Silage and the safety and quality of dairy foods: a review

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Together to the next level
Food safety responsibility

- EU Regulation 178/2002 - General Food Law
  - Regulation 852/2004 on hygiene of foodstuffs
  - Regulation 853/2004 on specific hygiene rules for food of animal origin
  - Regulation 183/2005 on feed hygiene

- Key obligations of food and feed business operators
  - ...  
  - **Responsibility**: Food/feed business operators are responsible for the safety of the food/feed which they produce, transport, store or sell  
  - ...  
  - **Prevention**: Operators shall identify and regularly review the critical points in their processes and ensure that controls are applied at those points: HACCP; ‘farm to table’-approach  
  - ...
How does silage fit into this ...

- Safety of silage for animals and food produced from products derived from these animals is a shared responsibility of the farmer and food operator.
Presentation outline

• Bacterial spores
  • *Clostridium*
  • *Bacillus / Paenibacillus*
  • Contamination pathway of raw milk

• Zoonotic pathogenic bacteria
  • *Listeria monocytogenes*
  • Shiga toxin-producing *Escherichia coli* (STEC, VTEC, EHEC)

• Mycotoxins

• Conclusions
Bacterial spores

*Clostridium* species

- Proteolytic group (e.g. *Clostridium sporogenes*); *Clostridium butyricum* group; *Clostridium tyrobutyricum*

- *Clostridium tyrobutyricum*
  - Not harmful to man and animals
  - Ferments lactic acid to butyric acid (*butyric acid bacteria*)
  - In silage: loss of preservation quality, palatability, nutritive value
  - In cheese: economic loss due to off-flavours and texture defects

- Factors controlling germination and outgrowth of *Clostridium tyrobutyricum* are the same in silage and cheese
  - pH, water activity, nitrate

- Occurrence spores of the pathogen *Clostridium botulinum* in silage is rare; no growth in silage; no outbreaks of foodborne botulism linked to dairy products
Contamination pathway spores from silage to milk

- Spores pass GI-tract cow; excreted with the faeces
- Contamination of udder/teats by ‘dirt’ is inevitable
- Teat-cleaning prior to milking removes dirt only partially
- Dirt that remains is rinsed off during milking operations
- Milk pasteurisation inactivates vegetative bacteria, but not spores
Butyric acid bacteria spores in silage

- **Classical view:**
  Growth of BAB associated with anaerobic instability due to insufficient pH decline in high BC/WSC crops, e.g. grass, alfalfa

- **More recent view:**
  Growth of BAB also associated with aerobic spoilage processes, for instance in maize silage

![Graph showing BAB spores in silage samples - means of 6 farms]
Distribution BAB spores in maize silage

Relation with silage density

Distribution BAB spores in maize silage

Relation with pH and yeasts&moulds counts

### BAB spores (log MPN/g)
- < 3.0
- 3.0 – 4.0
- 4.0 – 5.0
- 5.0 – 6.0
- > 6.0

### pH
- < 3.80
- 3.80 – 3.99
- 4.00 – 4.49
- 4.50 – 5.99
- > 6.00

### Yeasts&moulds (log cfu/g)
- < 5.0
- 5.0 – 6.0
- 6.0 – 7.0
- 7.0 – 8.0
- > 8.0

Together to the next level
Bacterial spores
*Bacillus and Paenibacillus* species

- Wide diversity of sources, physiology, spore heat resistance

- Source at dairy farm
  - Soil
  - Bedding materials
  - Feed: compound feeds; *silage*

- Relevance for dairy
  - spores of cold-tolerant strains in milk determine shelf life of pasteurised dairy products
  - highly heat-resistant spores of thermophilic strains cause non-sterility problems in UHT products
  - *Bacillus cereus* is a food-borne pathogen
Spores of aerobic sporeformers in silage

- Growth of aerobic sporeformers in silage is associated with aerobic spoilage processes.
Spores of *Bacillus cereus* in silage

- Silage is an important source of *B. cereus* spores, but concentrations are relatively low and not critical for milk quality/safety.

- Other sources (e.g., soil, bedding, milking installation) are probably more critical.

- Growth *B. cereus* less than other aerobic sporeformers.

*Graph depicting the growth of B. cereus spores, AS spores, Yeasts & moulds, and temperature over time (days)*
**Listeria monocytogenes**

- Listeriosis; rare, but serious food-borne disease
- High risk foods: raw and smoked fish; raw and cooked meat; prepared salads and sandwiches; soft/semi-soft cheeses from unpasteurised milk
- Growth at low T; high salt-tolerance, stress-tolerance
- Inactivated by pasteurization !!
- Listeriosis outbreaks in cattle, sheep, goats caused by contaminated silage !! Shedding to environment via faeces
- Occurrence/survival *Listeria monocytogenes* in silage associated with aerobic spoilage
E. coli O157:H7 and other STEC

- STEC = Shiga toxin producing Escherichia coli (syn. EHEC, VTEC)
- Healthy ruminants main natural reservoir
- Raw meat; vegetables; drinking water; raw milk and unpasteurised dairy products; direct contact with animals
- Inactivated by pasteurization !!
- No survival in well-fermented silage
- Possibly survival in poorly fermented silage and growth in aerobically deteriorating material
Major toxinogenic moulds and mycotoxins

*Distinction between mycotoxins formed before and after ensiling ‘field-derived’ vs. ‘ensilage-derived’*

