Mycofix®: The Definitive Protection against Mycotoxins

PM Mycotoxin Risk Management
Zanetta, Sander and Wolfgang

20-9-2023

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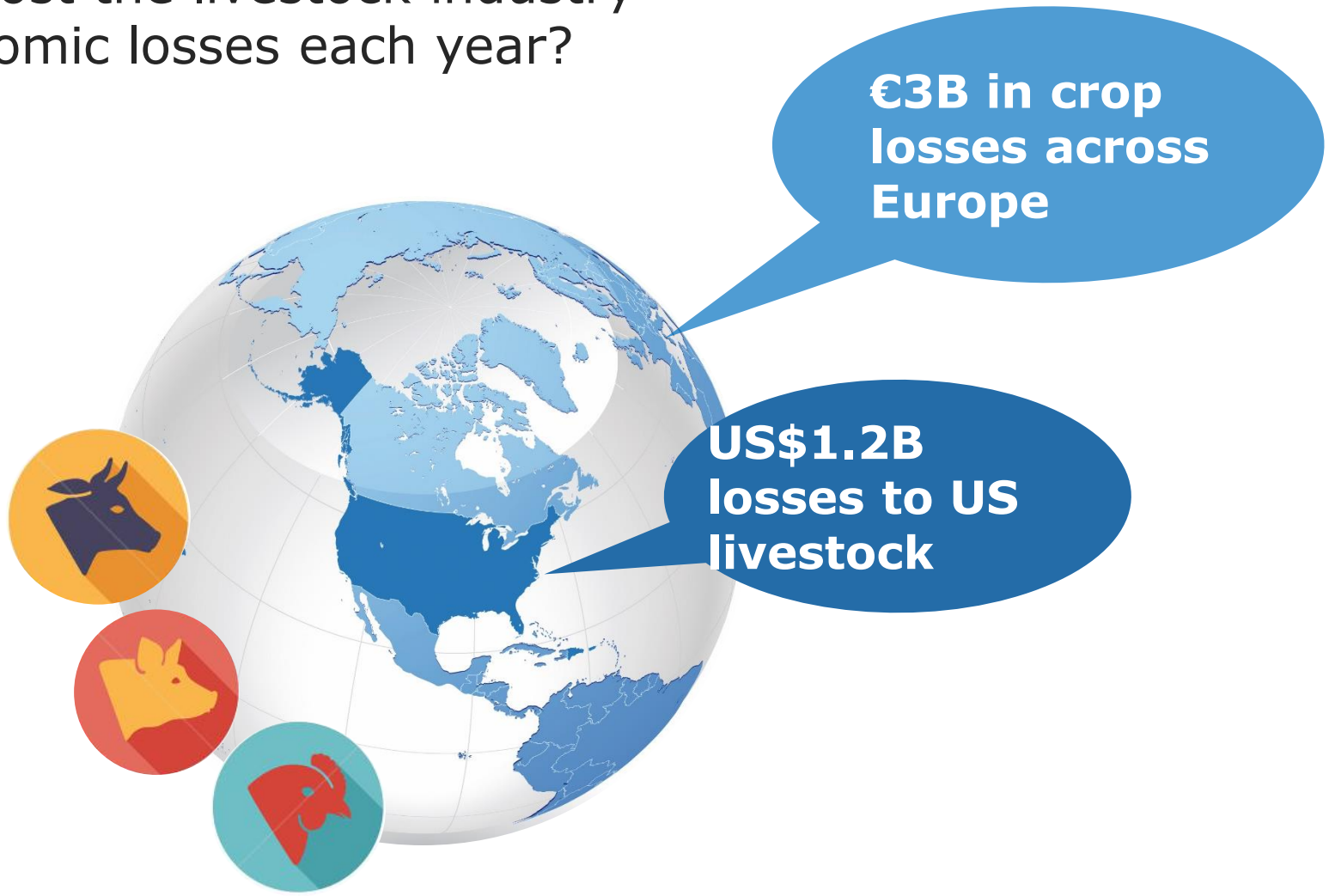
Did you know that…

… Mycotoxins cost the livestock industry billions in economic losses each year?



1.1 Analyse

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Mycotoxin Analytical Methods

ELISA
Quantification of specific mycotoxins in given matrices

- ✓ Fast
- ✓ Inexpensive
- ✗ Raw materials only

HPLC
Quantification of single toxins at low concentrations

- ✓ Fullfils legal requirements
- ✗ More time consuming
- ✗ More expensive

LC-MS/MS:
Spectrum 380® and Spectrum Top® 50
Simultaneous detection of multiple toxins in a wide variety of commodities

- ✓ **Sensitive method**
- ✓ **Suitable for various feed matrices**
- ✓ **Detection of masked & emerging mycotoxins**
- ✗ Highly qualified operator needed
- ✗ More expensive

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Grain Mycotoxin survey 2023 → We change the Method

ELISA[1]:

Quantification of specific mycotoxins in given matrices

- Inexpensive

- Raw materials only
- Many samples needed (only one ELSIA Day)
- No NIV ELSIA test
- Screening analyzes (no detailed Value)
- High LOD[3]

LC-MS/MS[2]: Spectrum Top®

Simultaneous detection of multiple toxins in a wide variety of grains

- 4 different Analysis packages
  - DON + ZEN
  - DON + ZEN + T2 + NIV
  - DON + ZEN + NIV + FUM B1/B2
  - DON + ZEN + AFLAs + FUM B1/B2
- Very Sensitive method
- Low LOD[3] (Limit of detection)
- Qualitative analysis
- Suitable for various feed matrices
- Less work (lead time 5 working days)

[1] **ELISA = E**nzyme-**l**inked **I**mmunosorbent **A**ssay
[2] **LC-MS/MS= Liquid Chromatography-Tandem Mass Spectrometry**
[3] **L**imit **o**f **D**etection

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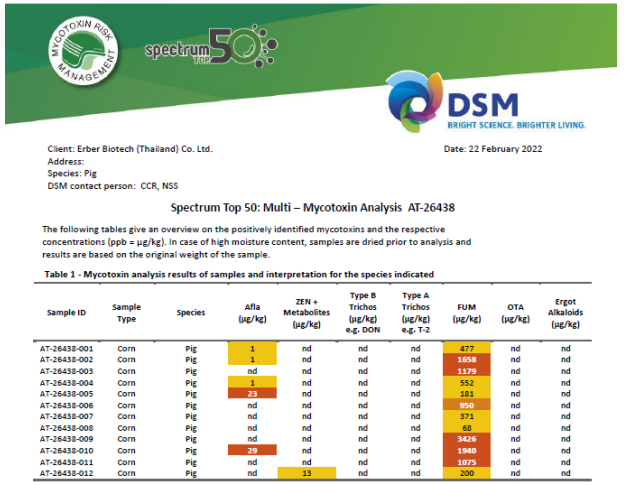
Spectrum Top® 50

- ✓Method was developed by Romer Labs
- ✓Analysis is done locally: Romer Labs Singapore
- ✓More than 50 different mycotoxins and metabolites
  - ✓ frequently occurring mycotoxins
  - ✓ masked mycotoxins
  - ✓ emerging mycotoxins
- ✓ Product package availabe
- ✓ Turn around time: 10 working days upon sample receival in Romer Labs SG
- ✓ Interpreted report with risk assessment (species-specific) and concise information about mycotoxins analyzed



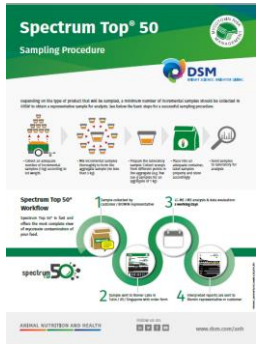
Client: Erber Biotech (Thailand) Co. Ltd. Date: 22 February 2022
Address:
Species: Pig
DSM contact person: CCR, NSS

Spectrum Top 50: Multi – Mycotoxin Analysis AT-26438

The following tables give an overview on the positively identified mycotoxins and the respective concentrations (ppb = µg/kg). In case of high moisture content, samples are dried prior to analysis and results are based on the original weight of the sample.

Table 1 - Mycotoxin analysis results of samples and interpretation for the species indicated

Sample ID	Sample Type	Species	Afla (µg/kg)	ZEN + Metabolites (µg/kg)	Type B Trichos (µg/kg) e.g. DON	Type A Trichos (µg/kg) e.g. T-2	FUM (µg/kg)	OTA (µg/kg)	Ergot Alkaloids (µg/kg)
AT-26438-001	Corn	Pig	1	nd	nd	nd	477	nd	nd
AT-26438-002	Corn	Pig	1	nd	nd	nd	1658	nd	nd
AT-26438-003	Corn	Pig	nd	nd	nd	nd	1179	nd	nd
AT-26438-004	Corn	Pig	1	nd	nd	nd	552	nd	nd
AT-26438-005	Corn	Pig	23	nd	nd	nd	181	nd	nd
AT-26438-006	Corn	Pig	nd	nd	nd	nd	950	nd	nd
AT-26438-007	Corn	Pig	nd	nd	nd	nd	571	nd	nd
AT-26438-008	Corn	Pig	nd	nd	nd	nd	68	nd	nd
AT-26438-009	Corn	Pig	nd	nd	nd	nd	3426	nd	nd
AT-26438-010	Corn	Pig	29	nd	nd	nd	1940	nd	nd
AT-26438-011	Corn	Pig	nd	nd	nd	nd	1075	nd	nd
AT-26438-012	Corn	Pig	nd	13	nd	nd	200	nd	nd



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Spectrum 380® - Get the full picture

- ✓Method was developed by our cooperation partners at the Center for Analytica. Chemistry at IFA-Tulln
- ✓Analysis is done in Tulln in Austria
- ✓More than 800 different mycotoxins and metabolites
  - ✓ frequently occurring mycotoxins
  - ✓ masked mycotoxins
  - ✓ emerging mycotoxins & fungal metabolites
  - ✓ plant toxins and metabolites (incl. phytoestrogens)
  - ✓ bacterial toxins and metabolites
  - ✓ *on request: veterinary drug residues, pesticides*
- ✓Turn around time: 10-15 working days upon sample receival at the university in Austria
- ✓2 reports: Interpreted report with risk assessment (species-specific) and an independent report of the university

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Spectrum Top 50 Procedure – how it work

Spectrum Top® 50 Workflow

Spectrum Top 50® is fast and offers the most complete view of mycotoxin contamination of your feed.

1 Sample collected by customer /DSM representative

2 Sample sent to Romer Labs in Tulln / US / Singapore with order form

3 LC-MS / MS analysis & data evaluation: **5 working days**

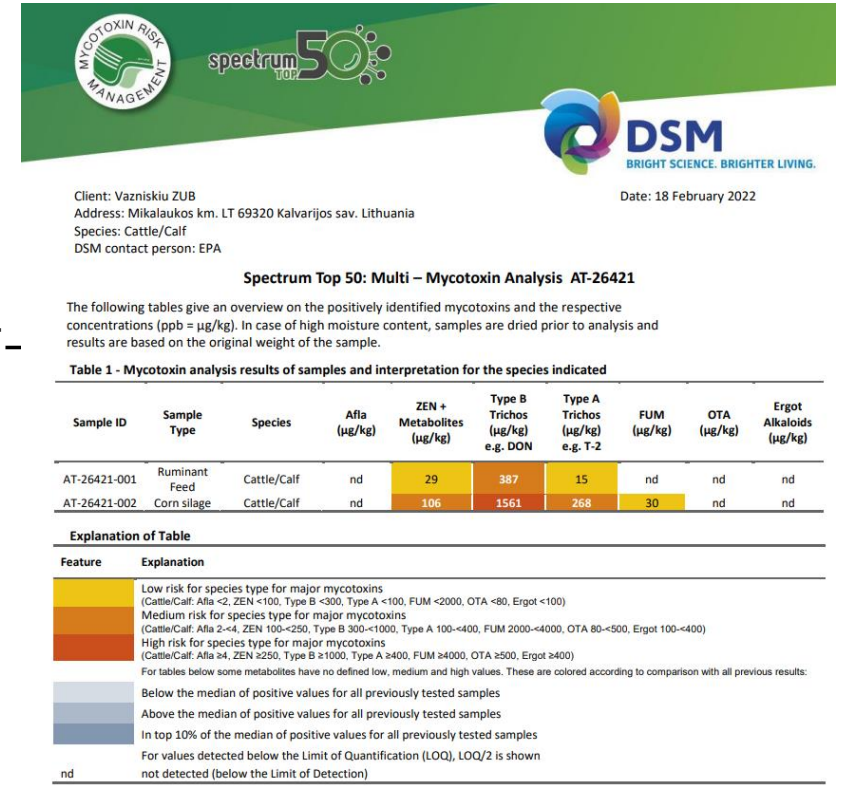
4 Interpreted reports are sent to DSM representative or customer

Facts in numbers

Results are represented in numbers and colors

Tested against > 50 different mycotoxins and metabolites

1. Frequently occurring mycotoxins
   - AFLA, DON, ZON, FUM, OTA, T-2, HT-2, Ergots
2. Masked mycotoxins
   - Glycosylated
3. Emerging mycotoxins
   - Not enough data yet to define thresholds (Moniliformin's ..)



Client: Vazniskiu ZUB
Address: Mikalaukos km. LT 69320 Kalvarijos sav. Lithuania
Species: Cattle/Calf
DSM contact person: EPA

Date: 18 February 2022

Spectrum Top 50: Multi – Mycotoxin Analysis AT-26421

The following tables give an overview on the positively identified mycotoxins and the respective concentrations (ppb = µg/kg). In case of high moisture content, samples are dried prior to analysis and results are based on the original weight of the sample.

Table 1 - Mycotoxin analysis results of samples and interpretation for the species indicated

Sample ID	Sample Type	Species	Afla (µg/kg)	ZEN + Metabolites (µg/kg)	Type B Trichos (µg/kg) e.g. DON	Type A Trichos (µg/kg) e.g. T-2	FUM (µg/kg)	OTA (µg/kg)	Ergot Alkaloids (µg/kg)
AT-26421-001	Ruminant Feed	Cattle/Calf	nd	29	387	15	nd	nd	nd
AT-26421-002	Corn silage	Cattle/Calf	nd	106	1561	268	30	nd	nd

Explanation of Table

Feature	Explanation
	Low risk for species type for major mycotoxins (Cattle/Calf: Afla <2, ZEN <100, Type B <300, Type A <100, FUM <2000, OTA <80, Ergot <100)
	Medium risk for species type for major mycotoxins (Cattle/Calf: Afla 2-<4, ZEN 100-<250, Type B 300-<1000, Type A 100-<400, FUM 2000-<4000, OTA 80-<500, Ergot 100-<400)
	High risk for species type for major mycotoxins (Cattle/Calf: Afla ≥4, ZEN ≥250, Type B ≥1000, Type A ≥400, FUM ≥4000, OTA ≥500, Ergot ≥400)
	For tables below some metabolites have no defined low, medium and high values. These are colored according to comparison with all previous results:
	Below the median of positive values for all previously tested samples
	Above the median of positive values for all previously tested samples
	In top 10% of the median of positive values for all previously tested samples
	For values detected below the Limit of Quantification (LOQ), LOQ/2 is shown
nd	not detected (below the Limit of Detection)

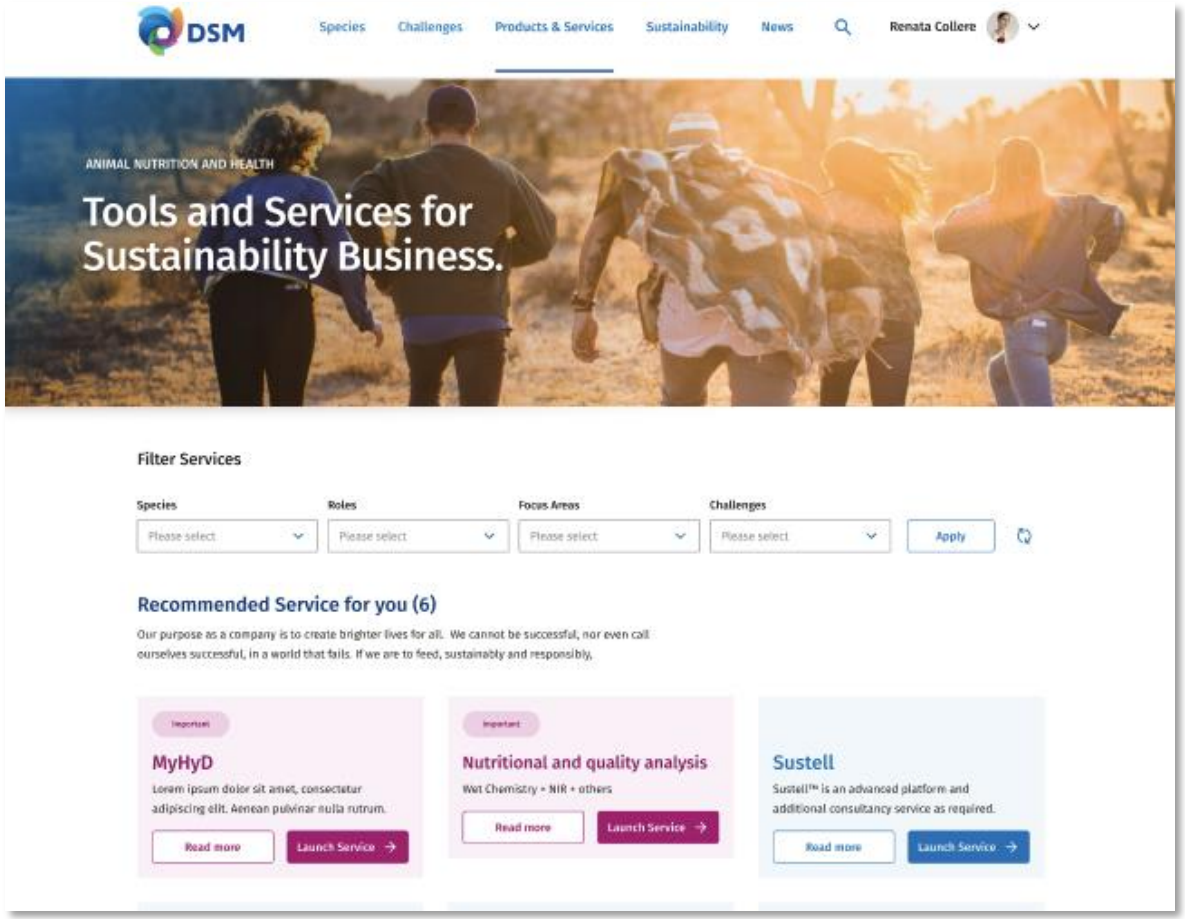
Sample: AT-26421-002 Cattle/Calf (Corn silage) VZ 02 MaysSI & Mays grain

Substance	Value (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	Description
Zearalenone and metabolites		(zearalenone, alpha-zearalenol, beta zearalenol, zearalanol)		
Zearalenone	106.0	5	25	Zearalenone is estrogenic, acting like the sex hormone estradiol thereby interfering with fertility and sexual development of animals. It is also hepatotoxic, hematotoxic, immunotoxic and genotoxic.
Type B trichothecenes		(deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, DON-3-glucoside, nivalenol, 15-acteoxyscirpenol, fusarenon X)		
Deoxynivalenol	1393.4	75	250	Deoxynivalenol induces emesis and feed refusal resulting in reduced weight gain. Other effects include immunotoxicity, hematotoxicity and myelotoxicity, as well as reproductive toxicity. It furthermore causes intestinal lesions and compromises the intestinal barrier function.
DON-3-glucoside	167.5	15	50	Deoxynivalenol-3-glucoside is a masked mycotoxin. It is converted back to deoxynivalenol in the gastrointestinal tract of mammals.
Type A trichothecenes		(T-2 toxin, HT-2 toxin, T-2 tetraol, T-2 triol, diacetoxyscirpenol, neosolaniol)		
HT-2 toxin	268.2	15	50	HT-2 toxin is a type A trichothecene and a metabolite of T-2 toxin. HT-2 toxin showed a high acute toxicity in mice and chickens with LD50 values in the same dose range as reported for T-2 toxin. HT-2 toxin was shown to induce feed refusal in mice. Haematotoxic, immunotoxic and cytotoxic effects of HT-2 toxin were observed in vitro.
Fumonisins		(fumonisin B1, fumonisin B2, fumonisin B3)		
Fumonisin B1	15	10	30	Fumonisins are hepatotoxic and nephrotoxic. High fumonisin doses cause the species specific fatal diseases porcine pulmonary edema in pigs and equine leukoencephalomalacia in horses. Fumonisin B1 has been classified as a group 2B carcinogen (possibly carcinogenic to humans) by the International Agency for Research on Cancer. Fumonisins were shown to be immunotoxic and to compromise gut health. They furthermore exert reproductive toxicity.
Fumonisin B2	15	10	30	
Alternaria Toxins		(alternariol)		
Alternariol	15.6	5	15	Alternariol showed no acute toxicity in published studies in animals. However, alternariol was cytotoxic, genotoxic and mutagenic to mammalian cell lines in vitro. Furthermore, negative effects of alternariol on the reproductive and immune system have been suggested by in vitro results.
Fusarium Toxins		(moniliformin)		
Moniliformin	39.6	10	30	Moniliformin was shown to be toxic to rodents and poultry. Toxic effects included damage to the heart muscle, respiratory distress, decreased feed intake and body weight gain and impaired immune function.
Beauvericin and Enniatins		(beauvericin, enniatin A, enniatin A1, enniatin B and enniatin B1)		
Enniatin A	3.8	0.5	2	Beauvericin and enniatins were toxic to different mammalian cell lines in vitro. According to published studies, acute exposure to beauvericin and enniatins was not toxic to animals. However, the effect of chronic exposure is currently unknown. According to the results of in vitro studies, beauvericin and enniatins may affect the immune system and the bioavailability of pharmaceuticals. Beauvericin and enniatins were shown to accumulate in the eggs of laying hens, but detected levels were likely no cause for concern.
Enniatin A1	9.8	0.5	2	
Enniatin B	308.5	1	3	
Enniatin B1	28.9	0.5	2	

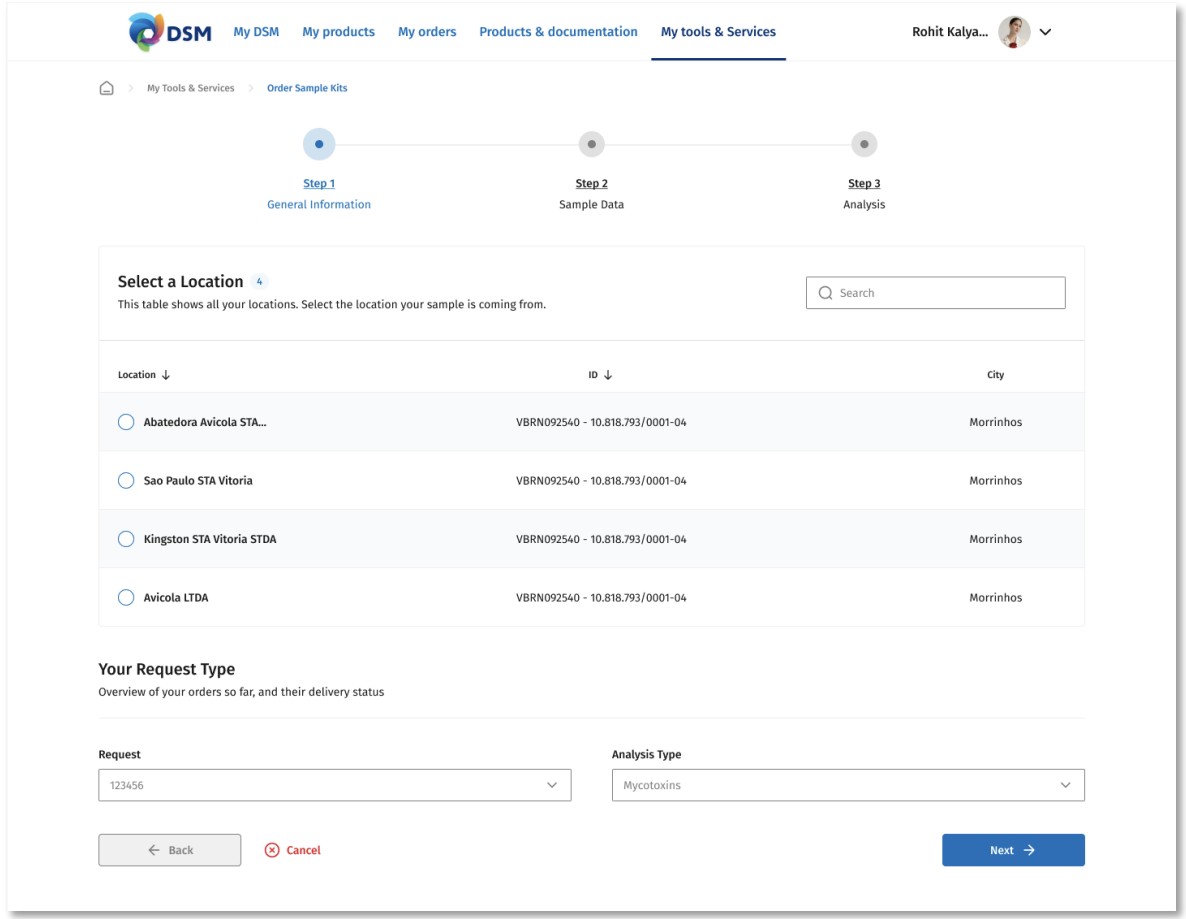
Customer Portal NEWS

A demo of the logged-in experience and service catalogue

Logged-in experience



Self service for lab and analytical (Spectrum TOP® 50) services



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1.2 Survey data Results

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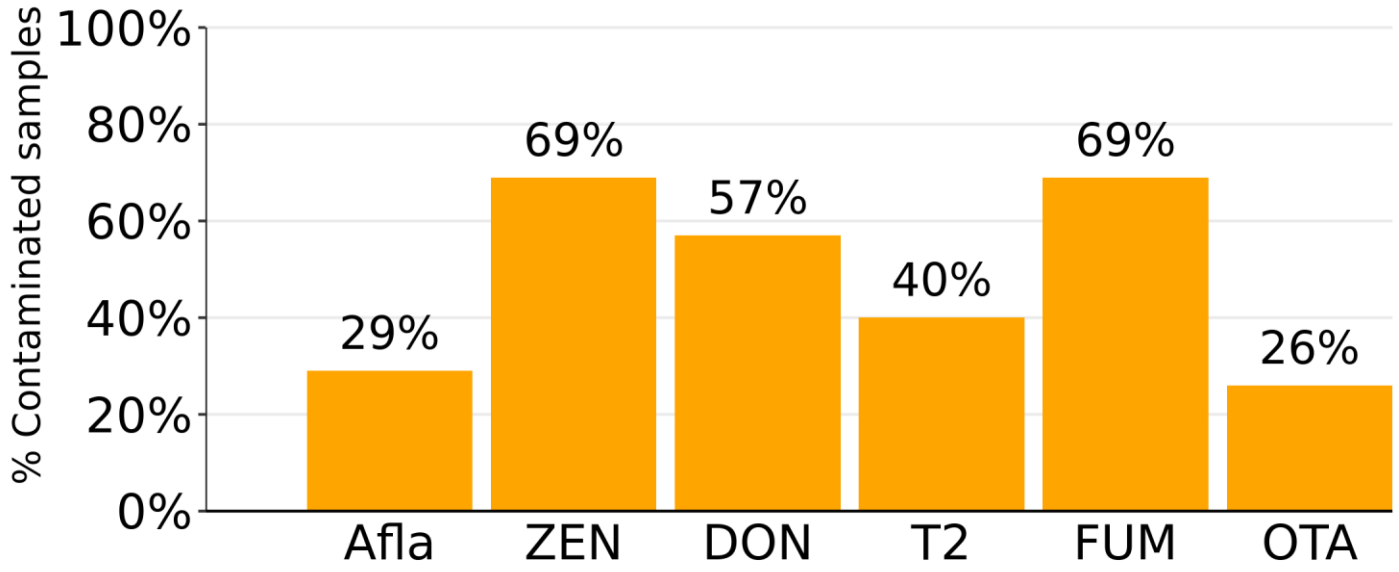
All commodities in Hungary from Jan 2023 to Sep 2023

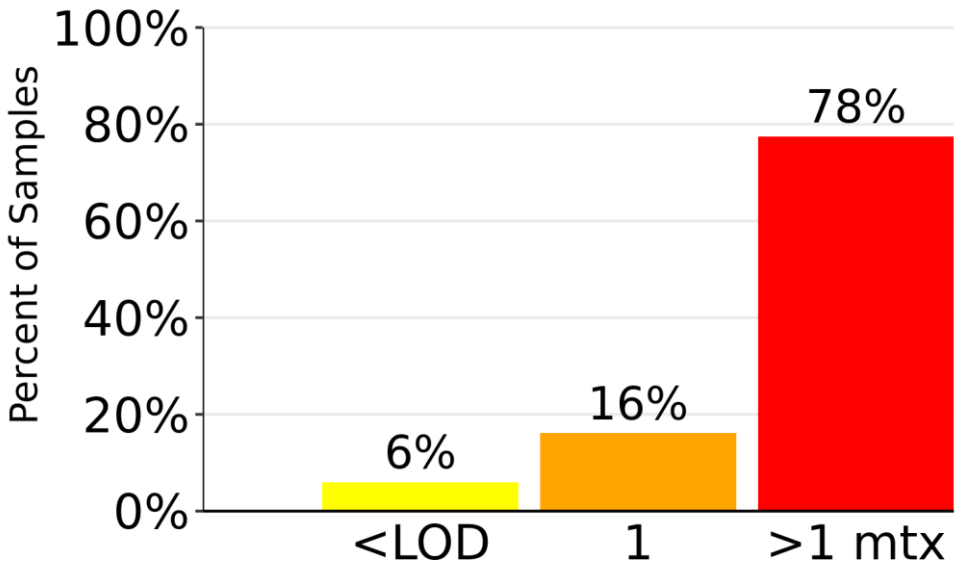
Parameter	Afla	ZEN	DON	T2	FUM	OTA
Number of samples	444	445	444	436	437	434
% Contaminated samples	29%	69%	57%	40%	69%	26%
% Above risk threshold	20%	16%	34%	8%	24%	6%
Average of positives (ppb)	19	44	529	36	551	14
Median of positives (ppb)	3	22	204	24	296	4
Maximum (ppb)	1362	884	9400	298	7754	294

