Euroopa Maaelu Arengu Põllumajandusfond: Euroopa Investeeringud maapiirkondadesse

Mycotoxins effects in Ruminants
For: Estonian Dairy Farmers

BIOMIN GmbH

Ignacio Artavia
Competence Center Mycotoxins

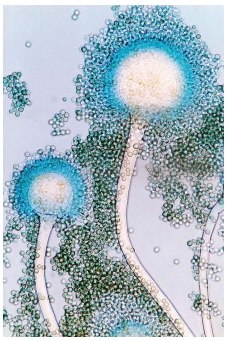
Mycotoxin Risk Management – Product Portfolio

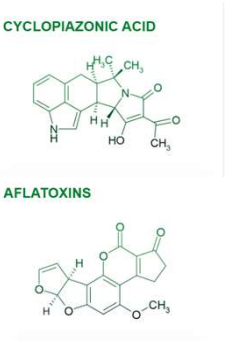
Product	Mycofix® 5.E	Mycofix® 5.0	Mycofix® 3.E	Mycofix® 3.0	Mycofix® MTV
Plus	✓ Blend of minerals (adsorbable toxins => Afla, ergots, endotoxins) ✓ Biomin® BBSH 797 (trichothecenes) ✓ Biological constituent (ZEN) ✓ FUMzyme® ✓ Biomin® Bioprotection Mix	✓ Blend of minerals (adsorbable toxins => Afla, ergots, endotoxins) ✓ Biomin® BBSH 797 (trichothecenes) ✓ Biomin® MTV (ZEN & OTA) ✓ FUMzyme® ✓ Biomin® Bioprotection Mix	✓ Blend of minerals (adsorbable toxins => Afla, ergots, endotoxins) ✓ Inactivated bioprotein (trichothecenes) ✓ Biological constituent (ZEN) ✓ Plant extracts ✓ Algae extracts	✓ Blend of minerals (adsorbable toxins => Afla, ergots, endotoxins) ✓ Biomin® BBSH 797 (trichothecenes) ✓ Biological constituent (ZEN) ✓ Plant extracts ✓ Algae extracts	✓ Blend of minerals (adsorbable toxins => Afla, ergots, endotoxins) ✓ Biomin® BBSH 797 (trichothecenes) ✓ Biomin® MTV (ZEN & OTA) ✓ Plant extracts ✓ Algae extracts
Select	✓ Blend of minerals ✓ Biomin® BBSH 797 (trichothecenes) ✓ FUMzyme® ✓ Biomin® Bioprotection Mix	✓ Blend of minerals ✓ Biomin® BBSH 797 (trichothecenes) ✓ FUMzyme® ✓ Biomin® Bioprotection Mix	✓ Blend of minerals ✓ Inactivated bioprotein (trichothecenes) ✓ Plant extracts ✓ Algae extracts	✓ Blend of minerals ✓ Biomin® BBSH 797 (trichothecenes) ✓ Plant extracts ✓ Algae extracts	✓ Blend of minerals ✓ Biomin® BBSH 797 (trichothecenes) ✓ Less Biomin® MTV (OTA) ✓ Plant extracts ✓ Algae extracts
Focus	Bentonite, FUMzyme®				
Secure	Bentonite				
Eco	Bentonite, Plant extracts				
PRO-tect	Bentonite, Biomin® Bioprotection Mix				

What are Mycotoxins?

Toxic secondary metabolite products of moulds often affecting commodities

- Invisible
- Tasteless
- Chemically stable
- Temperature resistant
- Storage resistant



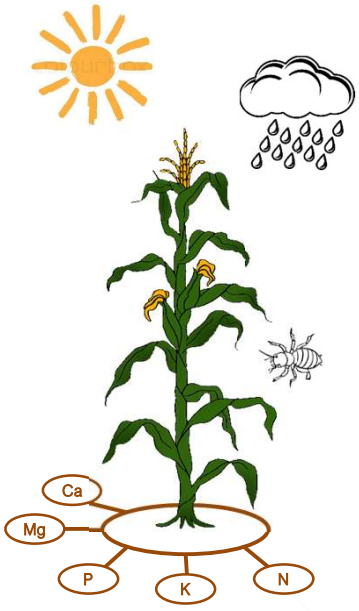


Aspergillus flavus responsible for producing Aflatoxins and Cyclopiazonic acid, among others

Mycotoxin Formation

Most important factors:

- Plant and environmental factors (e.g. soil fertility, water activity, pH)
- Competitive actions (e.g. insect and fungi attack)
- Climatic conditions (e.g. temperature, drought)



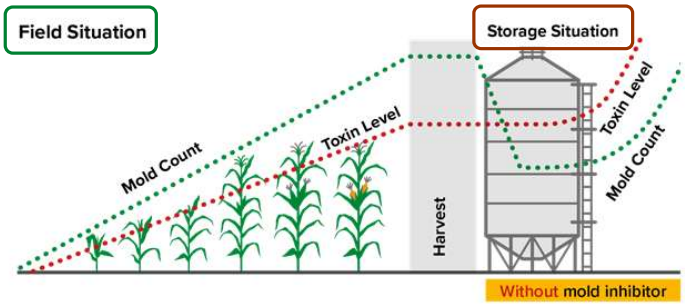
Where do mycotoxins come from?

- **Field:** 95% of mycotoxins in feed are produced on the field
- **Harvest:** toxin level remains steady
- **Storage:** toxin level may increase depending on fungi activity

Fusarium sp.

Aspergillus sp.

Penicillium sp.



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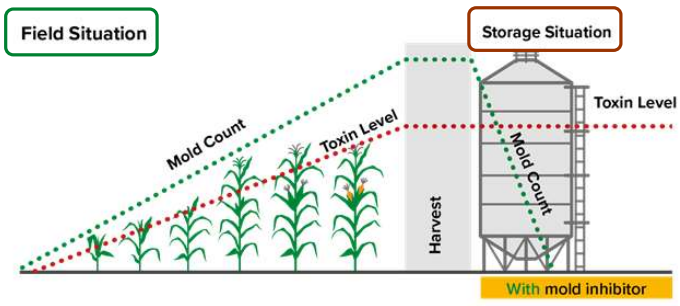

Naturally ahead
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Biomin

Mycotoxins can be (partly) responsible for:

Decrease in Milk production down/fluctuates – 1-5 kg

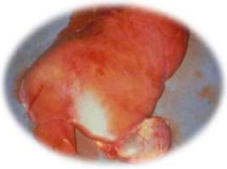
Diarrhea, dark stool, bloody mucous

Increase in mastitis - SCC

Sporadic, non-specific mortality

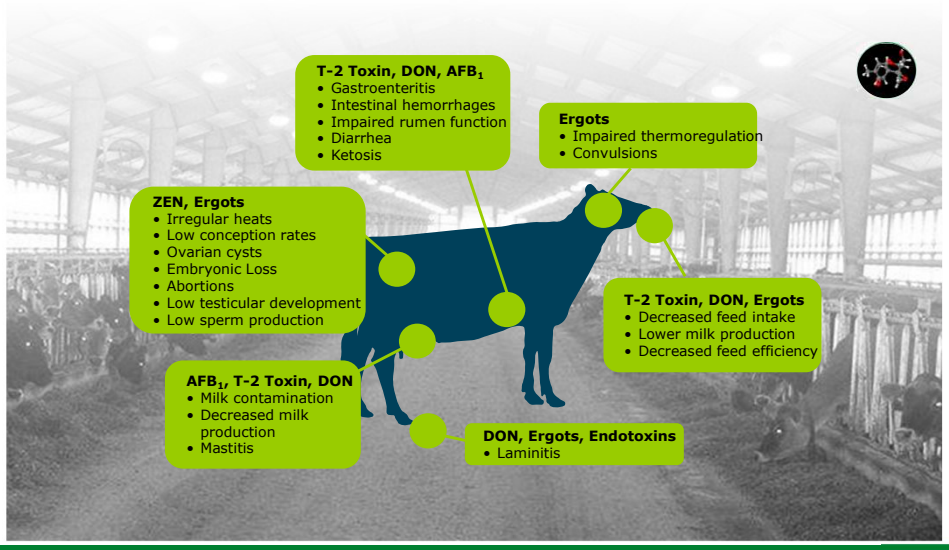
Increase in uterine infections post calving

More cows not conceiving, irregular cycles



Higher incidence of ketosis, DA's, laminitis

General overview of Mycotoxins' impacts in dairy cows



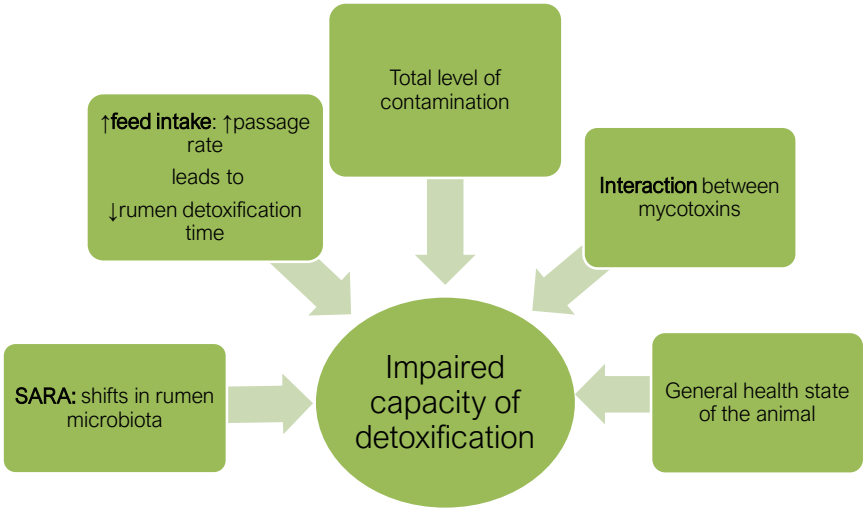
Higher passage rate reduces time for detoxification

Low Yielding Cow	High Yielding Cow
Feed Intake: 12-15 kg DM Time for Detoxification: 24 h **120 min/kg DM**	Feed Intake: 26 kg DM Time for Detoxification: 24 h **55 min/kg DM**

Ruminal Degradation of Mycotoxins

Mycotoxin	Degradation in the Rumen	No Degradation in the Rumen
Aflatoxin	0 - 42 % More toxic Aflatoxicol *(Engel and Hagemeister, 1978)*	58 – 100 %
Zearalenone	90% α- and β-Zearalenol *(Kiessling et al., 1984; Hagler et al. 1979)*	10 % metabolites more estrogenic
Deoxynivalenol	15 % - 99 % DOM-1 *(Cote et al., 1986; Kiessling et al., 1984, Debevere, 2020)*	1 - 85 %
T2 - Toxin	90 % → influence on protozoa	10 %
Ochratoxin A	Totally (Mobashar et al, 2010)	?
Fumonisin	No degradation (EFSA, 2018)	Unknown, not reported oral bioavailability
Enniantin B	1-25% (Debevere et al, 2020)	75%-99%

Detoxification Capacity Summarized



Exposure of dairy cows to mycotoxins through feed

Feed	Possible mycotoxins present
Concentrates	Aflatoxins, FUM, ZEA, DON, Trichothecenes, ergot alkaloids
Silages	Patulin, mycophenolic acid, roquefortines, fumitremorgens, cerruculogen, monacolines, etc…
Forages	Alternaria, Cyclopiazonoic acid, DON, other thrichothecenes, Mycophenolic acid, roquefortines, etc….

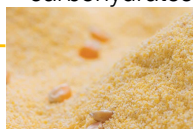
Subacute Ruminal Acidosis

↗VFAs
↗ Lactic acid
↘ pH

↘Protozoa (fiber-digesting bacteria)

↗Starch-digesting bacteria

Excess of Readily-fermentable carbohydrates

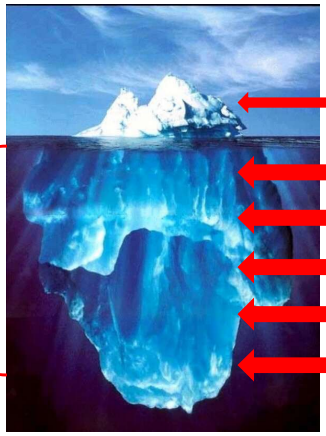


↘Mycotoxin cleavage is hampered

↗Mycotoxins bypassing intact to the intestine

Effects of Mycotoxins on Animal Health

- Lower Performance
- Lower farm profits



Clinical symptoms

↑mastitis incidence and ↑SCC

↓feed intake

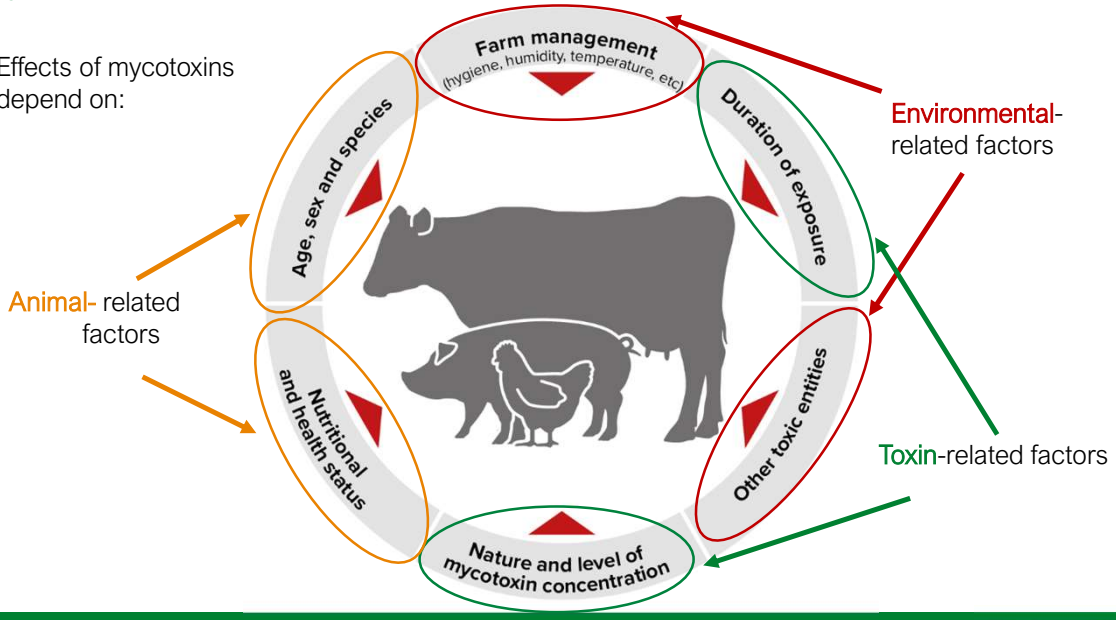
Reduction of production

Immunosuppression

Losses in fertility

Mycotoxins - General effects on animals

Effects of mycotoxins depend on:



Environmental-related factors

Animal- related factors

Toxin-related factors

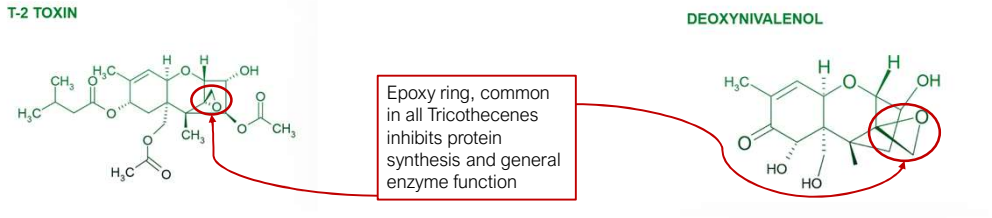
Fusarium Toxins

- *Fusarium* spp. most known metabolites:
  - Trichothecenes
  - Fumonisins
  - Zearalenone

  } Often co-ocurring
- Ubiquitous, highly prevalent on warm conditions
- Commonly found in:
  - Silage
  - Corn
  - Sorghum
  - Wheat

Trichothecenes

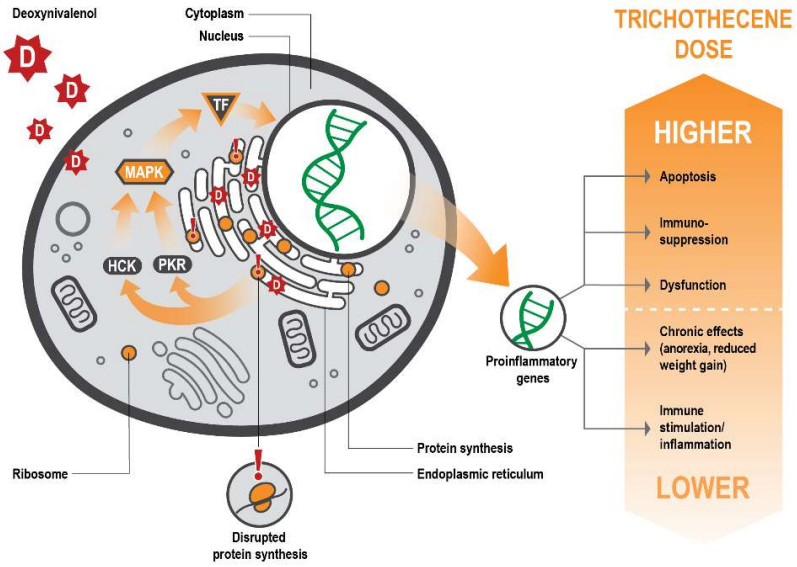
- A family with >200 mycotoxins
- Mode of Action: Inhibition of protein synthesis by disrupting DNA replication.



