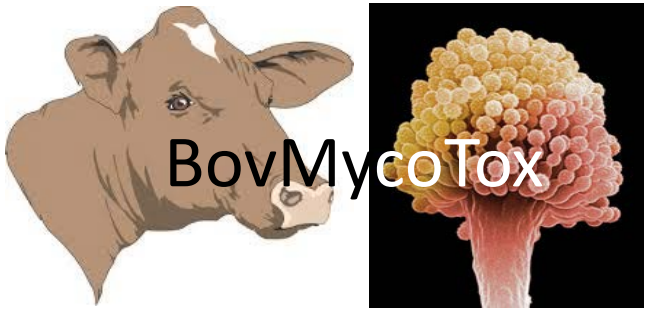
A metabonomic approach to identify biomarkers for bovine mycotoxicosis

‘BovMycoTox’

Healthy Silage Workshop – Duchy College

Tuesday 13th September 2016



The project is co-funded by

Meet the Consortium

Name	Organisation	Role
Victoria Morris	University of Bristol	Post Doctorate
Jennifer Bailey	University of Bristol	Post Doctorate
Michael Lee	University of Bristol and Rothamsted Res	Principal Investigator
Tristan Cogan	University of Bristol	Co-Investigator
Mick Bailey	University of Bristol	Co-Investigator
Jonathan Swann	Imperial College London	Principal Investigator
Michael Wilkinson	University of Nottingham	Consultant
Stephen Roderick	Duchy College	Consultant
Liz Norton	Micron Biosystems	Lead Industry
Chris Bartram	Mole Valley Farmers	Industry Partner
Jamie-Leigh Douglas	AB Vista	Industry Partner

Background - Mycotoxins

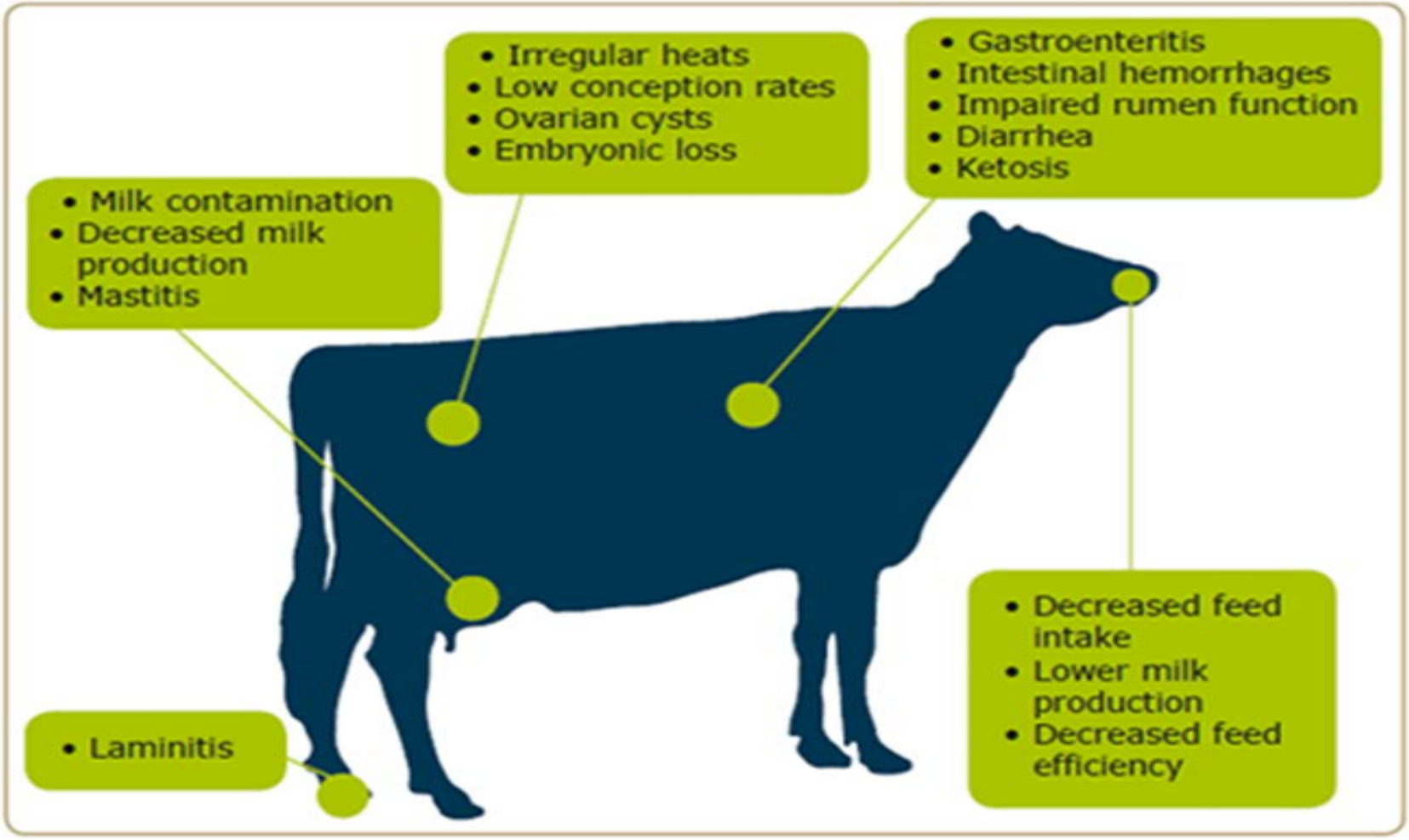
- Mycotoxins – fungal metabolites (Fusarium and Aspergillus)
- Found on cereals and conserved forage
- Little guidelines for eliminating mycotoxins in ruminant rations
- Recent survey results – issue relating to maize silage