Metabolite	Average	Maximum	Prevalence
Deoxynivalenol	315	1934	68%
Nivalenol	176	352	22%
Deoxynivalenol-3-Glucoside	44	108	20%
15-Acetyl-Deoxynivalenol	69	75	10%
3-Acetyl-Deoxynivalenol	1818	1818	2%

Prevalence of Mycotoxins Detected



No. of Mycotoxins per Sample



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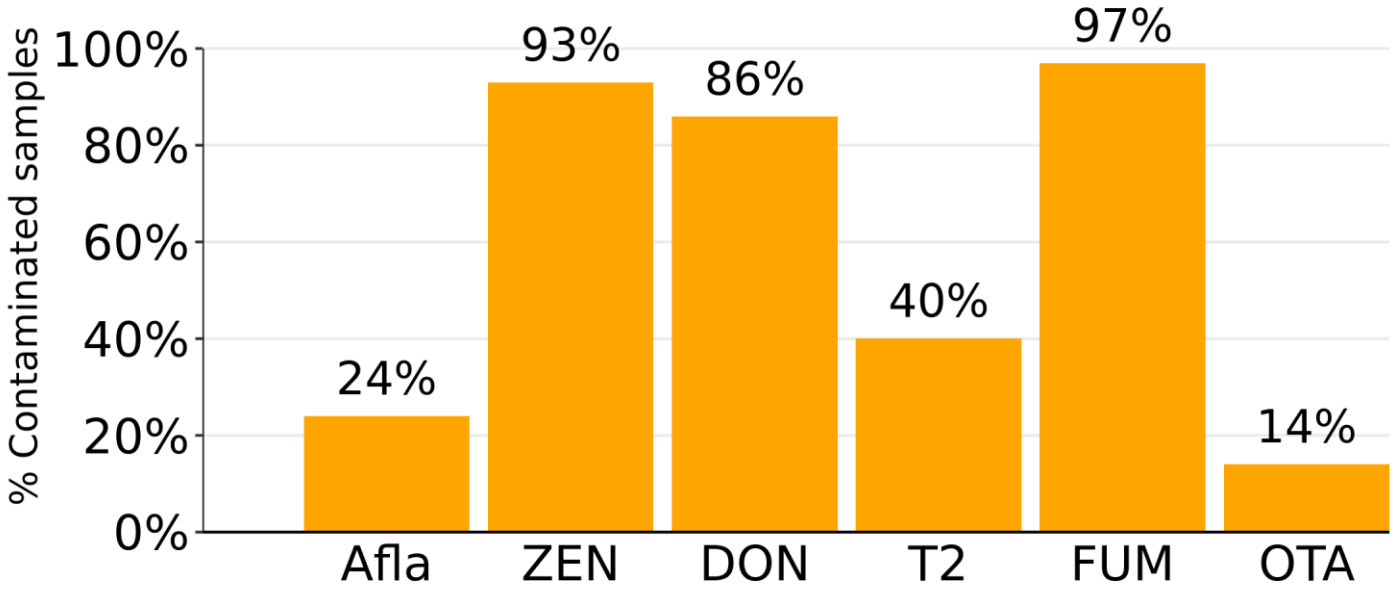
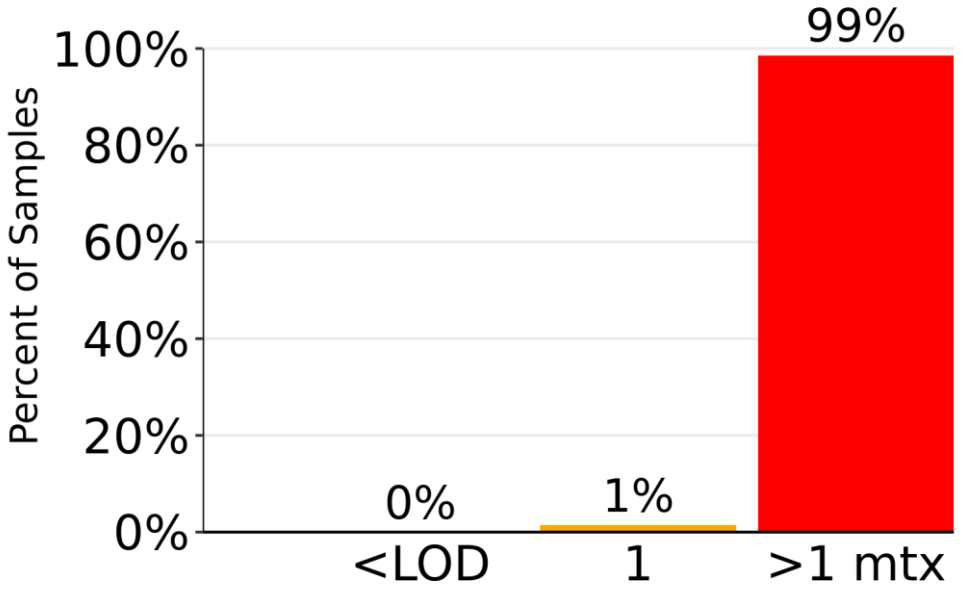
Finished Feed Poultry in Hungary from Jan 2023 to Sep 2023

Parameter	Afla	ZEN	DON	T2	FUM	OTA
Number of samples	70	70	70	70	70	70
% Contaminated samples	24%	93%	86%	40%	97%	14%
% Above risk threshold	10%	19%	56%	4%	10%	6%
Average of positives (ppb)	4	25	405	25	215	10
Median of positives (ppb)	2	16	211	20	129	7
Maximum (ppb)	26	97	2298	72	912	27

Prevalence of Mycotoxins Detected



No. of Mycotoxins per Sample



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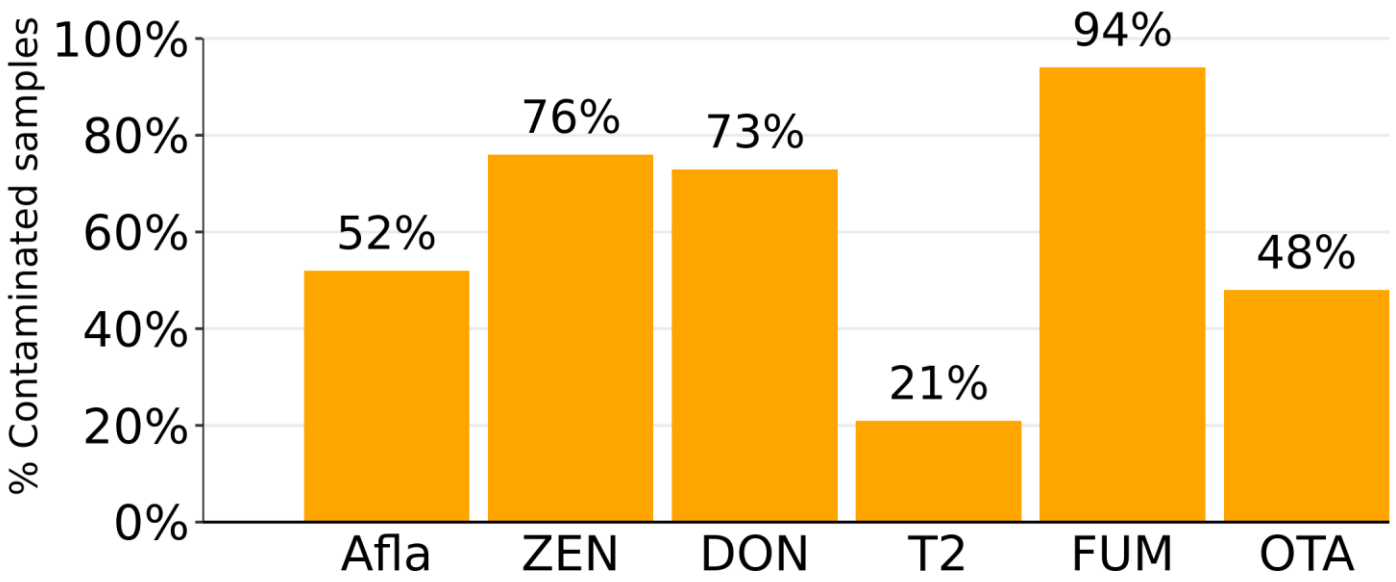
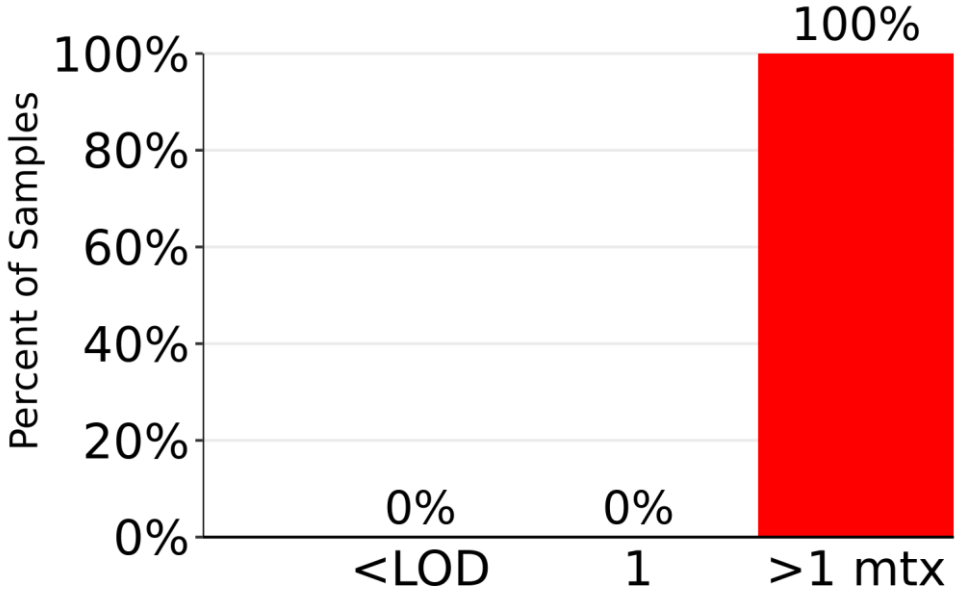
Finished Feed Swine in Hungary from Jan 2023 to Sep 2023

Parameter	Afla	ZEN	DON	T2	FUM	OTA
Number of samples	33	33	33	33	33	33
% Contaminated samples	52%	76%	73%	21%	94%	48%
% Above risk threshold	36%	12%	39%	0%	33%	15%
Average of positives (ppb)	13	25	387	23	490	14
Median of positives (ppb)	5	10	223	15	208	4
Maximum (ppb)	101	139	1255	46	3240	59

Prevalence of Mycotoxins Detected



No. of Mycotoxins per Sample



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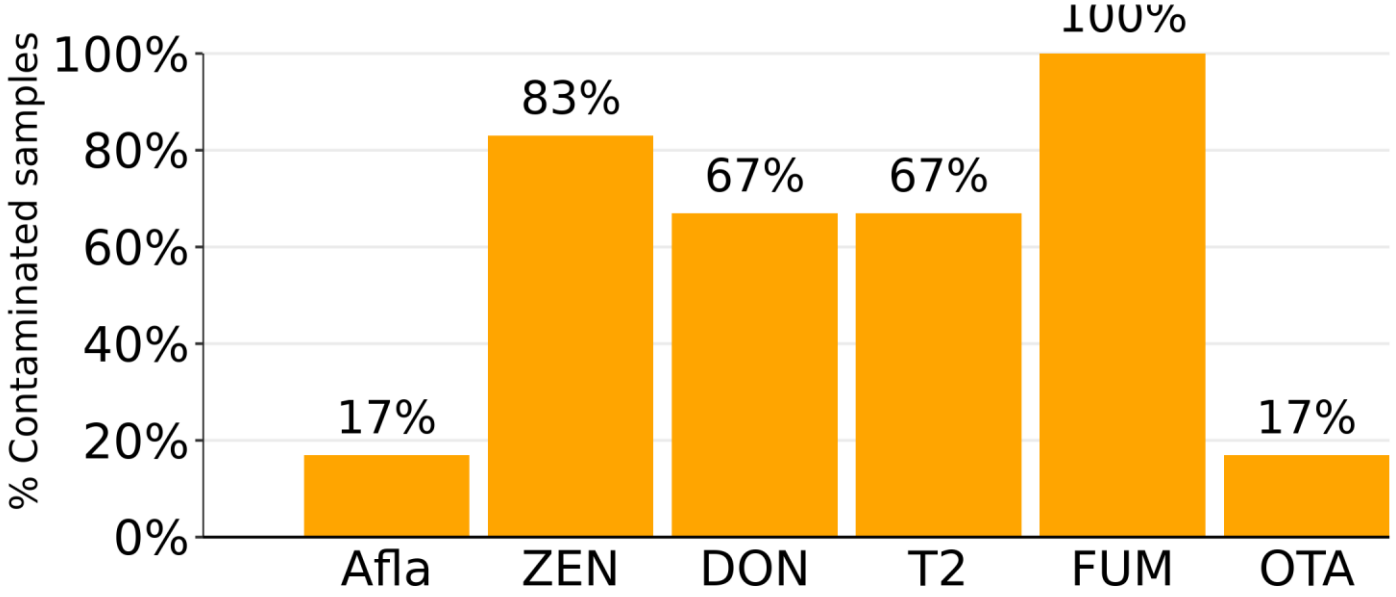
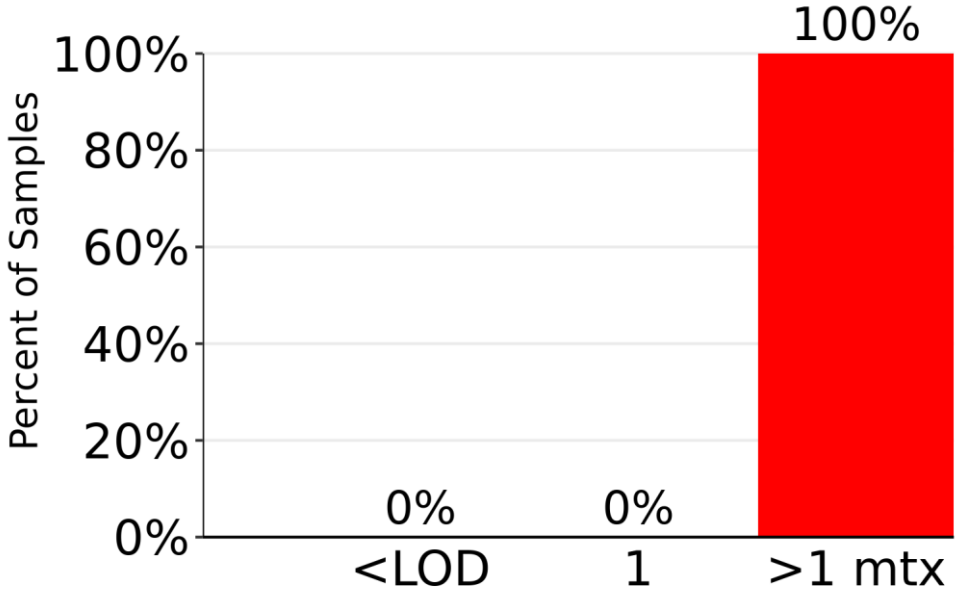
Finished Feed Ruminants in Hungary from Jan 2023 to Sep 2023

Parameter	Afla	ZEN	DON	T2	FUM	OTA
Number of samples	12	12	12	12	12	12
% Contaminated samples	17%	83%	67%	67%	100%	17%
% Above risk threshold	17%	58%	67%	0%	8%	0%
Average of positives (ppb)	6	69	434	6	149	7
Median of positives (ppb)	6	60	217	5	112	7
Maximum (ppb)	10	247	1546	15	524	8

Prevalence of Mycotoxins Detected



No. of Mycotoxins per Sample



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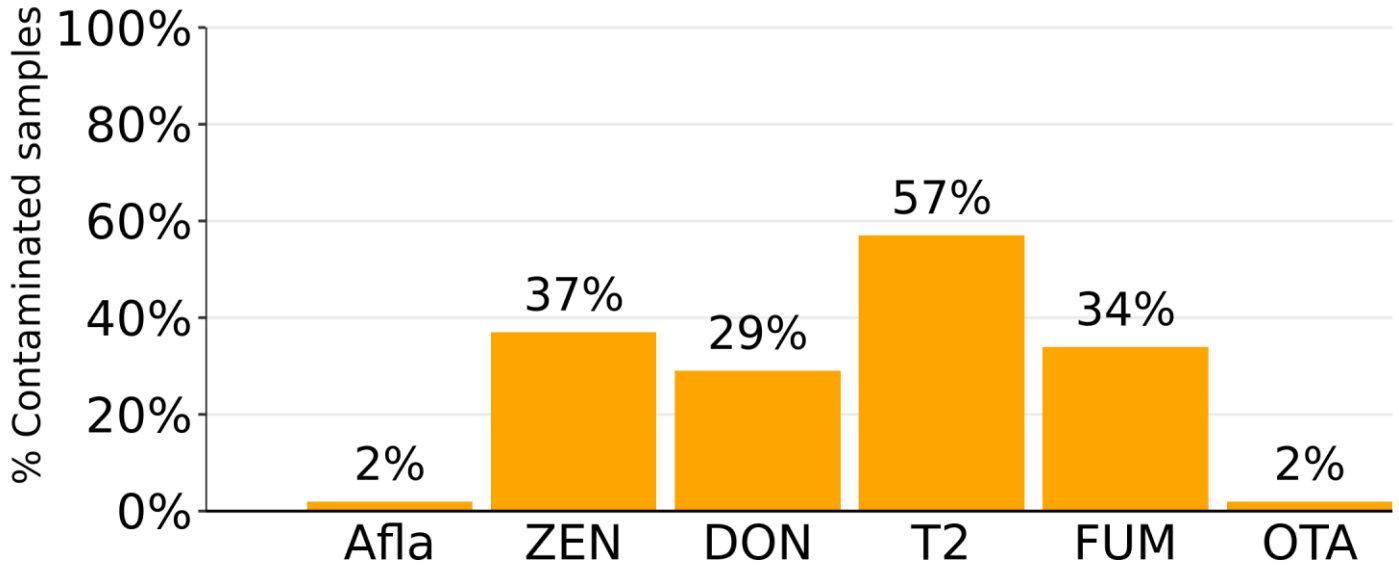
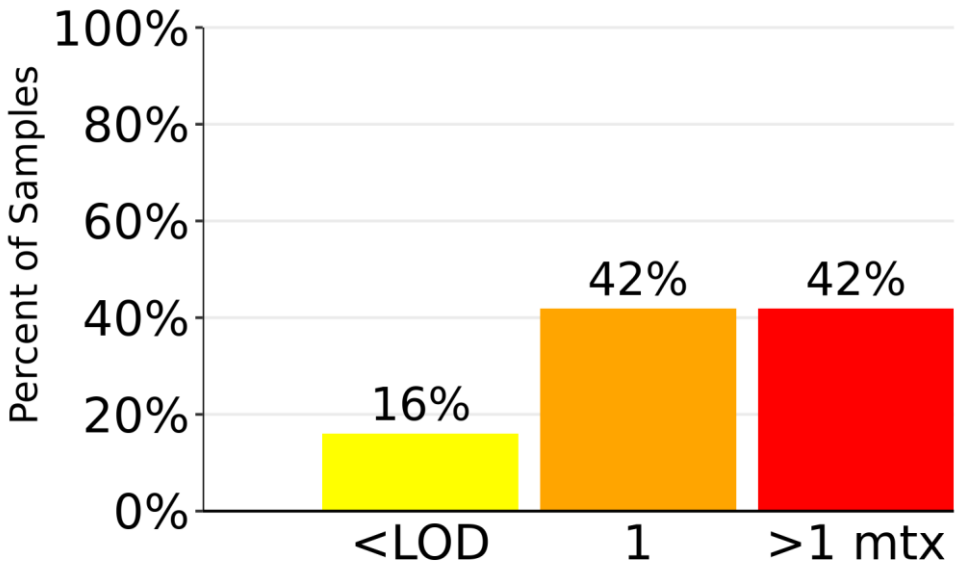
Wheat grain & Barley grain in Hungary from Jan 2023 to Sep 2023

Parameter	Afla	ZEN	DON	T2	FUM	OTA
Number of samples	62	63	63	53	61	52
% Contaminated samples	2%	37%	29%	57%	34%	2%
% Above risk threshold	2%	5%	19%	11%	15%	0%
Average of positives (ppb)	4	34	640	32	482	2
Median of positives (ppb)	4	31	218	27	375	2
Maximum (ppb)	4	98	6535	65	1272	2

Prevalence of Mycotoxins Detected



No. of Mycotoxins per Sample



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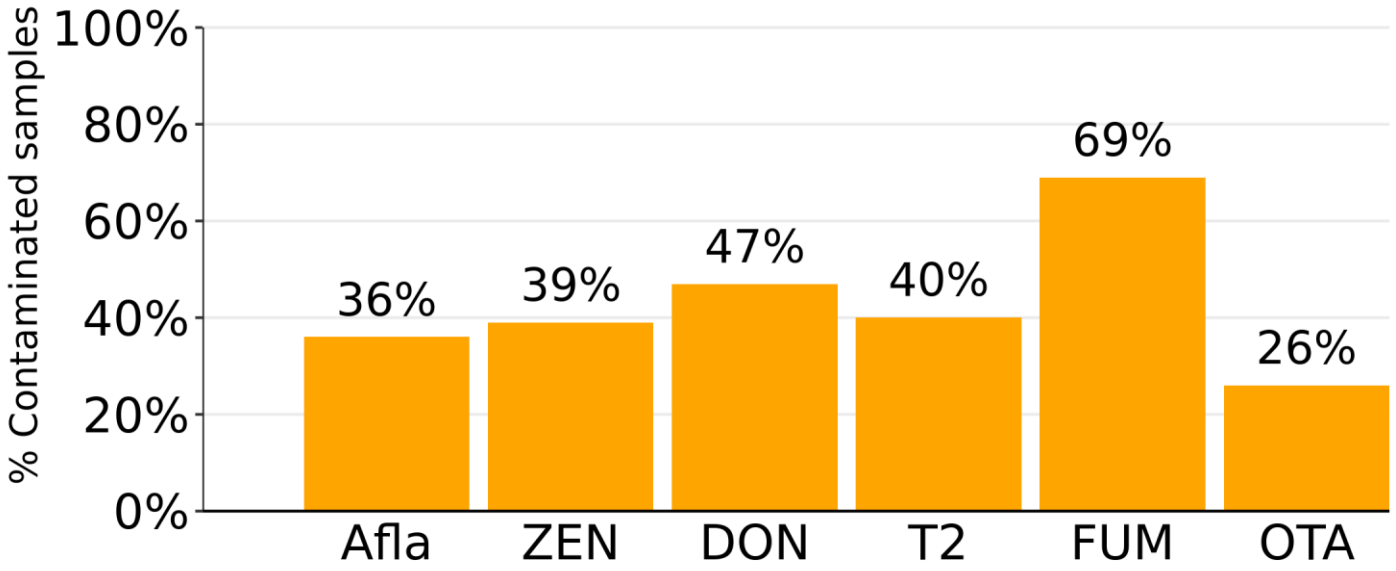
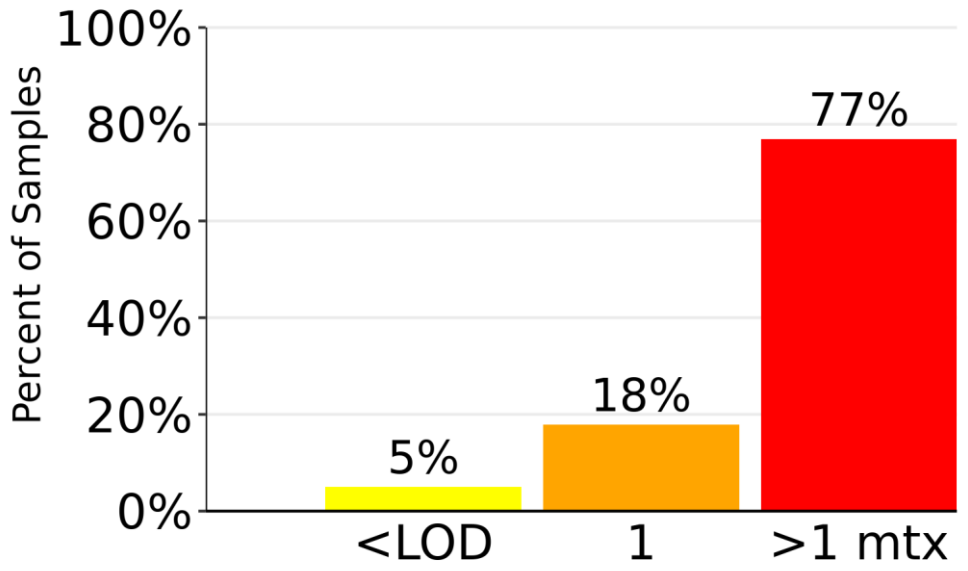
Corn kernels in Hungary from Jan 2023 to Sep 2023

Parameter	Afla	ZEN	DON	T2	FUM	OTA
Number of samples	39	38	38	40	35	39
% Contaminated samples	36%	39%	47%	40%	69%	26%
% Above risk threshold	36%	24%	47%	12%	60%	8%
Average of positives (ppb)	117	158	1951	64	1698	58
Median of positives (ppb)	11	63	1480	32	1032	6
Maximum (ppb)	1362	792	5826	231	7754	294

Prevalence of Mycotoxins Detected



No. of Mycotoxins per Sample



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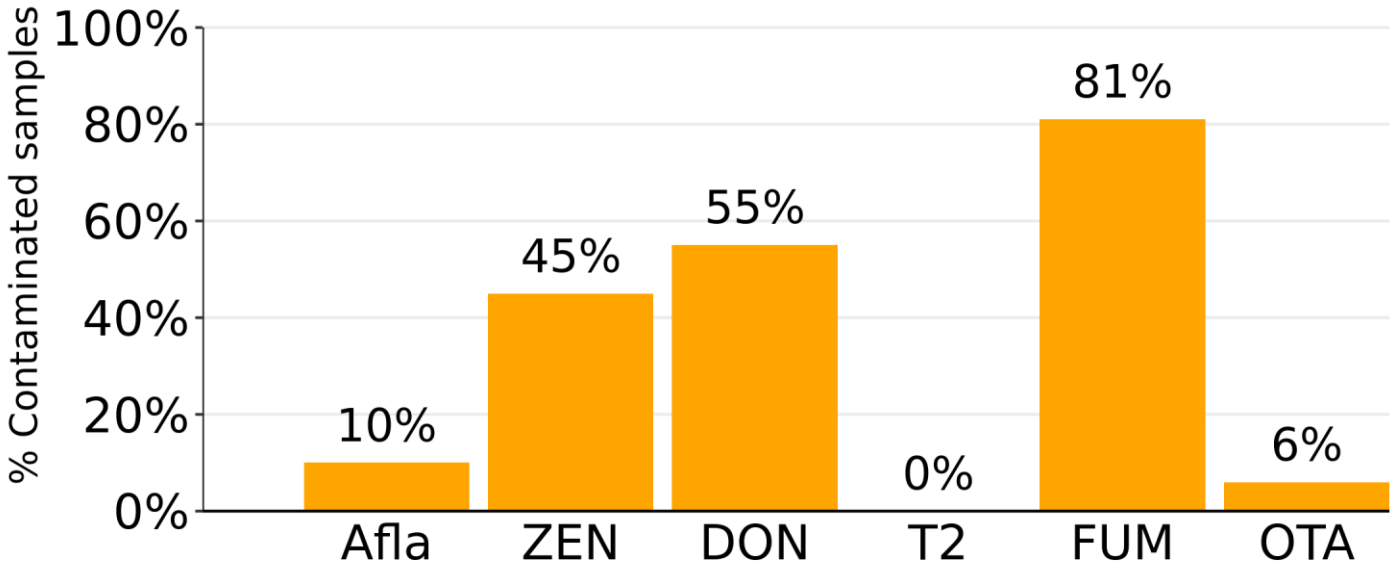
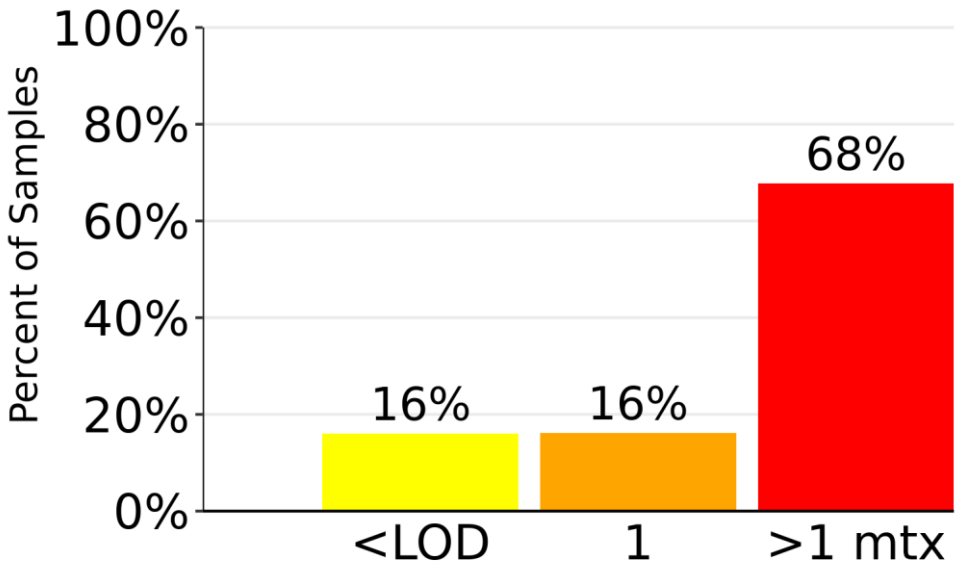
Corn Silage (all types) in Hungary from Jan 2023 to Sep 2023

Parameter	Afla	ZEN	DON	T2	FUM	OTA
Number of samples	31	31	31	31	31	31
% Contaminated samples	10%	45%	55%	0%	81%	6%
% Above risk threshold	6%	10%	35%	0%	26%	0%
Average of positives (ppb)	2	33	466		574	3
Median of positives (ppb)	3	23	233		297	3
Maximum (ppb)	3	107	1934	0	2956	3