- Affect actively diving cells like GIT, skin, lymphoid and erythroid (red and white) cells

Tricothecenes A	Tricothecenes B
HT-2 toxin, T-2 toxin, DAS	DON, NIV

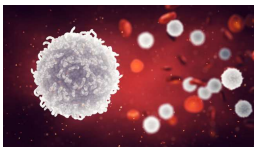
Mode of Action - Trichothecenes

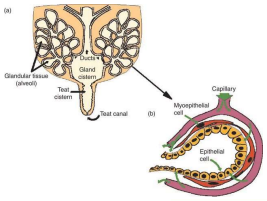


Trichothecenes

- Ruminal degradation is pH dependant
- Antimicrobial activity in the rumen → Reduced fermentative capacity
  - Decreased VFA production
  - Decreased digestibility
- Targets fast reproducing cells, such as:

Intestinal epithelial	Immune	Mammary epithelial	Etc.





Deoxynivalenol (DON)

- A.k.a “vomitoxin”
- “DON is considered a major cause of economic losses due to reduction of animal performance” – Gallo et al (2015)

Common issues related to DON:

- Feed refusal, lower DMI
- Impaired rumen fermentation
- Leaky gut
- Depressed immune system

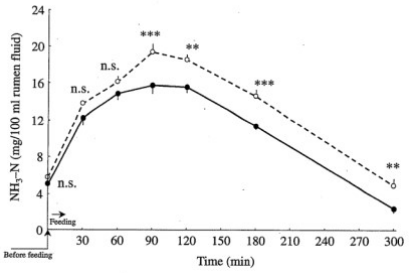
By Paige Gott/ TSM USA

DON impairs ruminal protein synthesis

- Cows fed a diet 50% Grass silage, 50% wheat.
  - Control cows were fed non-contaminated wheat
  - DON treatment received DON contaminated wheat, with a total concentration on the total diet of 3.1ppm.

Nutrient flows at the proximal duodenum

	Control	DON (3.1ppm)	% relative to control	Probability
Microbial Protein (g/d)	862	680	-21	0.122
Microbial Protein (g/MJ ME)	10.9	8.3	-24	0.087



Rumen ammonia levels post-feeding a control diet (dashed) or DON contaminated (bold) diet

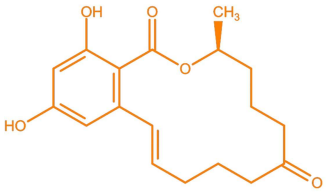
Source: Dänicke et al., 2005

T-2 and HT-2 Toxins

In cows can cause:
- Feed refusal
- Diarrhea
- Lower milk production
- Absence of oestrus cycles (Kegl and Vanyi, 1991)
- Abortions in midgestation (Osweiller, 2011)

In calves:
- Decreased DMI
- Weight loss
- Rough hair coats
- etc.

Zearalenone (ZEN) effects in Ruminants

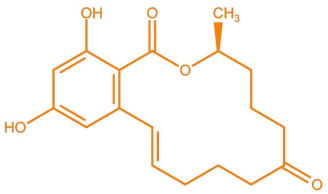


- Fusarium spp. produced mycotoxin
- Different effects:
  - **Estrogen binding**
  - Antimicrobial
  - Induces oxidative stress
  - Inflammatory

Estrogen receptors are located in:
- uterus
- mammary gland
- Hypothalamus
- Pituitary gland

It's degraded by ruminal microbiota, BUT:
- α-ZEL is **60x** as estrogenic as ZEN
- β-ZEL is **0.2x** as estrogenic as ZEN

Zearalenone: co-occurrence is the rule!

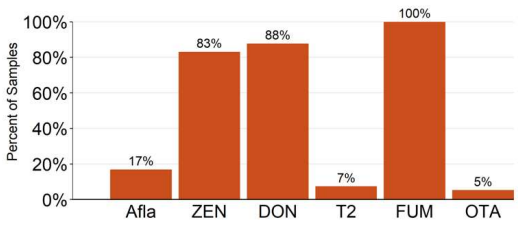


- *F. graminearum*
- *F. culmorum*
- *F. cerealis*
- *F. equiseti*
- *F. verticillioides*
- *F. incarnatum*

ZEN + DON, FUM, NIV, T-2, …

ASIA CORN Jan-Jun 2020

Prevalence of Mycotoxins Detected



A real life case from Dr. Marios Christoforou

Dr. Marios is a veterinary in Cyprus

Symptoms on more than ¼ of a 170 milking cows herd:

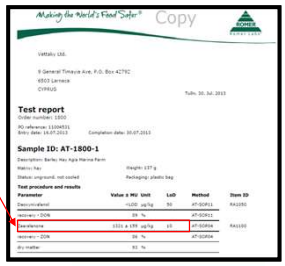
- Diarrhea
- Nymphomania (even when pregnant)
- Cows not responsive to hormonal treatment
- Vulva inflammation

With the pregnancy check, 30d after insemination:
- Abortion
- Difficulties to get in heat again
- Cystic ovaries (over 20 mm ⌀, observed with ultrasound)

Solution:

1. Sample the feed
2. Dilute the straw in the diet
3. Supplement Mycofix® Plus 3.E

Reproduction went back to normal after 2 weeks



Barley hay with
1380 ppb ZEN
Dose: 5 kg DM/d

In practice: What can ZEN cause in cows?

Clinical effects : Morphological changes in reproductive organs

- Vaginal secretions

- Genitalia hypertrophya

- Ovary cysts

In practice: What can ZEN cause in cows?

- Premature udder enlargement in heifers
  (associated with future milking problems)
- False estrus→ more difficult heat detection
- Unsuccessful insemination
- Abortions

Unpublished, NC State.

Zearalenone degradation in the rumen

- Ruminal microbiota degrades zearalenone:

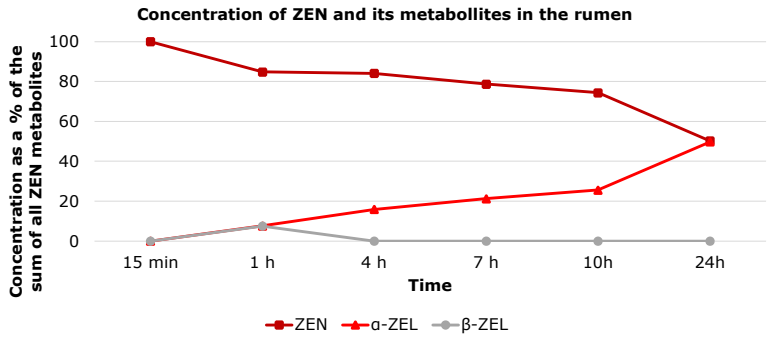
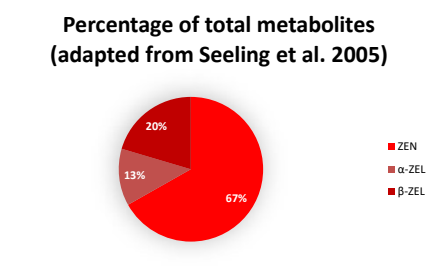


Figure. In vivo degradation of Zearalenone in rumen cannulated dairy cows (Gruber-Dorninger et al., submitted manuscript)

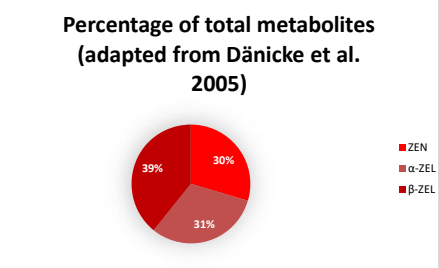
- α-ZEL is **60x** as estrogenic as ZEN
- β-ZEL is **0.2x** as estrogenic as ZEN

Zearalenone degradation in the rumen

- In vivo measurements at duodenum canula (Seeling et al. 2005):

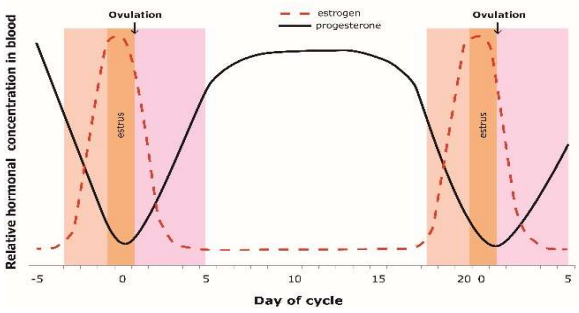


- In vivo measurements in digesta (Dänicke et al. 2005):



What are some effects of estrogens in the reproduction?

- Development and functioning of sex organs
- Prepares and determines the onset of estrus
  - Influences behaviour
  - Maintains thickness of vaginal wall and promotes lubrication
  - Makes the uterus more sensitive to stimulation and aids to transport of semen



Adapted from Reith et al. (2018)

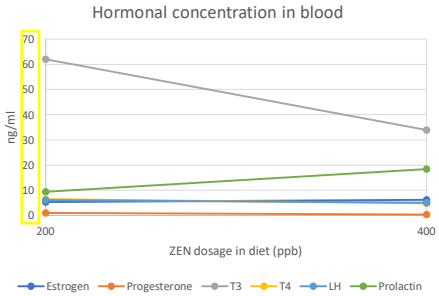
Hormonal imbalances as a result of ZEN

Assiut Vet. Med. J. Vol. 59 No. 138 July 2013

EFFECT OF MYCOTOXIN ON REPRODUCTIVE PERFORMANCE IN DAIRY CATTLE

MONA A. MAHMOUD*; GHATTAS T.A.*; AMAL Z.A. LEIL** and ABED ELAZIZ M.Z***
*Biology of Reproduction Dept., Giza.
**Field Investigation Dept., Giza.
***Pathology Dept. Animal Reproduction Research Institute, Giza.

	Treatment A	Treatment B
ZEN (ppb)	200	400
Afla (ppb)	5	20

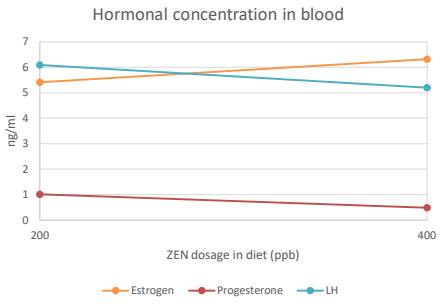


- Significant ($p<0.05$) changes in blood hormone concentrations

(Mahmoud et al., 2013)

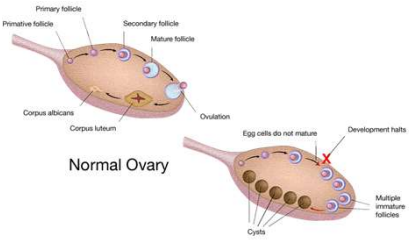
Hormonal unbalance as a result of ZEN

At a smaller scale:



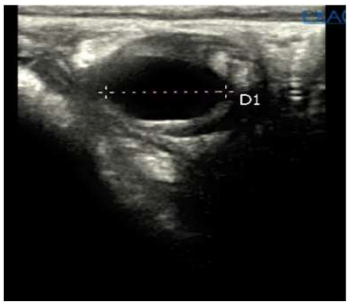
(Mahmoud et al., 2013)

- Progesterone (a.k.a. the pregnancy hormone) ensures that conditions for the embryo are kept if fertilization is achieved – a decrease in progesterone would increase risk of abortions
- Higher estrogen is produced by stimulation of ZEN – more follicle development
- LH stimulates the release of the oocyte – low LH would be a limitation for adequate ovulation and can lead to polycystic ovaries

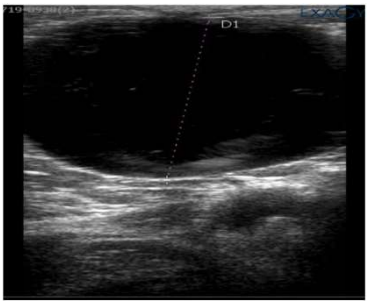


ZEN induced follicular cysts

200 ppb ZEN
Mean follicular diameter: 22.1±2 mm



400 ppb ZEN
Mean follicular diameter: 42.3±3 mm



(Mahmoud et al., 2013)

Economical impact:

- Extension of open period
- Cost of treatment
- Risk of elimination of cows from herd

ZEN as a cause for ovarian cysts

- **Follicullar cysts:** anovulatory folicular structures that are at least 2.5 cmø and persist for more tan 10d in the absence of a corpus luteum

Detection of zearalenone and its metabolites in naturally contaminated follicular fluids by using LC/MS/MS and in vitro effects of zearalenone on oocyte maturation in cattle

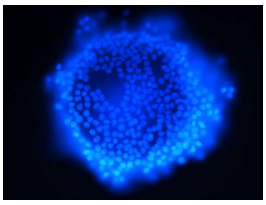
Mitsuhiro Takagi[a,*], Shuhei Mukai[a], Toshiyuki Kuriyagawa[a], Katsuhito Takagaki[a], Seiichi Uno[b], Emiko Kokushi[c], Takeshige Otoi[d], Agung Budiyanto[d], Koumei Shirasuna[e], Akio Miyamoto[e], Osamu Kawamura[f], Koji Okamoto[g], Eisaburo Deguchi[a]

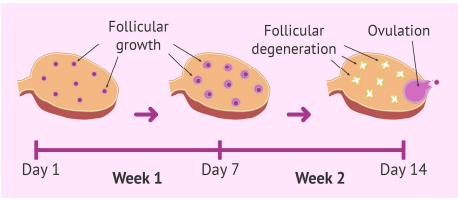
Condition of follicles	Number of samples	ZEN detected (%)
Cystic follicles	20	35
Normal follicles	32	19

Anti-Müllerian Hormone (AMH) – a biomarker for fertility in cows

- Produced by the granullosa cells of growing ovarian follicles
- Function: to control the number of follicles in the ovary and determine the dominant one during the folicular waves
- Without AMH, the recruitment of follicles is faster but ovarian folicular reserves exhaust faster without it
- It's an indicator of antral follicle population which relates to the fertility of the cow

Pig oocyte surrounded by granullosa cells (Wikipedia)





