

Challenges in evaluating mycotoxins in grass silages

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Introduction

Filamentous fungi (moulds) are found all over the world and in different climates.

Some fungal species produce toxic substances called mycotoxins.

Detrimental effects: crops, animals and humans, causing health problems and economic losses



Where can I find mycotoxins?

Feeds that can be contaminated at different stages in feeding chain

Soil → plant production → harvesting → preservation → feeding phase

Introduction

Typically, mycotoxin-producing fungi can be divided into 2 groups:

Produced on field while
plants are growing



***Fusarium,
Cladosporium and
Alternaria***

Produced during
storage period



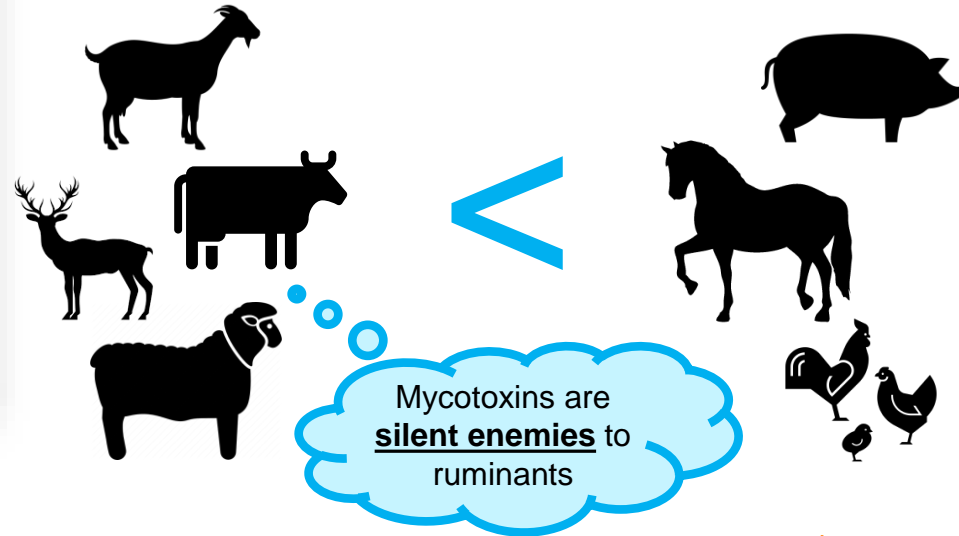
***Aspergillus and
Penicillium***

Introduction



Massive economic losses worldwide:
animal health + human health +
agricultural trade

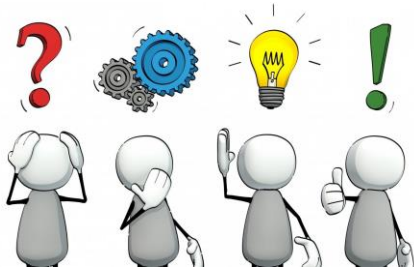
Mycotoxin sensitivity



Introduction

Accurate knowledge of type and amount of mycotoxins in feeds is important for decision making!

Identifying weaknesses of feed production + use of binders.



Currently there is no consensus among laboratories on methods for evaluation of mycotoxins in animal feeds.

More effort: cereals!



**But forages form the major part of cattle diets,
this information is also needed.**

Objectives



Evaluate the inter-laboratory variation with regard to mycotoxin analysis in grass silages produced in Finland in four different laboratories across Europe.

Materials and Methods

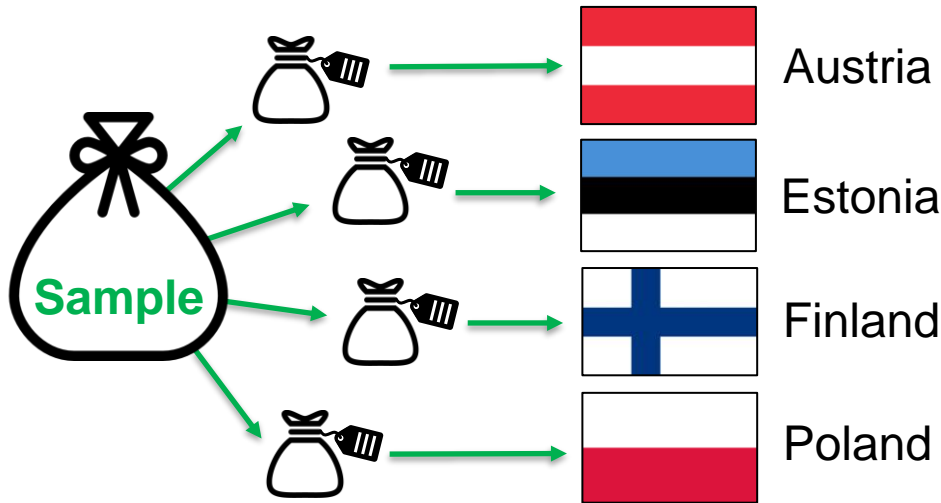
Ring-test: to evaluate the mycotoxin contents in a set of forages
10 samples (2 fresh grasses + 8 silages): wide range of hygienic quality