Prevalence of Mycotoxins Detected



No. of Mycotoxins per Sample



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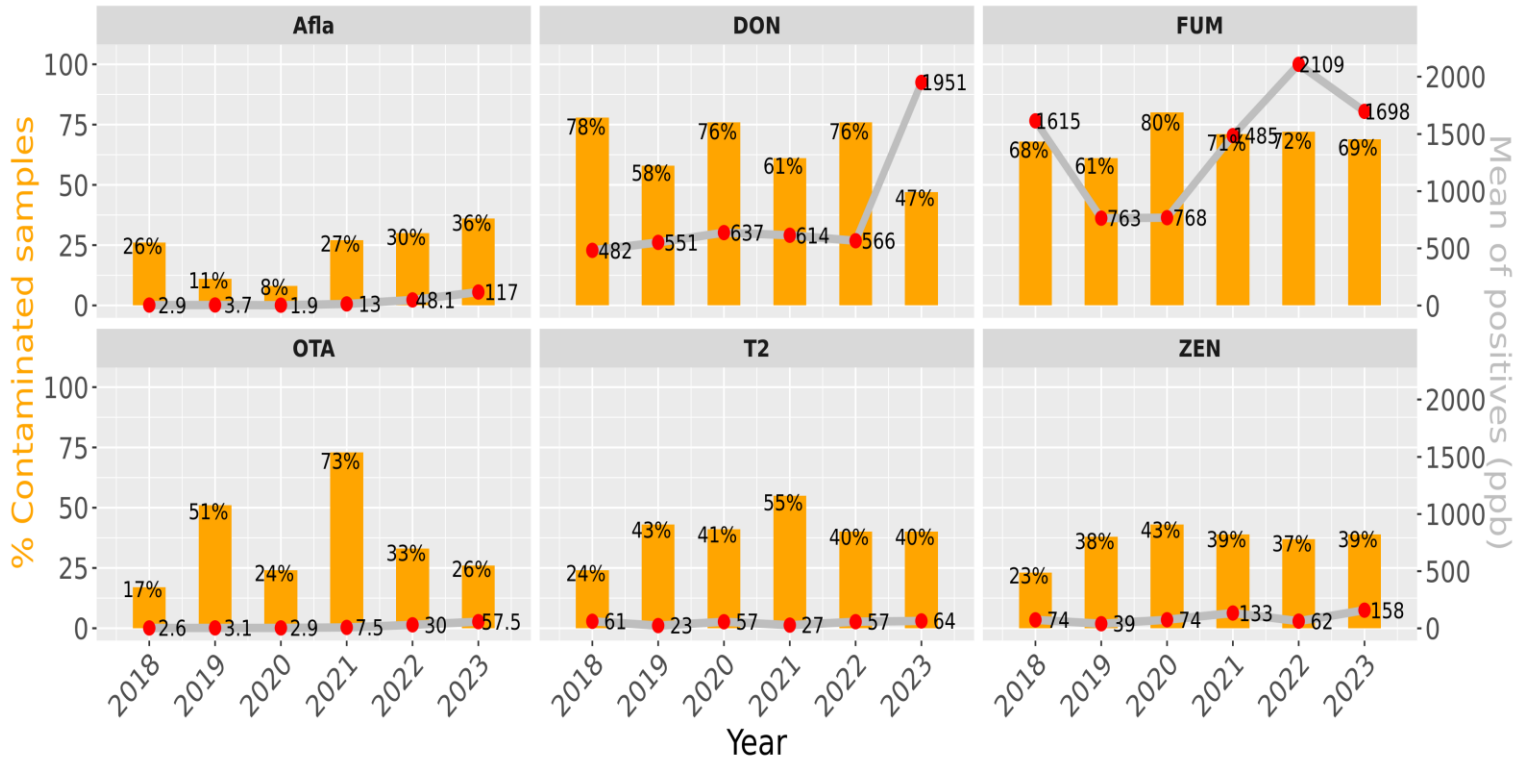
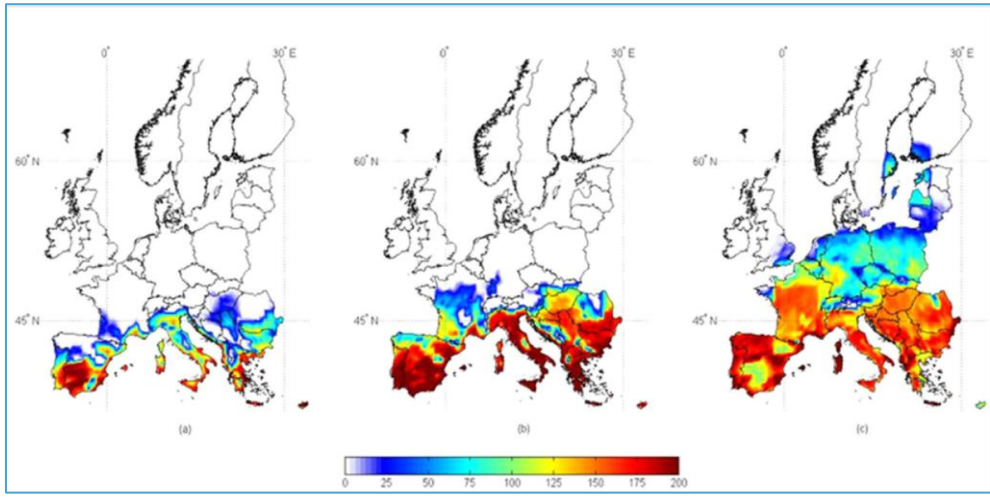
Trend for Corn kernels in Hungary from Jan 2018 to Dec 2023

prediction of aflatoxin contamination in maize and wheat crops, under a climate change scenario



Risk maps for aflatoxin contamination in maize at harvest in 3 different climate scenarios, present, +2 °C, +5 °C



Battilani *et al.* Nature, 2016. > Increased prevalence of Afla in EU at +2 or +5 degree Celsius.

Scientific report published: 12 April 2016

Authors:

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Wageningen University & Research

Antonio Moretti
Italian National Research Council

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1.3 Prediction data

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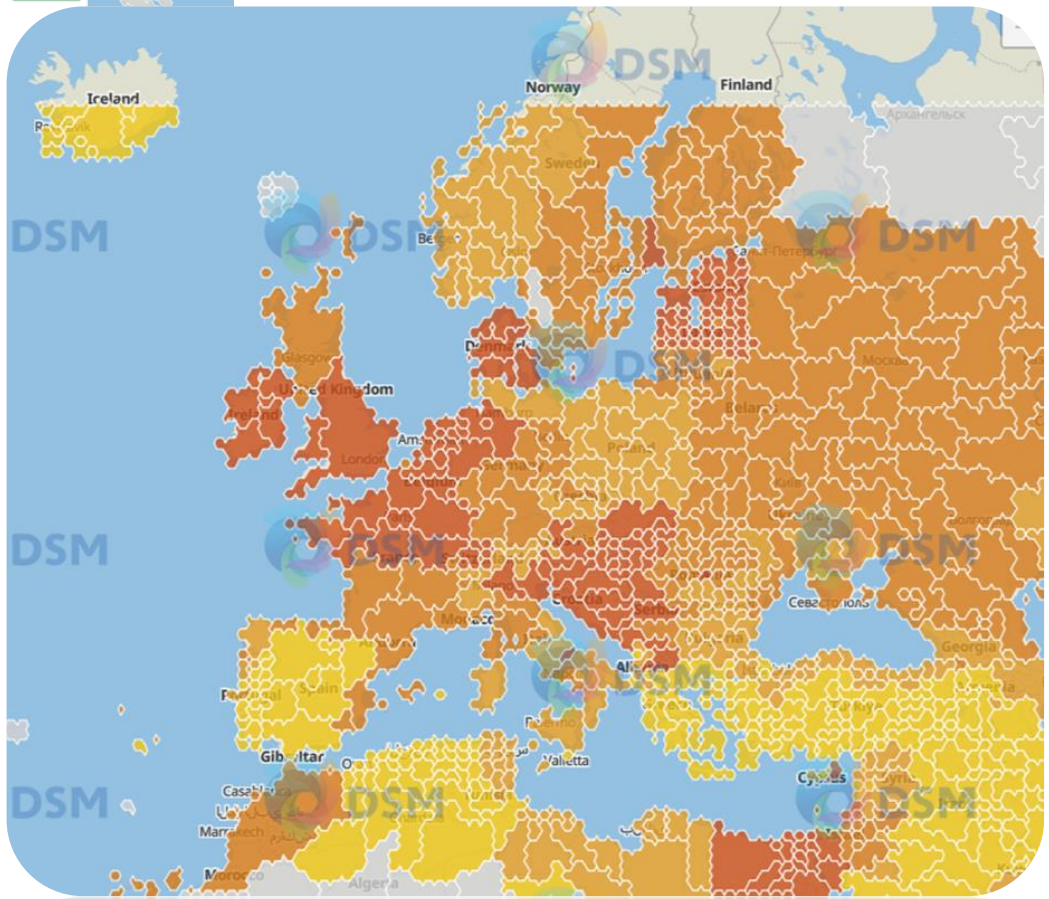
Mycotoxin Prediction Service

Biomin® mycotoxin prediction



- Since 2020
- Regional, (exporting) countries up to district level
  - **Corn**: DON, ZEN, FUM, AFLA
  - **Wheat**: DON, ZEN

UNIVERSITÀ CATTOLICA del Sacro Cuore

Battilani et al. (2003 & 2013)

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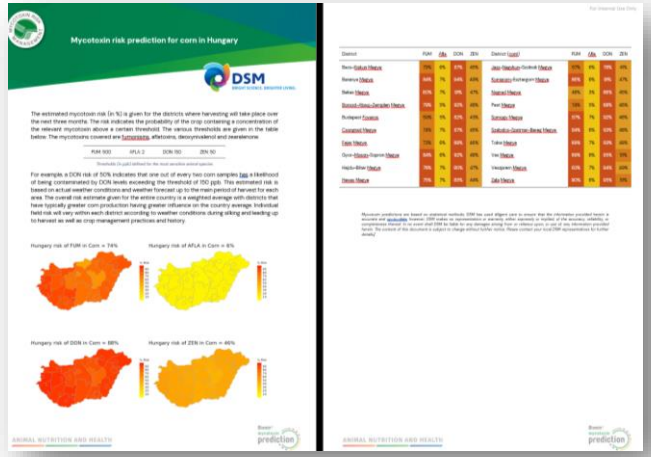
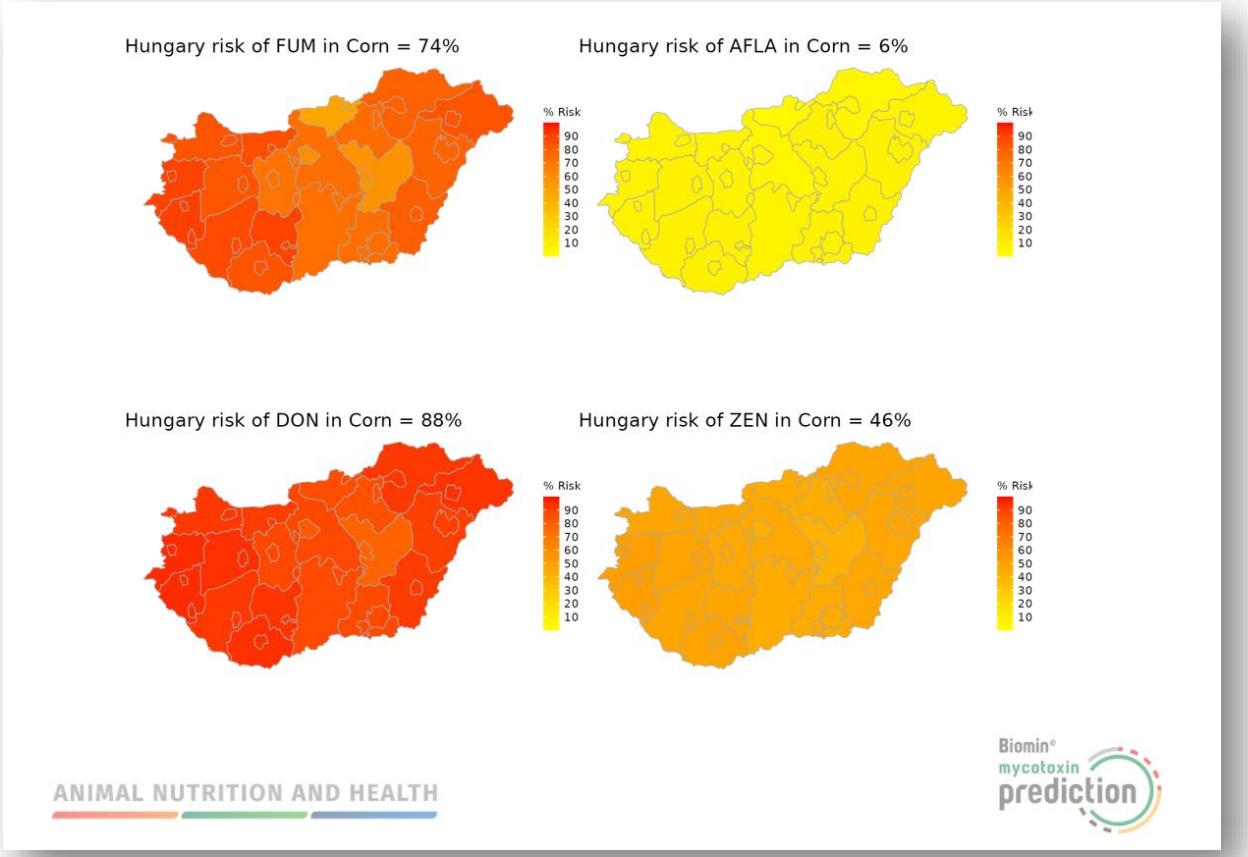
Mycotoxin Prediction Hungary

Corn, 4-9-2023





District	FUM	Afla	DON	ZEN	District (cont)	FUM	Afla	DON	ZEN
Bacs-Kiskun Megye	73%	6%	87%	45%	Jasz-Nagykun-Szolnok Megye	57%	6%	78%	41%
Baranya Megye	84%	7%	94%	49%	Komarom-Esztergom Megye	86%	6%	91%	47%
Bekes Megye	80%	7%	91%	47%	Nograd Megye	48%	3%	86%	45%
Borsod-Abauj-Zemplen Megye	79%	5%	92%	48%	Pest Megye	74%	5%	88%	46%
Budapest Fovaros	59%	5%	82%	43%	Somogy Megye	87%	7%	92%	48%
Csongrad Megye	74%	7%	87%	45%	Szabolcs-Szatmar-Bereg Megye	84%	6%	93%	48%
Fejer Megye	72%	6%	88%	46%	Tolna Megye	89%	7%	93%	48%
Gyor-Moson-Sopron Megye	84%	6%	92%	48%	Vas Megye	89%	6%	95%	51%
Hajdu-Bihar Megye	78%	7%	90%	47%	Veszprem Megye	83%	7%	94%	49%
Heves Megye	75%	7%	85%	44%	Zala Megye	90%	6%	95%	51%

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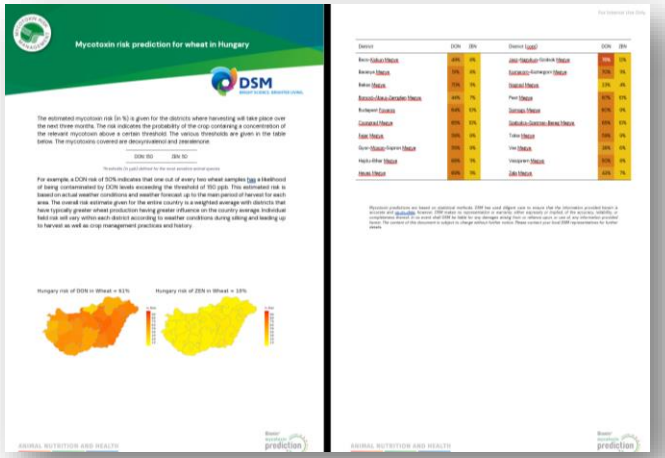
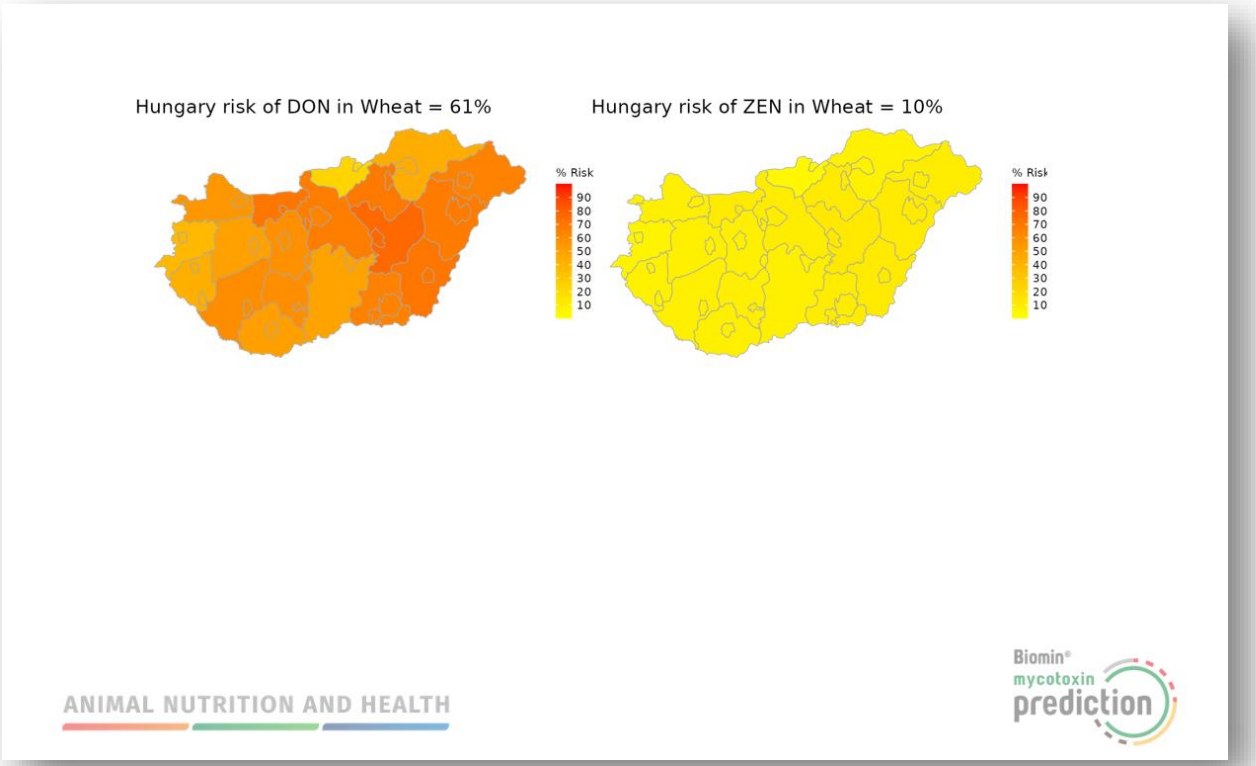
Mycotoxin Prediction Hungary

Wheat, 28-8-2023





District	DON	ZEN	District (cont)	DON	ZEN
Bacs-Kiskun Megye	49%	8%	Jasz-Nagykun-Szolnok Megye	76%	12%
Baranya Megye	51%	8%	Komarom-Esztergom Megye	70%	11%
Bekes Megye	70%	11%	Nograd Megye	23%	4%
Borsod-Abauj-Zemplen Megye	44%	7%	Pest Megye	67%	10%
Budapest Fovaros	64%	10%	Somogy Megye	60%	9%
Csongrad Megye	65%	10%	Szabolcs-Szatmar-Bereg Megye	65%	10%
Fejer Megye	58%	9%	Tolna Megye	59%	9%
Gyor-Moson-Sopron Megye	55%	9%	Vas Megye	38%	6%
Hajdu-Bihar Megye	68%	11%	Veszprem Megye	50%	8%
Heves Megye	69%	11%	Zala Megye	43%	7%

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Mycotoxin & (Future) Risk Exposure incl. young children

Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Food Science

Recent advances in assessing the effects of mycotoxins using animal models

Ana Flávia Furian[1,2,*], Michele R Fighera[3], Luiz Fernando F Royes[3] and Mauro S Oliveira[1]

Flurian *et al.* Food Science, 2022. > Broad consistent behavioral (CNS) impact.*

Exposure and Health
https://doi.org/10.1007/s12403-022-00514-z
ORIGINAL PAPER

Assessing the Mycotoxin-related Health Impact of Shifting from Meat-based Diets to Soy-based Meat Analogues in a Model Scenario Based on Italian Consumption Data

Octavian Augustin Mihalache[1] · Luca Dellafiora[1] · Chiara Dall'Asta[1]

Received: 3 July 2022 / Revised: 21 September 2022 / Accepted: 22 September 2022

Mihalache *et al.* Exposure and Health, 2022. > Disability adjusted life yrs meat (502) versus soy-based meat (12.080)**

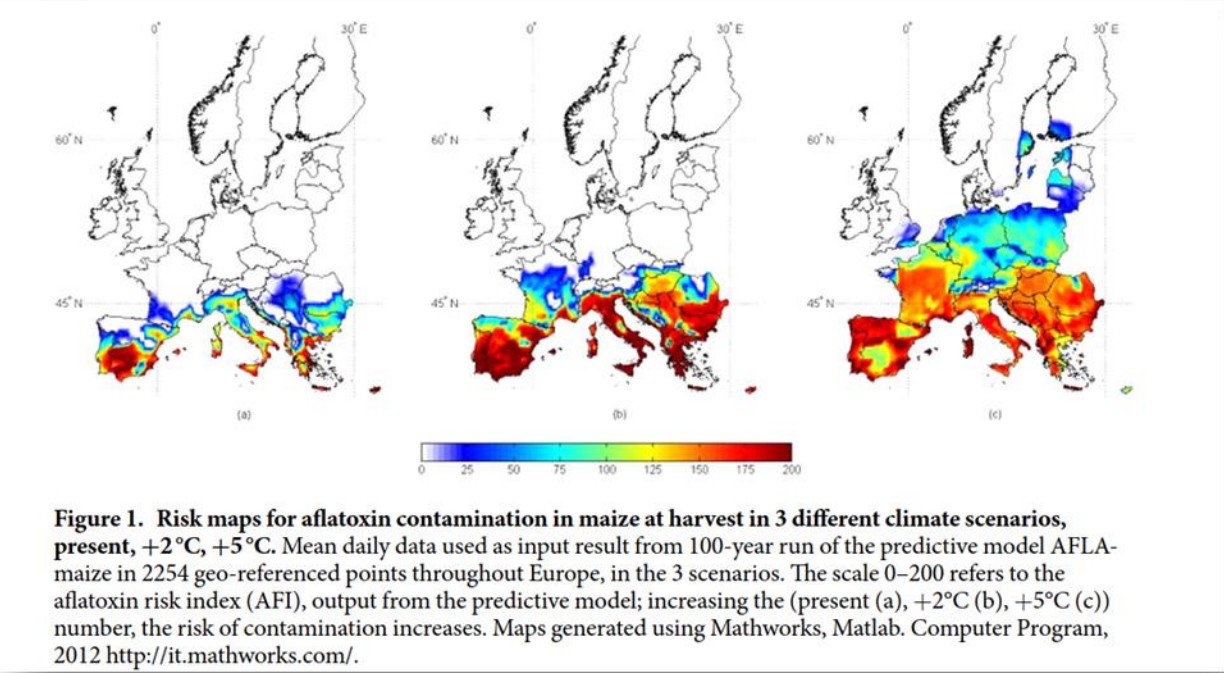


Figure 1. Risk maps for aflatoxin contamination in maize at harvest in 3 different climate scenarios, present, +2 °C, +5 °C. Mean daily data used as input result from 100-year run of the predictive model AFLA-maize in 2254 geo-referenced points throughout Europe, in the 3 scenarios. The scale 0–200 refers to the aflatoxin risk index (AFI), output from the predictive model; increasing the (present (a), +2°C (b), +5°C (c)) number, the risk of contamination increases. Maps generated using Mathworks, Matlab. Computer Program, 2012 http://it.mathworks.com/.

Battilani *et al.* Nature, 2016. > Increased prevalence of Afla in EU at +2 or +5 degree Celsius.

Toxicology Letters 277 (2017) 69–75
Contents lists available at ScienceDirect
Toxicology Letters
journal homepage: www.elsevier.com/locate/toxlet

Daily uptake of mycotoxins – TDI might not be protective for nursed infants

G.H. Degen[a], F. Partosch[b], K. Muñoz[c], U. Gundert-Remy[d,*]

Degen *et al.* Toxicology Letters, 2017. Nursed infants 29.2 fold above set TDI for OTA > impair kidney function

Dietary exposure to mycotoxins of 1- and 2-year-old children from a Dutch Total Diet Study

A.M. Pustjens[1], J.J.M. Castenmiller[2], J.D. te Biesebeek[3], T.C. de Rijk[1], R.C.J. van Dam[1] and P.E. Boon[3*]

[1]Wageningen Food Safety Research (WFSR), Wageningen University and Research, P.O. Box 230, 6700 AE Wageningen, the Netherlands; [2]Netherlands Food and Consumer Product Safety Authority (NVWA), P.O. Box 43006, 3540 AA Utrecht, the Netherlands; [3]National Institute for Public Health and the Environment (RIVM), P.O. Box 1, 3729 Bilthoven, the Netherlands; polly.boon@rivm.nl

Received: 8 December 2020 / Accepted: 23 March 2021

Pustjens *et al.* World Mycotoxin Journal, 2022. > In NL 1 to 2 yrs old children, look at 95 percentile, mycotoxin intake (AF, Alternaria, OTA, T-2/HT-2) may pose health risk based on HBGV or MOE.

2.0 Mycotoxins as a predisposing factor in animal diseaseas and production

„ If there is an issue with animal most probably it is at Gut level..”

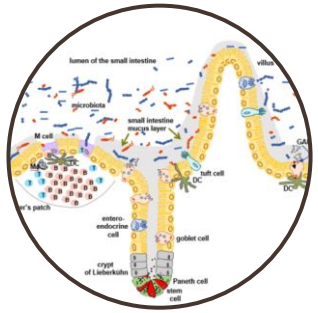
„The Good Gut health is the ability of the animal to adapt into the environmental stresses, that is put under .
The ability to reach 100% of its genetic potential.
The central check point of the healthy animal.

The no. 1 factor is diet
as the animal need to eat to grow and to perform”.

Dr. Michel Kogut, Microbiologist
Food and Feed Safety Research

Michael KOGUT, Research Microbiologist | Cited by 7909 | of United States Department of Agriculture, District of Columbia (USDA) | Read 270 publications

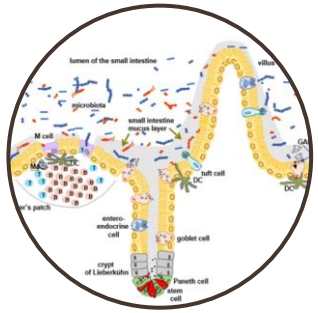
The first site of Mycotoxins contact in animal is the Gut and the Gut microbiota



Gut microbiome

Antimicrobial effect

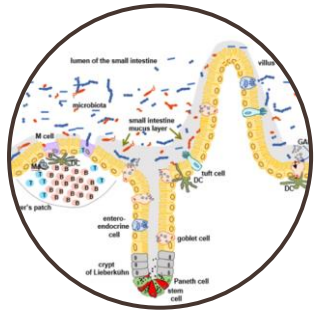
Mucus production



Gut integrity

Permeability increase

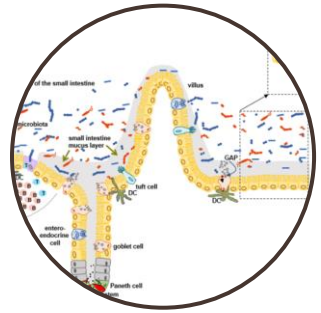
Pathogens translocation



Gut absorption

Villi reduction & damage

Transporter interference



Gut immunity

Antibody production suppress

Mucus immune respond suppress

Mycotoxin effects

The gut epithelium a selective barrier, facilitates the transport of molecules (through or between cells). Any damage results in increased permeability of the layer, leading to intestinal disorders.

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Ruminal mycotoxin degradation

Mycotoxin	Degradation in the Rumen	No Degradation in the Rumen
Aflatoxin	0 - 42 % More toxic Aflatoxicol *(Engel and Hagemeister, 1978)*	58 – 100 %
Zearalenone	50% α- and β-Zearalenol *(Gruber-Dorninger et al., 2021)*	50 % metabolites more estrogenic
Trichothecenes	15 % - 99 % DOM-1 *(Cote et al., 1986; Kiessling et al., 1984, Debevere, 2020)*	1 - 85 % pH dependent
Ochratoxin A	90-100% (Mobashar et al, 2010)	0-10%
Fumonisin	No degradation (EFSA, 2018)	Unknown, no reported oral bioavailability
Enniantin B	1-25% (Debevere et al, 2020)	75%-99%

AFLA Reduced animal health, performance, reproduction, weight loss, liver damage, decreased milk yield, (Whitlow L.W., Hagler)

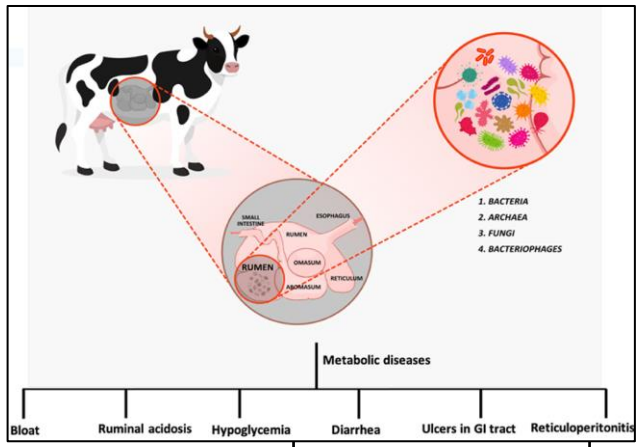
ZEN reproduction problems, reduced CR, ovarian Cysts (Mahmoud et al., 2013)

DON Iimmunosuppression, reduced growth rate, reproductive disorders, feed refusal, vomiting (Rocha O., Ansari K. 2005)

OTA Nephrotoxic, hepatotoxic, teratogenic, carcinogenic. (Yang S., Zhang H. 2015)

FUM Liver, kidney lesion damage (Gurung N., Rankins D)

Ruminal mycotoxin degradation



Rumen microbiota can break down mycotoxin…

1. Mycotoxin have antimicrobial properties

(FinkGremmels, 2008; Strickland et al., 2011)

2. Rumen degradation can lead to more toxic metabolites

(DeLorme et al. 2007).

