28 Mykotoxin Workshop
29-31 May 2006

Ignacy Paderewski Pomeranian Philharmonic
in Bydgoszcz

Programme
Abstracts
Participants

Organizers:

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&
Kazimierz Wielki University in Bydgoszcz
Institute of Biology and Environmental Protection
Department of Physiology and Toxicology
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BMVEL, Bonn
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PIW, Puławy
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CVUA, Stuttgart
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Agroscope FAL Reckenholz
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IOR, Rzeszów
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Institut für Ernährung und Lebensmittelwissenschaften, Bonn
Fusarium graminearum in competitive interaction with other fungi in suboptimal stored wheat

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IFA Tulln
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Eurofins, Hamburg
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Onabanjo University
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Uniwersytet Kazimierza Wielkiego, Bydgoszcz
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Großhandels und Lagerei Berufsgenossenschaft, Mannheim
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IRMM Geel
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Universität Münster
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CVUA, Stuttgart
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VetMed Universität, Wien
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UWM Olsztyn
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FAL Braunschweig
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IfaDo Dortmund
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UFZ, Leipzig
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University of Belgrade, Serbia & Montenegro
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UWM Olsztyn
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Uniwersytet Zielonogórski, Zielona Góra
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Lithuanian Veterinary Academy, Kaunas
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LCI, Köln
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3. **Raters M., Matissek R.**
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5. **Czerwiecki L., Wilczyńska G., Kwiecień A.**
IBPRS, Warszawa
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UWM Olsztyn
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National Institute of Agricultural Science and Technology, Suwon
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VetMed Universität, Wien
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12. **Meyer K., Matthes J., Krammer A., Engelhardt G., Bauer J.**
TU, Lehrstuhl für Tierhygiene, München
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SGS Germany GmbH, Hamburg
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TNO Quality of Life, Zeist
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Centre for Hygiene of Food Chains, Brno
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Sabine Kruse

Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz
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Mit der Verordnung (EG) Nr. 178/2002 zum Lebensmittelrecht werden Anforderungen an die Futtermittelsicherheit gestellt. Danach dürfen Futtermittel, die nicht sicher sind, nicht in den Verkehr gebracht oder an Lebensmitteltiere verfüttert werden. Ein Futtermittel gilt als nicht sicher, wenn es geeignet ist, die Gesundheit von Mensch und Tier oder die Leistungsfähigkeit von Tieren zu beeinträchtigen. Verantwortlich für die Einhaltung der Anforderungen an die Futtermittelsicherheit sind die Futtermittelunternehmer. Hat ein Futtermittelunternehmer Grund zu der Annahme, dass ein Futtermittel nicht sicher ist, so muss er Maßnahmen für deren Rückruf einleiten und die zuständigen Behörden unterrichten.

Futtermittelrechtlich sind Mykotoxine unerwünschte Stoffe, die in der Richtlinie 2002/32/EG über unerwünschte Stoffe in der Tierernährung definiert sind als *„Stoffe und Erzeugnisse, mit Ausnahme von Krankheitserregern, die in und/oder auf einem zur Tierernährung bestimmten Erzeugnis vorhanden sind und eine potentielle Gefahr für die Gesundheit von Mensch oder Tier oder für die Umwelt darstellen oder die tierische Erzeugung beeinträchtigen.*" Für bestimmte unerwünschte Stoffe werden Höchstgehalte oder Aktionsgrenzwerte festgelegt. Futtermittel, die die im Anhang der Richtlinie aufgeführten Höchstgehalte überschreiten, dürfen nicht in den Verkehr gebracht oder verfüttert werden. Ferner dürfen solche Futtermittel nicht mit anderen Futtermitteln zu Verdünnungszwecken gemischt werden. Unter bestimmten Bedingungen können jedoch geeignete Verfahren angewendet werden, die den Gehalt an unerwünschten Stoffen verringern oder entfernen. Gegenwärtig sind für Futtermittel Höchstgehalte für die Mykotoxine Aflatoxin B_1 und Mutterkorn festgelegt. Die Höchstgehalte für Aflatoxin B_1 wurden 2003 auf der Grundlage einer Stellungnahme der Europäischen Lebensmittelbehörde herabgesetzt. Für Mutterkorn in Futtermitteln wird gegenwärtig geprüft, anstatt (oder neben) der bisherigen auf einer mikroskopischen Bestimmung basierenden Regelung, eine Regelung auf der Basis einer analytischen Bestimmung von mindestens vier Leitalkaloiden zu treffen, damit auch verarbeitete Erzeugnissen einbezogen werden können.

Für weitere Mykotoxine (Deoxinivalenol, Zearalenon, Ochratoxin A und Fumonisine) sollen Orientierungswerte festgelegt werden. Für Futtermittel mit erhöhten Gehalten an diesen Mykotoxinen gelten die Bestimmungen der Richtlinie 2002/32/EG hinsichtlich des Verschneidungsverbotes nicht.

Ein Sonderfall sind die auf dem Markt anzutreffenden so genannten Mykotoxinbindemittel. Diese Erzeugnisse sind als Futtermittelzusatzstoffe einzuordnen und unterliegen damit den Bestimmungen der Verordnung (EG) Nr. 1831/2003 über Zusatzstoffe zur Verwendung in der Tierernährung. Sie sind deshalb zulassungsbedürftig. Im Rahmen der Zulassung muss die Sicherheit und die Wirksamkeit nachgewiesen werden. Für das Prüfungsverfahren werden von der Europäischen Lebensmittelbehörde entsprechende Leitlinien erarbeitet.

National programme of official control of animal feedingstuffs under surveillance of veterinary inspection in Poland

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Poland as a EC country was obliged in agree with Directive 95/53/EC to drawn up one, coherent and coordinated national programme of official inspections. Taking these facts into account it has been worked out the Polish National Programme of an official control conducted by the Veterinary Inspection in the field of animal feedingstuffs sector in Poland. The objective of the programme is to lay down the substantive scope of official inspections and laboratory control testing of animal feedingstuffs, appropriate for the surveillance exercised by the Veterinary Inspection. The programme is under implementation process which has begun in 2004. On drawing up the programme, the following has been taken into account important aspects i.e. the volume of feeds production, the assortment of the products manufactured, the areas of risk, etc. The programme lays down the general rules and procedures of official inspections of animal feedingstuffs determined by Directive 95/53/EC and Commission Recommendations issued at an annual basis, on the coordinated inspection programmes in the field of animal nutrition, which shall be carried out by the Veterinary Inspection authorities. The many sources of environmental chemicals may contaminate animal feed and constitute a hazard not only to animal health but also to human. It was recognized that, apart from environmental contaminants and many different hazard agents, feeds in Poland may contain mycotoxins which are secondary metabolites produced by fungi and can affect a wide range of food products. Taking this into account the following mycotoxins were included to the control programme in 2006: aflatoxin B_1, ochratoxin A, zearalenone, deoxynivalenol, fumonisins. In the programme other aspects such as: the methods of analysis; the method of sampling are regulated. In the case of targeted sampling, the samples should be feed materials suspected of containing higher concentrations of mycotoxins, such as cereal grains, oil seeds, oil fruits, their products and by-products, and feed materials stored for a long time or transported by sea over a long distance. In the case of aflatoxin $B_{1,}$ particular attention is also paid to compound feedingstuffs for dairy animals other than dairy cattle. In years 2004 and 2005 respectively: 816 and 933 samples were examined for the contents of different mycotoxins. Actually the laboratory analyses are performing in 9 Regional Veterinary Laboratories (ZHW) all over Poland. The scope of an official control in mycotoxin area will be expanding and will follow EC law. Base on Directive 2002/32/EC polish Regulation of Ministry of Agriculture of June 28, 2004 implemented maximum contents of aflatoxin B_1 in feedingstuffs established by this directive. Similar to Community law, there are no polish rules for other mycotoxins, such as ochratoxin A, zearalenone, deoxynivalenol, fumonisins, T-2 and HT-2 toxins. In our opinion gathering information on the presence of those mycotoxins through random sampling could provide useful data for an assessment of the situation with a view to the development of the legislation. Furthermore, certain feed materials such as cereals and oil seeds are particularly exposed to mycotoxin contamination because of harvesting, storage and transport conditions. As mycotoxin concentration varies from year to year, it is appropriate to collect data from consecutive years for all mycotoxins mentioned. Results obtained in this control programme in 2006 in mycotoxins area should be useful for the development of the suitable legislative provisions. Beside that it should be add that in Poland before 2003 the level of mycotoxins was limited undirectly because allowed total count of fungi was established on the level 200,000 cfu/g in animal feedingstuffs.

Aktuelle rechtliche Regelungen (Höchstmengen, Probenahme) zu Mykotoxinen in Lebensmitteln auf nationaler und EU-Ebene

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Die im Rahmen der Mykotoxinforschung, v.a. in den 90er Jahren gewonnenen Erkenntnisse (toxikologische Bewertung, Bildungsbedingungen und Vermeidungs-strategien, zuverlässigere und empfindlichere Analyseverfahren), führten in den letzten 5 Jahren sowohl auf nationaler, als auch insbesondere auf EU-Ebene zu einer Vielzahl von Richtlinien und Verordnungen in diesem Bereich.

Während im Jahr 2001 lediglich Aflatoxine in ausgewählten Matrizes reglementiert waren (Verordnung (EG) Nr. 466/2001 v. 08.03.2001), wurden in den neun mykotoxin-spezifischen Änderungsverordnungen zwischenzeitlich EU-weit Höchstmengen für nahezu alle wichtigen Mykotoxine in sämtlichen relevanten Matrizes festgesetzt. Hinzu kommen die nationalen Regelungen im Bereich der deutschen Mykotoxin-Höchstmengen-Verordnung.

Darüber hinaus wurden, jeweils parallel zum Erlass der EU-Höchstmengen, toxin-spezifische Anforderungen an die Probenahme (8 EU-Probenahme-Richtlinien), eine Leitlinie zur Probenahme bei Aflatoxinen sowie spezifische Vorgaben zur Durchführung von Einfuhruntersuchungen (8 EU-Entscheidungen) erlassen. In den jeweiligen EU-Probenahme-Richtlinien wurden ferner die Anforderungen an die jeweiligen Analyseverfahren sowie, zum ersten Mal im Bereich der natürlichen Kontaminanten, dezidierte Vorgaben zur Beurteilung der Analysedaten (u.a. Einbeziehung der Wiederfindungsrate und Berücksichtigung der erweiterten Messunsicherheit) formuliert.

Für die nicht im Bereich der EU-Kontaminanten-VO geregelten Mykotoxine (insbesondere Fusarientoxine) gelten bis dato nationale Höchstmengen (MHmV).

Im aktuellen Vortrag sollen u.a. die ab 01.07.06 geltenden EU-weiten Höchstmengen-Regelungen (u.a. für Fusarientoxine; EU-VO 856/2005) zusammenfassend vorgestellt sowie insbesondere die Auswirkungen auf die nationale Mykotoxin-Höchstmengen-Verordnung diskutiert werden.

Ebenfalls zum 01.07.06 tritt die neue EU-Probenahme-Verordnung (Verordnung (EG) Nr. 401/2006 v. 23.02.2006) in Kraft. Darin werden die bisherigen toxinspezifischen EU-RL nunmehr im Rahmen einer, unmittelbar in jedem Mitgliedsstaat geltenden, Verordnung zusammengefasst. Die hierdurch bedingten Änderungen werden vorgestellt sowie die nach wie vor bestehenden Fragen (u.a. Gegenprobenproblematik, Probenahme im Einzelhandel sowie bei großen Getreideschüttungen) diskutiert.

Official control of mycotoxin contamination in food of plant origin in Poland

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The basic principles of food control in Poland were comprised in Regulation (EC) No 882/2004 and Food and Nutrition Law of 11 of May 2001. To implement the tasks in the field of public health, in particular, supervising the conditions of food safety, The State Sanitary Inspection (PIS) was founded. The head of PIS – Chief Sanitary Inspectorate (GIS) – fixes priorities and directs the State Sanitary Inspection's activities in relation to the food safety. In cooperation with National Institute of Hygiene coordinating issues are performed, associated with the elaboration, implementation and functioning of food contamination monitoring. As well, analysis of the country food epidemiological situation and risk evaluation is carried on. GIS is managing and coordinating the Rapid Alert System for Food and Feed (RASFF).

Official control monitoring program in mycotoxin area settle the requirements for control of domestic market and import, including realization of Commission Decisions imposing special conditions on the import of certain products (pistachios from Iran, Brazil nuts from Brazil, figs, pistachios and hazelnuts from Turkey, peanuts from China and Egypt). It also contains a sampling plan for new mycotoxins and group of products that are not covered by legislation in the moment.

Laboratories of PIS carried the work in accordance with internationally approved procedures and basing on the criteria of performance standards as well applying validated analytical methods. Laboratories are equipped with ELISA sets (for screening purposes) and HPLC sets as well as equipment for homogenization of large samples.

By Regulation of Ministry of Health Laboratory of National Institute of Hygiene has been designated as national reference laboratory for contamination, including mycotoxins, of food products of plant origin. Main activities of reference laboratory are: validation of new analytical methods, organization of proficiency tests and training of laboratory staff for PIS laboratories.

Fusarium head blight and mycotoxins in cereals – potential strategies to control contamination under conservation tillage

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The occurrence of fusarium head blight (FHB) in cereals is strongly influenced by cultivation practices such as crop rotation, tillage, and choice of varieties. FHB caused by *Fusarium graminearum* (FG) and contamination of wheat with deoxynivalenol (DON) is more prevalent when wheat is grown after maize and especially with maize residues remaining on the soil surface (i.e. conservation or zero tillage). If these two risk factors co-occur with weather conditions favourable to infection, serious mycotoxin contamination can occur even with the most resistant wheat varieties presently grown. The current regulations on DON (e.g. EU: maximum value of 0.75 ppm in cereal flours; Switzerland: tolerance value of 1 ppm in milling products) underline the need for controlling FHB caused by FG. In order to avoid FHB and mycotoxins while protecting the soil with conservation tillage, combinations of several measures have to be developed (see also contribution by Musa et al.).

We assume that the risk for infection of the wheat crop could be reduced with an accelerated decomposition of maize residues, the main source of *Fusarium* inoculum. Since 2003, we have been conducting on-farm trials with winter wheat grown after maize and with management of maize residues under conservation tillage. On 4 sites, we are examining the effect of fine mulching with or without surface incorporation of the residues on the occurrence of FHB. Mulching is being performed using a multipurpose shredder equipped with forged hammer knives and counter blades whereas a rototiller is being used for residue incorporation. Wheat varieties are according to the choice of the local farmer. Collected data include visual disease assessment in the field, yield, incidence of different *Fusarium* species on wheat grains (whole seed agar plate method), as well as the amount of DON in grains and straw.

The weather conditions in 2004 were substantially more favourable for FHB infestation compared with those of 2005. The results from the first two years show that with fine chopping of maize residues and a less susceptible wheat variety such as Arina or Titlis it is possible to produce no-till wheat with low DON contents in both grains and straw. For example, in 2004 on a site with Arina, the mean DON content in grains from plots with no residue treatment was 1.8 ppm whereas grains from plots with fine mulching showed only 0.5 ppm. Nevertheless, inconsistent results between different trial locations demonstrate the need for further research. We suppose that the differing results are primarily due to variations in mulch quality such as the size of mulched debris and the homogeneity of dispersal, but also to soil activity, climatic conditions, or the given wheat variety.

For current and upcoming on-farm trials, we are focusing on further improving the mulching procedure as well as on evaluating the mulch quality and the subsequent decomposition of maize residues. The results of this research project on residue management will be important for both no-till and conservation tillage systems. Furthermore, it will contribute towards safe food and feed while respecting the environment.

Fusarium spp. occurrence and mycotoxins content in grain of genetically modified corn

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Fusarium species are contaminants of maize. Infection may be associated with yield reduction, but *Fusarium* species may also infect the plant without any symptoms. Several *Fusarium* species are also able to produce mycotoxins, with may be dangerous for both human and animal health. The aim of this work to study *Fusarium* spp. occurrence and mycotoxins content in grain of four traditional maize cultivars and four genetically modified maize cultivars. Two field experiments were conducted in 2005 in Poland. Infestation by insects or *Fusarium* sp. was exclusively natural, no manual treatment took place.

Just before harvest plants were the assessment of the *Fusarium* occurrence in grain performed according to scale: 1 – 9. Fungi species were identified on the basis of conidial morphology according to the method of Nelson et al. in Institute of Plant Protection, Local Experimental Station in Rzeszów. Fumonisins and DON were measured by using the method an HPLC column equipped with a fluorescence detector. The analysis of fumonisin B_1, fumonisin B_2, and fumonisin B_3 were measured in Chemisches und Veterinaruntersuchungsamt Stuttgart and the analysis of DON were measured in Kazimierz Wielki University, Institute of Biology and Environmental Protection in Bydgoszcz.

In all field experiments, mycological analysis revealed that the percentage of kernels infected by *Fusarium spp.* and mycotoxins content in grain was higher in traditional maize than in transgenic maize.

Fusarium graminearum in competitive interactions with other fungi in suboptimal stored wheat

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The phytopathogenic fungus *Fusarium graminearum* infects wheat plants and other crops already on the field and is well known as a potent mycotoxin producer. *F. graminearum* produces the trichothecenes, e.g. deoxynivalenol (DON) and zearalenone (ZEA). Interactions with other fungi are considered as a possible trigger for mycotoxin production. There are only few studies in the literature analyzing the influence of other fungi on *Fusarium* spp. concerning their growth and their mycotoxin production (e.g. Marin *et al*., 1998; Simpson *et al*., 2004, Prange *et al.*, 2005).

To investigate competitive interactions of *F. graminearum* with *Alternaria alternata*, *Penicillium verrucosum* and *Aspergillus ochraceus*, respectively, *in vivo*-cultivation experiments on wheat were performed over a storage period of 12 weeks. Experiments were conducted under suboptimal storage conditions (20% humidity, 20°C) to provide «optimal» conditions for the growth of the moulds and mycotoxin production. Three strains of *F. graminearum* were chosen including a *tri5*$^{-}$ knock out mutant, which is not able to synthesize DON as it lacks the key enzyme of trichothecene biosynthesis. The study includes two storage trials, one with sterile wheat kernels and one with wheat carrying its natural microbial flora. Growth of fungi was detected by means of quantitative PCR and with «classical» cultivation on agar plates. Mycotoxins (DON, ZEA, OTA) were determined using ELISAs. The results revealed that the chosen strains of *F. graminearum* did not produce significant amounts of DON, no matter to the fungus they compete with. Concerning ZEA in all interaction experiments (but also for one strain of *F. graminearum* which is cultured alone) increasing levels were detected. In the *in vitro* interaction models comparable tendencies for DON and ZEA were found, too, together with a clear inhibition of growth of interacting fungi. In the *in vivo* interaction experiments on wheat an inhibitory effect was observed in most cases, however, it has to be assumed that other parameters have influence, too, perhaps due to the more complex *in vivo*-system.

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Study of the metabolism of *Fusarium* mycotoxins in plants with HPLC – tandem mass spectrometry

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During growth of certain fungi on plant commodities toxic metabolites are formed. Living plants partly metabolize these xenobiotics. Humans and animals that consume the contaminated plants or products thereof are not just exposed to the native mycotoxins, but also to altered forms. Little is known about the metabolism, occurrence and bioavailability of mycotoxin metabolites, some of which are masked toxins.

The first part of this work reports on the natural occurrence of deoxynivalenol-3-glucoside (D3G) in wheat and maize [1] and its relevance for plant breeding [2]. In almost all investigated deoxynivalenol (DON) contaminated cereals also D3G was found. It was further examined whether the detoxification of DON to D3G could be used as a marker of plant resistance. Wheat ears of different cultivars have been treated with DON and analyzed for DON and D3G. It is shown that the D3G/DON ratio is closely linked to the DON resistance of the cultivars. As DON is a major virulence factor of *Fusarium sp*., the resistance towards the toxin is also correlated with resistance to the spread of FHB symptoms in the wheat ears.

The second part of this work describes an assay for monitoring, identification and quantification of the metabolic transformation of natural or xenobiotic compounds in plants using LC-MS/MS and the model plant *Arabidopsis thaliana* [3]. After treatment of seedlings with zearalenone (ZON) in liquid medium, both the medium as well as the extracted plant materials were analyzed for metabolites. Masses of potential analytes were calculated from known metabolic reactions. The identities of found metabolites were confirmed with product ion scans. In a time course experiment, ZON was metabolized by the plants almost completely after 24h and is rapidly transformed to a variety of products, including glucosides, sulfates, malonyl-glucosides, diglucosides, glucosyl-xyloses and other conjugates of ZON, α-ZOL and β-ZOL. In total, 17 different compounds were identified.

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Einfluss der Lagerdauer und des Feuchtegehaltes auf die Bildung von Mykotoxinen bei Körnerleguminosen aus ökologischem und konventionellem Anbau

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Über das Vorkommen der Mykotoxine Ochratoxin (OTA) und Citrinin (CT) in Proben aus der ökologischen Produktion liegen nur wenige Ergebnisse vor. Es ist aber davon auszugehen, dass diese Toxine bei gleicher Lagerhygiene nicht häufiger vorkommen als in konventionell erzeugten Produkten. Die zunehmende Bedeutung heimischer Eiweißfuttermittel insbesondere in ökologisch wirtschaftenden Betrieben veranlasste uns, die Toxinbildung bei der Lagerung zu untersuchen. Die verschiedenen Körnerleguminosen (Erbsen, Ackerbohnen) aus ökologischem Anbau und Erbsen aus konventionellem Anbau wurden auf Soll-Feuchtegehalte von 14 und 19 % eingestellt und wie von Abramson et al. 1992 und Richter 1996 beschrieben, gelagert. Unter diesen Bedingungen wurde die Entwicklung der Lagerpilze untersucht und die CO_2-Gehalte gemessen. Bei den Ansätzen mit einer Sollfeuchte von 14 % lag der CO_2-Gehalt im Bereich der intergranularen Luft in den Silos (0,02 Volumen %). Die Gehalte streuten zwischen 0,04 und 0,02 Vol.-%. Bei den konventionell angebauten Erbsen lagen die gemessenen Werte beider Parallelen zwischen 0,4 und 0,5 Vol.-%. Gegen Ende der Lagerdauer von 6 Monaten wiesen die Ansätze mit Feuchtegehalten von 19 % einen CO_2-Gehalt von über 0,1 Vol.-% und die Ansätze mit 14 % Feuchtigkeit 0,02 Vol.-% CO_2 auf. Dies deutet auf eine höhere mikrobielle Aktivität in den feuchteren Ansätzen hin, was auch zu erwarten war Bei den Varianten mit konventionell erzeugten Erbsen stieg der CO_2 Gehalt bei den 19 %- Varianten deutlich auf 0,3 % an, es konnte aber OTA nicht nachgewiesen werden. Bei den Versuchsvarianten mit konventionell erzeugten Erbsen war ein Einfluss der Lagerung auf die Bildung des Mykotoxins Ochratoxin A zu sehen. Nach einer Lagerdauer von 18 Wochen und 19 % Sollfeuchte konnten Spuren von OTA nachgewiesen werden. In allen Varianten mit 14 % Sollfeuchte war der Toxinnachweis negativ. Bei den biologisch erzeugten Erbsen und Bohnen konnte auch in den Versuchsvarianten mit 19 % Feuchte kein OTA gefunden werden. Wir schließen daraus, dass kein toxinbildender Pilz anwesend war oder die Bedingungen für eine Toxinbildung nicht gegeben waren. Daraus lässt sich auf keinen Fall ableiten, dass biologisch erzeugte Körnerleguminosen feuchter gelagert werden können. Bei den Versuchsvarianten mit 19 % Feuchte zeigten die biologisch erzeugten Produkte eine höhere Schimmelpilzbelastung als in den konventionellen Produkte. Es ist daher auf eine sachgerechte trockene Lagerung mit weniger als 14 % Feuchtegehalt Wert zu legen.

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Sampling variability – Investigation of variability associated with testing lots of wheat kernels for deoxynivalenol and Ochratoxin A

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In June 2005 the new EC-regulation 856/2005 for *Fusarium* toxins (deoxynivalenol, zearalenone, fumonisines, HT-2/T-2 toxin) has been published. In parallel a sampling procedure similar to the existing one for the investigation of Ochratoxin A (2002/26/EC) came out. *Fusarium* toxins such as deoxynivalenol and zearalenone are produced by several species of *Fusarium*. Wet and cold weather conditions during flowering promote infection, resulting in head blight in wheat, barley, oats and rye.

Since *Fursarium* infections occur during growth period the fursarium toxin distribution throughout a given lot of grain kernels should be much more even compared to ochratoxin, which is a prominent example for intoxication as result of fungi attack during storage. Toxins from storage fungi are often concentrated in spots, while for the variability of fursarium toxins stratification effects depending on handling and manipulation through the harvest process have probably the highest impact (Hart et al. 1998, Freese et al. 2000).
Beside the toxicological aspect which has to be considered for the food chain, grain producer are facing significant economic consequences in those years with excessive fursarium infections. Altogether, this leads to the need of a reliable sampling standard with the lowest possible variability. However, the sampling protocol mentioned above was primary developed to investigate storage toxins and recommends 100 incremental samples (100 g each) for a lot between 20 t and 50 t. The incremental samples have to be homogenized in total; from the resulting homogenate the laboratory sample is taken. The entire sampling and homogenation procedure is costly an especially time consuming. During harvest "time" is the most limiting factor.

Within the present study the distribution of deoxynivalenol and ochratoxin A infected kernels in a lot of 26 t of wheat was investigated to develop a practical sampling plan which can be used effectively to avoid storage of highly contaminated lots and to save time within the trade of grains. The study delivered clear results for the sampling variability for DON in comparison to ochratoxin A.

On the same occasion the sampling plan of the directive 2005/38/EC was compared to the procedure recommended by the ICC standards as well as GIPSA standards of the grain inspecting handbook. In north European countries the risk of fusarium toxins in freshly harvested cereals is much higher compared to ochratoxin A, therefore it is more desirable to have a fast screening assay to evaluate the deoxynivalenol concentration directly on site. Different commercially available membrane immunological methods were compared and validated against a reference method based on LC-MS/MS. All investigations were part of a project financed by Bayrisches Landesamt für Gesundheit und Lebensmittelsicherheit (LGL, Erlangen) in cooperation with Verband Deutscher Mühlen (VDM).

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Mycotoxin contamination of food systems in sub-Saharan Africa

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This report presents an overview on prevalence levels, acute and chronic effect of dietary exposures and management strategies currently under investigation against mycotoxin contamination in sub Saharan Africa. From the African perspectives, two classes of mycotoxins, aflatoxins and fumonisins have been established to be widespread in major dietary staples. While aflatoxins occur mostly in maize and groundnuts, the prevalence of fumonisins is 100% or close to it in all surveillance data that have been reported on maize from different parts of Africa. Limited surveys have also established the presence of ochratoxin A, while few data currently exist on the trichothecenes and zearalenone mycotoxins in Africa. The exposure to mycotoxins particularly aflatoxins have also been demonstrated by their detection in analysed samples of urine, blood, breast milk and faeces of people in the region. Epidemiological studies have shown a wide range of toxic effect of mycotoxins on Africans including the 2004 and 2005 acute aflatoxicoses episode in Kenya, carcinogenicity, growth retardation and reproductive toxicity. The prevailing hot and humid conditions in most parts of Africa provide excellent condition for growth of fungi including the toxigenic ones, making the production of mycotoxins unavoidable in major dietary staples consumed in the region, while other contributory factors to mycotoxin problem in the region include food insecurity and low literacy level. The 2003/2004 FAO enquiry shows that regulatory limits mostly for aflatoxins which are rarely enforced exist in 15 African countries covering 59% of the inhabitants in the region. The strategies currently under investigation to mitigate mycotoxin problem in Africa include pre and post harvest management strategies with emphasis on biological control and use of plant products to arrest fungal growth during storage, enterosorbent clay technology, and the search for traditional techniques that could significantly reduce/detoxify mycotoxins during food processing.

Attempts to make Japanese traditional fermented food and mushroom safe from mycotoxin contamination

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Miso (fermented soybean paste), shoyu (soy sauce), sake (rice wine) and katsuo-bushi (fermented dried bonito), which are traditional Japanese fermented foods, have been consumed for a long period of time and are considered to be the safest foods. However, fermented foods may be contaminated with mycotoxins. Fungi used for the first stage of fermentation of miso and shoyu consist of *Aspergillus oryzae* and *A. sojae*. Fungi used for the first stage of fermentation of sake consists of *A. oryzae*. Although *A. oryzae* and *A. sojae* belong to the *A. flavus* group, which is known to produce aflatoxins, none of the strains examined actually produced aflatoxins. Rice is used for the fermentation of miso and shoyu as one of the ingredient, and it is used for the fermentation of sake as ingredient. Aflatoxin contamination of rice, miso, shoyu and sake could not be detected. Aflatoxigenic fungi do not occur in areas with a mean temperature below 16°C. Since the mean temperature in most areas of Japan is lower than 16°C, it appears that food contamination with aflatoxigenic strains does not occur in most parts of Japan. Some koji molds are known to produce kojic acid (KA) and cyclopiazonic acid (CA). Production of KA and CA was examined and the producing strains were eliminated from the commercial fermented foods. The fate of KA and CA in shoyu fermentation was also examined and it became clear that the contents of KA and CA decreased during shoyu fermentation. Katuo-bushi is fermented bonito. Bonito is heated in hot water, dried, inoculated mainly with *Eurotium herbariorum* (dried bonito mold) and fermented. Then it becomes Katsuo-bushi. Formerly inoculation of fungi was made naturally. Therefore, contamination of mycotoxins was doubted, but aflatoxins, sterigmatocystin and ochratoxin A were not be detected. Nowadays inoculation of *E. herbariorum* is artificially made and katsuo-bushi fermentation is made in good condition without contamination. Therefore, katsuo-bushi is now safe from mycotoxin contamination.

