Current situation of mycotoxin contamination and novel approaches for mycotoxin detoxification

Gerd Schatzmayr

BIOMIN Research Center, Tulln, Austria
Molds…on the field
Mycotoxins in media – recent incidences with aflatoxins

Contaminated animal feed found in Germany

By AFP | March 1, 2013

BERLIN, Mar 1 – German officials said Friday that a carcinogenic substance had been discovered in animal feed delivered to more than 3,500 farms but stressed that any risk to humans was unlikely.

The contamination originated from a delivery of maize from Serbia which went into making animal feed for 3,560 farms in western Lower Saxony state, the region’s ministry for food, agriculture and consumer protection said.

The substance, Aflatoxin B1, is a substance with "a strongly carcinogenic effect" that comes from mould, it said.

However, preliminary investigations showed there was a negligible risk to humans either through milk from cows fed with the contaminated feed or from eating meat.
BIOMIN Mycotoxin Survey Program 2004-2012

- started in 2004
- more than 19,000 samples analyzed from 2004-2012
- more than 64,000 individual analyses
- analyzed for aflatoxins (AF), zearalenone (ZEN), deoxynivalenol (DON), fumonisins (FB) and ochratoxin A (OTA)
- samples were analyzed with HPLC or ELISA
Global results

SAMPLE TYPES:
Corn, Soybean Meal, Wheat/bran, Corn Gluten Meal, Rice/bran, DDGS, Feed, Straw/silage, Other feed ingredients

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Average Positives [µg/kg]</th>
<th>Maximum [µg/kg]</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF</td>
<td>57</td>
<td>6323</td>
</tr>
<tr>
<td>ZEN</td>
<td>286</td>
<td>26728</td>
</tr>
<tr>
<td>DON</td>
<td>1009</td>
<td>50289</td>
</tr>
<tr>
<td>FB</td>
<td>1647</td>
<td>77502</td>
</tr>
<tr>
<td>OTA</td>
<td>14</td>
<td>1589</td>
</tr>
</tbody>
</table>

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### Global results

<table>
<thead>
<tr>
<th></th>
<th>AF</th>
<th>ZEN</th>
<th>DON</th>
<th>FUM</th>
<th>OTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of tested samples</td>
<td>11967</td>
<td>15533</td>
<td>17732</td>
<td>11439</td>
<td>7495</td>
</tr>
<tr>
<td>Positive samples</td>
<td>3142</td>
<td>5797</td>
<td>9960</td>
<td>6204</td>
<td>1902</td>
</tr>
<tr>
<td>% Positive</td>
<td>26%</td>
<td>37%</td>
<td>56%</td>
<td>54%</td>
<td>25%</td>
</tr>
<tr>
<td>Average (ppb)</td>
<td>15</td>
<td>107</td>
<td>566</td>
<td>893</td>
<td>4</td>
</tr>
<tr>
<td>Average of positive (ppb)</td>
<td>57</td>
<td>286</td>
<td>1009</td>
<td>1647</td>
<td>14</td>
</tr>
<tr>
<td>Median of positive (ppb)</td>
<td>11</td>
<td>85</td>
<td>453</td>
<td>750,5</td>
<td>2,64</td>
</tr>
<tr>
<td>1st quartile of positive (ppb)</td>
<td>3</td>
<td>43</td>
<td>234</td>
<td>332</td>
<td>1,1</td>
</tr>
<tr>
<td>3rd quartile of positive (ppb)</td>
<td>40</td>
<td>225</td>
<td>972,25</td>
<td>1780,25</td>
<td>6,2</td>
</tr>
<tr>
<td>Maximum (ppb)</td>
<td>6323</td>
<td>26728</td>
<td>50289</td>
<td>77502</td>
<td>1589</td>
</tr>
<tr>
<td>Sample Origin</td>
<td>Myanmar</td>
<td>Australia</td>
<td>Central Europe</td>
<td>China</td>
<td>China</td>
</tr>
<tr>
<td>Sample Type</td>
<td>other feed (12)</td>
<td>Silage (07)</td>
<td>Wheat (07)</td>
<td>Finished Feed (11)</td>
<td>Finished Feed (11)</td>
</tr>
</tbody>
</table>
Mycotoxin occurrence Europe (2004-2012)

Northern Europe

- Northern Europe
- Positive samples
- Negative samples
- Sample number
- ZEN
- DON
- 22%
- 78%
- 64%
- 36%

Eastern Europe

- Eastern Europe
- Sample number
- AF
- ZEN
- DON
- FB
- OTA
- 8%
- 92%
- 13%
- 33%
- 49%
- 67%
- 67%
- 51%