ZEN and Mycofix® Plus in AMH

Application of mycotoxin adsorbent to cattle feed contaminated with zearalenone: urinary zearalenone excretion and association with anti-Müllerian hormone

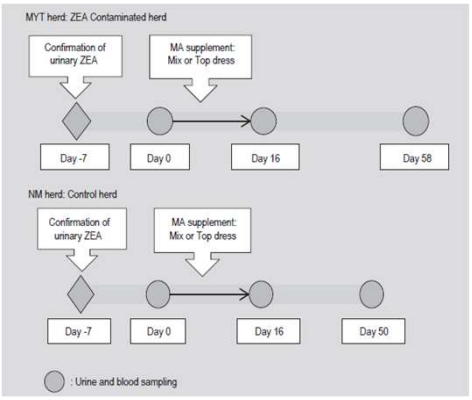
Y. Fushimi[1,2], M. Takagi[1*], H. Hasunuma[1,2], S. Uno[3], E. Kokushi[3], U. Watanabe[4], J. Liu[5], M.A. Marey[5], A. Miyamoto[5], T. Otoi[6], E. Deguchi[1] and J. Fink-Gremmels[7]

KAGOSHIMA UNIVERSITY

Experimental Design

		Mycofix Plus 3.0 dosing	
		50g	0g
ZEN contamination in complete diet	1.25 ppm		
	0.025 ppm		

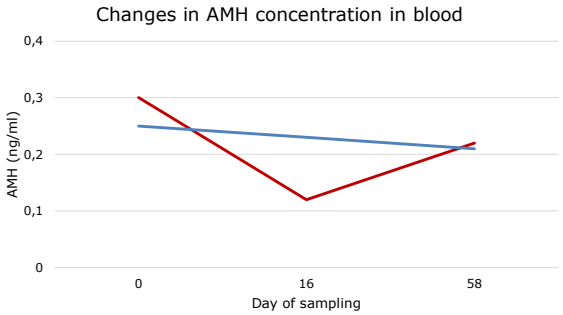
- Japanese Black beef cow



Effect of ZEN and Mycofix Plus 3.E in AMH concentrations

- The cows were fed the treatments from d0 until d16
- Unknown dosage of straw

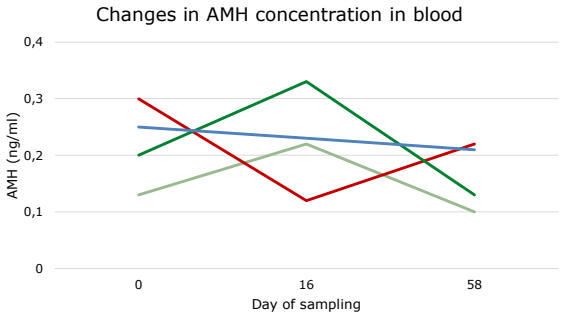
		Mycofix Plus 3.0 dosing	
		50g	0g
ZEN contamination in complete diet	1.25 ppm		
	0.025 ppm		



Effect of ZEN and Mycofix Plus 3.E in AMH concentrations

- Differences between farms were significant ($p<0.05$)
- ZEN concentration in the diet reduced AMH concentration which indicates a lower population of antral follicles

		Mycofix Plus 3.0 dosing	
		50g	0g
ZEN contamination in complete diet	1.25 ppm		
	0.025 ppm		

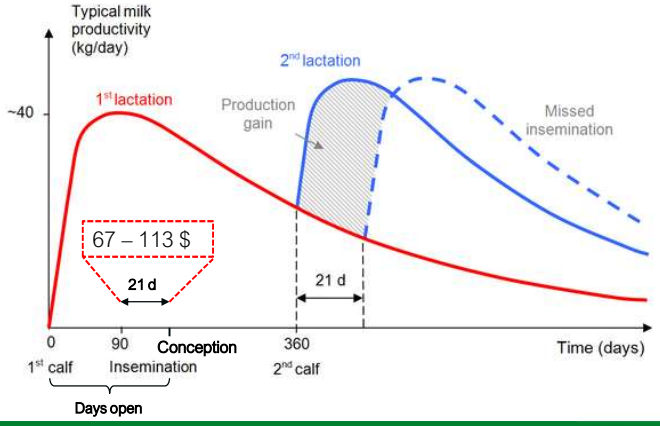


The cost of fertility problems

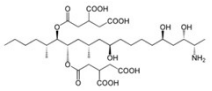
Days open: n° of days between calving and conception

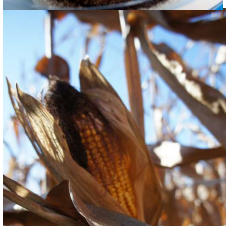
Cost per extra day open (per cow per vear) = 3.20 – 5.40 $ *De Vries A., 2006*

- **Herd average milk production**
- **Replacement price**
- Feed price
- Semen cost
- **Milk price**
- Cull cow price
- Veterinarian cost



Fumonisins





Produced by: *Fusarium verticilloides*
Fusarium proliferatum
Aspergillus niger

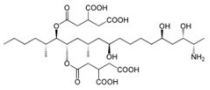
Hot or moderate climates

Contamination: maize, maize by-products, wheat
(From the field)

FB_1, FB_2, FB_3, FB_4, FB_5, FB_6
FA_1, FA_2

Originates maily from field contaminations

Fumonisins - Impact on ruminants

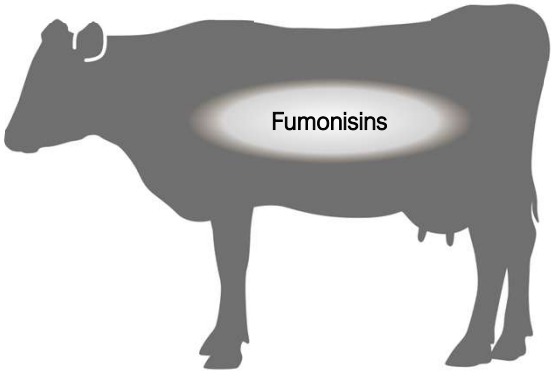


Acute symptoms

- Liver lesions
- Increased concentration of liver enzymes

Intestinal health

- Reduced tight junction strength
- Reduced mucin production

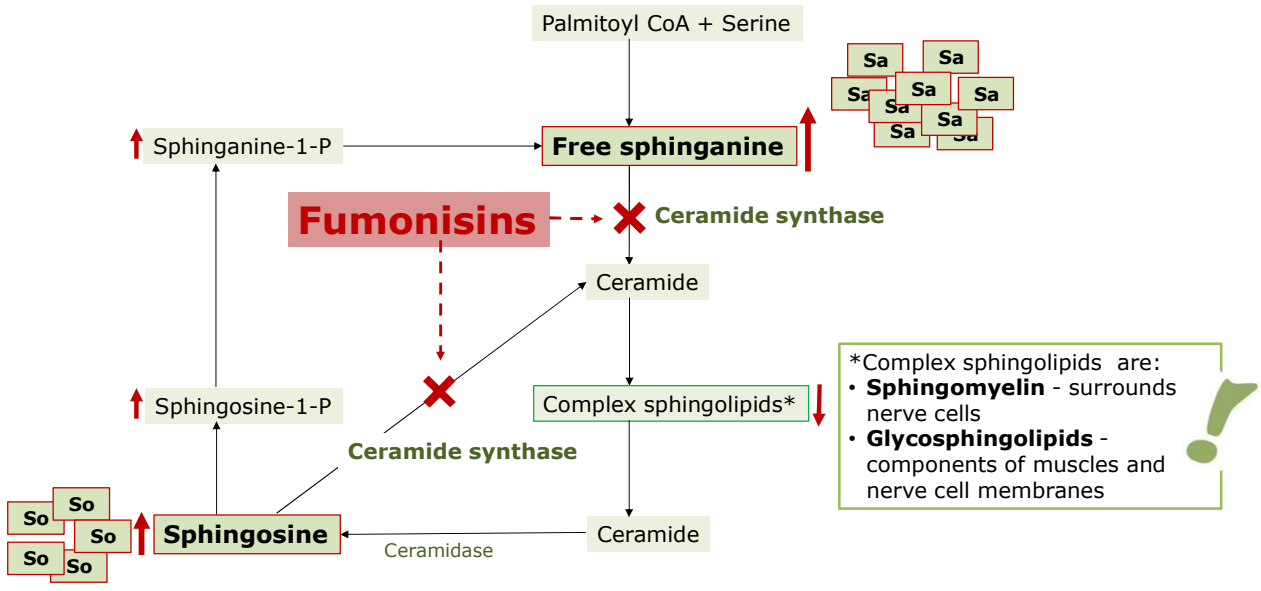


Decreased DMI

Chronic symptoms

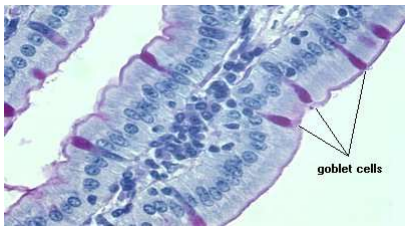
- Immune modulation

Fumonisins - Mode of action



Fumonisins

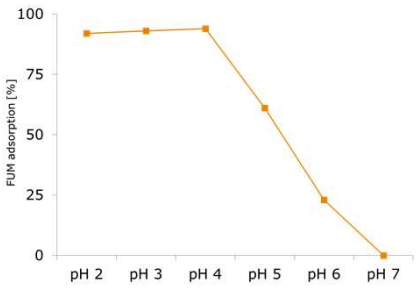
- Very low rumen degradation
- They share a similar structure with Sphingosine, a component of Sphingolipids, present in cell membranes.
- Fumonisins toxicity results from blockage of sphingolipid biosynthesis and degradation of tissues rich on sphingolipids, like:

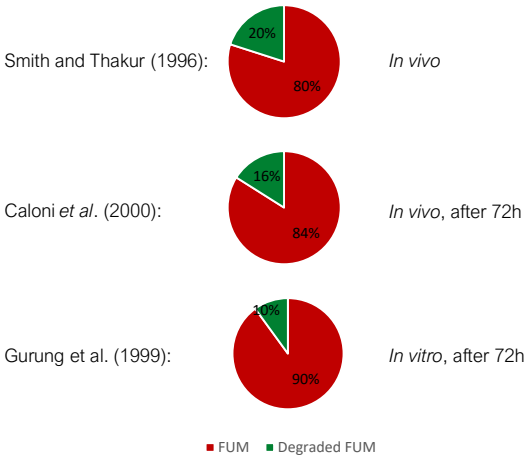


Goblet cells:
They produce mucus which act as a protection to epithelium. Its absence or inaction may lead to:
-Decreased mucin layer thickness
-Lower tight junction strength
-Potential invasion of pathogens or mycotoxins

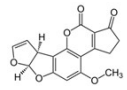
Ruminal degradation and binding of fumonisins

- Fumonisins are poorly degraded in the rumen
- Fumonisin-adsorption decreases in the intestinal tract (pH dependent)!





Aflatoxins



Produced by: ***A**spergillus **fla**vus*
Aspergillus parasiticus

Afla B_1, Afla B_2
Afla G_1, Afla G_2

Most toxic mycotoxin

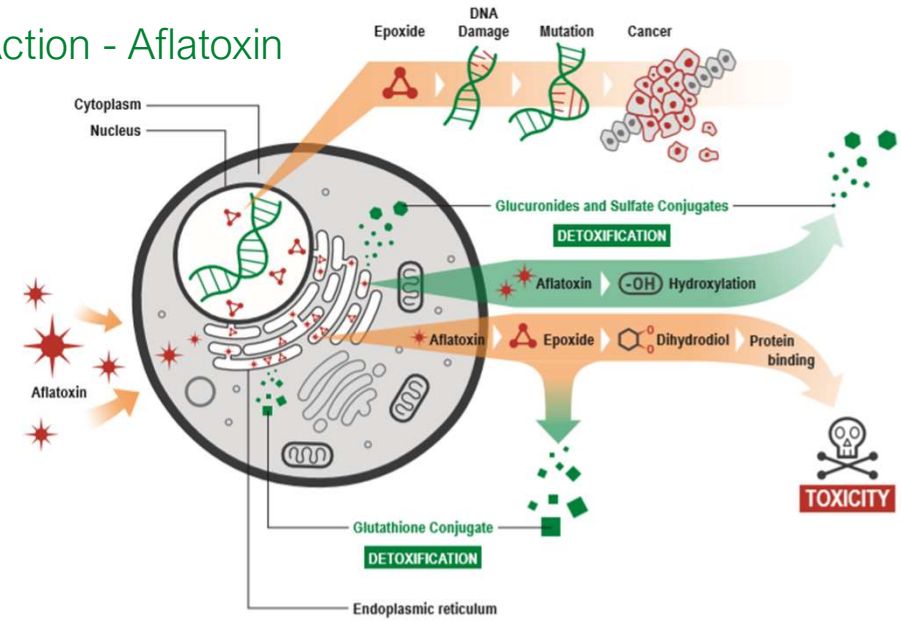
Contamination: maize, barley, sorghum, soy, milk, eggs

Prevention: storage at <12% humidity

Hot climates
Humidity during storage

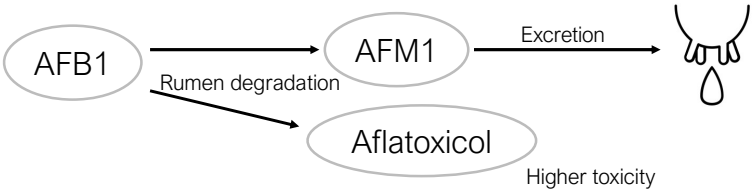
Mode of action: enters nucleus → reacts with DNA adducts → leads to mutations

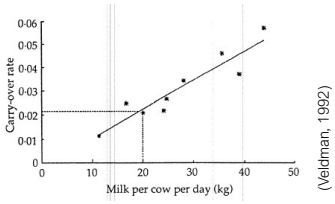
Mode of Action - Aflatoxin



Aflatoxins (AF)

- Produced by *Aspergillus* spp.
- Among most cancerigenous natural substances
- Mainly known for regulations in milk





(Veldman, 1992)

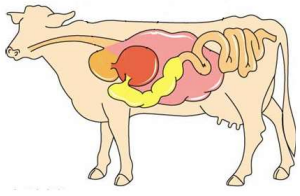
Carry over rate: 1-6%

Aflatoxins

Hepatotoxicity

Decrease liver enzyme activity, fatty liver

Carcinogenicity



Immune supression

Residues: liver and milk (Afla M_1)

Aflatoxins and legislation

- Specific regulations depending on the country or region

	EU - EFSA	US - FDA	Mycotoxin
Complementary and complete feed	10 µg/kg		Aflatoxin B1
Complete feed for dairy	5 µg/kg	20 µg/kg	Aflatoxin B1
Milk	0.5 µg/kg	0.5 µg/kg	Aflatoxin M1

Aflatoxins effect in rumen ammonia (N-efficiency)

	Control	50% replacement	100% replacement
Non-contaminated cotton seed	2kg	1kg	0kg
Contaminated cotton seed	0kg	1kg	2kg
AFB1 in whole diet	0	20ppb	40ppb
ZEA in whole diet	0	85ppb	170ppb

- The contaminated cottonseed may have reduced NH3-N utilization by microbes
- Nitrogen metabolism was affected probably due to effects of mycotoxins on the synthesis and degradation of AA (the involved mechanism may influence glucogenic nutrients and AA utilization.