Ex-type culture of *Spicellum roseum* isolated from silage in France has been known to produce some trichothecenes. As the causative agent of the pink mold damping-off disease, we isolated some strains of *S. roseum* from a cultivated mushroom *Flammulina velutipes* at Nagano, Japan. To examine whether the Japanese strains of *S. roseum* produce trichothecenes or not, the isolates were cultivated on rice medium and extraction was made for mycotoxin analysis. 8-Deoxy-trichothecin was accordingly detected and confirmed with GC/MS by means of EI and CI mode. However, deoxynivalenol, 3-acetyldeoxynivalenol, fusarenon-X and nivalenol were not detected in the extract, all of which are well known to be detected from scabby wheat.

As mentioned previously, mycotoxin contamination, which is presently very low, should be completely eliminated in future.

T-2 and HT-2 Toxins in Oats

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Trichothecenes produced by *Fusarium* occurs frequently in cereals grown and harvested in Europe. The main focus has been on deoxynivalenol in wheat, but nivalenol, HT-2 and T-2 toxin are also found with a relatively high incidence. They are more common in oats.
Trichothecens have been surveyed in Swedish oats and other cereals during several years 1986-98. The appearance of nivalenol, HT-2 and T-2 toxins differed from deoxynivalenol. They were more common during years with warm weather and early harvests. Nivalenol is produced by *Fusarium poae* and T-2/HT-2 toxins by *F. langsethia* and they probably differ from *F. graminearum/culmorum* in plant infection and toxin production.
High occurrences of HT-2 and T-2 toxins have been found in the oat harvest of 2005 in Norway, Sweden and Finland. The concentrations have normally been between 100 and 500 µg/kg, but levels above 1000 µg/kg have also been found. HT-2 toxin has dominated but T-2 toxin co-occurred in lower levels or traces. Deoxynivalenol and nivalenol have also been detected but mainly in lower concentrations. Traces of HT-2 toxin were also detected in winter wheat from fields with oats as the preceding crop. A new project to further investigate the agricultural conditions that promote occurrence of HT-2 and T-2 toxins in oats has started in Sweden.

The trichothecenes are mainly found in the husk of oats, which is mainly removed in food processing. Horse is the animal at risk due to the high feeding of whole oats. Very little is known about the toxicity of HT-2 and T-2 toxin in horses. There are case reports on disturbances describing stomatitis, ulceration or edema of lips, nostrils, stomach and intestinal haemorrhage. Colic has also been more common in horses on feed containing T-2 toxin.

Mycotoxins and moulds contamination of feedstuffs and their components in the years 2001-2005

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Microbiological quality of feeds and their components is evaluated in the respect of maximal acceptable fungi level (moulds and yeast) below 200 000 cfu/g (Polish norms) and the toxicological quantity of aflatoxins (AF), ochratoxin A (OTA), deoxynivalenol (DON) and zearalenon (ZEA), individual for each country according to its regulations.
Mycological and toxicological evaluation of cereals and feedstuff sold in Poland collected and tested in own studies within the past 5 years, was carried out.
Quality and quantity identification of moulds and yeast was done in accordance with PN ISO 7954: 1999 (inoculation on YGC medium – yeast extract, glucose, 100ppm chloramphenicol, incubation 5-7 days in 25°C).

The extraction of mycotoxins was done on the immunoaffinity columns: AlfaTest, ZearalaTest and DONTest by Vicam for aflatoxins, zearalenon and deoxynivalenol, as well as Donprep by R-Biopharm Rhőne Ltd for ochratoxin A and deoxynivalenol, respectively. The evaluation of deoxynivalenol was done with the Elisa Ridascreen Fast DON by R-Biopharm Rhőne Ltd in 2001-2004. Mycotoxins were evaluated with the HPLC method with fluroescent and UV detection. Cobra Cell was used for aflatoxins derivatization.
The average number of fungi in cereal grains in all the years varied between 2,2 x 10^4 (2002)-8,6 x 10^4 cfu/g (2005). The acceptable level of contamination was exceeded from 0% (2003) to 7,1% (2001) in the samples sent for analysis. Only quantity differences were noted in the mould ranges: both storage and field moulds were present (it proves that harvested and stored grains were used). Corn grains contained higher level of yeast and significantly higher level of field moulds from *Fusarium* genus than other cereals. The level of contamination of the mixed feeds was stable and ranged from 8,0 x 10^3 (2001) to 4,0 x 10^4 cfu/g (2002), 2,6% of all the samples of the mixed feeds was up to 200 000 cfu/g in 2004. Moulds constituted 17,5% (2001) and 81,6% (2004) of all the fungi.
When compared to the maize green fooder, in the silage from maize the number of moulds decreased 10^3 times and the moulds constituted only 1-36% of all the fungi.
Greater mycological stability was characteristic of silage from grass and the increase of the moulds' amount was 10^1-10^2 times, while moulds constituted 31-89% of all the fungi before and after the stability.

In the years 2001-2005 over 2000 samples of cereal, corn and mixed feedstuff, were analyzed for mycotoxin detection (approx. 700-AF, 300-OTA, 550-ZEA and 500-DON).
Aflatoxin evaluation showed low level of contamination with this toxin in the samples examined. Only in the case of corn harvested in 2001-2002 the increase of the aflatoxin contamination was observed.
Significant increase in the ochratoxin A level was noted in cereal grains (median 4,9) and corn grains (median 10,3) in 2004, respectively. As a result, higher level of this toxin was found in feedstuffs.

When observing the level of zearalenon and deoxynivalenol contamination of cereal grains the increase in 2004 followed by the toxins content decrease, were noted.
However, the analysis of those toxins found in corn and feedstuffs showed high contamination in the years 2001-2003, lower in 2004 and again higher in 2005.

Concentrations of airborne mycotoxins and toxicity of grain dust in grain handling companies

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Grain is known to contain mycotoxins at variable concentrations. During grain handling the employees are exposed to dust that may be contaminated by these mycotoxins. Informations about the extent of this potential burden are few, particularly for small grain handling companies, which are the dominating company size in Germany. The goal of our investigations was the determination of mycotoxin concentrations in the air, the cytotoxicity and if there is an uptake of mycotoxins by the exposed employees.

Investigations included the following parameters: airborne respirable and inspirable dust, airborne moulds, analysis of ochratoxin A (OTA), zearalenone (ZEA), deoxynivalenol (DON) and cytotoxicity in settled dust as well as ochratoxin A analysis in the blood of the exposed employees. The concentrations of the mycotoxins in the air are calculated on the basis of the airborne respirable dust concentrations and the mycotoxin concentrations in the dust.

Dust concentrations varied according to the tasks and the type of the storing facilities. High concentrations occurred mainly during the harvest period. Outside the harvest period the dust burden is substantially lower but is accompanied by peak expositions over time periods of several hours to several days i.e. when silos are cleaned.

According to the variation of dust concentrations in the air the calculated concentrations of OTA, ZEA and DON varied. Detailed results of the mycotoxin concentrations, concentrations of the airborne moulds and the cytotoxicity of the dust samples will be presented during the presentation. Results from biomonitoring of OTA in grain handlers are presented separately (see Abstract Degen et al.).

Biomonitoring of ochratoxin A in grain handlers

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The handling of commodities such as grains may result in an inhalation of mycotoxin containing dusts. A small pilot study in nine cargo workers had pointed to the possibility that inhalation exposure may result in higher blood levels of ochratoxin A (OTA) in persons who handle grains than in the general population, and it demonstrated the usefulness of biomonitoring for OTA in occupational contexts [1].

Now, a larger study was conducted on individuals who work in granaries of several grain handling companies in Germany. OTA was analyzed in blood samples obtained between July 2005 and november 2005 from male grain handlers (n=50) aged between 18 and 57 years; the cohort is of German origin except for three persons (two from Romania, one from Turkey). OTA analysis in blood samples was performed by HPLC with fluorimetric detection as described previously [1]; calibration curves were run for each batch of samples, and the level of detection was 0.05 ng/ml plasma. Results from our analyses were then compared to OTA-levels for the general German population that are known to result from dietary (background) exposure to this mycotoxin [2].

The OTA plasma levels of the fifty grain handlers ranged between 0.09 and 0.57 ng/ml. The mean (0.28 ± 0.12) and median value (0.26 ng/ml) for this group are similar to values reported previously for the general population in Germany and thus not higher than internal levels resulting from dietary OTA exposure alone. Additional blood samples obtained from a subgroup of eleven grain handlers between november 2005 and march 2006 confirmed this result: Their OTA levels range between 0.13 and 0.75 ng/ml plasma, the mean (0.30 ± 0.17) and median (0.25) values are close to those of the first sample.

The biomonitoring data do not provide evidence for a significant additional uptake of OTA by inhalation in grain handlers. Yet, OTA has been detected in dust samples collected at their workplace [3]. Since deoxynivalenol and zearalenone were also detected in several of these dust samples in part in higher concentrations [3], additional analyses in blood (and or urine) are indicated to assess internal levels of these mycotoxins.

[1] Degen GH, Meifort J, Blaszkewicz M (2005) Biomonitoring für Ochratoxin A bei Beschäftigten im Getreide- und Rohkaffee-Umschlag. (a) 27. Mycotoxin-Workshop, 13.-15. Juni 2005, Dortmund, Tagungsband, Seite 72; and (b) *Mycotoxin Research* (2006), in press

[2] Rosner H, Rohrmann B, Peiker G (2000) Ochratoxin A in human serum. *Archiv für Lebensmittelhygiene* 51: 104-107

[3] Mayer S, Gareis M, Usleber E (2006) Concentrations of airborne mycotoxins and toxicity of grain dust in grain handling companies. 28. Mycotoxin Workshop 29.- 31. Mai 2006, Bydgoszcz

Do we need certified calibrants for traceable mycotoxin analysis?

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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 trade barriers but also to avoid unnecessary transport of goods.

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 traceability to SI is not achievable. Traceability can only be guarantied if the analytic results are traceable step by step in an unbroken chain.

For mycotoxin analysis instrument calibration is an essential step in the analytical procedure. Instrument calibration allows correlating the instrument output (e. g. peak area) to the amount of analyte present. Therefore this step in the analytical procedure is important in the traceability chain.

Instrument calibration is not only an important step in the procedure regarding traceability of the analytical result but also in relation to trueness and precision. The analytic result can only be as good as the instrument calibration and never be better.

The easiest solution to tackle all these problems is the use of certified calibrants. Certified calibrants guarantee a certain analyte concentration (mass fraction) with a statement of its traceability and its uncertainty.

The certified solution allows calibrating the instrument without bias (trueness). Due to the stated traceability of the certified value the traceability chain stays unbroken and the given uncertainty of the concentration together with the precision of the calibration allows estimating an appropriate contribution to the overall uncertainty of the measurement result.

The Institute for Reference Materials and Measurements produces such certified calibrants for mycotoxin analysis. These calibrants are normally produced by dissolving the neat mycotoxin in a suitable solvent. The neat mycotoxin is therefore tested for its purity and identity. For the verification of identity nuclear magnetic resonance spectroscopy and elemental analysis are employed amongst other methods. The purity and its uncertainty are assessed using various independent and complimentary methods (e.g. chromatographic methods, differential scanning calorimetry, thermo gravimetric analysis, spectrometric methods).

The concentration (mass fraction) is certified on the basis of the gravimetric processing and allows therefore traceability to the relevant SI unit.

The uncertainty budget not only contains contributions from the gravimetric preparation and the purity of the neat substance but also contributions from the homogeneity and stability of the calibrant.

The degradation of zearalenone in typical food processing environments

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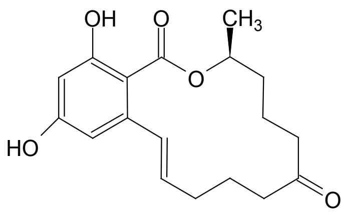
Zearalenone (ZON) is a nonsteroidal mycotoxin with estrogenic activity that can disrupt the function of the endocrine hormone estrogen in animals and possibly in humans. It is produced by *Fusarium* species and frequently found in all major cereal grains as well as in processed food [1]. Zearalenone, 6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)-β-resorcyclic acid lactone, is known to be quite heat stable despite its large lactone ring. However, stronger thermal treatment or extrusion cooking can reduce toxin levels by more than 80 % [2].



ZON

In an attempt to get more information about the fate of mycotoxins under food processing conditions, we recently developed heating experiments with model substances to investigate possible reaction mechanisms [3]. To study the formation of degradation and reaction products as well as the binding of ZON to food constituents, it was heated with the following model substances: α-D-glucose as sugar model, methyl-α-D-glucose as starch model and the amino acid derivates N-acetyl-L-lysine methyl ester and N-acetyl-L-cysteine methyl ester as protein models.

The reaction residues were analysed by gas chromatography/mass spectrometry (GC/MS) and high performance liquid chromatography/diode array detection (HPLC/DAD). Degradation products were isolated by preparative HPLC and characterized by mass spectrometry. Partial structural elucidation was achieved by subsequent 1D and 2D nuclear magnetic resonance spectroscopy.

We demonstrated that ZON can bind to free thiol groups of the cysteine model and to selected peptides by a Michael addition. A fast degradation of ZON was also achieved by amino compounds in a Maillard type reaction at typical food processing temperatures (175-200°C). Further heating experiments showed that ZON was not able to bind or react with the starch model. The degradation of ZON by reducing sugars was also only observed at higher temperatures (> 200°C).

The results achieved with the model system were confirmed by heating experiments with gluten, wheat starch and wheat flour, attributing a significant role to proteins in the degradation process of zearalenone.

[1] Opinion of the Scientific Committee on Food on *Fusarium* toxins, Part 2, 2000.

[2] Ryu, D.; Hanna, M. A.; Bullerman, L. B. Stability of zearalenone during extrusion of corn grits. *J. Food. Prot.* 1999, *62*, 1482-1484.

[3] Seefelder, W.; Knecht, A.; Humpf, H.-U. Bond fumonisin B_1: Analysis of fumonisin B_1 glyco and amino acid conjugates by liquid chromatography-electrospray ionization-tandem mass spectrometry. *J. Agric. Food Chem.* 2003, *51*, 5567-5573.

Determination of fumonisins in maize and maize-based food products depending on different clean-up procedures

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Fumonisins are a group of structurally related mycotoxins of *Fusarium verticillioides* (formerly *Fusarium moniliforme*), one of the most common moulds that is associated with grain and grain-based foods and feeds worldwide. These toxins are most frequently formed in preharvested maize-plants and less in other grains - thus fumonisins are mainly found in all kinds of maize-based foodstuffs.

In consideration of a possible carcinogenic potential for men, present and effective analytical methods for all relevant matrices have to be improved.

Nowadays the determination of o-phenylendiamine derivatives of fumonisin B1, B2 and B3 via HPLC with fluorescence detection is a reliable and effective method. For extraction different mixtures of organic solvents and water were used. Clean-up of the extracts was performed via immunoaffinity-columns (IAC). Especially the performance of IAC is predominant for accuracy of fumonisin determination in different matrices.

In this work, based on a CEN-Method (prEN 14352), commercially available IAC are compared and their capability regarding different elution procedures examined. Furthermore, noticeable aspects during sample preparation as well as during clean-up of different matrices are discussed.

According to EU-Regulations e.g. a maize batch is rejected only if the laboratory sample exceeds the maximum limit beyond reasonable doubt. Therefore, recovery rates have to be taken into account. Considering these facts, the work presents results of matrix-based recovery trials depending on spike levels and natural contamination.

Neue Ansätze in der Immunanalytischen Probenvorbereitung von DON

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Eine der wichtigsten Schritte in der Analyse von Mykotoxinen in komplexen Matrices wie Lebens- und Futtermitteln ist die Probenvorbereitung vor der eigentlichen Bestimmung mittels chromatographischer oder immunanalytischer Methoden. Eine der effektivsten Methoden in der Probenvorbereitung der Mykotoxinanalytik ist die Immunaffinitätschromatographie, da sie auf der selektiven Wechselwirkung zwischen den Mykotoxinen und immobilisierten Antikörpern beruht.

Bis zum heutigen Zeitpunkt sind Probenvorbereitungsmethoden, bei denen Antikörper eingesetzt werden, sehr teuer, da es sich bei den kommerziell erhältlichen Immunaffinitätssäulen um Einwegsäulen handelt, die auch nur zur Anreicherung eines Analyten geeignet sind.

Das Ziel dieser Arbeit war es, verschiedene Strategien zu entwickeln, um die Kosten der Analyse von Mykotoxinen zu senken.

Eine der Strategien ist die Anreicherung von DON mittels Immunultrafiltration kombiniert mit Ultrafiltration. Da generell sehr geringe Mengen von Antikörpern eingesetzt werden, ist diese kostengünstige Methode für die Analyse einer großen Zahl von Proben sehr gut geeignet. Verschiedene kommerziell erhältliche Ultrafiltrationseinheiten wurden auf ihre Anwendbarkeit getestet. Weiters wurde der Einfluss folgender Parameter auf die Wiederfindung von DON geprüft: Medium der Probenaufgabe, Inkubationszeit, Menge der Antikörper, Waschmedium, Waschvolumen, Elutionsmittel, Elutionsvolumen sowie die Bedingungen bei der Zentrifugation. Im Standardversuch wurden zufrieden stellende Ergebnisse erzielt.

Weiters wurden Immunaffinitätssäulen mittels der Sol-Gel Methode durch Einschluss der Antikörper in den Poren einer Silikatmatrix hergestellt. Die Sol-Gel Methode hat gegenüber anderen Immobilisierungsmethoden den Vorteil, dass der Einschluss der Biomoleküle unter sehr schonenden Bedingungen erfolgen kann und daher die Affinität der Antikörper zum Analyten kaum beeinträchtigt wird. Die Sol-Gel-Säulen zeichnen sich durch eine hohe Stabilität aus, sie können ohne Zusatz eines Bakteriostatikums auch bei Raumtemperatur gelagert werden. Die Wiederverwendbarkeit der Sol-Gel Säulen ist ein weiterer Vorteil verglichen mit den kommerziell erhältlichen DON Immunaffinitätssäulen.

Influence of standards, extraction-, clean-up- and detection-methods on the determination of trichothecenes

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Trichothecenes are secondary metabolites produced by several fungi of the *Fusarium* genus. Trichothecenes are responsible for a wide range of toxicity symptoms in animals, causing toxic effects on skin and mucous surfaces, feed refusal, weight loss, vomiting, immunosuppressive and haemorrhaging effects and inhibiting protein biosynthesis.

The Scientific Committee on Food (SCF) established a full tolerable daily intake (TDI) of 1 µg/kg bodyweight/day for deoxynivalenol and confirmed the combined TDI of 0.06 µg/kg bodyweight/day for T2 and HT-2. In the German national food law, tolerance levels for deoxynivalenol, as low as 100 µg/kg in grains and grain products for infants, have been established in February 2004. However limits for deoxynivalenol will be applied by the European Union (EU) from 1 July 2006 (200 µg/kg in infant-products). Further regulations in the µg/kg range for T-2 and HT-2 are currently under discussion and a maximum level will be fixed, if appropriate, before 1 July 2007.

Therefore collection of occurrence data and more investigations in the factors involved in the presence of T-2 and HT-2 in cereal and cereal products particularly in oats and oats products is necessary.

For this reason, the development of reliable and sensitive single and/or multi-toxin-methods is of high priority. In this context HPLC coupled with MS detection has recently become the method of choice, as it offers highest sensitivity, specificity and allows the determination of different trichothecenes within one run. However inherent problems of HPLC-MS analysis are matrix effects, which enhance or suppress the signal intensity of the analyte, depending on the respective sample. These matrix effects have to be compensated by effective clean-up procedures (e.g. IAC), via laborious matrix calibration and/or by the use of suitable internal standards.

In the actual presentation different procedures to determine Deoxynivalenol, T-2 and HT-2 toxin either by HPLC-FLD (after pre- or post-column-derivatization) or by HPLC-MS/MS are presented. Dedicated extraction- as well as clean-up procedures (e.g. IAC, Mycosep®, BondElut® Mycotoxin) are compared and depending on the toxin (e.g. availability of certified standards) respective assets and drawbacks are discussed.

In a laboratory of official authorities, according to EU-Regulations (Commission directive 2005/38/EC; Commission regulation 401/2006) a sample is rejected only if the laboratory sample exceeds the maximum limit beyond reasonable doubt (taking into account the measurement uncertainty and correction for recovery).

For this in the present work different possibilities for the determination of recovery-rates and measurement uncertainty are visualized. Furthermore their respective implication concerning the decision of compliance or not compliance with EU-Regulations is demonstrated.

Development of an isotope dilution mass spectrometry (IDMS) method for the analysis of deoxynivalenol (DON) and its acetylated derivatives

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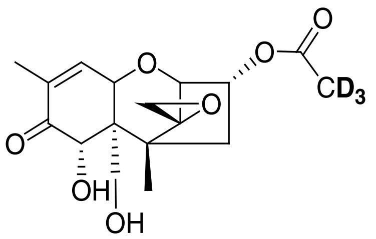
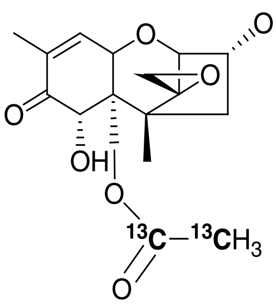
Trichothecenes are secondary metabolites produced by several fungi of the *Fusarium* genus during their growth period. They inhibit protein biosynthesis in eukaryotic cells resulting in numerous toxic effects such as diarrhoea, vomiting and gastro-intestinal inflammation. Considering its occurrence in food and feed, deoxynivalenol (DON) is one of the most important trichothecenes. Its biosynthetic precursors are the acetylated derivatives 3-acetyldeoxynivalenol (3-AcDON) and 15-acetyldeoxynivalenol (15-AcDON) [1].

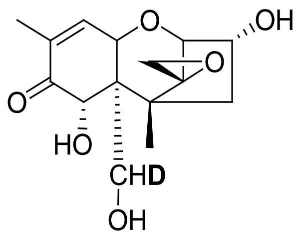
We report the synthesis of stable isotope labeled 15-d_1-deoxynivalenol (15-d_1-DON) [2], 3-d_3-acetyldeoxynivalenol (3-d_3-AcDON) [3] and 15-$^{13}C_2$-acetyldeoxynivalenol (15-$^{13}C_2$-Ac-DON) from 3-AcDON or DON.



3-d_3-AcDON



15-$^{13}C_2$-AcDON



15-d_1-DON

Furthermore, methods for the analysis of DON and 3-AcDON using high performance liquid chromatography – tandem mass spectrometry (HPLC-MS/MS) with stable isotope labeled 15-d_1-DON and 3-d_3-AcDON as internal standards will be presented.

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Effects of practically relevant doses of deoxynivalenol in pig diets

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Introduction: Deoxynivalenol (DON), a mycotoxin formed by several species of the genues *Fusarium*, is a frequent contaminant of grain and consequently of animal feed. Among farm animals pigs are considered to be the most sensitive animal species to the effects of DON. The most prominent effect is the reduction of voluntary feed intake resulting in reduced live weight gain. Moreover, immunomodulatory properties of DON are evidenced. At present the orientation value describing the critical concentration of DON in feeds for pigs is set at 1 mg/kg feed at 88%DM (BML, 2000). This orientation value is mainly derived from data published on the feed intake depressing effect of DON and thus should prevent the economical losses caused by DON contaminated feed in pig production. In practice problems have been also reported at levels below 1 mg DON/kg. Therefore, an experiment with low concentrations was carried out to scrutinize on the effects of DON at a contamination level of 0.5mg and 1.0 mg DON/kg feed.

Material and methods: The inclusion of *Fusarium* toxins into the diets was facilitated by a stepwise exchange of control wheat (0.04mg DON/kg) by contaminated wheat (2.2 mg DON/kg) at a constant wheat proportion in the diet of 50%. The wheat batches were harvested on the experimental station of the FAL, and were of the same variety grown at the same location but the contaminated wheat was inoculated. For a growth experiment a total of 96 pigs were allotted to floor pens and animals were randomly assigned to one of the three diet groups at a mean live weight of 27.3±2.4 kg. Diets and water were offered for *ad libitum* intake for each individual pig. Furthermore a two-phase feeding regime (grower diets from 27 – 72 kg live weight and finisher diets from 72 – 111 kg live weight) was chosen to account for the changing needs of protein of the pigs during this growth period. At a mean live weight of 111 kg 30 pigs (10 from each DON-level) were slaughtered to record organ weights and take samples for the analysis of DON residues and further analyses. A concomitant metabolism trial was carried out with 8 pigs in two balance periods (at mean live weights of 78.1±2.8kg and 90.0±2.6kg) investigating the digestibility of crude nutrients and metabolizable energy of the grower diets containing 50% control wheat or 50% contaminated wheat, applying standardized procedures.

Results and discussion: The overall performance of the pigs in the growth experiment was high with a mean live weight gain of 1.08±0.117 kg/d for all of the pigs. The increasing proportions of contaminated wheat in the diet had no effect on the voluntary feed intake of the pigs over the complete experimental period. But in the finisher-phase of the experiment the live weight gain of the group receiving the diets containing only contaminated wheat tended ($p=0.080$) to be decreased by 7%. However, the feed to gain ratio of the total experimental period was significantly affected by the factor wheat as it was 2.65, 2.62 and 2.73 kg/kg for the groups receiving diets containing the control wheat, control and contaminated wheat and contaminated wheat, respectively. In the metabolism trial the digestibility of organic matter, crude protein, crude fat, N-free extractives and crude fibre and also the metabolizable energy were not affected by the *Fusarium* contamination. Therefore, the effect on the feed to gain ratio observed in the growth experiment may not be ascribed to effects of the on the digestibility of nutrients.

Residues of DON could be detected in most samples of muscle, liver, kidney and serum of the two groups receiving diets containing contaminated wheat. Concentrations in the kidney were the highest (mean concentration for the highest dosed group 11 ng/g).

Influence of deoxynivalenol at low doses administred per os on histopathological image of selected organs of pigs

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Deoxynivalenol is one of the mycotoxins that are most frequently determined in animal feed manufactured in Poland. The examination of histopathological lesions concomitant with deoxynivalenol intoxication is difficult because of common, often synergistic, reaction of this mycotoxin with other Fusarium toxins, such as zearalenone or ochratoxin which has a strong nephrotoxic activity.

The aim of this research was to determine the extent of histopathological lesions in the selected organs of pigs consequent to the intoxication of the different amounts of pure deoxynivalenol.

The experiment was carried out on 12 nursery pigs of mixed breed (Polish White Large x Polish White Ear-pendent) with the average body weigh of 35±2,4 kg. The condition and nutrition status of the nursery pigs was good. During the research the animals were kept in the separate cages.

The experimental nursery pigs were divided into 3 groups: group I (n=4) – control; group II (n=4) – DON was administered at a dose of 0,2 mg/kg b.w.; group III (n=4) – DON was administered at a dose of 0,4 mg/kg b.w.

After the slaughter of the animals, the macroscopic examination was performed and the segments of duodenum, jejunum, ileum, liver and mesenteric lymph nodes were sampled and assigned for the histopathological examination. These segments were fixed in 10% neutralized formalin and embedded in the paraffin blocks. The microtome sections were treated with hematoxylin and eosin stain and then inspected in the light microscope.

In the results of the histopathological examinations of segments of the duodenum, the jejunum, the ileum, the liver and the lymph nodes our attention is attracted by the regressive lesions that are more expressed in the experimental group treated with the highest concentration of deoxynivalenol – vacuolar degeneration of the hepatocytes and necrosis of the individual hepatic cells appeared. It may suggest that the occurrence of the first regressive lesions in the histopathological image of the selected organs is particularly correlated with the liver as the organ that plays the major role in metabolic transformation of deoxynivalenol

Effects of the *Fusarium* toxin deoxynivalenol from naturally contaminated wheat on the *in vivo* protein synthesis of peripheral blood lymphocytes and plasma proteins in the pig

[Auswirkungen des *Fusarium* Toxins Deoxynivalenol auf die *in vivo* Synthese von Plasma und Lymphozytenprotein bei Schweinen]

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Trichothecenes, such as deoxynivalenol (DON), are known to inhibit the protein synthesis *in vitro* by binding at the 60S subunit of eukaryotic ribosomes. Therefore, cells and tissues with high protein turnover, such as lymphocytes and the liver (albumin & fibrinogen synthesis), were suggested to react most sensitively to DON. However, to the author's knowledge this observation was not proven *in vivo* in pigs, which are regarded as the farm animals most susceptible to DON.

A total of 31 castrated male, crossbred German Landrace x Pietrain pigs weighing approx. 40 kg were fed a DON contaminated diet (5.7 mg/kg) either acutely (single dose, n = 7) or chronically (≥ 4 weeks, n = 10) or a control diet (0.1 mg/kg, n = 7). In addition, one group (n = 7) received an intravenous injection of 53 µg DON/kg LW. One hour after feeding, a "flooding dose" of the stable isotope L-[$^2H_5$]-phenylalanine (125 mg/kg LW) was given and frequent blood samples (permanent catheter) were collected over a 60 min period. The molar percent excess (MPE) of plasma free and protein-bound phenylalanine were measured by GC/MS.

No differences could be observed in the plasma concentrations of total protein, albumin, fibrinogen and serum enzymes between the groups. On the other hand, fractional synthesis rates (FSR, %/d) of albumin were significantly decreased by 43, 45 and 26 % and FSR of lymphocytes declined by 27, 19 and 24 %, whereas fibrinogen was not significantly affected after chronic or acute oral and intravenous DON exposure, respectively. Additionally, the absolute synthesis rate (ASR, g/d) of albumin and the proportion of albumin to total body protein synthesis were reduced in the same manner, whereas the albumin secretion time ranged between 6.8 – 34.4 min and was not affected by treatment. In conclusion, the flooding dose technique appeared to be suitable for distinguishing DON-related effects on the protein synthesis, while determination of plasma protein concentrations seemed not to be an appropriate parameter.

Intoxication of premature female pigs with zearalenone – Are pathological investigations useful diagnostic tools?

