Monitoring of exposure to chemical substances from foodstuffs“ in the system of National Environment Health Monitoring in the Czech Republic in years 1999–2006. Its objective was to obtain information on the degree of the contamination of walnuts with toxigenic microfungi which could serve as the basis for the evaluation of the dietary exposure and health risk assessment.

Sample characterization:

The samples of walnuts were purchased from retailers in 12 locations of the Czech Republic (Prague, Brno, Ostrava, Plzen, Hradec Kralove, Usti nad Labem, Ceske Budejovice, Zdar nad Sazavou, Znojmo, Jablonec nad Nisou, Benesov and Sumperk) in years 1999–2006. In total, the study involved 84 randomly and not representatively collected samples of walnuts.

Mycological examination:

Mycological analyses were based on the valid standards (CSN ISO 7954) and recommendations of the International Commission for Food Mycology (ICFM) on the application of diagnostic nutrient media for the identification of toxigenic microfungi. The individual assays are elaborated into the form of standard operation procedures (SOP) and are accredited at the Czech Accreditation Institute. The identification of isolated strains of microfungi was carried out according to special mycological literature. The identification of *Penicillium crustosum* (dubious isolates) was independently confirmed. Biological activity of *Penicillium crustosum* strains (rot after experimental inoculation of apples) was tested according to Raper and Thom (1949).

Results and conclusions:

The determination of the total count of microfungi (CFU·g⁻¹) in walnuts is given in Table.

Table: Determination of the total count of microfungi (CFU·g⁻¹) in walnuts

Foodstuff	Number of samples <i>n</i>	Mean (CFU·g ⁻¹)	Median (CFU·g ⁻¹)	90 % percentile (CFU·g ⁻¹)	Range min – max (CFU·g ⁻¹)
Walnuts	84	22 108	1 200	30 900	5 – 600 000

Potentially toxigenic microfungi of *Penicillium crustosum*, the possible producer of the neurotoxin penitrem A, were detected in 29 (35%) samples of walnuts.

Occurrence of toxigenic microfungi, the producers of aflatoxins (e.g. *Aspergillus flavus*, *A. parasiticus*) and ochratoxin A (e.g. *Aspergillus ochraceus*, *Aspergillus carbonarius* and other *Aspergillus* section *Nigri*, *Penicillium veruocosum*) in walnuts were not determined.

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Distribution of a spectrum of 16 *Fusarium* toxins during dry milling of corn

Margit Schollenberger, Hans-Martin Müller, Winfried Drochner

University of Hohenheim, Institute of Animal Nutrition, Emil-Wolff-Str. 10, 70599 Stuttgart, Germany

Corn plants are susceptible to *Fusarium* infestation and a spectrum of *Fusarium* toxins has been described in the literature for corn kernels. The redistribution of nivalenol (NIV), deoxynivalenol (DON), T-2 Toxin (T-2) and in addition of zearalenone (ZEA) was investigated for wet milling that of ZEA for dry milling of corn. There is a lack of information about the fate of trichothecenes for dry milling process of corn.

In the present study the redistribution of 13 trichothecenes of the A and of the B-type as well as of ZEA, α - and β -zearalenol (α -ZOL and β -ZOL) during dry milling of corn was investigated. Raw corn and outcoming fractions belonging to two different batches were obtained from a corn processing factory. The samples were analysed for the B-type trichothecene toxins NIV, fusarenon-X (FUS-X), DON, 3 and 15-acetyldeoxynivalenol (3- and 15-ADON) as well as the A-type trichothecenes T-2, HT-2 toxin (HT-2), T-2 triol, T-2 tetraol, neosolaniol (NEO), 4,15-diacetoxyscirpenol (4,15-DAS), 15-monoacetoxyscirpenol (15-MAS) and scirpentriol (SCIRP) using GC/MS and for ZEA, α -ZOL and β -ZOL using HPLC/fluorescence detection. Detection limits ranged between 1 and 15 $\mu\text{g}/\text{kg}$ out of oil samples and between 1 and 19 $\mu\text{g}/\text{kg}$ for all other fractions of dry milling process.

In samples of raw corn from both batches 7 and 4 trichothecenes, respectively, as well as ZEA were detected. Further toxins were found in fractions of the dry milling process. In general toxins were accumulated in germ, bran and expeller and reduced in the fractions grit and flour, compared to the original substance. Concerning the redistribution between expeller and crude oil the polar alcohols NIV, DON, T-2 tetraol and SCIRP were exclusively located in the expeller, no toxin was found in crude oil fraction. Out of the partially acetylated toxins, FUS-X, 3-ADON and MAS, contained in the germ with concentrations below 100 $\mu\text{g}/\text{kg}$ were found in expeller but not in oil. 15-ADON present in germ with concentrations above 100 $\mu\text{g}/\text{kg}$ was found in expeller and in oil with relatively low concentrations in the latter fraction. HT-2, though present in germ in concentrations below 100 $\mu\text{g}/\text{kg}$ was detected in expeller and oil. T-2 and ZEA, due to their higher lipophilic properties, were found in crude oil in higher proportions compared to the more polar toxins.

Aflatoxin contamination of pearl millet during field and storage with reference to stage of grain maturation and insect infestation

Narsimha Bhumi Reddy, Gajjela Shobha Rani, Chinnam Raghu Raghavender

Mycology and Plant Pathology Laboratory, Department of Botany, UCS, Osmania University, Hyderabad, India

Aflatoxin contamination of pearl millet (*Pennisetum typhoides* (Burm. f.) Stapf & Hubb.) was studied by collecting the seed samples from field and storage conditions and evaluated the inter-relationships between various parameters such as stage of grain maturation in the field and insect pest infestation in storage on the production of aflatoxins.

Five different varieties of pearl millet viz., ICMH-451, ICMP-501, ICTP-8203, WCC-75 and high yielding composite variety ICMV-155 were selected for the study from Mahabubnagar, Medak and Nalgonda districts of Andhra Pradesh, India. In general, aflatoxin contamination was more in the seed samples collected from the fields during rainy season (*kharif*) than winter season (*rabi*). The *kharif* season coincides with the greater moisture content and further promotes the growth and development of different fungi including toxigenic ones. All major aflatoxins such as B₁, B₂, G₁ and G₂ were isolated from one or the other varieties of pearl millet. Whereas aflatoxin G₂ was not commonly observed in the seed samples collected during *rabi*. Among all the varieties tested, ICMH-451 seed samples showed higher percentage and greater amounts of aflatoxins whereas lowest level of aflatoxin was noticed in ICMV-155 variety. The higher levels of aflatoxins were observed in the matured seed samples followed by the seed samples collected at pre-matured stage. On the other, low levels of aflatoxins were reported in the samples collected at the milky stage. Among all the toxins reported in the field, aflatoxin B₁ was found in higher concentration followed by B₂. Whereas the levels of aflatoxin G₁ and G₂ were exceeding the tolerable levels (i.e. 30 ppb) only in few samples.

Higher levels of aflatoxin contamination were reported in the *kharif* seed samples after six months of storage. This may be attributed to the retention of more moisture content in the seeds of *kharif* and also due to high incidence of fungi including toxigenic *Aspergillus flavus*. The four major types of aflatoxins were reported from the *kharif* seed samples after six months of storage whereas aflatoxin G₁ was not observed in any variety of stored seed sample from *rabi*. The levels of aflatoxin B₁ and B₂ were always greater when compared to aflatoxin G₁ and G₂. The relationship between aflatoxin contamination and the grains with insect damage clearly revealed that the seed samples with 20–40% of insect damage contain higher amounts of aflatoxins. More or less similar results were observed in the seed samples with 45–65% insect damage. The lowest percentages of aflatoxin contamination with meagre amounts were reported in 0–15% insect damaged grains. It is clear from these results that insect infestation during storage facilitates the seed-borne fungi (including toxigenic ones) to easily enter the seeds through the cracks and pores made by the insects that subsequently lead to aflatoxin production.

Comparative Study of mycotoxin content on grain and straw in different cereals – first results

Anja Töpfer, W. Wagner, K. Michels, K. Mastel

Landwirtschaftliches Technologiezentrum Augustenberg, Neßlerstraße 23, 76227 Karlsruhe, Germany

An exposure of mycotoxins in feed might have grave negative effects on health and performance of farm animals. Especially pigs reveal high sensibilities. Therefore fusarium mycotoxins in particular deoxinivalenone (DON) and zearalenone (ZEA) are of major importance.

In practice piglets and breeding sows show common symptoms, which indicate an exposure of mycotoxins in feed. The analysis of such feed shows no or minor concentrations. However advanced analysis of straw used as litter or engage-material can indicate high mycotoxin content.

This was the inducement to start a comparative study of mycotoxin exposure in grain and straw. The aim was to explore whether the average exposure of mycotoxins in straw is higher than in grain.

Therefore grain and straw of different species and varieties were analysed. Samples originate from field trials to compare different varieties and from different farms in Baden-Württemberg. For all samples the concentrations of DON and ZEA were determined based on 88 % DM.

Table1: concentrations of DON und ZEA in grain and straw in different varieties of cereals

	species	DON mg/kg (88 % DM)		ZEA mg/kg (88 % DM)	
		grain	straw	grain	straw
Mean	all species	<lq ^a	0,80 ^b	<lq	0,038
SD		0,34	1,21	0,011	0,181
n		163	150	95	80
Mean	barley	<lq	<lq	<lq	<lq
SD		0,35	0,33	0,000	0,010
n		72	68	29	23
Mean	oat	<lq ^a	0,70 ^b	<lq	<lq
SD		0,27	0,62	0,000	0,000
n		12	8	5	3
Mean	triticale	0,40	1,96	0,006	0,171
SD		0,70	2,24	0,009	0,259
n		7	4	5	4
Mean	wheat	<lq ^a	1,37 ^b	<lq	0,039
SD		0,28	1,44	0,014	0,213
n		70	68	55	49

lq: limit of quantitation (lq for DON = 0.2 mg/kg; lq for ZEA = 0.005 mg/kg)

a,b: different letters show significant differences between grain and straw (Pr > F = 0.05)

The first results of this study show that there is a higher amount of mycotoxin in straw samples than in grain samples. In some samples in the grains mycotoxins were below limit of quantitation whereas in straw high concentrations of DON or ZEA were found. Calculating over all species, differences for DON between grain and straw were significant as well as the individual values for oat and wheat (Pr > F = 0.05). This tendency is found for DON in the other cereals and for ZEA in all species. Actually, an estimation of ZEA concentration in grain and straw is difficult due to the low ZEA levels in the season of 2006. For an adequate statistical analysis in oat and triticale higher numbers of analyses would be necessary.

To draw reliable conclusions further more investigations regarding also the climatic terms in different years are needed. Furthermore an evaluation of the different varieties of cereals with regard to the mycotoxin content should be carried out.

Ochratoxin A in blood serum in patients treated by dialysis

Magdalena Twaruzek, Jan Grajewski, Katarzyna Kuzminska, Joanna Buharowska

Kazimierz Wielki University, Institute of Biology and Environmental Protection,
Chodkiewicza 30, 85064 Bydgoszcz, Poland

Ochratoxin A (OTA) is supposed to induce renal diseases in men and animals and correlation between renal diseases and OTA concentration in blood serum is suspected.

The own studies showed that in patients subjected to nephrectomy the average level of OTA in blood serum was higher than in the control group. It is unclear at present whether the elevated OTA concentrations in the patients are the cause of renal disease or merely due to the impaired kidney function. Nonetheless, our data and other recently published data support the hypothesis that OTA may contribute to the development of renal disease. The aim of the study was to determine the level of ochratoxin A in blood serum in patients treated by dialysis. Serum samples from healthy individuals who had no known renal diseases served as control.

The patient's examined were between 23–85 years of age, both male ($n = 48$) and female ($n = 40$), with chronic kidneys' diseases, all from Wloclawek or the neighbouring area. The remaining 16 people - the control group (8 women and 8 men) were healthy, not subjected to dialyses people, at the age of 24–73. Moreover, a parathormone and other parameters such as: protein, urea, creatinine, albumin, calcium, sodium, potassium and phosphorus were identified in the blood serum.