Mycotoxicosis

- Fungal toxins produced in silage following aerobic deterioration
- Detoxified by rumen bacteria and protozoa but if pH drops then microflora lose the capability to detoxify (SARA)
- If suspected – stop feeding contaminated silage and add sodium bentonite as a binder to the feed

Mycotoxin	Toxicity level	Biological Activity
Aflatoxin	300-700 ppb	Hepatotoxic, Carcinogenic, Haemorrhagic, Immuno-suppresive
Deoxynivalenol (vomitoxin or DON)	300-500 ppb	Neurotoxic, Dermatotoxic, Haemorrhagic, Teratogenic, Antibiotic
T-2 toxin	100	Neurotoxic, Dermatotoxic, Haemorrhagic, Teratogenic, Antibiotic
Ochratoxin	NDA	Nephrotoxic, Immuno-suppresive, Carcinogenic, Teratogenic
Zearalenone (ZEN)	200-300	Oestorogenic, Antibiotic, Carcinogenic

Mycotoxicosis



Diagnosis

- Complications with SARA
- Need for unique identifier
- Metabolite associated with mycotoxicosis
- Metabonomic approach

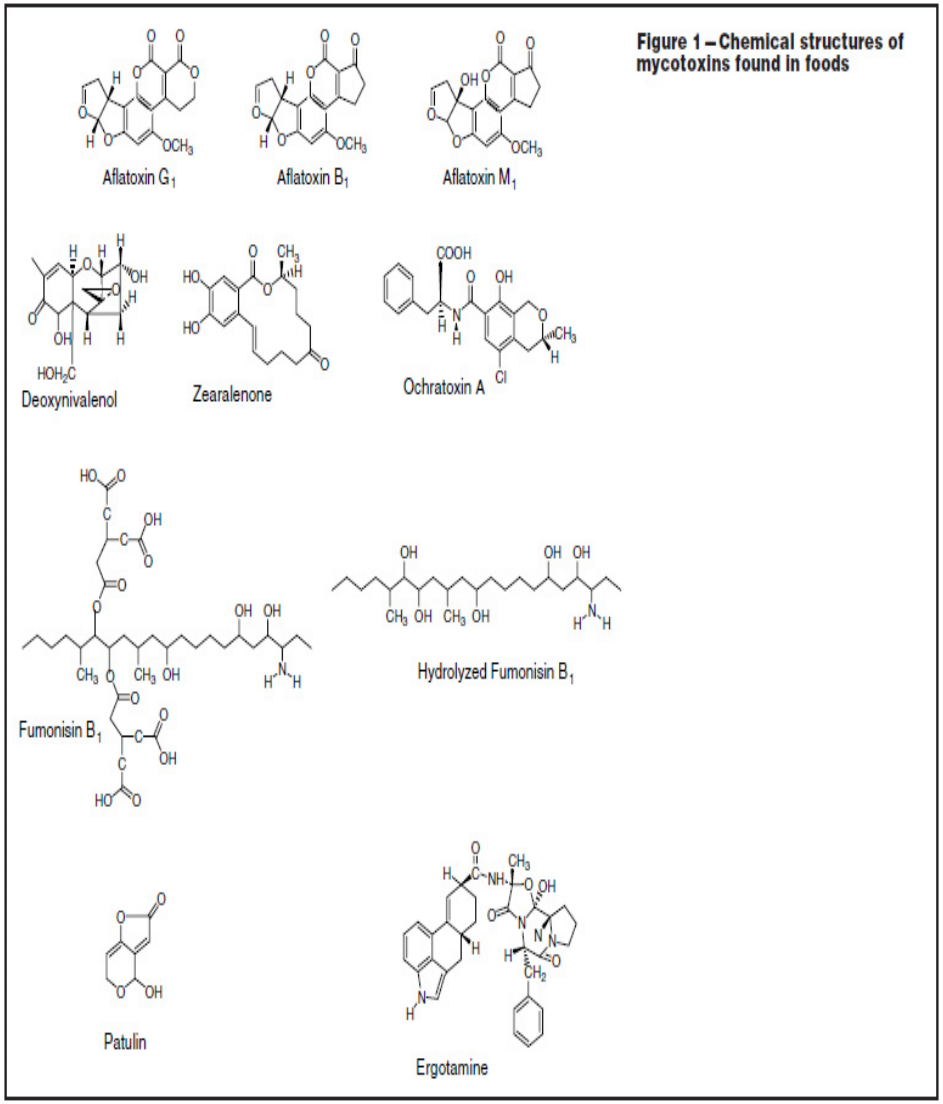


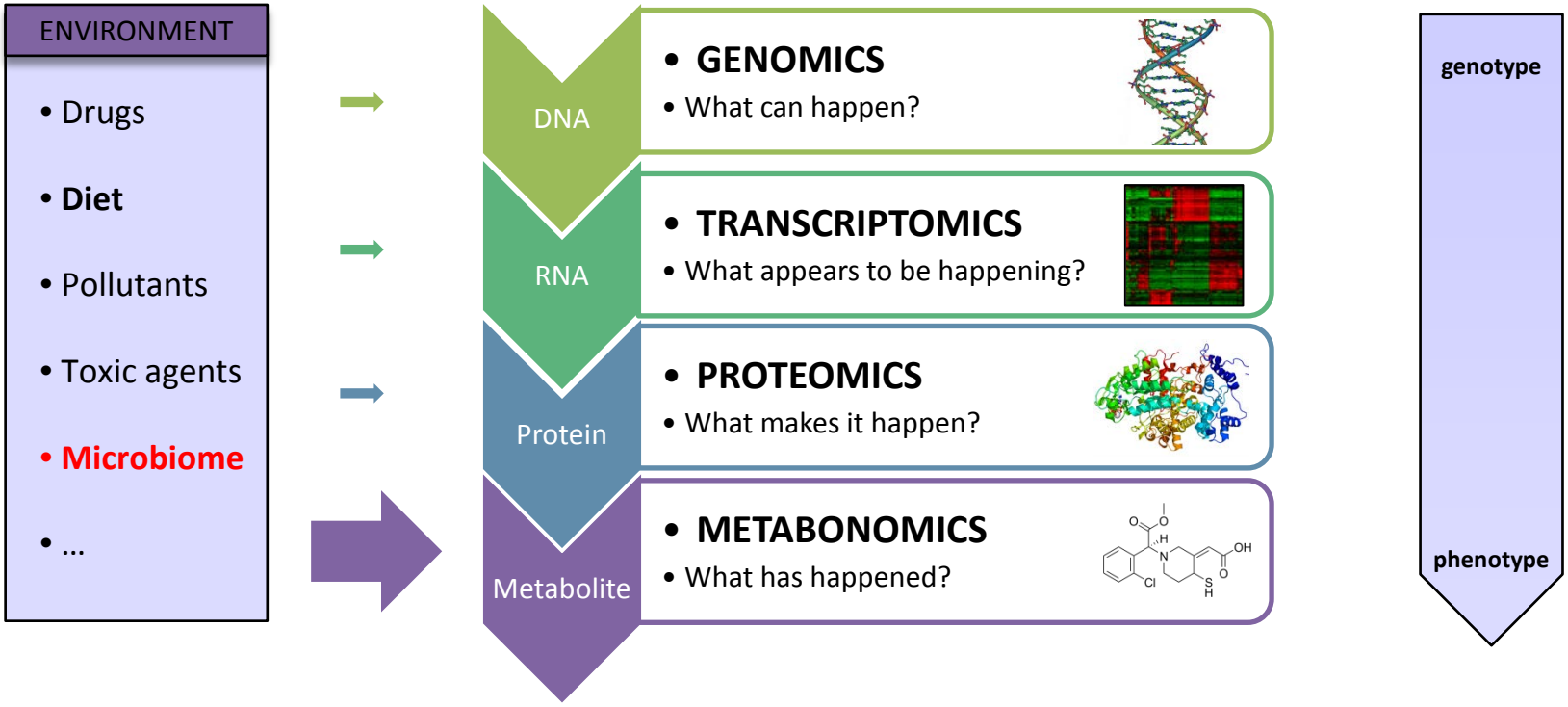
Figure 1 – Chemical structures of mycotoxins found in foods

Aims of BovMycoTox

- Impact of the mycotoxins and their metabolites on the gastro-intestinal cell
- Impact of diet and binders on mycotoxin detoxification, metabolite formation and microbial ecology
- Metabonomic and biomarker identification
- Knowledge exchange and dissemination to the feed industry, veterinarians and farmers