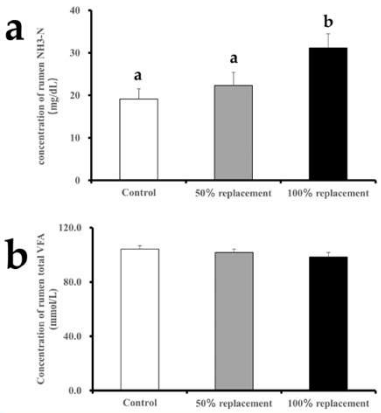
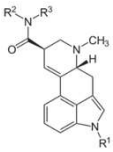


Figure 1 **Effects of cottonseed contaminated with mycotoxins on the concentration of NH_3-N (A) and total VFA (Volatile Fatty Acid) (B) in the cows rumen.** Notes: Control group = uncontaminated cottonseed; 50% replacement group = 50% of the uncontaminated cottonseed was replaced with cottonseed contaminated with mycotoxins; and 100% replacement group = 100% of the uncontaminated cottonseed was replaced with cottonseed contaminated with mycotoxins.

Full-size DOI: 10.7717/peerj.8742/fig-1

(Wang et al., 2020)

Ergot alkaloids



Produced by: *Claviceps purpurea*
C. paspali
C. fusiformis
C. africana

Contamination: rye, grasses, sorghum, wheat

Main metabolites: clavines
lysergic acids
lysergic acid amides
ergopeptines

Sclerotia

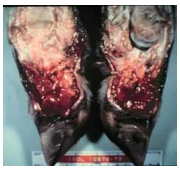
Ergot Alkaloids

- Effects on nervous system:
  - -Trembling
  - -Salivating
  - -Staggers
- Vasoconstriction:
  - -Decreased ability to thermo-regulate
  - -Laminitis

Ryegrass staggers cause by Lolitrem B

(Naudé et al, 2005)

Cows with thermoregulation problems at 22°C



Gangrene (Fescue foot)

Masked mycotoxins - An emerging issue for food safety?

Defense mechanism of plants:

Mycotoxins can be bound to certain molecules:
e.g. glucosides

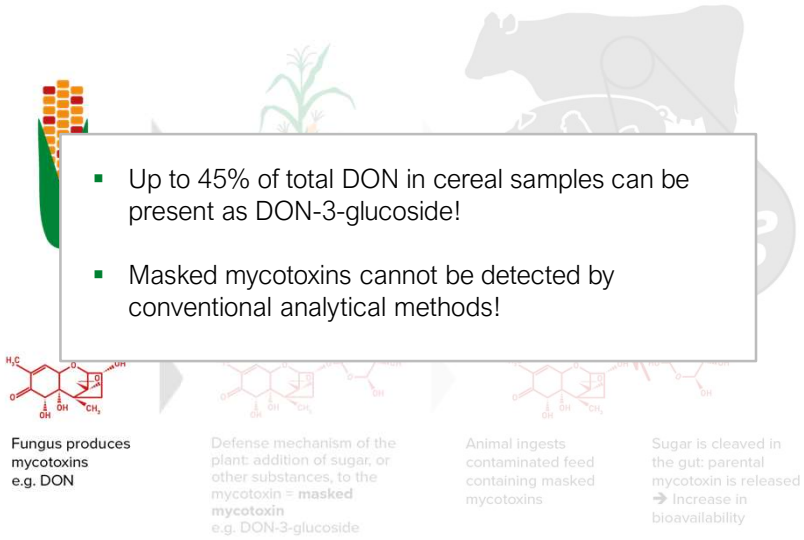
-> masked mycotoxins

In the gastrointestinal tract of animals:

mycotoxin-molecule bond might be cleaved

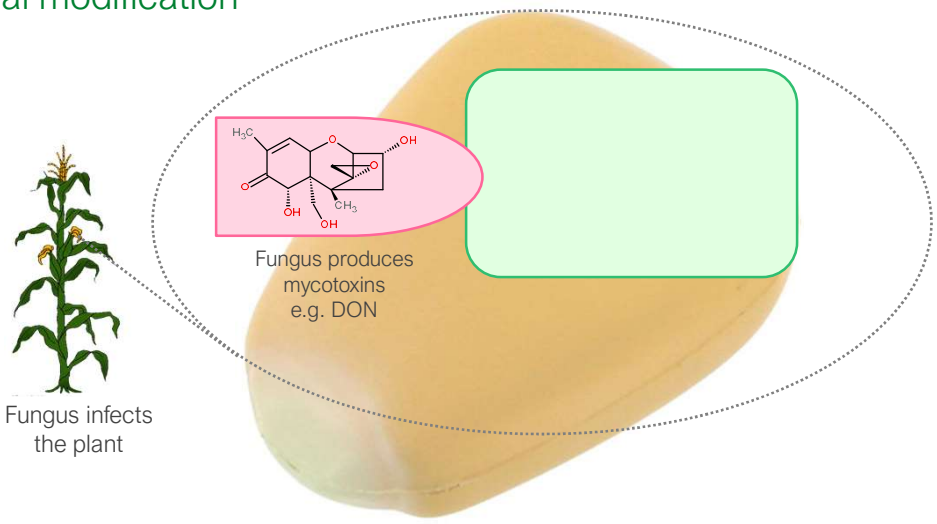
-> mycotoxin released

-> toxic effects

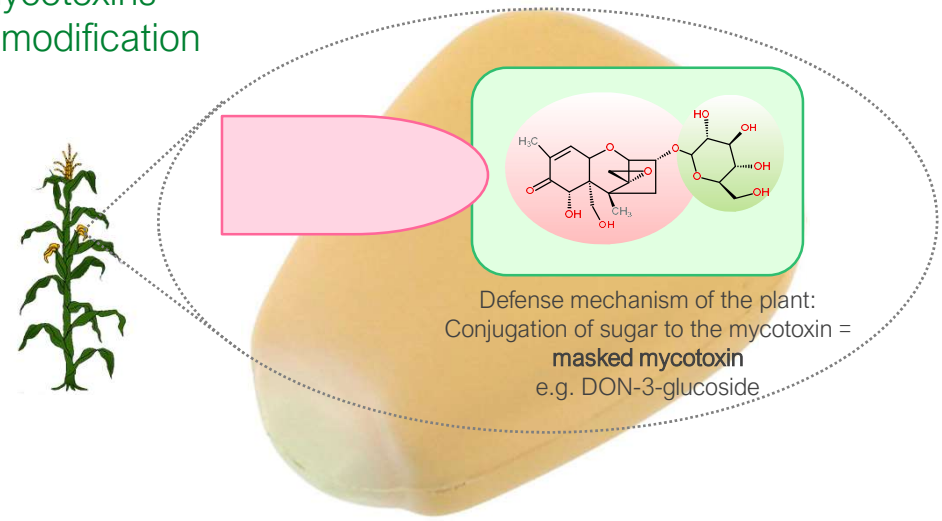


- Up to 45% of total DON in cereal samples can be present as DON-3-glucoside!
- Masked mycotoxins cannot be detected by conventional analytical methods!

Masked mycotoxins
Biological modification



Masked mycotoxins
Biological modification

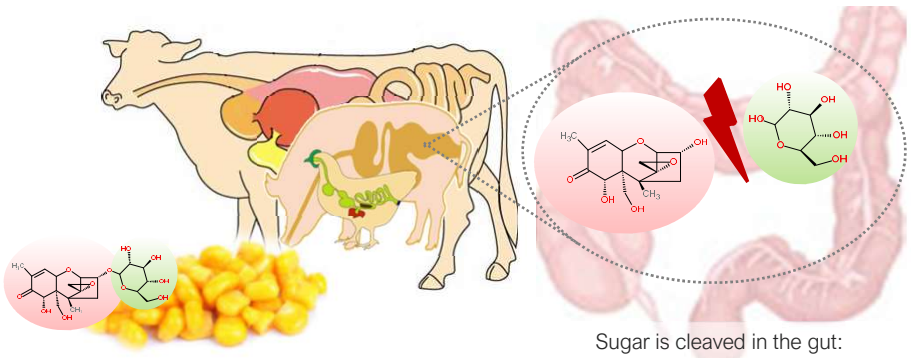


Defense mechanism of the plant:
Conjugation of sugar to the mycotoxin =
masked mycotoxin
e.g. DON-3-glucoside

Masked mycotoxins cannot be detected by conventional analytical methods!

Masked mycotoxins - Release in the gut

Up to 45 % of total DON in cereal samples can be present as DON-3-glucoside!



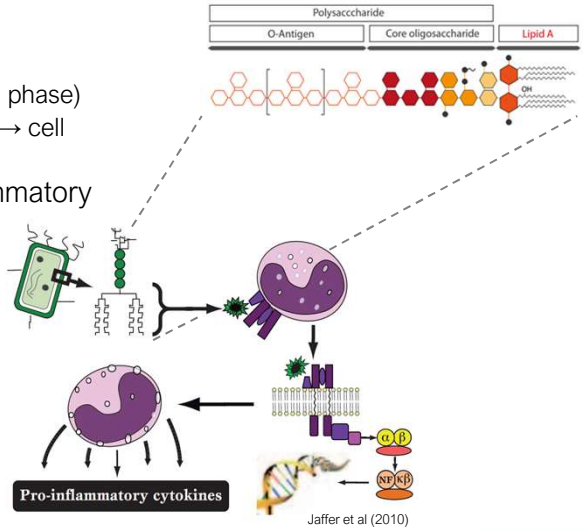
Animal ingests **contaminated feed**
containing masked mycotoxins

Sugar is cleaved in the gut:
parental **mycotoxin** is released
→ Increase in bioavailability

Masked mycotoxins cannot be detected by conventional analytical methods!

Endotoxins – Lipopolysaccharides (LPS)

- Gram negative cell wall components
- LPS as a result of:
  - Autolysis (during rapid growth bacteria phase)
  - Low rumen pH induce lysis of bacteria→ cell membrane LPS shear-off
- LPS induce a mass-release of proinflammatory cytokines (energy demanding)



Jaffer et al (2010)

How Endotoxins Affect the Liver?

Healthy animal

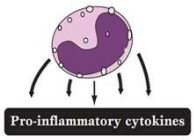
Performance

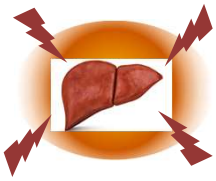
Immune system

How Endotoxins Affect the Liver?

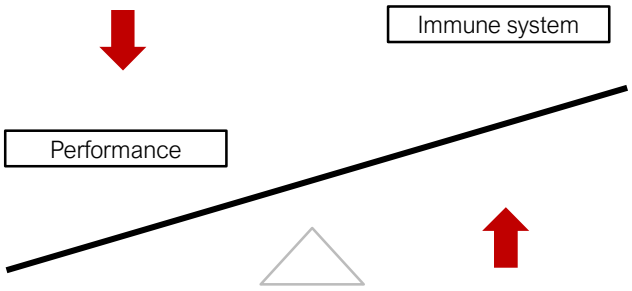
Mass release of:

+ ENDOTOXINS





- High energy expense
- Liver inflammation
- Hepatocytes degeneration
- Immune cells depletion

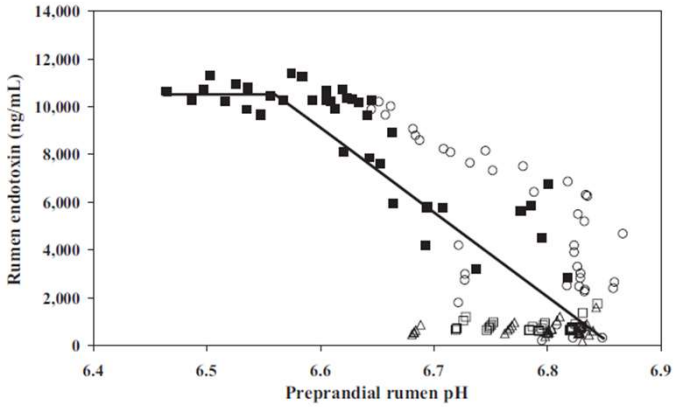


Endotoxins' Influence in Periparturient Diseases

- Mastitis
- Laminitis
- Retained placenta
- Metritis, endometritis, infertility
- Fatty liver
- Displaced abomasum
- Milk fever

(Eckel and Ametaj, 2016)

Endotoxin Release and Grain Portion in the Diet



Rumen pH of lactating primiparous Holstein cows fed concentrate proportions:

Δ= 0%

□=15%

○=30%

■=45%

(Emmanuel et al., 2008)

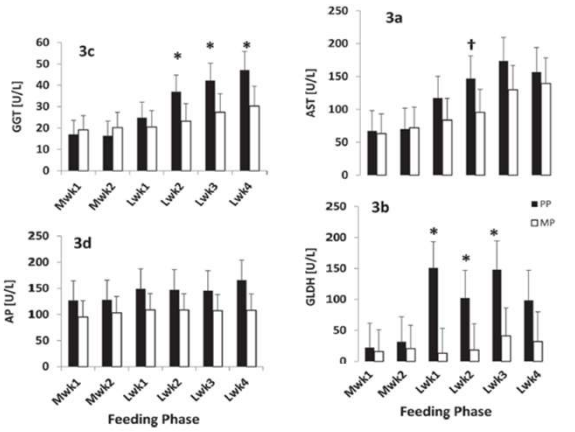
Effects of High Concentrate Feeding on Liver Health

J. Dairy Sci. 103
https://doi.org/10.3168/jds.2019-17760

Distinct responses in feed sorting, chewing behavior, and ruminal acidosis risk between primiparous and multiparous Simmental cows fed diets differing in forage and starch levels

A. Stauder,[1] E. Humer,[1] V. Neubauer,[1,2,3] N. Reisinger,[4] A. Kaltenegger,[1] and Q. Zebeli[1]*

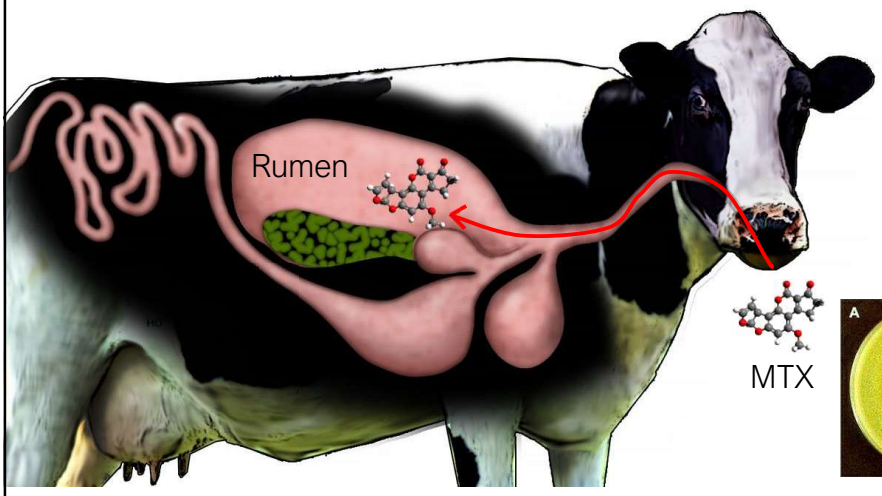
- 24 cows:
  - 8 primiparous: DMI 19.1±1.1kg
  - 16 multiparous: DMI 22.5±1.1kg
- 50±22 DIM

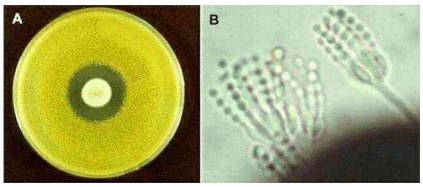


Mwk1	Mwk2	Lwk1	Lwk2	Lwk3	Lwk4
60% forage 40% concentrate		40% forage 60% concentrate			

Mycotoxins degradation by ruminal microbiota

The Mycotoxin Journey: the rumen





Lower in vitro rumen metabolization of mycotoxins at conditions of rumen acidosis and dry conditions

Sandra Debevere (UGent) , Siegrid De Baere (UGent) , Geert Haesaert (UGent) , Siska Croubels (UGent) and Veerle Fievez (UGent)

(2019) Book of abstracts of the 70th annual meeting of the European Federation of Animal Science. In Book of Abstracts 25. p.555-555

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Introduction

Dry cows:

- can't have too much grass silage to avoid milk fever risks, therefore MS is preferable (lower Ca and K)

High yielding cows:

- Need higher portions of MS in their ration
- Have higher passage rates
- Have higher incidence of metabolic disorders like SARA

This set of factors increase the risk of mycotoxins bypassing to the intestine

Materials and Methods

Contamination with mycotoxins:

12 ppm **Deoxynivalenol**
3 ppm **Zearalenone**

According to European guidance level for maize silage

1 ppm **Enniatin B**
6 ppm **Mycophenolic acid**
2 ppm **Roquefortine-C**
60 ppm **Nivalenol**

According to concentrations found in maize silage from Belgium and Netherlands

Incubators were fed maize silage

Buffers were used to simulate:
- Normal pH (6.8)
- SARA conditions, low pH (5.8)

Samples taken at hours of incubation:
- 1.5
- 3
- 6
- 24
- 48

Important note:
- "Normal pH" is not really representative of a "nowadays" cow, but represents an ideal scenario
- Incubations are done until 48h for research purposes but this does not relate to the retention time of a dairy cow.