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Infections of feedstuff with the mycotoxin zearalenone (ZON) and its derivates are considered to be important reasons for fertility problems in pigs due to the estrogenic properties of the toxin. Although pathognomonic alterations caused by a ZON-intoxication in pigs have not yet been found, increasing demands for pathomorphological investigations had to be satisfied in recent years in order to confirm the diagnosis of a ZON-mycotoxicosis. Therefore, the objective of this study was to investigate the effect of orally administered ZON on the pituitary, ovaries and uteri of 70-day-old female piglets. In order to characterize pathomorphological parameters which reflect the influence of ZON on the pituitary-ovary-uterus-axis, (immuno-) histological and morphometrical methods were used. For this purpose, 100 prepubertal female piglets were equally allotted in 5 treatment groups after weaning. Following an eleven-day familiarisation period with uncontaminated pre-trial diet, the piglets got fed for 35 days with diets containing increasing amounts of naturally contaminated maize receiving 0.01 (control group, I) to 0.46 (group V) mg ZON per kg diet. Subsequent to slaughtering, the pituitaries, ovaries and samples of the uterine horns were fixed in formalin and embedded in paraplast. Sections were stained with hematoxylin eosin for routine light microscopy. Immunohistological investigations were performed using antibodies against estrogen receptor (ER), progesterone receptor (PR), follicle stimulating hormone (FSH), luteinising hormone (LH), gonadotropin-releasing hormone receptor (GnRH-R), and proliferating cell nuclear antigen (PCNA). Furthermore, morphometrical examinations were carried out. In the control group all parameters investigated macroscopically and histologically showed a high variability. Compared to group I, the findings in the pituitaries, ovaries and uteri of the ZON-treated groups did not provide evidence for parameters which can be regarded as specific for ZON-intoxication. The high variability of the parameters examined may be caused by the likewise differing functional morphology of the prepubertal ovaries. Statistical analyses showed, that with rising activity of the ovaries, characterized by an increased number of tertiary follicles, the synthesis of ovarian estrogens amplifies, too. Therefore, despite the fact, that literature tells uterine edema to be characteristic for the influences of supraphysiological estrogens (e.g. ZON), areas of severe endometrial edema were visible even in the control group. An increase in the number of ovarian tertiary follicles and in the degree of endometrial infiltration with eosinophils, as a typical effect of ZON, could not be documented in the present study, either. Even with immunohistochemical methods in the pituitary (ER, FSH, LH, GnRH-R and PCNA), ovaries and uteri (ER, PR and PCNA), diagnostically useful, ZON-related alterations could not be found. Not until statistical analyses of the morphometrical parameters of the uterus (uterine calibre, thickness of the lamina muscularis and its structural parts), a ZON-related increase was documented in group IV and V (highest ZON concentrations in the diets). These findings can be explained by a stronger estrogenic effect. As tabulated, race and age dependent standard data of these parameters do not exist, morphometrical analyses of the uterus are inappropriate methods for routine diagnostical investigations. Summing up, pathology can not contribute in any way to the confirmation of the diagnosis of a ZON-mycotoxicosis in 70-day-old female piglets. The only reliable methods, so far, represent analyses of the diets as well as of mycotoxin residues in the animal (especially in bile fluid).

Ochratoxin A – a re- appraisal of mechanisms involved in renal toxicity

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Ochratoxin A (OTA) contamination of cereal products, pulses, coffee, beer, grape juice raisins and wine, as well as cacao products, nuts and spices is a worldwide food safety issue. Previously, two distinct pathological conditions have been associated with exposure to ochratoxin A (OTA): BEN (Balkan Endemic Nephropathy) described as progressive interstitial nephritis resulting ultimately in complete renal failure, and UTT (Urinary Tract Tumours). A recent review of previously published data from animal experiments revealed that in all *in vivo* experiments, renal toxicity preceded tumour development. Subsequently, in recent risk assessments (JECFA, 2001, ILSI, 2005, EFSA 2006), signs of renal toxicity were considered as most sensitive toxicological endpoint, which is in contrast to the previous hazard characterization in which the potential genotoxicity was used to perform a quantitative risk analysis. The decision to use the renal toxicity as basis in the establishment of a NOAEL was made also in consideration of data showing that oxidative stress is obviously the principal mechanisms leading to renal proximal tubule damage, including DNA damage and the formation of adducts as for example C-C8 and O-C8 OTA-dGMP. In addition, OTA has been shown to affect several cell signalling pathways and alters the expression of genes associated with cellular Calcium homeostasis and cellular defence mechanisms against oxidative stress. The latter include a reduction of HNF4α pathways, which are associated with renal carcinogenicity, whereas depletion of Nrf2–regulated enzyme expression is associated with GSH synthesis and the defence potential of a cell against oxidative stress. Whether or not these effects have to be ascribed to the parent OTA molecule alone, or to toxic intermediates originating from a bioconversion of OTA remains a matter of controversial debate.

Oxidative cellular injury is a thresholded process and hence long-term exposure and renal accumulation need to be reassessed. Due to its high protein binding, OTA has in many species, and particularly in humans, a very long elimination half-life. Renal accumulation is facilitated by organic anion transporter proteins, particularly by the SLC22A (formerly OATS) family, having a broad substrate specificity. Moreover, we recently could show that OTA is also a substrate for various efflux transporters (including BCRP), which also contribute to the tissue distribution and excretion. Food ingredients, pre-existing diseases, as well as therapeutic agents modulate the expression of all these transporters. Hence a quantitative risk assessment has to take into account a high degree of uncertainty associated with the individual (patho-)physiological variability in the renal accumulation of OTA.

Ochratoxin A in food: recent developments and significance. Food Additives and Contaminants, 2005; Vol. 22, Suppl 1, 1-107 – and references therein.

Induction of micronuclei by ochratoxin A is a sensitive parameter of its genotoxicity in cultured cells

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Ochratoxin A (OTA) is a well known nephrotoxin and rodent carcinogen with genotoxic properties. The frequent occurrence of OTA as food contaminant necessitates a careful risk assesment for this mycotoxin based also on information on its mode of action and dose-effect relationships. With respect to regulation of carcinogens in general and scientifically based limit values for OTA, it is important to distinguish between direct and indirect modes of genotoxicity and assess dose-response characteristics [1].

As part on an ongoing effort to better characterize the OTA-induced DNA damage and investigate factors which may modulate dose-effect relationships in cells [2], we have now studied the induction of micronuclei in urothelial cells (PUBEC) and in V79 cells: Cultured cells were treated in serum-free medium for about 1,5 cell cycles with graded concentrations of OTA and with DMSO as solvent control. Cytotoxicity of OTA was assessed in parallel cultures by neutral red uptake assay. The frequency of micronuclei in treated and control cultures was scored by fluorescence microscopy after DNA staining in at least 1000 cells per dose level as described previously [3].

OTA concentrations between 0.03 and 1 µM caused a dose-dependent increase of micronuclei in V79 cells (up to 4-fold compared to the control) whilst the lowest tested concentration of 0.01 µM OTA did not induce a higher frequency of micronuclei. Clear evidence for genotoxic effects was also observed in PUBEC cultures treated with OTA concentrations of 0.1 µM and more, although the dose-effect relationship in PUBEC was more variable for several freshly isolated batches of cells. Further investigations are underway to elucidate whether the chromosomal genotoxicity of OTA is primarily due to aneugenic or clastogenic mechanisms.

In conclusion: OTA can induce micronuclei at concentrations below those which are overtly cytotoxic. The shape of the dose-response curve at very low concentrations supports the existence of a threshold for this genotoxic effect. This observation is in accord with a study on the induction of oxidative DNA damage by OTA [4].

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Th2 reactivity and allergic reaction by patulin

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Background: There are evidences from epidemiological studies that an exposure to mold can lead to an increased allergy risk. Further data from experimental studies showing T helper 2 (Th2) mediating immunmodulatory effects of mycotoxins (secondary metabolic products of mold) let assume, that mycotoxins may be at least partly responsible for the allergy mediating effect of mold. In the present study we investigated the influence of the mycotoxin Patulin on Th1/Th2 regulation and for the allergic reaction important induction of IgE response. Patulin is produced by several *Penicillium* and *Aspergillus species*, indoor relevant and food contaminating mold species.

Methods: Periperal blood mononuclear cells (PBMC) of healthy donors were stimulated with monoclonal antibodies (anti-CD3/CD28/CD40) or with the birch pollen allergen Betv1 and exposed with serial dilution of Patulin. Culture supernatants were harvested after 24h and 7d for the measurement of Th1/Th2 cytokines and after 7d for immunoglobulin E (IgE) measurement using ELISA.

Results: Patulin suppressed the cytokine production of CD3/CD28/CD40-stimulated PBMC in concentrations below the cytotoxic range. After 24h the Th1 cytokine interferon-γ (IFN-γ) was stronger inhibited compare to the Th2 cytokines interleukin-4 (IL-4) and IL-13, whereas after 7d a stimulated IL-13 and decreased IFN-γ production was observed. Betv1-stimulated PBMC showed an increased IL-13 and a decreased IFN-γ release under Patulin exposure. An enhanced allergen specific IgE production was determined after 7d.

Conclusion: Patulin affects T cell function in terms of an adjuvant effect resulting in an altered Th1/Th2 balance and subsequent an induction of IgE production. Exposure to low concentrations of these mycotoxins therefore can lead to an increased risk for allergic sensitization.

Effects of broiler limited feeding by aflatoxin contaminated feed on performances

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Aflatoxin B_1 is a very potent carcinogen in many species, including nonhuman primates, bird, fish and rodents. In each species, the liver is the primary target organ of acute injury. It cannot only seriously affect animal health, but it may also have deleterious effects on performances. Poor condition of birds, decreased BW, decreased feed intake, and increased water consumption are described. Besides that, in recent years AFB_1 has received considerable attention due to residues of mycotoxins in poultry products.

The present study was, therefore, designed not only to examine the performances and presence of AFB_1 residues in the broilers fed with contaminated feed, but also to assess the feasibility of causative agents removal aimed at alleviating its harmful effects.

The forty-two days long experiment was conducted on a total of 1000 Arbor Acres broilers, divided into two groups. Both groups of broilers were fed with a commercial feed mixture which consisted of standard feedstuffs and contained enough nutrients regarding the requirements. During the first three weeks of trial, corn naturally contaminated with AFB_1 (0.05 ppm) was used in the amount of 20% in experimental group, while AFB_1 free corn was given to birds in control group. After the period of toxin administration until the end of the trial, broilers from both groups were normally fed with uncontaminated feed. Performances and health status were monitored during the trial, as well as liver injuries and determination of AFB_1 residues using TLC.

In the first phase of trial, broilers in the control groups had an average daily gain of 31, 90 g, average daily feed intake of 45, 24 g and a feed:gain ratio of 1, 42 kg. In the same time, experimental broilers achieved an ADG of 25, 62 g, ADFI of 35, 71 g and a FGR of 1,39kg. During the whole period of trial, control and experimental broilers achieved 49.12 g, 95.24 g and 1.94 kg and 39.71 g, 86.90 g and 2.19 kg, respectively.

For the experimental group the end of the trial was delayed due to lower performances till the weighing standard body weight. Trial lasted two weeks longer but overall performances were unsatisfied (ADG 37, 11 g, ADFI 121, 43 g and FGR 3, 27 kg).

After the period of using contaminated fed, the liver of treated broilers was enlarged, dark yellow colored and tender in consistence. In some cases punctoformes and maculoses extravasation could be seen. In all liver samples residues of AFB_1 were detected in amount of 0, 63±0, 12 ppb.

AFB_1 has negative effects on performances of broilers even in small amounts, but the removal of contaminated feed could alleviate its adverse effects but only to a certain extent.

Gene expression as an indication for mycotoxin biosynthesis

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Mycotoxins are secondary metabolites from filamentous fungi. The biosynthesis of mycotoxins is greatly dependent on environmental parameters like substrate composition, temperature, water activity or pH. This dependence indicates that the genes of mycotoxin biosynthesis are tightly regulated at the molecular level. Usually the mycotoxin biosynthesis genes are not constitutively expressed, but are induced for mycotoxin biosynthesis. At the molecular level, this induction can be determined some time before actual mycotoxin biosynthesis can be measured. However it is also known that not all genes of a pathway show the same correlation in terms of expression and mycotoxin biosynthesis. So if the relationships between gene expression and mycotoxin production of each gene is known, this approach is very well suited to determine very sensitively the influence of environmental parameter on gene expression and thereby on mycotoxin biosynthesis.

A prerequisite for the application of this approach is the availability of gene sequences of the mycotoxin biosynthesis genes. The genes for aflatoxin-, fumonisin- and trichothecene biosynthesis are present in public data bases. The genes for ochratoxin A biosynthesis specific for Penicillia have been determined.

Real Time PCR and microarray analysis have been performed to study the correlation between expression of mycotoxin biosynthetic genes and phenotypic mycotoxin production.

Strategien zur Reduzierung des Fusariumtoxinrisikos im Getreide durch variierte Bodenbearbeitungsverfahren und Sortenwahl

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Verfahren konservierender Bodenbearbeitung spielen bei der Umsetzung guter fachlicher Praxis im Bereich Bodennutzung eine entscheidende Rolle: organische Rückstände vermindern Verschlämmung und Erosion, schonende Lockerung beugt Bodenverdichtungen vor. Von Pflanzenresten der Vorfrucht geht jedoch ein erhöhtes Infektionsrisiko von pilzlichen Schaderregern, u.a. *Fusarium spp.* auf die Folgefrucht aus. Dieses Risiko ist besonders in engen Mais-Getreide-Fruchtfolgen von Bedeutung.

In einem mehrfaktoriellen Feldversuch wurde der Einfluss von gering und hoch gegenüber *Fusarium* anfälligen Silomais- und Getreidesorten in Kombination mit den Bodenbearbeitungsverfahren Pflug, sowie Mulchsaaten mit und ohne Hächseln bzw. mit Lockerung auf die Deoxynivalenol- (DON)-Belastung der Ernteprodukte in der Fruchtfolge Silomais-Winterweizen-Winterweizen untersucht. Die Kombination dieser Maßnahmen sollte aufzeigen, ob ein hohes Maß an Bodenschutz kombiniert werden kann mit der Produktion von einwandfreien Rohstoffen für die Lebensmittel- und Futtermittelproduktion.

Nach Anlage des Feldversuchs in 2002 waren im ersten Versuchsjahr 2003 aufgrund der außergewöhnlichen Trockenheit in den Weizenkörnern nur sehr geringe DON-Gehalte zu finden, die keine Rückschlüsse auf Einflüsse der Bodenbearbeitungsverfahren bzw. der Sorteneigenschaften zuließen.

In 2004 wurden bei der hoch anfälligen Weizensorte nach Vorfrucht Mais 2 bis 3-fach höhere DON-Gehalte (max. 1,78 mg/kg) festgestellt als nach der Vorfrucht Weizen. Bei der gering anfälligen Weizensorte wurden nur nach Vorfrucht Mais nennenswerte DON-Gehalte (max. 0,64 mg/kg) nachgewiesen. Nach Pflug wurden um ca. 40-50% geringere DON-Gehalte im Weizen gefunden als nach Mulchsaaten. Ohne Häckseln der Maisstoppeln wurden ca. 30-40% höhere DON-Gehalte beim Weizen festgestellt als mit Häckseln der Maisstoppeln. In 2005 wurden weitgehend ähnliche Tendenzen festgestellt, wobei das Niveau der Toxinbelastungen in den Weizenkörnern etwas niedriger lag als 2004.

Die Unterschiede in den Toxinbelastungen der Weizenkörner korrespondierten mit den Restmengen an Maisrückständen auf der Bodenoberfläche zum Zeitpunkt der Weizen-blüte. In den 3 Versuchsjahren variierten die Maisreste ohne Häckseln der Stoppeln zwischen 70 bis 120 g/m^2 Bodenoberfläche. Demgegenüber waren die Maisreste mit Häckseln der Stoppeln um 50 bis 60% verringert, so dass mit dieser Maßnahme eine deutliche Reduzierung des Infektionspotenzials erreicht wurde.

Das Risiko eines Überschreitens der EU-Höchstmengen für DON in unverarbeitetem Getreide (1250 µg DON/kg) war an diesem Standort nur beim Einsatz einer hochanfälligen Weizensorte nach Mais und Mulchsaat ohne Häckseln der Maisstoppeln gegeben. Bei Anwendung von Mulchsaatverfahren werden rottefördernde Zusatzmaßnahmen empfohlen.

Einfluß von Bodeneigenschaften, Bestandesfeuchte und Feldheterogenitäten auf den Mykotoxingehalt von Winterweizen

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Der Deoxynivalenol-Gehalt in erntefrischen Körnern des Winterweizens wird durch mehrere Faktoren unterschiedlich stark beeinflußt: Neben der Witterung zum Zeitpunkt der Blüte und in der Abreifungsphase sind das vor allem ackerbauliche Maßnahmen wie Bodenbearbeitung, Vorfrucht, Sorte und Pflanzenschutzmitteleinsatz. Dabei haben vielfältige Untersuchungen gezeigt, dass diese Faktoren eng miteinander gekoppelt sind und dadurch eine sichere Vorhersage der Mykotoxinbelastung erschweren.

Im Untersuchungsgebiet im Norden des Landes Brandenburg (Uckermark) sind die Winter-Weizenanbauflächen durch ihre Größe von oftmals 30-60 ha und durch ihre teilweise Relefierung gekennzeichnet. Die Kuppen und Senken innerhalb dieser großflächigen Felder zeigen durch ihre unterschiedlichen Bodeneigenschaften ein optisch unterschiedliches Pflanzenwachstum. Die Pflanzendichte, die Pflanzenhöhe, der Zeitpunkt der Blüte und die unterschiedliche Dauer der Abreife des Korns sind auf Kuppen und in Senken unterschiedlich. Die vorgestellten Untersuchungen gehen deshalb der Frage nach, ob es durch diese Feldheterogenitäten zu unterschiedlichen Belastungen des Winterweizens mit Mykotoxinen 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, Winterweizen, Mais) ausgewählt und auf diesen die Probenahmeorte auf den Kuppen, am Mittelhang und in den Senken festgelegt. An ausgewählten Punkten wurden Bodenproben entnommen und Bodenprofile bestimmt sowie die Bestandesfeuchte gemessen.

Die Auswertung der Deoxynivalenol-Analysen in den Winterweizenkörnern des Jahres 2004 zeigte den hauptsächlichen 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 teilweise 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.

Investigation of various adsorbents for their ability to deactivate Aflatoxin B_1

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The contamination of animal feed with mycotoxins represents a worldwide problem for the animal industry. Amongst the major mycotoxins affecting animal health is aflatoxin B_1 (AfB_1), a very potent mycotoxin commonly produced by *Aspergillus flavus* and *A. parasiticus.* The most applied method for protecting animals against aflatoxicosis is the utilization of clay minerals mixed into the feed in order to bind the mycotoxins efficiently in the gastro-intestinal tract. This strategy has been given considerable attention in the last years and HSCAS (hydrated sodium calcium aluminosilicates) are amongst the adsorbents reported to adsorb AfB_1 with high affinity and high capacity and to prevent animals from aflatoxicosis.

In the course of a research project about 60 different minerals, mainly naturally Ca-, Na- or acid treated- bentonites, were examined to obtain information concerning adsorption efficacy, specificity and the mechanism of the adsorption process. As a reference a HSCAS product, whose characteristics are very well described in literature, was used in parallel for all experiments.

Adsorption experiments were performed in buffer solutions in order to evaluate the ability to bind AfB_1 at various pH-values and to determine the equilibrium time. To investigate the strength of binding, already bound AfB_1 was extracted with methanol and based on the results the chemisorption index was calculated. Moreover isothermal analysis to determine values for the maximum adsorption capacity and the type of adsorption process (chemisorption/ physisorption) was performed. To get information on binding under physiological conditions and interactions with GI-matrix adsorption experiments in simulated gastrointestinal (GI) fluid and real gastric juice from swine was carried out. Special attention was paid to the formation of AfB_{2a} during experimental conditions. In addition, unspecific binding capability of the materials regarding selected vitamins was examined. The investigations resulted in the identification of an adsorbent with improved performance compared to the reference. Three other materials were comparable to the reference.

Physical parameters of the clay minerals such as cation exchange capacity (CEC), and particle size showed no correlation with the adsorption capacity. Mineralogical characterisation of the clays using X-ray diffraction (XRD) as well as simultaneous thermoanalysis (differential scanning calorimetry (DSC) and thermogravimetry (TG)) was conducted in order to find out a relationship between the capacity to bind mycotoxins and mineral composition. In some cases the mechanism of aflatoxin binding was investigated using Fourier Transform Infrared Spectroscopy (FTIR).

Based on the obtained results highly promising sorbent materials were ranked for further *in vivo* studies, since all mycotoxin binding agents should be tested for efficiency, safety and performance in sensitive animals before their inclusion in animal diets.

Initial microbiological assessment of school facilities

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In a large group of pupils that are the same age the risk of all infections transferable among individuals is very high. The status of air and walls in small rooms in which the children stay for many hours per day can pose a potential threat to their health and the adults working at school. As a consequence a study was made aiming at determination of the characteristics of toxins producing fungi in selected school facilities.

The study material consisted of fungi obtained from the air by sedimentation method and from walls by surface swabs technique. The samples were collected from facilities of two schools: the classroom, corridor, female and male toilets, changing room, sports changing room and shower. In total 321 samples were collected from 13 rooms. The study was carried out in May 2005 after the end of the heating season. Fungi were cultured on Chapek-Dox medium at three temperatures: 25, 37 and 40^{0}C. For MTT cytotoxicity test 33 isolates of fungi were selected.

The total of 379 isolates of mould fungi, yeasts and yeast-like fungi belonging to 32 genera were obtained from 312 samples. The most frequently isolated fungi belonged to genera: *Aspergillus, Penicillium* and *Cladosporium*. 72 species of fungi were identified in the test samples, 33 of them were isolated at least twice. It is possible to identify a group of dominating species with a similar frequency of occurrence. It includes: *Cladosporium herbarum, Aspergillus fumigatus Penicillium chrysogenum* and *P.citrinum*. The largest number of fungi was obtained at room temperature – 236, fewer by a half at human body temperature – 121 and only 22 isolates of fungi were obtained at 40^{0}C. Among 33 isolates subjected to MTT test, all showed production of toxins. Highly toxic isolates were obtained from corridors, shower and general changing room. The toxin of *A. crystalinus* was still cytotoxic in the 7th step of dilution at IC50 = 0,488 cm^2/ml. Similar cytotoxicity was shown by secondary metabolites of *P. chrysogenum*. On the other hand, *A. spinulosum* showed toxicity beyond the range, i.e. no safe dilution of the toxin was recorded.

Although the results obtained require formulation of further research tasks aiming at determination of conditions of toxin forming fungi appearance in the studied environments, one thing is sure – the selected school facilities represent a potential threat for health of school children and employees.

Micotoxicity of alergenic moulds in dwelling buildings

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Human organism can react to various mould's product like: proteins, β-(1,3)-D-glucans, microbial volatile organic compounds (MVOCs), micotoxins, also another microorganism exist with moulds, for example mite (*Acarina*). It's self-evident that most allergenic moulds are in possession of ability to synthesis secondary metabolites. More important still that almost micotoxins has immunosuppressive interaction on human immune system what make easier attacking organs like: kidneys, liver, peripheral nervous system.

On Lubuskie Province it was stated acting 76 moulds species in housing developments, therein 10 species with numerous attendance. From among of them can mention 6 species, which in order from the most allergenic caused state of inflammation: *P. chrysogenum, A.niger, C. herbarum, A. versicolor, A. alternata, A. flavus.*

Into biotoxicity research it was selected 4 species moulds therein 3 from genus *Aspergillus,* one from genus *Cladosporium* (Tab.1).The strains of selected moulds were examined biotoxicologically using the test with *Dugesia tigrina* Girard (*Plathelminthes, Turbellaria, Tricladida, Paludicola*).

Tab.1. The results of biotoxicological tests using *Dugesia tigrina* Girard

Mould species	240-h LC 50 (%)	240-h LC 50 (mg dm^{-3})
Aspergillus niger	0.56	69.5
Aspergillus versicolor	0.57	70.9
Aspergillus flavus	0.85	106.0
Cladosporium herbarum	1.24	155.2

All of the moulds extract were for *Dugesia tigrina* Girard toxic.

The most dangerous natural toxins synthetizing from moulds are from genus *Aspergillus*. This create a double threat from health people residing in dwelling apartments infected with moulds (toxic and allergenic).Tests results showed, that this moulds are the most toxic (Tab.1).

C.herbarum is able to synthesis cladosporic acid. From *Cladosporium sp.* it was isolated several metabolites with antifungial effects, plant growth regulators and other. This is species which often exist in dwelling buildings with numerous attendance. At the same time is near by *A. alternata* the most well known allergenic mould. In the investigations *C. herbarum* was the least toxic species mould. The examinations proved priority biotoxicicological tests over chromatographic analyses, because bioassays show complete toxicity. Conformed test with *D.tigrina* Girard proved good and competitive to other method to discovered toxic-productive strains and to state their productivity.

Posters

Mycotoxins levels in combined fodder

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Exposure of livestock to mycotoxins in fodder 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 temperate regions: Canada, USA and most European countries as Lithuania, aflatoxins (AFL) are not considered to be a major problem. But climate is getting warm and AFL content is increasing in Lithuanian grain. Deoxynivalenol (DON), zearalenone (ZEN), ochratoxins are found more frequently.

Fodder samples were taken from animal farms in the period of 2004-2005, when problematic cases had been detected. The above mentioned mycotoxins were found in combined fodder, also grains of winter and summer crop. Fodder can be contaminated with other toxins (bacterial) so fodder toxicity can evaluate with biological methods. There are used cytotoxicity screening assay. Contaminated fodder with mycotoxins was evaluated *in vitro* with differential mammalian cell cultures.

In 2004-2005, AFL content in combined fodder was from traces to 0.003 ppm, ZEN – from traces to 0,700 ppm, DON – from 0.050 to 1.00 ppm, T-2 toxin – from 0.005 to 0.125 ppm, ochratoxins were detected only traces. In grains mean AFL content was 0,001 ppm, ZEN – 0,450 ppm, DON – 0.300 ppm, T-2 toxin – 0.010 ppm, ochratoxins – 0.005 ppm.

Estrogenic and reproductive disorder effects were for pigs when ZEN content was 0.700 ppm in fodder. If DON content was 1.000-1,750 ppm in fodder, we could see result in toxicological interactions: loss of appetite, feed refusal, clinically obscure changes in growth, immunosuppression. Mycotoxicological analysis showed that mycotoxins are passed from fodder materials to combined fodder. Grind, mix of fodder materials can increase mycotoxins content in combined fodder. Mycotoxin contamination of fodder materials cannot be avoided under the current production methods. Any regulatory measures will affect only the quality of commercial fodder materials, but not on-farm products. Future attempts should focus on the development of detoxifications strategies.

New examinations of mycotoxin carryover to Cocoa Beans

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Mycotoxins are secondary metabolites which can form on various foodstuffs through the growth of certain fungi. Ochratoxin A and the aflatoxins B1, B2, G1 and G2 have been detected in low concentrations in cocoa and cocoa products [1-3]. Compared to other raw materials, the mycotoxin content levels found in cocoa are not only lower, but their distribution within a batch is distinctly more homogeneous. So-called "hot spots" were not determined in cocoa [2]. A key issue affecting distribution would seem to be the time point when contamination with mycotoxin-producing moulds and the formation of mycotoxins took place. It is assumed that in the case of cocoa the contamination is not concerning the individual beans but the whole fermentation units.

A model test was to provide information on the process by which a possible carryover of the above-mentioned mycotoxins to cocoa beans occurs during the fermentation process. For this purpose fresh cocoa beans were left to soak in an artificial mycotoxin-containing fermentation solution. The mycotoxin levels in the cocoa beans were regularly determined over a period of 12 days. The findings are presented.

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[3] Tafuri A, Ferracance R, Ritieni A, Ochratoxin A in Italian marketed cocoa products. Food Chemistry, 2004, 88, 487-494

No OTA in fresh Cocoa Beans

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Ochratoxin A (OTA) is a secondary metabolite of toxigenic moulds, particularly of the genus Aspergillus ochraceus. It typically occurs in native types of cereal, nuts, coffee, and spices, etc. In addition, positive findings of this mycotoxin have also been made in cocoa [1, 2]. There is no previously published data available on the source and development of ochratoxin A producing moulds and ochratoxin A itself in cocoa nor on the exact contamination timepoint within the cocoa extraction process or during cocoa processing.

Within the scope of a European (CAOBISCO) research project, the LCI examined a selection of fresh and undamaged cocoa pods from various growing regions for mycotoxin OTA content. Both the cocoa beans and the pulp clinging to them were examined. In addition, a small selection of damaged or mouldy cocoa pods were included in the examination. It was shown that the ripening phase of healthy cocoa pods from the tree up to being harvested was not a critical timepoint for the generation of the mycotoxin OTA. Mycotoxin ochratoxin A was not detectable in any of the analysed cocoa pods [3]. The findings are presented.

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The fate of fusarium toxins in the course of the synthesis of bio-ethanol from grain contaminated with fungi

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Bio-ethanol as a fuel has been produced in plants of our region from rye and wheat since 2005. As a co-product a feed-stuff reach in protein is available, which should be put up for sale. The farmers must fulfil some requirements concerning the quality of the offered grain. Especially the content of mycotoxins should be lower than the EU-regulation limit.

By means of a modelling experiment we followed the fate of Deoxynivalenol (DON) and Zearalenone (ZEA) in the production process. From the results of these investigations recomodations should be derived with respect to the maximum value of toxins that could be tolerated in the grain.

The experiment has been performed at the «Versuchs- und Lehranstalt für Brauerei in Berlin e.V.» 33 kg Triticale with a concentration of 3,2 mg/kg DON and 0,035 mg/kg ZEA were treated with water and enzymes which yielded the mash. Then the addition of yeast brought about the mixture to fermentation. Finally, the alcohol was obtained by destillation.