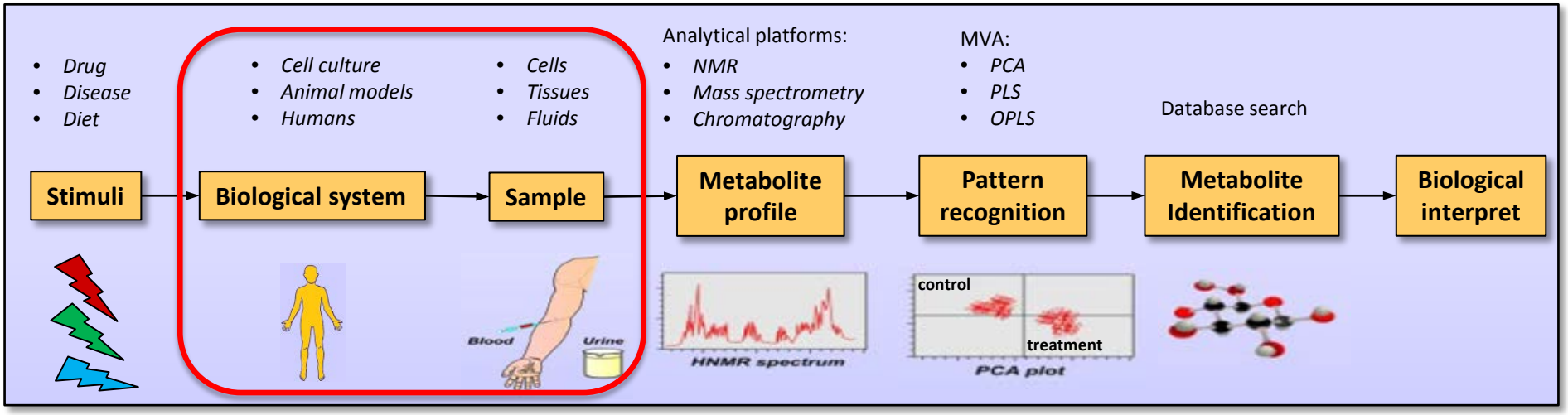
What is Metabonomics?

- Metabonomics: Quantitative measurement of time-related metabolic responses of multicellular systems to pathophysiological stimuli or genetic modification (Nicholson et al. Xenobiotica 29 (1999) 1181)
- Metabolite: Any organic molecule detectable in the body with a MW < 1500 Da



Changes in metabolome → BIOMARKERS of health/disease
Insights into pathogenesis (mechanisms)

