Animal Health, Nutrition and Mycotoxins

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ALIMENTATION
AGRICULTURE
ENVIRONNEMENT
Mycotoxins

- Fungal secondary metabolites that exert toxic effects on animals and human
- More than 1000 mycotoxins have been described
- The chemical structure of mycotoxins is very diverse
- Chemical structure and toxic properties of mycotoxins are conserved during both storage and processing/cooking of food or feed

Mycotoxins:

- Aflatoxin B1
- Deoxynivalenol - DON
- Fumonisin B1
- Zearalenone
# Importance and diversity of mycotoxins

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Mycotoxins</th>
<th>Raw material</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aspergillus</strong></td>
<td>Aflatoxins</td>
<td>Maize, peanuts, cotton seeds, rice, haricots, milk animal tissues, silage</td>
</tr>
<tr>
<td></td>
<td>Ochratoxin A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patulin</td>
<td></td>
</tr>
<tr>
<td><strong>Fusarium Gibberella</strong></td>
<td>Trichothecenes, Zearalenone</td>
<td>Wheat, maize, barley, rice, rye, oats, nuts</td>
</tr>
<tr>
<td></td>
<td>Fumonisins, Fusarin C</td>
<td></td>
</tr>
<tr>
<td><strong>Penicillium</strong></td>
<td>Patulin, Citrinin,</td>
<td>Fruits, fruit juice, wheat, rice cheese, nuts, animal tissues, silage, cheese</td>
</tr>
<tr>
<td></td>
<td>Ochratoxin A,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cyclopiazonic acid</td>
<td></td>
</tr>
<tr>
<td><strong>Byssochlamys</strong></td>
<td>Patulin</td>
<td>Fruits and fruit juice, silage</td>
</tr>
<tr>
<td><strong>Claviceps</strong></td>
<td>Ergot alcaloids</td>
<td>Rye, wheat</td>
</tr>
<tr>
<td><strong>Alternaria</strong></td>
<td>Alternariol, Ténuazonic acid</td>
<td>Fruits, vegetable apples and tomatoes</td>
</tr>
</tbody>
</table>
Mycotoxins have multiple effects
Example in pigs

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>AFB1</th>
<th>OTA</th>
<th>DON</th>
<th>T-2</th>
<th>FB1</th>
<th>ZEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anorexia</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Growth</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Liver damage</td>
<td>+++</td>
<td>+</td>
<td></td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney damage</td>
<td>+++</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abortion</td>
<td>+</td>
<td></td>
<td></td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infertility</td>
<td></td>
<td></td>
<td>+++</td>
<td></td>
<td></td>
<td>+++</td>
</tr>
<tr>
<td>Vulvovaginitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+++</td>
</tr>
<tr>
<td>Pulmonary oedema</td>
<td>+++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+++</td>
</tr>
<tr>
<td>Immunomodulation</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>
Pig: a target and a model species
to study mycotoxins

- Because of its cereal rich diet pig is exposed to mycotoxins
- Because of its similarity with human, pig is a good model for human
- Pig, is very sensitive to mycotoxins
• Effects of mycotoxins on immune response & impact on animal health

• Effects of mycotoxins on intestine & impact on animal health

Results obtained from swine
The immune system – A target for mycotoxins
The immune response

**Mycotoxin Symposium**
December 11, Quebec

**The immune response**

**Immune and non immune cells**
( epithelial & endothelial cells, macrophage, neutrophils, dendritic cells)

**Innate immune response**
*Rapid but not specific*

- Complement factor
- Phagocytosis
- Antigen presentation
- Cytotoxicity

**Acquired immune response**
*Specific but slow*

- Immune cells (lymphocytes)
- Cytokines
- Complement factor
- Reactive oxygen & nitrogen compound
- Reactive lipids
- Cytokines
- Antibody
Mycotoxins decreases the vaccinal immune response: experimental protocol

Dietary exposure to mycotoxins (4 weeks)

0 1 2 3 4 Weeks

Ovalbumin (OVA) vaccine

The cell-mediated acquired immune response
- Antigen-stimulated versus mitogen-stimulated lymphocytes proliferation

The humoral acquired immune response
- Antigen-specific versus total antibody production
T-2 toxin decreases the specific antibody response

T-2 toxin (mg/kg) for 4 weeks
Control 0.5 1.3 2.1 20 pigs

• Total antibody response

• Specific antibody response

(Meissonnier et al., 2008)
Mycotoxin Symposium
December 11, Quebec
Aflatoxin decreases the specific lymphocyte proliferative response