OTA identification was carried out at the Mycotoxin Lab of the Biology and Environment Protection Institute of the Kazimierz Wielki University in Bydgoszcz. The extraction of ochratoxin A from the samples was done on the immunoaffinity columns Ochraprep produced by R-Biopharm Rhone Ltd and identified by HPLC-FLD (produced by Merck-Hitachi) method. Biochemical identification was carried out at the Medical Analysis Lab „ALCO“ in Bydgoszcz. Parathormone analysis was performed by the immunoenzyme test, with the chemiluminescence method using the „Immulite 1000“ produced by DPC. Calcium was identified with the colorimetric method, using the cresolo-ftalein complex. Sodium and potassium were identified by the direct potentiometric method with ion selective electrodes, using the „Ciba Corning 614“ device. Urea was identified by the enzymatic method with urease. Creatinine was identified by the Jaffe's method with z alkaline picrate. Both tests were carried out, using the biochemical analyser „Autohumalyzer 900 S plus“. Phosphorus was identified by the colorimetric method, using phosphomolybdate. Albumin and protein were identified by the colorimetric method, using bromocresolic green and biuret, respectively.

The average concentration of OTA in the blood serum of the healthy person was 0.69 ng/ml in men and 0.72 ng/ml in women. The average concentration in patients treated by dialysis was 0.71 ng/ml and 0.78 ng/ml, respectively (in range 0.1–2.53 ng/ml in women and 0.1–2.776 in men). The average concentration of parathormone in the blood serum of the healthy person was 28.2 pg/ml in men and 27.6 pg/ml in women. The average concentration in patients treated by dialysis was 946 pg/ml and 780 pg/ml, respectively.

There was no significant difference between OTA concentration of healthy individuals and patients treated regularly by dialysis. Standard dialysis did not result in a decrease of the OTA level in the blood serum of patients regularly treated by dialysis.

Significantly increased levels of parathormone, urea, potassium and phosphorus were detected. Higher concentration of parathormone in patients treated by dialysis is a typical result of dialysis treatment. Increased levels of urea, potassium and phosphorus indicate progressive kidney disease.

Screening of Ochratoxin A in cereals products in Chile

Mario Vega, Ricardo Villegas, Carolina Sepúlveda, Katherine Muñoz, Ricardo Madariaga

Universidad de Concepción, Facultad de Farmacia Departamento de Bromatología, Nutrición y Dietética,
Casilla N° 237, Concepción, Chile

Ochratoxin A (OTA) is a Mycotoxin recognized as natural contaminant in cereals and by-products. This mycotoxin is produced by different mould species such as: *Aspergillus ochraceus*, *Penicillium verrucosum*, *Aspergillus niger*, among other. The Ochratoxin production is related with bad manufacturing practices, especially at storage and transportation stages with deficient conditions of temperature and humidity.

The Ochratoxins family involves 4 toxins, being Ochratoxin A the most toxic. The toxic effects of this mycotoxin are related with the kidney function, but also have been described adverse effects in the immune system and liver. The scientific literature has published carcinogenic and teratogenic effects as well.

During this study more than 150 flour samples were collected in the local market, the samples were analyzed using a solid phase extraction for purification of OTA, followed by an HPLC analysis with fluorescence detection. The confirmation was done by methyl ester formation. The results obtained from this study are summarized in the Table 1.

Table 1: Ochratoxin levels in cereal products in the Chilean market.

Zone and date of sampling	N° of samples	Average OTA ppb	Range
Flour samples			
South 2005	18 (16+)	0.200	0.06 – 0.45
Central coast 2006	12 (8+)	0.111	0.07 – 0.39
South 2006	13 (7+)	0.189	0.06 – 2.24
South 2006	13 (10+)	0.250	0.08 – 0.74
Central 2006	15 (11+)	0.31	0.07 – 2.10
North 2006	2 (2+)	0.36	0.02 – 0.71
Wheat grain			
South 2002	60	Negative	—
Rice			
2006	31 (13+)	0.56	0.01 – 12.51
Corn Starch			
2006	30 (12+)	0.09	0.03 – 1.23

This study showed that more than 70 % of the flour samples, 40 % of the corn starch and 42 % of the rice, were positive to OTA. In total were analyzed 192 samples, 61 % was negative to OTA and just 1.5 % of the samples were over the maximum allowed.

Acknowledgement

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Presence of Deoxinivalenol in Chilean wheat samples, study of 3 years

Mario Vega, Ricardo Villegas, Roberto Saelzer, Ricardo Madariaga

Universidad de Concepción, Facultad de Farmacia Departamento de Bromatología, Nutrición y Dietética, Casilla N° 237, Concepción, Chile

Deoxinivalenol (DON, vomitoxin) is a type B tricothecene. This mycotoxin occurs predominantly in grains such as wheat, barley, oats, and maize. DON is produced mainly by *Fusarium graminearum* (*Gibberella zeae*) and *Fusarium culmorum*, both are important plant pathogens which produce Fusarium head blight (FHB). A direct relationship between the incidence of FHB and DON contamination has been established.

In this research, three years of wheat harvest were studied. This study was developed in an important Mill located at the south of the country. The samples were collected following the European Union protocols for mycotoxin sampling. For DON quantification, two methodologies were validated. Both methodologies used Mycosep #225TM as clean up step. Quantitative analysis was done by HPTLC with Fluorescence detection and by Gas Chromatography with ECD detection. The results of this study are summarized in Table 1.

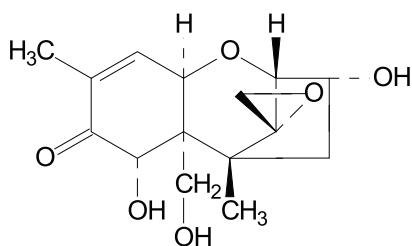


Figure 1: DON structure

Table 1: DON presence at three wheat harvest.

Harvest	Samples	DON ppm	Range ppm.
2002 – 2003	41	Negative	—
2003 – 2004	30	Negative	—
2003 – 2004 ^a	40	Negative	—
2004 – 2005	60	Negative	—
2004 – 2005 ^b	28 (22+)	0.052	0 – 0.090

^aSamples obtained from the truck out of the mill.

^bSamples collected at the central zone of Chile. The samples presented a high humidity level and evident plant damage.

The samples collected in the mill did not show DON contamination, the moistures levels were below than the limit maximum allowed by the local regulation. The positive samples found are below the maximum allowed by the current international regulation.

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Occurrence of Fumonisin in Polish corn lines infected by *Fusarium verticillioides* (Sacc.) Nirenberg*

Marcin Wit¹, Piotr Ochodzki², Roman Warzecha², Wojciech Wakulinski¹

¹Warsaw Agricultural University, Department of Plant Pathology, Nowoursynowska 159, 02-776 Warsaw, Poland

²Plant Breeding and Acclimatization Institute at Radzikow, 05-870 Blonie, Poland

Fusarium cob rot of corn is considered as one of the most important maize diseases throughout the world. Its etiology is complex and different *Fusarium* species from *Liseola*, *Discolor*, *Roseum* and *Sporotrichiella* section are listed as corn pathogens. Most recently, in Poland, *Fusarium verticillioides* was found as predominating causing factor. In 2005 and 2006 the fungus was isolated from 45 % and 69 % of infected cobs derived from four localities represented West, Central, East and South part of the country. On average, in both years occurrence of the fungus resulted in damage of 30 % and 29 % kernels per cob.

Exceptionally high and epidemic incidence of *F. verticillioides* and in consequence strong risk of corn contamination by secondary metabolites was the main reason to start studies which focused on evaluation of fumonisin level in naturally infected corn.

Corn samples for mycotoxin analysis were selected from materials collected during plant inspection at Radzików in 2005. *Fusarium verticillioides* infected cobs of 16 breeding lines exhibited various disease intensity were chosen.

Analysis of fumonisin B₁ (FB₁) was assayed by employing HPLC method. For this purposes ground maize kernels were extracted overnight with methanol:water (3:1) solution on orbital shaker. Subsequently aliquot were applied to conditioned SAX column, washed with 5 ml of methanol:water (3:1) and 3 ml of methanol, and eluted with 12 ml of 1 % acetic acid in methanol, and evaporated to dryness. Prior injected into HPLC analysis sample dissolved in MeOH, were derivatized with OPA solution. FB₁ was eluted with mobile phase methanol: 0.1 M phosphate buffer (77:23), pH = 3.35 at a flow rate 1 ml·min⁻¹ and detected with fluorimetric detector. Fluorescence of the OPA-derivatives was recorded at excitation and emission wavelengths 335 nm and 440 nm, respectively. FB₁ was identified by comparison of retention time with reference standard solution. Fumonisin B₁ content was calculated by comparison of the peak areas of sample and known amounts of standard solution. In performed studies ergosterol content was used as biochemical index for living mass of fungi.

In analyzed material fumonisin B₁ was detected in 15 out of 16 samples and mycotoxin content ranged from 1 to 263 ppm. The metabolite was present both in *Fusarium* damaged crenels as well as in healthy looking one. On average they contained 60 ppm and 5 ppm fumonisin B₁ respectively.

Moreover it was stated significant linear relationship between the level of fumonisin B₁ (fum) and ergosterol (erg) content described by equation $fum = 12.71 + 0.24 \cdot erg$

Modulation of the expression of genes involved in cell cycle control and progression through mitosis by ochratoxin A *in vivo*

Melanie Adler, Katja Müller, Eva Rached, Wolfgang Dekant, Angela Mally

Department of Toxicology, University of Würzburg, Versbacher Strasse 9, 97078 Würzburg, Germany

The mycotoxin and food contaminant ochratoxin A (OTA) is one of the most potent renal carcinogens studied to date, but little is known regarding the mechanism of OTA toxicity. Early histopathological alterations induced by OTA in rat kidney under conditions of carcinogenicity, which include single cell death, increased cell turnover and nuclear enlargement, suggest that disruption of mitosis may be the principal cause of cell death and subsequent trigger for cell proliferation to compensate for cell loss.

To gain further insight into the molecular changes involved in disruption of mitosis in kidneys of OTA treated rats, we used custom made RT-PCR arrays, which combine the quantitative performance of qRT-PCR with the multiple gene profiling capabilities of microarrays, to analyse the expression of 84 genes, selected based on their known function in cell proliferation and cell cycle control. Gene expression analysis was performed in kidney tissues obtained from rats treated with 0, 21, 70 and 210 $\mu\text{g}/\text{kg}$ b.w. for 90 days ($n=3/\text{group}$), as well as in livers of control and high dose animals. Results obtained show that OTA causes altered expression of several genes implicated in chromosome segregation and progression through mitosis, including Aurora B kinase, Polo-like kinase 1 (Plk1), cyclin dependent kinase 1 (Cdk1), cyclin B, cyclin A, cyclin E, the Cdk inhibitors p21^{cip/waf} and 14-3-3 σ (stratifin), topoisomerase II, securin, the spindle checkpoint protein Bub1. Changes in gene expression correlated with the severity of histopathological alterations and were evident in kidneys of rats treated with 70 and 210 $\mu\text{g}/\text{kg}$ b.w.. In contrast, no alterations in gene expression were detected in kidneys of rats treated with 21 $\mu\text{g}/\text{kg}$ b.w. or in the liver, which is not a target for OTA toxicity.

Taken together, these findings provide important new evidence in support of a mechanism of OTA carcinogenicity involving disruption of mitosis through interference with key regulators of chromosome separation and progression through mitosis, resulting in blocked or asymmetric cell division, accompanied by an increased risk of aneuploidy acquisition and subsequent tumor formation.

Silibinin Protects OTA-Mediated TNF- α Release from Perfused Rat Livers and Isolated Kupffer Cells

Lauy AL-Anati, Ernst Petzinger

Faculty of Veterinary Medicine, Institute of Pharmacology and Toxicology,
Justus-Liebig-University Gießen, Frankfurter Str. 107, D-35392 Gießen, Germany

OTA causes necrotic and apoptotic changes in the liver, and a single-chain breakdown of DNA in hepatocytes. New acute effects of OTA on the liver are the release of TNF- α from blood-free perfused rat livers, in particular from Kupffer cells. Since silibinin has a strong hepatoprotective activity against several liver toxins including phalloidin and CCl₄, we investigated its role on this OTA acute effect, namely on TNF- α release and the leakage of cytotoxic markers (GLDH, LDH) and compared them with LPS effects. For this purpose, isolated blood free rat livers were perfused with Krebs-Henseleit buffer containing 2% dextran for 90 min, and isolated pure Kupffer cells were incubated for 24 hrs under standard cultural conditions.

In the recirculation perfusion model, OTA at 2.5 μ mol/L released 2600 pg/ml TNF- α into perfusate at 90 min without a significant increase of cytotoxicity markers (LDH, GLDH). LPS at 0.1 μ g/ml induced 3000 pg TNF- α /ml at the end point, but caused significant leakage of GLDH and LDH. Under similar experimental conditions, the addition of 2.5 μ g/ml silibinin 10 min prior to OTA and LPS, reduced TNF- α by 50% and restored the basal levels of LDH and GLDH. At 12.5 μ g/ml, silibinin had a stronger effect and levels amounted to only 20% and 30% of OTA and LPS induced levels, respectively. At a high-dose of silibinin (12.5 μ g/ml), GLDH and LDH levels in the perfusate were completely restored.