METABONOMICS TYPICAL STRATEGY



In-vitro

Cell extracts
Culture media
Batch culture supernatants

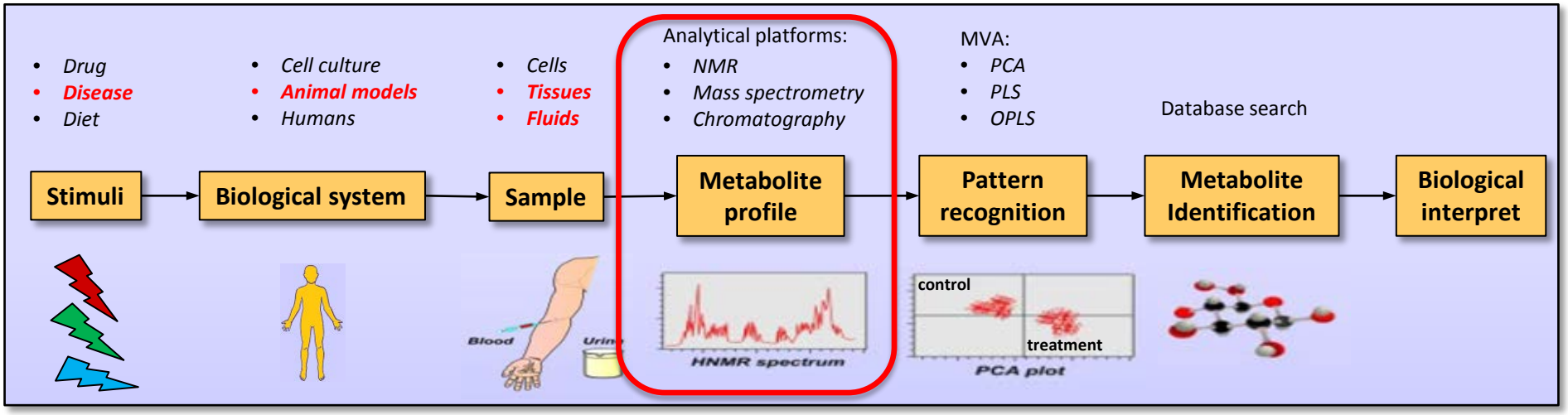
In-vivo

Biofluids:
Urine
Plasma/Serum
Saliva
Faecal water
Cerebrospinal fluid

Tissues:
Liver, kidney, heart, lung
Brain regions
Gut tissue

Other:
Vaginal wash/swab
Teeth
Hair
Milk

METABONOMICS TYPICAL STRATEGY



Sample → **Simultaneous and Non-selective** → **NMR & MS**

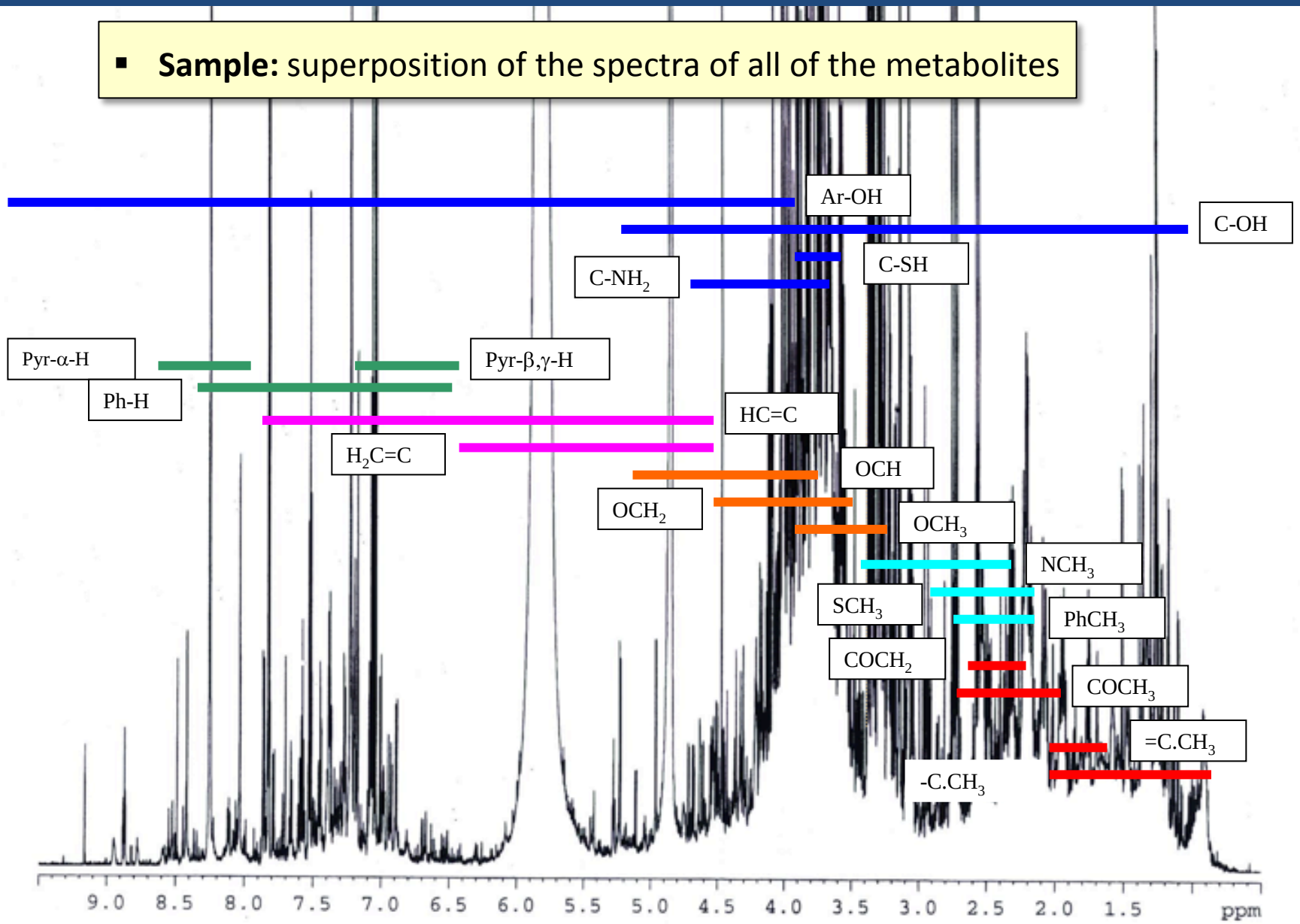
- Wide RANGE OF METABOLITES
- Minimal sample preparation
- Single experiment

METABOLITES:

Sugars	10s
Nucleosides	
Organic acids	100s
Amino acids	
...	1000s

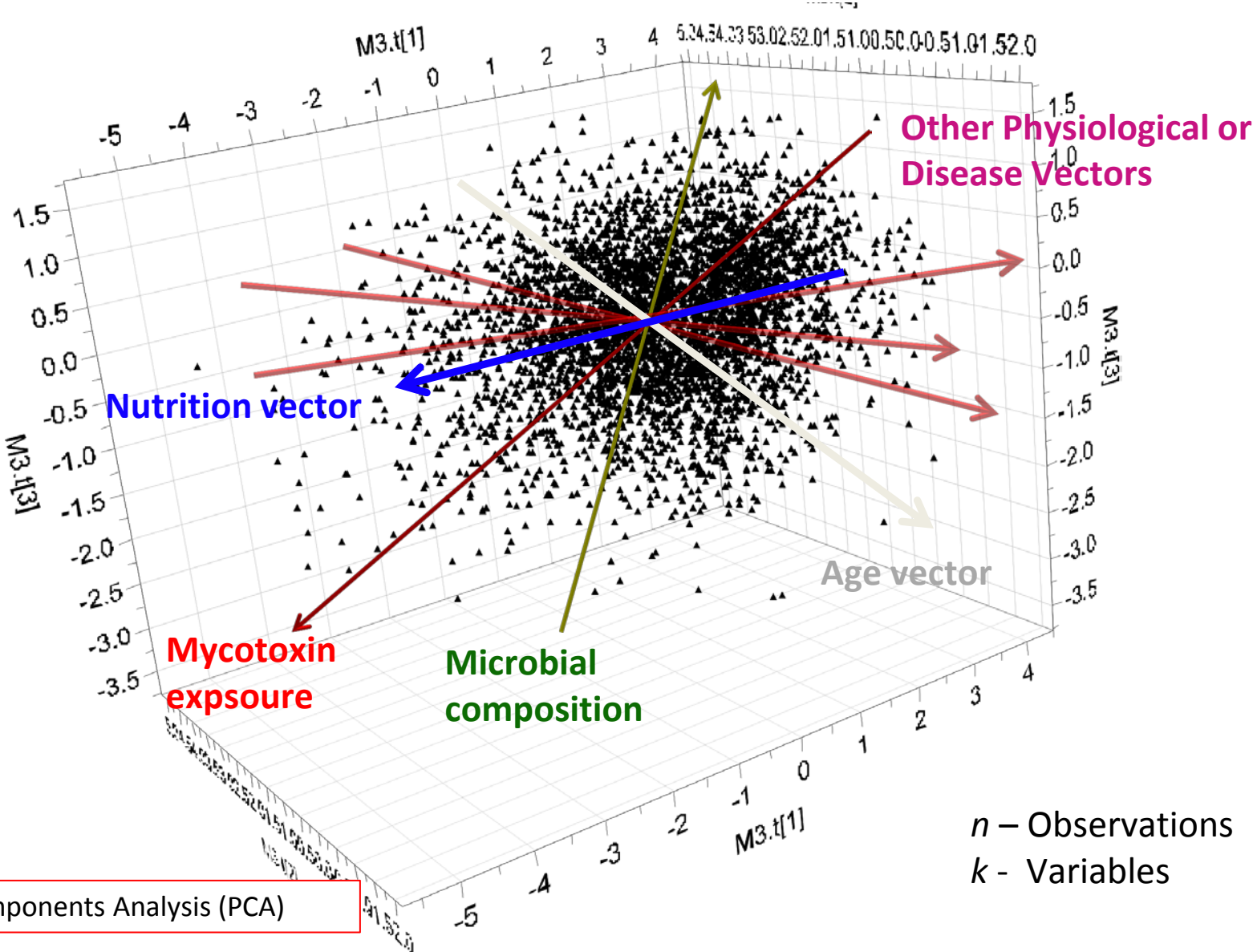
^{1}H NMR spectroscopy

- **Sample:** superposition of the spectra of all of the metabolites



900 MHz spectrum of human urine – 5-10 minutes to acquire

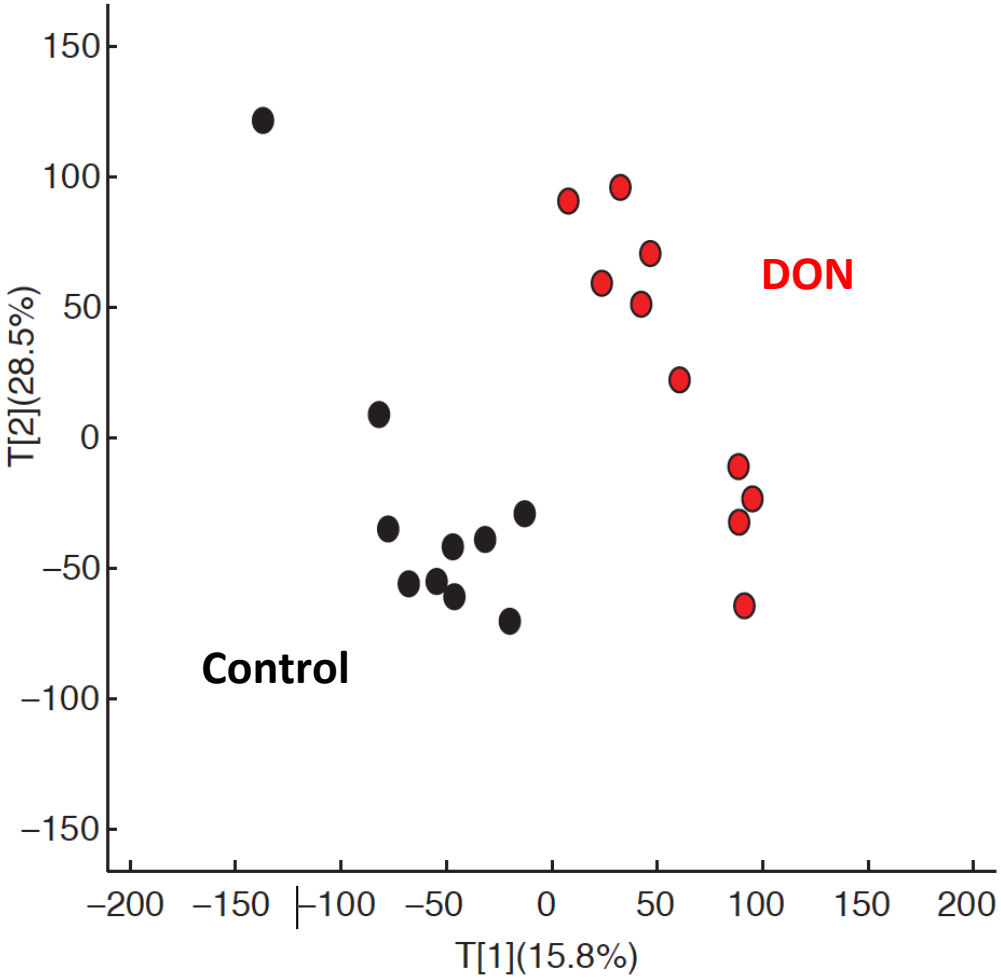
Metabolic Hyperspace Modelling: PCA and The "Influence Vector Concept"