<table>
<thead>
<tr>
<th>AFB1 (mg/kg)</th>
<th>Control</th>
<th>0.4</th>
<th>0.9</th>
<th>1.8</th>
<th>20 pigs</th>
<th>OVA vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>for 4 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Unspecific lymphocyte proliferation
- OVA-specific lymphocyte proliferation

Low doses of mycotoxins decrease the vaccinal immune response without altering the "global" immune response

- Need to stimulate the immune response to investigate the effects of mycotoxins
T-2 toxin only decreases the antibody response

<table>
<thead>
<tr>
<th>T-2 toxin (mg/kg)</th>
<th>0.0</th>
<th>0.2</th>
<th>0.4</th>
<th>0.6</th>
<th>0.8</th>
<th>1.0</th>
<th>1.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OVA vaccination</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

- **Cellular immune response**
- **Humoral immune response**

(Meissonnier et al., 2009)

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Aflatoxin only decreases the cellular response

Each mycotoxin acts differently the immune response (T-2 toxin humoral response, aflatoxin cellular response)

➢ Need to investigate different parameters of the immune response
What are the consequences of the modification of the immunity by mycotoxins on animal health?

- Increased susceptibility to infections
- Decreased vaccine efficacy
Aflatoxin B₁ decreases resistance to *Brachyspira hyodysenteriae* infection

**PROTOCOL**
- 24 piglets (4-6 week-old)
- Feed with aflatoxin contaminated feed (D0 to D22)
- Infection with *B. hyodysenteriae* (D4)

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Aflatoxin</th>
<th>B. hyo</th>
<th>AF + B. hyo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality</td>
<td>1/8 pig</td>
<td>1/8 pig</td>
<td>4/8 pigs</td>
</tr>
</tbody>
</table>

**Clinical response**

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubation</td>
<td>_</td>
<td>17.1 days</td>
<td>7.7 days</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>_</td>
<td>6/8 pigs</td>
<td>7/8 pigs</td>
</tr>
</tbody>
</table>

(Joens et al., 1981)
T-2 toxin decreases host resistance to infectious diseases

**Bacterial infections**
- *Mycobacterium bovis* (mice)
- *Salmonella* species (mice, chicken)
- *Listeria monocytogenes* (mice)
- *Staphylococcus aureus* (mice)

**Parasitic infections**
- *Cryptosporidium baileyi* (chicken)

**Fungal infections**
- *Aspergillus fumigatus* (rabbit)

**Viral infections**
- Herpes virus type 1 (mice)
- Intestinal reovirus (mice)
- Enteric reovirus (mice)
FB1 increases susceptibility to *Escherichia coli* infection in piglets

**Oral Intoxication (0.5 mg/kg bw)**
Crude extract or purified toxin

**Oral infection with *E. coli***

**In situ** visualization of bacteria in the colon by immunohistochemistry using an anti-O75 serum

(Oswald et al., 2003)
Ochratoxin A increases incidence of bacterial infection in pigs

(Stoev et al., 2000)

<table>
<thead>
<tr>
<th>Type of bacterial infection</th>
<th>Control</th>
<th>OTA 1 ppm</th>
<th>OTA 3 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella cholerasuis</em></td>
<td>0/6</td>
<td>2/6</td>
<td>6/6</td>
</tr>
<tr>
<td>(day 13)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Brachyspira hyodysenteriae</em> &amp; <em>Campilobacter coli</em></td>
<td>0/6</td>
<td>_</td>
<td>2/6</td>
</tr>
<tr>
<td>(day 47)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
What are the consequences of the modification of the immunity by mycotoxins on animal health?

• Increased susceptibility to infections
• Decreased vaccine efficacy
Aflatoxin reduces vaccine efficacy against viral infections in chicken

Gabal & Azzam 1998

640 chicks – 4 months with 0.2 mg AF/kg feed

**Vaccination** single or combined

**Challenge** to each viral infection

<table>
<thead>
<tr>
<th>Diet</th>
<th>Challenge</th>
<th>Antibodies titre</th>
<th>Mortality after challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control AF</strong></td>
<td>Newcastle disease</td>
<td></td>
<td>10% 30%</td>
</tr>
<tr>
<td><strong>Control AF</strong></td>
<td>Infectious bronchitis</td>
<td></td>
<td>0% 20%</td>
</tr>
<tr>
<td><strong>Control AF</strong></td>
<td>Infectious bursal disease</td>
<td></td>
<td>0% 0%</td>
</tr>
</tbody>
</table>
AFB₁ decreases vaccine efficacy in pigs