3. Divers' diet higher risk of mycotoxin

(Fink-Gremmels, 2008)

4. Rumen environment / pH dependent mycotoxin degradation

(A. Gallo 2017)

5. Mycotoxins alters rumen microbiota

(Q. Zebali, at all 2020)

High feed intake increase passage rate
Diagnostic challenge in multiple ingredients
Emerging & Mask mycotoxins detection issue
Interactions between mycotoxin additive, synergistic,
Specific & nonspecific symptoms

Diversified diet for ruminants

Feed	Possible mycotoxins present
Concentrates	Aflatoxins, FUM, ZEN, DON, Trichothecenes, ergot alkaloids
Silages	DON, ZEN, FUM, Patulin, mycophenolic acid, roquefortines, fumitremorgens, cerruculogen, monacolines, etc…
Forages	Alternaria, Cyclopiazonoic acid, DON, other thrichothecenes, Mycophenolic acid, roquefortines, etc….
By-products	Alfatoxin, ZEN, FUM, DON, T-2, HAT-2

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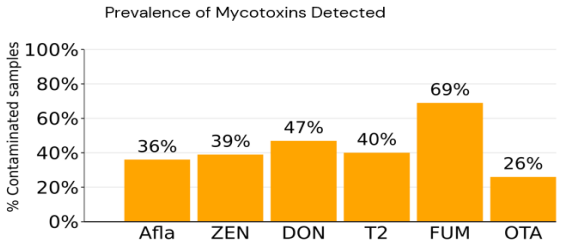
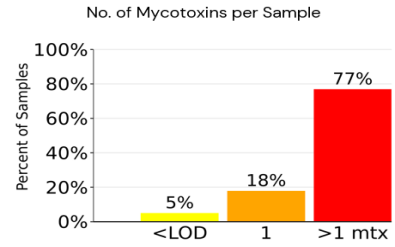
Maize silage main diet component >30% in TMR
Maize based diet ingredients >50% in the TMR

1794ppb x8kg =14.352ppb +4kgCR x1480>20ppM Trich.B/cow/day

Corn kernels in Hungary from Jan 2023 to Sep 2023

Parameter	Afla	ZEN	DON	T2	FUM	OTA
Number of samples	39	38	38	40	35	39
% Contaminated samples	36%	39%	47%	40%	69%	26%
% Above risk threshold	36%	24%	47%	12%	60%	8%
Average of positives (ppb)	117	158	1951	64	1698	58
Median of positives (ppb)	11	63	1480	32	1032	6
Maximum (ppb)	1362	792	5826	231	7754	294





MYCOTOXIN RISK MANAGEMENT

spectrum TOP 50

DSM
BRIGHT SCIENCE. BRIGHTER LIVING.

Client: DSM Nutritional Products Hungary Kft.
Address: Hungary 2367 Ujhartyan Japan fasor 4
Species: Unspecified
DSM contact person: Norbert Revesz

Date: 01 March 2023

Spectrum Top 50: Multi – Mycotoxin Analysis AT-30196

The following tables give an overview on the positively identified mycotoxins and the respective concentrations (ppb = µg/kg). In case of high moisture content (silages, TMR), samples are dried prior to analysis and results are based on the dried weight of the sample.

Table 1 - Mycotoxin analysis results of samples and interpretation for the species indicated

Sample ID	Sample Type	Species	Afla (µg/kg)	ZEN + Metabolites (µg/kg)	Type B Trichos (µg/kg) e.g. DON	Type A Trichos (µg/kg) e.g. T-2	FUM (µg/kg)	OTA (µg/kg)	Ergot Alkaloids (µg/kg)
AT-30196-001	Corn silage	Unspecified	nd	nd	nd	nd	1164	nd	nd
AT-30196-002	Corn silage	Unspecified	nd	93	2350	nd	297	nd	nd
AT-30196-003	Corn silage	Unspecified	nd	50	1114	nd	83	nd	nd
AT-30196-004	Corn silage	Unspecified	nd	60	1794	nd	2956	nd	nd

Explanation of Table

Feature	Explanation
	Low risk for species type for major mycotoxins (Unspecified: Afla <2, ZEN <50, Type B <150, Type A <50, FUM <500, OTA <10, Ergot <30)
	Medium risk for species type for major mycotoxins (Unspecified: Afla 2-<4, ZEN 50-<100, Type B 150-<200, Type A 50-<100, FUM 500-<1000, OTA 10-<100, Ergot 30-<300)
	High risk for species type for major mycotoxins (Unspecified: Afla ≥4, ZEN ≥100, Type B ≥200, Type A ≥100, FUM ≥1000, OTA ≥100, Ergot ≥300)
	For tables below some metabolites have no defined low, medium and high values. These are colored according to comparison with all previous results:
	Below the median of positive values for all previously tested samples
	Above the median of positive values for all previously tested samples
	In top 10% of the median of positive values for all previously tested samples
	For values detected below the Limit of Quantification (LOQ), LOQ/2 is shown
nd	not detected (below the Limit of Detection)

Sample: AT-30196-002 Unspecified (Corn silage) R601 Corn silage

Substance	Value (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	Description
Zearalenone and metabolites				(zearalenone, alpha-zearalenol, beta zearalenol, zearalanol)
Zearalenone	92.9	3	10	Zearalenone is estrogenic, acting like the sex hormone estradiol thereby interfering with fertility and sexual development of animals. It is also hepatotoxic, hematotoxic, immunotoxic and genotoxic.
Type B trichothecenes				(deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, DON-3-glucoside, nivalenol, 15-acteoxyscirpenol, fusarenon X)
Deoxynivalenol	1933.9	15	50	Deoxynivalenol induces emesis and feed refusal resulting in reduced weight gain. Other effects include immunotoxicity, hematotoxicity and myelotoxicity, as well as reproductive toxicity. It furthermore causes intestinal lesions and compromises the intestinal barrier function.
DON-3-glucoside	63.7	15	50	Deoxynivalenol-3-glucoside is a masked mycotoxin. It is converted back to deoxynivalenol in the gastrointestinal tract of mammals.
Nivalenol	352.4	25	75	Nivalenol induces emesis and feed refusal resulting in reduced weight gain. Other effects include immunotoxicity, hematotoxicity, reproductive toxicity and to kidneys and the gastrointestinal tract.
Fumonisins				(fumonisin B1, fumonisin B2, fumonisin B3)
Fumonisin B1	204.4	10	30	Fumonisins are hepatotoxic and nephrotoxic. High fumonisin doses cause the species specific fatal diseases porcine pulmonary edema in pigs and equine leukoencephalomalacia in horses. Fumonisin B1 has been classified as a group 2B carcinogen (possibly carcinogenic to humans) by the International Agency for Research on Cancer. Fumonisins were shown to be immunotoxic and to compromise gut health. They furthermore exert reproductive toxicity.
Fumonisin B2	92.2	20	60	
Alternaria Toxins				(alternariol)
Alternariol	36.3	5	15	Alternariol showed no acute toxicity in published studies in animals. However, alternariol was cytotoxic, genotoxic and mutagenic to mammalian cell lines in vitro. Furthermore, negative effects of alternariol on the reproductive and immune system have been suggested by in vitro results.
Beauvericin and Enniatins				(beauvericin, enniatin A, enniatin A1, enniatin B and enniatin B1)
Beauvericin	46.5	2	6	Beauvericin and enniatins were toxic to different mammalian cell lines in vitro. According to published studies, acute exposure to beauvericin and enniatins was not toxic to animals. However, the effect of chronic exposure is currently unknown. According to the results of in vitro studies, beauvericin and enniatins may affect the immune system and the bioavailability of pharmaceuticals. Beauvericin and enniatins were shown to accumulate in the eggs of laying hens, but detected levels were likely no cause for concern.
Enniatin B	7.1	1.5	5	
Enniatin B1	5.0	1.5	5	

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Animal respond to mycotoxin

Mycotoxins impact on ruminants reported from dairy practice

Feeding of mycotoxin-contaminated materials may lead to:

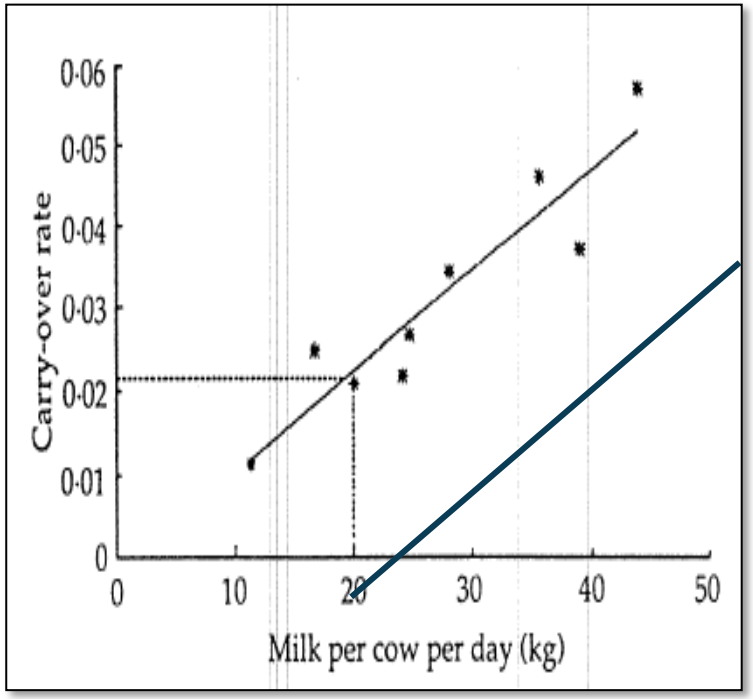
- Reduce feed intake
- Prolonged feeding time,
- Reduced rumen fill,
- Negative energy balance
- Gastrointestinal upset
- Poor feed digestion and conversion,
- Altered milk production or components
- Reproduction issues as early abortions

Mobilization of nutrient and fat stores that influence body condition and health.
Challenge ruminal microflora due to their antimicrobial, anti-protozoal and antifungal activity.

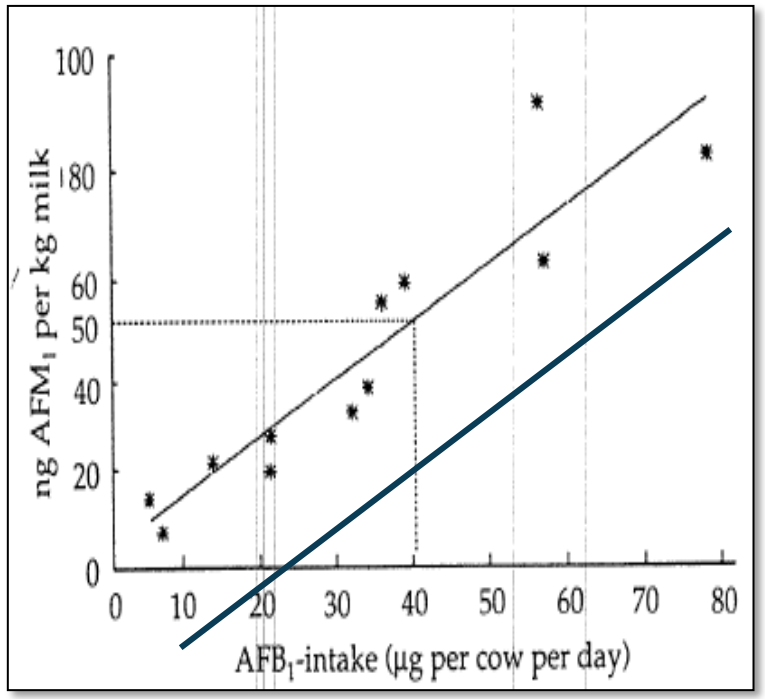
Aflatoxins – Carry Over into Milk

Carry-over rate of aflatoxin B_1 as aflatoxin M_1 varies from **1 to 6%** and increases linearly with:

Milk yield of the animal



Aflatoxin daily intake per animal



(Veldman et al, 1992)

Aflatoxins have the highest acute and chronic toxicity of all mycotoxins

Carry over % increased with modern genetics (1-2 % to above 8,6 %) Prof. Fink- Gremmels.

Negative affect on production, immune system and rumen metabolism in cattle (Hussein and Brasel, 2001)

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DON affected immune function of cows

Effects of feed naturally contaminated with Fusarium mycotoxins on metabolism and immune function of dairy cows, University of Guelph

J. Dairy Sci. 92:1585–1593
doi:10.3168/jds.2008-1267

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

S. N. Korosteleva,* T. K. Smith,* and H. J. Boermans†
*Animal and Poultry Science Department, Ontario Agriculture College, and
†Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Table 2. Mycotoxin content of feedstuffs and experimental diets (mg/kg)

Mycotoxin[1]	Corn	Wheat	Hay	Corn silage	Control TMR	Contaminated TMR
DON	7.5	2.4	—	0.31	0.5	3.5
15-acetyl-DON	0.9	—	—	—	—	—
ZEN	0.7	—	0.31	—	—	—

Effect of diets on neutrophil phagocytotic activity (%; overall means)

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

[1]Least squares means.
*$P < 0.05$.

Effect of diet on antibody response to ovalbumin (optical density)

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

[1]Least squares means.
*$P < 0.05$.

BW 630 kg, Milk 36 kg. DIM 90 – 150

Significantly decreased neutrophil phagocytosis in cows fed mycotoxin contaminated feed .
Increase primary IgG antibody response to OVA in animals fed the contaminated diet .
Trichothecenes posses ability to up- and down-regulate immune function

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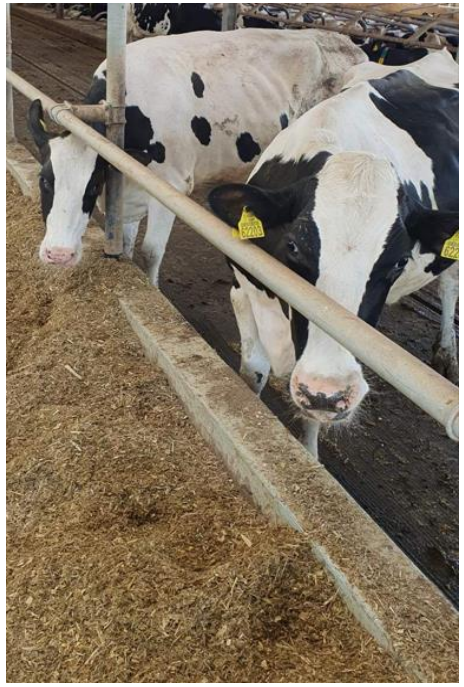
DON reduce feed intake

Induces release of anorexia mediators

DON
↓
CCK
↓
Satiety
↓
Feed Intake

DON induces the release of the satiety hormones, including cholecystokinin (CCK), which are critical mediators of anorexia



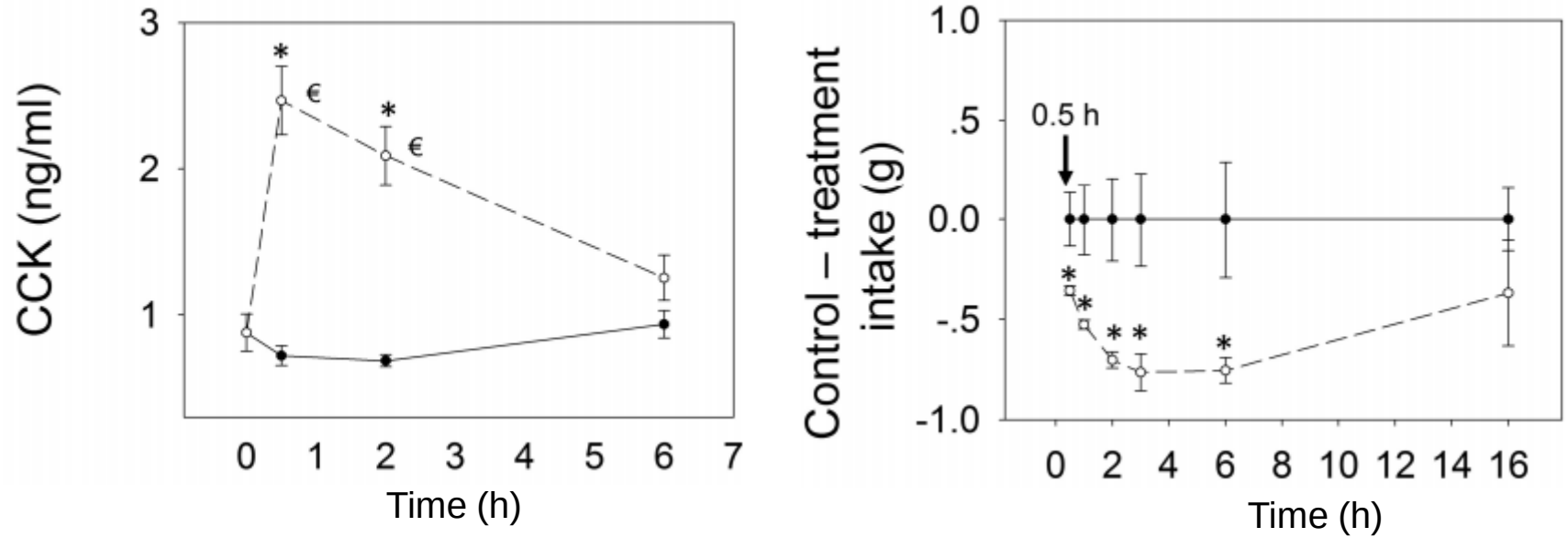


Figure. Relationship of DON exposure (-----) and a control diet (——) to the anorectic response (decrease in feed intake) mediated by CCK.

Wu et al., 2014; Toxicological Sciences

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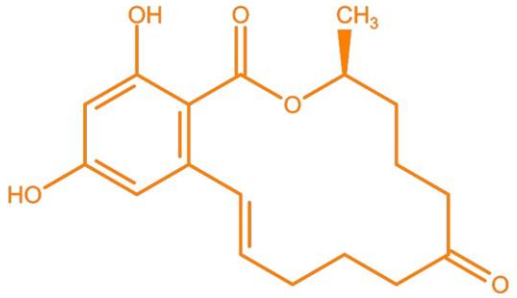
Zearalenone alters animal hormonal status



- Fusarium spp. produced mycotoxin
  (e.g. Fusarium graminearum, Fusarium culmorum)
- Different effects:
  - Antimicrobial
  - Induces oxidative stress
  - Inflammatory
  - **Estrogen's receptors binding**

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

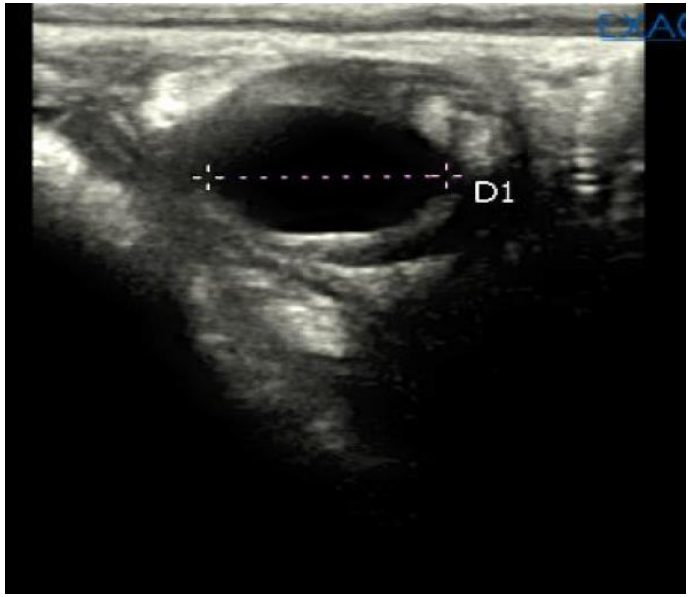
It's degraded by ruminal microbiota, BUT:

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

Zearalenone induced follicular cysts

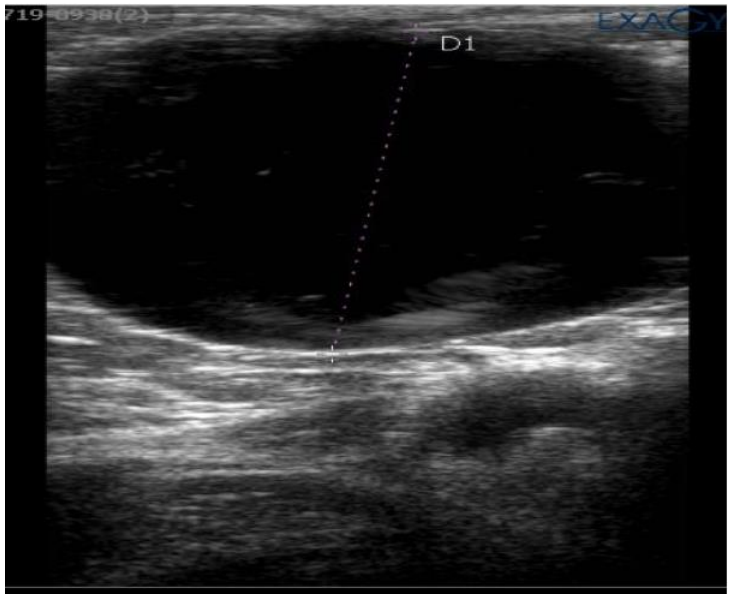
200 ppb ZEN

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**

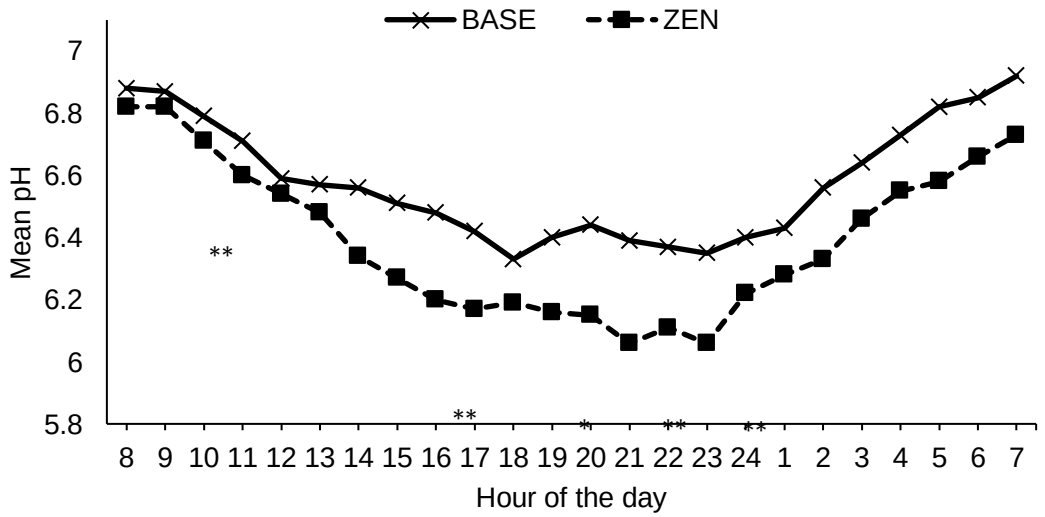
ZEN lowers rumen pH, alters rumen fermentation

Short-term exposure to zearalenone or fumonisins affects rumen fermentation and microbiota, and health variables in cattle, Hartinger et al. 2021

Hartinger, Thomas Dietmar Said, Dr.agr.
Institut für Tierernährung und funktionelle Pflanzenstoffe
E-Mail: Thomas.Hartinger@vetmeduni.ac.at
Zur Visitenkarte

Zebeli, Qendrim, Univ.-Prof. Dr.sc.agr.
Institut für Tierernährung und funktionelle Pflanzenstoffe
Institutsleitung
Veterinärplatz 1
1210 Wien
E-Mail: Qendrim.Zebeli@vetmeduni.ac.at
Zur Visitenkarte

pH:
ZEN decreased mean ruminal pH and the minimal pH
ZEN decreased hourly mean pH between 3pm and 7am



** p<0.05
* p<0.1

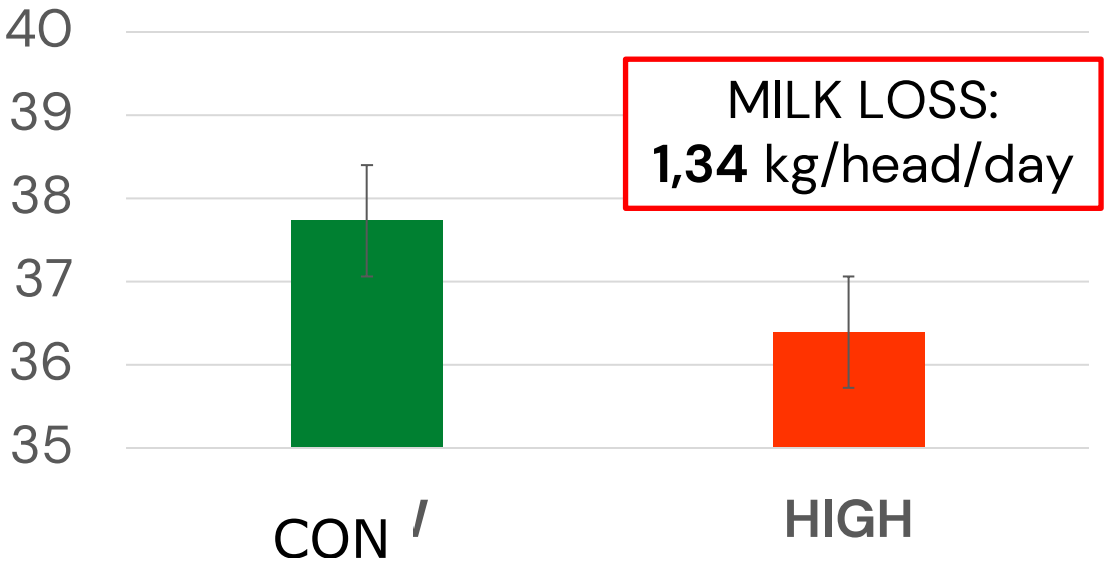
SCFA:
SCFA in total lowered in ZEN treatment at 0h and 3h
ZEN lowered Acetate concentration

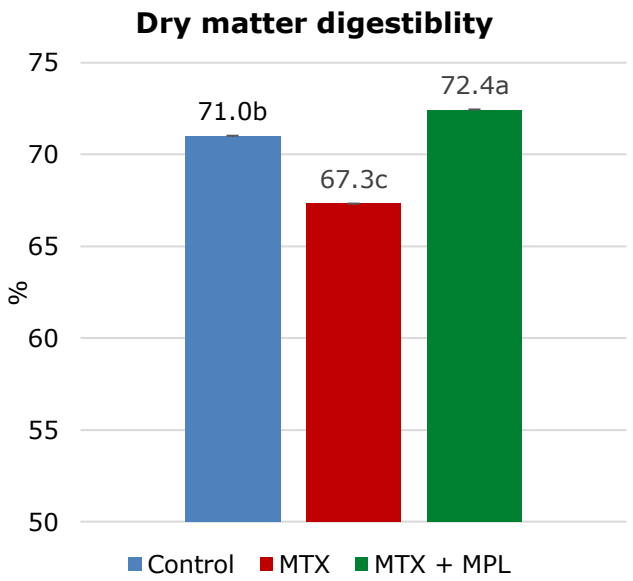
	BASE			ZEN		
	*	**	**			
Item	0 h	3 h	10 h	0 h	3 h	10 h
Total SCFA (mmol/L)	85.5^{A}	107.1^{A}	103.1	68.6^{Ba}	72.7^{Bab}	104.7^{b}
Acetate (%^{2})	63.5^{B}	62.7	63.1	66.8^{Aa}	64.1^{ab}	61.4^{b}
Propionate (%)	19.2	21.1	20.8	17.4	19.6	21.3
n-Butyrate (%)	12.9	11.5	10.9	11.7	10.9	12.4
n-Valerate (%)	1.27	1.56	1.60	0.94	1.65	1.60
Caproate (%)	0.62	0.94	1.33	0.51	1.09	1.07
Isobutyrate (%)	1.14	0.95	0.86	1.24	1.08	0.85
Isovalerate (%)	1.35	1.20	1.32	1.34	1.43	1.15

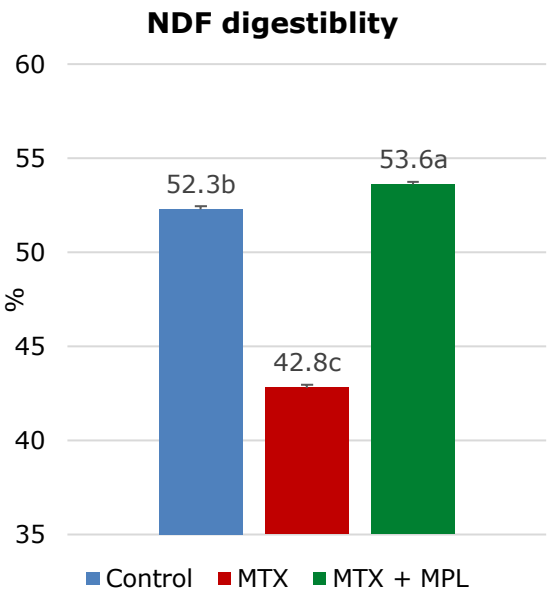
DON & FUM effects on health and performance Mycofix conteraction

A mycotoxin deactivating feed additive counteracts the adverse effects of low-level Fusarium mycotoxins in dairy cows
Significant reduction in milk yield after 21 days exposure to MTX (Antonio Gallo *et al.*, 2020)

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







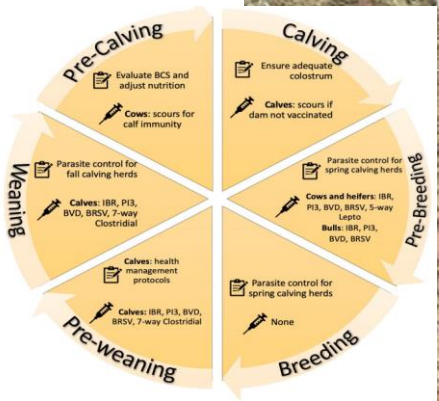
Mycotoxins, predisposing factor for intestinal and systemic infectious diseases

(Antonissen et al. 2014, 2015; Bouhet and Oswald 2005; Gallo et al. 2015; Pinton and Oswald 2014)

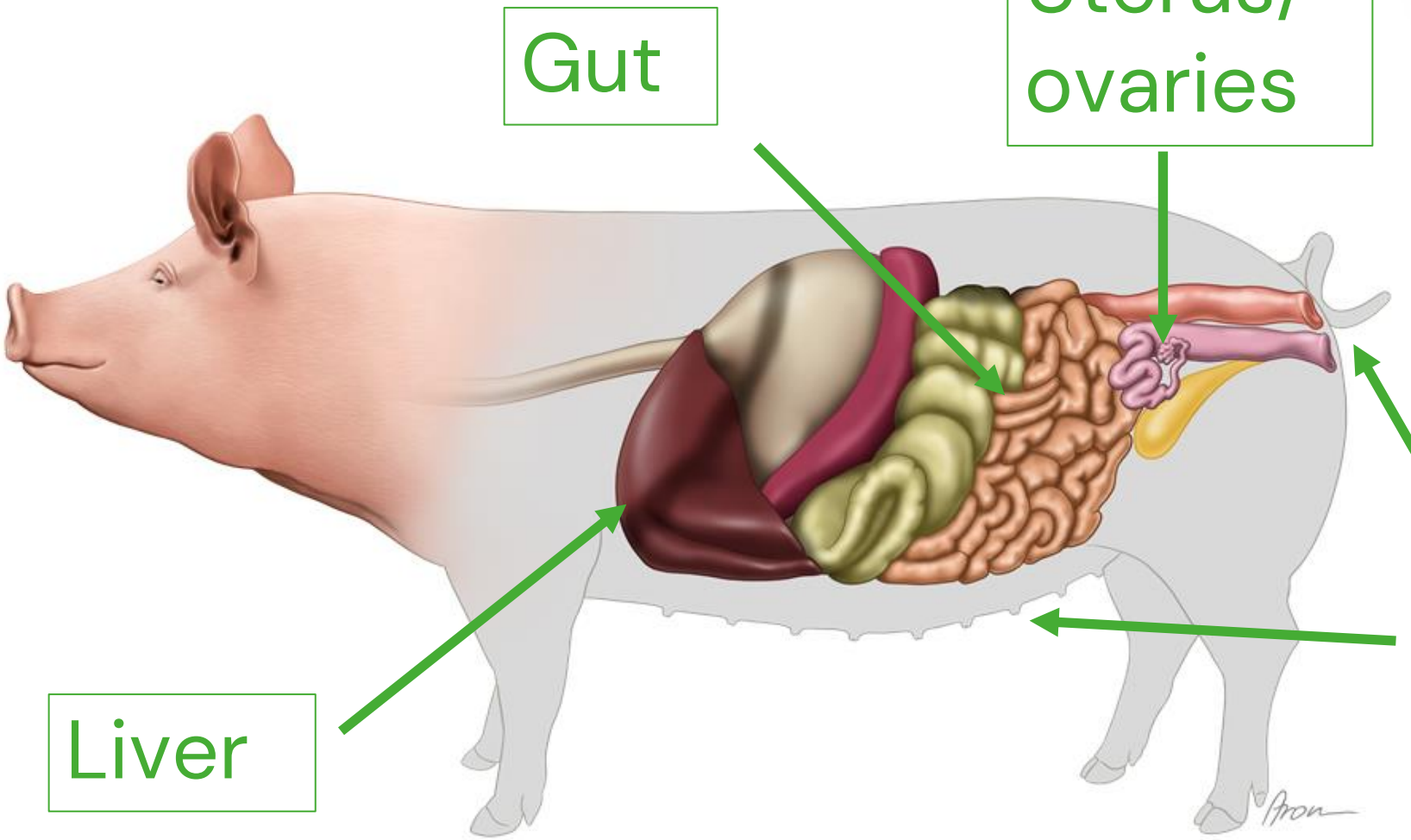
The presence of mycotoxins in feed is a predisposing factor for :

- The occurrence of diseases
- Treatment-related complications
- Failure of ongoing vaccinations,
- Reduced productivity and
- Reproductive problems.