Rumen retention time examples

Table 7. Passage kinetics of Yb-labeled corn silage and grass silage fiber administered to cows fed the respective forage; parameters are based on the G4G1 model (a 2-pool model consisting of an age-dependent and an age-independent pool), with treatments including corn silage and grass silage at forage:concentrate ratios of 50:50 and 75:25

Roughage : concentrate

Item[1]	Corn silage 50:50	Corn silage 75:25	Grass silage 50:50	Grass silage 75:25	SEM (n = 16)	*P*-value[2] F	R	F × R
C_2 (mg/kg)	18.3	19.9	58.7	64.3	16.1	0.001	0.70	0.80
λ (h^{-1})	0.599	0.527	0.470	0.345	0.097	0.033	0.14	0.72
k (h^{-1})	0.047	0.045	0.053	0.052	0.006	0.26	0.80	0.96
τ (h)	5.92	7.03	4.08	5.04	0.69	0.003	0.053	0.97
CMRT1 (h)	7.12	7.74	8.78	11.8	1.01	0.017	0.092	0.25
CMRT2 (h)	21.8	23.8	19.2	19.7	3.9	0.26	0.66	0.80
CMRT (h)	28.9	31.5	28.0	31.5	3.9	0.89	0.35	0.88
TMRT (h)	34.8	38.6	32.1	36.6	3.9	0.44	0.19	0.88
CMRT1:CMRT	0.247	0.255	0.312	0.375	0.035	0.013	0.26	0.40
$TMRT_{algeb}$ (h)	33.8	35.0	31.1	34.5	1.4	0.25	0.16	0.42

[1] C_2 = initial marker concentration in the second, age-independent pool; λ = fractional rate of passage from the age-dependent pool in a 2-pool model; k = fractional rate of passage from the age-independent pool in a 2-pool model; τ = time delay, time elapsed before first appearance of marker at the sampling site; CMRT1 = mean retention time in the age-dependent pool; CMRT2 = mean retention time in the age-independent pool; CMRT = mean retention time of marker in both pools, CMRT = CMRT1 + CMRT2; TMRT = total mean retention time, TMRT = CMRT + τ; and $TMRT_{algeb}$ = total mean retention time calculated algebraically (Thielemans et al., 1978).

[2] F = forage type, R = forage:concentrate ratio, F × R = interaction between F and R.

Smaller particle size (corn kernels)

Total mean retention time with two different ways of calculating it

(Kramer et al, 2013)

Results and Discussion

Deoxynivalenol can reach the small intestine!

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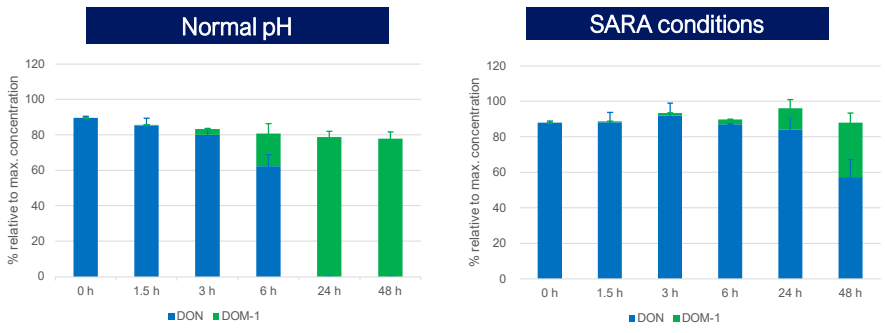

- DON is degraded and converted into DOM-1, its non toxic metabollite

Nivalenol can reach the small intestine!

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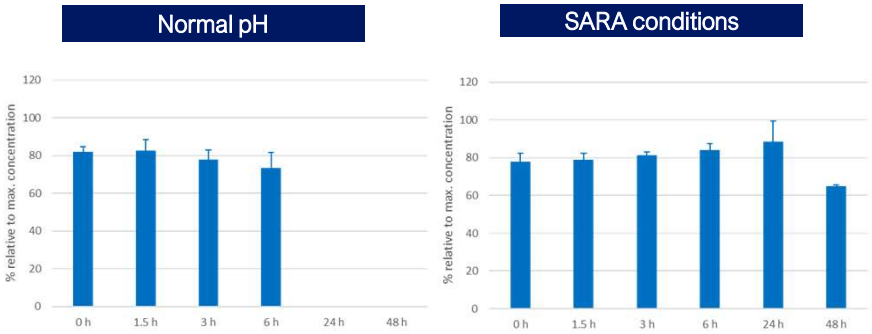

- NIV at low pH undergoes a very low degradation whilst at normal pH disappears after 24h.

Enniatin B can reach the small intestine!

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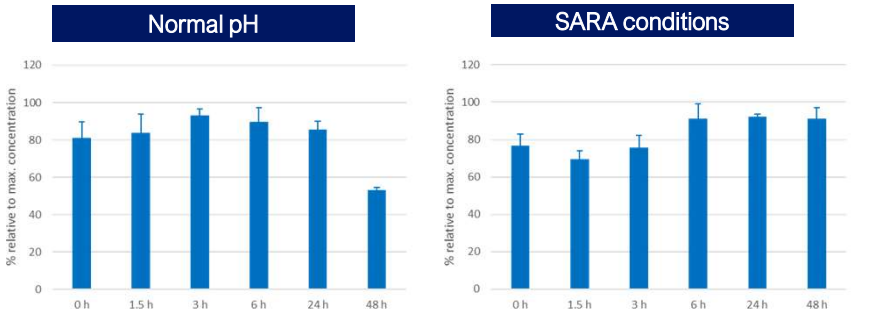
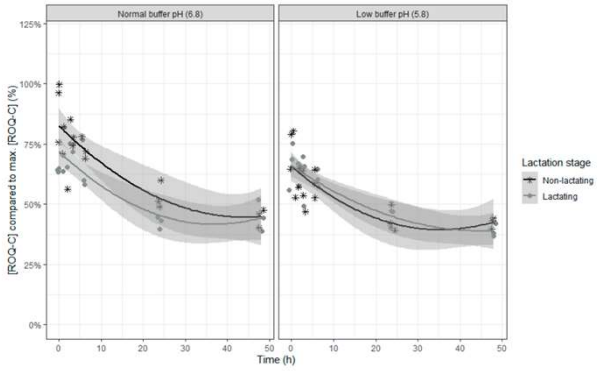


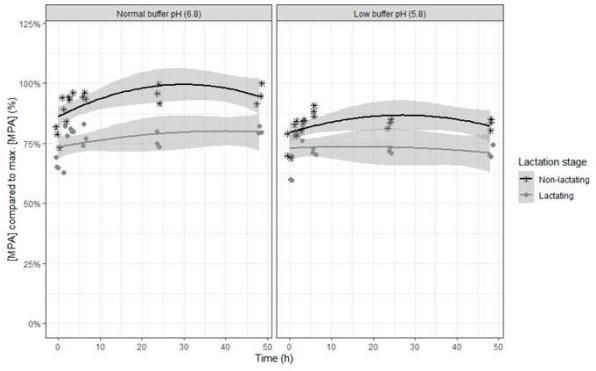
- A slight increase after beginning of incubation explained by a release of some ENN B adsorbed by feed particles or microbes at the beggining

Roquefortine C can reach the small intestine!



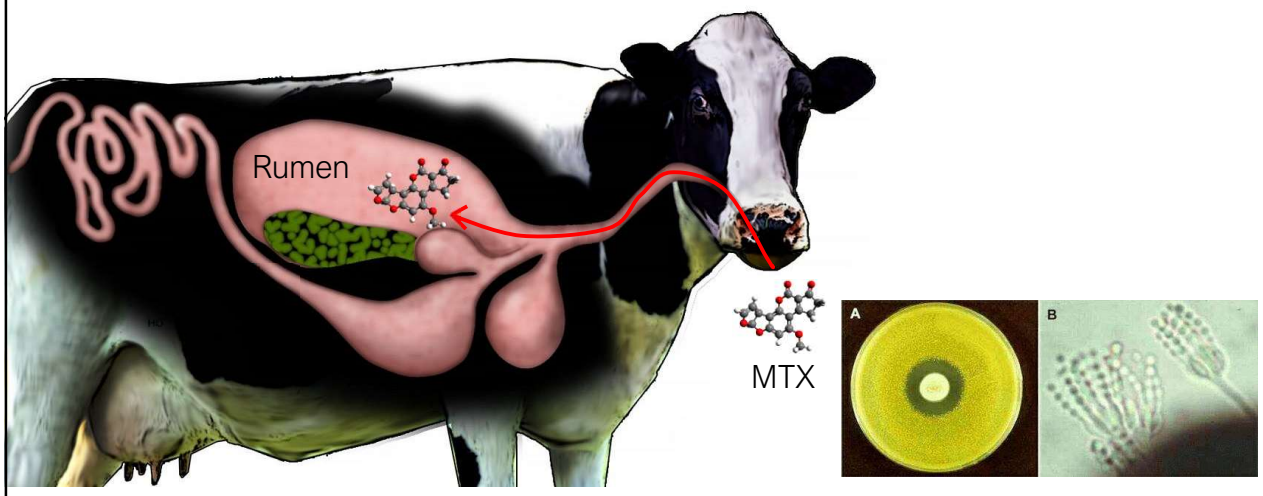
- Very limited degradation at either pH
- Slight effect by microbial activity

Mycophenolic acid would reach the intestine



- Overall, no relevant degradation of MPA through the incubation

The Mycotoxin Journey: the rumen



Mycotoxins effects in the Ruminal Environment

Mycotoxins effects (*in vitro*)

Table 4. Survey on the effects of mycotoxins on rumen microbiota tested by *in vitro* approaches from literatures.

Mycotoxins [a]	Media	Tested Dosages	Effects	References
AFB_1	rumen fluid	0, 300, 600, 900 ng AFB_1/mL buffered rumen fluid	↓ gas production, ↓ dry matter digestibility, ↓ NH_3-N concentrations	[141]
AFB_1	rumen fluid	1, 10 µg AFB_1/mL buffered rumen fluid	↓ dry matter digestibility	[142]
AFB_1	rumen fluid	9.5 ng AFB_1/mL buffered rumen fluid	no effects	[143]
AFB_1	rumen fluid	0, 320, 640, 960 ng AFB_1/mL buffered rumen fluid	↓ final gas production, ↓ rate of degradation, ↓ NH_3-N concentrations, ↑ isobutyrate, valerate and isovalerate molar proportions	[144]
DON	rumen fluid	0.36/0.46 or 5.76/6.90 mg of DON/kg diet	None, expect ↓ NDF digestibility	[145]
DON	rumen fluid	0.3 or 3.4/4.4 mg of DON/kg diet	None, expect ↓ NDF digestibility	[146]
DON	rumen fluid	40 µg DON/mL of rumen fluid	↓ gas production, ↓ VFA and NH_3-N concentrations	[147]
DON and fusaric acid	culture media		antimicrobial activity of fusaric acid against *Ruminococcus albus* and *Methanobrevibacter ruminantium*. No effect of DON	[148]
Gliotoxin	rumen fluid	0, 1, 2, 5, 10, 20, 40, 80 µg/mL buffered rumen fluid	< 80 µg/mL no effects. At 80 µg/mL ↓ DM degradation, gas and VFA productions	[149]
FB_1	rumen fluid	0, 50 or 100 mg/kg rumen fluid	none	[150]
OTA	rumen fluid	200 µg of OTA/l of rumen fluid	none	[151]
Patulin	rumen fluid	20, 100 and 300 µg of Patulin/mL rumen liquid	↓ Acetic acid production within 4 h and Inhibition of protein synthesis	[152]
Patulin	rumen fluid	0, 10, 20 and 40 mg of Patulin/mL rumen fluid	↓ dDM, VFA production, dNDF, dADF, dCHO, dCP and bacterial N flows ↑ NH3-N	[153]
Mycopenolic acid, Roquefortine C and PR toxin	rumen fluid	0.01, 0.30, 1.01, 1.71 and 2.00 µg of each mycotoxin/mL buffered rumen fluid	Mychopenolic acid and roquefortine C ↓ gas production, VFA production. No effect of PR toxin	[130]
Citrinin, Monacolin K, Pravastatin and Mevastatin	rumen fluid	5 or 20 µg of monacolin/mL rumen fluid; 5 or 20 µg of citrinin/mL rumen fluid; *Monascus* spp. contaminated rice	none, ↓Methane production	[154]

[a]: aflatoxin B_1, AFB_1; ammonia nitrogen, NH_3-N; dADF, digestible ADF; dCHO; digestible carbohydrates; dDM, digestible dry matter; deoxynivalenol, DON; DM, dry matter; dNDF, digestible NDF; fumonisin B_1, FB_1; ochratoxin A, OTA; VFA, Volatile fatty acids.

Lower DM digestibility

Lower VFA production

Lower NDF digestibility

Lower MCP production

Less nutrients available for the cow

CSIRO PUBLISHING

Animal Production Science, 2012, **52**, 832–841
http://dx.doi.org/10.1071/AN11205

Effect of mycotoxin deactivator product supplementation on dairy cows

K. Kiyothong[A,E], *P. Rowlinson*[B], *M. Wanapat*[C] *and S. Khampa*[D]

Mycotoxin	Concentration [ppb]
AFB1	38
ZON	541
DON	720
FB1	701
T-2[1]	270
OTA	74

Ruminal fluid collection

[1] Thin-Layer Chromatography. All the others performed with HPLC.