The residue (destiller`s dried grains with solubles = DGGS) was dried by centrifugation to a contents of 25 % dry matter and put in silage tubes. In each part of the whole process test samples are taken and the contents of DON and ZEA was determined. After three month the silage were scraped out from the tubes and analyzed, too. The mycotoxins were analyzed by HPLC with DAD and HPLC with Fluorescence, corresponding to the VDLUFA-methods.

Results :

The balance of the mycotoxin contents showed no losing DON in the process. But for the ZEA resulted a defizit of about 60 % after the destillation. Because of the mass loss during the starch fermentation (33 kg grain yield 8 kg draff (DGGS)) the mycotoxin contents grows with a factor of 2 or 3.

The DON is well resolved in water an is therefore partially washed out by filtration of the mash. But, because the whole process is performed in practice as a cycle, the filtrate with the DON is permanently recycled in the brewery process.

The DGGS is mainly used for cattle feeding as a part of the daily ration in amounts of about 15%.

Assuming the lowest recommended boundary values of DON : 2 mg/kg and ZEA : 0,25 mg/kg for the cattle, a DON concentration of about 4,4 mg/kg and a ZEA concentration of about 0,5 mg/kg could be tolerable in grain under the supposition that the rest of feed is free from mycotoxins.

Because one cannot assume, that the rest of the feed (without DGGS) is free from mycotoxins, and taking in considerations an input of mycotoxins in the brewering process through contaminated filtrates (cycle), the mycotoxin concentration in the original grain should not exceed the legal EU boundary values of 1,25 mg/kg DON and 0,1 mg/kg ZEA.

But, we also decided that is no reason to demand mycotoxin concentrations below these values to produce a DGGS of high quality wich fulfil the demands of feed laws.

Mycotoxins in several Polish food products in 2004-2005

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In 2004-2005, 389 samples of several selected Polish foods collected from Warsaw market and taken from food producers were analyzed on presence of aflatoxin B_1, B_2, G_1, G_2 (Afla), ochratoxin A (OTA), zearalenone (ZEA) and deoxynivalenol (DON). After clean-up of extracts on immunoaffinity columns, RP-HPLC using fluorescence and UV detectors was performed.

Investigation in 2004
Aflatoxins in nuts and spices (80 samples): aflatoxins were detected in 18 (23.7%) out of 76 samples of nuts (hazel nuts, peanuts, walnuts etc.) at 0.02 (near LOD) to 7.8 µg/kg. The mean concentration of aflatoxin B_1 was 0.13 µg/kg. The highest level of aflatoxin B_1 was 7.8 µg/kg (one peanuts sample). In case of culinary spices (mixes) all samples – 4, showed aflatoxins in ranges 0.02 – 0.4 µg/kg.

Aflatoxins in cereal products (21 samples): in 3 (14.3 %) of samples of cereal products (flour, grain, bread, cereal breakfast etc.) aflatoxins were detected, at the levels near the LOD of analytical method. The maximum concentration of Afla B_1 was 0.02 µg/kg.

Ochratoxin A in cereal products, raisins and coffee (53 samples): *o*chratoxin A occurred in the majority of cereal products – 73 % (flour, cereal flakes, bread etc.) at concentrations ranged from 0.02 to 1.71 µg/kg with mean 0.14 µg/kg. All coffee and raisins samples (16) showed OTA at 0.02- 5.7 µg/kg – this maximum level, refers one coffee sample.

Investigation in 2005
Aflatoxins in nuts, spices, cereal products and others (155 samples): aflatoxins occurred in 17 (25 %) out of 68 samples of nuts (hazel nuts, peanuts etc.) at concentrations ranged from 0.02 to 1.16 µg/kg (Afla G_1). The mean level of Afla B_1 was 0.04 at maximum concentration of 0.78 µg/kg (peanuts). In 15 % of samples all aflatoxins were detected. Read paprika (dried) showed aflatoxins in 14/15 of samples at concentrations ranging from 0.02 to 8.16 µg/kg (Afla $B_{1)}$ – with the mean 2.4 µg/kg. In case of pepper and curry, 25 % of samples were contaminated by aflatoxins but the levels of contamination were low, 0.1 – 0.62 µg/kg (Afla B_1). Concentrations of aflatoxins in spices (mixes) varied from 0.04 to 6.35 µg/kg (Afla B_1 in one peanut sample) at higher frequency of contamination – 80 % of samples were positive. Cereal products including flour, bread, cereal breakfast etc. showed a moderate level of contamination – in 18.5 % of samples the concentrations of Afla B_1 were at 0.04-1.35 µg/kg. In the case of the vegetables, raisins, fruits (dried), aflatoxins were detected in 4 out of 22 samples at 0.02-0.48 µg/kg.

Ochratoxin A in cereal products, raisins, fruits, coffee and cocoa (66 samples): ochratoxin A was detected in 40 samples of cereal products (74 %) out of 54 investigated samples, its concentrations varied from 0.02 to 8.26 µg/kg (one wheat bran sample). Higher concentrations of OTA showed one sample of rye flour – 4.46 µg/kg and one sample of bread- 5.83 µg/kg. The highest levels of OTA occurred in one sample of coffee, 11.9 µg/kg and cocoa, 5.6 µg/kg. In products such as raisins and dried fruits, levels of OTA were low, near 0.02 µg/kg.

Zearalenone and deoxynivalenol in cereal products (14 samples): ZEA was detected in 4 out of 9 samples of wheat flour, its concentration ranged from 5.7 to 19.8 µg/kg. DON was detected in one out of five samples of flour at 29.5 µg/kg (rye flour).

In general, almost all investigated foods in 2004-2005 showed very low concentrations of mycotoxins – below of maximum levels permitted in EU countries.

Deoxynivalenol the most often mycotoxin dedected in the fodder in 2005 year

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The aim of researches were showing the frequency following five mycotoxins in fodders in 2005 year: deoxynivalenol (DON), aflatoxins total (AfT), ochratoxine A (OTA), zearalenone (ZEN) and Toxine T-2 (T-2). The results were gathered in Laboratory of Veterynary Diagnostic in Olsztyn. Mycotoxins were dedected by HPLC method using affinitive columns by Vicam® Company. Totally among 560 samples which were dedected only 9,3% were free from mycotoxins, 41,8 % had one mycotoxine, 48,9% from two o three mycotoxines. In the samples, the most often was present the DON (in 86,6% ofsamples) and ZEN (in 78,0% of samples). The concentration of mycotoxines showed in table 1.

Table1. Concentration of mycotoxines in fodders in 2005 year (μg/kg)

Mycotoxine	No of samples	$\bar{x}$	sd	max. conc.
DON	485	164,7	228,4	1831,0
OTA	363	0,4	5,9	111,1
ZEN	437	27,2	87,0	1373,0
AfT	175	0,3	4,0	53,4
T-2	14	87,7	69,9	206,0

Exepted DON the all mycotoxines had low average concentrations. However the maximal concentrates indicate that each kind of mycotoxine sometimes can be danger for animals. July in 2005 was cold and rainly. Such the climate condition is optimal to deweloping of fusarium fungi. Hence the higher concentration of DON mainly in III and IV quarters of year was noted. Seasonal the higher concentration of DON was noted in 2003 too (104 μg/kg; sd 156). Similar phenomena was observed in ZEN in 2002 in grains (166,6 μg/kg; sd 424).

A Survey of Aflatoxin and Deoxynivalenol in Rice and Barley in South Korea

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This study was conducted to monitor of aflatoxin and deoxynivalenol from rice and barley in south Korea. 126 brown rice samples were collected at harvest from 30 different paddy fields in 2004. 80 polished rice and 55 barley were purchased from commercial markets in 2005. Samples were ground using Romer Series ®™ mill. The presence of mycotoxins were determined by Enzyme-Linked Immunosorbent Assay (ELISA), and the positive samples from ELISA were confirmed using High Performance Liquid Chromatography (HPLC). The limit of quantitation for aflatoxin was 1.0 ppb in ELISA and 0.3 ppb in HPLC, and the recovery rate was ranged from 73 to 109% in ELISA and from 72 to 87% in HPLC, respectively. The result of ELISA showed that 6 out of 126 brown rice samples, 1 out of 80 polished rice samples were positive. However, aflatoxin was not detected in the 7 positive samples by HPLC. The limit of quantitation for deoxynivalenol was 250 ppb in ELISA and 100 ppb in HPLC, and the recovery rate was 92% in ELISA and 80% in HPLC. The result of ELISA showed that 2 out of 126 brown rice samples and 1 out of 55 barley samples were positive. However, deoxynivalenol was detected only 1 barley sample in the 3 positive samples by HPLC. It was discussed that more systematic and periodic monitoring is needed.

Investigation on species of *Fusarium* on Wheat and Rye in Bavaria (Germany) since 2003

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In the year 2003 a monitoring program in Bavaria has started. 190 samples of wheat and 80 samples of rye were examined yearly for infections of *Fusarium* species and desoxynivalenol (DON) content. For identification of the species 200 surface disinfected kernels per sample were plated on different media. After 10 days of incubation at 22°C under black light the number of kernels infected with *Fusarium* spp. were determined. Identification of the species was carried out by microscopy. With an infection rate up to 72% *F. graminearum* is the most dominant DON producing fungus on wheat. In the case of rye *F. graminearum* and *F. culmorum* occurred on nearly the same level of 65%. Percentage of kernels infected with *F. graminearum* ranged from 0% – 18,5% among the samples of wheat and 0% - 8,5% among the samples of rye. Other predominant species isolated from grain were *F. poae*, *F. avenaceum*, *F. equiseti*, *F. tricinctum* and *Microdochium nivale.* Studies in the 1990`s support our results that *F. graminearum* is the most important DON producing species concerning of *Fusarium* head blight (FHB) on wheat in Bavaria. One reason for this observation is that crop rotations of wheat and maize which is also an excellent host for *F. graminearum* are widely practiced in Bavaria favouring the propagation of this fungus.

Production of mycotoxins by selected *Fusarium graminearum* and *F. culmorum* isolates cultured on rice and wheat

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Five isolates of *Fusarium culmorum* and 5 isolates of *F. graminearum* were cultured on whole wheat and polished rice grain. These isolates were previously used for inoculation of cereal heads and showed high pathogenicity to wheat and triticale. Isolates were grown on Petri plates with PDA medium and next transferred to Erlenmeyer flasks with autoclaved grain. Flasks were incubated over one month in darkness at temperature about 25°C and shaken daily to ensure uniform growth of mycelium in grain medium.

After incubation period grain overgrown by *Fusarium* mycelium was autoclaved, lyophylised and finely ground. One gram of ground material was extracted with 25 ml of acetonitrile:water (84:16 v/v). Five millilitres of extract was purified on Mycosep SPE column (Romerlabs) and analysed using TLC and HPLC methods for *Fusarium* mycotoxins: deoxynivalenol (DON), 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, nivalenol (NIV) and zearalenone (ZEA).

Fusarium isolates cultured on wheat medium produced more abundant mycelium and some sporulation was observed. On rice medium mycelium was less abundant and no sporulation was observed. Weight of samples after four weeks of incubation reduced twofold to six fold (on rice).

All isolates produced DON on both media, and some produced also 3-acetyl derivatives of DON. Only 1 isolate of *F. graminearum* produced both DON and NIV on wheat medium. ZEA was produced in considerable amounts by 4 isolates of *F. culmorum* and 3 isolates of *F. graminearum*. ZEA was produced in higher concentrations on wheat grain compared to rice, whereas opposite situation was observed for DON production.

High variability in amount of produced mycotoxin was observed. The highest content of DON produced by *F. graminearum* and *F. culmorum* on rice were 10400 ppm and 9000 ppm in sample (weight after incubation), respectively. The highest concentration of ZEA observed for *F. graminearum* and *F. culmorum* cultured on wheat were similar (ca. 1000 ppm).

Untersuchungen zu DON-Gehalten in Getreideerzeugnissen aus dem deutschen Einzelhandel im Jahr 2006

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Deoxynivalenol (DON) ist in Europa eines der am häufigsten vorkommenden Mykotoxine in Getreide und Lebensmitteln auf Getreidebasis. Die Festlegung von tolerierbaren Tageshöchstaufnahmen (TDI: 1 µg/kg Körpergewicht und Tag) durch den wissenschaftlichen Ausschuss für Lebensmittel der Europäischen Union führte in 2004 zur Einführung von Höchstmengen für dieses Toxin in Lebensmitteln in Deutschland und ab 2006 in der Europäischen Union. Die in den letzten Jahren durchgeführten Untersuchungen auf DON in Lebensmitteln auf Getreidebasis in Deutschland haben gezeigt, dass Erzeugnisse aus Weizen, insbesondere aus Hartweizen, relativ häufig eine hohe DON-Belastung aufweisen. Im Zeitraum Januar bis April 2006 wurden 81 Proben (Weizenmehle, Weizengrieß, Teigwaren) aus dem Einzelhandel im Raum Giessen mittels Enzymimmuntest (EIA) untersucht. Die Nachweisgrenze in diesem Testsystem lag bei 10 µg/kg, die Wiederfindungsraten lagen bei 81 bis 110%. Die Ergebnisse zeigten - im Vergleich zu früheren Jahren - eine deutliche Reduktion der DON-Gehalte in allen untersuchten Getreideerzeugnissen. Die Medianwerte für DON in Weizenmehl, -grieß und in Teigwaren lagen jeweils unter 50 µg/kg. DON wurde in keiner Probe über der zulässigen Höchstmenge nachgewiesen.

Determination of zearalenone and its metabolites in urine, plasma and faeces of horses by LC-MS

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A method for the sensitive and selective determination of zearalenone and its metabolites in urine, plasma and faeces of horses by high performance liquid chromatography and atmospheric pressure chemical ionisation (APCI) mass spectrometry (MS) has been developed. A one step sample clean-up by an immunoaffinity column was sufficient for plasma samples, whereas urine and faeces samples had to be prepared by a combination of a solid-phase extraction (SPE) and an immunoaffinity column (IAC). The method allows the simultaneous determination of Zearalenone (ZON) and all of its metabolites; α-Zearalenol (α-ZOL), β-Zearalenol (ß-ZOL), α-Zearalanol (α-ZAL), ß-Zearalanol (ß-ZAL) and Zearalanone (ZAN). Dideuterated zearalanone (D_2-ZAN) was used as internal standard. Recovery rates between 56 and slightly above 100% were achieved in urine samples, and more than 80% in plasma and faeces samples. The limits of detection ranged from 0.1-0.5 µg/L or µg/kg, the limits of quantification from 0.5-1.0 µg/L or µg/kg. An effective sample preparation clearly enhances the sensitivity of the method. This, however, depends on the nature and complexity of the matrix. Although a one step clean up by IAC was sufficient for plasma samples its limitations became apparent for other complex biological samples like urine and faeces which could only be analyzed after a clean-up combining SPE prior to IAC.

Occurrence of Conjugates of Deoxynivalenol, Nivalenol and Zearalenone in wheat

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Field samples of wheat naturally contaminated with *Fusarium* spp. were analysed for conjugates of the mycotoxins deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZON). In particular, the proof of glucosilated compounds was of major interest. The study was accomplished by metabolism studies of plant cell cultures. In addition, standards of the glucosides were produced to quantify all samples by LC-MS/MS analysis.

Metabolism studies with a variety of cell cultures have demonstrated that plant cells were able to take up and metabolise deoxynivalenol, nivalenol and zearalenone. For each of the three mycotoxins the formation of the glucoside was proven by characteristic MRM-transitions. In addition, after the separation of the glucosides by semipreparative chromatography the samples were digested by β-glucosidase, resulting in the appearance of the aglucon and the disappearance of the glucoside peak. Furthermore the structure of zearalenone- and deoxynivalenol-glucoside was characterized by ^{1}H NMR and ^{13}C NMR. This result supports the assumption that glucosides of the three mycotoxins were initially formed by plant cells.

All glucosides detected in plant cell culture metabolism studies were found in wheat samples (n=190) as well. Their retention times and ion fragmentation patterns in LC-MS/MS analysis were identical to those of the standards which had been produced by plant cells. The frequency of glucoside detection in wheat samples varied between 14 % and 79 %. However, concentrations of glucosides were generally very low compared to the aglucon contents. A correlation between deoxynivalenol and deoxynivalenol-glucoside could be shown (r^2= 0,91; b=0,023).

Finally some wheat samples were digested by β-glucosidase or α-amylase or both to cleave the mycotoxins or their glucosides from starch components of the cell walls. However, no increases in deoxynivalenol, nivalenol or zearalenone content could be observed in this experiment.

Content of Mycotoxins (Aflatoxins, Ochratoxin A, Fusariatoxins) found in grains of European origin over the last 7 years

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Introduction

The quality of cereals is of great importance for consumers and producers of flour and bakery products because of their consumption in high quantities. As the quality of the cereals can be affected by occurrence of certain contaminants, the German Mill Association (VDM) initiated a monitoring program on contaminants in cereals for their members in 1998.

The VDM entrusted the SGS Laboratory Hamburg with the performance of an analytical monitoring program of contaminants for German and other European mills for cereals and flours. Within this program a wide range of mycotoxins were analysed in over 3000 samples of whole wheat, wheat flour, rye, barley, spelt and durum wheat. Following mycotoxins were analysed: Aflatoxin B_1,B_2,G_1,G_2; Ochratoxin A, Fusariatoxins like Deoxynivalenon (DON), 3-Acetyl-DON, Zearalenon (ZON), Nivalenol (NIV), HT2-Toxin, T2-Toxin, Diacetoxyscirpenol (DAS), FX-Toxin. The results of the mycotoxin levels from the collected data of the last 7 years are presented.

Methods

Aflatoxin B1,B2, G1,G2: DIN EN 12955, HPLC-FLD

Ochratoxin A: DIN EN 14132, HPLC-FLD

Fusariatoxins: Inhouse method, extraction and clean up with Romer columns, ZON with immunoaffinity columns, measurement by GC/MS

Materials

The monitoring data are based on unprocessed whole grains. Each participant (mill) is sending at minimum one sample per 12.500 tons of cereals for the analysis. The grain samples are coming from more than 150 mills and are mostly of German and Austrian origin. In addition the German DRV and BVA are participating in this program. In general the samples are drawn from representative loadings in responsibility of the sender.

Results

Analyses, data processing, anonymization, statistical evaluation and reporting are carried out by SGS. Selected parts of the monitoring results are presented.

The detailed results show that the content of Aflatoxins and Ochratoxin A are rather low in the analysed samples over all years. The contamination of DON and ZON in wheat decreased in the last years because of continuous survey, less rainy summers and GMP instruments used in agricultural practise.

Conclusion

The monitoring program of the European mills is a perfect instrument to survey the quality of the grains concerning the mycotoxin content. The development of the contamination level can be tracked easily and necessary actions can be taken in time.

Aflatoxin M1 and M2 in Dutch infant formula

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Aflatoxin B1 and B2 contaminated feedstuffs can result in milk (products) contaminated with aflatoxin M1 and M2. Conversion ratios vary from 0.3 to 1%. Aflatoxin M1 is less toxic than aflatoxin B1 (LD50 0.46 mg/kg bw; 1 day old duckling) however, this mycotoxin is a source of concern for the infant food industry.

In most countries legislation exist regarding Aflatoxin M1. Only a few countries have legislation regarding Aflatoxin M2 in milk and milk products.

EU regulation 683/2004 allows a maximum level of 0.025 µg aflatoxin M1/kg in milk ready to drink. Equivalent to this, is approximately 0.15 µg aflatoxin M1/kg in dry milk powder.

Recently TNO was faced via international trade with demands concerning maximum levels of aflatoxin M2 in infant formula. As a result, a fast method was developed by TNO, involving immuno affinity clean-up.

After extraction, the extract is cleaned up by Aflaprep M1 columns (R-Biopharm Rhone), which have also a strong cross reaction with aflatoxin M2. After the cleaning step, the extract is eluted and diluted. Separation and detection take place at an HPLC system with fluorescence detection.

A baseline separation was obtained between aflatoxin M1 and M2.

A successful validation was performed in infant formula (powder).

Hereafter an investigation was performed, 22 infant formulae both powder and ready to drink products were analyzed. One powder sample appeared to be positive for both aflatoxin M1 and M2. The amount was for M1 0.67 µg/kg and for M2 0.10 µg/kg. Converted to ready to drink amounts we calculated a contamination of 0.11 µg/kg (M1) and 0.02 µg/kg (M2).

This exceeds the maximum allowed concentration in the EU, which underlined the necessity of monitoring.

Analysis of grape fresh juice, must and young wine for *Alternaria* toxins

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Alternaria alternata is a frequently occurring species and probably the most important mycotoxin-producing species that occurs in cereals, sunflower seeds, oilseed rape, lentils, olives, wine grapes, cranberries and various fruits etc. *A. alternata* produces a number of mycotoxins, including alternariol (AOH), alternariol monomethyl ether (AME), altenuene (ALT), altertoxins I, II, III (ATX-I, -II, -III), L-tenuazonic acid (TeA) and other less-toxic metabolites. Alternaria mycotoxins have been detected in various foodstuffs, including grape must and wine. Wine grapes are good substrates for production of AOH and AME by *A. alternata.* AOH, AME, and ATX-I were stable in fruit juices and white wine over 20 days or at 80° C after 20 min.

Sample characterization
In total twenty two samples of wine grapes („red and white") were sampled to sterile plastic bags during harvesting at the beginning of October 2004 in the ZNOJMO WINE REGION. One sample of wine grapes varieties was represented by three subsamples of wine grapes, which were sampled in left, middle and right parts of vineyard. The samples were transported to mycological laboratory for immediate processing. Grape fresh juice (twenty two samples) was prepared under laboratory conditions by wringing. Must (thirteen samples) and young wine (thirteen samples) corresponding to wine grape variety were sampled in a winery Znovin Znojmo.

Alternaria mycotoxins determination
An HPTLC method for quantification of *Alternaria* mycotoxins (alternariol /AOH/, alternariol monomethyl ether /AME/, altenuene /ALT/, and tenuazonic acid /TeA/) in grape fresh juice, must and wine was used. The limit of quantification (LoQ) was 1.5 μg/l for AOH, AME, ALT and 7.5 μg/l for TeA, respectively. Validation of the method was performed according to the principles used for HPTLC methods (ICH Guideline for planar chromatography).

Results and conclusions
Occurrence of *Alternaria* mycotoxins in grape fresh juice, must and wine were not determined. All the results were below the limit of quantification (LoQ).

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The level of ochratoxin A in patients after nephrectomy

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The occurrence of ochratoxin A (OTA) in food commodities has recently been of interest of numerous scientists and the subject of studies for mycotoxin detection. The studies aimed at determining the influence of OTA on animals have frequently been carried out and proven the possibilities of accumulation of this toxin in tissues. Ochratoxin A is also known for its cancerogenic, nephrotoxic and immunotoxic properties.

The aim of the study was to determine the level of ochratoxin A in patients with nephropathy.

The research has been carried out in cooperation with the Urology Department of the Biziel's Hospital, where the sample materials (kidney tissues and blood serum) were taken. The examined patients were between 52-68 years of age, both male and female, with kidney tumour detected clinically (19) and kidney cirrhosis (1), all from Bydgoszcz or the neighbouring area. The survey containing 21 questions concerning the dietary intake of food products with possible content of OA, was carried out among the patients. The control samples were the blood serum from people without symptoms of nephropathy (6). OTA identification was done at the Mycotoxin Lab of the Institute of Biology and Environmental Protection 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. The average concentration of OTA in the blood serum of the control was 0,4ng/ml in men and 0,37ng/ml in women. The average concentration in patients with kidney diseases was 0,58ng/ml and 0,68ng/ml, respectively. The content of ochratoxin A in kidneys of the examined patients was 0,25ng/ml and 0,19ng/ml, respectively.

The studies proved higher concentration of OTA in blood serum and kidneys in women than men. After the analysis of the patients' diet it was concluded that women eat more products that might contain OTA at high level, such as: coffee, wheat products (pastas and bread). The preliminary studies showed that the high level of ochratoxin A in blood serum might be the cause of nephropathy in people. The survey results confirmed that the kind of dietary food devoured might influence the illness.

Mycological and mycotoxicological contamination of nuts sold in Poland

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Nuts are frequently polluted by fungi, because of nutrient substances, conditions of storage, and the climate of the countries that import nuts. International directives WE 2073/2005 do not determine the microbiological levels of contamination. Till now only the level of aflatoxin B_1 and other aflatoxins has been the only parameter required to determine the quality of nuts.

The aim of the study was the estimation of the contamination level of nuts using microbiological, biological (MTT bioassay) and toxicological methods of analysis.

The mould and yeast quality and quantity were identified in samples of nuts, taken from supermarkets, according to PN ISO 7954:1999. Additional medium: AFPA (separation of A.flavus group) and DG18 (isolation of xerophile fungi) were applied.

Cytotoxicity of nuts was determinated using MTT test, with swine kidney target cells (SK) sensitive to a wide range of mycotoxins (Hanelt, 1994). The initial concentration on MTT test plate was 1200 mg/ml. In case of isolated moulds the first concentration on MTT test plate was 31,25 cm^2/ml.

Extraction of mycotoxins was carried out using immunoaffinity columns: AflaTest by Vicam for aflatoxins, and Ochraprep by R-BIOPHARM RHÔNE LTD for ochratoxin A, respectively. The identification was done with the HPLC method with fluorescent detection (HPLC-FLD), in case of aflatoxins, and Cobra Cell was used for derivatization.

The level of contamination in walnuts varied between 2,5 x 10^3cfu/g DM (imported nuts) and 4,3 x 10^5cfu/g DM (walnuts from Poland). Fungi from *A.flavus* species were found in imported walnuts. No aflatoxins were found in samples. Fungi from *Penicillium* species constituted 1% – 96% of all moulds. The following strains were isolated: *P. brevicompactum* (22-41% of all *Penicillium*), *P. expansum* (74-53% of all *Penicillium*), *P. palitans, P. cyclopium*, all the isolated strains were tested with the cytotoxicity MTT test. The highest level of cytotoxicity was detected in *P. brevicompactum* (highest cytotoxicity in 37°C), *P. expansum* – moderate level of cytotoxicity (impact of temperature was not detected)

No toxic substances were detected in nuts with the MTT cytotoxicity test, and the estimation of aflatoxins and ochratoxin A levels, in spite of the quantity of moulds being up to 10^5/g (DM). It may be assumed that further storage of nuts in temperatures stressful for fungi (+4°C, +37°C) could initiate the production of toxic substances.

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FusaProg-a tool for the prediction of fusarium head blight and deoxynivalenol contamination in winter wheat

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Fusarium head blight (FHB) caused by different *Fusarium* species is an important disease on cereals worldwide. To evaluate the risk of FHB infection in Switzerland, we performed a survey from 2001 to 2003 by examining close to 300 wheat samples from Swiss farms for the occurrence of FHB fungi and for deoxynivalenol (DON) content. Wheat samples were grouped by factors previous crop (maize, others), tillage (ploughed, no tillage), and the susceptibility of the variety to *Fusarium graminearum* (FG) (low or medium to high). The most frequent *Fusarium* species isolated was FG, followed by *F. poae* and *F. avenaceum*. Disease incidence with FG and DON-contamination was highest in samples from fields with corn as previous crop, no-tillage, and varieties with medium to high FG susceptibility. The level of DON-contamination was closely correlated with the incidence of FG on wheat grains.

To avoid critical mycotoxin contamination, farmers need efficient cultivation measures to prevent infections by fusaria and to control FG with fungicides if necessary. Decision support systems (DSS) are useful tools for a targeted control of pathogens. Therefore, we are developing the DSS FusaProg to predict FHB and DON in winter wheat. Our DSS considers the key factors which influence the development of FG, namely pathogen, host, and weather. Based on the results from our Swiss survey, we developed a modified disease forecast approach: instead of a FG inoculum, we use the final disease incidence at harvest, representative for one of the eight different cropping systems investigated in our survey. In addition, and based on the high correlation between FG incidence and DON, we replaced the disease incidence value by the DON-contamination This basic DON-value is subsequently corrected with other pathogen or DON-influencing factors: straw management, seedbed tillage, pre-previous-crop, FG susceptibility of the wheat variety, growth stage, and prevailing weather to obtain a situation and cropping-system related DON-value. To take the weather based infection risk into account, weather conditions of the past three days including temperature, precipitation, and relative humidity are analysed. Anthesis is considered as the most critical period for infection, hence, the DON prediction model runs from ear emergence until the end of flowering. As a result, the predicted DON-value is a function of the basic DON-value, the field specific crop management, and the weather conditions.

To evaluate and calibrate the DSS, solely plant protection officers can access and use FusaProg at www.fusaprog.ch as of yet. The program supplies information about the regional and the current weather based infection risk. For plot specific risk assessments, participants have to register their plots, and to include characteristics of the crop, its management, and the current growth stage.

First results show that FusaProg is a helpful tool to display the local and regional weather based infection risk, although the DON prediction model still needs to be improved. We assume that within two or three additional validation years, FusaProg will be ready to support Swiss farmers to control FHB. In addition, we are convinced that our DSS is a good tool to develop management strategies that reduce the risk of DON-contamination under conservation tillage.

Effect of chemical control on *Fusarium* spp. and mycotoxins occurrence on spring and winter wheat

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Fusarium head blight (FHB) is a widespread disease of cereals caused mainly by *Fusarium graminearum, F. culmorum, F. avenaceum, F. poae* and *Microdochium nivale.* FHB is of particular concern because of the ability of the *Fusarium* species to produce mycotoxins in the grain that are harmful to human and animal consumers. *F. culmorum*, *F. graminearum* and *F. poae* can produce different trichothecenes. *F. avenaceum* produces moniliformin. In the paper two fungicides were applied in different doses and dates to control *Fusarium* head blight of two cultivars of spring wheat and two cultivars of winter wheat. The PCR was used to detect and identify the species present in ears of wheat. And also the contents of DON, NIV and MON were defined in the grain after harvest of wheat. The predominant species in all tested cultivars were *Fusarium avenaceum* and *Fusarium graminearum*, and also *Fusarium poae* but only in cultivars of winter wheat. The used fungicides inhibited growth of some *Fusarium* species and as a consequence of this lower production of mycotoxins or their lack were observed. The ability to accumulation of mycotoxins in the grain and plant reaction on chemical control depended on cultivar of wheat.