MTX prevention, part of disease prevention



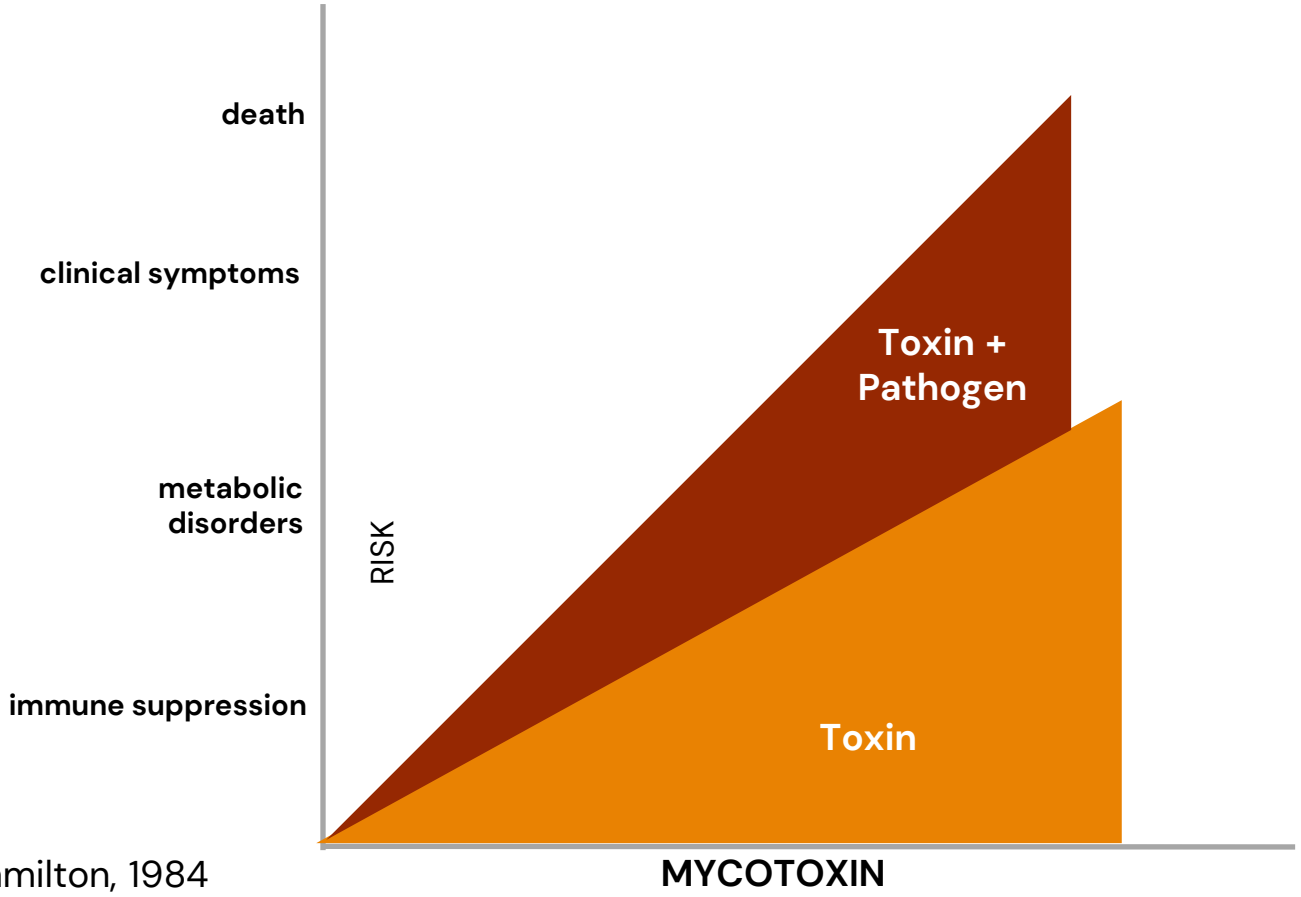
The four main organs sensitive to mycotoxins



Mycotoxin-Related Problems

There are no safe levels: major effects can be observed in the immune system at low mycotoxin levels

Severe economic losses!



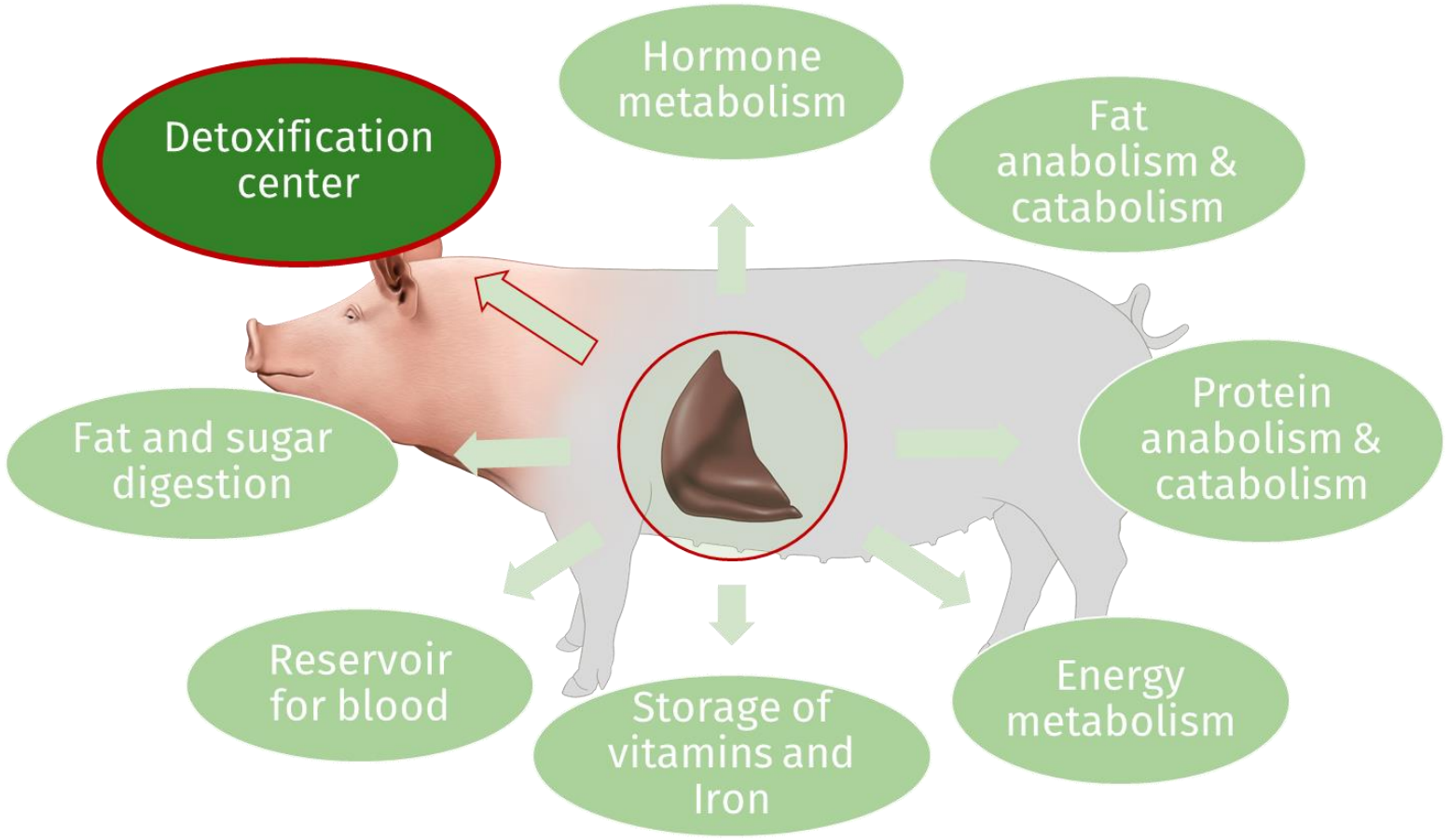
Hamilton, 1984

Consequences of immune suppression

- increased risk of infections
- more severe disease processes
- therapies become more difficult
- impaired vaccination response
- activation of tumor formation

Effects of mycotoxins on the liver

- *Impair the detoxification functions of the liver through histological changes*



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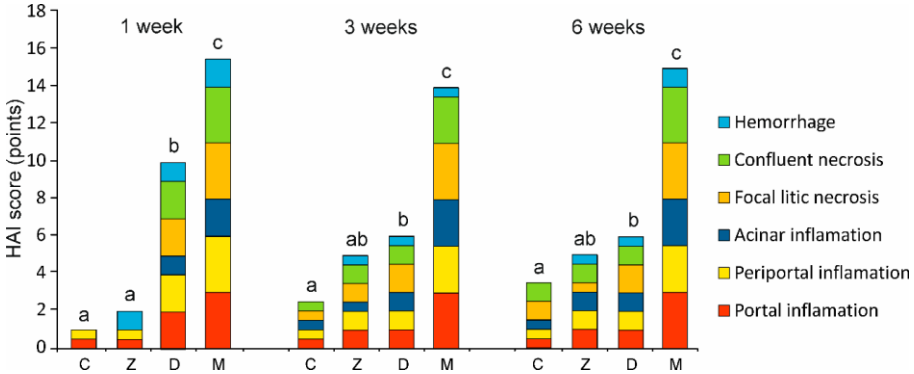
Problems on Farm & Associated Cost

Sow longevity

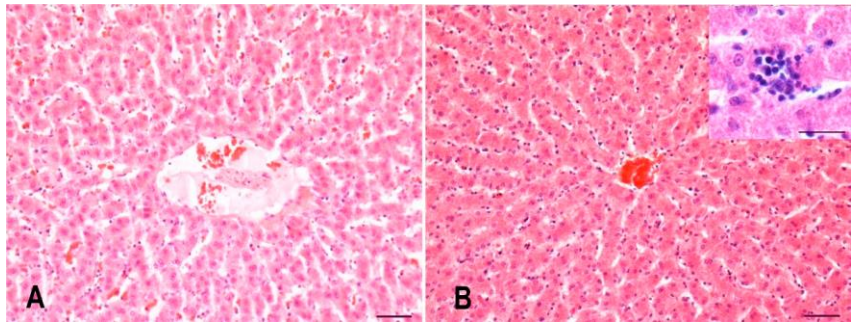


DON and ZEN affect the ultrastructure and histology of pig liver. Histopathological score of the examined livers according to the histology activity index (HAI). *Skiepko et al., 2020*

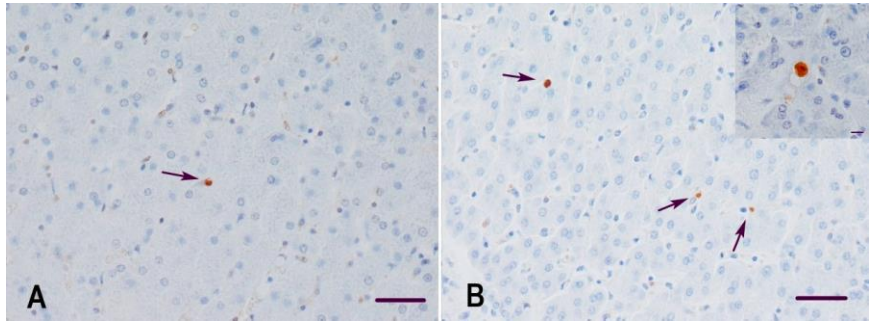
Mycotoxins are targeting major organs and tissues, affecting their functionality and integrity

- *Organ and tissues disfunction can lead to mortality and involuntary culling*

Dolenšek et al., 2021



Sinusoidal leukocytosis (Increased numbers of leukocytes) in pregnant gilts fed **mycotoxins** (B), indicating **inflammatory process in liver**



Pregnant gilts fed mycotoxins (B) had increased number of **apoptotic cells in liver**

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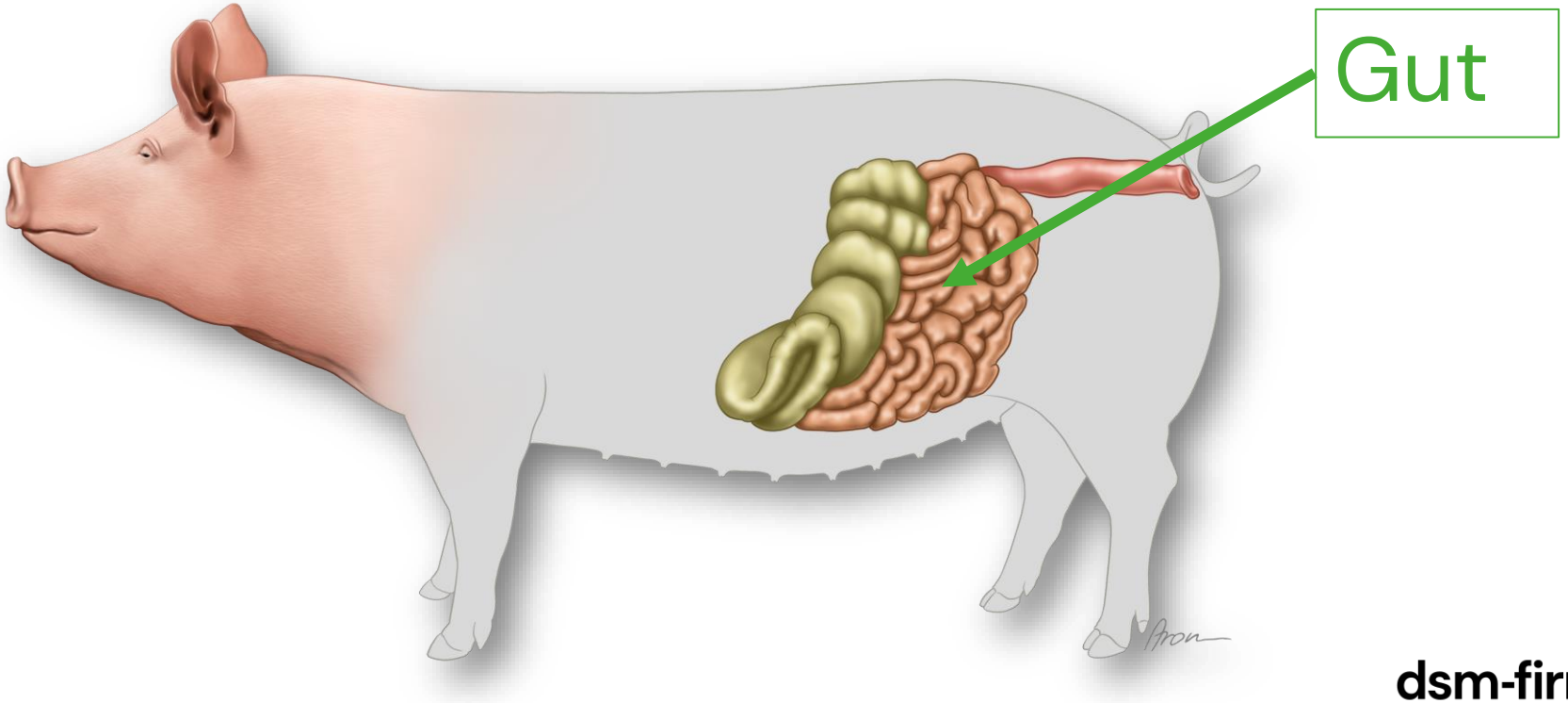
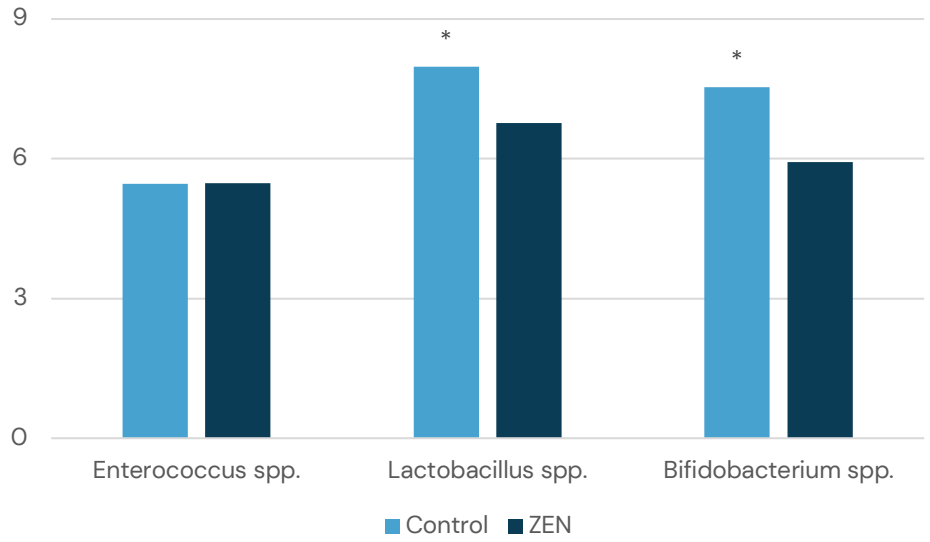
Effects of mycotoxins on the gut

- *Negative effects on microbiota*
- *Impacts on villus height and tight junction function*
- *General effect on feed intake*



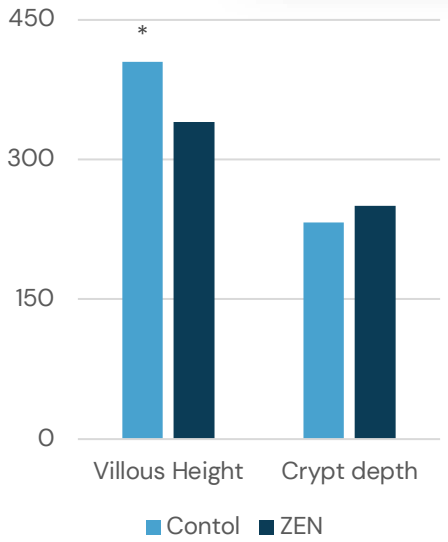
Microbiota and Villi formation

Effect of ZEN in GIT of pregnant sows



Bacterial numbers in cecal digesta of sows

- ✓ Negative effect on microbiota
- ✓ Negative effect on GIT structure
- ✓ Negative effect oxidative stress markers in the jejunum of sows
- ✓ Upregulate gene expression of (TNF)-α Interleukin (IL)-1α; IL-6
- ✓ Downregulated gene expression of IL-8



Villous height and crypt depth (µm) in jejunum of sows

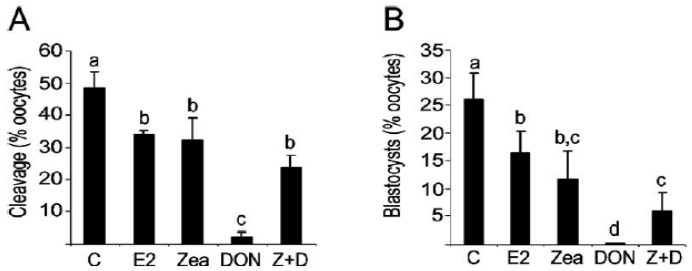
ZEN 2.17 ppm; duration day 35-70 of pregnancy; samples collected @ day 70

Liu et al., 2017

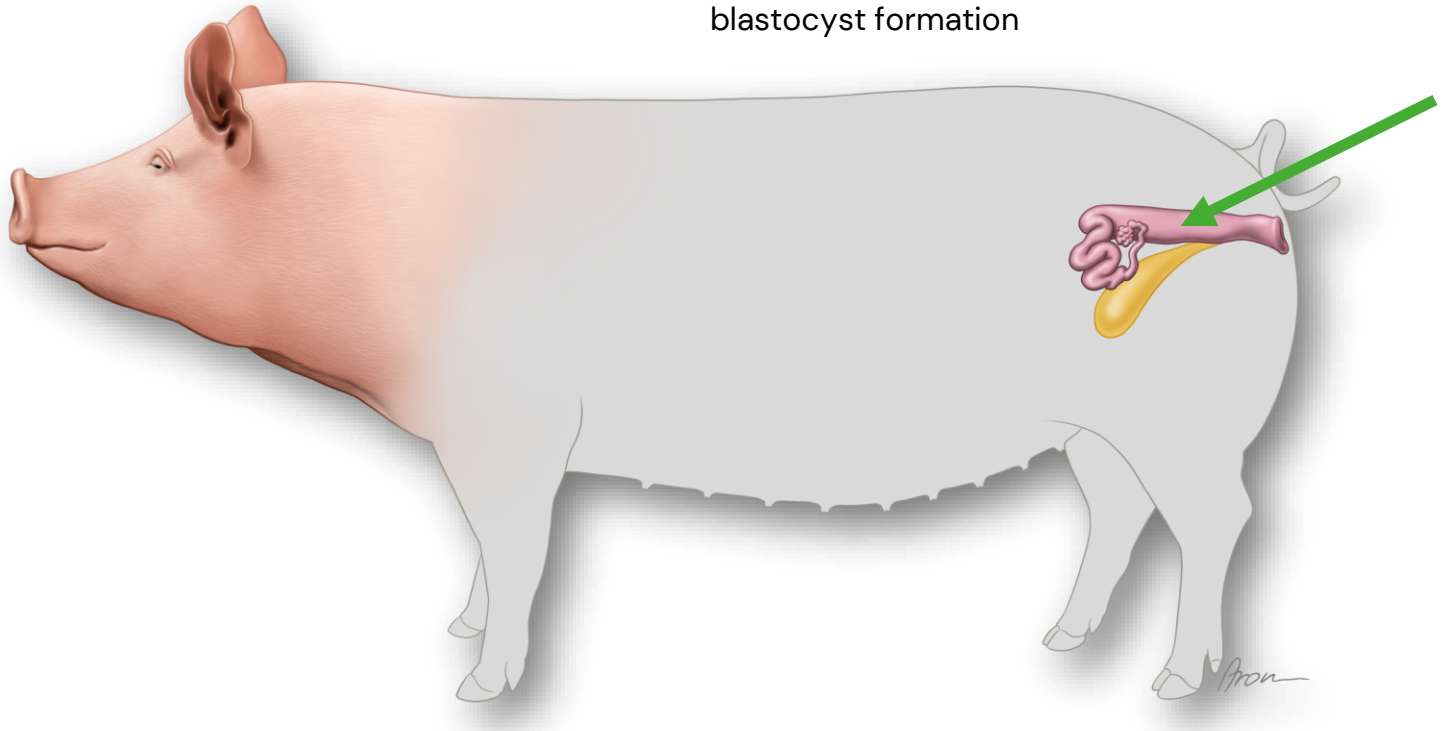
Mycotoxins impair digestion!!!

Effects of mycotoxins on the uterus/ovaries

- *Impacts on oocyte development*
- *Disruption of pregnancy hormones*



Mycotoxins Reduce the Developmental Capacity of Oocytes by delaying cleavage process and blastocyst formation



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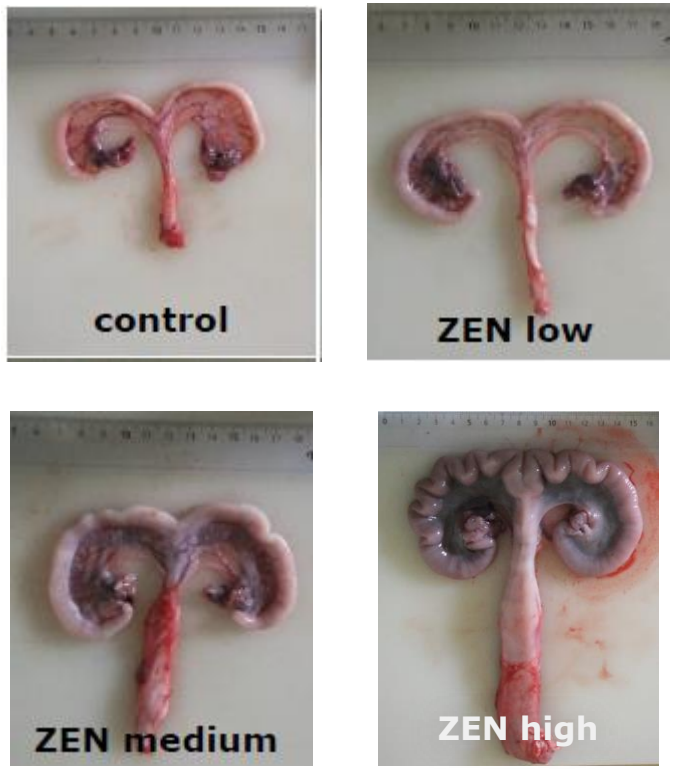
Effect of mycotoxins on the uterus

ZEN exposure increases the weight of the reproductive tract

→ The longer the ZEN exposure period, the more negative is the effect on the weight of the reproductive tract.