Treatment groups

	Negative Control	Trial 1	Trial 2	Trial 3
Basal diet	X	X	X	X
Mycofix Plus [g/cow/day]	-	15	30	45

Blood collection jugular venipuncture

Rumen ecology, BUN and MUN

	Negative Control	15g/cow/day	30g/cow/day	45g/cow/day
Temperature [ºC]	40.1	39.0	38.9	39.0
Ruminal pH	6.1a	6.6b	6.6b	6.7b
NH3 – N [mg/dl]	12.4a	16.9b	17.6c	17.8c
BUN [mg/dl]	7.7a	9.4b	9.6b	9.8b
MUN [mg/dl]	17.8	17.4	18.2	18.0

[a,b,c] Values on the same row with different superscripts differ (*P<0.05).*

VFA Production

	Negative Control	15g/cow/day	30g/cow/day	45g/cow/day
Total VFA [mM]	110.2^{a}	119.2^{b}	119.6^{cb}	119.2^{b}
Acetate [mol/100mol]	71.6^{a}	68.0^{b}	66.8^{b}	66.3^{b}
Propionate	20.0^{a}	23.4^{b}	23.7^{b}	25.4^{c}
Butyrate	9.3	9.4	10.7	9.8
Acetate:propionate	3.6^{a}	3.0^{b}	2.9^{b}	2.7^{b}

Ruminal population

	Negative Control	15g/cow/day	30g/cow/day	45g/cow/day
Rumen microbes-bacteria [x10^{12} cell/ml]	7.0^{a}	13.9^{b}	15.4^{b}	15.8^{b}
Rumen microbes-Protozoa [x10^{5} cell/ml]	4.0^{a}	2.1^{b}	2.0^{b}	1.8^{b}
Total viable bacteria [x10^{9} CFU/ml]	4.9^{a}	7.0^{b}	8.7^{c}	9.4^{d}
Amylolytic [x10^{6} CFU/ml]	2.3^{a}	4.5^{b}	5.3^{b}	5.9^{b}
Proteolytic [x10^{6} CFU/ml]	1.5^{a}	2.1^{b}	2.7b^{c}	2.8^{c}
Cellulolytic [x10^{9} CFU/ml]	2.4^{a}	3.7^{a}	7.4^{b}	7.6^{b}

Digestion coefficients, feed intake and BW

	Negative Control	15g/cow/day	30g/cow/day	45g/cow/day
Crude protein digestibility [%]	70.0[a]	74.0[b]	74.4[c]	74.4[c]
Neutral-detergent fiber digestibility [%]	52.3[a]	57.4[b]	58.3[c]	58.2[c]
DM intake [kg/d]	10.2[a]	12.1[b]	13.4[c]	13.6[c]
LW Initial [kg]	430.2	420.8	426.1	425.8
LW Final [kg]	435.0	426.6	431.4	430.9
Liveweight change	0.11	0.10	0.08	0.10

[a,b,c] Values on the same row with different superscripts differ (*P<0.05).*

Milk production, composition and quality

	Negative Control	15g/cow/day	30g/cow/day	45g/cow/day
Milk yield [kg/cow/day]	12.6[a]	14.7[b]	14.7[b]	14.9[b]
Fat [g/kg]	34.1	37.2	37.2	36.4
Protein [g/kg]	31.0[a]	34.2[b]	34.3[b]	36.1[b]
AfM1 [ppb]	0.7	nd	nd	Nd
Somatic Cell Count [x10^3 cell/ml]	547[a]	385[b]	346[c]	346[c]

[a,b,c] Values on the same row with different superscripts differ (*P<0.05).*
nd – not detected (detection limit 0.06 ppb)

Hematology parameters

	Negative Control	15g/cow/day	30g/cow/day	45g/cow/day
Red blood cells [x10^6 cell/ml]	7.0[a]	10.0[b]	10.0[b]	9.0[c]
White blood cells [x10^3 cell/ml]	7.0[a]	12.1[b]	12.5[b]	12.4[b]
Lymphocytes [%]	54.1[a]	72.8[b]	73.4[b]	74.0[b]
IgA [g/l]	0.16[a]	0.32[b]	0.36[c]	0.37[c]
IgG [g/l]	1.01	1.02	0.99	0.98
IgM [g/l]	0.16	0.11	0.13	0.13

[a,b,c] Values on the same row with different superscripts differ (*P<0.05).*
nd – not detected (detection limit 0.06 ppb)

Conclusions

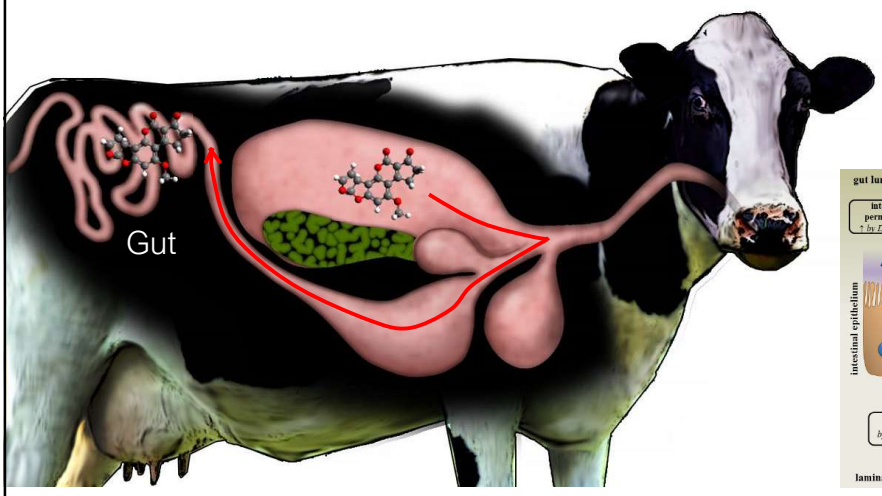
Mycofix had positive impacts on:

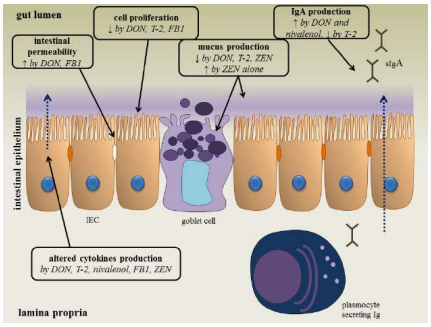
- Ruminal pH (maintenance of normal range, whereas control was below 6.2);
- Ruminal NH3-N (↑ NH3-N → ↑ microbial protein synthesis, ↑ feed intake);
- Ruminal microflora (↑ count viable bacteria, ↓ protozoa);
- Ruminal fermentation efficiency and microbial protein synthesis;
- Feed Intake (↑);
- Milk yield (2 kg ↑), milk protein and milk fat;
- Milk quality (↓ SCC);
- Immune function (↓ WBC, ↑ IgA);

and ...

Aflatoxin M_1 was found in non-supplemented cows but in NONE of the groups where Mycofix was supplemented!!!

The Mycotoxin Journey: the gut





Mycotoxins effects in the intestine

Once the rumen is passed, the intestine follows

toxins

MDPI

Article

Mycotoxin Occurrence in Maize Silage—A Neglected Risk for Bovine Gut Health?

Nicole Reisinger [1], Sonja Schürer-Waldheim [1], Elisabeth Mayer [1], Sandra Debevere [2,3], Gunther Antonissen [2,4], Michael Sulyok [5] and Veronika Nagl [1,*]

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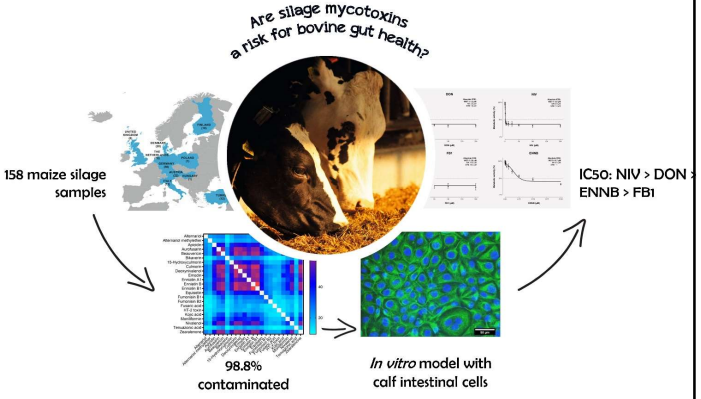
[2] Department of Pharmacology, Toxicology and Biochemistry, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium; sandra.debevere@UGent.be (S.D.); gunther.antonissen@UGent.be (G.A.)
[3] Department of Animal Sciences and Aquatic Ecology, Faculty of Bioscience Engineering, Ghent University, Coupure links 653, 9000 Ghent, Belgium
[4] Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium
[5] Institute for Bioanalytics and Agro-Metabolomics, University of Natural Resources and Life Sciences, Vienna (BOKU), Konrad Lorenz-Straße 20, 3430 Tulln, Austria; michael.sulyok@boku.ac.at
* Correspondence: veronika.nagl@biomin.net; Tel.: +43 2272 81166 0

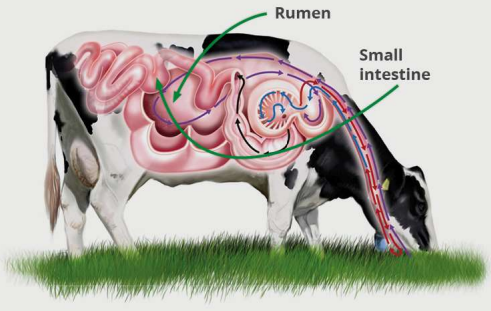


Mycotoxins effects in the intestine

- Gut barrier damage
- Reduced mucin production
- Altered Microbiota composition
- Weaken local immune system

LEADING TO

- Decreased digestibility and nutrient uptake
- Immunosupression



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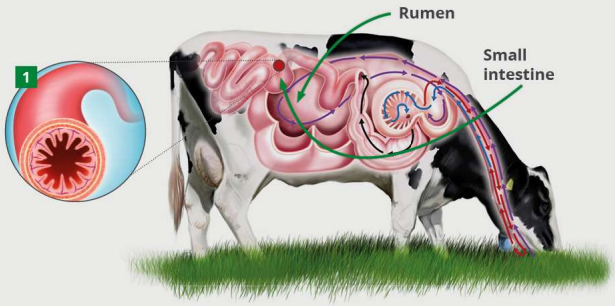

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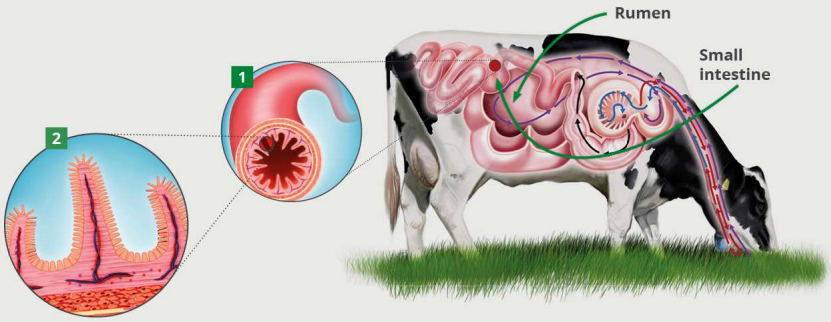

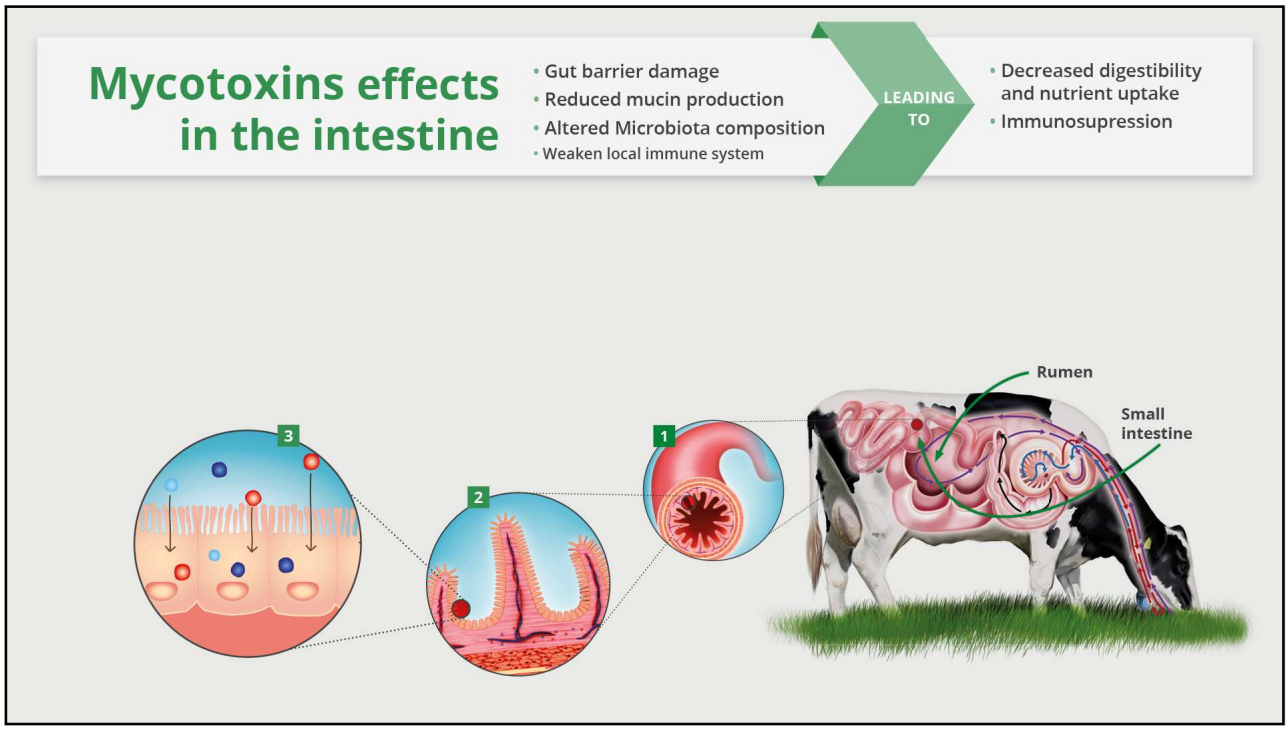
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LEADING TO
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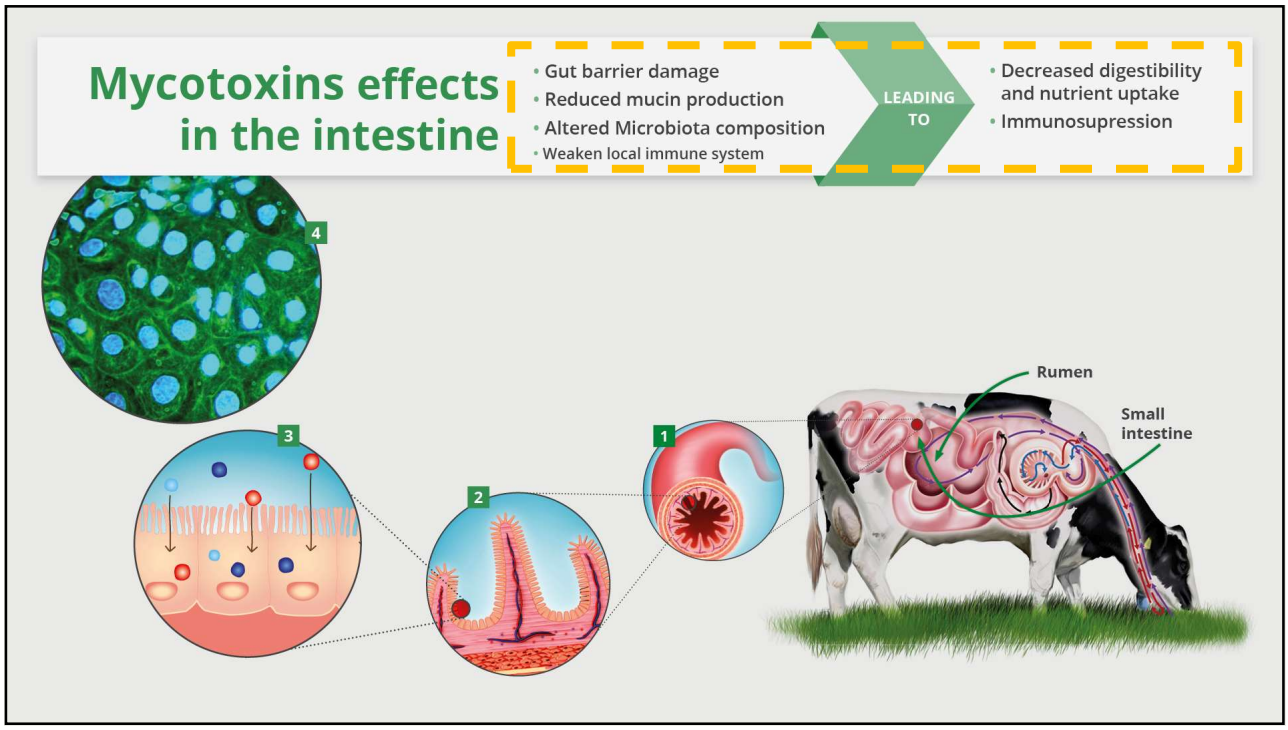
Rumen
Small intestine
1
2
3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Rumen
Small intestine
1
2
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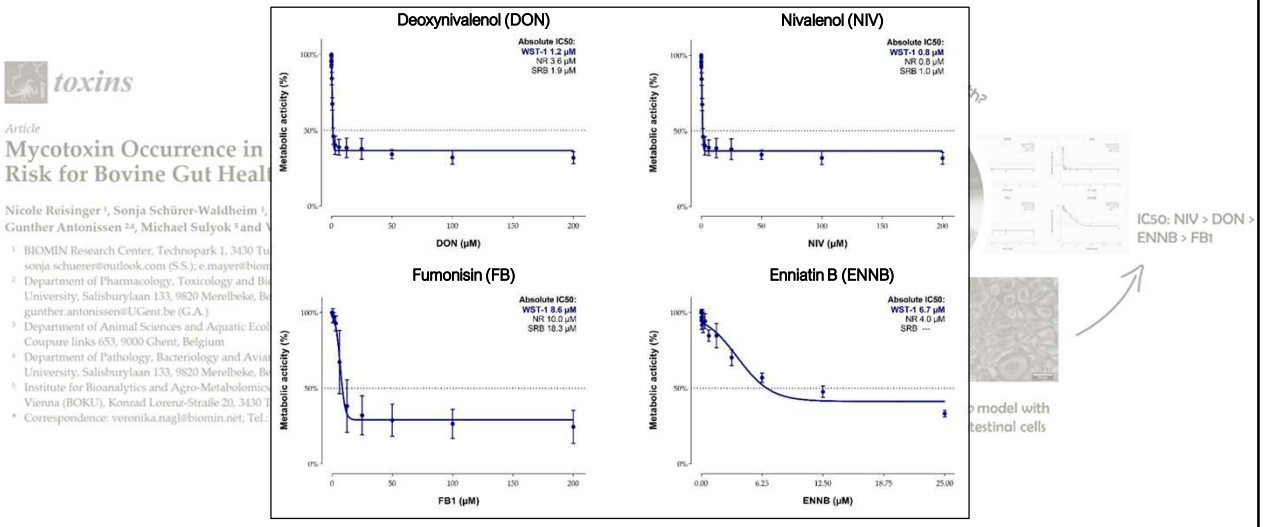
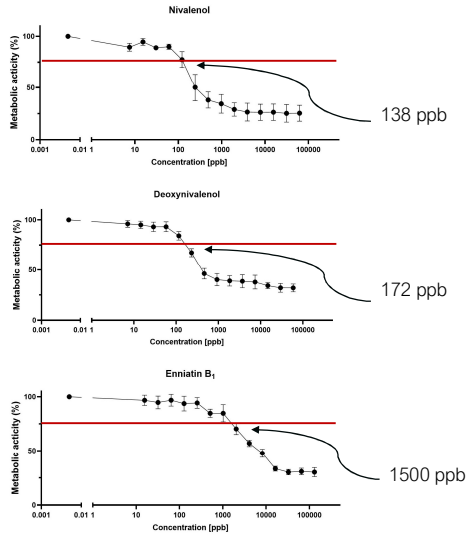
4