**PROTOCOL**
- 23 piglets (15-19 Kg)
- Vaccination with *Erysipsela* bactrin
- Challenge with a virulent strain

*(Cysewski et al., 1978)*

<table>
<thead>
<tr>
<th>Vaccination status</th>
<th>Type of feed</th>
<th></th>
<th>+ AFB₁</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>67% suscep.</td>
<td>100% suscep.</td>
</tr>
<tr>
<td></td>
<td>33% part. resis.</td>
<td>0% resistant</td>
<td>0% resistant</td>
</tr>
<tr>
<td></td>
<td>0% resistant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Vaccinated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaccinated</td>
<td>17% suscep.</td>
<td>80% suscep.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>33% part. resis.</td>
<td>20% part. resis.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50% resistant</td>
<td>0% resistant</td>
<td></td>
</tr>
</tbody>
</table>
Aflatoxin reduces vaccine efficacy

Aflatoxin B1 interferes with the development of acquired immunity

- Rabbit: *Bordetella bronchiseptica*
- Swine: *Erysipelothrix rhusiopathiae*
- Chicken & Turkey: *Pasteurella multocida*
- Chicken & Turkey: Viral diseases (Marek, Newcastle, infectious bursal diseases)

The presence of low level of mycotoxin in the food/feed can lead to a breakdown in vaccinal immunity and may therefore lead to the occurrence of disease even in properly vaccinated human population or animal flocks.
Effect of mycotoxins on the immune response: conclusion

• Mycotoxins can act on the different steps of the immune response
  - Innate immune response (epithelial cells, neutrophils, macrophages)
  - Acquired immune response (B cells and antibody production, T cells and cytokine production)

• This have consequence in terms of human and animal health
  - Increase susceptibility to infections
  - Reactivation of chronic infections
  - Decreased vaccine and/or drug efficacy

However, in most cases, the underlying mechanisms, still need to be elucidated
Intestine another target for mycotoxins
Mycotoxins and the intestinal epithelium

The gastro-intestinal tract is the first barrier against food contaminants

The intestinal epithelium can be exposed to high concentrations of mycotoxins upon ingestion of contaminated food
Working hypothesis

Low concentrations of mycotoxins

Alteration of the intestinal epithelium structure/function

Increased susceptibility to pathogenic microorganisms

APPROACHES

In vivo model
experimental intoxication

Ex vivo model
intestinal explants

Cellular model
intestinal epithelial cell line
DON alters intestinal tissues

- **Ex vivo data** (Explants, 10µM, 4 hours)

- **In vivo data** (piglets, 2.3 ppm, 4 weeks)

(Kolf-Clauw et al., 2009; Pinton et al., 2012)
DON decreases intestinal cell proliferation

**In vitro data**
IPEC-1 cells, 24 hours

**Ex vivo data**
Explants, 4 hours

**In vivo data**
3 ppm, 4 weeks

Consequences for nutrients absorption

(Kolf-Clauw et al., 2009; Bracarense et al., 2012; Pinton et al., 2012)
DON decreases animal growth

- One experiment (Piglets, 4 weeks)

<table>
<thead>
<tr>
<th>DON conc (µg/Kg feed)</th>
<th>Control</th>
<th>DON</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>2.3</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Animal weight gain (gram per day)</th>
<th>Control</th>
<th>DON</th>
</tr>
</thead>
<tbody>
<tr>
<td>616 ± 59</td>
<td>564 ± 55</td>
<td></td>
</tr>
</tbody>
</table>

(Pinton et al., 2012)

- Meta analysis

![Graph showing the relationship between dietary DON levels (ppm) and feed intake (% control)](image)

(Grosjean et al., 2003)
Effect of DON on intestinal barrier function

1. Analyze the effect of DON on the intestinal barrier function
   - Measure of the trans electrical epithelial resistance (TEER)
   - Passage of macromolecules and bacteria

2. Identify the underlying mechanisms
   - Expression of tight junction proteins
   - Involvement of the MAPkinase signaling pathway

Several approaches: *in vitro* (intestinal epithelial cell cultures)
*ex vivo* (intestinal explants)
*in vivo* (animals feed contaminated diet)
DON decreases trans-epithelial electrical resistance (TEER)

(Pinton et al., 2009)
DON increases trans-epithelial passage

Passage of dextran

DON (48h) + dextran

DON concentration (µM)

Apparent permeability (nmol.h⁻¹.cm⁻²)

0 5 10 20 50

0 5 10 15 20

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DON increases trans-epithelial passage

**Passage of dextran**

**Passage of bacteria**

DON (48h) + bacteria

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DON increases passage of dextran across intestinal explants

DON and dextran

skeletal compartment
mucosal compartment
intestinal tissue

DON concentration (µM)

Apparent permeability (nmol.h⁻¹.cm⁻²)

0 µM
5 µM
20 µM
50 µM

1h
2h

a
b

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DON decreases the barrier function of the intestinal epithelium

What are the underlying mechanisms?