ZEN had a negative influence on the reproductive tract weight
average reproductive tract weight (g per kg body weight)*100:

51.8 ± 20.6	Control group,
55.8 ± 17.2	ZEN low group
121.4 ± 43.4	ZEN medium group,
353.4 ± 110.6	ZEN high group

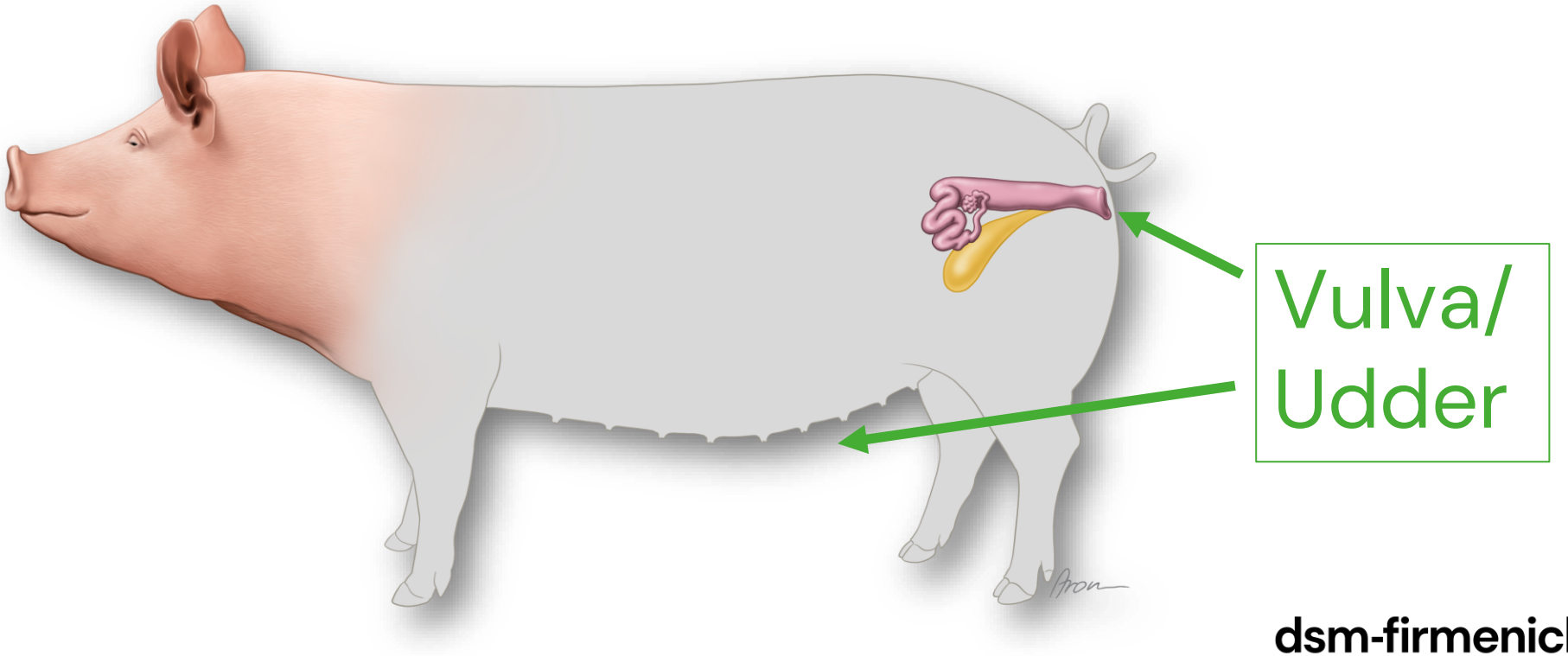


Reproductive tract at d27 (in cm)

ZEN is estrogenic and has a direct effect on fertility and embryo viability

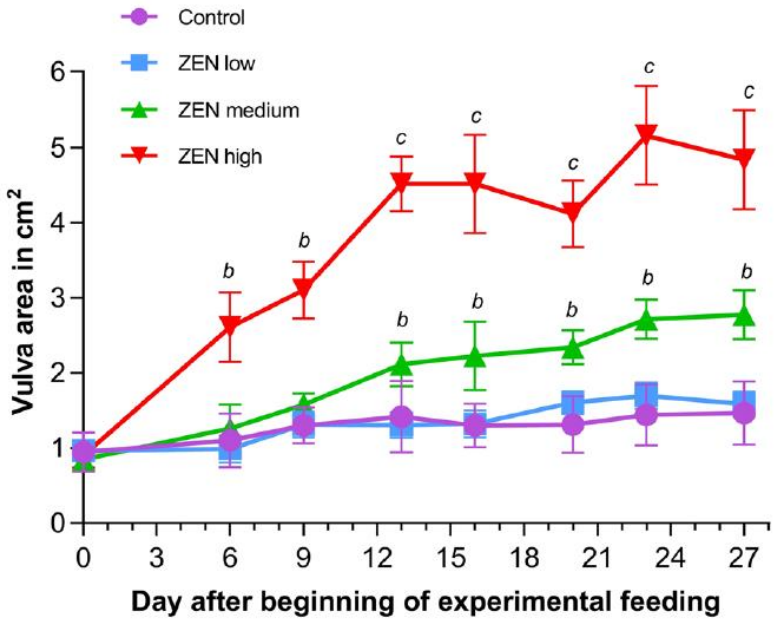
Effects of mycotoxins on the vulva/udder

- *Enlargement of the vulva*
- *Cell death (apoptosis) in udder*



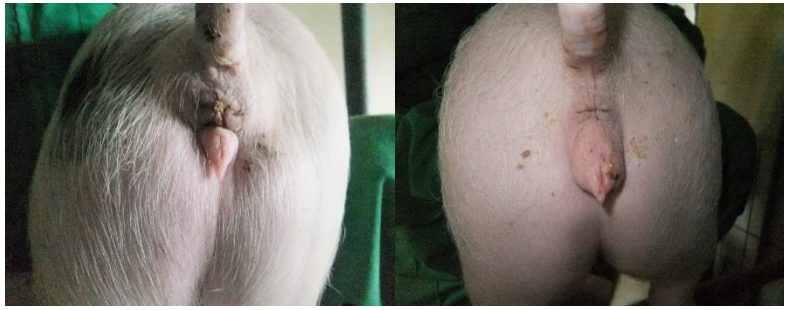
Effect of ZEN on vulva size

ZEN exposure increases vulva size



Control
Zearalenone low

Zearalenone
high



→ ZEN exposure causes a dose-dependent increase of the vulva size.

- **Vulva was significantly enlarged** compared to negative control from
  - from d 6 onwards for the ZEN HIGH group and
  - D 13 onwards for the ZEN MEDIUM group
- **On d 27, the vulva was enlarged by the factor 1.9 and 3.3 in the ZEN MEDIUM and ZEN HIGH group, respectively**
- No significant changes were observed in the ZEN LOW group compared to negative control

Transfer of Deoxynivalenol & Zearalenone in Swine

1993 – ZEN-Transfer via Milk [1]

2007 – Deoxynivalenol (DON)-Transfer via Placenta [2,3]

ZEN-Transfer via Placenta in the last trimester [2,3]

2018 – Case reports with Zearalenon (ZEN) [4]

Deoxynivalenol (DON)-Transfer via Placenta and milk [5]

2020 – DON- Transfer via milk [6]

DON & ZEN influence the immune system of neonatal piglets [7,8, 9]

2021 – DON- & ZEN-Transmission from sows to piglets cause gut inflammation [10]

[1] Vanyi et al., 1993; Acta Vet Hung 42; [2] Goyarts et al. 2007, Toxicol Letters 171; [3] Dänicke et al. 2007, Food & Chem Toxicol 45; [4] Henning-Pauka et al. 2018, Porcine Health Management 4; [5] Savyari et al. 2018, Toxins 10; [7] Stepanova et al. 2020, Toxins 12; [8] Ujčič-Vrhovnik et al. 2020, Acta Veterinaria Hungarica 68(2); [9] Ferret-Bernard et al. 2020, Nutrients 12; [10] Benthem de Grave et al. 2021, Toxins 13

Placenta transfer of mycotoxins

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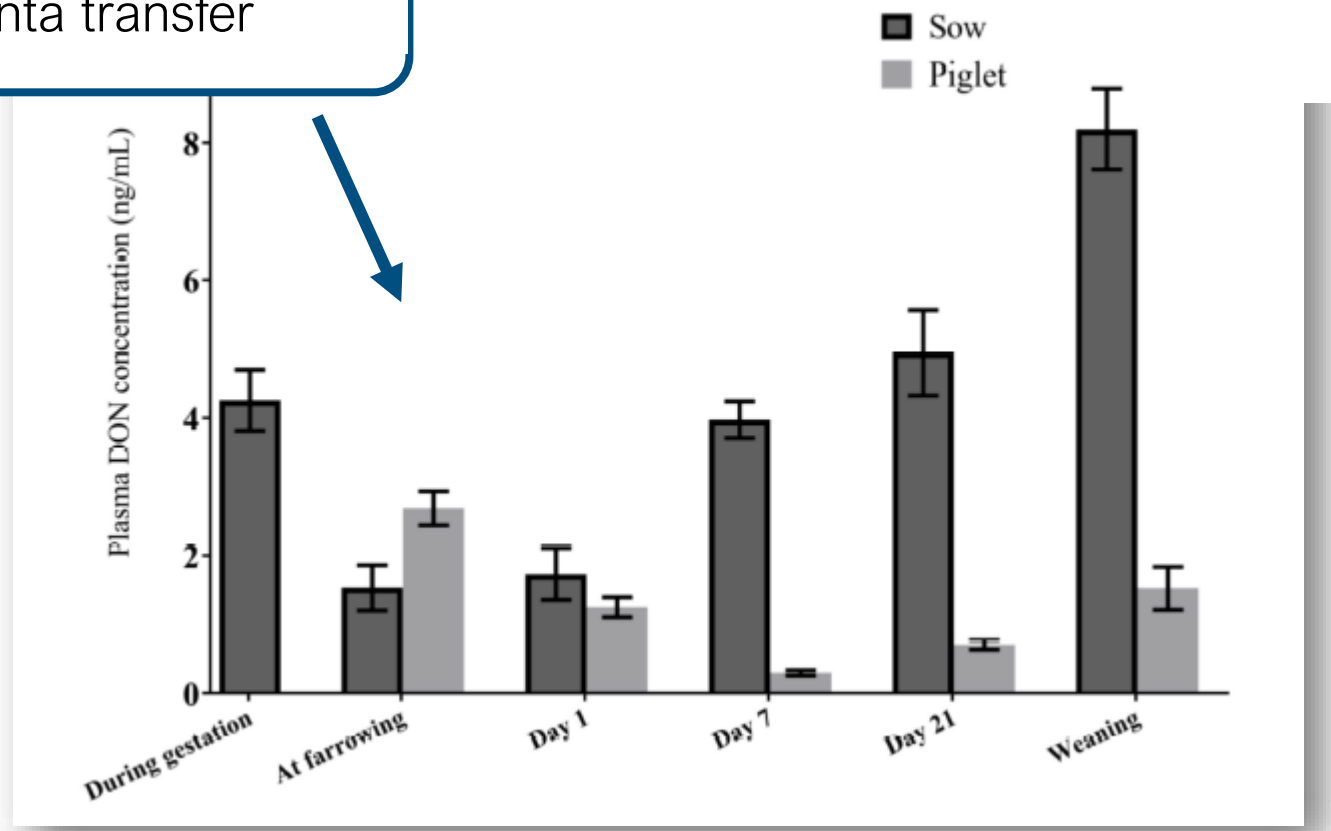
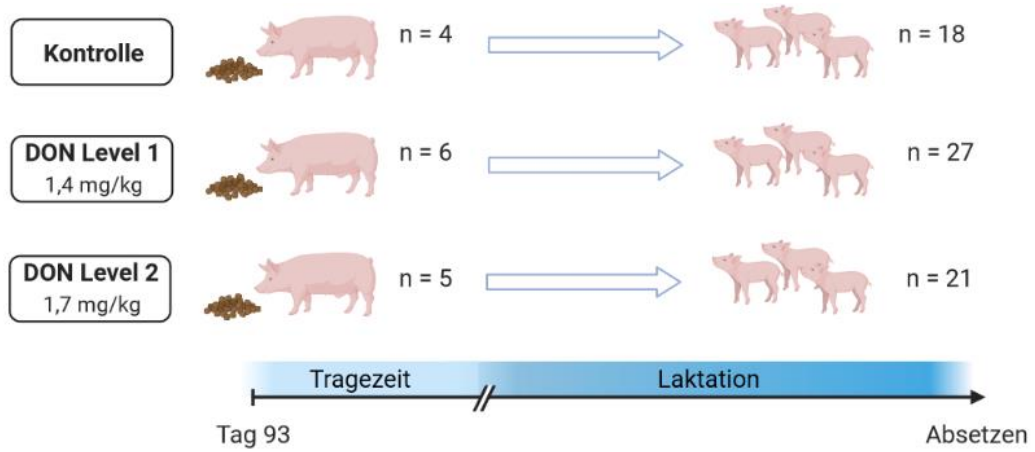
Placenta-Transfer of DON

Before colostrum intake→
Placenta transfer





Sayyari et al. 2018, Toxins 10

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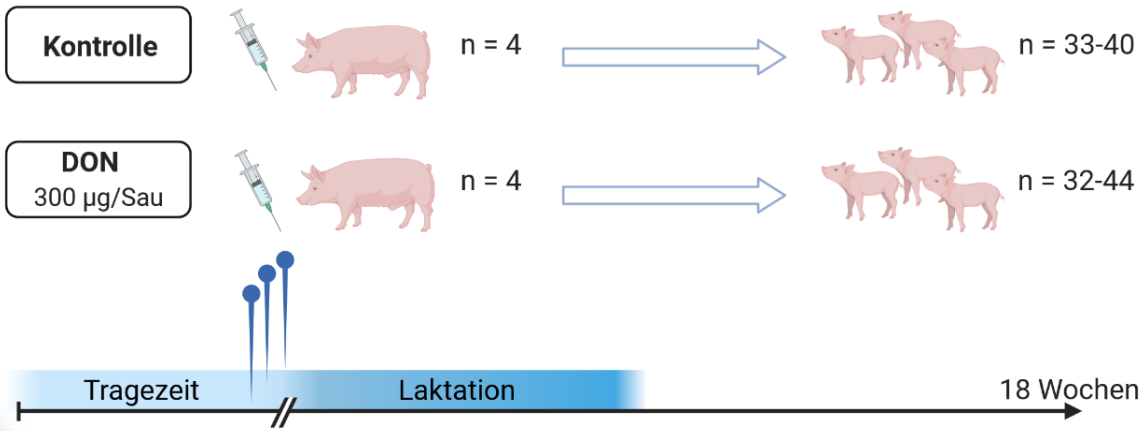
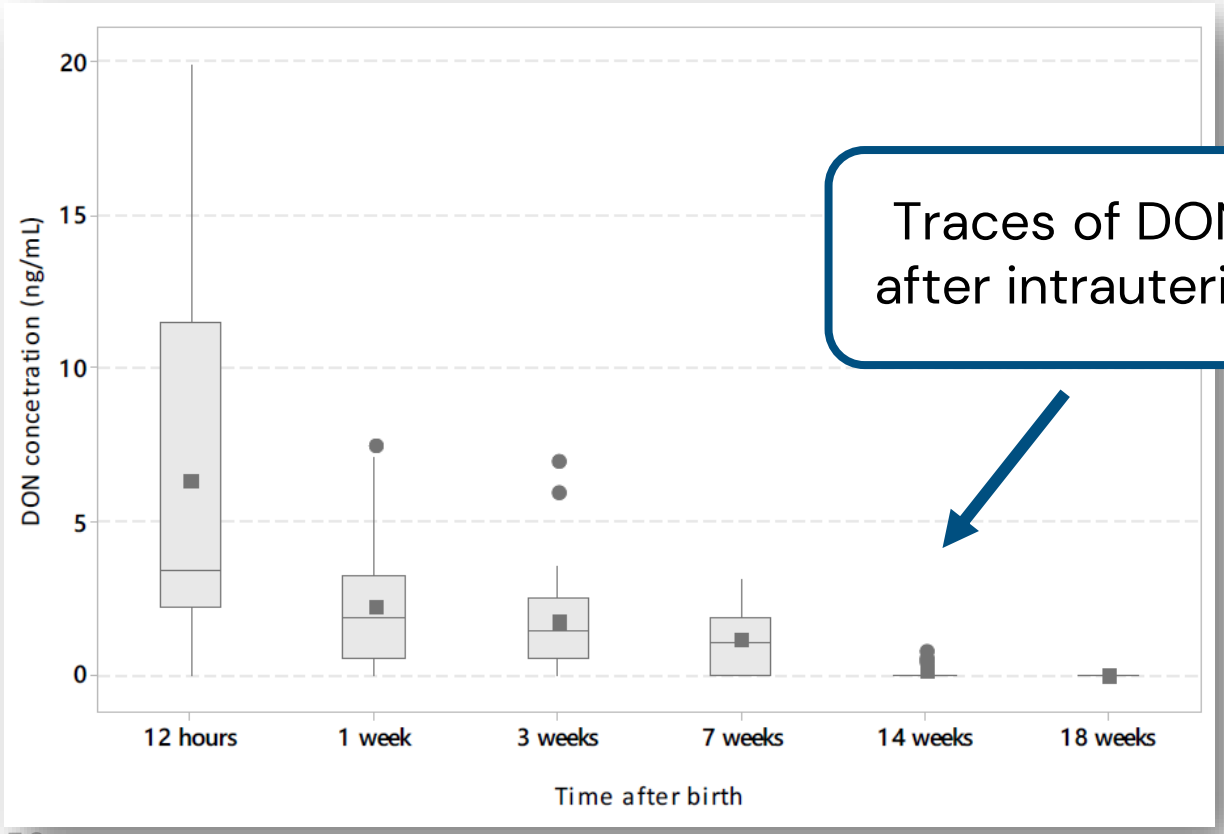
Placenta-Transfer of DON





Traces of DON 14 weeks after intrauterine transfer

Stepanova et al. 2020, Toxins 12

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Key messages

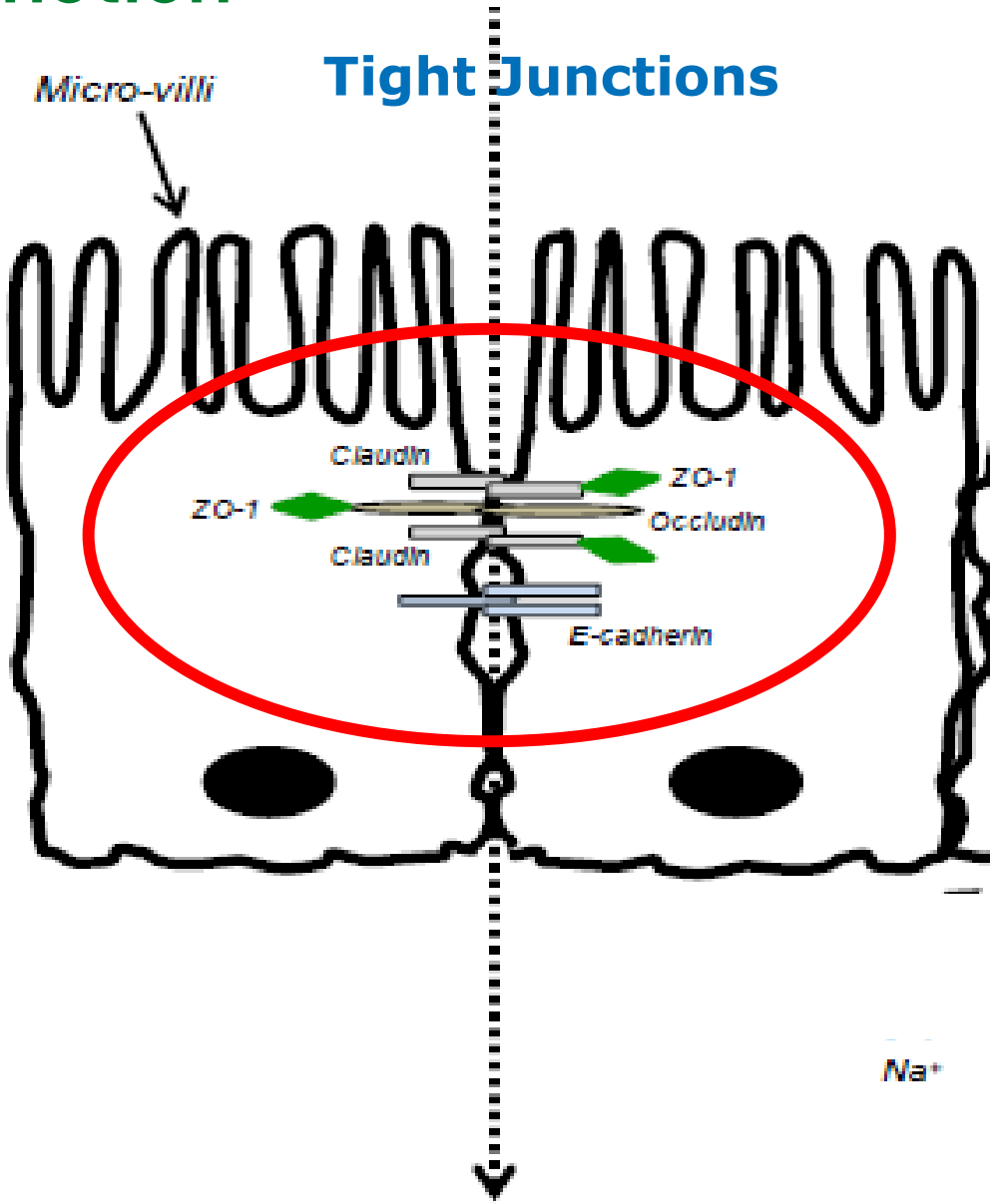
- DON and ZEA are being (successfully) transmitted via placenta
- DON plasma levels of piglets are almost identical with sow plasma levels
- DON persists up to 14 weeks in piglets

Milk transfer of mycotoxins

Key messages

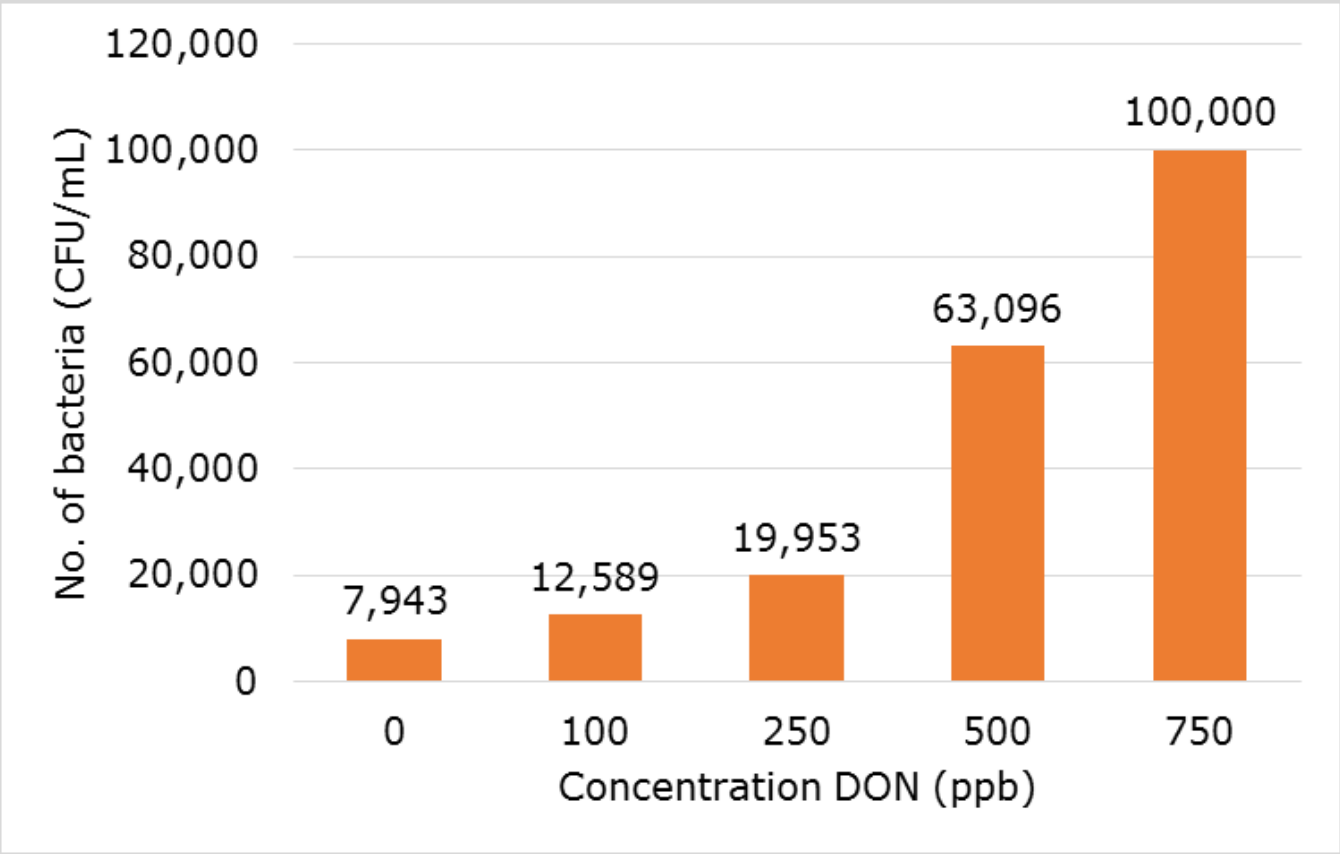
- Colostrum/milk transfer of DON and ZEN is possible
- Residues in milk are rather low compared to the rate of placenta transfer
- [Be careful with the interpretation of milk residues!]

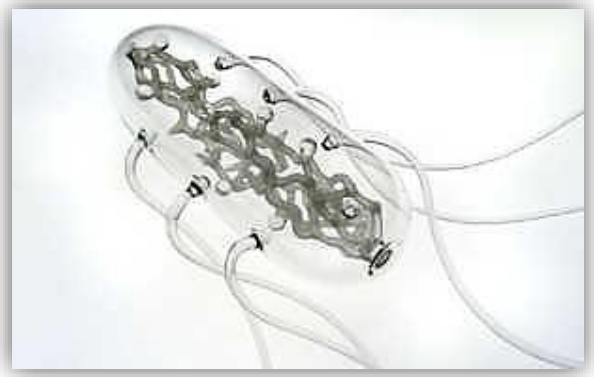
Mycotoxins decrease intestinal barrier function



- multiprotein complexes
- network near luminal surface
- seal paracellular pathway
- prevent transport of stressors (bacteria, viruses, toxins, antigens)

DON increases transepithelial passage of *Salmonella* Typhimurium





OPEN ACCESS Freely available online

PLoS one

The Mycotoxin Deoxynivalenol Potentiates Intestinal Inflammation by *Salmonella* Typhimurium in Porcine Ileal Loops

Virginie Vandenbroucke[1]*, Siska Croubels[1], An Martel[2], Elin Verbrugghe[2], Joline Goossens[1], Kim Van Deun[2], Filip Boyen[2], Arthur Thompson[3], Neil Shearer[3], Patrick De Backer[1], Freddy Haesebrouck[2], Frank Pasmans[2]

1 Department of Pharmacology, Toxicology and Biochemistry, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium, 2 Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium, 3 Institute of Food Research, Norwich Research Park, Norwich, United Kingdom

Impact of non-cytotoxic DON-concentrations on transepithelial passage of *Salmonella* Typhimurium using IPEC-J2 cells

- After exposure to just 100 ppb DON → increase in passage of *Salmonella* Typhimurium
- Exposure to 500 and 750 ppb DON → significant increase in passage

(Adapted from Vandenbroucke *et al.* 2011)

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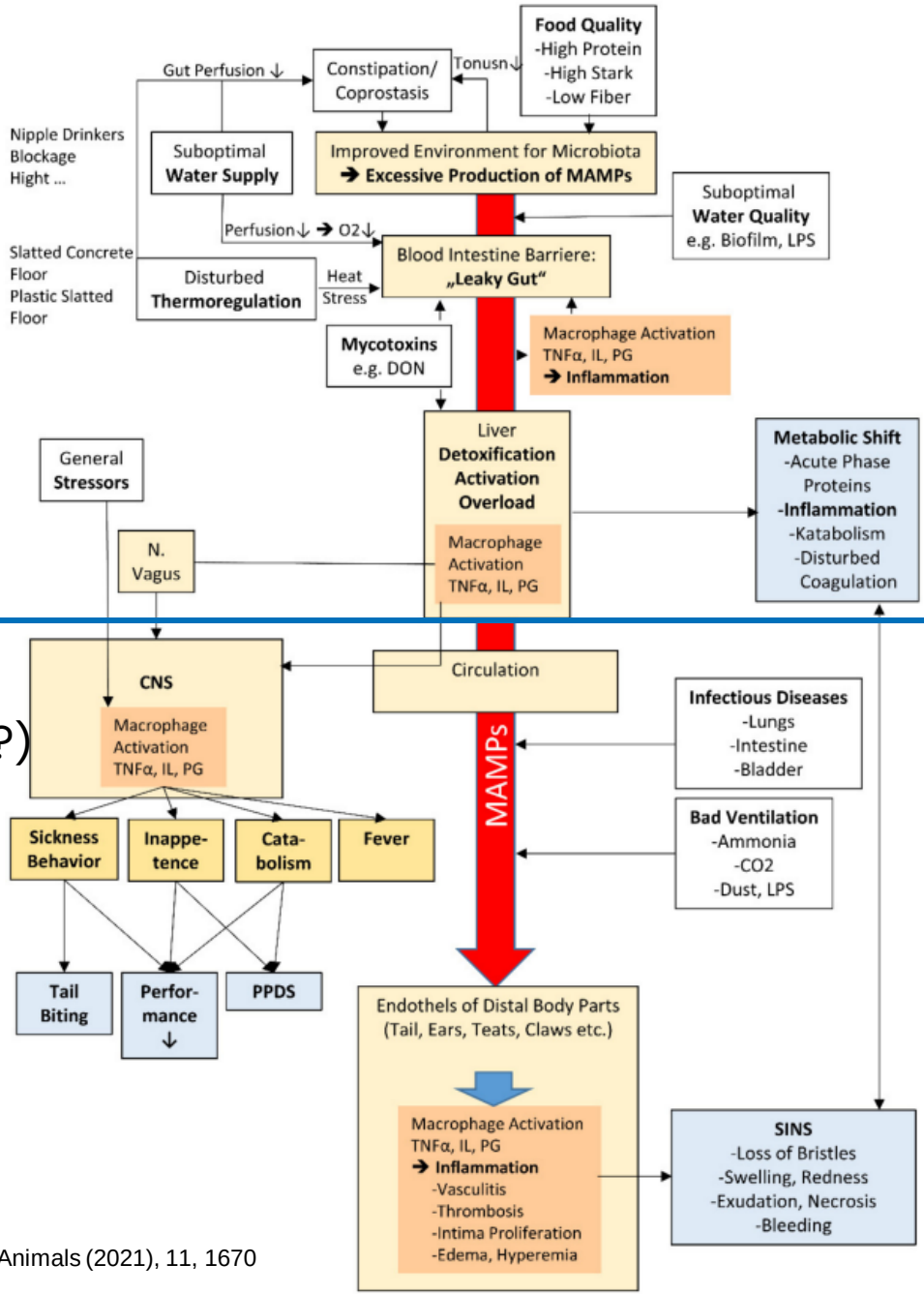
Tail docking, Tail biting and SINS

Environmental factors

Genetic factors (inflammatory responses?)