Mycotoxins can affect gut health in ruminants

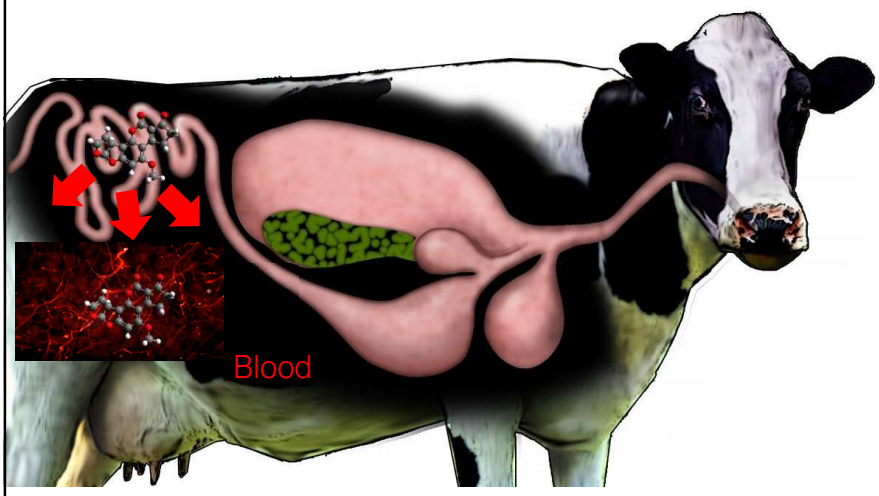


Bovine intestinal cell damage by DON, NIV and ENN B



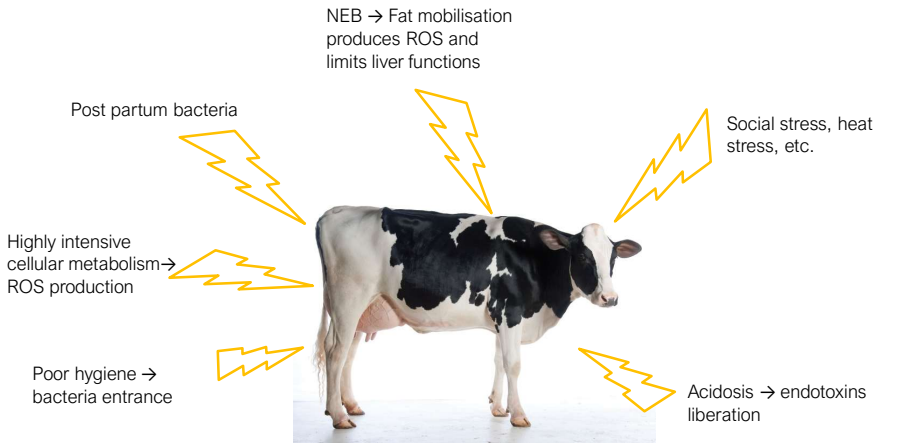
- Metabolic activity indicates the percentage of cells that survive under a certain exposure to toxins
- If 25% of cells die or inactivate, the intestinal function is severely compromised
- Toxicity: NIV>DON>ENN B

The Mycotoxin Journey: the blood stream



Mycotoxins' effects on Inflammation and Immunity

Stressing factors during periparturition



The role of mycotoxins

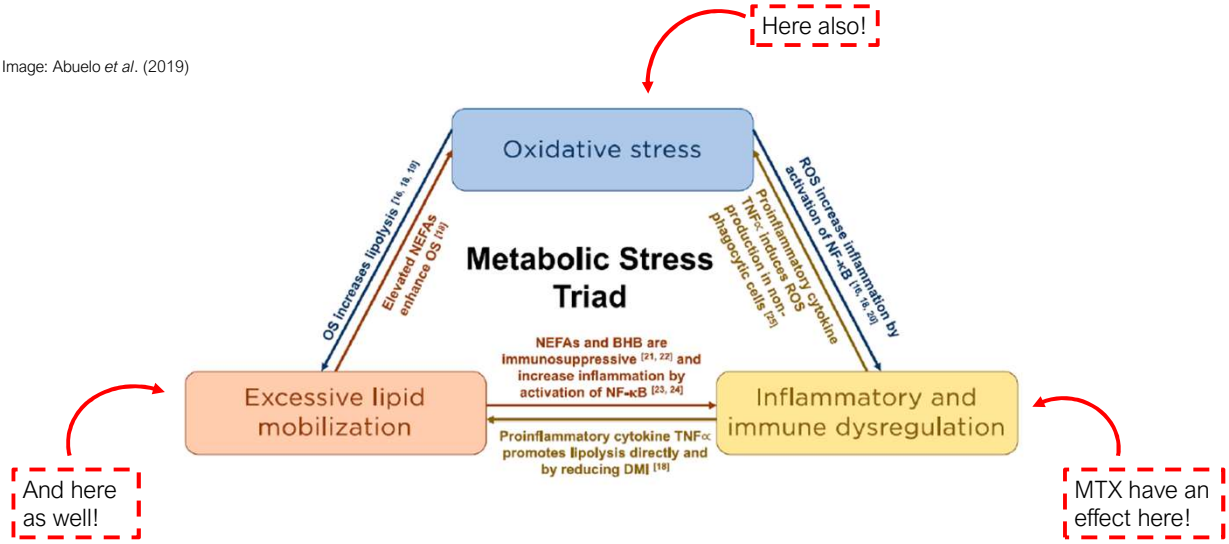
- Trichothecenes (e.g. DON, NIV, DAS, T-2, etc.)
- Zearalenone

Effects at cellular level:
- Inflammation
- ROS production
- Reduced cell viability

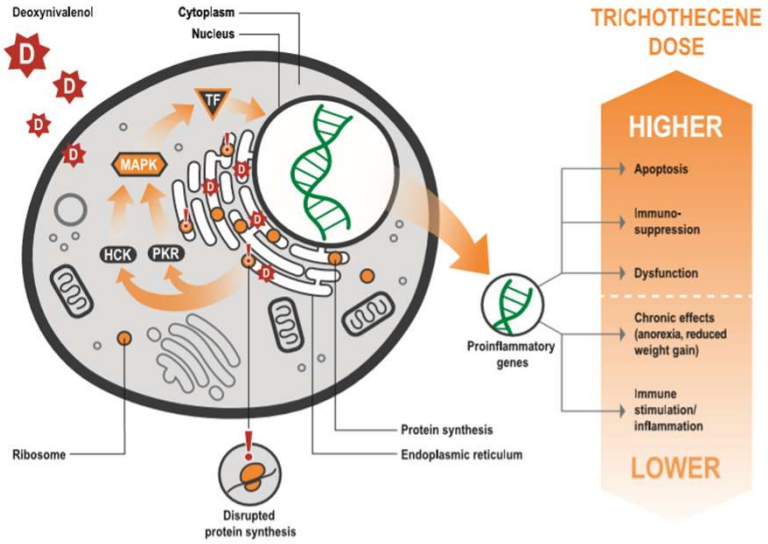
Target cells are fast reproducing ones like:
- Intestinal epithelium
- Immune
- Mammary epithelium

Inflammation does not come alone…

Image: Abuelo *et al*. (2019)



Mode of action - Trichothecenes



Mammary epithelial cells (MEC) as an example

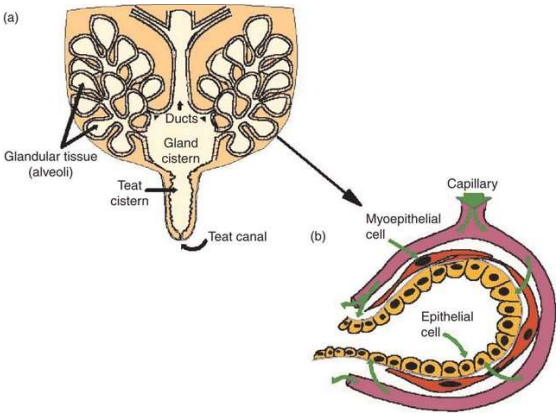
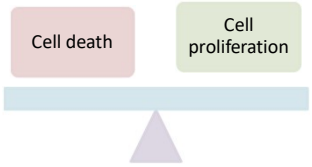


Image: Herve *et al.* (2017)

- Milk is synthesized by MEC
- Milk yield is determined by the metabolic activity and number of MEC in the mammary gland
- The number of MEC depends on the balance between cell proliferation and apoptosis



Oxidation and Inflammation induced by DON (*in vitro*)

ORIGINAL ARTICLE
Ruminants

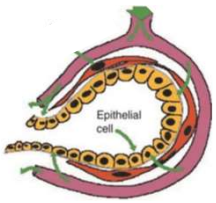
WILEY

Deoxynivalenol induces oxidative stress, inflammatory response and apoptosis in bovine mammary epithelial cells

Junmei Wang | Yongcheng Jin | Shunlu Wu | Hao Yu | Yun Zhao | Hengtong Fang | Jinglin Shen | Changhai Zhou | Yurong Fu | Ruihua Li | Rui Wang | Junxiong Wang | Kexin Zheng | Qingsong Fan | Bojiong Chen | Jing Zhang

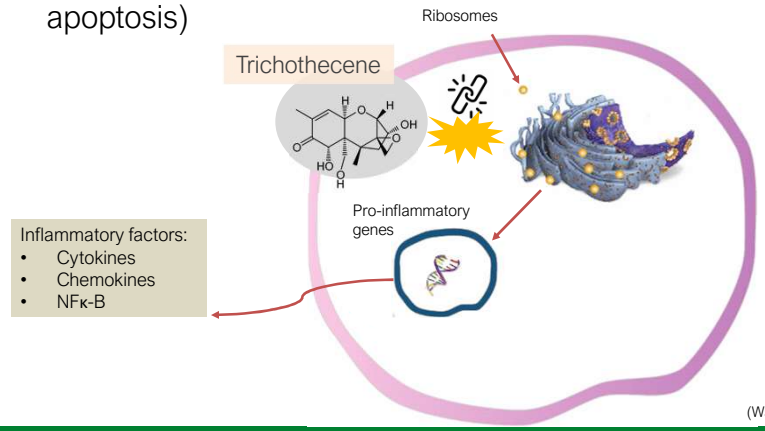
(Wang et al., 2019)

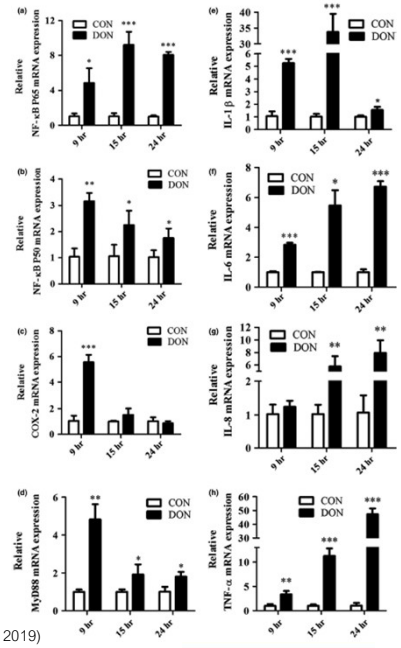
- MAC-T cells exposed to **250 ppb DON**
- Parameters evaluated:
  - Oxidative Stress
  - Inflammation
  - Apoptosis
- Material: MAC-T (Bovine Mammary Epithelial Cells)



How do Trichothecenes cause inflammation?

1. DON when binding with ribosomes, triggers a ribotoxic stress
2. Pro-inflammatory gene up-regulation (and/or apoptosis)





(Wang *et al*., 2019)

How do Trichothecenes cause inflammation?

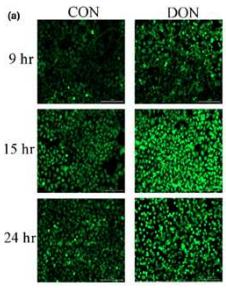
3. DON reduces antioxidant capacity, leading to increased ROS production

4. Intracellular ROS induce inflammation by producing:

Inflammatory factors:
- Cytokines
- Chemokines
- NFκ-B

} Similar reaction as with bacterial toxins (e.g. mastitis)

(Wang *et al*., 2019)



Effect of DON on ROS levels in MAC-T detected by fluorescent dye DCFDA ($p<0.001$)

How do Trichothecenes cause inflammation?