Central Europe

- Central Europe
- Sample number
- ZEN
- DON
- AF
- FB
- OTA
- 26%
- 58%
- 19%
- 51%
- 29%
- 74%
- 42%
- 81%
- 49%
- 71%

Southern Europe

- Southern Europe
- Sample number
- AF
- ZEN
- DON
- FB
- OTA
- 34%
- 21%
- 51%
- 70%
- 46%
- 66%
- 79%
- 49%
- 30%
- 54%
Co-contamination

- All samples
  - 39% co-contaminated

- Compound feed
  - 59% co-contaminated

- Not considered in setting up of mycotoxin limit-/guidance values

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Co-contamination: Synergistic and additive effects of mycotoxins

Mycotoxin co-contamination of food and feed: meta-analysis of publications describing toxicological interactions

B. Grenier\textsuperscript{1,2} and I.P. Oswald\textsuperscript{1}

\textsuperscript{1}INRA, UMR 1331 ToxAlim, 180 chemin de Tournefeuille, BP 93173, 31027 Toulouse Cedex 3, France; \textsuperscript{2}BIOMIN Research Center, Technopark 1, 3430 Tulln, Austria; isabelle.oswald@toulouse.inra.fr

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..most studies show \textbf{synergistic} or \textbf{additive} interactions regarding adverse effects on animal performance (Grenier and Oswald 2011)

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Multi-mycotoxin method – IFA-Tulln – 300+ metabolites

M. Sulyok, F. Berthiller, R. Krska, R. Schuhmacher
LC-MS/MS „multi-mycotoxin“ method

- **83 feed samples** were subjected to multi-mycotoxin HPLC-MS/MS analysis

- **Mainly compound feed**, maize, wheat and silage samples

- **340 mycotoxins and other secondary metabolites**: incl. “emerging” and “masked” mycotoxins
“Emerging” and “masked” mycotoxins

- **139 different metabolites** out of 340 were detected in the 83 feed samples
- All of the samples were co-contaminated with **7 to 69** different metabolites
- Co-occurrence of **28 metabolites** was most frequent:
“Emerging” and masked mycotoxins

<table>
<thead>
<tr>
<th>Mycotoxin/Metabolite</th>
<th>n (Pos)</th>
<th>Pos [%]</th>
<th>Median [μg/kg]</th>
<th>Max [μg/kg]</th>
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</thead>
<tbody>
<tr>
<td>Beauvericin</td>
<td>81</td>
<td>98</td>
<td>6.7</td>
<td>2326</td>
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<tr>
<td>Summe Enniatins</td>
<td>80</td>
<td>96</td>
<td>30</td>
<td>5441</td>
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<tr>
<td>Deoxynivalenol</td>
<td>74</td>
<td>89</td>
<td>122</td>
<td>25928</td>
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<tr>
<td>Emodin</td>
<td>74</td>
<td>89</td>
<td>9.8</td>
<td>1570</td>
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<tr>
<td>Equisetin</td>
<td>72</td>
<td>87</td>
<td>23</td>
<td>13680</td>
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<tr>
<td>Zearalenone</td>
<td>72</td>
<td>87</td>
<td>14</td>
<td>5326</td>
</tr>
<tr>
<td>Aurofusarin</td>
<td>70</td>
<td>84</td>
<td>85</td>
<td>17659</td>
</tr>
<tr>
<td>Alternariol methyl ether</td>
<td>68</td>
<td>82</td>
<td>1.4</td>
<td>733</td>
</tr>
<tr>
<td>Alternariol</td>
<td>66</td>
<td>80</td>
<td>2.8</td>
<td>221</td>
</tr>
<tr>
<td>Tentoxin</td>
<td>66</td>
<td>80</td>
<td>3.9</td>
<td>76</td>
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<tr>
<td>Moniliformin</td>
<td>63</td>
<td>76</td>
<td>45</td>
<td>12236</td>
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<tr>
<td>DON-3-Glucosid</td>
<td>62</td>
<td>75</td>
<td>15</td>
<td>7764</td>
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<tr>
<td>Culmorin</td>
<td>61</td>
<td>63</td>
<td>195</td>
<td>44616</td>
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<tr>
<td>Nivalenol</td>
<td>61</td>
<td>63</td>
<td>17</td>
<td>1760</td>
</tr>
<tr>
<td>Tryptophol</td>
<td>59</td>
<td>71</td>
<td>267</td>
<td>99040</td>
</tr>
<tr>
<td>Apicidin</td>
<td>55</td>
<td>66</td>
<td>1.9</td>
<td>160</td>
</tr>
<tr>
<td>Brevianamide F</td>
<td>54</td>
<td>65</td>
<td>69</td>
<td>2043</td>
</tr>
<tr>
<td>Tenuazonic acid</td>
<td>54</td>
<td>65</td>
<td>68</td>
<td>1983</td>
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<tr>
<td>15-Hydroxyculmorin</td>
<td>52</td>
<td>63</td>
<td>49</td>
<td>15620</td>
</tr>
</tbody>
</table>

