Mycotoxin Detection & Solutions

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Mycotoxins

• Mycotoxins are metabolites of molds

• Stresses on the mold generally trigger mycotoxin production

• More than 250 different mold toxins have been identified

• Deoxynivalenol (DON), zearalenone (ZEA), and ergot alkaloids are generally the toxins of primary concern for swine producers in Canada and the northern US

• There are no totally safe levels with mycotoxins present
Mycotoxin-Producing Molds

- **Fusarium** - Most economically significant on a global basis
  - Zearalenone
  - Fumonisin
  - Trichotheccenes including DON and many others
- **Aspergillus** - Primary producer of aflatoxins, the most potent mycotoxins
  - Aflatoxin
  - Ochratoxin
- **Penicillium** - Can produce 27 different mycotoxins
  - Aflatoxin
  - Ochratoxin
- **Claviceps** - Produce sclerotia (ergot bodies) in grains
  - Ergot alkaloids
Interactions

• It is common to find more than one mycotoxin present in grains and grain by-products

• Effects of many toxin combinations are at least additive and may be synergistic

• Combinations of ZEA and DON are very common and major concern for sow health and performance
Masked Mycotoxins

- Plants alter mycotoxins to protect themselves

- Types include
  - Largely irreversible conjugates
  - Potentially reversible (glycosylation or others)

- Generally, not detected by HPLC and other lab methods

- Immunochemical methods, e.g., ELISA may or may not

- Can lead to under-estimation of mycotoxin risks

- Effects in animals depend on the toxin and type of masking
Deoxynivalenol (DON)

- Feed refusal or reduced feed consumption from neurochemical response of the brain
- Vomiting may occur with high dosage, acute exposure
- Weight loss, poor lactation performance
- Chronic exposure can produce ulcers on mucous membranes, destroy tight junctions leading leaky gut and predisposing animals to enteritis (diarrheas)
- Immune suppression aggravates effects of other stressors
- Reduces antioxidant status of swine
Zearalenone (ZEA)

- Limited impact on health or performance of growing animals

- Mimics the effect of the female hormone estrogen binding with estrogen receptors and activating all the estrogen metabolic pathways

- May increase the size or early maturity of reproductive organs and disrupt reproduction

- May produce abortions, stillbirths or other effects depending on timing and level of toxin exposure
### General Swine Toxin Risks

#### Mycotoxin levels in complete diets

<table>
<thead>
<tr>
<th></th>
<th>Low</th>
<th>Medium-High</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DON (ppb)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pig (&lt; 20 kg)</td>
<td>&lt;250</td>
<td>250-2000</td>
<td>&gt;2000</td>
</tr>
<tr>
<td>20 kg to market</td>
<td>&lt;1000</td>
<td>1000-4000</td>
<td>&gt;4000</td>
</tr>
<tr>
<td>Sows</td>
<td>&lt;750</td>
<td>1000-4000</td>
<td>&gt;4000</td>
</tr>
<tr>
<td><strong>Zearalenone (ppb)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pig (&lt; 20 kg)</td>
<td>&lt;250</td>
<td>250-1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>20 to 70 kg</td>
<td>&lt;500</td>
<td>500-2000</td>
<td>&gt;2000</td>
</tr>
<tr>
<td>70 kg to mkt</td>
<td>&lt;1000</td>
<td>1000-3000</td>
<td>&gt;3000</td>
</tr>
<tr>
<td>Developing Gilts &amp; Sows</td>
<td>&lt;250</td>
<td>250-500</td>
<td>&gt;500</td>
</tr>
</tbody>
</table>
Analysis Methods

• Laboratory based
  • HPLC, GC, LC-MS
  • Standards defined; reference methods

• Rapid tests
  • Immunoochemical (ELISA, Fluorometric)
  • GIPSA-approved methods are quantitatively accurate
  • Effectiveness depends largely on sampling and extraction efficiency
  • Generally, lacking for complete feed and forages
Improving Risk Assessment

• Heterogenous distribution

• Increase sample size or number of samples of a given size

• Represent the lot evenly

• Increase the number of subsamples

• Increase the test sample size

• Grind the samples into smaller particles

• Use more accurate analytical method

Pre-harvest distribution of Aflatoxin B1

RIKILT RD project, Wageningen U, 2015
• Annual survey of the corn crop delivered (or rejected) at Cargill facilities of AgHorizons, Corn Milling, and Animal Nutrition

• 2,530 samples total analyzed for one or more mycotoxins (aflatoxin, fumonisin, DON, ZEA, T-2, ochratoxin)

• 1,377 samples analyzed for DON, 407 for ZEA

• Selecting levels to report as cautionary or maximum is challenging
  • Animals of different species, age or physiological classes respond differently to various types of mycotoxins
• The levels below represent generalized, cautionary and maximum levels for sensitive classes and species of animals