**Typical ‘field-derived’ moulds/mycotoxins**

<table>
<thead>
<tr>
<th>Mould/Mycotoxin</th>
<th>Typical Products</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Fusarium sp.</em></td>
<td>DON, T2, zearalenone, fumonisins</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em></td>
<td>Aflatoxins</td>
</tr>
<tr>
<td><em>A. ochraceus, P. verrucosum</em></td>
<td>Ochratoxin A</td>
</tr>
<tr>
<td><em>Neotyphodium sp.</em></td>
<td>Lolitrem B, ergovaline</td>
</tr>
</tbody>
</table>

**Typical ‘ensilage-derived’ moulds/mycotoxins**

<table>
<thead>
<tr>
<th>Mould/Mycotoxin</th>
<th>Typical Products</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Penicillium roqueforti, P. paneum</em></td>
<td>Roquefortin C, mycophenolic acid</td>
</tr>
<tr>
<td><em>Aspergillus fumigatus</em></td>
<td>Fumigaclavines, gliotoxin</td>
</tr>
<tr>
<td><em>Monascus ruber</em></td>
<td>Monacolin K, citrinin</td>
</tr>
<tr>
<td><em>Byssochlamys nivea</em></td>
<td>Patulin</td>
</tr>
</tbody>
</table>
Stability of mycotoxins in silage

- Contradictory information in literature
- Some are stable, some are degraded, some are partially degraded

**Stable**
DON, zearalenone, fumonisins, roquefortin C, mycophenolic acid

**Partially degraded**
Aflatoxin B1, ochratoxin A, alkaloids

**Degraded**
PR-toxin (*Penicillium roqueforti, P. paneum*), patulin (*Byssochlamys nivea, P. paneum*)
Occurrence field-derived mycotoxins

- **DON & zearalenone**
  - Global occurrence, highest incidence in temperate climate

- **Maize silage main source of DON & zearalenone in dairy cow diet**
  - 81-83% of total dietary intake (*Driehuis et al. J. Dairy Science 91:4261, 2008*)

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Positive samples</th>
<th>Avg. concentration (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DON (7 surveys)</td>
<td>42 - 100% (80%)</td>
<td>0.6 - 1.9 (1.1)</td>
</tr>
<tr>
<td>ZEA (6 surveys)</td>
<td>32 - 96% (54%)</td>
<td>0.05 - 0.45 (0.17)</td>
</tr>
</tbody>
</table>

- **Fumonisins**
  - High incidence in maize in warm climate areas
  - 97% in Midwestern USA 2001-2002, 1.4% in the Netherlands 2002-2004

- **Aflatoxins**
  - Associated with (sub)tropical and warm temperate climates
  - Generally field-derived; but may also be formed in silages with extensive aerobic spoilage
Occurrence ensilage-derived mycotoxins

- Associated with aerobic spoilage of silage
  *Dependent on infiltration of oxygen during storage or feeding-out*

- *Penicillium roqueforti* predominant mould
  *growth at low O$_2$, tolerates low pH and high CO$_2$*

- Heterogeneous distribution
  *Surface, ‘blue balls’*

![Incidence and Concentration Graphs](image)

*Driehuis et al. J. Dairy Science 91:4261 (2008)*
## Metabolism & carry-over into milk

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Detoxification in rumen</th>
<th>Carry-over into milk</th>
<th>Health risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxins</td>
<td>No</td>
<td>High (1 – 6%)</td>
<td>Human</td>
</tr>
<tr>
<td>DON</td>
<td>Yes</td>
<td>Low (&lt; 0.02%)</td>
<td>Animal</td>
</tr>
<tr>
<td>Zearalenone</td>
<td>No</td>
<td>Low (&lt; 0.03%)</td>
<td>Animal</td>
</tr>
<tr>
<td>Fumonisins</td>
<td>No</td>
<td>Low (&lt; 0.001%)</td>
<td>Animal</td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td>Yes</td>
<td>Low (&lt; 0.03%)</td>
<td>Animal</td>
</tr>
<tr>
<td>Lolitrem B</td>
<td>unknown</td>
<td>Low (&lt; 0.02%)</td>
<td>Animal</td>
</tr>
<tr>
<td>Roquefortin C</td>
<td>No</td>
<td>Low (&lt; 0.005%)</td>
<td>Animal</td>
</tr>
<tr>
<td>Mycophenolic acid</td>
<td>No</td>
<td>Low (&lt; 0.005%)</td>
<td>Animal</td>
</tr>
</tbody>
</table>
Prevention strategies

Field-derived mycotoxins
- Reduction infection pressure (crop rotation, removal residues)
- Use of plant varieties with increased mould-infection resistance
- Avoid plant stress and plant damage

Ensilage-derived mycotoxins
- Minimize exposure to air; prevention of aerobic spoilage
  - High packing density
  - Adequate sealing
  - High feed-out rate
- Removal of moulded silage before feeding
- Additives: aerobic spoilage inhibitors
Conclusion - mycotoxins

- Field-derived vs. ensilage-derived mycotoxins
  \textit{DON, ZEA, roquefortin C and mycophenolic acid have highest incidence}

- Maize silage most important source

- Different prevention strategies required

- Aflatoxin B$_1$/M$_1$ only mycotoxin of concern for food safety. Other mycotoxins mainly of concern for animal health and productivity
Conclusion – bacteria and spores

- Contamination pathway: via faeces and bedding
- Spores of butyric acid bacteria associated with anaerobic and aerobic instability; maize silage important source
- Spores of Bacillus/Paenibacillus associated with aerobic instability; silage not a critical source of Bacillus cereus
- Silage important source of Listeria monocytogenes, causing listeriosis in cattle; associated with aerobic instability
- Silage minor source of E. coli O157:H7
- Listeria monocytogenes and E. coli inactivated by pasteurization
Thank you for your attention