In further experiments, isolated Kupffer cells were pretreated with 0.02, 0.1, 0.5, 2.5, and 12.5 μ g silibinin/ml 30 min prior to OTA or LPS. Silibinin at 0.02 and 0.1 μ g/ml reduced the OTA-induced TNF- α level to 90% and 70% at 4 hrs, respectively. Higher silibinin concentrations reduced to 25% at 4 hrs, but abrogated any TNF- α release at 24 hrs. The presence of silibinin (0.02, 0.1, 0.5, 2.5, 12.5 μ g/ml) prior to LPS caused a reduction of the LPS-induced TNF- α level at 4 hrs to 71%, 57%, 18%, 22%, 18%, respectively. However, at the end of the incubation time, the silibinin effects vanished and TNF- α partially recovered into the incubation medium under LPS.

In summary, silibinin has hepatoprotective effects against OTA- or LPS- mediated TNF- α release and also reduces the cytotoxicity of both toxins. Isolated Kupffer cells are even more sensitive to the protective effect of silibinin and respond to very low concentrations of silibinin with a strong inhibition of toxins mediated TNF- α release.

The effects of a dietary prebiotic supplementation (inulin) on the electrophysiological indices in the presence or absence of deoxynivalenol of intestinal mucosa of broilers

Wageha Awad¹, Josef Böhm¹, Ebrahim Razzazi-Fazeli¹, Khaled Ghareeb², Jürgen Zentek¹

¹Institut für Ernährung, Veterinärmedizinische Universität Wien, Veterinärplatz 1, A-1210 Vienna, Austria

²Institut für Tierhaltung und Tierschutz, Veterinärmedizinische Universität Wien, Veterinärplatz 1, A-1210 Vienna, Austria

Exposure to deoxynivalenol (DON) is a permanent health risk assessment issue for both humans and farm animals. The detoxification of DON is of major practical interest, and the use of feed additives with *Fusarium* toxin degrading properties might be one method for accomplishing this. Therefore, the objective of the present work was to study the in vitro effects of dietary inulin on the electrophysiological parameters in presence or absence of the mycotoxin deoxynivalenol of the gut by using the Ussing chamber technique.

Materials and Methods: Forty 1-d-old broilers were randomly divided into 2 groups. Chicks in group A were fed a basal diet and group B was fed the basal diet with 1.0% inulin. The diets were provided ad libitum for a period of 5 weeks. At the end of the feeding period, 5 birds from each group were killed by stunning and bleeding. Intestinal segments were immediately taken from the mid-jejunum and colon. The epithelial sheets were mounted in modified Ussing chambers. Isolated epithelia were incubated in D-glucose-free buffer mucosally and serosally in Ussing chambers for at least 30 min. Thereafter, 5 mmol/L glucose was added to the mucosal side. After 1-min incubation with glucose, DON were added to the buffer solution. In further experiment, D-glucose was added to the luminal side after prior incubation of the tissues with 10 µg DON /mL. Data were compared by a paired t-test to evaluate the effects of both substrates before and after their addition on I_{sc} and R_t. Independent samples t-test was used for groups comparisons.

Results: In the first experiment, the addition of D-glucose on the luminal side of the jejunum and colon increased the I_{sc} in the control and inulin-supplemented groups compared to basal values, while it decreased after addition of DON. The R_t was higher ($p < 0.05$) in the jejunum after DON addition in control group ($426 \pm 19 \Omega \cdot \text{cm}^2$) compared with the basal values ($337 \pm 15 \Omega \cdot \text{cm}^2$) and the values after addition of glucose ($355 \pm 26 \Omega \cdot \text{cm}^2$) while in inulin-supplemented group the R_t remained unchanged. In the second experiment, the I_{sc} was not affected in jejunum and colon in the control group ($p > 0.05$) by the addition of glucose after preincubation of tissues with DON. However, in the dietary inulin supplemented group the addition of glucose after preincubation of tissues with DON increased the I_{sc}, suggesting that the dietary inulin supplementation could alleviate the alterations caused by DON.

Conclusion: The present study verified that the jejunum and colon of the chicken's gut are able to absorb glucose via an electrogenic mechanism, sodium glucose cotransporter (SGLT1). The effect of D-glucose on I_{sc} was either decreased or prevented by the addition of DON of the jejunum and colon. Dietary inulin increase glucose absorption after preincubation of the tissue with DON. Therefore, the dietary inulin supplementation may be useful in counteracting the toxic effects of DON on intestinal glucose transport.

***in vitro* effects of different B-trichothecenes on the electrophysiological responses to D-glucose of isolated jejunal mucosa of broilers**

Wageha Awad, Josef Böhm, Ebrahim Razzazi-Fazeli, Jürgen Zentek

Institut für Ernährung, Veterinärmedizinische Universität Wien, Veterinärplatz 1, A-1210 Vienna, Austria

In chickens, D-glucose and amino acid absorption occurs via carrier-mediated transport. Cereals are commonly used in feed, and farm animals may therefore consume relatively high amounts of trichothecenes. In our earlier studies, the proximal small intestine, feeding of DON was shown to decrease the absorption of D-glucose. The objective of the present work was to investigate whether the other B-trichothecenes (NIV, 15-Ac-DON, and FUS X) have similar effects on sodium glucose-cotransporter (SGLT1) as DON.

Materials and Methods: Broilers, 6–8 wk of age, weighing 2.5–3 kg, were used in the present study. Birds were killed by stunning and bleeding. Intestinal segments were immediately taken from the mid-jejunum. The epithelial sheets were mounted in modified Ussing chambers. Isolated epithelia were incubated in D-glucose-free buffer mucosally and serosally in Ussing chambers for at least 30 min. D-glucose-induced currents were investigated with DON, NIV, 15-Ac-DON; and FUS X (10 µg/ml) added either before or after the addition of D-glucose (5 mM). Data were compared by a paired t-test to evaluate the effects of both substrates before and after their addition on I_{sc} and R_t.

Results: The R_t was higher ($p < 0.05$) in the tissues exposed to DON, NIV, and 15-Ac-DON (428 ± 36 , 307 ± 40 , $290 \pm 47 \Omega \cdot \text{cm}^2$) compared with the basal values (348 ± 33 , 267 ± 38 , $243 \pm 41 \Omega \cdot \text{cm}^2$), respectively (Table 1). Furthermore, FUS X tended to increase ($p < 0.1$) the R_t ($382 \pm 32 \Omega \cdot \text{cm}^2$) compared with the basal values ($325 \pm 31 \Omega \cdot \text{cm}^2$). The addition of D-glucose on the mucosal side produced an increase in I_{sc} (ΔI_{sc}) in all tissues. DON, NIV, 15-Ac-DON, and FUS X decreased the current in all tissues. The addition of D-glucose on the mucosal side after preincubation of the tissues with DON, NIV, and 15 Ac-DON increased the R_t (408 ± 63 , 346 ± 25 , $373 \pm 29 \Omega \cdot \text{cm}^2$, respectively), and it was numerically higher for the FUS X ($300 \pm 40 \Omega \cdot \text{cm}^2$) compared with the basal values. The addition of D-glucose after preincubation of the tissues with DON, NIV, 15-Ac-DON and FUS X had no effect on I_{sc} ($p > 0.05$) in all tissues.

Conclusion: In the present study, the effect of D-glucose on short circuit-current (I_{sc}) was either decreased or inhibited by the addition of DON, NIV, 15-Ac-DON, and FUS X in the jejunum of broilers. The results indicate that the glucose co-transporter activity appears to be more sensitive to DON, NIV, and 15-Ac-DON suppression than FUS X. From this study it can be concluded that trichothecene mycotoxins (DON, Nivalenol, and 15-Ac-DON) impaired the jejunal function relating to glucose absorption. These results suggest that the trichothecenes might cause specific damage in the active transport system for monosaccharides.

Effects of deoxynivalenol on electrical properties across isolated jejunal epithelium of laying hens are dose dependent

Wageha Awad, Josef Böhm, Ebrahim Razzazi-Fazeli, Jürgen Zentek

Institut für Ernährung, Veterinärmedizinische Universität Wien,
Veterinärplatz 1, A-1210 Vienna, Austria

Feeds contaminated with mycotoxins pose a health risk to animals and, as a consequence, may cause big economical losses due to lower efficacy of animal production. The most significant mycotoxins in contaminated foods and feeds are *Fusarium* mycotoxins. Among the *Fusarium* toxins, deoxynivalenol (DON) plays an important role, because it can occur in concentrations which are of toxicological relevance for farm animals. The aim of the present studies was to evaluate the effects of DON on electrophysiological parameters in laying hens' jejunum mounted in Ussing chambers. In vitro studies were performed to measure the effects of different luminal concentrations of DON (0.5, 1, 5, 10 $\mu\text{g}/\text{mL}$) on the electrical tissue resistance (R_t) and electrogenic ion flux rates (short-circuit current, I_{sc}) across the isolated gut mucosa.

Materials and Methods: Lohmann Brown laying hens, 48 wk of age, were used in the present study. Birds were killed by stunning and bleeding. Intestinal segments were immediately taken from the proximal-jejunum. The epithelial sheets were mounted in modified Ussing chambers. Isolated epithelia were incubated in D-glucose-free buffer mucosally and glucose-containing buffer serosally in Ussing chambers for at least 20 min. Thereafter, 5 mL of Ringer solution on the luminal side were replaced by 5 mL of Ringer buffer containing different concentrations of DON (0, 2.5, 5, 25, 50 μg DON/5 mL of Ringer solution). Furthermore, other experiments were performed to investigate the mechanisms of action of DON. Amiloride (1 mmol/l), a specific inhibitor of Na^+ transport (Mall *et al.*, 1998), was added to the luminal side 15 min after addition of 5 μg DON/mL. In a second experiment, 5 mmol/L glucose was added to the mucosal side. After 1 min incubation with glucose, DON or phlorizin (a specific inhibitor of SGLT1) were added to the buffer solution. Data were compared by a paired t-test to evaluate the effects of both substrates before and after their addition on I_{sc} and R_t .

Results: The tissue resistance ($317 \pm 39 \Omega \cdot \text{cm}^2$, $325 \pm 41 \Omega \cdot \text{cm}^2$, $371 \pm 38 \Omega \cdot \text{cm}^2$) was higher ($p < 0.05$) in the tissues exposed to 1, 5, 10 μg DON/mL, respectively, compared with the basal values ($229 \pm 17 \Omega \cdot \text{cm}^2$). The addition of 1, 5, 10 μg DON/mL to the mucosal compartment was followed by an immediate and steady decrease ($p < 0.05$) in I_{sc} compared with the basal values. DON had a dose-dependent effect on I_{sc} , with a decrease of I_{sc} to -12 ± 19 , -20 ± 18 and $-32 \pm 11 \mu\text{A}/\text{cm}^2$, respectively, compared with the basal values ($42 \pm 11 \mu\text{A}/\text{cm}^2$). The I_{sc} was not affected ($p > 0.05$) by the addition of amiloride after incubation of tissues with DON, which did not have any further inhibitory effect. The addition of DON or phlorizin to the mucosal solution after glucose addition decreased the current.

Conclusion: The main effect of DON was a dose-dependent decrease in the short-circuit current (I_{sc}). The decrease in I_{sc} is apparently caused by the inhibitory effect of DON on Na^+ -transport; this is similar to the effect of amiloride, an inhibitor of sodium ion transport. The addition of D-glucose on the luminal side of the isolated mucosa increased I_{sc} , and this effect was reversed by phlorizin and DON. This finding indicates that the inhibition of Na^+ -transport and Na^+ -D-glucose co-transport are the important mechanisms of DON toxicity in the intestine of laying hens.

Cytotoxicity of mycotoxins in forages

Bronius Bakutis, Violeta Baliukoniene

Lithuanian Veterinary Academy, Department of Food Safety and Animal Hygiene,
Tilzes 18, 47181 Kaunas, Lithuania

Exposure of livestock to mycotoxins in animal feeds materials and the resulting clinical symptoms are a threat to animal health and productivity throughout the world. In certain geographical areas of the world, some mycotoxins are produced more readily than others. In colder more temperature regions: Canada, USA and most European countries as Lithuania, aflatoxins (AFL) are not considered to be a major problem. Deoxynivalenol (DON), zearalenone (ZEN), ochratoxins are found more frequently. These mycotoxins may potentially affect animal health and productivity.