Effect of severity of *Fusarium* head blight and kernel infection with *Fusarium culmorum* on mycotoxin content in grain of winter wheat cultivars

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Resistance of 38 common cultivars of winter wheat to *Fusarium* head blight (FHB) was tested in a field experiment. Wheat heads were inoculated using deoxynivalenol-producing isolates of *Fusarium culmorum*. Resistance traits such as FHB index (combination of FHB incidence and severity scored 3-times at 7-day intervals), number of *Fusarium*-damaged kernels (FDK) and reductions of yield components (grain yield per head, TKW, specific kernel weight) were scored. Wheat grain was analysed for *Fusarium* mycotoxins: deoxynivalenol (DON), 3-Acetyldeoxynivalenol, 15-acetyldeoxynivalenol, nivalenol and zearalenone using HPLC technique. Five grams of ground grain was extracted with 25 ml of aqueous acetonitrile (84:16 v/v) and purified on Mycosep (Romerlab) SPE columns. Purified extract was analyzed on HP 1050 HPLC fitted with LiChrospher 100 RP-18 250x4 mm (5 µm) column and UV detector. Results were corrected for losses during sample preparation.

Winter wheat cultivars were severely infected by *F. culmorum.* Average FHB index was 37.7% at a range 18.7 – 64.3 %. Resistance variability of cultivars was statistically significant. The most resistant were cvs Liwilla (18.7 %), Turnia (19.0 %) and Piko (20.7 %). The most susceptible were cvs Clever (64.3 %), Maltanka (64.2 %) and Slade (58.7 %). Percentage of FDK was 58.1 %, at a range 32.8 – 94.5 %. The highest FDK exhibited cvs Slade (94.5 %) and Clever (92.8 %), the lowest - cvs Liwilla (32.8%) and Piko (33.8%). Average reduction of grain yield per head, TKW and specific kernel weight were 60.0, 32.8 and 15.4, respectively. Cultivars differ statistically significant for reductions of all yield components.

Large amounts of DON, up to almost 80 mg/kg, were found in grain of wheat cultivars. Only traces of acetyl-derivatives of deoxynivalenol and zearalenone were found. Nivalenol was not detected in any sample. Wheat cultivars varied significantly for DON content in grain. DON concentration ranged from about 3.7 mg/kg to 78.6 mg/kg.

Resistance traits and DON concentration in grain were correlated to find relationships between them. FHB index significantly correlated with DON concentration and determination coefficient was r^2 = 47.0%. Correlation coefficient for FDK versus DON was also significant and higher than for FHB index. Determination coefficient amounts r^2 = 67.4 %. However, we found some non-linear relationship between these variables. Application of log transformation on the data resulted in better fit of the regression curve. Coefficient of determination increased to r^2 = 75.5 %. Regarding reductions of yield components, correlations coefficients were significant for grain yield per head (r^2 = 60.0 %) and specific grain weight (r^2 = 81.2 %) versus DON concentration. No correlation was found between TKW reduction and DON concentration.

Finding of significant correlation coefficients in studied population of winter wheat cultivars would allow prediction of possible DON contamination of grain based on head infection or kernel damage.

Mycotoxins in German cereals in 2005

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In Germany the act "Agrarstatistikgesetz" requires the annual determination of the processing quaility and the content of contaminants of cereals ("Besondere Ernteermittlung" = "BEE"). For this aim samples from statistically selected fields of each federal state are collected. Beside the content of pesticides and heavy metals, the content of mycotoxins is of great relevance in regard to the implementation of maximum levels for certain mycotoxins in the EU. With this set of samples, it is possible to get representative information about the content of mycotoxins under natural growing conditions. In 2005, a total number of 1036 samples was analysed: 496 wheat, 265 rye and 275 oat samples. The mode of choosing the wheat samples ensured also statistically validated results for each of the federal states of Germany. The oat samples were separated in two groups: oat for human consumption and feed oats. As in the past four years, all samples were analysed for the Fusarium toxins deoxynivalenol (DON) and zearalenone (ZEA). In 2005, ergot alkaloids have been determined in a set of rye samples, having in mind that little is known about the average content in German rye on the one hand and about ratio of the individual ergot alkaloids to each other on the other hand.

The results show that in comparison to the past years the content of DON is quite low in 2005. The lowest levels were found in oat, especially in oat for human consumption. Referred to the median it has been the lowest DON content in wheat since 2001. The analysis of ergot alkaloids demonstrated that the individual ergots are distributed very heterogeneously. Thus it is not possible to specify in rye a general ratio of the individual ergot alkaloids to each other, which would be useful in the discussion of orienting levels or maximum levels for ergot alkaloids.

Comparison of different extraction, clean-up and quantification methods for the determination of ergot alkaloids in rye flour and delivered bread

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Ergot, caused by the fungus *Claviceps purpurea*, is a disease of cereal crops and grasses. Intoxications induced by ergots alkaloids have been known in Europe for many centuries, described as St.Anthony´s fire or Holy fire. The term ergot refers to fungal structures from Claviceps species (*Claviceps purpurea*) replacing kernels on grain ears or seeds on grass heads. Infections occurred on rye, wheat, barley and in general grass due to bad weather conditions. Sklerotia contain different classes of alkaloids: The most prominent being ergometrine, ergotamine, ergosine, ergocristine, ergocryptine and ergocornine. The total alkaloid content of central European ergots is in the range between 0.20 % up to 0.25 %. According to EU-regulation 824/2000 ergot should not exceed 0.05 % in precleaned cereals. Normally, ergot is removed during the first cleaning process of grain in the mill. But during the last 3 years a few samples of rye flour contained high concentrations of ergot alkaloids. Additionally, the EFSA stated in April 2005 that there is a special concern related to feed. Altogether, this leads to the necessity for validated analytical methods for the quantification of ergot alkaloids in feed material (EFSA 19.4.2005).

Before milling grain can be rapidly checked for ergot by stereo-microscopy, which is done during the routine dockage analysis according regulation 824/2000. Products from cereals such as flour, feed mixtures or bakeries can be only analysed chemically.

The determination of ergot alkaloids is often carried out using HPLC with fluorescence detection, a few methods uses GC-MS including a derivatisation step. Nevertheless, no technique to determine ergot alkaloids ever reached the stage of formal inter-laboratory validation, so that the performance characteristics are not fully known. Certified matrix reference materials are not available for ergot alkaloids.

To get an analytical method for this group of compounds, which could be used for routine monitoring of ergot alkaloids in food and feed samples, different extraction and quantification modes were compared. Furthermore, results obtained by an extremely extensive HPLC-flurescence method with clean up are compared with a LC-MS/MS method without a clean-up step. Additionally, a group of transformation products of derivatives of lysergic and iso-lysergic acid are investigated.

Validation HPLC method of determination ochratoxin A

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Ochratoxin A, 7-carboxyl-5-chloro-8-hydroxyl-3,4-dihydro-3R-methylisocumarin-7-L-β-phenylalanineis a lactone containing secondary metabolite of some toxigenic species of *Aspergillus* and *Penicillium* [1]. Ochratoxin A contamination of food, feed, animal tissues and even human blood is a worldwide problem [2].

The term "validated" refers to a method, which has been statistically evaluated and accepted as suitable. The guidelines for the acceptance are often defined in normative standard and legislation that concern the use of the method. In the case of official control for the monitoring of mycotoxin in Europe, the required method specifications are given in the relevant EU Directives [3,4].

The aim of the study was the validation method of determination ochratoxin A in food and feed by high performance chromatography (HPLC) to official control and monitoring system under surveillance of veterinary inspection in Poznań (Poland).

A HPLC method in combination with an immunoaffinity clean-up step and fluorescence detection is described for the analysis of ochratoxin A at low ppb level in samples of artificially contaminated feed and food.

Standard material was injected six times for 0.05 to 1.5 µg/ml and regression calculated to be 0.9999. Recoveries of ochratoxin A added to matrices (feed, wheat, corn, soybean, rice, raisins) ranged from 79.9 to 100.7 % for three levels 5, 10, 20 µg/kg assay. Additionally, a certified references material (CRM) ochratoxin A in wheat was tested. An average recover of 92.6% (n = 20, CV = 3.64) was achieved for CRM. Limit of detection 0,032 µg/kg and limit of determination 0,042 µg/kg.

Specifically the detected concentration of ochratoxin A was lower than the recommended limit 0.5 µg/kg (for infant food [4,5]).

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[1] K. J. van der Merewe, P. S. Steyn, L. Fourie, B. de Scott, J. J. Theron, *Nature* 205 (1965) 1112-1113.
[2] R. R. Marquardt, A. A. Frohlich, J*. Animal Science* 70 (1992) 3968-3988.
[3] Commission Directive 2002/27/EC of March 2002 amending Directive 98/53/EC laying down the sampling methods and the methods of analysis for the official control of the levels for certain contaminants in foodstuffs. Official Journal L 075, 16/03/2002, 44-45.
[4] Commission Directive 2002/26/EC of March 2002 laying down the sampling methods and the methods of analysis for the official control of the level ochratoxin A in foodstuffs. Official Journal L 075, 16/03/2002, 38-43.
[5] Commission Regulation (EC) No 466/2001 of March 2001 setting maximum levels for certain contaminants in foodstuffs Official Journal L 077, 16/03/2001.

Determination of DON and Deepoxy-DON in liquid manure of biogas production

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Every year batches of wheat are harvested which are not suitable for consumption because of high contamination with *Fusarium* and *Fusarium* toxins, particularly with the trichothecene deoxynivalenol (DON). Therefore methods of safe and economically effective disposal of these grain charges are urgently needed. In a research approach at the University of Hohenheim under financial support of the German FNR (Fachagentur für Nachwachsende Rohstoffe) the prerequisites of a potential deactivation of *Fusarium* spores as well as of a degradation of DON during biogas production are evaluated.

A precondition of this study was the capability of a selective and sensitive determination of DON and its degradation product, Deepoxy-DON, in liquid manure of biogas production. Due to the aqueous matrix of the liquid manure, a sample preparation using liquid/liquid partitioning on kieselguhr material was developed.

Measurement of toxins was done with two different methods. First DON and Deepoxy-DON were determined by GC/MS after derivatisation with trifluoraceticanhydride (Schollenberger et al., 2005). Derivatisation procedure and measurement conditions were modified to allow for selective and sensitive analysis of these two substances as well as of a spectrum of further trichothecenes of the A- as well as of the B-type out of liquid manure. This could be of interest when substrates other than wheat are used in biogas production. Furthermore a rapid method for the determination of DON with HPLC as described by Sano et al (1987) and Lepschy von Gleissenthall (2000) was adapted to fit for analysis of DON and DeepoxyDON out of liquid manure of biogas production. The toxins were separated by reversed-phase chromatography and measured using fluorescence detection after post-column derivatisation with NaOH and methylacetoacetate. Correlation between both methods was satisfactory.

Lepschy von Gleissenthall, J. (2000). Nachweis eines Pilztoxins (Deoxyndivalenol) mittels HPLC und Nachsäulenderivatisierung. GIT Laborfachzeitschrift 3/2000.

Sano, A., Matsutani, S., Suzuki, M, Takitani, S. (1987). High-Performance liquid chromatographic method for determining trichothecene mycotoxins by post-column fluorescence derivatization. Journal of Chromatography 410, 427 - 436.

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Fast Centrifugal Partition Chromatography (FCPC) as an Efficient Tool for the Preparative Isolation and Purification of Mycotoxins

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Studies on the toxicity, stability and mode of action of mycotoxins require large amounts of the respective compounds. Despite the commercial availability of most toxins, the high expenses limit the realization of research projects. Thus, the biosynthetic production of mycotoxins by moulds in laboratory scale is a favorable alternative. Although simple techniques for the toxin production with commercially available mould strains are described in literature, the subsequent time and solvent consuming purification of the mycotoxin is a major disadvantage.

Facing these problems we developed easy and efficient preparative clean-up methods for the isolation and purification of deoxynivalenol, fumonisins, ochratoxin A and zearalenone using fast centrifugal partition chromatography (FCPC). This separation technique is based on partition chromatography by the distribution of solutes between two immiscible liquid phases. The fast centrifugal partition chromatograph is a one axis centrifugal rotor, with a system of more than 1000 linked small chambers (called "loculi") arranged around its center. On rotation (800-1000 rpm), a permanent gravitational field keeps the stationary phase in the loculi while the mobile phase is pumped through the system. During this process, the different distribution of the sample constituents in the mobile and stationary phase results in a chromatographic separation.

As a basic clean up approach, extraction and concentration are the only steps prior to FCPC. After separation, the compounds are crystallized under appropriate conditions. The purity of the isolated products ranged between 90 and 98 percent. Methods for the isolation and purification of deoxynivalenol, fumonisin B_1, ochratoxin A and zearalenone are successfully established using this technique.

Ergot effects on health and performance of piglets with special consideration of liver function

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Introduction: Ergot (sclerotium of *Claviceps purpurea*) contains several alkaloids which are known as substances derogating performance and health of animals. Ergot alkaloids can affect a number of physiological processes. In addition to the vasoconstrictive action of ergotamine, ergot alkaloids could cause also pathological insults in liver tissue[a]. Therefore, we suppose that liver function could be influenced by ergot alkaloids as well. We measured the liver function by a ^{13}C-methacetin breath test which is widely used in human medicine. In addition, selected serum parameters indicative for liver health were determined.

Materials and Methods: 72 crossbred piglets (36 gilts and 36 barrows) with an initial live weight of 9.2 ± 1.0 kg were housed in pens (four piglets per pen) with free access to feed and water. Two groups received ergoty diets formulated to contain similar alkaloid concentrations of 16.8 mg/kg* diet from two different ergot source and were compared with a control group (Table 1). Feed consumption and body weight were recorded weekly. Blood from 8 piglets of each group was collected for determination of clinical chemical parameters. Liver function was measured with a ^{13}C-methacetin breath test. ^{13}C-methacetin was given at a dose of 2 mg/kg live weight, and breath samples were taken after 0/15/30/45/60/90/120/180/240/300 and 360 minutes. Several parameters, for example the cumulative dose recovery after 30 min ($cPDR_{30}$), could be calculated[b].

Results: The ergot-fed groups showed significantly lower daily feed intakes and weight gains compared to the control group. There were no clinical signs of ergotism. Whereas the serum parameters revealed no significant changes, $cPDR_{30}$ of the group fed with Ergot Type A differed significantly from the control.

Table 1: Cumulative daily feed intake and weight gain, aspartate aminotranferase (ASAT), alanine aminotransferase (ALAT), glutamate dehydrogenase (GLDH) and cumulative ^{13}C-methacetin recovery ($cPDR_{30}$) after 3 weeks on experimental diets

	Calculated alkaloids [mg/kg]	Feed intake [g DM/d]	Weight gain [g/d]	ASAT [U/l]	ALAT [U/l]	GLDH [U/l]	$cPDR_{30}$ [% ^{13}C]
n		24	24	8	8	8	8
Control group	0	554^{a}	424^{a}	29.4	24.2	2.1	13.3^{a}
1.2% Ergot Type A	16.8	303^{b}	177^{b}	27.3	24.3	1.9	7.1^{b}
2.2% Ergot Type B	16.8	282^{b}	181^{b}	24.5	26.3	1.5	8.7^{ab}

a, b: Means within a column not sharing a common superscript are significantly different at $p<0.05$

Conclusions: Ergot in feed with a calculated alkaloid concentration of 16.8 mg/kg diet altered the performance significantly whereas serum parameters remained unaffected. However functional liver capacity seemed to be reduced in the group fed with Ergot A with a similar trend for Ergot B. There were no significant differences of the measured parameters between the two ergot sources.
*sum of ergometrine, -tamine, -sine, -cryptine, -cornine, -cristine and inine-isomers

[a] Dignean, M. A. et al., 1986. J. Vet. Med. A (33), pp. 757-766
[b] Armuzzi, A. et al., 2002: Aliment Pharmacology Therapy 16, pp. 1977-1996

Multimethode zur Bestimmung von Mykotoxinen in Speiseölen mittels LC-MS-MS

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In den zur Speiseölherstellung verwendeten Rohstoffen wie Mais, Weizen und Erdnüssen, aber auch Sonnenblumenkernen, Raps oder Oliven sind häufig Rückstände an Fusarientoxinen, Aflatoxinen sowie Ochratoxin A nachweisbar. Die meisten Mykotoxine (Aflatoxin B1, B2 und G1; die Fumonisine B1, B2 und B3; Zearalenon; Ochratoxin A sowie die Typ B Trichothecene HT 2 Toxin, T 2 Toxin, T 2 triol, 4,15-Diacetoxyscirpenol und 15-Acetoxyscirpenol) besitzen einen positiven Oktanol-Wasser-Verteilungskoeffizienten (logP) [1]. Es ist folglich nicht auszuschließen, dass diese Mykotoxine bei der Ölgewinnung in die Fettphase übergehen und – sofern sich nicht weitere Raffinationsschritte anschließen, die zu einem Abbau führen – auch im Endprodukt noch nachweisbar sind.

Über das Vorkommen von Mykotoxinen in Speiseölen wurde in der Vergangenheit schon von einigen Autoren berichtet. Kappenstein [2] stellt hohe Zearalenon-Befunde in Maiskeimöl sowie geringere Befunde in Sojaöl und Weizenkeimöl fest. Finoli [3] und Papachrisou [4] weisen in italienischem bzw. griechischem Olivenöl Spuren an Ochratoxin und Aflatoxinen nach. Diop schließlich [5] findet in Erdnussöl aus dem Senegal sehr hohe Gehalte an Aflatoxinen.

Vor diesem Hintergrund war die Frage, ob nicht noch andere Mykotoxine - insbesondere die o. g. Trichothecene und Fumonisine - im Öl nachweisbar sind. Ziel war es daher, eine routinetaugliche Multimethode zu entwickeln, mit der ohne großen apparativen und personellen Aufwand bei der Probenvorbereitung Speiseöle auf Mykotoxinrückstände untersucht werden können.

Mit Hilfe des pH-abhängigen Oktanol-Wasser-Verteilungskoeffizeinten (logD) [1] lassen sich die Mykotoxine zunächst in zwei „pH-Wert-Gruppen“ aufteilen. Der logD der Trichothecene und der Aflatoxine ist vom pH-Wert unabhängig, wohingegen der logD der Fumonisine, von Ochratoxin A und Zearalenon im alkalischen deutlich kleiner ist als im Neutralen. In Vorversuchen hat sich dann auch bestätigt, dass im Neutralen nicht alle Toxine zufriedenstellend aus dem Öl extrahierbar sind, sondern dass insbesondere Zearalenon im alkalischen extrahiert werden muss. Zum Nachweis aller o. g. Mykotoxine sind daher zwei getrennte, jedoch sehr schnelle Probenaufarbeitungen erforderlich:

Das Öl wird im Reagenzglas mit Hexan versetzt und anschließend mit einem Methanol-Wasser-Gemisch bzw. mit einem alkalischen Methanol-Wasser-Gemisch mit Hilfe eines hochtourigen Reagenzglasschüttlers extrahiert. Nach der Phasentrennung können die Methanol-Wasser-Phasen, ggf. noch nach zusätzlicher Aufkonzentrierung, direkt zur Messung eingesetzt werden. Zur Chromatographie wird eine Umkehrphasensäule wie die Gemini C18 (Phenomenex) verwendet. Die Messungen erfolgen an einem Triple-Quad-Massenspektrometer (API 3000) mit einer TurboIonSpray-Quelle je nach Mykotoxin im positiven oder negativen MRM-Modus.

Das Probenvorbereitungsverfahren einschließlich der Messbedingungen aller genannten Mykotoxine sowie die Untersuchungsergebnisse diverser Speiseöle werden vorgestellt.

[1] Entnommen aus der Datenbank des SciFinder (Berechnet mit Advanced Chemistry Development (ACD/Labs) Software)

[2] O. Kappenstein et al.: Mycotoxin Research, 2005, 21(1), 79-82
[3] C. Finoli et al.: Industrie Alimentary, 2005, 44(447), 506-514
[4] A. Papachristou, P. Markaki: Food Additives and Contaminants, 2004, 21(1), 85-92
[5] Y. Diop et al.: Annales Pharmaceutiques Francaises, 2000, 58, 470-474

Multimethode zur Bestimmung von Alternaria-Toxinen mittels LC-MS-MS

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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) [1] 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 ein Analysenverfahren entwickelt, das weitgehend automatisierbar ist und sich aufgrund der LC-MS-MS sowohl als schnelle Screening- aber auch als Bestätigungsmethode eignet. Die routinetaugliche Multimethode umfasst derzeit die Bestimmung der Alternaria-Toxine Alternariol (AOH), Alternariol-monomethylether (AME), Altenuen (ALT), Tentoxin (TEN) sowie Tenuazonsäure (TEA), die Einbindung weiterer relevanter Alternaria-Toxine ist in Bearbeitung.

In neueren Arbeiten wurde erstmals über die Bestimmung einzelner Alternaria-Toxine mittels LC-MS-MS berichtet. So erwähnt Scott [2] eine SPE-Methode zur Bestimmung von AOH und AME mittels LC-MS-MS. Horiuchi [3] beschreibt die simultane Detektion von Tentoxin mittels APCI, ESI sowie FAB-MS. Solfrizzo [4] schließlich hat ausführlich eine Methode zur Bestimmung von Alternaria-Toxinen in Karotten vorgestellt. Er extrahierte zunächst TEA, AME und AOH sowie Radicinen und Altertoxin-I aus der Probenmatrix und teilte dann den Extrakt zur weiteren Aufreinigung über eine C18- sowie eine Oasis-HLB-Kartusche; die Detektion erfolgte jedoch am DAD.

Ausgehend von der Probenvorbereitungsmethode von Solfrizzo [4] wurde eine SPE-Methode entwickelt, mit der die o.g. fünf Alternaria-Toxine gemeinsam bestimmt werden können. Die homogenisierte Matrix wird mit einem Acetonitril-Methanol-Wasser-Gemisch bei pH 3 mittels Schüttelmaschine oder Ultra-Turrax (je nach Probenmatrix) extrahiert. Nach Zentrifugation wird ein Aliquot des Überstandes mit einer Natriumdihydrogenphophatlösung pH 3 verdünnt. Dieser verdünnte Extrakt wird über eine Oasis-HLB-Säule gegeben, nach dem Waschen mit Wasser wird mit Methanol eluiert. Das Eluat wird im Vakuum zur Trockene gebracht, der Rückstand mit einem Methanol-Wasser-Gemisch aufgenommen und in ein Mikrovial überführt. Zur Chromatographie wird eine Gemini C18 (Phenomenex) eingesetzt. Die Verwendung eines Wasser/Methanol-Gradienten unter Zusatz von Ammoniumbicarbonat ergibt für alle Toxine eine gute Peakform sowie eine gute Retention. Die Messung erfolgt an einem Triple-Quad-Massenspektrometer (API 3000) mit einer TurboIonSpray-Quelle im MRM-Modus.

Durch die Verwendung einer automatisierten Festphasenextraktionseinheit (ASPEC von Gilson) und eines Vakuum-Konzentrierungssystems (Beta-RVC von Christ) können von einer Person ca. 30 Proben in 1,5 Tagen aufgearbeitet werden.

Bislang wurden Apfel-, Birnen- und Traubensäfte sowie Kartoffel- und Tomatenerzeugnisse untersucht. In allen Tomatenerzeugnissen war Tenuazonsäure nachweisbar. Die weiteren Untersuchungsergebnisse sowie die ausführlichen Validierungsdaten werden vorgestellt.

[1] Stellungnahme des BfR vom 30. Juli 2003 zur toxikologischen Bewertung von Alternaria; http://www.bfr.bund.de/cm/208/alternaria_toxine_in_lebensmitteln.pdf
[2] P. Scott: Journal of AOAC, 2001, 84, 1809-1817
[3] M. Horiuchi et al: Chromatography, 2003, 24(3), 109-116
[4] M. Solfrizzo et al .: Journal of AOAC, 2004, 87, 101-106

Monitoring of FHB using PCR for qualitative and quantitative detection of Fusarium spp.

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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 (1stubble/m^2) 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 with qualitative PCR and quantitative Real-time PCR for different *Fusarium* species: *F. avenaceum, F. culmorum, F. graminearum, F. moniliforme var. subglutinans, F. poae, F. pseudograminearum, Microdochium nivale* var. *major* and *M. nivale* var. *nivale*. In addition expression of tri5 gene will be studied using a RT-PCR system recently developed in our lab. In parallel concentrations of DON in ear samples are determined. Parts of results concerning PCR investigations and *Fusarium* incidence in the samples of 2004 and 2005 will be presented.

Determination of trichothecenes type A in cereals and cereal products by LC-MS/MS

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Trichothecenes type A like T-2 and HT-2 toxin are known as a group of cytotoxic and immunosuppressive mycotoxins produced by genera of *Fusarium*. Among cereals oats, maize, wheat, and rye are considered to be the most contaminated with these toxins. According to mean intake data provided by a study of the Scientific Cooperation (SCOOP 2003) the ingestion of trichothecenes type A especially by infants and children could exceed the temporary TDI for the sum of T-2 and HT-2 proposed by the Scientific Cooperation on Food (0.06 µg/kg bw; SCF 2002). In order to set appropriate maximum levels till 1 July 2007 the European Commission claimed for more occurrence data of these toxins especially in oats and oat products (Commissions Regulation No 856/2005).

A sensitive LC-MS/MS method for the determination of T-2 and HT-2 toxin, 4,15-diacetoxyscirpenol (DAS), 15-monoacetoxyscirpenol (MAS), neosolaniol (NEO), T-2 triol, T-2 tetraol, and verrucarol (VOL) has been developed using an API 4000 triple quadrupole mass spectrometer (Applied Biosystems). Ground cereal samples were extracted with acetonitril/water (84/16, v/v) using a high-speed Ultra-Turrax homogenizer and the extracts were purified with MycoSep® columns. The limits of quantitation (LOQ) ranged from 0.1 µg/kg for MAS, 0.4 µg/kg for T-2 and HT-2 toxin to 1 µg/kg for VOL.

The samples analysed were either purchased at retail stage or requested from wholesale stage or from producers/mills according to Commissions Regulation No 401/2006. Results on contamination of oats and other cereal samples from conventional or organic agriculture will be presented and discussed.

Commissions Regulation (EC) No 401/2006 (23 February 2006) laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs

Commissions Regulation (EC) No 856/2005 (6 June 2005) amending Regulation (EC) No 466/2001 as regards *Fusarium* toxins

Scientific Committee on Food (2002): Opinions of the Scientific Committee on Food on *Fusarium* Toxins, Part 6, European Commission, DG Health and Consumer Protection

Scientific Cooperation (SCOOP, 2003): SCOOP Task 3.2.10: Collection of occurrence data of *Fusarium* toxins in food and assessment of dietary intake by population of EU Member States

Monitoring the expression of the trichothecene biosynthetic genes in correlation to toxin formation

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A microarray, which covers most of the known relevant mycotoxin biosynthesis genes has been developed and implemented to monitor the expression of the mycotoxin biosynthesis genes under different growth conditions to be able to predict mycotoxin biosynthesis. Several experiments have been made to show that this array is applicable to predict mycotoxin production in different natural food matrices. Mycotoxins like trichothecene, ochratoxin, aflatoxin, fumonisin and patulin are produced by different species of *Fusarium, Penicillium* or *Aspergillus.* Contamination of different foods with mycotoxins represents a significant hazard to human and animal health and has attracted increasing attention from food safety authorities and legislators. Mycotoxins are secondary metabolites which are produced only at certain growth phases and under certain growth conditions. Toxin production varies according to environmental factors like substrate, pH, temperature and water activity. The phenotypic response to environmental signals is regulated at the genetic level. An understanding of the regulation of the mycotoxin biosynthesis genes in correspondence to environmental parameters will lead to measures to minimize mycotoxin occurrence in foods.

Most of the genes, selected for the microarray are available in public data bases except the ochratoxin biosynthesis genes which have been identified by us. We have used the microarray to study the influence of type of substrate and growth phase on the expression of trichothecene biosynthesis genes. Our preliminary results indicate that it is possible to monitor expression of mycotoxin biosynthesis genes in relation to environmental parameters in different food matrices and that a certain expression profile is indicative for trichothecene biosynthesis. In addition it will be possible to use the microarray to differentiate between mycotoxin producing species using control genes, which are specific for each species. In case of trichothecene producing species, it will be possible to differentiate between *Fusarium* species producing type-A trichothecenes like the T-2 toxin or type-B trichothecenes like nivalenol or deoxynivalenol by monitoring the differential expression of the genes *tri*7 and *tri*13. In the present study this microarray has been used to analyse the regulation of toxin biosynthesis genes under different environmental influences like pH, water activity and temperature in correlation to trichothecene formation.
According to the experiments, the microarray seems to be suitable as a quality assurance system for improving food safety and for minimising toxin production in the food chain to prevent consumer from mycotoxin exposure.

New rapid and cost-effective SPE Procedure for the Clean-up of Fusarium toxin contaminated Cereals and Cereal-based Foods

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Fusarium fungi are probably the most prevalent toxin-producing fungi of the northern temperate regions and are widely distributed in the food chain in America, Europe and Asia. The main sources of intake are products from cereals, in particular wheat and corn. Fusarium toxins can produce a wide range of mycotoxins including several type A and type B trichothecenes, which can be harmful to humans and animals. Suitable sensitive and reliable multitoxin methods for the analysis of trichothecenes and other Fusarium toxins are therefore required.