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Cautionary Level (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin</td>
<td>5</td>
</tr>
<tr>
<td>Fumonisin</td>
<td>3600</td>
</tr>
<tr>
<td>Vomitoxin (DON)</td>
<td>850</td>
</tr>
<tr>
<td>T2 Toxin</td>
<td>100</td>
</tr>
<tr>
<td>Zearalenone</td>
<td>360</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Regulatory or CAN Maximum Level (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin</td>
<td>20</td>
</tr>
<tr>
<td>Fumonisin</td>
<td>5000</td>
</tr>
<tr>
<td>Vomitoxin (DON)</td>
<td>2000</td>
</tr>
<tr>
<td>T2 Toxin</td>
<td>500</td>
</tr>
<tr>
<td>Zearalenone</td>
<td>500</td>
</tr>
</tbody>
</table>
DON Results

Map showing DON results across the United States.
Zearalenone Results
2017 Cargill Corn Survey Results

Zearalenone Tests

- Yellow: Zearalenone Cautionary
- Red: Zearalenone Max
- Green: Zearalenon Below Advisory

States: Alberta, Arkansas, Colorado, Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, Ohio, Ontario, Quebec, South Dakota, Tennessee, Wisconsin
Reducing Mycotoxin Impact

• Screen moldy grains to remove fines more likely to harbor mycotoxins

• Remember stored grain can become more contaminated with mycotoxins if mold grows before use in diets or processing
  • Dry grain down to 13% moisture

• Store moldy grain separately

• Feed suspect grain before the temperature of stored grain begins to rise in the spring

• Avoid feeding to more sensitive, young, or reproducing animals

• Consider utilizing a product that mitigates mycotoxin impacts
Common commercial products shown to reduce some effects of mycotoxins by binding or destroying some toxins

- Clays or aluminosilicates
- Yeast cell walls or other yeast derivatives
- Plant extracts
- Enzymes
- Preservatives
- Blends of the above
PROMOTE® Defusion® Prime

PROMOTE® Defusion® Prime is a blend of feed preservatives
Published Studies Comparing Mycotoxin Mitigation Products
Product Types

• Live yeast
• Yeast Cell Wall
• Hydrolyzed yeast
• Bentonite
• Aluminosilicate
• Defusion® preservative blends
Experiment 1

4 ppm DON
180 pigs fed products for 21 days
Initial body weight 10.3±0.2 kg

Kansas State University

*Denotes significant change from NC, P<0.05

Average Daily Gain

G:F

% change vs NC

% change vs NC

-5.0 0.0 5.0 10.0 15.0 20.0

-2.39 -0.24 11.93*

1 kg/MT Blended Pdt 1.5/5 kg/MT Yeast Deriv+Bentonite 1 kg/MT Defusion Plus

4 ppm DON

180 pigs fed products for 21 days
Initial body weight 10.3±0.2 kg

Frobose et al., 2015
Experiment 2

3 ppm DON

1,080 pigs fed products for 24 days

Initial body weight 12.5±0.3 kg

Average Daily Gain

<table>
<thead>
<tr>
<th>Product</th>
<th>Value</th>
<th>% change vs NC</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 kg/MT Defusion</td>
<td>3.83*</td>
<td></td>
</tr>
<tr>
<td>5 kg/MT Defusion</td>
<td>2.51*</td>
<td></td>
</tr>
</tbody>
</table>

G:F

<table>
<thead>
<tr>
<th>Product</th>
<th>Value</th>
<th>% change vs NC</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 kg/MT Defusion</td>
<td>2.37</td>
<td></td>
</tr>
<tr>
<td>5 kg/MT Defusion</td>
<td>0.14</td>
<td></td>
</tr>
</tbody>
</table>

*Denotes significant change from NC, P<0.05

Frobose et al., 2015
4 ppm DON
904 pigs fed products for 21 days
Initial body weight 8.5±0.1 kg

Average Daily Gain

5 kg/MT Defusion: 34.67*
3 kg/MT Yeast Deriv: 6.67
4 kg/MT Blended Pdt: -1.33

G:F

5 kg/MT Defusion: 9.59*
3 kg/MT Yeast Deriv: 3.90
4 kg/MT Blended Pdt: -2.44

*Denotes significant change from NC, P<0.05

Mahan et al., 2010
4 ppm DON

60 pigs fed products for 14 days

Initial body weight 6.0 kg

Average Daily Gain

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% change vs NC</th>
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</thead>
<tbody>
<tr>
<td>1 kg/MT Yeast Deriv</td>
<td>1.82</td>
</tr>
<tr>
<td>1.5 kg/MT Blended Pdt</td>
<td>19.55</td>
</tr>
<tr>
<td>2.5 kg/ton Aluminosilicate</td>
<td>7.27</td>
</tr>
<tr>
<td>2.5 kg/MT Defusin</td>
<td>60.00*</td>
</tr>
</tbody>
</table>