Numerous bioassays have been developed for screening of mycotoxins in fodders. The most commonly applied bioassay for detection mycotoxins is the use of *in vitro* mammalian cell culture systems.

The purpose of the this study was to screen the cytotoxicity of the most commonly found mycotoxins DON, ZEN, ochratoxins in grains and fodder extracts, using human hematopoietic cells K-562, mice-derived hepatoma MH-22A, and swine kidney SPEV cell lines.

The cytotoxicity of mycotoxins was determined by measuring different endpoints such as inhibition of protein and DNA synthesis, plasma membrane integrity and reduced metabolic activity. The crystal violet was used to evaluate of apoptosis cells. The metabolic activity of viable cells was measured by using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide) bioassay.

In a screening assay, duplicate samples of samples a total of 40 extracts of wheat, barley, triticale, pease and composite forages at the concentrations of 500–875 mg/ml were incubated in triplicates with K-562, MH-22A, SPEV cells. The % inhibition of cell proliferation and IC₅₀ value for cytotoxic compound were calculated.

Comparison of predicted and tested grains and forages extracts effects resulted in higher toxicity of DON in K-562 cells. The tested grains and forages contaminated with ZEN resulted in high toxicity in K-562 and MH-22A cells. The grains and forages with ochratoxins resulted in higher toxicity in K-562 and SPEV.

Cell culture systems can be more sensitive and more reproducible than tests involving intact animals. These cell culture assays can be used for the screening of toxicity of mycotoxins. The comparison of toxic responses obtained with each bioassay may orient to its toxicological mechanism.

Novel oxidative *in vitro* metabolites of zearalenone-type mycotoxins

Georg Damm, Erika Pfeiffer, Anja Heyting, Manfred Metzler

Institute of Applied Biosciences, Chair of Food Chemistry, University of Karlsruhe,
Adenauerring 20a, D-76131 Karlsruhe, Germany

Zearalenone (ZEA) is a resorcylic acid lactone produced by *Fusarium* species infesting mostly corn but also other grains. We have recently disclosed that rat liver microsomes are capable of transforming ZEA to two major and six minor monohydroxylation products, which have not been previously reported as mammalian metabolites (Pfeiffer et al., *Mol. Nutr. Food Res.*, in press). The aim of the present study was to compare the *in vitro* metabolism of ZEA with that of α -zearalanol (α -ZAL), which is a derivative of ZEA and extensively used as a growth promoter for cattle in several non-EU countries.

Upon incubation of 50 μ M ZEA or α -ZAL for 40 min at 37 °C with 1 mg microsomal protein (obtained from the liver of non-induced male Sprague-Dawley rats) and a NADPH-generating system, 46% of ZEA and 80% of α -ZAL were metabolized. When the metabolites of α -ZAL were analyzed by HPLC and subsequently by GC-MS after trimethylsilylation, four mono-hydroxylation products of α -ZAL and one monohydroxylated zearalanone (ZAN) were identified. The major hydroxy- α -ZAL metabolite accounted for 27% and hydroxy-ZAN for 15% of the total α -ZAL metabolites. Although the positions of the new hydroxyl groups could not be unequivocally derived from the mass spectra of the metabolites, our current interpretation of the fragments suggests that the hydroxylation has occurred at the aliphatic moiety rather than at the aromatic ring.

As hydroxylated metabolites of α -ZAL have not been reported before, our study has provided evidence for the formation of several novel oxidative metabolites of the growth promoter α -ZAL *in vitro*. Further studies are now needed to elucidate their complete structures and biological activities, and to clarify whether they are also formed *in vivo*.

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Researches Regarding The Nutritive Value Of Some Alimentary Products For Animals And Human, Correlated With The Mycotoxicological Charge

Zoe Dancea¹, Maria V. Morar¹, Andrei Szakacs¹, Adrian Macri¹, Vlad Schlezak², Winfried Drochner³

¹University of Agricultural Science and Veterinary Medicine,
Cluj-Napoca Calea Manastur Nr. 3-5, 400372 Cluj-Napoca, Romania

²University of Medicine and Pharmacy, Cluj-Napoca, Romania

³University of Hohenheim, Institute for Animal Nutrition, Emil-Wolff-Str. 10, D-70599 Stuttgart, Germany

The paper presents the results of the research realised upon 20 samples of feed/food, from which: 12 TMR destined for different morpho-productive categories of poultry (meat chicken, layer hens, reproduction hens); 2 – TMR for feeding pigs; 1 – concentrated feeding stuff for dairy cows; 7 – simple feed/food (wheat flour, maize meal, carrots, beet). The majority of the analysis were made at the request of some farmers from the area of Transylvania, as a result of the occurrence of some health disturbances in animals, being doubted that in their etiology was implicated the feed quality.

The working procedures foresaw: the epizootic investigation in the farms from which the samples were taken, the macroscopic and stereo-microscopic examination of the feed, the raw chemical composition (after Weende methodology), the estimation of the nutritive value based on the raw chemical composition data and expressed through feed units oat, the mycological examination (cultivation on solid Sabouraud medium, counting of the colonies from the medium surface after 3 days and from the profoundness after 6 days of incubations at the temperature of 24 °C) and the identifications of the dominant mycets species by the examination of the cultural and morphological characters in slide preparations coloured with cotton bleu. Simultaneously there were determined the fats freshness (acidity value) in 13 samples and the proteins freshness (free ammonia content) in 3 samples.

The obtained results emphasised the followings: the water content was between 13.45 % and 17.28 % in 14 of the total 17 samples of feed/concentrated food. The nutritive value was variable, being dependently of the dry matter content and of the structure of the feed. The myco-bacteriological charge was increased, being in the 17 samples upon the limits foreseen by the quality norms. The dominant mycets species belonged to *Fusarium*, *Aspergillus*, *Penicillium*, *Mucorales* (known as potentially mycotoxigenic). In these feed the fats quality was very reduced, the acid value varying between 75 – 199,9 mg KOH p.c. feed. The conclusion revealed that in the most of the cases, the feed quality was involved in the alteration of the animal health. According to these there were made recommendation regarding the cultivation, harvesting, storage and control on the processing chain of the feed (corresponding to the principles HACCP system).

Alternariol acts as a topoisomerase poison

Markus Fehr, G. Pahlke, J. Fritz, D. Marko

Institute of Applied Bioscience, Section of Food Toxicology,
University of Karlsruhe (TH), Adenauerring 20a, 76131 Karlsruhe, Germany

The mycotoxin alternariol (AOH) is found in food and feed infected by *Alternaria alternata*. AOH has been reported to possess cytotoxic, genotoxic and mutagenic properties *in vitro*. In the present study, we investigated the genotoxic effect of AOH in human colon carcinoma cells with special emphasis on the potential mechanisms of action. Interference with human topoisomerases can cause profound DNA damage. During the catalytic cycle topoisomerases introduce transient breaks in the phosphodiester backbone of the DNA, enabling the release of torsion stress and thereby regulating the topology of the DNA during all essential processes of DNA metabolism, such as transcription, replication, chromosome condensation and segregation. In mammals two major classes of topoisomerases exist, classified due to their mode of action. Topoisomerase I introduces a transient single strand break in the DNA double helix. In contrast, topoisomerase II, an ATP-dependent enzyme, performs a transient double strand break, through which a second DNA double helix is passed. During these processes a transient covalent enzyme-DNA-intermediate is formed, the so-called cleavable-complex. The majority of compounds targeting topoisomerases affect the stability of the cleavable complex. As a consequence the collision with an approaching replication fork might lead to fatal DNA strand breaks.

We investigated the DNA strand breaking potential of AOH in human colon carcinoma cells (HT29) by single cell gel electrophoresis. Within one hour of incubation, AOH significantly increased the rate of DNA strand breaks at concentrations $\geq 1 \mu\text{M}$ indicating substantial genotoxic potential. In a cell-free system, AOH was found to inhibit the catalytic activity of topoisomerase I at concentrations $\geq 50 \mu\text{M}$. The catalytic activity of topoisomerase II α and II β was significantly suppressed by AOH at $\geq 25 \mu\text{M}$ and $\geq 150 \mu\text{M}$, respectively, indicating a preference of this mycotoxin towards the II α isoform. Furthermore, the question was addressed whether AOH acts as a pure catalytic inhibitor or as a topoisomerase poison, stabilising the cleavable complex. In HT29 cells, the inhibition of topoisomerase I and II activity was associated with the stabilisation of the topoisomerase I- and II-DNA-complexes thus identifying AOH as a topoisomerase I and II-poison. In summary, AOH was identified as a topoisomerase I and II poison which might cause or at least contribute to the impairment of DNA integrity in human colon carcinoma cells.

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***Fusarium* contaminated bedding straw and pig fertility**

Andreas Gutzwiller, Jean-Louis Gafner

Agroscope Liebefeld-Posieux, Research Station ALP, 1725 Posieux, Switzerland

Swiss pig farmers are encouraged to provide straw bedding to sows for welfare reasons. However, straw is generally more heavily contaminated with *Fusarium* toxins than the corresponding grains. Since sows like to chew fresh straw, they may swallow mycotoxins which contaminate its surface even if actual straw intake is low. Many farmers and veterinarians therefore consider straw as a risk factor for fertility problems in sows.

Five tons of *Fusarium* contaminated wheat straw from one field were used for our investigation. Twelve samples drawn from 12 different bales were analysed for deoxynivalenol (DON) and zearalenone (ZON) using RIDASCREEN[®] FAST ELISA kits. The mycotoxin concentration in the 12 straw samples, expressed as the median, lowest and highest values, was 1.4 (0.4–5.6) mg DON/kg and 0.9 (<0.15–8.1) mg ZON/kg, respectively. Aliquots of 6 samples were also analysed after soaking and kneading for 3 minutes in an electrolyte solution resembling saliva. Soaking and kneading the straw in the electrolyte solution reduced the median DON level of these 6 samples from 1.2 to 0.3 mg/kg ($p < 0.05$) and tended to reduce the median ZON level from 0.58 to 0.51 mg/kg ($p = 0.10$). The *in vitro* study suggests that pigs may indeed ingest DON by merely chewing straw.

In a preliminary animal trial, 16 sows were kept either on contaminated ($n = 8$) or uncontaminated straw ($n = 8$) for two reproductive cycles and until pregnancy detection during the third cycle. Since no treatment effects on health and fertility were observed in this study, in a second study a larger number of sows were kept on contaminated (**C**; $n = 40$) and uncontaminated straw (**UC**; $n = 40$) during one gestation period and the following 35 d lactation period. In each treatment four sows were not pregnant after mating in two consecutive oestrus periods and were excluded from the trial. No signs of mycotoxicosis, such as abortion, splayleg, vulva swelling or increased piglet mortality, were observed. The fertility of the sows and the growth performance of the nursing piglets did not differ between the two groups (Table 1).

Table 1. Fertility of the 80 sows of the second study and performance of their nursing piglets

	Treatment		<i>p</i> -value
	C	UC	
Pregnant after 1 / 2 matings, n	36/36	35/36	1.00
Piglets weaned per litter, n	8.2 ± 4.4	8.3 ± 3.2	0.88
Weight gain of nursing piglets, g/d	236 ± 48	243 ± 43	0.18

Thus, the use of contaminated straw as bedding material for the pregnant and lactating sows did not affect their fertility. These findings are in contrast to reports from veterinary practitioners and pig breeders of fertility problems attributed to straw which had *Fusarium* toxin contaminations comparable to those of the load used in the present investigations.

The results of our experiments suggest that bedding straw of fair quality that is contaminated with DON and ZON should not markedly affect the fertility of healthy well-fed sows.

Novel cytotoxic and mutagenic mycotoxins from *Aspergillus nidulans*

Claudia Handrich¹, Jürgen Büniger², Götz Westphal¹, Ernst Hallier¹, Michael Müller¹

¹Department of Occupational and Social Medicine, Georg-August-University of Göttingen, Waldweg 37, 37073 Göttingen, Germany

²BGFA, Ruhr-University of Bochum, Bürkle-de-la-Camp-Platz, 44789 Bochum, Germany

High concentrations of filamentous fungi of the genera *Aspergillus* and *Penicillium* are found in composting plants and other waste treatment facilities. Many of these moulds are able to produce one or more mycotoxins as secondary metabolites. Thus, in workplaces with exposure to decaying organic compounds the respiratory uptake of mycotoxins present in airborne dust and bioaerosols plays a crucial role. Acute and chronic pulmonary diseases were reported after inhalation of these agents. One of the most frequent toxigenic moulds in composting plants is *Aspergillus nidulans*. The mutagenic and carcinogenic mycotoxin sterigmatocystin was detected in the extract of this fungus. In addition, the extract of *A. nidulans* caused serious toxic effects in A-549 pneumocytes, that could not be explained completely by its content of sterigmatocystin [1]. Using a structure activity-approach the presence of further toxic principles in *A. nidulans* was shown.