Traditional sample preparation for trichothecene analysis typically involves extraction with acetonitrile/water and clean-up on polar columns. Analytical interfering substances are retained while trichothecenes are not adsorbed on the packing material. As the trichothecenes differ a lot in polarity and solubility, recoveries of the more polar analytes are often compromised with this approach. Another approach is the use of Immunoaffinity columns (IAC). These provide highly selective extractions with high recoveries, but separate IAC columns are needed for each toxin. To overcome the limitations of these methods there was a need to develop an extraction and clean-up method for the simultaneous determination of several trichothecenes with high recoveries for the polar toxins by minimizing the matrix effects.

This poster shows the optimized method extraction and single column clean-up step of 12 type A- and B-trichothecenes [deoxynivalenol (DON), nivalenol (NIV), 3-acetyldeoxynivalenol (3ADON), 15-acetyldeoxynivalenol (15ADON), fusarenon-X, T-2 toxin, HT-2 toxin, neosolaniol, monoacetoxy-scirpenol, diacetoxyscirpenol,T-2 triol and T-2 tetraol] and zearalenone (ZEA) in cereals and cereal based food on Bond Elut® Mycotoxin, a newly developed solid phase extraction sorbent.

Average recoveries and RSD obtained for 12 trichothecenes and ZEA from spiked wheat, corn, durum, oats, bread, muesli and cereal infant food samples after clean-up with Bond Elut Mycotoxin columns were calculated. The recoveries especially for the polar toxins DON, NIV, 3ADON and T-2 tetraol were increased up to 31% when compared to the extraction method on polar charcoal-aluminia cartridges. To calculate the amount of ZEA, the extracted matrices were spiked with a defined amount of Zearalanone (ZAN) standard solution before the clean-up step on Bond Elut Mycotoxin. Using ZAN as internal standard, the recovery of ZEA was about 100%.

The trichothecene content of six naturally contaminated samples after 3 different clean-up methods shows that up to 43% higher values were achieved in the analysis of naturally contaminated samples for the polar toxins DON, NIV, 3ADON, 15ADON and T-2 tetraol in comparison to the charcoal-alumina based method. If the determination of DON alone is of interest, then the highest content can be achieved with an extraction of 100% water and clean-up with IAC; however for the determination of 12 trichothecenes with different polarities the Bond Elut Mycotoxin provides comparable results.

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 other commonly used standardized methods.

Sample Preparation for Analyzing of Deoxynivalenol by HPLC in Serum from Fattening Pigs after Feeding with DON-Contaminated Feed

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A selective sample preparation with immunoaffinity column (IAC) was performed for analyzing deoxynivalenol (DON) in pig serum. Combination of an extraction cartridge and IAC has been a preferable sample preparation for analyzing DON and its metabolite in biological samples[1)]. This study emphasizes in improvement strategy of clean-up with only one step sample preparation and intensive washing after application serum sample in IAC.

Serum samples were incubated with ß-glucuronidase overnight and applied to IAC afterwards. IAC procedure was modified in order to optimize the quality of obtained HPLC chromatograms. IAC was washed intensively with phosphate buffer saline (PBS) and HPLC-water to remove any matrix interferences. Two ml of eluate was evaporated and reconstituted in 150µl mobile phase consisting of water:acetonitrile:methanol (82:9:9). The separation and analyzing of DON were conducted in polar reversed-phase column and ultraviolet detector, respectively. In order to insure the analytical results, the method was validated. Day-to-day and within day reproducibility as well as recovery of the sample preparation were determined.

In the preliminary result, incubation of ß-glucuronidase provides 21–43% higher concentrations of DON that also indicates the rate of glucuronidation in the range mentioned. In contrast to previous reports, a clean-up with only IAC gave an appropriate separation of DON from matrix interferences in serum. Only after intensive washing step with 14 ml of PBS and 6 ml of HPLC-water a better quality of chromatogram could be reached. The reproducibility and recovery of the investigated sample preparation were measured in the range of 2–19% RSD and 87–94%, respectively. The limit of detection and quantification were determined being approximately 2 and 6 ng DON/ml serum, respectively. Piglets were fed with DON contaminated feed (2.5mg DON/kg feed; feed intake was 4% of their body weight per day). Serum was taken after 3.5 hours and DON was measured as described above. DON concentrations in serum were in the range of 12–19 ng DON/ml serum. These investigated sample preparation was also performed as clean-up for analyzing DON from pig's serum with ELISA. Preliminary results of ELISA are 2–3 times higher in correlation to HPLC results. An explanation of the higher ELISA results can not be given. Other objective of this study will be to provide a reliable sample preparation for ELISA.

1) S. Dänicke *et al*. (2004) : Arch. Anim. Nutr. 58 (1), 1-17

Stabilität von mittels der Sol-Gel Methode hergestellten DON-Immunaffinitätssäulen

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Die Probenvorbereitung ist einer der wichtigsten Schritte bei der Bestimmung des DON – Gehaltes in Lebens- und Futtermitteln. Eine der selektivsten Methoden zur Matrixabtrennung ist die Immunaffinitätschromatographie, da sie auf den hochselektiven Wechselwirkungen zwischen dem Analyten und immobilisierten Antikörpern beruht.

Die Immobilisierung von Biomolekülen ist ein kritischer Schritt, da dadurch die Bindungseigenschaften der Proteine beeinflusst werden können. So kann z.B. eine kovalente Bindung von Biomolekülen an ein festes Trägermaterial zu einer Änderung der Proteinkonformation führen, die ihrerseits wieder die Bindungseigenschaften des Proteins beeinträchtigt. Dieser Nachteil kann weitgehend verhindert werden, wenn die Biomoleküle physikalisch in eine poröse Matrix eingeschlossen werden. Eine schonende Methode, bei der Proteine unter milden Bedingungen bei Raumtemperatur in den Poren einer Silikatmatrix eingeschlossen werden, ist die Sol-Gel Methode.

In der vorliegenden Arbeit wurde die Stabilität von mittels der Sol-Gel Methode hergestellten DON-Immunaffinitätssäulen geprüft. Es wurden folgende Parameter getestet: die Abhängigkeit der Wiederfindung von DON von der Lagerungsdauer und der Temperatur beim Lagern der Säulen sowie von der Zahl der aufgereinigten Realproben und der Gesamtzahl der durchgeführten Versuche (Standardlösungen und Realproben). Weiters wurde der Einfluss des pH-Wertes der Aufgabelösung auf die Wiederfindung von DON geprüft.

Aus den Ergebnissen geht hervor, dass mit den hergestellten Sol-Gel Säulen mehr als 20 Proben (Mais und Weizen) aufgearbeitet werden können, ohne einen signifikanten Verlust an der DON-Wiederfindung zu beobachten. Die Säulen können sogar bei Raumtemperatur mehrere Wochen ohne Zusatz eines Bakteriostatikums gelagert werden, ohne dass es zu einer Herabsetzung der Wiederfindung von DON kommt. Wenn sich der pH-Wert der Aufgabelösung zwischen 6 und 8 bewegt, wird die Retention von DON in der Sol-Gel Immunaffinitätssäule nicht beeinträchtigt.

Optimierung der Immun-Ultrafiltrationsmethode zur Probenvorbereitung von Deoxynivalenol

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Bedingt durch die Höchstmengenverordnung für DON in der EU, gewinnt der DON-Nachweis immer mehr an Bedeutung. Lebensmittel- und Futtermittelproben werden in regelmäßigen Intervallen stichprobenartig untersucht, um eine Kontamination der Lebensmittel- und Futtermittel durch DON zu vermeiden.

Die konventionellen analytischen Verfahren zur Probenaufreinigung sind jedoch häufig kostenintensiv und benötigen viel Zeit für die Probenextraktion. Um diesen Aufwand zu verringern, galt es eine neue Probenvorbereitungsmethode zu entwickeln. Diese Methode sollte selektiv, sensitiv, einfach zu handhaben und kostengünstig sein.

Immunfiltration (IF) in Kombination mit der Ultrafiltration erwieß sich diese Probenaufreinigungsmethode als sehr einfach und zeitsparend.

Die IUF (=Immunultrafiltration) ist ein Verfahren, bei dem geringe Mengen Antikörper zur Probenvorbereitung für DON-Analysen eingesetzt werden. Bildung eines Antigen-Antikörper-Komplexes. Nach der Komplexbildung, werden Probenbestandteile, die nicht an den Antikörper gebunden wurden, mittels Ultrafiltration, durch ein Filter mit einer geeigneten Porengröße, abgetrennt. Es folgt ein Waschschritt um unspezifisch gebundene Matrixkomponenten zu entfernen. Der Antigen-Antikörper-Komplex wird durch die Zugabe von organischem Lösungsmittel vollständig dissoziiert. die Abtrennung des Antigens erfolgt durch abzentrifugieren der Lösung. Das organische Lösungsmittel wird im Stickstoffstrom entfernt. Das Antigen kann dann direkt mittels HPLC-UV analysiert werden. Die Vorteile dieser Methode liegen in ihrem geringen Antikörper-Verbrauch sowie in ihrer Schnelligkeit und Einfachheit.

Resultate wurden durch die Optimierung der folgenden Reaktionsbedingungen erzielt, als da wären Inkubation bei Raumtemperatur, Elution mit 80%igen ACN/H_2O (v/v) und ein Elutionsvolumen zwischen 3ml und 4ml.

Durch einhalten der Versuchsbedingungen wurden Wiederfindungen bis zu > 90% (bei Standard) erzielt. Die Dauer der IUF hängt von den jeweiligen Matrixbedinugngen ab, überschreitet aber im Durchschnitt nicht ~50 Minuten.

Identification of unknown toxic mycotoxins in *Aspergillus nidulans* using a structure-activity approach

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Mycotoxins are secondary metabolites of filamentous fungi, that can cause various toxic effects in humans, including cancer, immune suppression and teratogenicity. Exposure to mycotoxins occurs mostly by ingestion, but also by inhalation and skin contact. The respiratory uptake of mycotoxins plays a special role in waste treatment facilities, where toxic mycotoxins are present in organic airborne dust and bioaerosols. Acute and chronic pulmonary diseases were reported after inhalation of these agents. One of the most frequent toxigenic fungi in composting plants is *Aspergillus nidulans*, which produces the mutagenic and carcinogenic mycotoxin sterigmatocystin. The extract of *A. nidulans* caused serious toxic effects in A-549 pneumocytes. Since the observed cytotoxicity could not completely be explained by its content of sterigmatocystin, the presence of additional mycotoxins or other toxic prinpiples was assumed. [Bünger et al. (2004) Toxicology 202, 199] In addition mutagenicity tests indicated the presence of at least another genotoxic agent in the extract of *A. nidulans*. These unknown cytotoxic and mutagenic mycotoxins and their structures shall now be identified by a structure-activity approach.

In previous work the growth conditions and a mycotoxin extraction procedure for *A. nidulans* have been established. An HPLC-diode array method (gradient elution with water and acetonitrile) was used to separate the ~ 80 components in the extract within 50 minutes. Aliquots of the extract were chromatographed and 5 minutes-fractions were collected. These fractions were pooled, freezed and lyophilized. After determination of the dry weights of the fractions, aliquots of the residues were redissolved and rechromatographed to examine the accuracy of the 5 minutes-cuts.

Currently, the cytotoxicity and mutagenicity of these fractions are estimated using the neutral red assay (NRU assay) and a variation of the Ames test. Pilote work focussed on the validation of the Ames test with the diagnostic mutagens methyl methanesulfonate, daunomycin, 2-aminofluorene and sterigmatocystin. In order to find a suitable solvent for the fractions in the NRU assay, the solubility of sterigmatocystin was systematically tested in different organic solvents. These solvents were also examined for their cytotoxicity in different established cell lines (A-549, L-929 and Hep-G2). A promosing solvent mixture with a low cytotoxicity was ethanol/dichlormethane (1:1, v/v).
The 5-minutes-fractions exhibiting the strongest mutagenicity and cytotoxicity in these screening systems will further be analysed to identify the underlying unknown toxic principles.

Solid phase extraction on aminopropyl columns for determination of citrinin and ochratoxin A in cereals

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Ochratoxin A and Citrinin are nephrotoxic mycotoxins produced by *Aspergillus* and *Penicillium* spp.. Both toxins may have additive or synergistic effects and might be one cause of the Balkan endemic nephropathy of human in southeast europe and the nephropathy of swine occuring particulary in Denmark.

According to Meister [1] we developed a analytical method for determination of citrinin and ochratoxin A in cereals by aminopropyl clean-up and high performance liquid chromatography.

The extraction and quantification of ochratoxin A and citrinin in rice, maize and barley were carried out simultaneously. At the first step the toxins were extracted with acidified dichlormethan. The filtrate were clean-up on aminpropyl columns and the quantification achieved by HPLC-FD. The determination of the toxins in rye and wheat differ because of matrix effects. Therefore the toxins were not eluted simultaneously but consecutively from the aminopropyl columns and determinated by HPLC-FD.

According to DIN 32645 the detection limit of this method for Citrinin and Ochratoxin A amount 1-2 µg/kg resp. 0,2-1µg/kg with mean recovery rates of 69-85% resp. 83-91%.

[1] Meister, Ute (2004): New method of citrinin determination by HPLC after polyamide column clean-up. Eur. Food Res. Technol. 218: 394-399.

Production of certified reference materials for mycotoxins: Ochratoxin A

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One of the most important objectives of European Union policies is caring about the protection of human health and the environment. The progress of the European integration process and a huge increase in international trade of food products has induced an urgent need for the global comparability of analytical results [1].

A key element to ensure the accurate, repeatable and reproducible implementation of analytical procedures and obtaining reliable analytical results is the regular use of high quality Certified Reference Materials (CRMs). Suitable CRMs allow the correct implementation of Community regulations, written standards, the validation of newly developed analytical procedures and ensure the comparability of results and their metrological traceability. Furthermore, they provide a tool for the harmonisation of proficiency testing schemes and laboratory accreditation [2].

Mycotoxins, which are hazardous to human health, have been in the focus of Community research for a long time. The tangible results of this activity are the development of analytical procedures and CRMs or pure calibrants for various mycotoxins. Reports from all over the word show that it is still a problem, to control mycotoxin contents in food and to protect consumers from their harmful effects.

Hence, the aim of this project is to develop a CRM for ochratoxin A (OTA) analysis in wheat, to replace BCR-472, which is out of stock. This nephotoxic, carcinogenic and teratogenic mycotoxin is frequently found in cereals and cereal products, but also in raisins, coffee beans, cocoa, beer and wine. In addition it occurs in animal products by transmission to animals exposed to contaminated feeds based on cereals, such as wheat [3]. The importance of OTA as a food safety hazard is reflected in the existence of maximum permissible levels (e.g.: 5 $\mu g\ kg^{-1}$ in raw cereal grains, 5 $\mu g\ kg^{-1}$ in roasted coffee beans, 2 $\mu g\ kg^{-1}$ in wine) established by national and European regulations (e.g. Commission regulation (EC) No 123/2005, 26 January 2005).

The project realisation includes a number of steps, which will be briefly presented in the poster: selection of a suitable analyte/matrix combination, implementation and validation of analytical methodology for OTA determination, planning of the processing of the candidate CRM, homogeneity and stability studies of the CRM by using validated test method, organising the collaborative study to characterise the ochratoxin A content in the CRM, statistical evaluation and documentation of the certification data.

[1] B.M. Gawlik, F. Ulberth, H. Langenkamp, G. Bidoglio, M. Grasserbauer, “On the role of environmental reference materials in the development of horizontal standards for environmental protection” Accred. Qual. Assur. (2004) 9:221-225.
[2] F. Ulberth, H. Emons “Reference materials: are they fit-for-purpose for food analysis?” Anal. Bioanal. Chem. (2005) 381:99-101.
[3] International Agency for Research on Cancer, website: http://www.iarc.fr/

Determination of Patulin by online-SPE-LC

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Introduction

Patulin is a mycotoxin produced by several species of *Penicillium* and *Aspergillus*. Its occurrence is mainly attributed to fruits and fruit products like juice or puree. Especially apples and apple products can carry significant levels of patulin. Legal maximum levels in the EU are set within the EU regulation 466/2001.
Official methods (like ISO[1] 8128-1:1993, ASU[2] § 64 LFGB L 31.00-20) for the determination of patulin levels include a liquid-liquid partitioning step with ethyl acetate, incorporating an alkalic washing step. The subsequent identification and quantification is carried out by liquid chromatography (LC) with UV detection.
In contrary to most other important mycotoxins, no immunoaffinity material is commercially available yet. Therefore the required purification has to be reached on another way. Beside the described extraction with ethyl acetate, methods incorporating a multifunctional column (MycoSep, Romer) clean-up or SPE (solid phase extraction) steps are published. In addition, LC-MS, GC and TLC methods are described.

Materials and methods

The method presented here is based on the principles of the official ISO/ASU § 64 LFGB methods. The homogenised sample is dissolved resp. diluted with phosphate buffer and then extracted with ethyl acetate. The extract is evaporated and re-dissolved with acetate buffer under ultrasonification. This solution is ready for online-SPE-LC analysis with classic UV detection.

The required purification is reached with the help of the online-SPE instrument SP 3000, which is equipped with two easy-to-fill cartridges filled with MycoSep 228 resp. SDB (Styrene-Divinylbenzene copolymer, Baker) material.

The autosampler injects the sample solution via the MycoSep column onto the SDB column. On this material, patulin is trapped and enriched. While stuck on this material, the washing step with carbonate solution is automatically carried out.

Subsequently the SP 3000 instrument switches and the LC eluent flows over the SDB cartridge, thereby transferring the analyte onto the LC separation column (Polaris C18 A, Varian). The final detection is carried out by UV at 277 nm.

Results

This method allows the determination of patulin at a quantification limit of 15 µg/kg (mean recovery: 97.5 %; method of matrix calibration) in clear apple juice, but its performance was also proven for other fruits and fruit products like peaches, strawberries or apple puree.

Main advantage of the described method is the significant decrease in employed man-power in the analysis of sample series, combined with a reduction in analysing time. Additionally, a multiple use of expensive SPE and MycoSep material is possible.

The automated SPE step results in increased reproducibility and delivers a recovery correction for sample and standard solutions as both are analysed over the identical cartridges. The online-SPE device may also be coupled to an LC-MS system, thereby reaching even lower quantification limits.

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[1]International Organization for Standardization; [2]Amtliche Sammlung von Untersuchungsverfahren

Novel Fluorescence polarization (FP) immunoassays for several mycotoxins

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Novel applications of FP immunoassays to fumonisins ZEA, DON, or AFLA in grain, wheat, maize and other samples has been developed by AOKIN.
Fluorescence polarization (FP) immunoassays have an advantage in that separation of bound and unbound label is not required, a property that is useful for improving the speed of the assays. We report here the development of an FP immunoassay for ZEA in grain, wheat, maize and other samples. Combined with a rapid extraction technique, the assay can detect as little as 0.1 microgram ZEA/g within 10 min. These are potentially significant advantages over traditional ELISA techniques.

Enzyme-linked immunosorbent assays (ELISAs) for mycotoxins have existed for many years and are commercially available. Most such assays rely upon the competition between a toxin-labeled enzyme and toxin, from a sample, for a limited amount of antibody attached to a solid surface. After a washing step to remove unattached enzyme, and addition of substrate, a colored product is produced that is inversely proportional to the amount of toxin present.

Because of the low molecular weight mycotoxins can be detected in solution without the need for separating „bound" and „unbound" label, and the amplification step of ELISAs is unnecessary. FP immunoassays can be conducted using either the rate of association (kinetic assays) or the traditional endpoint of equilibrated mixture (batch or equilibration assays).

The FP immunoassay compared favorably to a liquid chromatographic method for the analysis of several naturally contaminated samples and samples amended with culture material. The results indicate the FP immunoassay as a rapid method for quantitatively screening for ZEA.

The potential speed of FP assays combined with the portability of available devices, demonstrates this technology is useful for mycotoxin screening, particularly in situations where samples need to be analyzed fast and quantitatively.

Development of a HPLC-MS/MS Based Method for a Simultaneous Determination of The Most Relevant Types of Mycotoxins in Wheat and Maize

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Toxic metabolites produced by miscellaneous species of fungi growing on plants include a variety of structurally different species. This has lead 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 [1,2], resulting in the search for multiresidue methods for the simultaneous screening of different classes of mycotoxins.

The development of such a method is, however, a difficult task, as both ionic (moniliformin is an acid with a pKa value of 1.7) as well as very apolar compounds (such as the enniatins) are included in the list of analytes. As a consequence, the conditions during sample preparation and chromatographic separation are a compromise and may be far from optimal concerning certain target substances. Furthermore, LC/MS, which is the only method capable of a selective and simultaneous detection of a large number of analytes in complex matrices, is prone to suffer from unpredictable signal suppression due to matrix effects.

This work describes the development of a method for the extraction, separation and the detection of 40 mycotoxins including trichothecenes, zearalenone derivatives, fumonisins, aflatoxins, ochratoxins, enniatins and ergot alkaloids in wheat and maize at the µg/Kg-level. The optimization of the analytical HPLC-MS/MS method is described as well as the performance of different solvent mixtures applied for extraction. Matrix effects are discussed for both wheat and maize and the feasibility of the direct injection of diluted crude extracts is demonstrated. Finally, the analytical figures of merit of the validation of the final method for both matrices are presented.

[1] D'Mello JPF, Placinta CM, MacDonald AMC, *Anim. Feed Sci. Technol.* 80 (1999), 183
[2] Speijers GJA, Speijers MHM, *Toxicol. Lett.* 153 (2004), 91

Immune modulation by *Fusarium* mycotoxins – single substance and mixture related effects

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The aim of the study was to investigate the effects of *Fusarium* mycotoxins (deoxynivalenol/DON, nivalenol/NIV, fusarenon X/FUS-X, Zearalenone/ZON) on the T helper cell type 1 (Th1-)-/ T helper cell type 2 (Th2-)-axis with regard to possible allergy facilitating, inflammatory or immune suppressive actions. Further research focused on defined mixtures of the single substances to determine potential additive and super-additive properties, respectively, as well as mechanisms of action influencing important functions (cytokine production) of peripheral blood mononuclear cells (PBMC).

Therefore, freshly isolated and subsequently CD3/CD28-stimulated PBMC were exposed to serial dilutions of the pure compounds. Following 24 h incubation culture supernatants were harvested and measured for the Th1-related cytokine interferon-γ (IFN-γ) as well as the Th2-related cytokines interleukin-4 (IL-4) and interleukin-13 (IL-13) by the enzyme-linked immuno-sorbent assay (ELISA). The vitality of the exposed cells was determined using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide]-assay.

In case of certain *Fusarium* mycotoxins (NIV, Fus-X, ZON) and dependent on the used mycotoxin concentration ELISA-measurements revealed differential inhibitions for IFN-γ on the one hand and IL-4/IL-13 on the other hand. Thereby, the Th2-cytokine production in the cell culture system was inhibited to a significantly lesser extent than that of IFN-γ. Thus allergy-facilitating effects cannot be totally excluded and need to be clarified trough further investigations. But generally, and also in contrast to relatively low impact on cell vitality (MTT-assay), the tested mycotoxins caused a strong inhibition of all measured cytokines. Hence, the single substances primarily act in a immune suppressive way. Consequently, EC_{50}-values were calculated for each compound concerning inhibition of the apparently most sensitive cytokine IFN-γ. Toxicity was in the order FUS-X>NIV> DON>ZON. The relationships between the ascertained values of EC_{50} for the mycotoxins DON, NIV and ZON were used to define concentration ratios from which serial dilutions of different mycotoxin compositions (DON/NIV, DON/ZON, NIV/ZON, DON/NIV/ZON) were generated and included in the subsequent two- and three-compound mixture experiments according to the design described above (culture assays with single substances). The results externalised that all mixtures affected cytokine- and especially IFN-γ – production in a way that corresponds to the so called model of concentration addition rather than to that of response addition (see e.g. Könemann and Pieters, 1996). Therefore, it is supposable that similar molecular mechanisms of action – at least particularly caused by structure relationships of the tested mycotoxins – account for the detected mainly immune suppressive effects on PBMC.

aided by the Deutsche Bundesstiftung Umwelt (DBU), http//www.DBU.de

Mycotoxin quick tests as a tool for the risk assessment in the evaluation process of wheat lots

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Cereals and cereal products are the most important staple foods. Mycotoxins can occur in all cereals. Therefore it is important, to protect the consumer against the intake of cereal food with high levels of mycotoxins. Starting in July 2006, maximum levels for certain Fusarium toxins in unprocessed cereals and many cereal products become effective (856/2005/EU).

For unprocessed wheat the maximum level for deoxynivalenol (DON) will be 1250 µg/kg.

During harvest, a huge number of cereal lots has to be evaluated for acceptance at storehouses and mills in a very short time. Thus it is not possible to use time consuming analytic procedures for checking DON levels. At these stages of the cereal processing chain, there is a need for quick tests as a tool for risk assessment. Such tests often would be used by persons, who have no laboratory experience. Therefore quick tests must be simple and robust. Furthermore, the test results must allow for clear-cut interpretation and must be reliable, to avoid entry of highly contaminated wheat lots in the food processing chain.

During the last year, we gained experience with the commercially available quick tests for DON using naturally contaminated wheat samples. Main objectives were: 1. evaluating the suitability of the tests for use by persons without laboratory experience, 2. identifying possible difficulties with the interpretation of the results and 3. estimating the reliability of the results. Our findings indicate that, with both available test systems, highly contaminated wheat lots can be identified with a relatively high degree of certainty. But it is emphasised that the quick tests tried out cannot replace an analysis with a HPLC or a mass spectrometer.

Determination of Aflatoxin M1 at trace levels in accordance with current Community legislation

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Commission Regulation (EC) No. 466/2001 sets a maximum residue level (MRL) of 0.05 µg/kg for Aflatoxin M1 in milk. This specification is amended by Regulation (EC) 683/2004 defining an MRL of 0.025 µg/kg for milk which is intended to be used for infants' food. Regulation 683/2004 also mentions that this limit should be reduced to 0.01 µg/kg if there is sufficient evidence that the analyte can be determined reliably at this low level. Performance criteria for analytical methods used by official laboratories are laid down in Commission Decision 2002/657/EC with respect to food of animal origin; for the analysis of mycotoxins Regulation 401/2006 will apply from 1st July 2006.
Official food control is mainly focused on the analysis of cow's milk; in Germany, the samples required by the National Residue Control Plan are only referring to this matrix.
Apart from the fact that cow's milk is consumed predominantly, it should be kept in mind that milk e.g. of goats might be of major importance for small children who have developed an intolerance against cow's milk.
In two validation studies (each comprising 40 samples) performed according to the requirements laid down in Decision 2002/657, it could be shown that the well-established procedure[1,2] for the determination of Aflatoxin M1 by immunoaffinity chromatography and fluorescence detection is, with regard to current legislation, appropriate for the analysis of milk of several species. The requirements referring to recovery rates and reproducibility that are in force at present and will be valid in the near future, are fulfilled. However, it should be mentioned that the criterion for reproducibility is the value calculated from the Horwitz equation, which gives unrealistic broad limits when applied to concentrations at trace level.
Also at lower concentration ranges, a quantitative evaluation can be done reliably, which can be seen from the signal-to-noise ratios that were taken from the chromatograms.

Kind of milk	S/N (M1 in matrix, 0.01 µg/kg)	Mean recovery (0.01 µg/kg)	RSD_R	2002/657 (Recovery 50-120%)	401/2006 (Recovery 60-120%)
Buffalo (natural fat content	20 - 52	75 %	7.2	✓	✓
Goat (natural fat content)	47 - 85	82 %	7.7	✓	✓
Cow 3,5% fat	27 - 72	79 %	12.5	✓	✓
Cow 1,5% fat	23 - 57	79 %	8.7	✓	✓

When testing the matrices for the validation studies, we noticed a contamination with Aflatoxin M1 in several samples, but the concentrations found were below the validated range beginning at 0.01µg/kg. However, this underlines that the method is suitable for the analysis of real samples with regard to the intended lowering of the MRL for children's food.

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Determination of macrocyclic trichothecenes in mouldy indoor materials by LC-MS/MS

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Macrocyclic trichothecenes produced by *Stachybotrys* species are highly toxic compounds associated with the so called “sick building syndrome”. These mycotoxins like satratoxin G and H could cause headaches, fatigue, nausea, vomiting, bleeding from mucosal membranes, depression, sleep disturbances, anxiety, vertigo, and memory-loss [1].

A sensitive LC-MS/MS method was developed for determining the macrocyclic trichothecenes roridin A, verrucarin A, satratoxin G and H in wall paper or gypsum board samples. Samples were extracted with acetonitrile/water (84/16, v/v) and cleaned up by standard purification steps. Preliminary results showed concentrations of satratoxin G and H up to 3,0 µg/cm^2. Furthermore, S*tachybotrys* was isolated from these materials and cultivated on rice [2]. After two weeks of incubation (dark, ambient temperature) rice media were extracted and analysed for the toxins. The extracts were cleaned up by preparative chromatography to obtain standard solutions of the toxins.

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Carry-over of deoxynivalenol and de-epoxy-deoxynivalenol into edible tissues as well as into blood serum and bile fluid of growing bulls

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The carry-over of deoxynivalenol (DON) and of the metabolite de-epoxy-deoxynivalenol (de-epoxy-DON) into edible tissues as well as into blood serum and bile fluid of growing bulls was examined within a feeding experiment with *Fusarium* contaminated wheat (10 mg DON per kg dry matter). The control group received uncontaminated wheat. The feeding experiment with bulls (n = 14 per treatment) covered the live weight range between 244 kg and 460 kg. The respective wheat batches were included in the concentrate portion at 65%, resulting in DON concentrations per kg DM (dry matter) of 7.8 mg (DON group) and 0.06 mg (control group), respectively. Concentrate supply was restricted to 2.3 kg/d – 2.8 kg/d (depending on the live weight ranges) whereas maize silage (DON concentration: 0.4 mg/kg DM) was offered for *ad libitum* consumption.