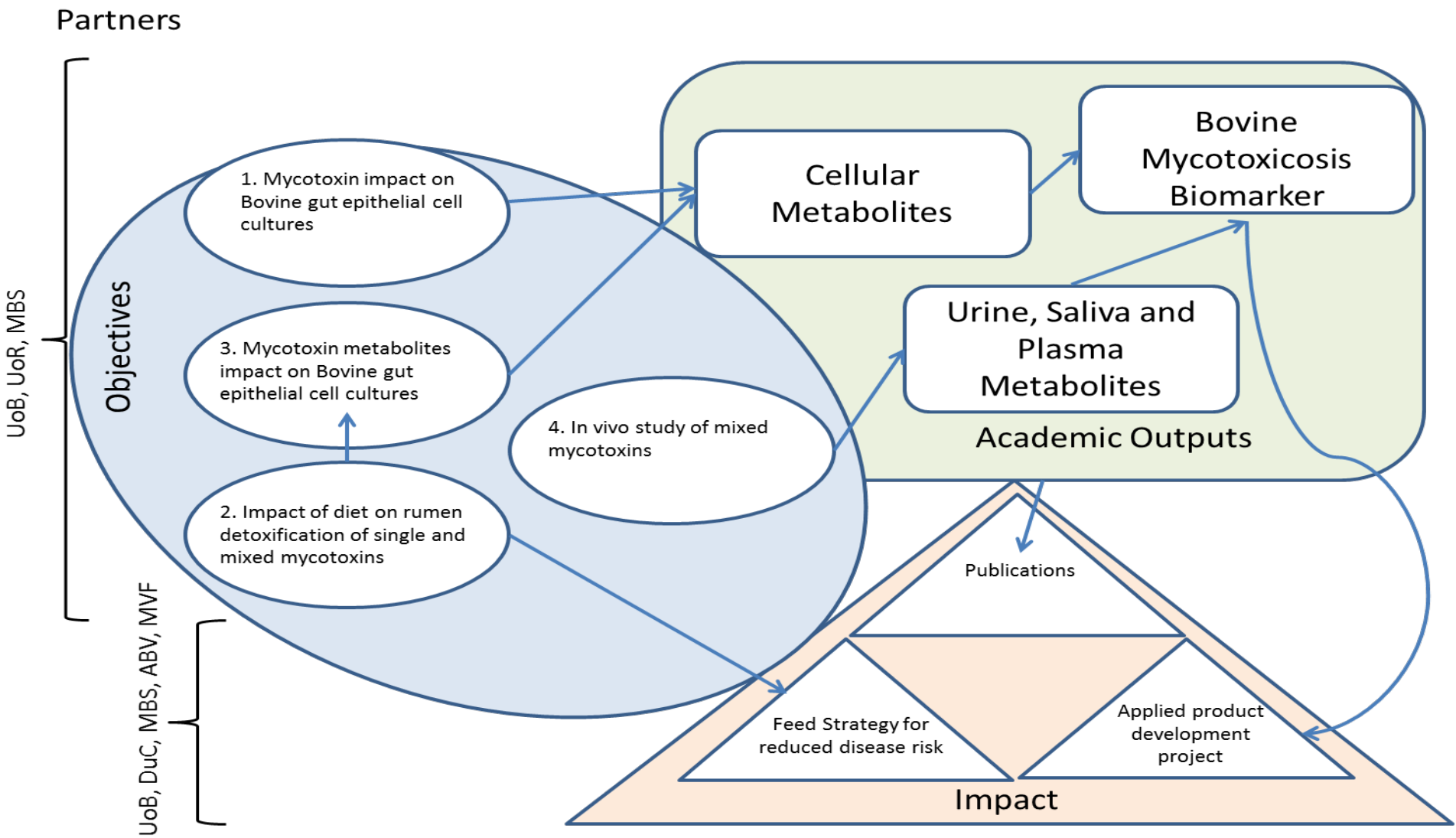
n – Observations
k - Variables

Principal Components Analysis (PCA)

Example PCA – cells dosed with DON



Project Overview

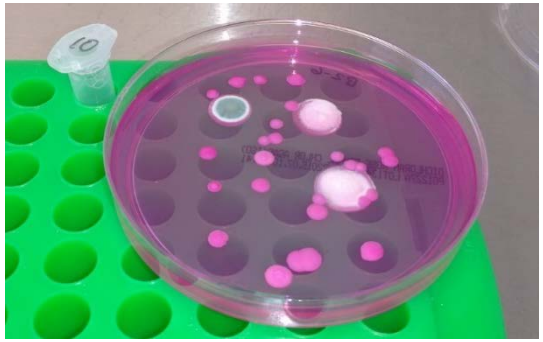


‘Growing’ Mycotoxin in the Lab

Mycotoxins are needed for subsequent parts of the project. Specifically DON and ZON are required as these are the most common found in maize silage.