An HPLC-diode array detector method was used to separate and characterize the components of the *A. nidulans*-extract within 50 minutes/analysis. Aliquots of the extract were chromatographed and nine 5-minute-fractions (0–45 min) were collected and lyophilized. Rechromatography of aliquots of the residues confirmed the accuracy of the 5-minute-cuts. Sterigmatocystin was identified in fraction 8 (35–40 min) with a content of up to 28 %. The fractions were tested for cytotoxicity in three established cell lines (A-549, L-929 and Hep-G2) using the neutral red assay (NRU assay). The HPLC-fractions were dissolved in ethanol/dichloromethane (1:1, v/v), a suitable solvent mixture with a low cytotoxicity. Fraction 9 (40–45 min) showed the strongest cytotoxicity in A-549-pneumocytes.

In addition, the mutagenicity of the 5-minute-fractions was determined using the Salmonella typhimurium/mammalian microsome assay (Ames test) with the strains TA 98 and TA 100. The tests were performed with and without metabolic activation by a microsomal mixed function oxidase system (S9 fraction). Fractions 1–7 did not significantly increase the number of revertants, while fraction 8 and 9 in low concentrations caused a significant dose-dependent increase of revertants in both tester strains after S9 activation. It was shown that the indirect mutagenic effect of fraction 8 is predominantly due to sterigmatocystin. In addition, a direct mutagenicity was observed testing the whole *A. nidulans*-extract in high concentrations.

The test results indicate novel cytotoxic and mutagenic mycotoxins in *Aspergillus nidulans*. Especially fraction 9, which does not contain sterigmatocystin, will be further investigated to identify the structures of these unknown toxic principles.

Reference

- [1] Büniger *et al.* (2004): *Toxicology*, **202**, 199

Ochratoxin A induces differential gene expression in HepG2 liver cells

Christoph Hundhausen¹, Christine Bösch-Saadatmandi¹, Ralf Blank², Siegfried Wolffram², Gerald Rimbach¹

¹Institute of Human Nutrition and Food Science, Christian Albrechts University Kiel, Hermann-Rodewald-Str. 6, D-24098 Kiel, Germany

²Institute of Animal Nutrition and Physiology, Christian Albrechts University Kiel, Hermann-Rodewald-Str. 9, D-24098 Kiel, Germany

The mycotoxin ochratoxin A (OTA) is a frequent contaminant of food and feedstuff and exerts nephro- and hepatotoxicity in man and animals. The International Agency for Research on Cancer (IARC) classified OTA as a putative human carcinogen. Even though various cytotoxic effects of OTA have been described in numerous studies, the exact molecular mechanisms of OTA-induced toxicity have not been fully elucidated. Therefore, the aim of this study was to investigate the effect of OTA exposure in cultured liver cells on large-scale gene expression using Affymetrix GeneChip[®] technology.

HepG2 cells were cultivated under standard conditions and incubated with medium containing 0, 0.25, 1.00 or 2.50 $\mu\text{mol/l}$ OTA for 24 h. These concentrations were non-toxic for the hepatocytes as determined by the neutral red assay. RNA was isolated and further processed for the microarray analysis. Data analysis was performed with a standard software tool (dChip 2006).

OTA treatment dose-dependently altered gene expression in HepG2 cells: out of 14 500 annotated genes 13 (0.9 %) and 606 (4.2 %) were regulated at least twofold by 0.25 or 2.50 $\mu\text{mol/l}$ OTA, respectively. Two third of these regulated genes were downregulated. Generally, many genes encoding proteins which are centrally involved in stress response, antioxidant defence, protein biosynthesis, detoxification, inflammation, energy metabolism, cell cycle, erythropoiesis, and apoptosis were differentially regulated by OTA. Genes encoding mitochondrial enzymes, such as oxidoreductases, hydrolases and transferases, were exclusively downregulated as well as all genes encoding proteins affecting the cytoskeleton. Furthermore, OTA treatment resulted in a significant decrease in mRNA levels of genes encoding key enzymes of glucose and fatty acid metabolism, for example phosphoglycerate kinase 1 and stearoyl-coenzyme A desaturase 1. In contrast, the proapoptotic bh3 interacting domain death agonist (BID) gene was significantly upregulated.

To summarize, our data suggest that microarrays can be considered as an efficient tool for revealing potentially relevant mechanisms regarding the toxicity of OTA in cultured cells.

Development of a proteomic approach to monitor protein expression in mycotoxin producing moulds

Judith Milles¹, Johannes Krämer¹, Alexander Prange^{2,3}

¹University of Bonn, Institute for Nutrition and Food Sciences, Food Microbiology and Hygiene, Meckenheimer Allee 168, D-53115 Bonn, Germany

²Niederrhein University of Applied Sciences, Microbiology and Food Hygiene, Rheydter Strasse 277, D-41065 Mönchengladbach, Germany

³Louisiana State University, Center for Advanced Microstructures and Devices (CAMD), 6980 Jefferson Hwy, Baton Rouge, LA 70806, USA

The reasons for mycotoxin production of moulds occurring on crops are not elucidated finally. Physiological factors and interactions with other competing moulds are discussed. Studying protein expression under different physiological parameters can give information about triggering factors for mycotoxin production and at the same time can open new insights in directly (e.g. enzymes for synthesis) or indirectly (e.g. transporters) in the mycotoxin metabolism involved proteins. A useful method to investigate complex mixtures of proteins or whole proteomes is the two dimensional (2D-) gel electrophoresis which consists of two steps [1]. In the first dimension the proteins are separated in a pH gradient according their specific isoelectric point. The following second dimension separates the proteins according their mass by a SDS-Polyacrylamid gel electrophoresis. The result – a specific pattern of protein spots – is often used when a comparison between two different types of metabolism is needed.

Only few studies can be found in literature concerning fungi, mostly *Saccharomyces cerevisiae*, and none for *Fusarium* spp.. Therefore, it was necessary for our model organism *Fusarium graminearum* to establish a protocol for lysing the cells and extracting and cleaning the proteome. Lysis of hyphens harvested from liquid culture was successful with ultrasonification, french press or by grinding in a mortar with liquid nitrogen added. The last method was coupled with low protein degradation and stable protein patterns. Proteins were extracted with TCA/acetone and then cleaned with the 2D-Cleanup-Kit (Fa. Amersham). These isolated proteins were separated in a 2D-gel electrophoresis system (Fa. BioRad) using different pH-gradients.

Furthermore the preparation technique was tested with other filamentous fungi like *Aspergillus ochraceus* or *Penicillium verrucosum* and was suitable, too, so it may be generally applicable for a variety of moulds.

Reference

- [1] Görg A., Weiss, W., Dunn, M.J. (2004): *Proteomics*, **4**, 3665–3685.

Disruption of sister chromatid cohesion and mitosis by Ochratoxin A

Katja Müller¹, Gaetano Pepe², Michaela Bekteshi¹, Eva Rached¹, Wolfgang Dekant¹,
Pasquale Mosesso², Angela Mally¹

¹Department of Toxicology, University of Würzburg, Versbacher Strasse 9, 97078 Würzburg, Germany

²Dipartimento di Agrobiologia e Agrochimica, Università degli Studi della Tuscia, Viterbo, Italy

Ochratoxin A (OTA) is an ubiquitous mycotoxin and natural food contaminant, which occurs in a variety of food items, resulting in chronic human exposure. OTA is nephrotoxic and a potent renal carcinogen, with male rats being most susceptible to renal tumor formation by OTA. It is now widely accepted that OTA is not a mutagenic, DNA-reactive carcinogen, but the primary events leading to tumor formation by OTA are still poorly understood.

Using immortalized human kidney epithelial cells (IHKE) we have previously shown that OTA blocks metaphase/anaphase transition and leads to the formation of aberrant mitotic figures and giant cells with abnormally enlarged and/or multiple nuclei, sometimes still connected by chromatin bridges. Here, we show that the same effects occur in V79 fibroblasts, which do not represent target cells of OTA *in vivo*, suggesting that OTA mediated toxicity is not a cell-type specific effect. In addition, condensed and abnormally separated metaphase chromosomes were frequently observed in both IHKE and V79 cells treated with OTA for up to 48 h. Sister chromatids of metaphase chromosomes were clearly separated but remained in close proximity. These data indicate that premature separation of sister chromatids in the presence of OTA may be a key event in OTA toxicity and may provide the initial trigger for aberrant cell division and cell death at mitosis. It is now known that cohesion between sister chromatids is controlled by several mitotic kinases, e.g. or Polo-like-kinases (Plk), and aberrant activity of these key regulators of mitosis has been associated with malignant transformation. We, therefore, investigated the effects of OTA on the expression of Cdk1/cyclin B1, aurora B kinase and Plk1 in IHKE cells treated with OTA (0, 10, 25 and 50 μM) for up to 48 h.

Expression of cyclin B1 was decreased in a concentration-dependent manner as early as 4 h after exposure OTA. Cdk1 was also decreased following treatment with 25 μM and 50 μM OTA, although significant effects were only evident after 24 and 48 h. Analysis of aurora B expression demonstrated a significant decrease after 24 h and 48 h, but not at earlier time-points, suggesting that altered expression of aurora B is unlikely an initial event in OTA mediated disruption of mitosis. Immunodetection of Plk1 in IHKE cells treated with OTA revealed increased expression of Plk1 at OTA concentrations of 10 μM and 25 μM . However, higher concentrations lead to a decrease in Plk1 expression, which may be related to the decreased number of mitotic cells in OTA treated cell cultures.

Taken together, these experiments demonstrate that OTA causes premature sister chromatid separation and subsequent mitotic defects, presumably through modulation of key molecules involved in chromosome condensation and cohesion. Recent evidence has shown that cyclin B1 mediated inhibition of separase plays a critical role in the regulation of chromosome disjunction, suggesting that untimely destruction of cyclin B1 may be an important event in OTA toxicity.

Alternaria* toxins: DNA strand-breaking activity in mammalian cells *in vitro

Erika Pfeiffer, Sandra Eschbach, Manfred Metzler

Institute of Applied Biosciences, Chair of Food Chemistry, University of Karlsruhe,
Adenauerring 20a, D-76131 Karlsruhe, Germany

Fungi of the genus *Alternaria* infest numerous food items but also grow on other materials, e.g. soil, wall papers and textiles. *Alternaria alternata* is the most abundant of more than forty *Alternaria* species and produces several toxins including alternariol (AOH) and alternariol methyl ether (AME). These *Alternaria* toxins are frequently detected in moldy wheat and other grains, in pecans, in various fruits, e.g. tomatoes, olives, melons, apples and raspberries, and in processed fruit products such as apple and tomato juice. Consumption of food contaminated with *Alternaria* toxins has been associated with an increased incidence of esophageal cancer, and there are several reports on the mutagenicity and genotoxicity of AOH and AME. In most assays, AOH appears to exhibit a more pronounced genotoxicity than AME.

In the present study, the DNA strand-breaking activity of the two *Alternaria* mycotoxins has been compared in three cell lines with different metabolic capabilities, i.e. Chinese hamster V79 and human HepG2 and HT-29 from liver and intestine, respectively. DNA single strand breaks induced by the test compounds were determined by using the technique of alkaline unwinding.

AOH und AME were found to induce strand breaks in a concentration-dependent manner in all three cell lines. The incidence was about equal for AOH and AME in V79 cells which do not metabolize the mycotoxins, and exceeded the incidence observed in HepG2 and HT-29 cells. The human cells were not capable of oxidative metabolism but exhibited UDP-glucuro-nosyltransferase (UGT) activity. AOH was more extensively glucuronidated than AME in HepG2 cells, resulting in a higher incidence of strand breaks upon treatment of these cells with AME. HT-29 cells were much more efficient than HepG2 cells in glucuronidating AOH and AME. For example, 24 h after incubating HepG2 cells to AOH or AME, about 50 % of the dose was still present as unconjugated compound, but the rate of strand breaks was similar to a 1 h incubation. In contrast, AOH and AME were completely glucuronidated in HT-29 cells after 24 h, and strand breaks were no longer detectable at this time point. Our results suggest that glucuronidation of AOH and AME suppresses the genotoxic activity of these mycotoxins. In support of this assumption is the observation that curcumin, a known inhibitor of UGT activity, increased the incidence of AOH- and AME-induced strand breaks in HT-29 cells.