Ten animals of each group were slaughtered at the end of the experiment. Liver, kidney, muscle sample from *musculus longissimus dorsi*, back fat (sample of the corresponding subcutaneous fat), blood serum and bile fluid of eight animals of the toxin group and of four animals of the control group were analysed for DON and de-epoxy-DON using a previously described HPLC-UV method (Valenta, H., Dänicke, S. and Döll, S., Mycotox. Res. 19, 2003, 51-55) with slight modifications. All samples were incubated with ß-glucuronidase before extraction in order to cover glucuronide conjugates. Liver, kidney and muscle samples were lyophilized, extracted with acetonitrile-water and purified with immunoaffinity columns (IAC) after a pre-cleaning step. Fat samples were treated according to the method for muscle, but without freeze-drying. Blood serum and bile fluid were extracted with ethyl acetate on ChemElut® cartridges and the extracts were purified with IAC. The detection limits of both toxins were 4 ng/g in freeze-dried liver, kidney and muscle, 4 ng/g in fresh fat, 2 ng/ml in serum and 4 ng/ml in bile fluid.

Referring to the edible tissues, neither DON nor de-epoxy-DON could be detected in any of the muscle and fat samples. Very low concentrations of de-epoxy-DON below 10 ng/g (relating to freeze-dried samples) were found in four liver samples and three kidney samples of the toxin group. Only trace concentrations of de-epoxy-DON were measured in four serum samples of the toxin group, as well. In contrast to these results, de-epoxy-DON was detected in all bile samples of the toxin group (concentration range 0.008 – 0.040 µg/ml, mean value 0.024 µg/ml) and of the control group (range 0.007 – 0.017 µg/ml, mean value 0.012 µg/ml). The results underscore the low significance of carry-over of DON into edible tissues of bulls. Moreover, only the far less toxic metabolite de-epoxy-DON was detected.

Investigations on Mycotic Load in Seeds of Oleaginous Plants and in Residues Resulting from their Processing and Utilized as Animal Feeds

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Among species of oleaginous plants grown in Romania, a high-ranking position is held by varieties of sun flower. During cultivation, harvesting, storing and processing the risk of mycotoxicological pollution is present.
Another of the problems is that sun-flowers are utilized in the cold extraction of cooking oil on low- or average output pressing devices were the ongoing process is not always under control.

The aim of our research work has been the study of seed- and residue quality (cakes), residues resulting from processing with several privately owned units located in Transylvania.

Material and Method
There were drawn a number of 20 samples (10 of seed and 10 of oilcakes), and submitted to certain investigations: taste characteristics; gross chemical composition (performed by means of classical methods); freshness of the proteins – free ammonia; freshness of fats-contents in free fatty acids and, examinations for germs (NTG/g produce); also, for mycetes (load and identification of prevailing species).
Data on the chemical composition were helpful in establishing the feeding values.

The results obtained have led to the following *conclusions*:
a number of 14 samples have displayed various alterations in quality macroscopically detectable (low purity and faulty taste); then, in chemistry also (high water content, i.e., above 12 p.c. with twelve samples). The high water content correlated with the mycotic load of well over 200 thousand colony-forming units (CFU)/gram. The prevailing mycetes were yeasts and species of *Penicillium, Fusarium, Alternaria genera*, of high mycotoxicogenic potential.
The feeding value in six samples of oilcake was below the limits provided by literature data.

Finally, suggestions for measures to be taken towards lowering the alimentary risks both in man and in animal are also made.

References: with the authors

Investigations Regarding the Mycotic Load in Several Products Obtained from Plants Developing In the Transylvanian Spontaneous Flora, Used in Treating Various Diseases in Man and Animal

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Literature data, starting with ancient authors up to our days, offer innumerable evidence of so-called miracles in medicine. On the other hand, it is a proven fact that remedies found in plants cannot be easily replaced in the treatment of diseases, in health maintenance and in preserving longevity.

The Flora of Romania is very rich in medicinal plants and the population's interest in its therapeutic potential is ever increasing. Mostly during this last decade or so, besides the struggle for developing naturist medical practices, one can encounter numberless misdemeanors in so far as the legal and moral norms are concerned. However, many so-called *healers* have recently shown up and their scientific knowledge is either wanting or downright fraudulent. Moreover, some of the *practices* manage to escape the net of the law or, are consequences of faulty legislation.

Our research work have focused on the fruit quality of Hippo*phaë rhamnoides L.*, i.e. sea-buckthorn, plant growing in the spontaneous flora of this country, yielding considerable quantities of foodstuffs and medicinal products.

Material and Method. Within the 15th and 20th of October, there were drawn a number of 15 samples of Hippo*phaë ructus.* Of these, 5 samples of fruit dried at 50-60^{0} (BBM drier, Germany), packed in paper bags and stored in view of pharmaceutical processing and 10 samples of fresh fruit, available on the free market and sold for the purpose of preparing juice, syrup, jam, wine, liqueur, jelly or marmalade.

These were submitted to several determinations:
-taste analyses; hectoliter weight; gross chemical composition (in accordance with the Weende scheme); fatty-acids profile in oil by having used gas-chromatography; bacteriological examinations (NTG/g) by inseminations on agar broth incubated at 37^{0} and, mycological examination (inseminations on solid Sabouraud medium, incubation at 24^{0}, followed by reading and result interpretation within three days, and 6 days respectively from incubation).

Results and their Discussions. Water content of fresh sea-buckthorn fruit extended between 70 and 80 p.c., and in the half-dried ones, between 13-14 p.c.; the hectoliter weight was of 28-29 kg gross chemical composition. The oil structure of O*leum* Hippo*paë* was close to values given by literature.

The gross chemical composition, as well as oil structure (*Oleum* Hippo*phaë*) displayed values close to those in literature, with the exception of gross fat, which displayed lower values (25-26 p.c., of the dry matter). There has been established that the oil contains notable quantities of essential fatty acids, mostly of those belonging to the group of F vitamins. However, there was noted that the mycotic load in fresh fruit was higher than the limits in norms (over 300 thousand/g) with eight of the ten samples drawn. The prevailing mycetes belonged to the genera such as *Penicillium, Aspergillus, Fusarium,* which possess high mycotoxigenic potential.

One reaches to the conclusions derived from the results of our investigations that there is to be found a deleterious effect on the consumers' health from the part of the products available on the free market. Thus we suggest that steps towards quality-control should be taken right on the technological flow (harvest, packing, storing, transport, marketing), in accordance with the principles in the HACCP-system.

References: with the authors

Hämatologische und biochemische Untersuchungen bei Intoxikation mit DON bei Mäusen

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Es wurde mit 4 Gruppen weiblicher juveniler Mäuse gearbeitet. Gruppe 1 : 4 Mäuse intraperitoneal (i.p.) belastet mit je 7,5 mg DON pro kg Lebendmasse (LM), Tötung nach einer Woche; Gruppe 2 wie Gruppe 1, Tötung jedoch erst nach zwei Wochen; Gruppe 3: 4 Mäuse belastet (i.p.) mit 25 mg DON/kg LM, Tötung nach zwei Wochen; und Gruppe 4: Placebo, 10 Mäuse injiziert i.p. mit physiologische Kochsalzlösung.

Wir bemerkten ein Absinken der Gesamtleukozyten und der meisten „Leukozytenkategorien", etwas Moderater in der Gruppe 1 (getötet nach einer Woche) und deutlicher bei den Gruppen 2 und 3 (getötet nach zwei Wochen) (Johnson-Delaney, 1996). Dennoch, gegen unsere Erwartungen, konnte keine Korrelation zwischen der Dosis und der Schwere der Leukopenie bei den Gruppen 2 und 3 nachgewiesen werden, weil sowohl die Dosis von 7,5 mg/kg als auch die Dosis von 25 mg/kg vergleichbare Läsionen hervorriefen, anders gesagt hat DON auch in kleiner Dosis, nach einer Zeit von 2 Wochen eine drastische Nekrose – Wirkung, insbesondere des Knochenmarkes, verursacht.

Die Minderung der gesamten Anzahl der Leukozyten bei den Mäusen der Gruppen 2 und 3, (getötet 2 Wochen nach der Verabreichung von DON), erfolgte im Wesentlichen auf Kosten der Neutrophilen Granulozyten und der Lymphozyten , und weniger auf Kosten der Eosinophilen. Das Blutbild der Gruppen 2 und 3 ist eine Folge der Wirkung von DON auf das Knochenmark, auf die Lymphknoten, der Milz und des Thymus (Macri, 2005).

In einer ersten Phase der Toxinwirkung werden bei der Verabreichung von DON die freien Lymphozyten in den Organen intensiv gespeichert (Leber, Magen-Darm-Schleimhaut, Lungen, usw), was deren Verminderung in dem peripherischen Blut erklären kann.

Man kann also feststellen, dass die Läsionen, hervorgerufen durch DON in den lypmhatischen Organen, Veränderungen des Blutbildes hervorrufen, mit einer statistischen Relevanz bei gegebener Dosierung und 14 Tage nach der Verabreichung und dies bei beiden Dosierungen (sowohl bei der Dosis von 25 mg /kg DON als auch bei 7,5 mg/kg DON).

Das Serumprotein, wie auch die Proteinfraktionen (Albumin, Globulin α_1, α_2, β und γ), zeigten keine bedeutenden Veränderungen in Abhängigkeit von der DON-Belastung.

Diese hämatologischen Resultate, ermittelt als Folge der Verabreichung von DON bei Mäusen, stehen in Übereinstimmung mit den ermittelten Läsionen lymphatischen Geweben und Organen. Die Leukopenie registriert bei den Gruppen 2 und 3 kann durch die drastischen Läsionen des Knochenmarks, der lymphoiden Gebilde und die „Umverteilung" der weißen Blutkörperchen in verschiedene Gewebe und Organe erklärt werden.

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Effects of different ergot alkaloid levels in concentrate on health and performance of calves

[Dosis-Wirkungs-Studie zu den Auswirkungen verschiedener Mutterkorn-konzentratonen im Kraftfutter auf Gesundheit und Leistung von Kälbern]

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Ergot and its alkaloids are produced by *Claviceps purpurea.* It is found mostly on rye, but also on wheat, triticale and different grasses. Ergot alkaloids are known to be harmful for farm animals. During the last years the ergot contamination problem arised again due to the extensive cultivation of rye hybrids which are more susceptible to *C. purpurea* infection than the so-called population rye varieties. A number of studies dealing with the effects of ergot and ergot alkaloids on health and performance of poultry and pigs were reported in the past but only a few studies and field reports are available for ruminants. Therefore, a dose-response study was carried out with calves as this cattle category was considered as especially sensitive to ergot.

31 male Holstein bred calves were randomly assigned to 3 feeding groups after three weeks of feeding milk replacer. Hay was offered for ad libitum consumption during this period. The mean initial live weight of the calves was 49,4 ± 5,7 kg at the beginning of the experiment. There was one control group fed an ergot-free concentrate (n = 11), one group with an ergot proportion of 0.1 % in the concentrate (n = 10) and another one which was fed a concentrate containing 0.5 % ergot (n = 10). Hay, grass silage and water were available for *ad libitum* consumption, whereas the daily concentrate portion was restricted to 2 kg. Live weight, health parameters and feed intake were monitored over the experimental period of 84 days. In addition, blood samples were taken from the *Vena jugularis* at the beginning and at the end of the experiment and analysed for ergot alkaloids and liver parameters.

Live weight gain, feed to gain ratio, health and liver parameters, such as total bilirubin, GLDH, AST, γ-GT and CK were not significantly influenced by increasing ergot proportions in the concentrate. Concentrations of the individual ergot alkaloids in serum were lower than the detection limits of the applied HPLC-method.

However, in dry matter intake there was a trend for an ergot related decrease during the first 6 weeks of the experiment. After this period of time, it seemed that calves got used to the presence of ergot in the concentrate and were able to adjust their intake to the level of the control group.

In conclusion it can be assumed that an ergot contamination of the concentrate up to 0.5 % resulted in a transient depression of dry matter intake by the calves. However, no significant effects on health and performance could be detected when the entire test period of 84 days was considered.

Studies on toxicity of selected aquatic fungi and fungus-like organisms (FLO)

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Fungi and fungus-like organisms (FLO) inhabiting bodies of water exhibit a high level of biochemical activity which enable dead organic matter hydrolysis. This activity underlies the fact that aquatic microorganisms are absolutely crucial for the process of water self-purification. The trophic groups of aquatic fungi (zoopathogenic, phytopathogenic, or saprotrophic fungi) also exhibit a wide range of biodiversity. From the practical point of view, besides learning this biodiversity, also research on the toxicology of aquatic fungi should bring valuable information. Namely, cytotoxic properties of aquatic fungi have not been studies so far, although these pose a real health threat to the fish.

Hence, this study was carried out to investigate into the biological activity of the most common aquatic fungus-like organisms: *Saprolegnia ferax* (Gruith.) Thuret, *S. lapponica* (Tiesenhausen) Lund., and *Achlya americana* Humphrey. For this purpose, cytotoxicity assay (MTT) was applied using porcine kidney cell cultures. This assay has been so far used in testing the quality of food products and livestock feeds, as well as in evaluation of biological impact of living quarters fungal infestation. No reports have been published so far concerning an application of the MTT assay for aquatic fungi toxicity evaluation. Introductory toxicological studies on these microorganisms have been performed on 3-week cultures of aforementioned FLO grown on multicomponent agar medium (maize flour, pepton, yeast extract, glucose, and starch).

It has been demonstrated that all tested FLO species show a low level of cytotoxicity (15.625 IC 50 cm^2/ml). This implies that aquatic fungi, although having low cytotoxic activity, represent a significant health hazard for both juvenile and adult fish due to their massive occurrence in the environment. Consequently, their properties may disturb the biological balance of both aquaculture ponds and natural bodies of water.

In vitro effects of deoxynivalenol on intestinal glucose uptake in chickens

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One important aspect of DON toxicity is an injury of the gastrointestinal tract. It has been experimentally shown that DON inhibited intestinal absorption of essential nutrients such as D-glucose. Furthermore, DON selectively modulated the activities of intestinal transporters in human intestinal epithelial cells. DON appeared to alter the gut function and impair ion transport also in the chicken jejunum. Recently, it has been concluded that an inhibition of Na^+ transport and Na^+-D-glucose cotransport are important mechanisms of DON toxicity in the intestine of laying hens. However, these conclusions were based on indirect electrophysiological measurements. Therefore, the objective of the present work was to directly study the in vitro effects of DON on the glucose uptake of isolated jejunum mucosa of chickens by using the Ussing chamber.

Materials and Methods

Lohmann Brown laying hens, 18 wk of age, were used. The birds were slaughtered by stunning and bleeding. Intestinal segments were immediately taken from the mid-jejunum. The intestine was rinsed with ice-cold buffer and transported in ice-cold oxygenated incubation buffer to the laboratory. The intestinal segments were opened along the mesenteric border and washed free of any remaining intestinal contents with buffer solution at 4 °C. The underlying serosal layer was stripped off and the epithelial sheets were mounted in modified Ussing chambers. Isolated epithelia were incubated in D-glucose-free buffer mucosally and glucose containing buffer (10 mM) serosally in Ussing chambers for at least 30 min. Thereafter, 200 µmol/L [^{14}C] glucose was added to the mucosal side. After 1-min incubation with glucose, epithelia were washed three time with ice-cold glucose-free buffer solution. After tissue lysis by ice-cold NaOH for 3 min, glucose [^{14}C] was measured by scintillation counting. Data were compared by Friedman repeated measures analysis of variance on ranks. Student-Newman-Keuls method was used for the multiple comparison of different treatments (control, DON, Phlorizin, DON+Phlorizin).

Results

Glucose uptake under control condition was 3.28 ± 0.53 nmol/cm^2. The contribution of SGLT-1 to total glucose uptake was estimated by inhibiting SGLT-1 with phlorizin (100 µmol/L). In the presence of phlorizin, glucose uptake was reduced ($P < 0.05$) to 1.21 ± 0.19 nmol/cm^2. While DON decreased ($P < 0.05$) the glucose uptake in the absence of phlorizin to 1.81 ± 0.24 nmol/cm^2, it had no additional effect on the glucose uptake in the presence of phlorizin (0.97 ± 0.17 nmol/cm^2).

Conclusion

From this study it can be concluded that treatment with DON (10 mg/L) decreases glucose uptake almost as efficiently as phlorizin. The remarkable similarity between the effects of phlorizin and DON on glucose uptake evidences their common ability to inhibit Na^+-D-glucose co-transport. Our data shed some light on the mechanism by which DON could induce diarrhoea in animals. An inhibition of the intestinal SGLT-1 transporter by DON would result in a decrease of glucose-associated water absorption, in an increase of water content in the intestinal lumen and, consequently, in osmotic diarrhoea. In the longer term, these inhibitory effects of DON on SGLT-1 would be complemented by a decrease of intestinal absorptive area. We reported previously that DON affected small intestinal morphology, especially in the duodenum and the jejunum, as evidenced by shorter and thinner villi. The decrease in villus area and specific inhibition of SGLT-1 by these mycotoxins would commonly impair digestive and absorptive functions.

Effects of the *Fusarium* toxin deoxynivalenol on tissue protein synthesis in pigs

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The *Fusarium* toxin deoxynivalenol is known to be a potent protein synthesis inhibitor according to research in different in vitro and in vivo systems. However, the significance of this toxic feature was not yet evaluated for the pig. Therefore, an experiment was carried out to measure porcine tissue protein synthesis employing the so-called flooding dose technique using [$^{2}H_{5}$]-phenylalanine as tracer. A total of 25 male castrated pigs (initial weight of 23 kg) were used. Seventeen pigs were fed the control diet (control) and 8 the *Fusarium* toxin contaminated diet (chronic oral DON) for ~4 wks. Pigs recruited from the control group were treated with acute oral (n=7) and intravenous (i.v.) DON (n=5), and were exposed to the toxin once on the day of protein synthesis measurement. The DON dose given 60 min prior to the beginning of protein synthesis measurement amounted to 2, 77, 83 and 53 µg DON/kg live weight, respectively.

Protein synthesis expressed as fractional synthesis rate (FSR) was significantly reduced in kidneys, spleen and ileum of DON-exposed pigs with effects being less pronounced in the acute oral DON group. This was presumed to result from a higher degree of glucuronidation of the circulating DON. FSR of liver, skeletal and heart muscle, mesenteric lymph nodes, duodenum, jejunum, jejunal mucosa cells, pancreas and lung were not affected by DON.

Further studies should more specifically consider the degree of DON glucuronidation when examining DON effects on protein synthesis in the pig.

The dietary exposure of deoxynivalenol in The Czech Republic

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Deoxynivalenol (DON) is a mycotoxin produced by several field fungi, including *Fusarium graminearum* and *Fusarium culmorum*. An occurrence of DON is common in cereals and grains, particularly in wheat, barley and maize. The actual DON concentration in foodstuffs varies considerably depending on climatic, seasonal and geographic conditions, as well as on genetic predisposition of individual hosting crops, and on the applied good agricultural practice (GAP).

The WHO presented a comparative estimate of human exposure in Europe, Far East, Latin America and the Middle East. This comparison demonstrated, that approximately 80% of the total DON intake in Europe is associated with the consumption of wheat, whereas wheat and rice are equally important as source of DON exposure in the Far East (WHO, 2001).

DON is rapidly metabolised by de-epoxidation and glucuronidation, yielding less toxic products. There is no evidence for teratogenicity and genotoxicity of DON and its metabolites, neither in laboratory animal species nor in target animals. Transfer of DON and its metabolites into edible tissues, milk and eggs is very low.

Human exposure to DON occurs predominantly via ingestion of cereals and grains, and therefore animal products do not significantly contribute to this exposure. Considering the year-to-year variability of the contamination of cereals and grains with DON in Europe, an exposure of consumers close to the PTDI of 1000 ng/kg b.w./day is possible, as concluded by Scientific Committee on Food (1999 and 2002) and confirmed by the recent SCOOP task (EC, 2003). The dietary intake of DON is close to the PTDI for the group of young children and adolescents.

However, at present the available data on exposure via foodstuffs are incomplete in the Czech Republic. The estimation of dietary exposure dose of DON for the Czech population (for average person 64 kg b.w.) was for cereal and cereal products 730 ng/kg b.w./day and for beer 150 ng/kg b.w./day in the 90th years.

The validated and accredited immunochemical method ELISA (ELISA Ridascreen® Deoxynivalenol) was used for quantification of DON in foodstuffs. The limit of quantification 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).

Average dietary exposure will be calculated by multiplying of DON concentration data for specific foodstuffs (246 samples of cereal products) with consumption rate per kg of b. w. per day (Czech Food basket, 1997). The estimation of dietary exposure dose of DON for the Czech population (for average person 64 kg b.w.) was 420 ng/kg b.w./day for cereal products (e.g. bread = 249 ng/kg b.w./day, rolls = 96 ng/kg b.w./day, wheat flour = 62 ng/kg b.w./day).

This estimate can then be compared to known PTDI to assess if dietary exposure represents a risk to consumer health.

Supported by the FP6 project SAFE FOODS – Promoting Food Safety through a New Integrated Risk Analysis Approach for Foods.

Effekte hoher Fumonisin B_1–Konzentrationen im Mais auf Leistungsparameter und Gesundheitsstatus beim Mastschwein

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Einleitung:
Als Stoffwechselprodukte der Pilzgattung Fusarium sind Fumonisine hauptsächlich in Mais zu finden. Weltweit sind schätzungsweise 59% der Mais- oder Maisprodukte mit Fumonisinen kontaminiert, von denen Fumonisin B_1 (FB_1) am giftigsten ist.

Fragestellung:
1. Effekte hoher Fumonisin B_1–Konzentrationen (43 ppm FB_1) über einen Zeitraum von 6 Wochen auf Mastleistung und Gesundheitsparameter beim Mastschwein.
2. Effizienz eines oberflächenmodifizierten Dreischicht-Phyllosilikats (FIXAT®, Süd- Chemie AG) zur Kompensation der Fumonisin initiierten Effekte.

Ergebnisse:
Im Labor mit *Fusarium verticillioides* bebrüteter Mais wurde künstlich mit Fumonisin B_1 kontaminiert.

Effekte: Die hohen FB_1-Konzentrationen führten in der positiven Kontrollgruppe (T-1) zu signifikant verringerten Lebendmasse-Zunahmen um 12-19% (ca. 4-6 kg reduzierte Durchschnittszunahme), die durch Einsatz von FIXAT® (T-3, T-4) vollständig kompensiert werden konnten. Gleichzeitig verbesserte sich auch die Futterverwertungsrate. Beginnend in der ersten Woche, reagierten in der T-1 Gruppe am Ende des Versuchs 75% der Tiere mit Durchfallgeschehen und erhöhter Atem- und Herzschlagfrequenz. Dies ging einher mit deutlich erhöhten Konzentrationen coliformer Keime im Zwölffingerdarm. Die biochemische Analyse (Tab. 1) der Leber ergab signifikante Erhöhungen der Harnstoff- und Creatinin-Konzentrationen, als Indiz für einsetzende Niereninsuffizienz. Der statistisch gesicherte Anstieg der Leberenzyme ASAT und ALAT gab einen deutlichen Hinweis auf eine akute Hepatitis. Deutlicher als an Leber und Niere waren die histopathologischen Schädigungen an Lunge und Herz durch FB_1. Gewebeverdichtung und Kapillarblutung der Lunge, Flüssigkeitsansammlung im Herzbeutel und Organvergrößerung waren in der T-1 Gruppe bei allen Tieren zu beobachten. dienen. Das deutlich erhöhte Sphinganine : Sphingosine Verhältnis (Tab. 2) im Harn der Positivkontrolle (T-1), als diagnostischer Marker für eine Fumonisinexposition, weist auf eine Hemmung des Enzyms Ceramid-Synthetase hin.

Effizienz des Adsorbers: Durch Einsatz des Mykotoxinadsorbers FIXAT® (T-3, T-4) konnten alle untersuchen Parameter dosisabhängig, annähernd auf Normalniveau (T-2) gehalten werden und damit eine renal- und hepatoprotektive Wirkung gegen hohe FB_1 – Konzentration nachgewiesen werden. Histopathologische Schädigungen an Lunge und Herz konnten ebenfalls fast vollständig vermieden werden.

Tabelle 1: Biochemische Nieren- und Leber-Analysen

	T-1	T-2	T-3	T-4
mg/100 ml	Positivkontrolle FB_1 (43 ppm)	Negativkontrolle ohne FB_1	**FIXAT**® 2kg/to + FB_1 (43 ppm)	**FIXAT**® 4 kg/to + FB_1 (43 ppm)
Harnstoff	58,5^a	32,5^b	37,0^b	22,0^b
Creatinin	1,7^a	0,8	0,9	0,7^b
ASAT[1)]	216^a	145^b	160^b	139^b
ALAT[2)]	132^a	85^b	101^b	98^b

a) b) = Differenz mit p<0,05 gesichert, n=8 1)Aspartat-amino-transferase 2)Alaninaminotransferase

Tabelle 2: Verhältnis Sphinganine:Sphingosine Referenzwert

Referenzwert <1,0	1,9	0,12	0,5	0,2

Bioavailability of the *Fusarium* toxin deoxynivalenol (DON) from naturally contaminated wheat for the pig

[Zur Bioverfügbarkeit des *Fusarium* Toxins Deoxynivalenol aus natürlich kontaminiertem Weizen beim Schwein]

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Experiments were carried out with 16 castrated male pigs (41.5 ± 2.0 kg) to examine the toxicokinetics of deoxynivalenol (DON) from naturally *Fusarium* contaminated wheat (16.6 mg DON/kg) after chronic exposure or a single oral dose (acute). The systemic absorption (bioavailability) of DON was estimated based on the area under the curves (AUC) after dietary exposure (chronic or acute) to naturally *Fusarium* contaminated wheat and intravenous application of pure DON (53 µg/kg LW). Additionally, a balance study was conducted to quantitatively trace the DON metabolism.

After intravenous (IV) DON application (n = 5), serum DON concentrations decreased biphasically with terminal elimination half-lives ($t_{1/2}\beta$) of between 4.2 and 33.6 h. DON was rapidly absorbed following oral exposure and reached maximal plasma concentrations (C_{max}) of 21.79 and 15.21 ng DON/ml serum after (t_{max}) 88.4 and 99.1 min in the chronic (n = 5) and acute (n = 6) fed group, respectively. Thereafter serum DON levels declined slowly with an elimination half-life ($t_{1/2}\beta$) of 6.28 and 5.32 h for both oral groups. Glucuronide conjugation of deoxynivalenol was found in serum samples after oral exposure, but not after intravenous application. The mean bioavailability (F) of free (unconjugated) and total (free + conjugated) DON was 89 ± 27 % and 112 ± 24 % for the chronic group and 54 ± 17 % and 92 ± 27 % for the acute oral group, respectively. DON was highly distributed in all groups, with an apparent volume of distribution (V_d) higher than the total body water.

Dietary DON caused a significant increase in DON concentrations of urine and faeces, whereby the metabolite de-epoxy-DON was found only in the trials with a pre-period of longer than 4 weeks. The total recovery was 66.6 ± 39.0 and 54.0 ± 9.7 % for the control and the chronic DON groups, respectively, with urine being the main excretory route.

In conclusion, orally administered DON was quickly absorbed to nearly 100 % (total DON), highly distributed and only poorly metabolized. Twenty-four hours following oral dosing, DON could not be detected in the serum, except in one chronically fed pig at the level of the detection limit.

The impact of low – dose and short – term zearalenone mycotoxicosis on the pathological picture of the ovaries in a bitch

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The study was carried out on 9 bitches (cross – bred) within anoestrus stage which were divided into three groups (two experimental ones [n=3 bitches] and a control one [n=3 bitches]). Group I – bitches that were receiving zearalenone (ICN Pharmaceuticals Inc.) at a dose of 25 µg/kg b.w. orally for the period of 100 days (D I); group II - bitches that were receiving zearalenone at a dose of 50 µg/kg b.w. orally for the period of 100 days (D II); control group – bitches that weren't administered zearalenone (K). On the last day of the study the bitches were submitted to ovariohysterectomy. The ovaries were evaluated morphometrically and gross description was made. The specimens of the ovaries were preserved in neutralized, 7,4 pH buffered 10% formalin and subsequently fixed in paraffin blocks which were then processed into microtome segments for PCNA detection and HE and PAS (according to Mc Manus) staining. The ovaries of the experimental bitches were dark red in colour without apparent follicles the size of bean seed. On the surface of the ovaries a few follicles the size of mustard seed were observed. On some ovaries numerous firm cysts were noted whereas on some others – the cysts located on the ligament. Within group K the ovaries were intensively hyperaemic and preovulatory follicles were noted on their surface. The evaluation of the PCNA stained segments, derived from the ovaries of the bitches from both experimental groups, revealed what follows: a few PCNA positive cells in immature ovarian follicles and the destruction of the membrana granulosa in maturing ovarian follicles and the lack of PCNA positive cells. In the group K, among others, properly developing small ovarian follicles with numerous PCNA positive cells were observed. In both of the groups D the pathomorphological examination revealed: the extensive extravasation and the presence of small immature ovarian follicles; the hyperaemia and oedema of the ovaries; the cysts with retained elements of the membrana granulosa and the extravasation; the atrophy or damage of the membrana granulosa with retained oocytes; the hyperaemia of the core; the presence of abnormally distributed large oocytes in the follicles and the necrosis of the oocytes and the degeneration of the membrana granulosa in the follicles. No pathological lesions were observed in the group K. The presented results serves as the basis for an assumption that the presence of zearalenone in feeds for bitches causes deficient function or dysfunction of the ovaries.

Forensic mycotoxicosis investigation of an infant death case

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A forensic investigation of a six week old infant death case was conducted to detect evidence of a lethal mycotoxin intoxication. Routine liver and blood autopsy materials were analyzed for the presence of mycotoxins.

The infant death occurred in a home (Texas, USA) with significant water damage and microbial contamination with *Stachybotrys* spp., *Aspergillus* spp., *Penicillium* spp. and *Chaetomium* spp., among others. The only forensic pathological findings were a „moderate to severe vascular lung congestion“and „focal macrovascular steatosis of the liver“; routine toxicological blood tests (i.e., drug screen, alcohol and medications) were negative. The liver and blood from the infant was analyzed by use of HPLC for the presence of ochratoxin A [1, 2] and none was detected.