Central points:

- Bacterial degradation products
- overload of intestine and liver

MAMPs = microbe- associated molecular patterns

Source: Reiner, G., et al. Swine Inflammation and Necrosis Syndrome. Animals (2021), 11, 1670

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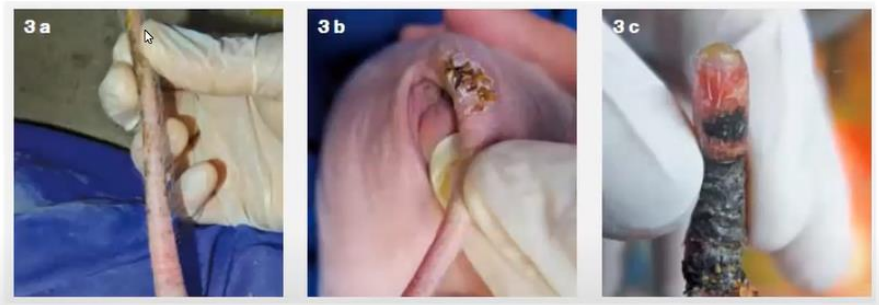
Tail necrosis

Tail necrosis

Tail injuries **without any “help” from other animals.**

(Hutura et al. 1938, Penny et al. 1971, Jademus et al. 2002, Blowey and Done, 2003, Santi et al. 2008, Meyer, 2015, Lechner et al. 2015, Langbein et al. 2016)

Klinisches Bild



Schwanznekrosen bei Saugferkeln innerhalb der ersten Lebenstage
– kein Zutun anderer Ferkel, keine Bisse

Klinisches Bild

Ringabschnürungen an Ferkelschwänzen;
Nekrose der distalen Bereiche; kein Zutun anderer Schweine

Source: Article Prof. Dr. G. Reiner

Swine Inflammation and Necrosis Syndrome (SINS)

JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN

Pictures provided by Mirjam Lechner

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Swine Inflammation and Necrosis Syndrome

JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN

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Source: Article by Dr. G. Reiner. Entzündungs- und Nekrosesyndrom beim Schwein (SINS). Deutsches Tierärzteblatt (2019); 67 (3)

Mycotoxin Thresholds, Limits, Orientation levels

DON (mg/kg)						
	Moderate	Medium	High	EU GUIDANCE VALUES*	EFSA NOAEL	EFSA LOAEL
Piglet	<0,15	0,15-0,20	>0,20	0,90	0,70	2,8
Gilt	<0,25	0,25-1,00	>1,00	0,90	0,70	2,8
Sow	<0,20	0,20-0,90	>0,90	0,90	0,70	2,8
Fattening Pig	<0,25	0,25-1,00	>1,00	0,90	0,70	2,8
ZEN (mg/kg)						
Piglet	<0,05	0,05-0,1	>0,1	0,10	0,22	0,42
Gilt	<0,05	0,05-0,1	>0,1	0,10	1,00	5,0
Sow	<0,05	0,05-0,1	>0,1	0,25	1,00	5,0
Fattening Pig	<0,1	0,1-0,25	>0,25	0,25	-	-
FUM (mg/kg)						
Piglet	<0,75	0,75-1,00	>1,00	5,00	1,0	5,0
Gilt	<0,75	0,75-1,00	>1,00	5,00	1,0	5,0
Sow	<0,75	0,75-1,00	>1,00	5,00	1,0	5,0
Fattening Pig	<1,0	1,0-2,0	> 2,0	5,00	1,0	5,0
AFLA B1 (mg/kg) * EU						
Piglet	<0,002	0,002-0,004	>0,004	0,01	-	-
Gilt	<0,005	0,005-0,010	>0,010	0,02	-	-
Sow	<0,005	0,005-0,010	>0,010	0,02	-	-
Fattening Pig	<0,005	0,005-0,010	>0,010	0,02	-	-

- NOAEL (no observed adverse effect level)
- LOAEL (lowest observed adverse effect level)

- NOAEL = of 0.7 mg/kg feed was
- reported for reduced feed intake.

- LOAEL = consider the critical acute effect in pigs at the concentration of 2.8 mg/kg feed for vomiting.
- Reference point (RP) = “estimated NOAEL”
- Orientation Values = ensure that feed business operators use in their HACCP system the guidance values referred to in determine the critical limits at CCP from unacceptability, for the prevention, elimination or reduction of identified hazards.
- AFLA = (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs

IN SUMMARY…

- Mycotoxins have been showed to decrease feed intake and impair sow body condition during lactation, which can worsen some **subclinical disease by pathogens and negatively affect milk production**
- **Mycotoxins can affect the mammary gland functionality** and milk production **via induction of cells apoptosis** and oxidative stress
- **Mycotoxins can be transferred via placenta, colostrum and milk to piglets, affecting their health and resilience (SINS)**

Mycotoxins can act at different organ levels, impairing the ovary function, hormonal status of sows, mammary gland functionality, affecting the genetic potential of sows, from ovulation, to placental development ➔

Welfare, longevity and productivity issues

Mycotoxins Risk Management is crucial to optimize modern sows productivity, longevity and welfare

2.3 Poultry

&

Finished Feed vs new EFSA opinion

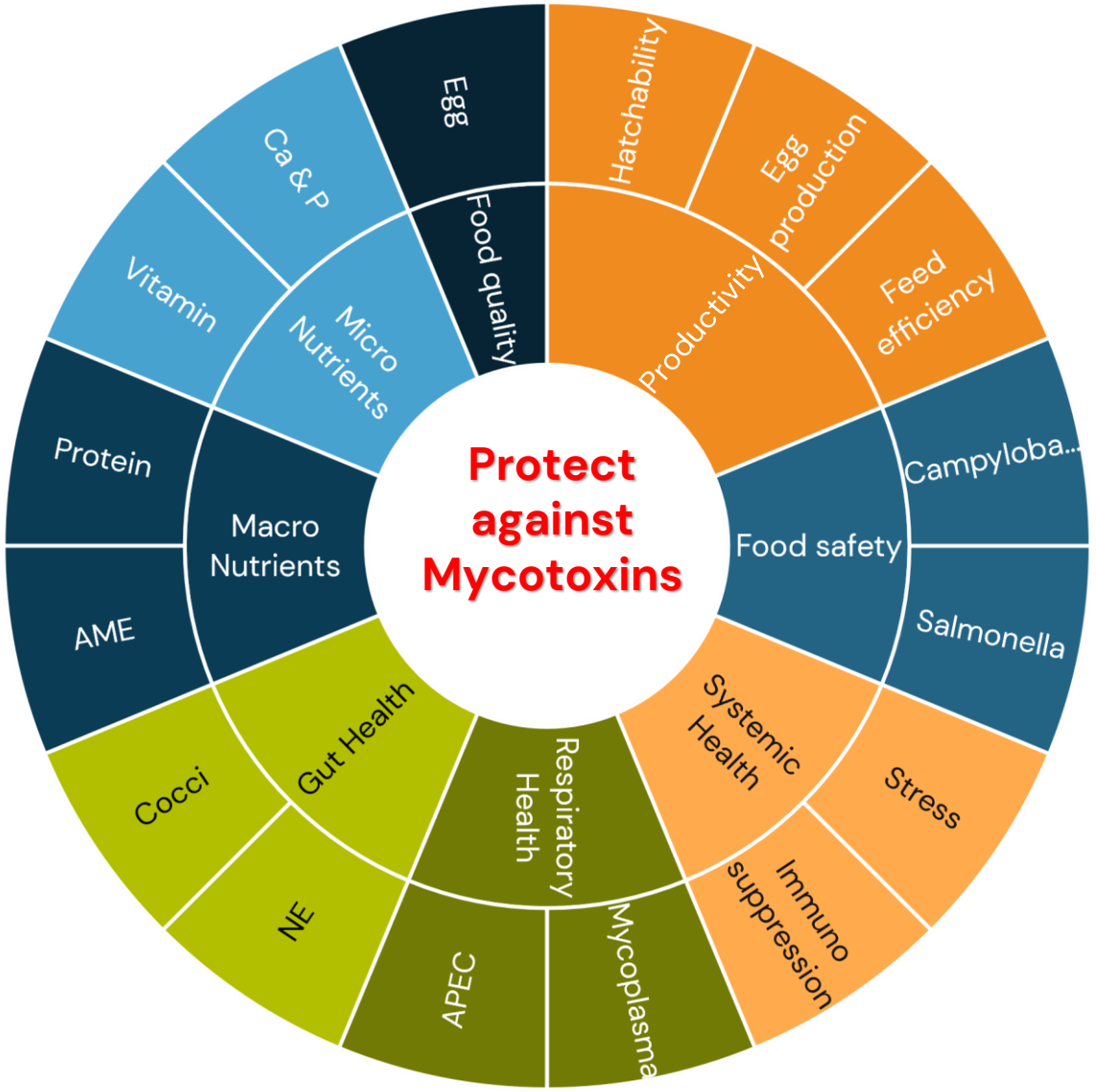
Mycotoxins, Predisposing Factor on Animal Health and Production

Safeguard your feed to protect your birds

Reduce the risk of **fungal growth** and mycotoxins development in **storages**

Deactivate and prevent the harmful effects of **mycotoxins from feedstuff** using a wide range of toxin management technologies

… **to prevent** the **negative** immunity, fertility and growth inhibitory **effects of toxins**



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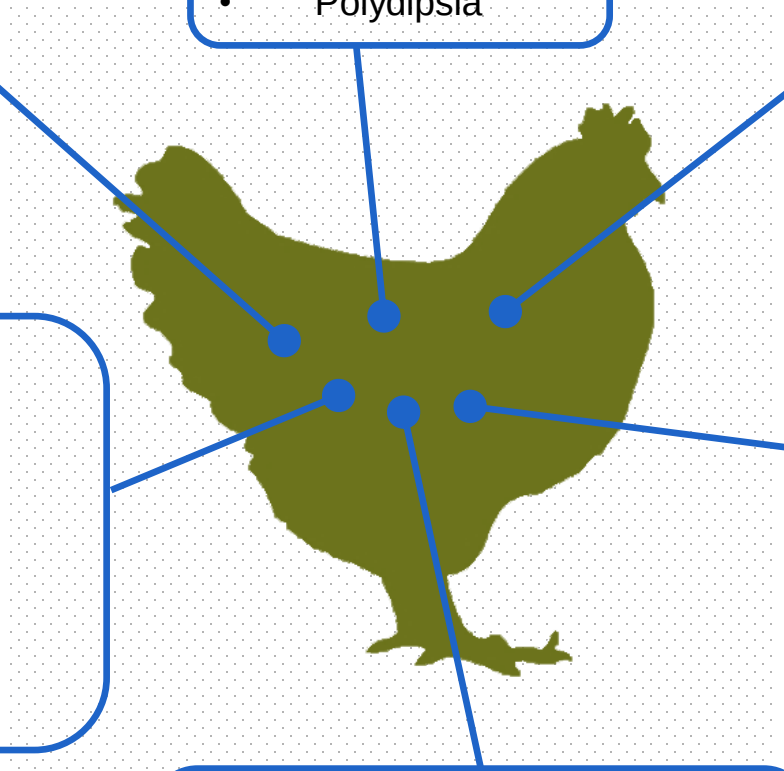
Effect of Mycotoxins in Poultry Health

ZEN, DON, T-2, DAS, Ergot alkaloids
- Decreased egg production
- Decreased hatchability
- Ovarian cysts
- Embryonic loss
- Delayed sexual maturation

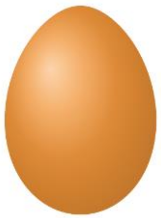
OTA
- Kidney damage
- Polydipsia

T-2, HT-2, NIV, DON, DAS, AFB1, Ergot alkaloids
- Oral and dermal lesions
- Inflammation of mucousmembrane of oral cavity
- Respiratory difficulties



AFB1, FUM, T-2, DON, NIV, DAS, OTA
- Immunosuppression
- Fatty liver
- Inhomogeneous flocks
- Impaired feathering
- Organ damage
- Impared performance
- Impaired intestinal health

T-2 and DON
- Gizzard lesions
- Feed refusal
- Decreased feed intake
- Diarrhea
- Vasoconstriction (necrosis)



AFB1, OTA, T-2, DON, ZEN
- Residues
- Poor egg shell quality
- Blood and meat spots
- Creamy yolk

DON: deoxynivalenol
NIV: nivalenol
ZEN: zearalenone
AFB1: aflatoxin B1
FUM: fumonisins
T-2: T-2 toxin
HT-2: HT-2 toxin
DAS: diacetoxyscirpenol
OTA: Ochratoxin A

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Clinical / Classical effects of Mycotoxins – Poulty

AFLA, Tricho's & FUM

Fatty liver, immunomodulation, lower performance

ZEN

Cystic oviduct

T2, OTA, ZEA

Egg shell changes

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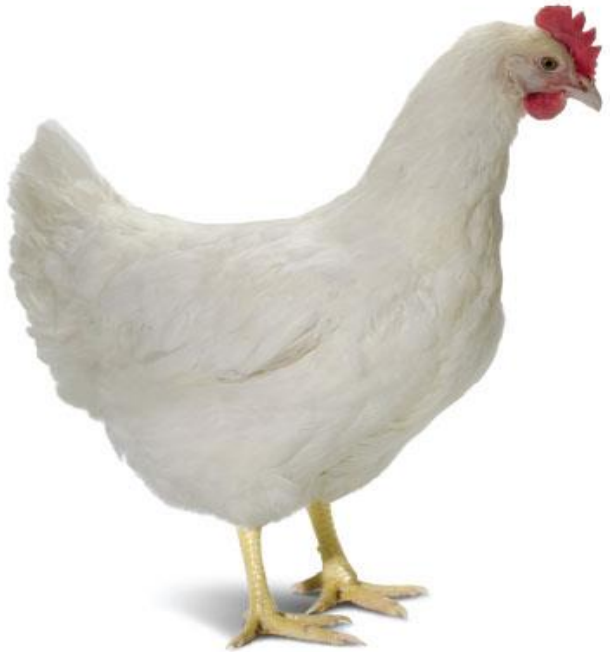
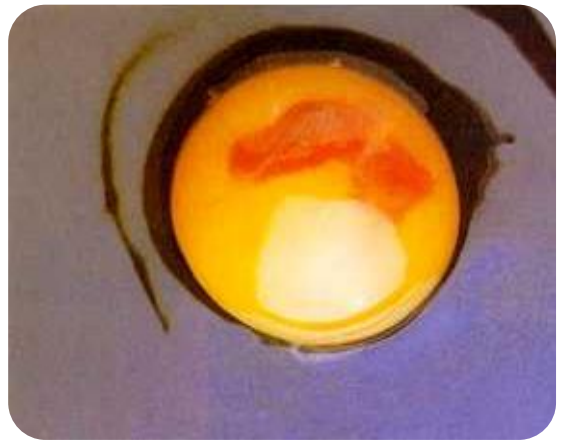
Clinical / Classical effects of Mycotoxins – Poultry

Oral lesions



OTA



Blood spots in yolk

Bad feathering

T2, HT2

Gizzard erosions

Kidney degeneration, wet litter

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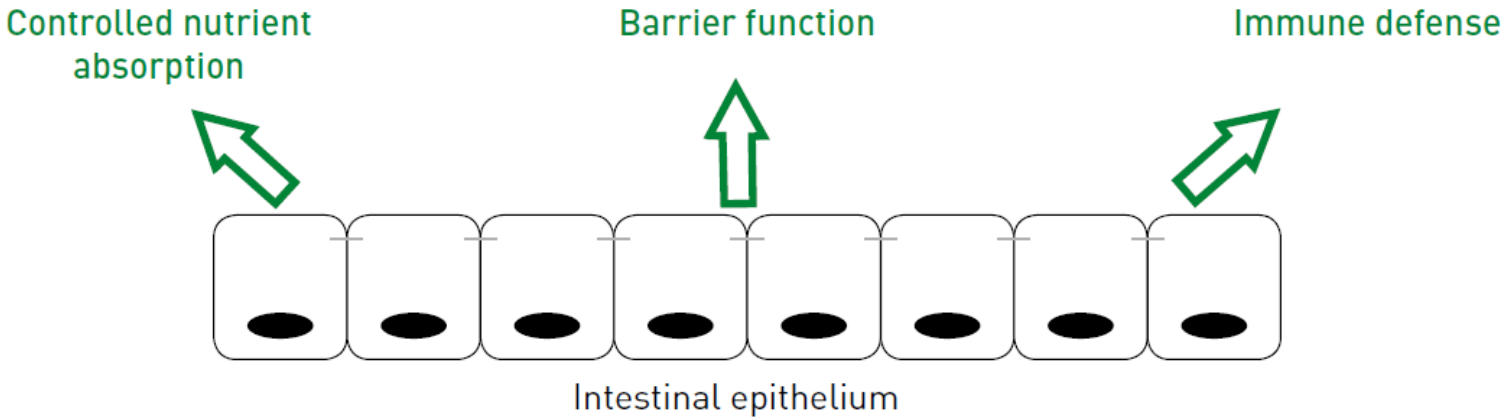
Impairs the animals to express their maximum genetic potential

Chronical lower health > performance >profitability

Consequences

- Lower absorption of:
  - Nutrients
  - Vitamins
  - Minerals
- Impairs tissues development and funcionality

- Increases disease susceptibility:
  - *Salmonella Typhimurium*
  - *E.* coli
  - Campylobacter
  - Necrotic enteritis
  - Coccidiosis
- Dirty eggs



- Decreases vaccination efficacy
- Redirects the energy of protein deposition for immune/inflammatory response
- Chronical effects

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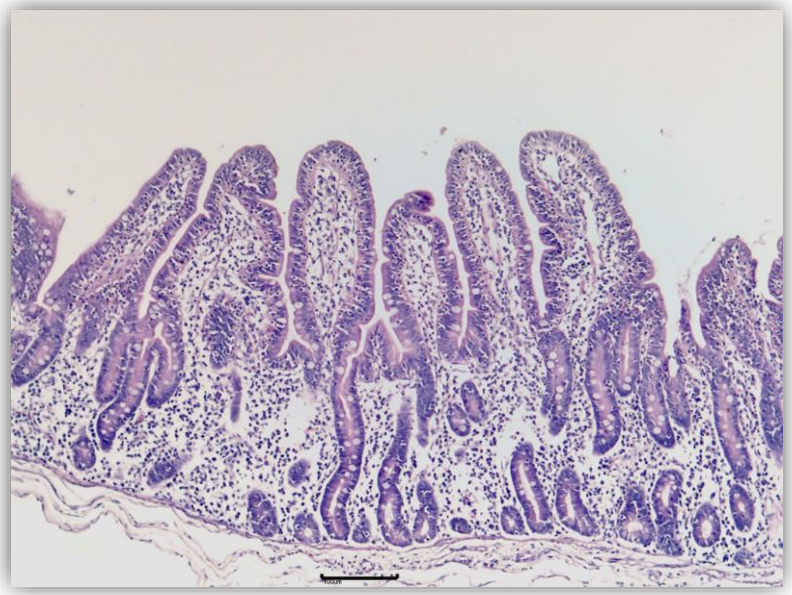
Fusarium toxins reduce surface for nutrient absorption

Universidade Estadual de Londrina

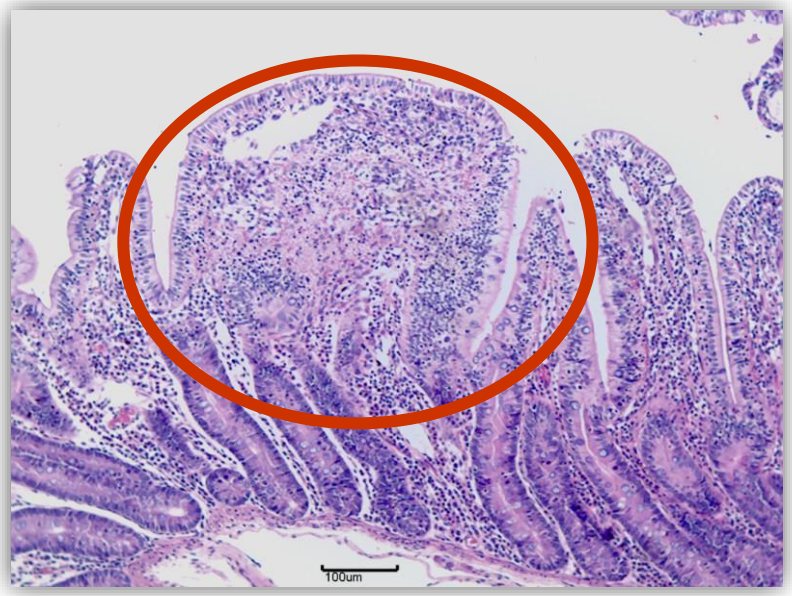
- *Fusarium* toxins > Changes the intestinal morphology with villi fusion consequently the absorption of nutrients

Healthy jejunum



Bracarense et al., 2011; Grenier et al., 2011

DON (3ppm) & FUM (6ppm) = fusion of the gut vili



*Jejunum of Fum treated group – **fusion of the vilosities**. HE. Barr 100µm.*

FUM+DON predispose to Necrotic Enteritis

OPEN ACCESS Freely available online

PLOS ONE

The Mycotoxin Deoxynivalenol Predisposes for the Development of *Clostridium perfringens*-Induced Necrotic Enteritis in Broiler Chickens

Gunther Antonissen[1,2*], Filip Van Immerseel[1*], Frank Pasmans[1*], Richard Ducatelle[1], Freddy Haesebrouck[1], Leen Timbermont[1], Marc Verlinden[1], Geert Paul Jules Janssens[3], Venessa Eeckhaut[1], Mia Eeckhout[4], Sarah De Saeger[5], Sabine Hessenberger[6], An Martel[1*], Siska Croubels[2*]

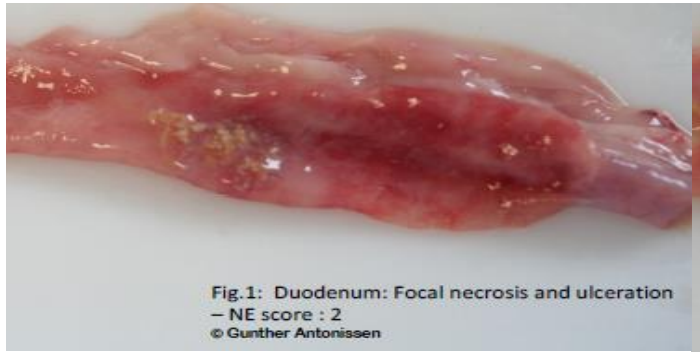


Fig.1: Duodenum: Focal necrosis and ulceration – NE score : 2
© Gunther Antonissen

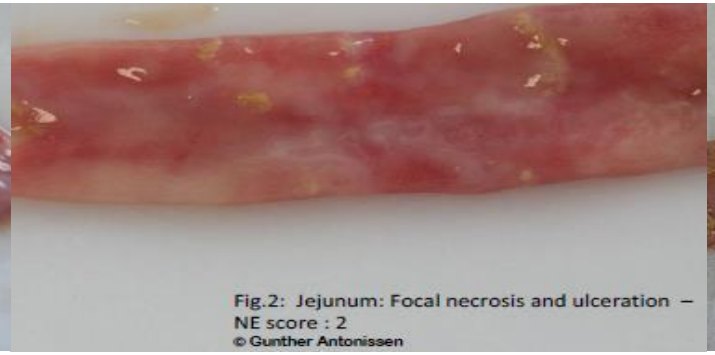
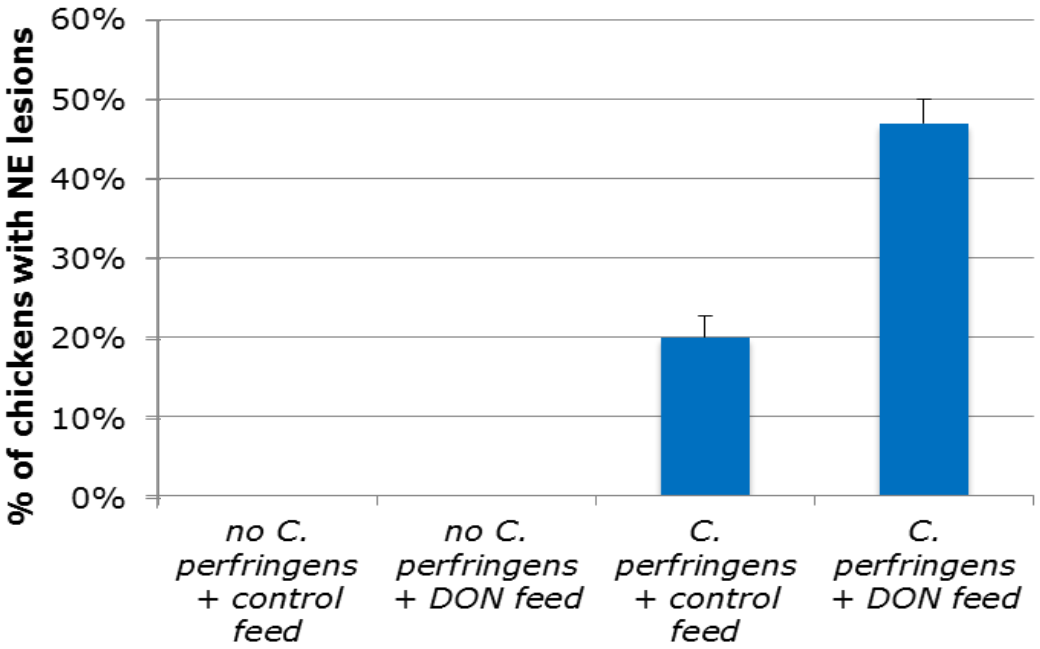
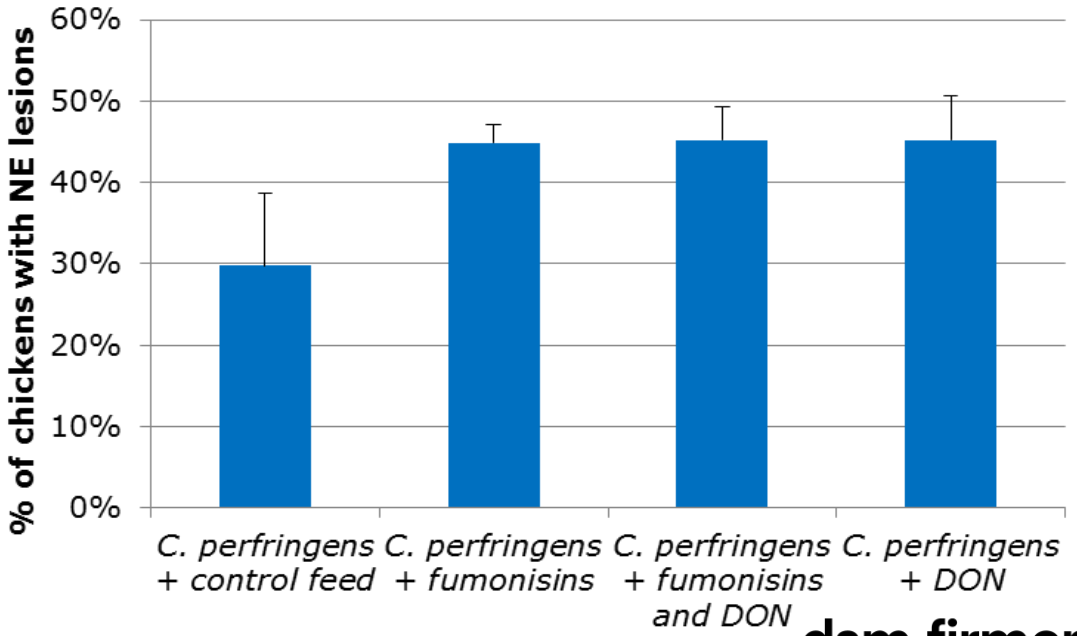


Fig.2: Jejunum: Focal necrosis and ulceration – NE score : 2
© Gunther Antonissen

Fig.3: Diffuse necrosis – score : 6
©Leen Timbermont





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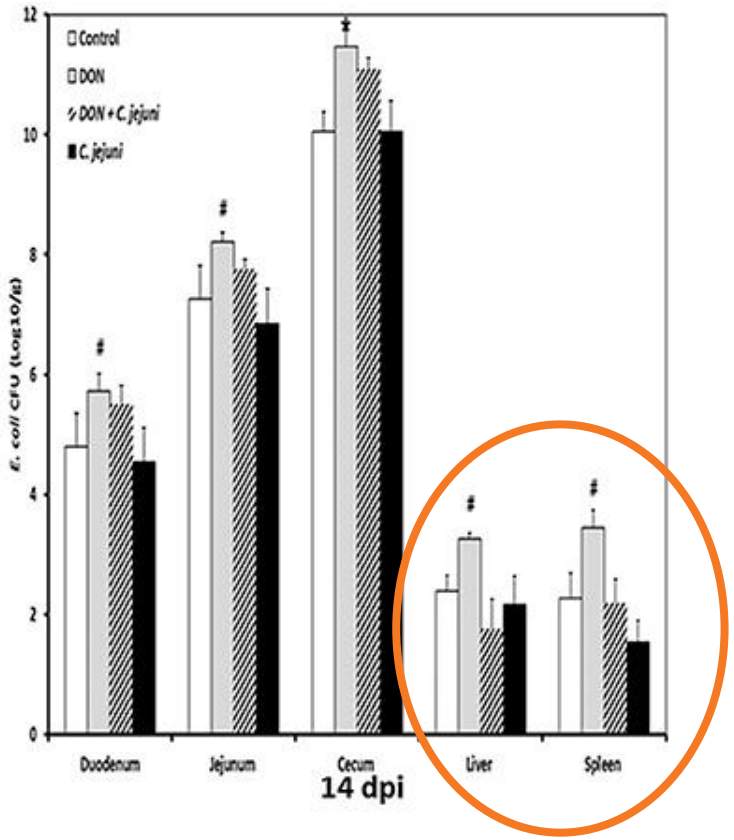
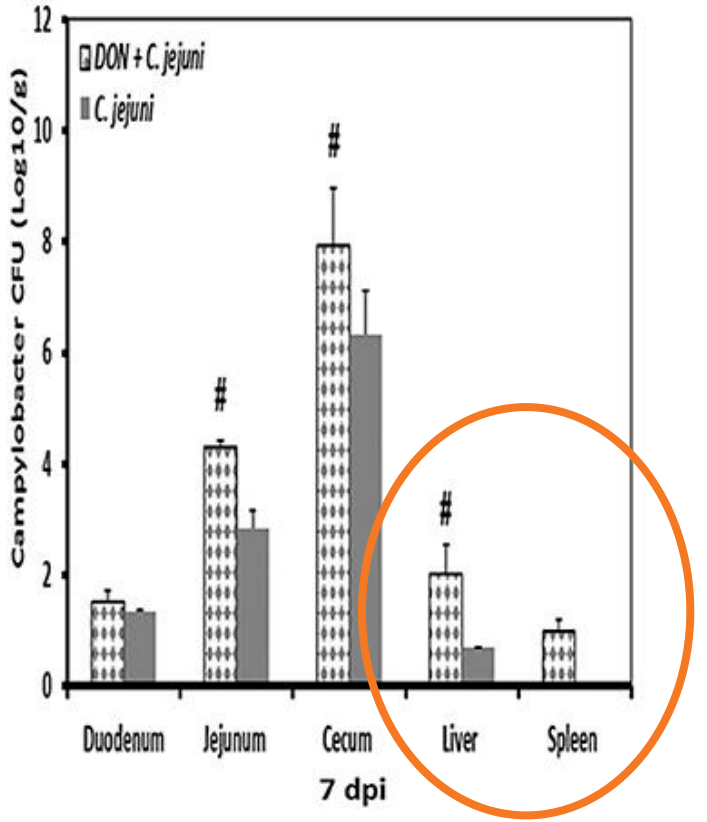
Impairment of intestinal epithelial integrity

DON 5 mg/kg

Effect on bacterial translocation in broilers





e.g. DON ↑ translocation of *Escherichia coli* and *Campylobacter jejuni*

Ruhnau, D., Hess, C., Grenier, B., Doupovec, B., Schatzmayr, D., Hess, M., & Awad, W. A. **(2020)**. The Mycotoxin Deoxynivalenol (DON) promotes Campylobacter jejuni multiplication in the intestine of broiler chickens with consequences on bacterial translocation and gut integrity. *Frontiers in Veterinary Science*, *7*, 1027.