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Application of the *Dugesia tigrina* Girard bioassay in mycotoxicological investigation. Sterigmatocistin (ST)

Marlena Piontek

University of Zielona Góra, Institute of Environmental Engineering,
Podgórna 50, 65–246 Zielona Góra, Poland

The estimation of people and animal risk of the mycotoxin absorption requires the toxicological researches: analytical (physicochemical analysis) and bioanalytical (biotoxicological analysis). The determination of the degree moulds metabolites toxicity is carrying out with the use of animals as trout, ducks, chicks, mice, rats, rabbits, guinea - pigs, monkeys, dogs, sheep and other. There are also known simpler and cheaper biological laboratory tests. During the realization of toxicity tests of moulds metabolites, most frequently the larvae of brine shrimps *Artemia salina* were applied. The presence of mycotoxins could be detected by the cell cultures (cytotoxicity), MTT bioassay. Good results are obtained in tests on the cellular lines of the swine kidneys (SK) and on the mice fibroblasts. In the toxicological laboratory of Department of Applied Ecology UZ are led the toxicology researches which use the tests with *Dugesia tigrina* Girard. All extracts from the biomasses of isolates (*Aspergillus versicolor* Tiraboschi) synthesize sterigmatocistin (ST) were toxic for the planarian (*Plathelminthes*, *Turbellaria*, *Tricladida*; *Paludicola*). In this method have been researched 27 strains of *A. versicolor* coming from the natural infested construction materials of different housing building. *Dugesia tigrina* is a sensitivity bioindicator of sterigmatocistin (ST). In the air-dry mould biomasses with the laboratory medium were detected the existense of sterigmatocistin in the quantities measured from 0.0 to over 600.0 mg·kg⁻¹ ST. Toxicity values of the extracts prepared from the same biomasses were containing in a wide range of LC 50 counting from non toxic, through the weakly toxic (100–1000 mg·dm⁻³), next medium toxic (10–100 mg·dm⁻³) and very potent (1–10 mg·dm⁻³). The comparison between the toxicity of the *Aspergillus versicolor* strains, based on the physicochemical analysis (HPLC) and the results LC 50 of the biotests with *Dugesia tigrina* proved the convergence which permitted to ascertain that chemical analysis unfriendly for environment could be replaced by biological analysis. It is difficult to require the realization of physicochemical analysis (HPLC) in all samples of moulds coming from buildings. Those analysis should be done in the case of high level toxicity of the sample.

Probabilistic assessment of exposure doses to mycotoxins: Example case of DON in foodstuffs

Jiri Ruprich, Vladimir Ostry, Jarmila Skarkova

National Institute of Public Health in Prague, Centre for the Hygiene of Food Chains,
Brno, CZ-612 42, The Czech Republic

The use of probabilistic modelling techniques within the field of exposure assessment is a subject of increasing interest. This entry is aimed at illustrating the rationale for their practical use - example case on probabilistic dietary exposure assessment of the deoxynivalenol (DON).

DON is a trichothecene mycotoxin produced by microfungi of the *Fusarium* genus, particularly *F. graminearum*. DON is highly toxic, producing a wide range of immunological disturbances. The major dietary sources of DON are cereals (e.g. wheat, barley and maize) and cereals products. Human exposure to DON occurs predominantly via ingestion of cereal products.

The validated and accredited immunochemical method ELISA (ELISA Ridascreen[®] Deoxynivalenol) was used for quantification of DON in foodstuffs. The limit of detection (LoD) of the method was 18.5 µg/kg. The results of determination of DON in selected samples with level of DON higher than 200 µg/kg were confirmed by an independent analytical method of instrumental high-performance thin-layer chromatography (HPTLC). Certified reference material (CRM 379) was used.

Total 254 samples of 17 different cereal products (e.g. bread, rolls, flour, pasta, rice, muesli) were sampled in the Czech retail. 197 samples were positive in analyses for DON. Results below LoD were considered as a value equal to 1/2 LoD during calculations. The individual food consumption data (twice repeated 24 h recall) for 2 560 persons with age 4–90 years were taken from the National study on individual food consumption (SISP 04) for the probabilistic assessment of chronic dietary exposure doses of the DON. The MCRA software (Rikilt, NL) has been used for calculation of particular percentiles of exposure doses for population groups. Discrete/semiparametric (Nusser) model, power transformation followed by spline transformation has been used for data transformation. Bootstrapping has been used to evaluate an uncertainty. Exposure doses estimated for 99 percentile of the Czech population was about 0.9 µg/kg b.w./day (confidence limit 97.5 %). The most important source of exposure were bread (47 %) and rolls (25 %). Probability of everyday DON intake from cereal product was about 90 %. The highest exposure doses, up to 1.3 µg/kg b.w./day (confidence limit 97.5 %) were observed for 99 percentile of boys 4–10 years old and DON intake was estimated for 97 % of them every day.

When PTDI was established by the Scientific Committee for Food of European Commission (EU SCF, 2000) to 1 µg/kg b.w./day, then mainly young population groups can be exposed to doses which are considered as not tolerable for longer time. About 5 % of boys aged 4–10 years can be exposed by higher doses of DON. The result supports an idea that even processed cereal foods on the market can represent certain health risk of DON intake for consumers and therefore should be under regular food control.

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Metabolic profile of estrogenic mycotoxin, zearalenone in horses

P. Songsermsakul¹, J. Böhm², J. Zentek², Ch. Aurich³, E. Razzazi-Fazeli⁴

¹Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand

²Department of Veterinary Public Health, Institute of Nutrition, University of Veterinary Medicine, Veterinärplatz 1, A-1210 Vienna, Austria

³Clinic for Obstetrics, Gynecology and Andrology, University of Veterinary Medicine, Veterinärplatz 1, A-1210 Vienna, Austria

⁴VetOMICS / Proteomics and Metabolomics Centre, University of Veterinary Medicine, Veterinärplatz 1, A-1210 Vienna, Austria

Six fertile Haflinger mares with a mean body weight of 450 kg were included into the feeding study. The feed per day consisted of 7 kg hay and 3 kg zearalenone (ZON)-contaminated (1 mg/kg) oat. ZON and its metabolites α -zearalenol (α -ZOL), β -zearalenol (β -ZOL), α -zearalanol (α -ZAL), β -zearalanol (β -ZAL) and zearalanone (ZAN) were determined in plasma, urine, and faeces samples by high performance liquid chromatography and atmospheric pressure chemical ionisation mass spectrometry. In plasma, ZON and β -ZOL were detected in the mean ratio of 3:1 on day 1 of the feeding study. The results of the urine samples showed that ZON was transformed mainly into β -ZOL and α -ZOL in a mean ratio of 1.4:1. α -ZAL, β -ZAL and ZAN were identified in slight amount. In contrast, α -ZOL was predominantly found in faeces samples with a mean ratio of α -/ β -ZOL of 2:1. α -ZAL, β -ZAL and ZAN were found in a mean concentration of 1 μ g/kg. The main excretion route of zearalenone has been found to be urine (50 %) and faeces (30 %). The degree of glucuronidation was established in all 3 sample types. In urine and plasma samples, the degrees of glucuronidation were approximately 100 % for all analytes, whereas the low percent of glucuronidation was found in faeces samples (4–15 %). The present study shows the main conversion of ZON into β -ZOL in horses. Since β -ZOL possesses less estrogenic activity, this finding could explain why horses are less susceptible to ZON compared to swine.

The Effect of Thermal Treatment on DON Mycotoxin

Mihaela Nicoleta Avram¹, Valeria Gagi¹, Petru Niculita², Nastasia Belc¹

¹Institute of Food Bioresources, Dinu Vintila, 6, Bucharest, Romania

²University of Agronomic Sciences and Veterinary Medicine, Faculty of Biotechnology, Marasti, 59, Bucharest, Romania

Mycotoxin DON belongs to trichothecenes group produced by fungi from *Fusarium* genera. The main important characteristic of mycotoxins is thermal stability. Trichothecenes group presents thermal stability at 120 °C, they are moderately stable at 180 °C and decompose within 30–40 min at 210 °C. For testing we used three samples of wheat whole flour naturally contaminated with high concentrations of DON. From each sample we obtained nine mixtures (wheat whole flour and water). We have tested the following parameters: temperatures 160 °C, 180 °C and 200 °C and baking time: 25, 30, 35 minutes. For determination of DON mycotoxin we used ELISA test kit specific for DON (Ridascreen[®] DON, R-Biopharm) which have a detection limit of 18.5 ppb. The measurement of absorption was made with Tecan Sunrise reader at 450 nm and Rida Win soft allowed to calculate the results and get up the calibration plot. The results obtained in this experiment emphasized a reduction of DON mycotoxin level at temperatures presented by comparison with reference sample. The best results were obtained at time baking 30 minutes and temperature 200 °C. The most important reduction was of 26 % at temperature/time baking 200 °C, respectively 30 minutes.

Evaluation of the most suitable AfB₁-adsorbent by multicriteria analysis

Sebastian Fruhauf¹, Elisabeth Apfelthaler¹, Gerd Schatzmayr², Elisavet Vekiru¹, Rudolf Krska¹

¹Christian Doppler Laboratory for Mycotoxin Research, Center for Analytical Chemistry, Department for Agrobiotechnology (IFA-Tulln), University of Natural Resources and Applied Life Sciences Vienna, Konrad Lorenz Str. 20, 3430 Tulln, Austria

²BIOMIN Research Center, Industriestrasse 21, 3430 Tulln, Austria

Aflatoxin B₁ (AfB₁) is one of the most potent carcinogens which is produced by two ubiquitous occurring fungi (*Aspergillus flavus* and *Aspergillus parasiticus*). Because of the widespread occurrence of AfB₁ in feed and food commodities, it is impossible to avoid exposure to animal and humans.

The most applied method for preventing aflatoxicosis in animals is the application of binding agents mixed into contaminated feed. These enterosorbents should be indigestible and they should have the capability to bind AfB₁ efficiently in the gastro-intestinal tract before the toxin gets resorbed. The specificity of this binding is important. On the one hand the enterosorbent has to bind the AfB₁ in the complex gastro-intestinal juice very efficiently, on the other hand dietary nutrients should not be bound to avoid reduction of the nutritional value of the feed. Different kinds of materials (clay minerals like bentonites, kaolinites, organoclays and yeast cell products etc.) were reported to be more or less effective in adsorbing AfB₁ [1].

The aim of this research project was to examine different minerals *in vitro* not only for their binding capacity to adsorb AfB₁, but also for their strength of binding and their binding selectivity e.g. by performing adsorption tests in real gastric juice or in presence of vitamins, mineral nutrients and trace elements.

The performance of a well known commercially available HSCAS product was evaluated in all tests in parallel to the samples to ensure the repeatability of the experiments and to identify products with improved performance compared to the reference. In order to optimise the evaluation procedure all experimental results were subjected to a multicriteria analysis. This allowed an objective evaluation of the examined adsorbents and resulted in a ranking of their suitability as AfB₁ enterosorbents.

Reference

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Adsorption of ochratoxin A by *Saccharomyces cerevisiae* yeasts cells

Małgorzata Piotrowska, Zofia Żakowska

Technical University of Lodz, Institute of Fermentation, Technology and Microbiology
Wolczanska 171/173, 90-924 Lodz, Poland

Contamination of food and feeds with ochratoxin A is a serious economic problem and primarily a phenomenon threatening the consumers' health. In order to prevent it, special precautions should be applied, such as following the guidelines of good practice in agriculture, storing raw products in proper humidity and temperature conditions, and applying proper measures while selecting raw products for production purposes and during the production of food and fodders itself. Such actions are not always effective. Therefore, some supplements are added to fodders, e.g. probiotics, that bind toxins in a digestive track of farm animals, which reduces their absorption into the blood circulation system.

Saccharomyces cerevisiae yeasts are organisms commonly used in numerous biotechnological processes such as bakery, wine-making and brewing. They are also used as additives for fodders. Our previous research demonstrated that these organisms are capable of eliminating ochratoxin A from plant raw material during fermentation and chromatographic analysis did not show any products of OTA metabolism, which proves that it was not the case of biodegradation. The research presented hereby attempts to discover the mechanism of this process and verify the literature data claiming that the reason for this phenomenon is adsorption of the toxin to the cell wall.