% change vs NC

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% change vs NC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 kg/MT Yeast Deriv</td>
<td>-8.62</td>
</tr>
<tr>
<td>1.5 kg/MT Blended Pdt</td>
<td>6.90</td>
</tr>
<tr>
<td>2.5 kg/ton Aluminosilicate</td>
<td>6.90</td>
</tr>
<tr>
<td>2.5 kg/MT Defusin</td>
<td>36.21*</td>
</tr>
</tbody>
</table>

G:F

*Not different from PC, P>0.10

Guay et al., 2015
Development and Dosing Studies
• Exposure to mycotoxins can induce oxidative stress
  • Glutathione (GSH) responsible for cellular redox status and regulator of gene expression has been shown to decrease following exposure to DON, T-2 toxin, Aflatoxin, Fumonisin, and Ochratoxin

• Reduction of endogenous antioxidants including tocopherols, lutein, carotenoids, and ascorbic acid have been observed in presence of mycotoxins

• Antioxidant enzyme activities including glutathione reductase, glutathione peroxidase, catalase, and superoxide dismutase are affected by exposure to DON, Fumonisin, and Aflatoxin

• Lipid peroxidation causing tissue damage has been shown in studies with DON, T-2, Aflatoxin, Fumonisin, and Ochratoxin
• Immune system suppression by mycotoxins
  
  • DON, T-2 and HT-2 appear to be similar and second to aflatoxin as immune system suppressors.

  • DON has been shown cell death of lymphocytes and thymocytes, especially in the presence of LPS (E. coli) challenge.

  • When mycotoxin challenges are present along with other stressors (crowding, disease, cold/heat) the immune suppression may be further exacerbated.
1,822 pigs, initial body weight 12.5 kg, on test diet for 18 days.

Naturally-contaminated corn and corn DDGS were used. Average level of DON in the complete feed was 3.2 ppm. Clean control had 1.1 ppm DON.

Treatments:

- Corn-SBM and clean DDGS (16.5%) (Clean)
- Corn and DDGS (16.5%) with DON (QC) + 2.5 kg/MT preservative (P)
- QC + P + 1X Antioxidant Support (Defusion - D)
- QC + P + 2X Antioxidant Support (Defusion Prime - DP)
- QC + P + Immune Support (IS)
- QC + D + IS
- QC + DP + IS
Main effects of Antioxidant Support and Immune Support on ADG and ADFI

**Antioxidant Support**

<table>
<thead>
<tr>
<th>ADG:</th>
<th>ADFI:</th>
</tr>
</thead>
<tbody>
<tr>
<td>P vs D/DP; <em>P</em> &lt; 0.01</td>
<td>P vs D/DP; <em>P</em> &lt; 0.01</td>
</tr>
<tr>
<td>D vs DP; <em>P</em> &lt; 0.05</td>
<td>D vs DP; <em>P</em> &lt; 0.10</td>
</tr>
</tbody>
</table>

**Immune Support:**

<table>
<thead>
<tr>
<th>ADG:</th>
<th>ADFI:</th>
</tr>
</thead>
<tbody>
<tr>
<td>None vs IS; <em>P</em> &lt; 0.01</td>
<td>None vs IS; <em>P</em> = ns</td>
</tr>
</tbody>
</table>

**Main effects of Antioxidant Support**

- **ADG**: 0.520 vs 0.693
- **ADFI**: 0.432 vs 0.601

**Main effects of Immune Support**

- **ADG**: 0.445 vs 0.617
- **ADFI**: 0.464 vs 0.623

*Note: * denotes statistical significance.
Main Effects of Antioxidant and Immune Support on G:F

**Antioxidant Support:**
- P vs D/DP; *P* < 0.01
- D vs DP; *P* = ns

**Immune Support:**
- None vs IS; *P* < 0.01

<table>
<thead>
<tr>
<th></th>
<th>Antioxidant Support</th>
<th>Immune Support</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLEAN</td>
<td>0.758</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.719</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>0.736</td>
<td></td>
</tr>
<tr>
<td>DP</td>
<td>0.743</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0.721</td>
<td></td>
</tr>
<tr>
<td>IS</td>
<td>0.744</td>
<td></td>
</tr>
</tbody>
</table>

Main effects of Antioxidant Support

Main effects of Immune Support
Main Effects of Antioxidant and Immune Support on Final BW

**Antioxidant Support:**
- P vs D/DP; \( P < 0.01 \)
- D vs DP; \( P < 0.10 \)

**Immune Support:**
- None vs IS; \( P < 0.05 \)

### Main effects of Antioxidant Support
- CLEAN: 22.1 kg
- P: 20.4 kg
- D: 20.8 kg
- DP: 21.1 kg

### Main effects of Immune Support
- None: 20.6 kg
- IS: 20.9 kg
Defusion® titration in GF pigs

1,080 pigs, initial body weight 30 kg, on test.