Conclusion:

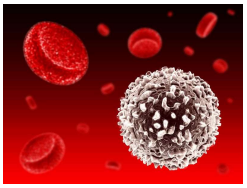
DON caused oxidative stress, inflammatory response and apoptosis in MAC-T

Possible implications:

- Reduction of milk yield
- Higher incidence of infections
- Lower quality of milk
- Reduced productive lifespan of cows

Mycotoxins & Ruminant Immune System

- DON interferes in protein synthesis and damages quickly proliferating cells e.g. immune cells
- Consequences:
  - Immuno-supression
  - Vulnerability to infection
  - Reactivation of latent infections
  - Decreased vaccine efficiency



toxins

MDPI

Article

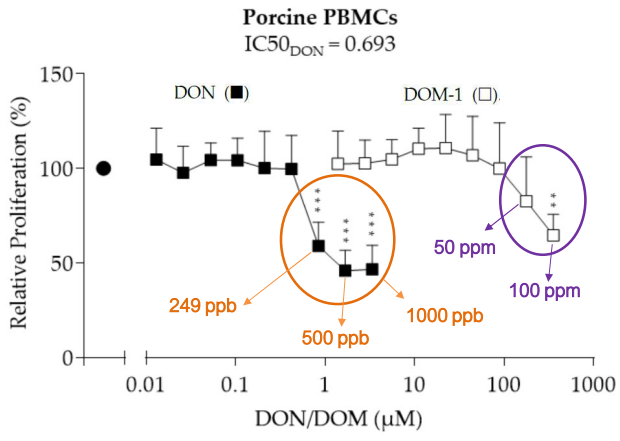
Bovine Peripheral Blood Mononuclear Cells Are More Sensitive to Deoxynivalenol Than Those Derived from Poultry and Swine

Barbara Novak [1], Eleni Vatzia [2], Alexandra Springler [1], Alix Pierron [2], Wilhelm Gerner [2], Nicole Reisinger [1], Sabine Hessenberger [1], Gerd Schatzmayr [1] and Elisabeth Mayer [1,*]

Lymphocytes, including:
- T-cells
- B-cells
- NK-cells

Responsible for adaptive immune response

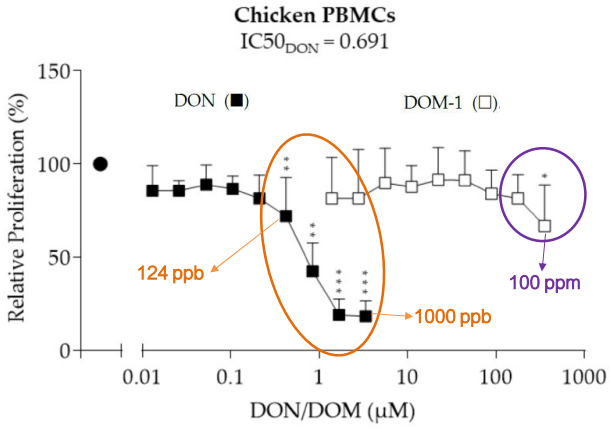
Mycotoxins & Ruminant Immune System



Relative proliferation [%] of porcine PBMC's

DON:
- reduction in proliferation starting at 124 ppb
- significant reduction at:
  - 249 ppb => - 41%
  - 500 ppb => - 54%
  - 1000 ppb => - 53%

Mycotoxins & Ruminant Immune System

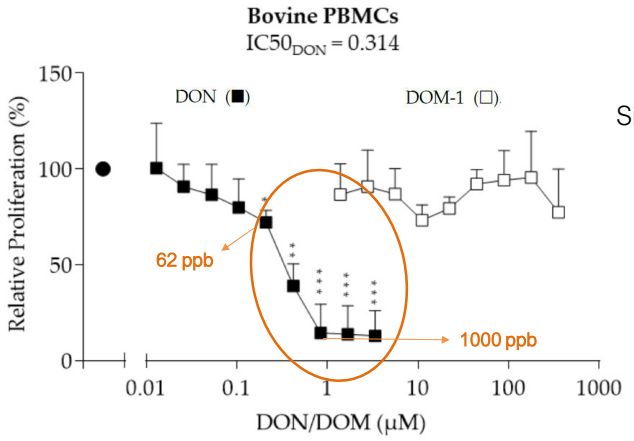


DON:

- reduction in proliferation starting at 62 ppb
- significant reduction at:
  - 124 ppb => - 28%
  - 1000 ppb => - 83%

Relative proliferation [%] of chicken PBMC's

Mycotoxins & Ruminant Immune System



Significant reduction at:

- 62 ppb => - 28%
- 270 ppb => -86%

Relative proliferation [%] of bovine PBMC's

DON affected immune function of cows

Effects of feed naturally contaminated with *Fusarium* mycotoxins on metabolism and immunity of dairy cows

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Journal of Dairy Science

- High yielding dairy cows fed 3.5 ppm DON in TMR
- Results:

Table 6. Effect of diets on neutrophil phagocytotic activity (%; overall means)[1]

Group	Phagocytosis activity
Control	64.0
Contaminated	53.3*
SEM	2.7
Control vs. contaminated (*P*-value)	0.0261

Table 7. Effect of diet on antibody response to ovalbumin (optical density)[1]

Group	Primary response	Secondary response
Control	0.86	1.20
Contaminated	1.15*	1.30
SEM	0.075	0.060
Control vs contaminated (*P*-value)	0.0285	0.4631

- Neutrophils serve as phagocytes and play an important role in nonspecific immunity

Effects of Mycotoxins(low levels) and Mycofix® in health and performance

UNIVERSITÀ CATTOLICA del Sacro Cuore

A mycotoxin deactivating feed additive counteracts the adverse effects of low-level Fusarium mycotoxins in dairy cows
(Antonio Gallo *et al*., 2020)

Published
Journal of Dairy Science

- Scientific Dairy Trial by Gallo et al.
- 12 lactating Holstein Frisian cows

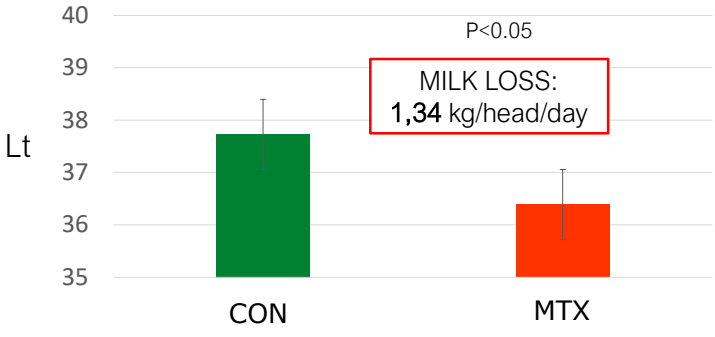
Treatments	DON (ppb)	FUM (ppb)	MPL (g/head/day)
Control	300	100	0
MTX	800	1100	0
MTX+MPL	800	1100	35

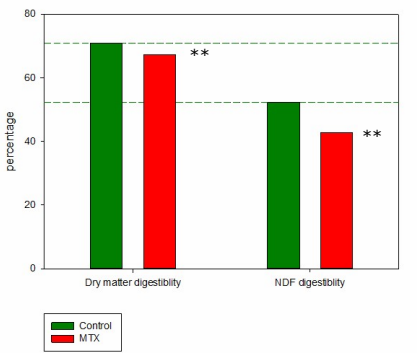
Trail design: latin square

		treatments		
period	**lenght**	**Control**	**MTX**	**MTX + Mycofix®**
Adaptation	14 days			
Study period 1	21 days			
Washout	14 days			
Study period 2	21 days			
Washout	14 days			
Study period 3	21 days			
Total	105 days			

Corn
natural contamination

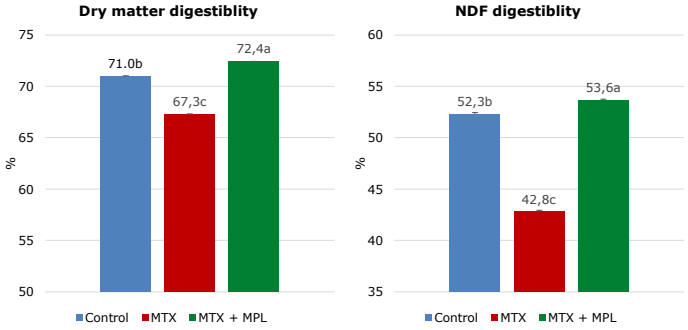
Effects of low level mycotoxins in milk production





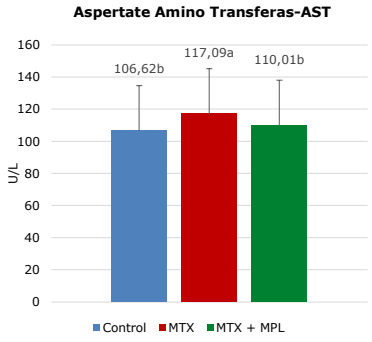
	Control	MTX	p-value treatment	SEM
Dry matter digestibility %	71.0	67.3	<0.05	0.037
NDF digestiblity %	52.3	42.8	<0.05	0.165

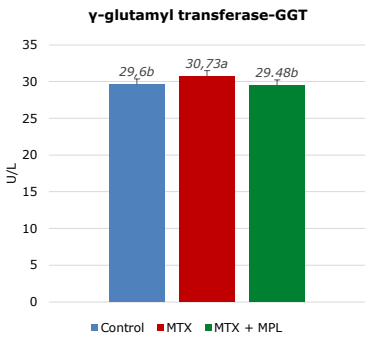
Mycofix® effects at low mycotoxin contamination - Digestibility



- Significant reduction in DM and NDF digestibility due to mycotoxins [P<0.05]
- ✓ Significant improvement by the addition of Mycofix®
  - ✓ Even better results than in control (still had some mycotoxins in it)

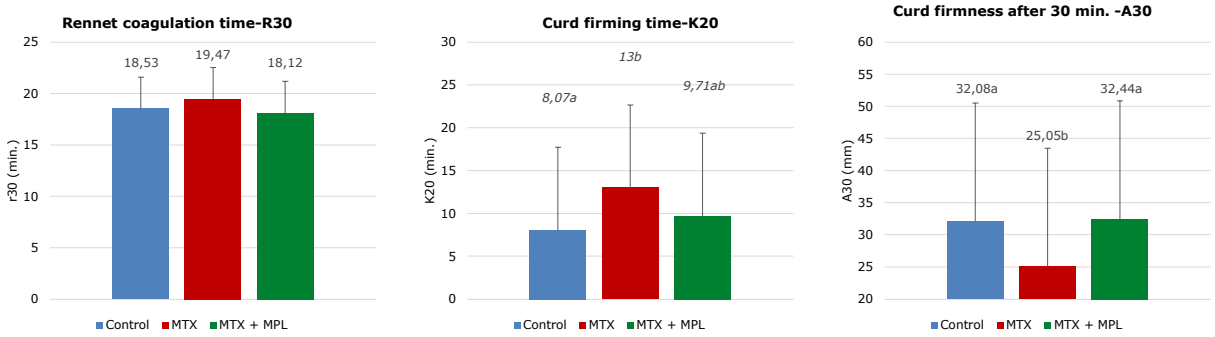
Mycofix® effects on liver health





- Enzymes analysed in blood to indicate liver health conditions
- Significant increases in both indicators by MTX contamination (although low)
- ✓ Alleviated effects by Mycofix®

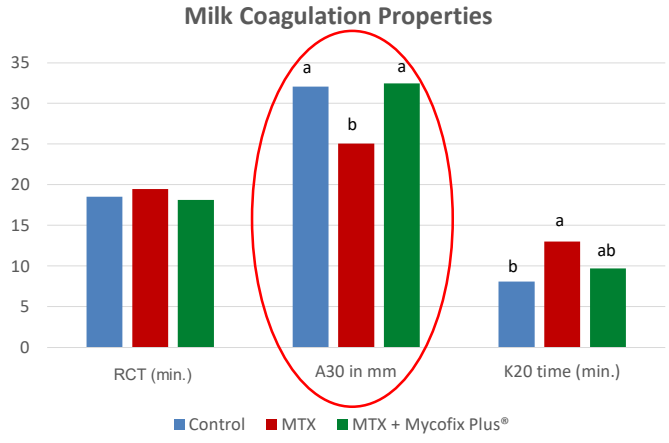
Milk processing (coagulation) parameters

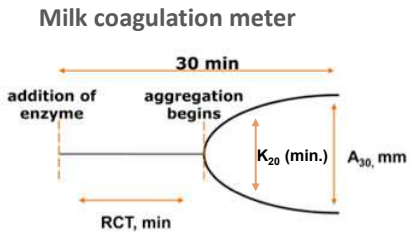


- Detrimental effects of mycotoxins on quality: Milk coagulation parameters

✓ Mycofix® can counteract those effects

Mycofix® effect on milk coagulation properties



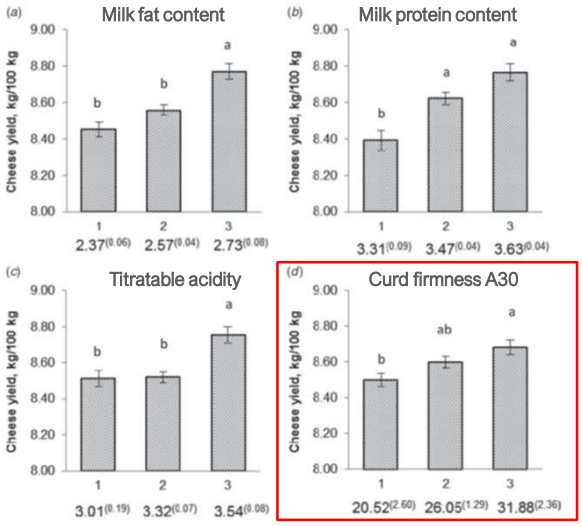


RCT = Rennet Clotting Time

A_{30} = Curd Firmness

K_{20} = Curd clotting speed

And then what??



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Effect of milk composition and coagulation traits on Grana Padano cheese yield under field conditions

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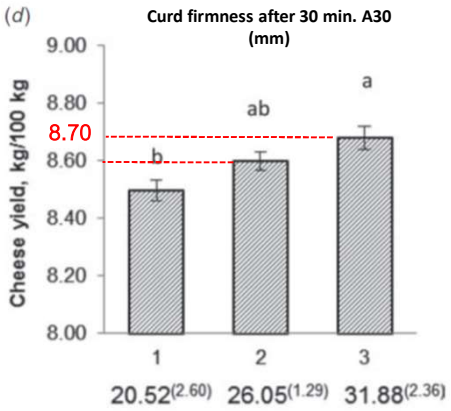
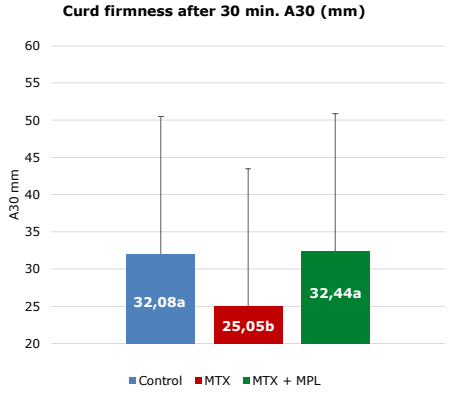
And then what??





100g of Grana Padano

≈ 0,70 €

How big is the gain?

every 100 kg milk

+ 100 g

12 cows * 36 kg/day * 21 days = 9000 kg/milk

⇩

+ 56 € ⇦ + 9 kg cheese

…but how much did we spend?

12 cows * 35 g/day * 21 days = 8.82 kg/MPL * 5 €/kg = - 44 €

…how much milk do 1000 cows make in one year?

13.140.000 kg

⇩

+ 92.000€

Conclusions

Low MTX contamination:

1. can impair performance
2. can negatively affect milk coagulation

Mycofix®

1. can increase diet digestibility
2. can restore milk coagulation attitude leading to higher cheese yield

Thank you for your attention!