The biological material was bakery yeasts from active, 24-hour cultivation and dead yeasts thermically deactivated in autoclave. It was checked if the presence of the cell wall was essential in order for the toxin elimination to take place. Thus, protoplastisation of yeast cells was conducted, accompanied by the toxin elimination process with the use of protoplasts. Three yeast biomass samples of various densities were used (1, 5 and 50 mg/ml). The tests were carried out in a PBS buffer contaminated with 1 g/ml of ochratoxin A. The experiments were conducted both in static conditions and during shaking. The firmness of the bond between the toxin and the biomass was evaluated during water rinsing. The changes in the ochratoxin A concentration in the buffer as well as the OTA concentration in the yeast biomass were evaluated with the use of ELISA method and AgraQuant tests by ROMER. The results were given as the percentage of the toxin eliminated in relation to its initial amount.

The amount of ochratoxin A removed by bakery yeasts after 24-hour contact equalled from 20 to 29 % for the biomass of lower density, and 75 % for 50 mg s.m./ml. The process of adsorption proved to be very fast; immediately after mixing the cells with the toxin its amount significantly decreased, and lengthening the contact up to 24 hours did not bring further notable changes. The presence of physiologically active cells is not necessary in order to remove the toxin; the dead biomass also removed OTA from the buffer and the amount of the toxin removed was much bigger than in the case of the active biomass. In case of the density 5 mg/ml, 54 % of the toxin was adsorbed, i.e. twice more than in case of the active biomass. As for the density 50 mg/ml the differences between dead and active cells were not so significant. The application of dead microorganisms to eliminate ochratoxin A is highly advantageous since such biomass does not change organoleptic features of end products.

Adsorption of ochratoxin A is closely related to the components of the cell wall of the microorganisms examined. It was proved that the level of toxin in buffer was the same during the experiment carried out with the use of yeast protoplasts.

Considering the influence of the cultivation conditions on the amount of the eliminated ochratoxin A added to the substratum, it was stated that incubation of the samples examined in static conditions is more effective than incubation with shaking. It was demonstrated that a part of the toxin attached to the biomass is rinsed out, which indicates that the bond between the toxin and the cell wall is not very firm and is of surface character. The bond with the dead biomass is less firm than it is in the case of live biomass.

Biodegradation of Aflatoxin B₁ by *Neurospora* sp. Isolated from Oncom

F.M.C.S. Setyabudi², Sardjono¹, J. Böhm², E. Razzazi-Fazeli²

¹Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Gadjah Mada University, Bulaksumur, 55281 Yogyakarta, Indonesia

²Department of Veterinary Public Health and Food Science, Institute of Nutrition, University of Veterinary Medicine Vienna, Veterinärplatz 1, 1210 Vienna, Austria

Aflatoxin B₁ (AFB₁) is the most common mycotoxin found in foods in tropical countries like Indonesia. Detoxification would be the important strategy step in food processing to reduce or eliminate AFB₁ since aflatoxins are relatively resistant to common processing conditions. Microorganisms such as molds (*Rhizopus oligosporus*, *Rhizopus oryzae*, *Aspergillus oryzae*) and bacteria (*Flavobacterium aurantiacum*, *Lactobacillus*, *Propionibacteri*) have been reported to degrade aflatoxins. Oncom is an Indonesian fermented food produced from fermentation of peanut press cake by the mold *Neurospora* sp. It is usual that peanut has high contamination of AFB₁, therefore research on the ability of *Neurospora* sp. to degrade AFB₁ is very important in order to minimize its toxic effect.

Direct plating on Dichloran Rose Bengal Chloramphenicol Agar (DRBC) was used for mold isolation. After identification, the isolate was transferred on Potato Dextrose Agar (PDA) slant for working culture. Evaluation of their ability to degrade AFB₁ was performed by mold inoculation into Czapek Yeast Extract (CY) broth that was already contaminated by AFB₁. The mycelium was disrupted by using sonicator afterwards the concentration of AFB₁ in medium and the binding AFB₁ in mycelium were analyzed by HPLC-fluorescence detector. Bioassay of degradation product was performed by *Bacillus megaterium* shape in order to determine their toxicity.

This trial indicates that decreasing AFB₁ in the medium was fast during the four days of their growth, correlated with the rate of increasing the biomass during the logarithmic growth phase. The lower growth of the biomass in medium spiked with AFB₁ may due to the fact that AFB₁ affecting their ability to grow. However, the analyses of dried biomass in medium spiked with AFB₁ were relatively lower in the range of 3.7 % – 29.3 % compared to control. Small amount of AFB₁ was bound to the cells after 8 days incubation. *Neurospora* sp. isolated from oncom was able to degrade and detoxify AFB₁. It was indicated by the results of bioassay that *Bacillus megaterium* was not intoxicated by degradation products. The mechanisms of bioreduction may be due to the binding of AFB₁ in the mycelium or/and might be due to the extracellular enzymes and should be investigated in future experiments.

Inactivation of ochratoxin A by probiotic preparation for broiler chicken

Katarzyna Śliżewska, Małgorzata Piotrowska

Institute of Fermentation Technology & Microbiology, Technical University of Lodz,
Wólczańska 171/173, 90-924 Lodz, Poland

The aim of this study was to determine the influence of spontaneous fermentation and with the use of probiotic preparation on reduction of ochratoxin A concentration during fermentation and the microflora pattern during fermentation. The probiotic preparation is a natural product containing bacteria resistant to gastric juice and bile: *Lactobacillus casei/paracasei* ŁOCK 0920, *Lactobacillus brevis* ŁOCK 0944, *Lactobacillus plantarum* ŁOCK 0945, as well as live yeasts cultures *Saccharomyces cerevisiae* ŁOCK 0140 of a high fermenting ability. The above mentioned strains come from the Pure Cultures Collection of Industrial Microorganisms (ŁOCK 105), Technical University of Lodz.

We conducted spontaneous fermentation and one with the use of probiotic bacteria and yeast in previously optimized conditions (37 °C, 24 h). In order to show that detoxication is a result of activity of microorganisms, we used, as a control sample, typical mixed feed for broiler chicken previously subject to radiation sterilization to which we added the doses of ochratoxin A. The control sample was incubated at the same conditions that accompanied fermentation.

The fermentation medium was typical mixed feed for broiler chicken with water in 1:1.5 proportion. To the medium we added ochratoxin A in the amount of 1 and 5 mg/kg (ppm). Mycotoxin reduction was estimated after 6 hours (time of intestine passage in chickens), after 12 and 24 hours of fermentation with the use of immunoenzymatic method (ELISA). The sample was analyzed for ochratoxin A using OchraQuant[®] tests (Tigret Sp. z o.o., Poland) after extraction with 70/30 methanol – water solution.

The development of microflora (total number of bacteria, *coli* group, *Pseudomonas sp.*, aerobic spore forming, lactic acid bacteria, yeast and moulds) was controlled at the same time. The analysis was carried out according to the standard of PN – ISO.

After 6-hour fermentation with addition of probiotic cultures the amount of ochratoxine A decreased of 55 %, in case low concentration of ochratoxin A (1 mg/kg). In case of high concentration ochratoxin A (5 mg/kg) the loss of ochratoxin A was more and equaled about 72 %. The same relation was observed after 12 and 24-hour.

Comparing the microflora pattern during feed fermentation we stated that addition of probiotic cultures not only influences the effective detoxication of ochratoxin A examined, but it also allows for proper development of desired microflora during fermentation, such as lactic acid bacteria and yeast. At the same time, it hampers development of *coli* bacteria, aerobic spore forming, *Pseudomonas* bacteria and moulds.

The probiotic bacteria and yeast applied creates starter culture for flour fermentation that has a stable feature of detoxication of ochratoxin A. Using probiotic starter allows the proper fermentation microflora pattern and reduces the risk of development of undesirable and harmful bacteria and moulds.

Adsorption of ochratoxin A by *Saccharomyces cerevisiae*

Isil Var, Zerrin Erginkaya, Bulent Kabak

Cukurova University, Agricultural Faculty Department of Food Engineering, 01330 Adana, Turkey

Ochratoxin A (OTA) is a well-known nephrotoxic agent and has been associated with human kidney disease, referred to as Balkan Endemic Nephropathy. In animal experiments, this toxin was identified as genotoxic, teratogenic, hepatotoxic, immunotoxic, and carcinogenic, but these have not been identified in humans. Several studies have shown that some chemical agents such as activated carbon, aluminosilicates can bind efficiently and remove mycotoxins from aqueous solution. However, the decontamination of food products such as wine with chemical agents is not permitted in either the EU or the USA. Therefore, the main aim of this study was to evaluate the ability of *Saccharomyces cerevisiae* to bind OTA in natural and artificially contaminated white wine.

Material and methods: Two *S. cerevisiae* strains at two different concentrations were tested for their ability to remove OTA from contaminated phosphate-buffer-saline (PBS) and white wine. The samples were analysed for OTA on a high performance liquid chromatography system (Agilent 1100) consisting of an isocratic pump, a manual injector and a fluorescence detector. Data were recorded on a personal computer equipped with Chemstation software. The mobile phase was water/acetonitril/acetic acid (51/47/2) eluted at a flow rate of 1 ml·min⁻¹. The fluorescence detector was set at excitation and emission wavelengths of 333 and 470 nm, respectively. The retention time was about 13 min for OTA.

Results: Using two different concentrations (10⁶ and 10⁸ CFU·ml⁻¹) of *S. cerevisiae*, adsorption abilities ranging from 0.83 % to 19.08 % in spiked white wine (5, 10 and 20 ng·ml⁻¹), depending on contamination level and incubation periods, were determined. On the other hand, a slight decrease was observed in the percentage of OTA removal by *S. cerevisiae* in PBS when compared to its activity in contaminated white wine. Effects of *S. cerevisiae* on the removal of OTA from naturally contaminated white wine are summarised in table 1. White wine was contaminated with 0.618 ng·ml⁻¹ OTA.

Table 1. Adsorption of OTA from naturally contaminated white wine (0.618 ng·ml⁻¹) by *S. cerevisiae*.

Yeast	Concentration (CFU·ml ⁻¹)	OTA detected (ng·ml ⁻¹ , means ± sD, n = 3)	Adsorption % (means ± sD, n = 3)
<i>S. cerevisiae</i> 1	10 ⁶	0.561 ± 0.019	9.22 ± 3.07
	10 ⁸	0.435 ± 0.009	30.20 ± 1.95
<i>S. cerevisiae</i> 2	10 ⁶	0.562 ± 0.017	9.00 ± 2.79
	10 ⁸	0.441 ± 0.015	28.64 ± 1.87

The addition of *S. cerevisiae* at 10⁸ CFU·ml⁻¹ resulted in a reduction to a maximum of 30.20 %, with respect to the control.

Conclusions: This work indicated that the adsorption abilities of *S. cerevisiae* varied, however and depended on both OTA and cell concentrations. Further research is required to determine adsorption abilities of more yeasts isolated from wine-grapes.

Removal of aflatoxin B₁ by *Lactobacillus* and *Bifidobacterium* strains

Isil Var, Bulent Kabak, Zerrin Erginkaya

Cukurova University, Agricultural Faculty Department of Food Engineering, 01330 Adana, Turkey

Among aflatoxins, aflatoxin B₁ (AFB₁) is the most naturally occurring compound and was classified by the International Agency for Research on Cancer of WHO as group 1A (carcinogenic) agent in 1993. Because of its detrimental effects a number of strategies have been developed to prevent the growth of mycotoxigenic fungi, as well as to decontaminate and/or detoxify mycotoxin contaminated foods and animal feeds. Recently, there has been an increasing interest in the hypothesis stating that absorption of mycotoxin in consumed food could be reduced by microorganisms in the gastrointestinal tract. Therefore, the aim of this study was to determine the ability of more dairy strains of lactic acid bacteria and bifidobacteria to bind AFB₁.

Material and methods: Four dairy strains of lactic acid bacteria, *Lactobacillus acidophilus* NCC 12, *Lb. acidophilus* NCC 36, *Lb. acidophilus* NCC 68, *Lb. rhamnosus*, and four bifidobacteria, *B. bifidum* NCC 381, *B. bifidum* Bb 13, *B. longum* NCC 135 and *B. longum* BI24 were tested for their ability to bind AFB₁. All tested strains except *Lb. rhamnosus* (purchased from Ezal, France), were obtained from Nestle-Switzerland Culture Collection as a freeze-dried powder. AFB₁-binding abilities of *Lactobacillus* and *Bifidobacterium* strains were determined by a competitive ELISA procedure as described by R-Biopharm GmbH. The percentage of AFB₁ bound to the bacteria was calculated using the formula: AFB₁ binding % = AFB₁ recovery rate of 1 µg·ml⁻¹ control % - unbound AFB₁ in supernatant %.