Grass	Abbr.	Legume	Abbr.
1. Timothy grass	Tim	6. Red clover grass	Rc
2. Tim ensiled untreated with additive	TimUT	7. Rc ensiled untreated with additive	RcUT
3. Tim ensiled with formic acid	TimFA	8. Rc ensiled with formic acid	RcFA
4. TimUT contaminated with soil	TimUTsoil	9. RcUT contaminated with soil	RcUTsoil
5. TimFA contaminated with soil	TimFAsoil	10. RcFA contaminated with soil	RcFAsoil

Materials and Methods

Ring-test: to evaluate the mycotoxin contents in a set of forages

10 samples (2 fresh grasses + 8 silages): wide range of hygienic quality



Laboratories were asked to analyse the samples using their current protocol.

Different quantification methods:

- LC-MS/MS API4000 by Sciex
- EN 17280 using LC-MS/MS
- Enzyme immunoassay for the quantitative determination of deoxynivalenol and zearalenone
- QuEChERS extraction and dSPE clean-up with identification and quantification by UPLC-MS/MS with MRM technique

Results





17 ≠ types of mycotoxins (although not all laboratories analysed all mycotoxins)

Beauvericin	Ergocornine	Moniliformin
Deoxynivalenol	Ergocorninine	Nivalenol
Enniatin A1	Ergosine	Roquefortine C
Enniatin B	HT2 toxin	Sterigmatocystin
Enniatin B1	Mycophenol acid	Zearalenone

Diacetoxyscirpenol and **T2 Toxin** were not detected in any of the samples or laboratories involved in this collaborative study

Results





DON: Deoxynivalenol

	Lab 1 (n = 4) 	Lab 2 (n = 2) 	Lab 3 (n = 1) 	Lab 4 (n = 2) 
Tim	HT2, ZEA	ZEA, BE, EA, EB, EB1, STE	DON, ZEA	BE
Rc	HT2, ZEA	BE, EA, EB, EB1, ERC, ERN, ERG	DON, ZEA	BE, EA, EB1
TimUT	ZEA	BE, EA, STE	DON, ZEA	BE, ROC
TimFA	HT2, ZEA	ZEA, EA, EB, EB1, STE	DON, ZEA	BE, EA, EB1
TimUTsoil	HT2, ZEA, DON	-	DON, ZEA	BE
TimFAsoil	ZEA	ZEA, EB1, STE	DON, ZEA	BE, ROC
RcUT	HT2, ZEA	ZEA, BE, EA, EB, EB1, ROC	DON, ZEA	BE, EA, EB1, ROC, MA
RcFA	HT2, ZEA, DON, NIV	ZEA, BE, EA, EB, EB1	DON, ZEA	BE, EA, EB1, ROC
RcUTsoil	ZEA	BE, EA, EB, EB1	DON, ZEA	BE, EA, EB1
RcFAsoil	ZEA	BE, EA, EB, EB1, MO	DON, ZEA	BE, EA, EB1

BE: Beauvericin; DON: Deoxynivalenol; EA: Enniatin A1; EB: Enniatin B; EB1: Enniatin B1; ERC: Ergocornine; ERN: Ergocorninine; ERG: Ergosine; HT2: HT2 toxin; MA: Mycophenolic acid; MO: Moniliformin; NIV: Nivalenol; ROC: Roquefortine C; STE: Sterigmatocystin; ZEA: Zearalenone.

Results





ZEA: Zearalenone – Labs 1 and 3 detected it in all samples; Lab 2 detected in half of the samples; Lab 4 did not detect.