In addition, an immuno-chemical assay for the presence of *Stachybotrys chartarum* derived trichothecenes mycotoxins was conducted (QuantiTox Kit for Trichothecenes, EnviroLogix, Portland, USA) on the autopsy materials and control samples. The test assay kit was designed for the quantitative laboratory detection of trichothecenes including Roridin A, E, H and L-2, Satratoxin G and H, Isosatratoxin F, Verrucarin A and J, and Verrucarol. The assay’s quantitative range is from 0.2 to 18 parts per billion (ppb) of Roridin A in the sample extract.

In an untreated infant liver specimen a mean 11.68 ppb Roridin A-equivalent/g was detected. Control matrix effects (positive/negative controls) were tested on pig liver specimens; these tests were all satisfactory, excluding a potential matrix effect. A mass spectrometric confirmation of the assays findings (by the Technical University of Denmark) was not achievable due to technical problems; the remaining test sample quantity and the recovery rate of this method were not sufficient (<10%). The formalin treated lung specimen suggested a positive finding of trichothecenes (EIA-result (3.05 ppb)); however, the formalin treated negative control showed that this was a false-positive result.

In conclusion, these findings strongly suggest a causal relationship between an exposure to trichothecenes producing *Stachybotrys* fungi and the trichothecenes findings in the liver of the deceased infant. For a mass spectrometric confirmation of the ELISA findings routine autopsy materials are not adequate. Frozen specimens without chemical preservation are sought in forensic mycotoxicosis investigation.

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Production of mycotoxins by filamentous fungi on building materials

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Filamentous fungi are a dominant microorganisms responsible for biodeterioration in indoor environment. These organisms can be found on water damaged building partitions, building and finishing materials. The consequence of their development is destruction of building materials and air contamination by fragments of mycelia and spores carrying fungal metabolites – mycotoxins. The moulds isolated from domestic dwellings included toxigenic species such as *Aspergillus versicolor*, *Aspergillus flavus*, *Penicillium auriantogriseum*, *Stachybotrys chartarum*, capable of producing mycotoxins: sterigmatocystin, aflatoxin B_1, ochratoxin A and trichotecens respectively. The aim of the presented studies was to the estimation of the frequency of 3 toxigenic moulds isolated from dwellings, screening of mycotoxins production by these strains and demonstrate to what extent the type of building material on which fungi grow modifies the ability to produce mycotoxins.

Moulds – *Aspergillus flavus, A.ochraceus* and *A.versicolor* isolated from surfaces of building partitions in dwellings with visible fungal growths (33 buildings in Lodz) and controls dwellings (without visible moulds growth – 20 buildings). Also the same toxigenic strains from culture collections were investigated. TLC was used as a screening method for mycotoxins production (aflatoxin, ochratoxin, sterigmatocystin) on building partitions in dwellings, next Elisa test was used in particular model research. Mycotoxins production on model study was estimated on YES or MEA medium, on building and finishings materials (gypsum-cardboard plate, gypsum-plaster, cementous adhesive, cement, wallpaper, leather, syntetic leather, PCV lining, carpet). The level of growth on building and finishing materials was estimated by determination of ergosterol.

Results of research show that, the frequency of presence *A.versicolor* on building partitions in mouldy buildings is about 12% level, this strain was isolated from surface of plaster, gypsum-cardboard plate, wallpaper only in mouldy buildings, where humidity of the air was above 60%. Frequency of isolation of *A.flavus* and *A.ochraceus* in buildings was 2-3%, but humidity of air in these dwellings was 40-50%. Studies with the screening method TLC did not reveal the presence of the analysed mycotoxins in any of the examined building partitions. In model study none strain *A.flavus* was capable of producing aflatoxin, ochratoxin was produced by *A.ochraceus* on natural building and finishing materials like – gypsum-cardboard, leather, wood, wallpaper and on YES medium, sterigmatocistin was produce by *A.versicolor* on gypsum-cardboard, wallpaper and YES.
It was obserwed that production of mycotoxins by moulds didn't corelate with high level of mycelium growing building and finishing materials.

The air quality in the microbiological laboratory after sterilization with UV lamps

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Microbiological cleanness of the air is one of the most important factors having impact on the quality of research results in a microbiological laboratory. International norms, like ISO 7218:1996, determine the number of microorganisms: bacteria, moulds, yeast at the level of 200cfu/m^3. The outdoor air and the samples of biological material in the laboratory are the sources of the pollution of the indoor air. Sterilization of the air is possible with HEPA/ULPA filters with efficiency >99,9999% for 0,12µm particles or by irradiation with ultra-violet rays with wave length between 100-340nm.

The microbiological air quality is checked once a week and the elaboration of the data from 2005 is to determine the efficiency of air sterilization with germicidal lamps. The data does not refer to the examination of air quality under the laminar box, where the number of microorganisms is 0 cfu/m^3.

UV lamps work in 12-16 hour - cycles, every 48 hours excluding unsociable hours (weekends and days off). The work conditions and the manner of dealing with biological material are in accordance with ISO 7218:1996.

The air samples were taken using the air sampler MAS100, produced by Merck with a striking method on agar medium YGC (Yeast extract, Glucose, 100ppm Chloramphenicol) and nutrient agar by Merck.

The acceptable level of contamination was noted down only in the rooms remote from the laboratory room, where no microbiological works were done and the maximum contamination of the air exceeded 2-3,6 times the permissible level (June, August, September, respectively). The contamination found in the sterile material preparatory room exceeded from 1,2 to 4,6 times the acceptable level of 200cfu/m^3 (excluding September – long holiday break). Microbiological contamination of the air exceeded the permissible level from 3,5 to 6,8-times (max.10,8-times in August) in the remaining sterilized rooms, where the biological material was analyzed and stored and in the rooms without germicidal lamps.

The effectiveness of the UV lamp sterilization is limited and not sufficient in case of high contamination of the outdoor air. Laboratory rooms' location and the number of the samples have significant impact on the indoor air's quality. To improve the quality of work it is necessary to install another system of sterilization.

Chemical indicators for fungi in house dust: ergosterol and NAGase

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Air and house dust may serve as materials to detect an indoor contamination with fungi. They are mostly identified and quantified as colony forming units (CFU), i.e. the count of culturable spores. But this method is rather time consuming as it lasts some days till viable spores have grown to colonies. Chemical analysis of compounds that are indicators for fungi may be an alternative. Among these chemical markers are ergosterol, a fungus specific membrane steroid, and NAGase (N-acetyl-β-D-glucosaminidase), an enzyme expressed by diverse fungi. The aim of our study was to investigate whether there is a correlation between the ergosterol content and the NAGase activity in house dust, respectively, and the number of CFU.

Material and Methods: 37 house dust samples were collected in the springtime by vacuum cleaners. The contents of the vacuum cleaner bags were sieved to ≤ 63 µm to discard fibre and coarse material. For ergosterol analysis dust was saponified with 10% KOH in methanol. The sample was centrifuged, water was added and extracted with n-hexane. The hexane extract was dried and the residue dissolved in methanol. Quantification was performed using HPLC by separation on a C18-column (solvent: methanol) and UV-detection at 282 nm (7-dehydrocholesterol served as an internal standard). For evaluating NAGase activity dust was extracted with a 0.9% NaCl solution containing 0.1% of Triton X100. After centrifugation the enzyme activity in the supernatant was analysed with 3-cresolsulfonphthaleinyl-N-acetyl-beta-D-glucosaminide as a substrate. To obtain the number of CFU house dust was suspended in 0.9% NaCl solution containing 0.1% of Triton X100 and plated on DG18 (containing 0,1% of chloramphenicol to prevent bacterial growth) and on malt extract (ME) agar. Plates were incubated at 20-22°C up to 10 days. The CFU were counted and the genera identified.

Results and Discussion: Ergosterol was present in all house dust samples, concentrations were: <5-480 mg/kg (median: 30; mean 55 mg/kg). Activities of NAGase were 2.5-420 U/kg (median: 80; mean 110 U/kg). CFU amounted from <1 to 2,300 CFU/mg for ME agar (median: 55, mean 260 CFU/mg) from 3 to 1,900 for DG18 agar (median: 85, mean: 270 CFU/mg), respectively. *Penicillium sp*. were most frequently observed, i.e. about 88% of all the colonies belonged to this genus. About 5% of the CFU were *Aspergillus sp*., about 3,5% *Cladosporium sp*. All the other genera also present (*Acremonium sp., Alternaria sp., Curvularia sp., Mucor sp., Paecilomyces sp., Pithomyces sp., Rhizopus sp., Trichoderma sp., Wallemia sp*.) did not account for more than 1% for each genus. Neither concentrations of ergosterol nor of CFU revealed normal distributions. Thus for statistical analysis non-parametric tests had to be used (Spearman rang correlation). As expected there was a close correlation of CFU grown on DG18-agar compared to those grown on ME (r_s=0.8639; P<0,001). There also was a weak but significant correlation of ergosterol concentrations to the number of CFU grown on DG18 agar (r_s=0.4212; P<0,01) to CFU grown on ME agar (r_s = 0,3487; P<0,05), respectively. These rather poor correlations may be explained by ergosterol in non-viable spores, i.e. spores forming no colonies (ergosterol is rather stable if protected form sunlight). There were no associations between NAGase activities in house dust and its ergosterol content and there also was no association between NAGase and CFU (neither for DG18 nor for ME agar). This may be due to the fact, that age and condition of spores affect the concentration and activity of enzymes.

Stachybotrys spp. in Stroh und Heuproben aus Pferdeställen

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Der cellolytische Schimmelpilz *Stachybotrys* ist in der Lage eine Reihe toxischer Sekundärmetabolite zu bilden, darunter die hochtoxischen makrozyklischen Trichothecene. Unter den Nutztieren gelten Pferde als besonders anfällig für Intoxikationen durch diese Mykotoxine. Obwohl die als Stachybotryotoxikose bezeichnete Erkrankung noch bis etwa vor 50 Jahren ein ernsthaftes Problem in Pferdebeständen darstellte, ist diese Mykotoxikose sowohl bei den Tierhaltern als auch innerhalb der Veterinärmedizin nahezu in Vergessenheit geraten.

Ziel dieser Arbeit war es daher festzustellen, ob toxinogene *Stachbotrys* spp. in Pferdeställen vorkommen und möglicherweise ein Gefahrenpotential darstellen.

Zu diesem Zweck wurden je 50 Heu- und Strohproben aus insgesamt 46 Pferdeställen gesammelt und zunächst mykologisch auf die Präsenz von *Stachybotrys* spp. untersucht. Bei positivem Nachweis wurden die *Stachybotrys*-Stämme isoliert und auf ihr Trichothecenbildungsvermögen mittels PCR über den Nachweis des Tri5- Gens überprüft. Parallel dazu wurde die Zytotoxizität der Kulturen mit dem MTT- Zellkulturtest gemessen und Toxinnachweise mittels ELISA durchgeführt. Ausgewählte Stämme wurden zusätzlich mit HPLC und LC- MS analysiert.

Insgesamt konnten 32 verschiedene *Stachybotrys*-Stämme (elf aus sechs Heu-, 21 aus 14 Strohproben) isoliert und näher charakterisiert werden.

Das Tri5-Gen wurde bei vier bzw. acht der *Stachybotrys*- Isolate aus Heu (= 36,4%) und Stroh (= 38,1%) festgestellt. Neun Kulturextrakte der zwölf Tri5-Gen positiven Isolate waren im Zellkulturtest hochtoxisch (IC_{50}-Werte zwischen 0,38 bis 24,41 mm^2 Agaroberfläche/ml Zellkulturmedium) und wiesen sehr hohe Toxinwerte im ELISA-Screening auf (3.447,1 bis 174.605,2 ng Roridin A-Äquivalent/g). Mittels HPLC und LC-MS wurde die Produktion von Satratoxin G und H in fünf untersuchten Isolaten nachgewiesen (1,46 bis 52,88 µg/ml).

Die restlichen 23 Isolate konnten als nur gering bzw. nicht zytotoxisch (IC_{50}-Werte zwischen 97,66 und 1.562,5 mm^2 Agaroberfläche/ml Zellkulturmedium) eingestuft werden. Die entsprechenden ELISA-Resultate ergaben Werte im Bereich von 1 bis 113 ng Roridin A-Äquivalent/g.

Die Ergebnisse dieser Arbeit zeigen, dass *Stachybotrys*-Isolate mit der Befähigung zur Toxinbildung in Heu- und Strohproben aus Pferdeställen vorkommen können und damit ein Gefährdungspotential darstellen. Insbesondere bei unklaren Erkrankungs- und Todesfällen sollte die Stachybotryotoxikose differentialdiagnostisch einbezogen und entsprechende Untersuchungen des Futters und der Einstreu veranlasst werden. Aufgrund der möglichen Präsenz von toxischen und nichttoxischen Isolaten reicht der ausschließliche mykologische Nachweis allerdings nicht aus, eine Kontamination mit Mykotoxinen abzuleiten.

The *Aspergillus versicolor* Tiraboschi sterigmatocistin (ST) synthesizing strains and dependent on their productivity in housing buildings mycotoxic threat differentiation

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In statistically significant quantity of samples (1000 samples) taken from buildings' barriers with visible mould infesting in over 250 habitable rooms were executed:
– mycological qualitative analysis (the number of mould species occurrence in housing buildings),
– mycological quantitative analysis (occurrence frequency calculation of particular species in buildings).

For chosen isolates, toxicological, biotoxicological, physical and chemical analyses investigations were executed onto presence some mycotoxin.

On base of moulds isolated in dwelling buildings occurrence frequency was determined. Proportional occurrence were expressed in five grade scale (1-5). Ten fungi species with frequency of 3-5, mostly colonized walls in flats on the terrain of the Lubuskie Province. With high frequency 3 microfungal genera *Aspergillus: A. versicolor, A. flavus* and *A. niger* occured among them.

Consider the mycotoxins synthesized in buildings by referred fungi, it was possible to find, that largest threat for staying persons in mouldy buildings' objects in Lubuskie Province and probably in Poland made *A. versicolor* which often colonizing building barriers. Some people says, that this mould consistently and with large efficiency synthesizes the ST on buildings' materials.
What percentage of strains is mycotoxin - positive and what they are extremely productive were examined due to large threat of occupants health of houses infected by *A. versicolor*. To this end 20 samples biomasses of microfungi isolates were analyzed in HPLC method. In three of them the ST presence was not affirmed (<0.03 $mgkg^{-1}$,detectability limit) one strain was atoxic with (0.072), 7 strains were poor toxic (1.3-8.6), 6 strains were intense toxic (10.0-84.0). However 3 strains were very intense toxic synthesizing (>100) ST. Their productivity were 107.4; 120.7 and 534.4 $mgkg^{-1}$ air dry biomass of culture. This shows that particularly one strain produced a lot of ST, over 500 $mgkg^{-1}$ and created largest mycotoxic threat.

Recapitulating it is possible to ascertain, that no all strains *A. versicolor* synthesized ST, about 50% of strains were low-productive and 15% were dangerous. Because ST is nefrotoxic, hepatocancerogenic and immunosuppressive mycotxin for man, investigations will be continued.

Mycotoxins in House dust: Comparison of three different analytical methods for the determination of Ochratoxin A in House dust

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In industrial developed countries persons stay up to 90% of their time in indoor environments. In northern latitudes for energy saving reasons increasing actions are taken for thermal insulation of houses and for the prevention of uncontrolled indoor air exchange. If these actions are not correctly performed, increasing infestations of indoor molds can be the consequences. Previous studies regarding these phenomenon are basically limited on the determination of germ total in indoor air and on the analysis of mycotoxins in moldy construction materials or interiors. Especially the determination of germ total is merely some kind of snap shot because of the short sampling time. For example this value can be distorted easily by aerating the room. In contrast to this house dust accumulates over a long period of time and hence buffers short term events. It is already used for the routine screening of indoor pollutants like polychlorinated biphenyls or biocides. Although it represents a difficult and highly variable analytical matrix, the analysis of mycotoxins might be a possible approach for the fast detection of hidden mold infestations in indoor environments.

Because house dust is inhaled and swallowed additionally the concentrations of mycotoxins especially with carcinogenic and mutagenic properties or reproductive toxicity should be examined. This is essential for the prediction of possible risks connected to the inhabitation in moldy indoor environments.

Three different analytical methods based on ELISA, HPLC-FLD and LC-MS/MS for determination of ochratoxin A in house dust were developed and compared.
At present a LC-MS/MS multimethod for the simultaneous analysis of further mycotoxins in house dust relevant in indoor environments is in progress. First results will be presented.

Detoxification of mycotoxin by probiotic preparation for broiler chickens

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Contamination of feed with mycotoxins threatens food safety, healthiness and productivity of farm animals. Decontamination methods regarding products containing mycotoxins can be divided into physical, chemical and biological. Physical and chemical methods are only partly effective in case of mycotoxins with polarized function groups (e.g. aflatoxins).

The research aim was to define the influence of fermentation process, with the use of probiotic vaccine (*Lactobacillus paracasei/casei* ŁOCK 0920, *L. brevis* ŁOCK 0944, *L. plantarum* ŁOCK 0945, *Saccharomyces cerevisiae* ŁOCK 0142), on the reduction of toxin concentration in feed for broiler chickens.

The fermentation medium was feed composed of barley flour (50%), wheat flour (45%) and corn flour (5%) mixed with water in proportion 1:1.5. To the medium we added aflatoxins (B1, B2, G1, G2) in the amount of 4 and 40 [μg/kg], or ochratoxin A in the amount of 5 and 50 [μg/kg]. Mycotoxins were marked after 0.6 (time of intestine passage in chickens) and after 24 hours of fermentation with the use of immunoenzymatic method (ELISA). We conducted spontaneous fermentation or one with the use of probiotic vaccine in previously optimized conditions (37^0C, 24h). In order to show that detoxication is a result of activity of microorganisms, we used, as a control sample, flour solutions previously subject to radiation sterilization to which we added the above-mentioned doses of aflatoxins and ochratoxin A. The control sample was incubated in the same conditions that accompanied fermentation.

The reduction of aflatoxins in the control sample where no fermentation took place equaled 28 – 30%, and in case of ochratoxin A it equaled 8 – 10%. After 6 hours of fermentation with the addition of probiotic cultures the amount of aflatoxins reduced of 18 – 33% in relation to the concentration in the control sample, and the amount of ochratoxin A dropped of 29– 49%. During spontaneous fermentation biodegradation of mycotoxins was much lower; the reduction of aflatoxin equaled 6 – 8% in relation to the control sample and in case of ochratoxin A it equaled 12 – 19%.

The research conducted proves that the probiotic vaccine used creates a starter culture characterized by active feature of mycotoxin degradation, especially in relation to ochratoxin A.

Reduction of ochratoxin A level in raw materials and model media by *Saccharomyces cerevisiae*

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Ochratoxin A (OTA) is well-know mycotoxin, which most frequently occurs in the countries of Central Europe, also in Poland. The toxin is produced by numerous fungal species belonging to the genera *Aspergillus* and *Penicillium*. Ochratoxin A is detected in raw materials and food products such a cereals, bread, coffee, dried wine fruits, also in beverages *e.g.* beer, grape juice and wine. Mycotoxins contamination of food and animal feed is a serious health problem, because of their carcinogenic, nephrotoxic, genotoxic and teratogenic properties.

Several possibilities are available for the detoxification of mycotoxins, including physical and chemical methods. Few of these have practical application. Biological methods with using microorganisms and enzymes for elimination of toxins have created new opportunities. Several studies showed that some bacteria, moulds and yeasts such a *Flavobacterium auriantiacum, Corynebacterium rubrum*, lactic acid bacteria (*Lactobacillus acidophilus*, *L.rhamnosus*, *L.bulgaricus*), *Aspergillus niger* and *Rhizopus nigricans, Saccharomyces cerevisiae*, *Candida sp*. and *Kluyveromyces* sp. are able to detoxification of mycotoxins.

In the presented study several strains of *Saccharomyces* yeasts were examined for their ability to ochratoxin A remove from liquid media and raw materials. Bakery and wine strains were used. The OTA removal capacity of yeast was undertaken in YPG medium and in wheat flour medium (bakery strains) and in natural commercial white grape juice (wine strains) initially contaminated with OTA.

Sample clean up for OTA analysis in liquid media and wine after fermentation was performed by the use of immunoaffinity column OchraStar® (ROMER). The ELISA method (Agra Quant Ochratoxin A ROMER) was applied to estimate of OTA in supernatant after removing the biomass.

The results of research demonstrated differences between strains. The greatest ability (30%) to remove ochratoxin A from liquid medium possessed bakery strain BS. The fermentation carried out by this strain in flour medium was resulted of about 40 percentages decrease of OTA after 24 hours.

The next test was carried out with wine strains. A decrease of ochratoxin A concentration, about 25%, was observed in YPG medium. On experiment with grape juice the inhibitory effect of toxin on yeast growth and alcohol fermentation yield was confirmed. Only 20% of initial OTA was transferred from juice to wine, the rest was removed by yeast during fermentation.

The presented results showed that the application of selected strains of yeasts in biotechnological processes involving raw materials contaminated with OTA might reduce the health risk related to human exposure of this toxin.

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The biodegradation of ochratoxin A in the technological process of alcoholic fermentation in agricultural distillery

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Nowadays starch products for distilleries such as wheat kernels are very often goods which cannot be used as animals' feeds, due to the improper storing conditions (too high humidity). They are characterized by high level of pathogenic moulds and bacteria contamination, and therefore the risk of the increased level of mycotoxins' presence is especially high.

After the bioconversion with the *Saccharomyces cerevisiae* of previously hydrolyzed starch, present in the products used for alcohol production, followed by its distillation, the post-distillation stock is left. The evaluation of the level of mycotoxins or their metabolites contamination is very difficult.

The research aimed at determining how possible is acquiring the reduction of the level of ochratoxin A in the technological process of alcoholic fermentation according to Henze, has been carried out.

RP–HPLC method with fluorescence detection joined with samples cleaning on the immunoaffinity columns OchraPrep was used for mycotoxins identification. The samples were taken from the distillery at the starch's enzymatic hydrolysis stage, after previous steam-heat processing, aimed at freeing starch from the cells' cultures and immediately after completing the fermentation and alcohol distillation (mash removal) processes.The kernel that underwent bioconversion into ethanol was collected from the field of increased level of humidity and stored in the warehouse with no additional drying. The result of which was so called 'self-heating' of the product in the next layers and moulds growth: *Aspergillus candidus, Eurotium glaucus, Absidia corymbifera, Penicillium and Cladosporium.* In the raw material ochratoxin A at the level below 0,2 ppb and ZEA- below 5 ppb were detected. Neither DON nor aflatoxins were found.

After completing the steam-heat processing (5 atm., 140-150^{o}C) and enzymatic hydrolyzes of the starch present, ochratoxin A was found in the mash at the level of 0,083 ppb. The level of ochratoxin A in the post-distillation stock decreased to approx. 0,021 ppb after three-day fermentation and alcohol distillation.

Degradation of fumonisins by new bacterial strains isolated from soil samples

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The fumonisins are a group of cancerogenic mycotoxins that are produced by *Fusarium verticillioides*, which is a pathogen especially of maize worldwide. Their molecular mechanism of action is based on inhibition of the enzyme ceramide synthase, thus disrupting sphingolipid metabolism. Acute symptoms include pulmonary edema in pigs and leucoencephalomalacia in horses. The cancerogenic effect of fumonisin B_1 (FB_1), the most important fumonisin, was studied in rats and suspected by epidemiological evidence in humans. Due to the ubiquitous occurrence of fumonisins, a technology for the detoxification of contaminated animal feed is highly desirable. Since chemical and physical decontamination strategies remained unsatisfactory, we are working on a biological detoxification strategy that is based on microbial degradation of fumonisins. The technological goal is to provide fumonisin-metabolising microorganisms as a feed additive that can detoxify the mycotoxins in the intestinal tract of animals.

We screened samples from pig intestines, bovine rumen, soil and contaminated maize for fumonisin-degrading microorganisms. Samples were enriched by incubation in various FB_1-containing growth media, and screened for fumonisin transforming activity. Degradation of the mycotoxin was detected in soil and maize samples, and several pure cultures were isolated from the enriched cultures. These strains were individually tested for their FB_1 degradation capability. Among positive strains, those with the fastest detoxification kinetics were chosen for a more detailed characterisation, which included phylogenetic analysis of partial 16S rDNA sequences. Most of the fumonisin-metabolising strains belonged to the alpha-Proteobacteria. In order to evaluate the technological potential of strains, their detoxification properties at various FB_1 concentrations, temperatures, pH, oxygen concentrations and in complex matrices were investigated. Contrary to previously described *Exophiala* yeast strains, the bacterial strains metabolised fumonisins also in the presence of other carbon sources and over a wide range of concentrations. For the strain with the fastest and most robust detoxification characteristics, an intermediate of the degradation pathway was found by analytical methods to be hydrolysed FB_1 which lacks the carboxylated side chains. The final degradation products were found to be non-toxic in a phytotoxicity bioassay with *Lemna minor*. The potential of the most interesting strain to degrade fumonisins in the intestinal tract of animals is being investigated at present.

Potential Deactivation of Fusarium Spores by Fermentation in Biogas Plants

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Almost every crop supports an indigenous population of moulds. Examining various occurrences, it appears that the most significant plant pathogenic fungus is Fusarium. The Food and Agricultural Organisation of the United Nations (FAO) classified 25% of the world's crops as contaminated with these catabolic products. Fusarium spores can therefore be regarded as problematic and should be inactivated within cereals. Consequently, the European Commission issued a legal Regulation ((EC) No 856/2005 [1]), which sets maximum levels of pollutants in foodstuffs. In order to achieve this, the regulation requires the disposal of affected wheat. Therefore, the most feasible, economical and environmentally sound option for the disposal of contaminated batches is to be sought. This inspired current research to create new, innovative and safe methods of disposal. One pioneering research approach at the University of Hohenheim is the investigation of the prerequisites for the potential deactivation of Fusarium spores by fermentation. The project is founded by the German FNR (Agency for renewable resources).A subsequent reduction of the contained Mycotoxin (DON) and a deactivation of the fertile parts of Fusarium throughout the process is prospective.

Method: Hohenheim Biogas Yield Test (HBT)

Due to the physiological character of rumen, it is capable of inactivating or transforming amounts of DON by means of the contained infusoria (KING et. al., 1984 If the microbiological environment of rumen provides a good basis for the process of deactivation, the respective micro organisms are proposed to be found in manure as well. Proving a potential respective Biogas production, determination of the resulting methane yield of the specimens is imperative and carried out by the HBT. One of the trials determining microbiological activity rate is characterised by the fermentation of wheat at different levels of contamination (6.700µg/kg, 20.000µg/kg, and 46.100µg/kg). The external infestation of the whole kernel could be ascertained by sealing the proportion of intact cells.

The aborticide of *Fusarium* through biogas fermentation could be proven with these initial test results. The infection of unfermented material comprises a rate of 100 percent, which is caused by the rapid domination of Fusarium on the respectively inoculated kernels. After a retention time of 0.5 days, samples showed no proof of *Fusarium* spores. Consequently, inactivation could be achieved after a short period of exposure (Frauz, 2005). The process yield was not discovered significantly different regarding the addition of standard substrate and contaminated specimen. Concluding it can be stated that the biogas forming process (HBT) is not adversely affected by the addition of a microbiologically contaminated charge. The comparatively high return of the seriously contaminated kernels is found due to the diversification of ingredients.

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Effect of zearalenone and modified glucomannans on blood parameters and antioxidant system of quails

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The aim of this study was to evaluate effects of modified glucomannan (Mycosorb^{TM}) on blood parameters and antioxidant profile of quails liver after inclusion of zearalenone in the diet. Fifty-four day-old Japanese quails were divided into three groups and were fed a corn-soya diet balanced in all nutrients *ad libitum* during 30 days. The diet of the experimental quail was supplemented with zearlanone at the level of 1,6 ppm or zearalenone plus Mycosorb^{TM} at 1 g/kg feed. Samples of quail liver were collected after 30 days of experiment. The main carotenoids, α-tocopherol, γ-tocopherol, ascorbic acid were analysed by HPLC-based methods. Inclusion of zearalenone in the diet was associated with changes in blood parameters (level of white blood cells, red blood cells, hemoglobin was significantly reduced due to zearalenone supplementation to the diet) Inclusion of modified glucomannan (Mycosorb^{TM}) in the toxin-contaminated diet provided a significant protective effect against changes in blood parameters and antioxidant composition quail liver. It is suggested that a combination of mycotoxin adsorbents and natural antioxidants could be the next step in counteracting mycotoxins in animal feed.

Effect of modified glucomannans in layers fed low levels of naturally contaminated grain

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Mycotoxicological analysis of feed from 20 poultry farm in Ukraine during last 8 years showed that 214 from 399 samples were contaminated with mycotoxins. Every fifth sample contained T-2 toxin in concentastions: 20-100 mcg/kg – 54, 100-200 mcg/kg - 21 and >200 mcg/kg – 8 samples. When contaminated feed wan fed to layers it was observed the reducing of egg production and increasing of mortality rate, and very often the sign of necrotic stomatit were found. The aim of our work was to elucidate effect of Mycosorb on egg production and mortality rate in poultry farm in Ukraine. With this aim, two poultry houses on poultry farm "Gorlitsa", Sumy region, with 10130 and 10320 hen in each house were fed naturally contaminated grain (results of mycotoxicological analysis suggested low level (trace) of T-2 toxin). Egg production and morality rate during two months were measured. Hens of experimental group were fed ration with addition of Mycosorb (1kg/T). Mortality rate in hens fed Mycosorb was reduced by 5% in comparison with control group hens. Addition of Mycosorb to the ration of laying hens fed naturally contaminated feed resulted in obtaining of 2355 eggs more in comparison with control group. Results of experiments suggested that even low level of mycotoxins presents in feed can reduce performance of poultry and modified glucomannans prevent their negative effect.

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