Most often detected in 83 samples analyzed (Streit et al., 2013)
Are mycotoxins in food less prevalent compared to feed?
Mycotoxin occurrence in food

Canada, Food: DON 9%, FB1 100%, OTA 27%, ZEN 943.
Europe (SCOOP): DON 59%, FB1 82%, OTA 31%, ZEN 33%.
Brazil, corn-based prod.: DON 56%, FB1 100%, OTA 801, ZEN 256.
Europe (SCOOP), cornflakes: DON 57%, FB1 54%, OTA 25%, ZEN 32%.
Europe (SCOOP), maize: DON 46%, FB1 31%, OTA 25%, ZEN 32%.
Europe (SCOOP), white wheat flour: DON 16%, FB1 16%, OTA 25%, ZEN 32%.

CFIA target survey 2010/2011; SCOOP report task 3.2.10 (2003); Martins et al. (2012)
More detailed information…

- **Current Situation of Mycotoxin Contamination and Co-occurrence in Animal Feed—Focus on Europe**
  - Elisabeth Streit, Gerd Schatzmayr, Panagiotis Tassis, Eleni Tzika, Daniela Marin, Ionelia Taranu, Cristina Tabuc, Anca Nicolau, Iuliana Aprodu, Olivier Puel and Isabelle P. Oswald
  - Toxins 2012, 4, 788-809

- **Multi-Mycotoxin Screening Reveals the Occurrence of 139 Different Secondary Metabolites in Feed and Feed Ingredients**
  - Elisabeth Streit, Christina Schwab, Michael Sulyok, Karin Naehrer, Rudolf Krska and Gerd Schatzmayr
  - Toxins 2013, 5, 504-523
Although prevention strategies are used mycotoxin contamination occur.

Initially 40% of raw materials were rejected at farmer level!

Previous Crop
« Inoculum »
⇒ No maize

Variety
« Resistance »
⇒ Resistant variety

Harvest
« Cleaning & Humidity »
⇒ Material & timing

Soil Techniques
« Inoculum »
⇒ Tilling

Fungicides
« Protection »
⇒ Triazoles

Weather
« Inoculum »
⇒ Rain at flowering
⇒ Geographic location

Storage
« Adequate storage conditions »
⇒ Humidity, T°, cleanliness


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Detoxification of mycotoxins in animal nutrition is needed

Mycotoxin deactivator

Gastrointestinal detoxification
Variation in molecular structures require a combination of different detoxification strategies

Aflatoxins

Ergot alkaloids

Trichothecenes

Deoxynivalenol

T-2 Toxin

HT-2 Toxin

Zearalenone

Ochratoxins

Fumonisins

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Dietary mycotoxin control strategies

**ADSORPTION**

*Elimination of toxin*

...reduction of mycotoxin - bioavailability

**BIOTRANSFORMATION**

*Elimination of toxicity*

...mycotoxin detoxification prior to resorption

**BIODEGRADATION**

**BIOPROTECTION**

*Elimination of toxic effects*

...elimination of toxin related effects

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Adsorption (binding of mycotoxins)

Very efficacious against aflatoxins
Underlying mechanism of aflatoxin adsorption to bentonites

Polarity, functional groups and planarity
Adsorption of mycotoxins

Aflatoxins
Ergot Alkaloids
Ochratoxins
Fumonisins
Zearalenone
T-2 Toxin
DON


Gerd Schatzmayr, FOODSEG Mycotoxin Workshop 2013
Binding of aflatoxin –
commercial products in buffer

<table>
<thead>
<tr>
<th></th>
<th>pH 3.0</th>
<th>pH 6.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean [%]</td>
<td>71.91</td>
<td>77.05</td>
</tr>
<tr>
<td>Median [%]</td>
<td>90</td>
<td>94</td>
</tr>
<tr>
<td>Minimum [%]</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Maximum [%]</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Number of products</td>
<td>85</td>
<td></td>
</tr>
</tbody>
</table>

Gerd Schatzmayr, FOODSEG Mycotoxin Workshop 2013
Binding of deoxynivalenol – commercial products in buffer