	Lab 1 (n = 4) 	Lab 2 (n = 2) 	Lab 3 (n = 1) 	Lab 4 (n = 2) 
Tim	HT2, ZEA	ZEA, BE, EA, EB, EB1, STE	DON, ZEA	BE
Rc	HT2, ZEA	BE, EA, EB, EB1, ERC, ERN, ERG	DON, ZEA	BE, EA, EB1
TimUT	ZEA	BE, EA, STE	DON, ZEA	BE, ROC
TimFA	HT2, ZEA	ZEA, EA, EB, EB1, STE	DON, ZEA	BE, EA, EB1
TimUTsoil	HT2, ZEA, DON	-	DON, ZEA	BE
TimFAsoil	ZEA	ZEA, EB1, STE	DON, ZEA	BE, ROC
RcUT	HT2, ZEA	ZEA, BE, EA, EB, EB1, ROC	DON, ZEA	BE, EA, EB1, ROC, MA
RcFA	HT2, ZEA, DON, NIV	ZEA, BE, EA, EB, EB1	DON, ZEA	BE, EA, EB1, ROC
RcUTsoil	ZEA	BE, EA, EB, EB1	DON, ZEA	BE, EA, EB1
RcFAsoil	ZEA	BE, EA, EB, EB1, MO	DON, ZEA	BE, EA, EB1

BE: Beauvericin; DON: Deoxynivalenol; EA: Enniatin A1; EB: Enniatin B; EB1: Enniatin B1; ERC: Ergocornine; ERN: Ergocorninine; ERG: Ergosine; HT2: HT2 toxin; MA: Mycophenolic acid; MO: Moniliformin; NIV: Nivalenol; ROC: Roquefortine C; STE: Sterigmatocystin; ZEA: Zearalenone.

Results

Laboratory 1





	Lab 1 (n = 4) 	Lab 2 (n = 2) 	Lab 3 (n = 1) 	Lab 4 (n = 2) 
Tim	HT2, ZEA	ZEA, BE, EA, EB, EB1, STE	DON, ZEA	BE
Rc	HT2, ZEA	BE, EA, EB, EB1, ERC, ERN, ERG	DON, ZEA	BE, EA, EB1
TimUT	ZEA	BE, EA, STE	DON, ZEA	BE, ROC
TimFA	HT2, ZEA	ZEA, EA, EB, EB1, STE	DON, ZEA	BE, EA, EB1
TimUTsoil	HT2, ZEA, DON	-	DON, ZEA	BE
TimFAsoil	ZEA	ZEA, EB1, STE	DON, ZEA	BE, ROC
RcUT	HT2, ZEA	ZEA, BE, EA, EB, EB1, ROC	DON, ZEA	BE, EA, EB1, ROC, MA
RcFA	HT2, ZEA, DON, NIV	ZEA, BE, EA, EB, EB1	DON, ZEA	BE, EA, EB1, ROC
RcUTsoil	ZEA	BE, EA, EB, EB1	DON, ZEA	BE, EA, EB1
RcFAsoil	ZEA	BE, EA, EB, EB1, MO	DON, ZEA	BE, EA, EB1

BE: Beauvericin; DON: Deoxynivalenol; EA: Enniatin A1; EB: Enniatin B; EB1: Enniatin B1; ERC: Ergocornine; ERN: Ergocorninine; ERG: Ergosine; HT2: HT2 toxin; MA: Mycophenolic acid; MO: Moniliformin; NIV: Nivalenol; ROC: Roquefortine C; STE: Sterigmatocystin; ZEA: Zearalenone.

Results

Laboratory 2: BE, EA, EB, EB1, MO, STE detected





Laboratory 4: BE, EA, EB1 < LOQ

	Lab 1 (n = 4) 	Lab 2 (n = 2) 	Lab 3 (n = 1) 	Lab 4 (n = 2) 
Tim	HT2, ZEA	ZEA, BE, EA, EB, EB1, STE	DON, ZEA	BE
Rc	HT2, ZEA	BE, EA, EB, EB1, ERC, ERN, ERG	DON, ZEA	BE, EA, EB1
TimUT	ZEA	BE, EA, STE	DON, ZEA	BE, ROC
TimFA	HT2, ZEA	ZEA, EA, EB, EB1, STE	DON, ZEA	BE, EA, EB1
TimUTsoil	HT2, ZEA, DON	-	DON, ZEA	BE
TimFAsoil	ZEA	ZEA, EB1, STE	DON, ZEA	BE, ROC
RcUT	HT2, ZEA	ZEA, BE, EA, EB, EB1, ROC	DON, ZEA	BE, EA, EB1, ROC, MA
RcFA	HT2, ZEA, DON, NIV	ZEA, BE, EA, EB, EB1	DON, ZEA	BE, EA, EB1, ROC
RcUTsoil	ZEA	BE, EA, EB, EB1	DON, ZEA	BE, EA, EB1
RcFAsoil	ZEA	BE, EA, EB, EB1, MO	DON, ZEA	BE, EA, EB1

BE: Beauvericin; DON: Deoxynivalenol; EA: Enniatin A1; EB: Enniatin B; EB1: Enniatin B1; ERC: Ergocornine; ERN: Ergocorninine; ERG: Ergosine; HT2: HT2 toxin; MA: Mycophenolic acid; MO: Moniliformin; NIV: Nivalenol; ROC: Roquefortine C; STE: Sterigmatocystin; ZEA: Zearalenone.