Corn and corn DDGS naturally-contaminated with DON were used.

Treatments:

- Corn-SBM, low DON, no Defusion®
- DON with 0 kg/MT Defusion®
- DON with 2.50 kg/MT Defusion®
- DON with 3.75 kg/MT Defusion®
- DON with 5.00 kg/MT Defusion®
Effects of Defusion® (30 to 90 kg)

**ADG:**
- Corn-SBM vs DON + 0, $P < 0.01$
- Corn-SBM vs DON + 2.5, $P < 0.05$
- Linear, $P < 0.01$
- Quadratic, $P < 0.01$

**ADFI:**
- Corn-SBM vs DON + 0, $P < 0.01$
- Corn-SBM vs DON + 2.5, $P < 0.05$
- Linear, $P = ns$
- Quadratic, $P = ns$

**Gain:Feed:**
- Corn-SBM vs DON + 0, $P = ns$
- Corn-SBM vs DON + 2.5, $P < 0.01$
- Linear, $P = ns$
- Quadratic, $P < 0.01$
Effects of Defusion® (30 to 90 kg)

Final BW:
- Corn-SBM vs DON + 0, *P* < 0.01
- Corn-SBM vs DON + 2.5, *P* < 0.05
- Linear, *P* < 0.01
- Quadratic, *P* < 0.05

Bar graph showing final body weight in kg for different Defusion® additions (kg/Mt): 93.7 for Corn-SBM, 84.8 for 0.00 kg/Mt, 90.3 for 2.50 kg/Mt, 89.4 for 3.75 kg/Mt, and 88.8 for 5.00 kg/Mt.
PROMOTE® Defusion® 501 is a modified clay production aid.
In vitro Adsorbent Evaluation
**In vitro evaluation**

- % Adsorption = % toxin bound at pH2
- % Desorption = % bound toxin released at pH 6
- % Efficiency = % Adsorption - % Desorption

- General principle – if it does not bind *in vitro*, it will not bind in the animal either

- Key consideration – *in vitro* doses of toxins and binders should reflect commercial dosing; some results do not.
In vitro evaluation of binders

- Defusion 501 1 kg/MT
- Std Clay 5 kg/MT
- Competing Modified Clay 1 kg/MT
- Carbon Binder 2 kg/MT
- Cell Wall 1 kg/MTT

- Aflatoxin 4000 ppb
- Zearalenone 5000 ppb
- Fumonisin 3000 ppb
- Ochratoxin 2500 ppb
Defusion® 501 in Zearalenone Challenge
150 gilts were fed diets naturally contaminated from 21 to 61 days of age.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Analyzed Zearalenone (ppb)</th>
<th>Product (kg/mT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>ZEA Challenged</td>
<td>940</td>
<td>-</td>
</tr>
<tr>
<td>ZEA + Defusion 501</td>
<td>979</td>
<td>1.5</td>
</tr>
<tr>
<td>ZEA + Combination product</td>
<td>820</td>
<td>1.5</td>
</tr>
</tbody>
</table>
### Defusion® 501 in ZEA Challenge

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Length (cm)</th>
<th>Cervix diameter (cm)</th>
<th>Volume of Vulva</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>31.18 A</td>
<td>0.825 A</td>
<td>5.24 A</td>
</tr>
<tr>
<td>ZEA Challenged</td>
<td>40.25 B</td>
<td>1.925 B</td>
<td>9.40 B</td>
</tr>
<tr>
<td>ZEA + Defusion 501</td>
<td>34.23 AB</td>
<td>1.275 A</td>
<td>8.60 B</td>
</tr>
<tr>
<td>ZEA + Combination Product</td>
<td>36.48 AB</td>
<td>1.325 AB</td>
<td>8.85 B</td>
</tr>
</tbody>
</table>

*AB Values without common superscript differ (p<.05).*
Reducing Impact of Mycotoxins

While no animal feed can be guaranteed to be free of toxins, key principles can be applied to reduce the risk and impact of mycotoxins:

1. Monitor raw materials to understand contamination risk

2. Manage materials to reduce risk of further toxin formation and minimize feeding of higher risk ingredients or feeds to more vulnerable animals (young pigs and reproducing sows)

3. Utilize appropriate ingredients to ameliorate the effects of contamination when risk is unavoidable