Whilst growth of the fungi Fusarium is easy in the lab, getting it to produce mycotoxins in the lab has proven to be quite tricky.

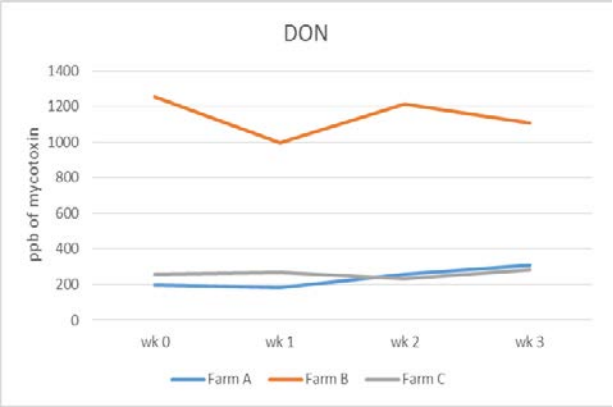
Trying to replicate the complexities of conditions in the field and the reaction of the maize to invasion by Fusarium is challenging.

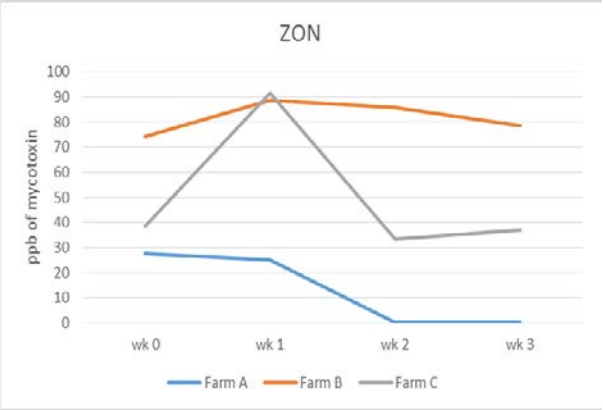


Factors that are being tested to induce mycotoxin production include:
Temperature
Moisture
pH
Nitrogen sources
Light

‘Growing’ Mycotoxin in the Lab

- Levels of DON and ZON from maize silage when incubated aerobically in the lab at 23°C. No significant differences occurred over time.





- Levels of patulin increased at two weeks of incubation, then depleted suddenly. Further investigation required as to what caused this depletion.

Patulin	Farm A	Farm B	Farm C
2 weeks	317.03ppm	141.67ppb	0
3 weeks	26.51ppm	0	0

Impact on Rumen Metabolism

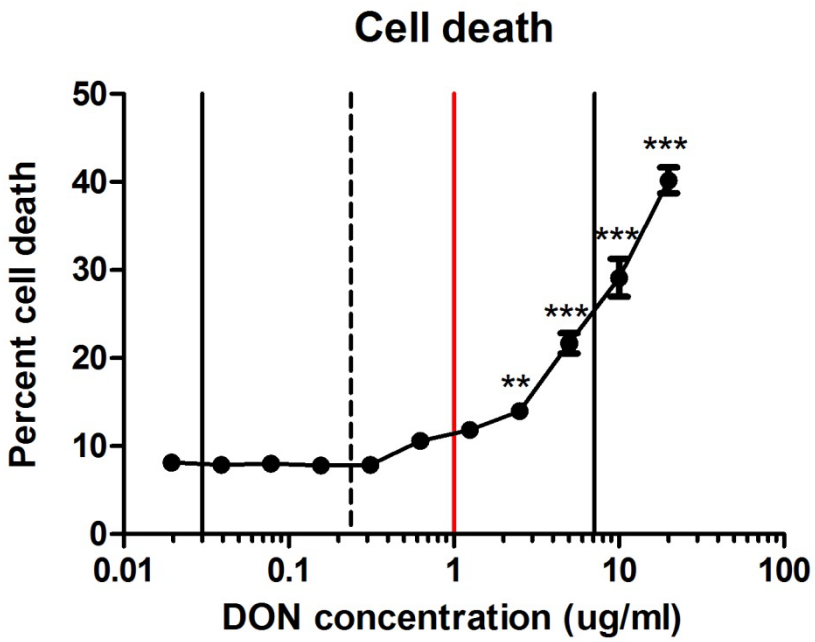
To test the effect of mycotoxins on the flora of the rumen, batch culture experiments are being conducted. These experiments use rumen content which is then incubated at 38°C with DON, ZON, a mixture of DON and ZON with and without a commercial binder added. The following analysis is being carried out:

- Transcriptomics – which microbes are more or less active when dosed with mycotoxins
- Fibre degradation
- Ammonia
- Fatty acids
- Metabonomics
- pH
- Mycotoxins and metabolites

A miniature rumen (80 ml)

To determine the effect of mycotoxins on bovine epithelial cells

Levels of mycotoxins found in silage are sufficient to kill epithelial cells.



Metabonomic results – so far!

Low levels of mycotoxins do not kill cells but do induce changes in the metabolites produced by them.

We are investigating the possibility of detecting these altered metabolites in biological fluids such as urine or saliva.