<table>
<thead>
<tr>
<th></th>
<th>pH 3.0</th>
<th>pH 6.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean [%]</td>
<td>5.85</td>
<td>5.07</td>
</tr>
<tr>
<td>Median [%]</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Minimum [%]</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Maximum [%]</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td>Number of products</td>
<td>63</td>
<td></td>
</tr>
</tbody>
</table>

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Enzymatic deactivation (Biotransformation)

Mycotoxin

Non toxic metabolite

Non toxic metabolite

Very efficacious against non adsorbable mycotoxins
Biotransformation - Microbials

Trichothecenes

Eubacterium BBSH 797

Zearalenone

T. mycotoxinivorans (MTV)

Ochratoxin A

T. mycotoxinivorans (MTV)

Schatzmayr, et. al, Mycotoxin Research, 2003

Molnar, et al; Syst. Appl. Microbiology, 2004

Politis, et al; British Poultry Science, 2005

Schatzmayr, et al; Molecular Nutrition and Food Research, 2006

Vekiru et al, Applied and Environmental Microbiology, 2011
BBSH 797 – positive EFSA opinion

Scientific Opinion on the safety and efficacy of micro-organism DSM 11798 when used as a technological feed additive for pigs

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The additive, based on a single bacterial strain (DSM 11798), is intended for use in feeds for pig to reduce the epoxide group of contaminating trichothecene mycotoxins to a less toxic metabolite. To establish an identity for the strain a phylogenetic analysis was made based on the genomic sequence of the strain and 24 genome sequences available for the family Coriobacteriaceae. The three approaches used consistently suggested an assignment to a new taxonomic unit within the family Coriobacteriaceae. The Panel accepts that a new species and/or genus may have to be established. In the interim the deposition of the bacterium in a European culture collection is sufficient to ensure that any authorisation would apply only to the additive under application. Based
EFSA opinion on Biomin® BBSH 797

Worldwide FIRST positive EFSA opinion for mycotoxin biotransformation

EFSA concluded that the strain BBSH 797 is SAFE for animals, humans and the environment

and confirmed EFFECTIVENESS in the target species to reduce the epoxide group of trichothecenes to produce less toxic thus harmless metabolites
Enzymatic fumonisin detoxification

Colony morphology of Isolate MTA #144
Fumonisin detoxifying enzyme


Fumonisin B₁  FUMzyme®  detoxified form

Hartinger & Moll, 2011, World Mycotoxin Journal
**Feeding trials with piglets – to find optimal dose of FUMzyme**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>c (FB₁ and FB₂) in feed</td>
<td>5 and 1 mg/kg</td>
</tr>
<tr>
<td>Type of contamination</td>
<td>Natural</td>
</tr>
<tr>
<td>Range of FUMzyme dosage</td>
<td>10-160 U/kg feed</td>
</tr>
<tr>
<td>No. of piglets/group</td>
<td>6</td>
</tr>
<tr>
<td>Duration</td>
<td>6 weeks</td>
</tr>
<tr>
<td>Sampling times</td>
<td>Day 0, 42</td>
</tr>
<tr>
<td>Samples taken</td>
<td>Serum, faces, urine</td>
</tr>
<tr>
<td>Parameters analyzed</td>
<td>Sa/So ratio, fumonisin concentrations</td>
</tr>
</tbody>
</table>

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Biomarker: FB1 increase Sa/So ration by inhibition of ceramide synthase

- **Sphinganine** accumulation
- **Dihydroceramide**
- **Sphingosine**

**Biomarker: FB1 increase Sa/So**

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Feeding trials with piglets
Sa/So in serum (6 weeks)
Feeding trials with piglets
Fumonisins concentrations in feces

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Feeding trials with piglets
Fumonisin concentrations in urine

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Take home message

- Mycotoxin Survey Program reveals that mycotoxins occur quite frequently in animal feed (ingredients)
- More toxicology data on emerging mycotoxins are important to set safe levels
- Mycotoxin deactivators are needed to neutralize toxic effects
- Very specific approaches have to be used as mycotoxins greatly vary in their structures
Take home message

- selected microorganisms or enzymes can be used for **specific detoxification** of mycotoxins
- recently positive EFSA opinion for BBSH 797 on efficacy and safety
- recent invention: recombinant enzyme for fumonisin degradation (FUMzyme)
- **Biomarkers** (of exposure and of effects) were used during development of this enzyme and to determine respective dose
www.mycotoxins.info
gerd.schatzmayr@biomin.net

Free App!