Results: AFB₁-binding ability of *Lactobacillus* and *Bifidobacterium* strains was found to be matrix and bacteria specific. The binding abilities of AFB₁ by viable test strains were found to range from 15.6 % to 52.5 % and from 14.6 % to 43.3 % for PBS and food model, respectively. There was a great difference ($p < 0.05$) in the removal of AFB₁ both from PBS and food model by test strains. Among the bacteria, *B. bifidum* Bb13 was found to be the most efficient binder to AFB₁ both in PBS and food model. The removal of AFB₁ from PBS by test strains was high when compared their activity in food model. The average AFB₁ recoveries from spiked PBS and food model were 91.1 % and 70.3 %, respectively.

Conclusions: This study demonstrated that specific dairy strains of lactic acid bacteria and bifidobacteria are able to bind AFB₁. However, more studies must be conducted to characterize the binding mechanisms.

Identification of a bacterial metabolite protecting the yeast *Saccharomyces cerevisiae* against DON toxicity

Hanna Weindorfer¹, Günther Ellersdorfer¹, Angela Sessitsch², Naser Safaie³, Markus Gorfer¹, Joseph Strauss¹, Iris Bogad⁴, Wolfgang Kneifel⁴, Marc Lemmens⁵, Antje Potthast⁶, Gerhard Adam¹

¹Institute of Applied Genetics and Cell Biology, Department of Applied Plant Sciences and Plant Biotechnology, BOKU - University of Natural Resources and Applied Life Sciences Vienna, Muthgasse 18, A-1190 Vienna, Austria

²Biogenetics-Natural Resources, ARC Seibersdorf research GmbH, A-2444 Seibersdorf, Austria

³Department of Plant Pathology, College of Agriculture, University of Tarbiat Modarres, P.O. Box 14115-111, Tehran, Iran

⁴Division of Food Microbiology and Hygiene, Department of Food Sciences and Technology, University of Natural Resources and Appl. Life Sciences Vienna, Muthgasse 18, A-1190 Vienna, Austria

⁵Institute for Biotechnology in Plant Production, Department of Agrobiotechnology (IFA Tulln), BOKU - University of Natural Resources and Applied Life Sciences Vienna, Konrad Lorenz Strasse 20, A-3430 Tulln, Austria

⁶Department of Chemistry, BOKU - University of Natural Resources and Applied Life Sciences, Vienna, Muthgasse 18, A-1190 Vienna, Austria

We have developed a yeast strain (YZGA515) which is highly sensitive to the trichothecene toxin deoxynivalenol (DON) by inactivating genes encoding three ABC transporter proteins (*PDR5*, *10*, *15*) and a gene encoding an acetyltransferase (*AYT1*). The ABC transporters cause pleiotropic drug resistance by active drug efflux, while the Ayt1 protein is able to convert DON into less toxic 3-acetyl-DON. With strain YZGA515 we developed a bioassay and used it to screen bacteria for the ability to detoxify DON. In a first step bacteria were grown for 4 days in the presence of DON. After centrifugation the supernatant was collected and combined with an equal volume of inoculated yeast medium, which also contained ampicillin, tetracycline and kanamycin to prevent regrowth of the bacteria. The initial DON concentration was chosen in a way, that unless DON degradation takes place the concentration in the second step is 3-fold higher than the minimal inhibitory concentration for the yeast strain YZGA515.

Overall we screened more than 1 200 strains that were either isolated specifically for this project (e.g. from artificially *Fusarium* infected corn, compost sites), or were provided by the partners (e.g. epiphytic bacteria isolated from Iranian wheat, collections of soil bacteria, intestinal bacteria, streptomycete strains, respectively). Two strains that allowed growth of the indicator yeast were identified. One strain (to be described elsewhere) was able to degrade DON, based on disappearance of the DON spot in a TLC assay.

For the other strain (#69) we found that the protective activity is constitutively present in the culture supernatant of the strain. Protease and heat resistance, and the ability to pass through a ultrafiltration membrane (3500 Da) indicates that the active principle is not an enzyme or binding protein, but a small molecule acting as „safener“ in yeast. The compound could be extracted with ethylacetate and displayed a biphasic dose response curve. In the presence of otherwise inhibitory amounts of DON low concentrations of the safener allowed yeast growth, whereas higher concentrations were toxic also in the absence of DON. Bioassay guided fractionation of the culture filtrate extract by column chromatography and analysis of the peak fractions by GC-MS revealed a few candidate compounds. The most abundant substance was commercially available. The substance was obtained and displayed protecting activity like the original extract, which strongly indicates that the active principle was identified. The mode of action of this compound in yeast is currently under investigation.

Counteracting mycotoxin contamination: the effectiveness of *Saccharomyces cerevisiae* cell wall glucans for sequestering mycotoxins

Alexandros Yiannikouris

Alltech Inc, 3031 Catnip Hill Pike, Nicholasville, KY 40356, USA

The chemical mechanisms involved in the sequestering activity of *Saccharomyces cerevisiae* cell wall components towards aflatoxin B1 (AFB1), deoxynivalenol (DON), zearalenone (ZEA), patulin (PAT) and ochratoxin A (OTA) were investigated *in vitro*. After reaction during 90 min at 37 °C under orbital agitation, quantification of the sequestered mycotoxins was achieved using HPLC. The interaction affinity was evaluated using Hill's equation. The comparison of several sources of yeast cell wall differing in their relative glucan/mannan/chitin content was carried out.

The chemical interaction was approached using molecular mechanics investigation to evaluate the impact of the sequestrant 3D-structure. Together with complementary NMR and X-ray structural data analysis, molecular modeling was used to generate and evaluate a statistical significant probability of existence of the modeled structures. The site-specific docking of a mycotoxin in the sequestrant was explored in all its conformational possibilities.

β -D-glucans of yeast cell wall proved their strong efficacy for AFB1, ZEA, DON and PAT sequestration. Complementary work showed a significant efficacy for sequestering T-2 toxin, and endophytes associated toxins. Molecular mechanics investigations demonstrated the importance of single and/or triple helix organized structure of β -D-glucans. Chemical interaction involved weak chemical hydrogen and van der Waals bonds between hydroxyl and cyclic groups of mycotoxins and β -D-glucans. Several *in silico* models proposed a realistic view of mycotoxin molecules caged inside β -D-glucans due to high geometric similarities.

The plasticity of the structure of β -D-glucans exhibiting diverse stereochemistry was undoubtedly responsible for the affinity on a large range of mycotoxins. Affinity rates varied widely between toxins due to their structural and physico-chemical disparities but were optimal for aflatoxin, deoxynivalenol and zearalenone-like structures.

Air borne mycotoxin producing moulds in working areas of food production and processing companies

Selamait Ghirmai¹, Regina Nattmann¹, Helder Correia¹, Elena Glaser¹, Alexander Prange^{1,2}

¹Niederrhein University of Applied Sciences, Microbiology and Food Hygiene,
Rheydter Strasse 277, D-41065 Mönchengladbach, Germany

²Louisiana State University, Center for Advanced Microstructures and Devices (CAMD),
6980 Jefferson Hwy, Baton Rouge, LA 70806, USA

The investigation and quantification of air borne moulds is an important issue in quality safety systems in food producing and processing companies. Although air borne moulds and especially mycotoxin producing moulds are frequently present in the air and can be a serious risk as contaminants in food, criteria and parameters for assessing the air hygienic status concerning mycotoxin moulds with special respect to specific „company-types“ are lacking. Therefore, it was the aim of this study to investigate air samples from three different „company-types“ (mill, catering Cook & Chill kitchen; canteen kitchen) and to determine the quantity and to identify the genera of moulds. Samples were taken weekly over period of six months using a MAS-100 VWR air sampler and cultivated using standard techniques and media. Moulds were identified using macroscopic and microscopic criteria. Identification of some (potentially) mycotoxin producing moulds were confirmed by PCR (ITS region, sequencing of PCR products). Derived from these results, company-type specific assessment criteria on air hygienic status of (mycotoxin producing) moulds will be presented.

Stachybotrysbefall im Klinikbereich nach Leitungswasserschaden

Reinhard Keller, Klaus Senkpiel

Institut für Medizinische Mikrobiologie und Hygiene der Universität zu Lübeck,
Ratzeburger Allee 160, 23538 Lübeck, Germany

Nach einem Wasserrohrleitungsschaden der Heizung im Klinikbereich, der längere Zeit unentdeckt blieb, kam es zum Schimmelpilzbefall des Estrichs und der Rigipsplatten, deren Ständerwerke bis auf die Betonsohle reichten. Große Bereiche der Gipskartonwände waren durchfeuchtet und massiv mit Stachybotrys-Wildstämmen befallen. Durch mikrobiologische Luft- und Materialuntersuchungen wurden die Sanierungsschritte begleitet, um eine mögliche Ausbreitung von Luftkeimen über das eingehauste Sanierungsobjekt hinaus zu erkennen und sofortige Gegenmaßnahmen einzuleiten. Eine Wirksamkeitsprüfung des eingesetzten Flächendesinfektionsmittels (Mikrobac forte, Fa. Bode) wurde durchgeführt.

Die Luftkeimuntersuchungen von mesophilen und thermotoleranten Schimmelpilzen wurden nach der quantitativen kulturellen Methode und die Luftpartikelzählungen (Sporenanzahl) mittels PS 30 Partikel-sammler (Fa. Holbach) vorgenommen. Die Mykotoxinbestimmungen nach Sporenaufschluß wurden mittels Trichothecen-ELISA sowie GC/MC-Analytik durchgeführt. Für den qualitativen Stachybotrysnachweis von Baumaterialien kamen mikroskopische Methoden zur Anwendung.

Nach Einhausung (Unterdruck: ca. 20 Pa) und vor Beginn der Demontage lagen die mesophilen Schimmelpilz-Sporenkonzentrationen außerhalb der Folienabschottung bei max. 370 KBE/m³, wobei keine Stachybotrys-Sporen feststellbar waren. Im eingehausten Sanierungsbereich wurden nach Beendigung der Demontage in der Innenraumluft 0–80 Stachybotrys-Sporen/m³ (arithm. M) und maximal 1 600 Sporen/m³ ermittelt. Der isolierte Stachybotrys-Wildstamm enthielt Satratoxin H mit einem Roridin-Äquivalent Toxin von 6 198 ppb (Trichothecen-ELISA)*.

Die Schimmelpilzbelastung infolge eines Wasserrohrdefektes im Fußbodenbereich wurde aufgezeigt, das Gefährdungspotential nachgewiesen und geeignete Schutz- bzw. Präventionsmaßnahmen vorgenommen [1].

Acknowledgement

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Reference

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Frequency and species distribution of gliotoxin-producing isolates of *Aspergillus* and *Candida* species isolated from clinical specimens

Claudio Kupfahl¹, Anna Michalka¹, Cornelia Lass-Floerl², Guido Fischer³, Gerhard Haase⁴, Thomas Ruppert⁵, Gernot Geginat¹, Herbert Hof¹

¹Medical Microbiology and Hygiene, University Heidelberg, Germany

³Hygiene and Environmental Medicine, RWTH Aachen; Germany

⁴Medical Microbiology, RWTH Aachen; Germany

⁵Zentrum für Molekulare Biologie der Universität Heidelberg (ZMBH), Germany

Background: The secondary metabolite gliotoxin is produced by several fungi including human pathogenic species like *Aspergillus fumigatus*. Gliotoxin is well known for its immunosuppressive activity *in vitro* and therefore gliotoxin is discussed as a virulence factor for human pathogenic fungi.

Methods: Clinical ($n = 111$) *Aspergillus* strains as well as clinical isolates of *Candida* species ($n = 100$) were cultured in RPMI medium for 5 or 7 days at 35 °C. Sterile filtered culture supernatants were extracted using a liquid phase extraction protocol followed by quantification of gliotoxin with HPLC. To assure specificity of the HPLC, representative samples were confirmed using tandem mass spectroscopy.

Results: Gliotoxin was detected in 98 % of clinical *A. fumigatus* ($n = 53$), in 37 °C of *A. terreus* ($n = 27$), in 56 % of *A. niger* ($n = 16$) and in 13 % of *A. flavus* ($n = 15$) strains. Highest gliotoxin concentrations were detected in *A. fumigatus* strains. In contrast, none of the *Candida* species tested showed gliotoxin production.

Conclusions: Frequency of gliotoxin production is variable among clinical *Aspergillus* isolates. Highest frequencies and concentrations of gliotoxin were found for *A. fumigatus*. In contrast, *Candida* species fails to produce gliotoxin in this study.

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