Effect of DON on tight junctions proteins

Effect of DON on signaling pathways
DON decreases the intestinal expression of the junction proteins

(Pinton et al., 2009)

IN VITRO EXPOSURE
(IPEC-1 cells, 30µM, 72h)

IN VIVO EXPOSURE
(piglets, 5 weeks, 3 ppm)

Claudin-4

β-actin

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DON acts through MAPKinase activation

(Pinton et al., 2010)

DON

inhibitor (U0126)

activate

MAPK

control

Tight junction proteins

regulate

Barrier function

Expression of Claudin-4

Trans-epithelial electrical resistance

Control U0126 DON DON+ U0126

(Pinton et al., 2010)
Comparative effects of DON and its acetylated derivatives

• DON is produced together with two acetylated derivatives 3-ADON and 15-ADON, which can reach 10 to 20% of the DON content

• There is very little data concerning the comparative toxicity of these toxins

Several approaches
  - *in vitro* (intestinal epithelial cell cultures)
  - *ex vivo* (intestinal explants)
  - *in vivo* (animals feed contaminated diet)

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Comparative effects of DON and A-DONs on intestinal morphology

(Pinton et al., 2012)

- **Ex vivo data**
  Explant treated for 4 hours with 10µM toxins
  
  - Control
  - 3-ADON
  - DON
  - 15-ADON

- **In vivo data**
  Animal feed with contaminated feed
  2.3 mg DON or 2.1 mg DON + 15-ADON
  
  - Control
  - DON
  - DON + 15-ADON

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### Comparative effects of DON and A-DONs

**Conclusion**

DON and A-DONs have differential intestinal toxicity

15-ADON > DON > 3-ADON

<table>
<thead>
<tr>
<th></th>
<th>In vivo data</th>
<th>Ex vivo data</th>
<th>In vitro data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellular proliferation</td>
<td>15-ADON&gt;DON&gt;&gt;3-ADON</td>
<td>not evaluated</td>
<td>15-ADON&gt;DON</td>
</tr>
<tr>
<td>Intestinal structure / Barrier function</td>
<td>15-ADON&gt;&gt;DON&gt;3-ADON</td>
<td>15-ADON&gt;&gt;DON=3-ADON</td>
<td>15-ADON&gt;DON</td>
</tr>
<tr>
<td>Claudins</td>
<td>15-ADON&gt;&gt;DON=3-ADON</td>
<td>not evaluated</td>
<td>not evaluated</td>
</tr>
<tr>
<td>MAPK</td>
<td>15-ADON&gt;&gt;DON=3-ADON</td>
<td>15-ADON&gt;DON=3-ADON</td>
<td>15-ADON&gt;DON</td>
</tr>
</tbody>
</table>

(Pinton et al., 2012)
Cytokines and intestinal immune response

- Cytokines are soluble proteins and peptides which act as humoral regulators at nano- to- picomolar concentrations

- They behave like hormones. They act at a systemic level, inflammation, septic shock, acute phase reactions…

(Rescigno et al, 2009)
DON induces a Th17 intestinal inflammatory response

- Expression of inflammatory cytokines

- Expression of Th17 cytokines

Consequences of inflammation for intestinal health

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Multiple effect of mycotoxins on the intestine

(Bouhet and Oswald, 2005)

Intestinal epithelium

Consequences for animal health

- Nutrient absorption
- Cell proliferation
- Barrier function
- Cytokines
- Immuno-globulin

- Deoxynivalenol
- Fumonisin B1
- Patulin
- Ochratoxin
- T-2 toxin
- Aflatoxin B1
- Gliotoxin
- Nivalenol

Multiple effect of mycotoxins on the intestine leads to various consequences for animal health.
Further needs:
Better assess the consequences of an altered intestinal barrier function

- Increased susceptibility to enteric infection
- Impaired absorption of nutrient (reduced growth)
- Increased passage of xenobiotics (including other mycotoxins)
- Predisposition to chronic intestinal inflammatory diseases
- Altered intestinal functions
Mycotoxins have multiple effect on animal, among them

• Mycotoxins decrease the immune response
  - Each mycotoxin have a specific effect
  - This have consequence in term of animal health
    (susceptibility to infection, reactivation of infection, vaccine efficacy…)

• Mycotoxins target the intestine
  - The consequence still need to be determined

The underlying mechanisms, still need to be elucidated
COLLABORATIONS

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Veterinary School, Toulouse, France
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Bruno Barillet

Université Carleton
Ottawa, Canada
David Miller
Thank you for your attention