Results





Laboratory 2: ergocornine, ergocorninine, ergosine and moniliformin

	Lab 1 (n = 4) 	Lab 2 (n = 2) 	Lab 3 (n = 1) 	Lab 4 (n = 2) 
Tim	HT2, ZEA	ZEA, BE, EA, EB, EB1, STE	DON, ZEA	BE
Rc	HT2, ZEA	BE, EA, EB, EB1, ERC, ERN, ERG	DON, ZEA	BE, EA, EB1
TimUT	ZEA	BE, EA, STE	DON, ZEA	BE, ROC
TimFA	HT2, ZEA	ZEA, EA, EB, EB1, STE	DON, ZEA	BE, EA, EB1
TimUTsoil	HT2, ZEA, DON	-	DON, ZEA	BE
TimFAsoil	ZEA	ZEA, EB1, STE	DON, ZEA	BE, ROC
RcUT	HT2, ZEA	ZEA, BE, EA, EB, EB1, ROC	DON, ZEA	BE, EA, EB1, ROC, MA
RcFA	HT2, ZEA, DON, NIV	ZEA, BE, EA, EB, EB1	DON, ZEA	BE, EA, EB1, ROC
RcUTsoil	ZEA	BE, EA, EB, EB1	DON, ZEA	BE, EA, EB1
RcFAsoil	ZEA	BE, EA, EB, EB1, MO	DON, ZEA	BE, EA, EB1

BE: Beauvericin; DON: Deoxynivalenol; EA: Enniatin A1; EB: Enniatin B; EB1: Enniatin B1; ERC: Ergocornine; ERN: Ergocorninine; ERG: Ergosine; HT2: HT2 toxin; MA: Mycophenolic acid; MO: Moniliformin; NIV: Nivalenol; ROC: Roquefortine C; STE: Sterigmatocystin; ZEA: Zearalenone.

Results

ROC: Roquefortine C

	Lab 1 (n = 4) 	Lab 2 (n = 2) 	Lab 3 (n = 1) 	Lab 4 (n = 2) 
Tim	HT2, ZEA	ZEA, BE, EA, EB, EB1, STE		BE
Rc	HT2, ZEA	BE, EA, EB, EB1, ERC, ERN, ERG		BE, EA, EB1
TimUT	ZEA	BE, EA, STE		BE, ROC
TimFA	HT2, ZEA	ZEA, EA, EB, EB1, STE		BE, EA, EB1
TimUTsoil	HT2, ZEA, DON	-		BE
TimFAsoil	ZEA	ZEA, EB1, STE		BE, ROC
RcUT	HT2, ZEA	ZEA, BE, EA, EB, EB1, ROC		BE, EA, EB1, ROC , MA
RcFA	HT2, ZEA, DON, NIV	ZEA, BE, EA, EB, EB1		BE, EA, EB1, ROC
RcUTsoil	ZEA	BE, EA, EB, EB1		BE, EA, EB1
RcFAsoil	ZEA	BE, EA, EB, EB1, MO		BE, EA, EB1

Lower than LOQ

Lower than LOQ

BE: Beauvericin; DON: Deoxynivalenol; EA: Enniatin A1; EB: Enniatin B; EB1: Enniatin B1; ERC: Ergocornine; ERN: Ergocorninine; ERG: Ergosine; HT2: HT2 toxin; MA: Mycophenolic acid; MO: Moniliformin; NIV: Nivalenol; ROC: Roquefortine C; STE: Sterigmatocystin; ZEA: Zearalenone.

Conclusions



- According to this collaborative study to investigate mycotoxins in grass silages produced in Finland, the results of mycotoxin analyses are not in a consensus across laboratories.
- To be able to cope with the potential risks of mycotoxins in grass silages, emphasis should be given to develop the analytical systems to be more reliable and in line with each other.

Thank
you!



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