FUM+DON enhances coccidial lesions

Subclinical doses of DON and FUM in broiler chickens challenged with *Eimeria* species (coccidiosis)

- 42 animals/treatment
- Treatments: control; DON (D); FUM (F); DON + FUM (D+F) – same 4 diets challenged with Coccivac-B 25X (mix of 4 strains of *Eimeria*) at 14d, evaluations at 21d

toxins

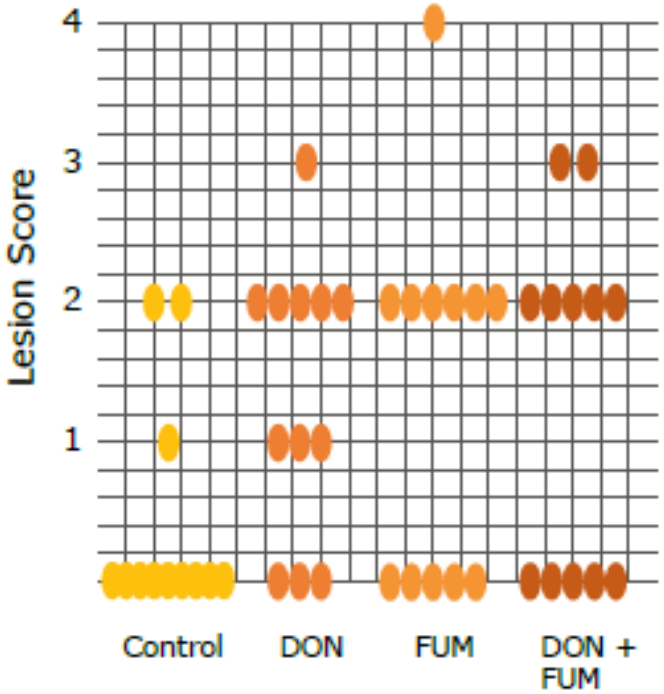
MDPI

Article

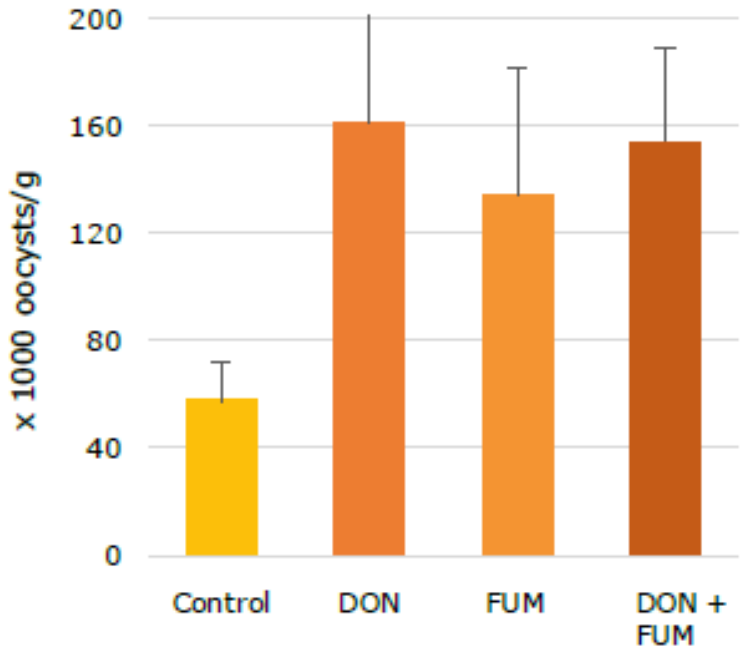
Susceptibility of Broiler Chickens to Coccidiosis When Fed Subclinical Doses of Deoxynivalenol and Fumonisins—Special Emphasis on the Immunological Response and the Mycotoxin Interaction

Bertrand Grenier [1,2], Ilse Dohnal [2], Revathi Shanmugasundaram [3], Susan D. Eicher [4], Ramesh K. Selvaraj [3], Gerd Schatzmayr [2] and Todd J. Applegate [1,5,*]

PURDUE UNIVERSITY



Cecum lesion score (One dot represents one sample)



Number of oocysts (x 1000) found in intestinal mucosa

rmenich

Reduction of Feed Conversion

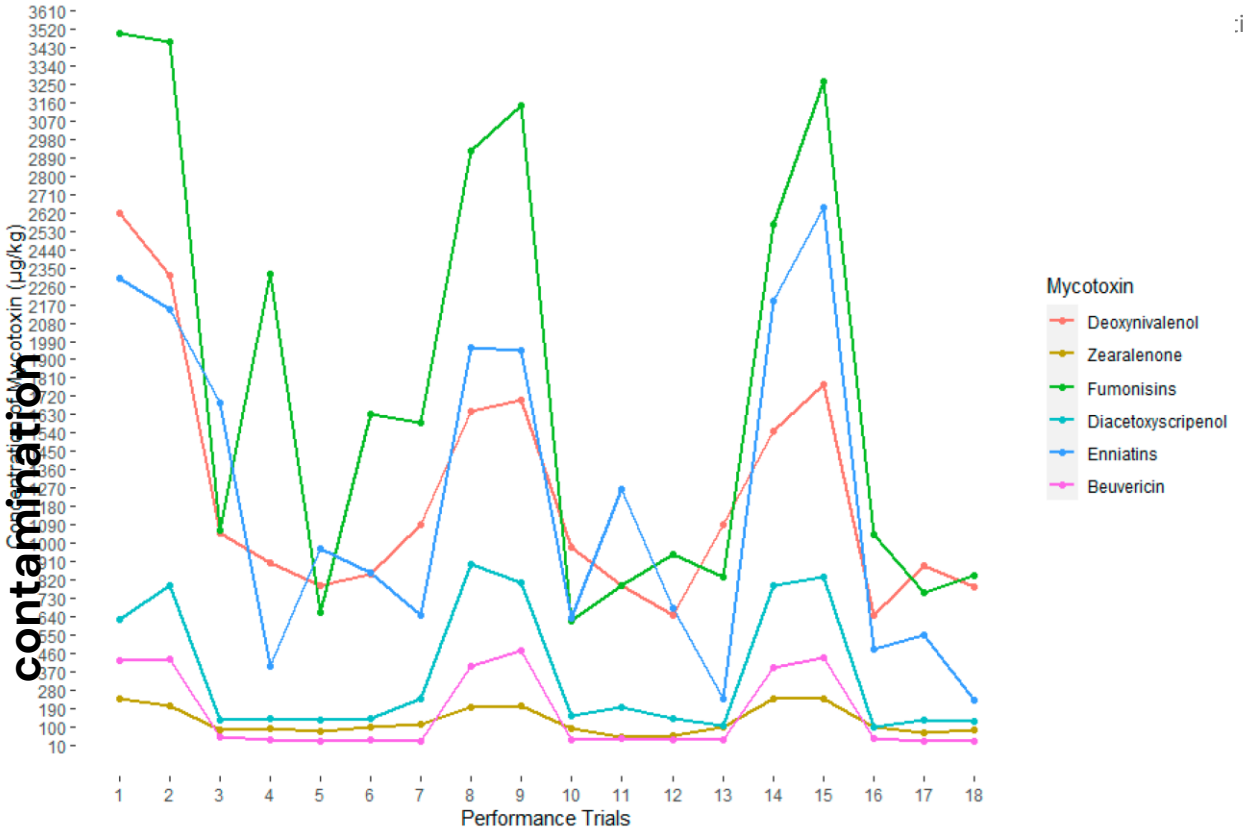
Kolawole, O., Graham, A., Donaldson, C., Owens, B., Abia, W. A., Meneely, J., ... & Elliott, C. T. (**2020**). Low doses of mycotoxin mixtures below EU regulatory limits can negatively affect the performance of broiler chickens: A longitudinal study. *Toxins*, *12*(7), 433.

- 18 successive broiler performance trials (2200 Ross 308 broilers / experiment)
- **low doses** of **mycotoxin mixtures** – naturally contaminated feed

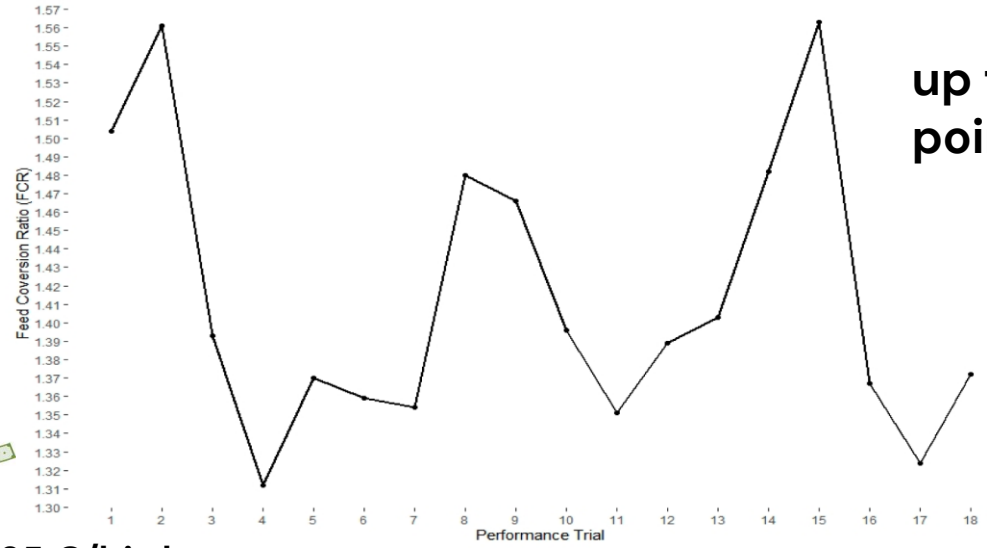
Positive relationship between FCR and

- DON (R^2=0.85)
- FBs (R^2= 0.53)
- diacetoxyscirpenol (R^2= 0.86)
- ZEN (R^2= 0.92)
- enniatins (R^2= 0.60)
- beauvericin (R^2= 073)

mycotoxin contamination



FCR



up to 26 points

Approx. 0,25 to 0,35 €/bird

Impairment of immunity

Humoral immunity:

↓ antibody response to vaccination infectious bronchitis virus, NDV, Marek's disease, …

e.g. broiler chickens 10 mg DON/kg feed (D1-D35)

→ serum antibody titer against IBV

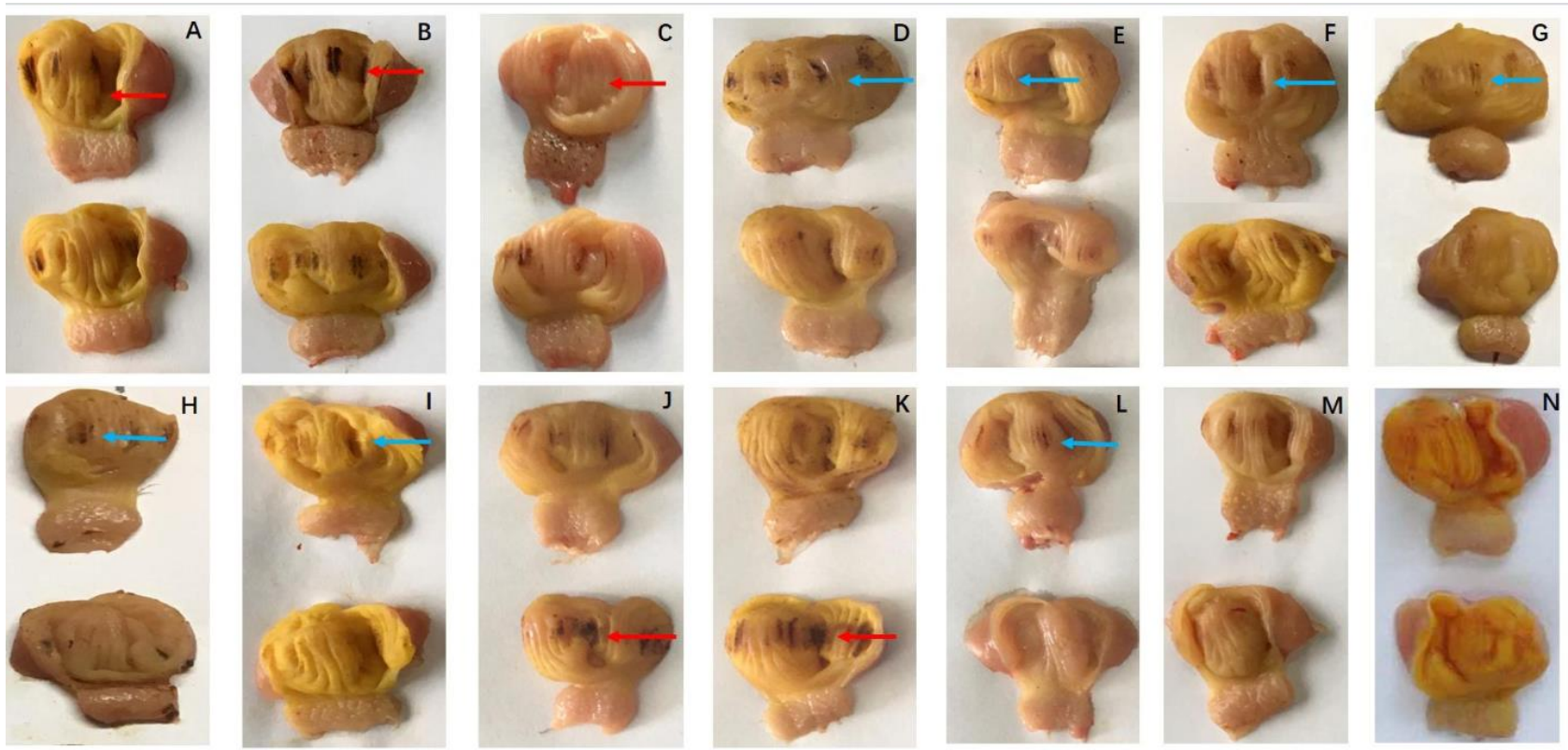
Control	DON
3123 ± 694 IU	2030* ± 900 IU

Ghareeb, K., et al. "Deoxynivalenol in chicken feed alters the vaccinal immune response and clinical biochemical serum parameters but not the intestinal and carcass characteristics." *Journal of animal physiology and animal nutrition* 100.1 (2016): 53-60.

*P<0.05

Vertical transfer to egg

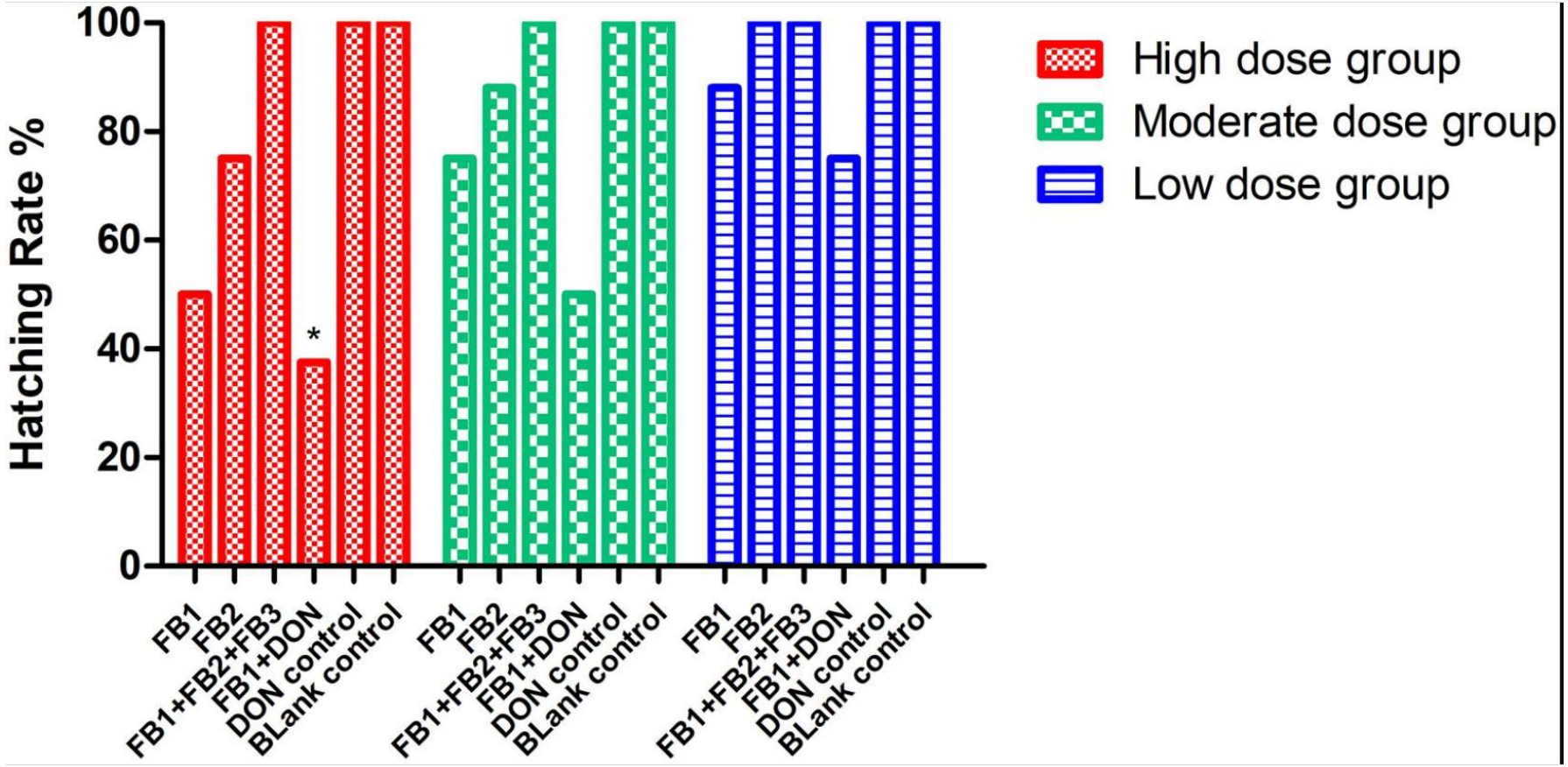
- Egg safety
- Reduced hatchability
- Gizzard ulceration (offspring)



Gizzard ulcerations of chicken progenies post-inoculation with FB or DON, or synergetic inoculation.

(A) FB_1 6 µg; **(B)** FB_1 12 µg; **(C)** FB_1 24 µg; **(D)** FB_2 12 µg; **(E)** FB_2 24 µg; **(F)** FB_2 48 µg; **(G)** FB_1 3.6 µg + FB_2 1.2 µg + FB_3 1.2 µg; **(H)** FB_1 7.2 µg + FB_2 2.4 µg + FB_3 2.4 µg; **(I)** FB_1 14.4 µg + FB_2 4.8 µg + FB_3 4.8 µg; **(J)** FB_1 3 µg + DON 0.1 µg; **(K)** FB_1 6 µg + DON 0.1 µg; **(L)** FB_1 12 µg + DON 0.1 µg; **(M)** DON 0.1 µg; **(N)** Control group. Severe gastric lesions were marked with a red arrow, and moderate lesions were labeled with a blue arrow. Peeling and shedding of the gizzard membranes were evident both in the high FB1 group and the high FB_1 + DON group. Additionally, severe hemorrhagic lesions were observed in the above two groups.

Reduced hatchability



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New EFSA Scientific Opinion – Reviewed Papers

FUM (July 13th 2022)

Table 3: New studies on adverse effects on poultry which have become available since the 2018 Opinion (EFSA CONTAM Panel, 2018a)

N/group, breed gender	Dosage and duration	Endpoint(s)	NOAEL/LOAEL (mg/kg feed)	Reference
10, male Cobb 500	0, 600 mg/kg diet 10 days	BW, WG↓ serum and liver oxidative stress	LOAEL 600 mg/kg feed	Galli et al. (2020)
36, male Ross 708 broilers	0, 11 mg/kg diet for 14 days	BW, WG, FCR unchanged Sa/So ratio in serum and liver ↑ Intestinal cytokines ↑	TBD	Grenier et al. (2017)
20, male Cobb 500	0, 2.5, 5 or 10 mg FB_1/kg diet from day 12 to day 21 (10 days)	Feed intake, weight gain↓ Serum and liver oxidative stress parameters↑ Liver, gut, spleen and lung histology Villi height and crypt depth↓	LOAEL 2.5 mg/kg feed for decreased crypt depth; NOAEL 5 and LOAEL 10 mg/kg feed for decreased weight gain	Sousa et al. (2020)
126, 1-day-old male Ross 308 broilers	0, 20 mg FB_1/kg diet (+ FB_2) for 21 days with 3 different diets (starter, grower, finisher)	Body weight gain and feed intake ↓ GENE expression of antioxidant response, stress, inflammation, and integrity of different enteric segments ↓	LOAEL 20 mg/kg feed	Paraskeuas et al. (2021)
8, 9-week-old Isa Brown hens (chicken reared for laying)	0, 1.0, 4 or 10.9 mg/kg bw of the FB_1 + FB_2 extract for up to 21 days (intracrop) 1 mg/kg bw à 20 mg/kg diet, 4 mg/kg bw à 80 mg/kg diet 10.9 mg/kg bw à 218 mg/kg diet	WG ↓ enteric villi and crypt height ↓ liver histological changes, changes in bone structure and composition	LOAEL 20 mg/kg feed	Tomaszkievska et al. (2021)

N: Number; LOAEL: Lowest Observed Adverse Effect Level; NOAEL: No Observed Adverse Effect Level; Sa/So: Sphinganine/Sphingosine; BW: body weight; WG: weight gain; FCR: feed conversion ratio.

DON (Dec 15th 2022)

Table 3: New studies on adverse effects on broilers chickens which have become available since the 2017 Opinion (EFSA CONTAM Panel, 2017a)

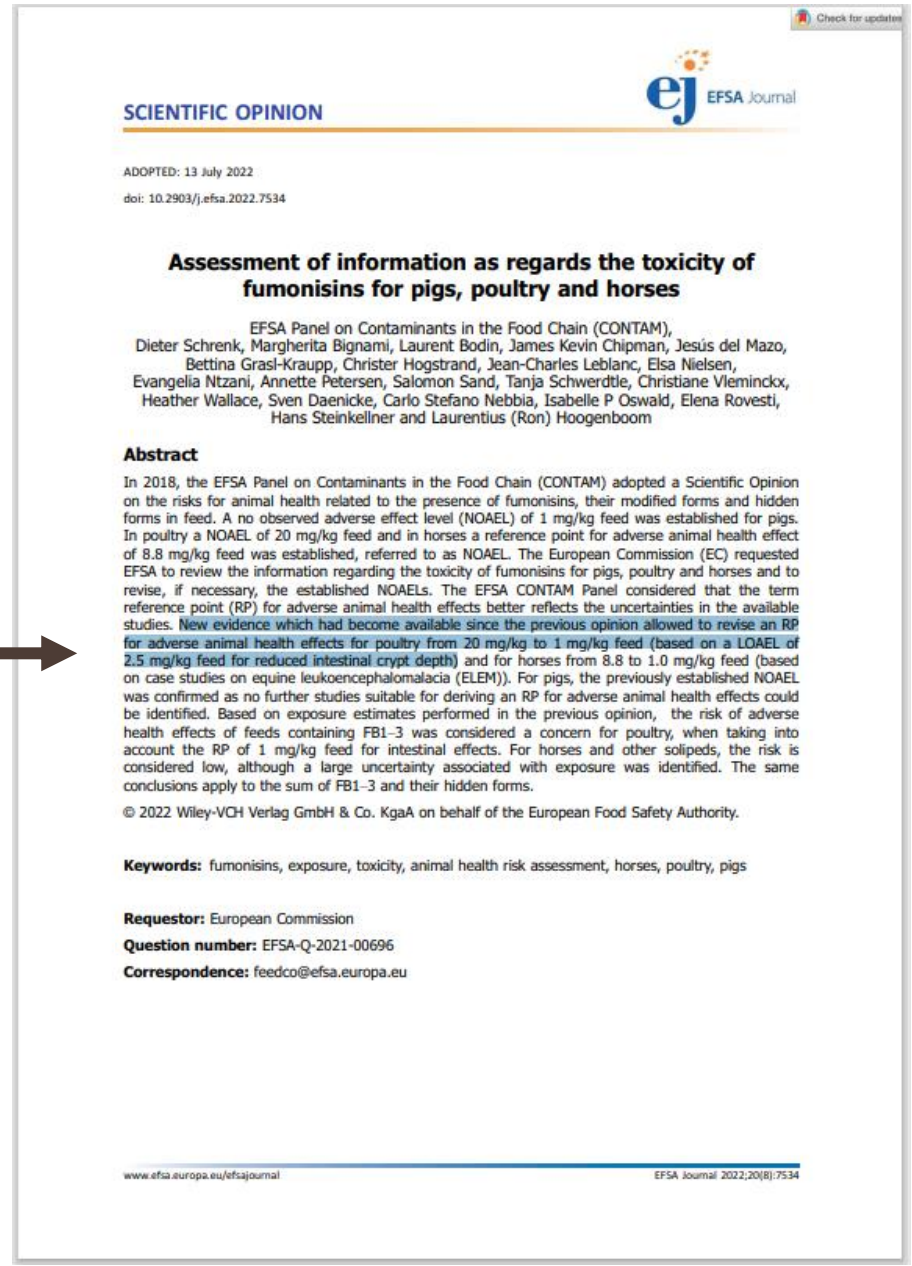
N[$]/group, breed gender	Dosage and duration (mg/kg feed or mg/kg bw)	Endpoint(s)	Adverse effect concentration (mg/kg feed)**	References
84, 1-day-old Ross 708 male broilers	0, 1.6 mg/kg for 20 days	• No significantly different villus height • No effect on BWG	No effects at 1.6 mg/kg*	Grenier et al., 2016
320 (plus 80 used for controls) Cobb- Cobb male broilers	DON (1.3, 4.3 mg/kg) for 15 days and the respective nitrogen free diets (NFD) (NFD Control; NFD DON 1.4 mg/kg and NFD DON 3.7 mg/kg) for 6 days	• Decreased digestibility of tyrosine. • No impact on BWG.	No effect at 4.3 mg/kg	Liu et al., 2020
452, 1-day-old male Ross 308 broilers	0, ~5[$] mg/kg for 39 days	• Reduction of BWG • Modulation of intestinal oxidative stress, detoxification, inflammation and integrity *Of note the authors used a challenge diet formulation*	Effects at ~5[$] mg/kg feed*	Paraskeuas et al., 2021
45, 1-day-old male broilers (Ross 308)	0, 4.65 and 15.12 mg/kg for 42 days.	• Increase in absolute and/or relative weight of thymus and gizzard weight • Decrease in the absolute and relative weight of the colon and the small intestine • Increased length and decreased density of the small intestine • Decrease in BWG at	Effects at 4.65 mg/kg	Riahi et al., 2020, Riahi et al., 2021b
60, 81-day-old Ross-308 male and female broilers	0, 5*** mg/kg for 5 weeks	• Decrease in BWG • Increased paracellular permeability and bacterial translocation • Increased susceptibility to infection by *Campylobacter jejuni*.	Effects at 5 mg/kg*	Ruhnau et al., 2020
60, 1-day-old Ross-308 broilers	0, 5*** mg/kg for 5 weeks	• Decrease in BWG • Increased paracellular permeability • Increased susceptibility to infection by *Campylobacter jejuni*.	Effects at 5 mg/kg*	Ruhnau et al., 2021
60, one-day-old male Ross 308 broilers	0, 3.95 mg/kg naturally and 3.86 mg/kg artificially contaminated diet for 14 and 28 days	• Reduction of villus height (day 14). • Severe damage of the jejunum villus (naturally and artificially contaminated, 28 days) abdomen, cysts in the liver, hydropericardium and enlargement of kidneys in 7/20 birds (naturally contaminated, 14 days). • No effect on body weight	Effects at 3.86 mg/kg*	Santos et al., 2021
120, 20-day-old male Ross 308 chickens	0.085 (control), 2.27 (low) and 5.84 (high) mg/kg for 6 days	• Increase in feed to gain ratio • No effect on body weight	Effects at 2.27 mg/kg	Wang et al., 2019
420, newly-hatched Ross 308 male broilers	0, starter diet: 6.62 mg/kg, (1–21 days of age), grower diet:7.9 mg/kg, (22–34 days of age).	• Suppressed growth of birds fed with grower diet. • Lower body weight and average daily gain. Higher feed to gain ratio in birds fed with DON grower diet. • Decreases in ileum villus height and depth of all DON treated birds.	Effects at 6.62 mg/kg	Wang and Hogan, 2019
36, 1-day-old Ross 308 broiler chickens	0, 5*** mg/kg for 4 weeks	• Shorter villus and decrease in ratio of villus height and crypt depth in duodenum • Reduced FCR • No effects on body weight and FI • Increased expression of *COX-2* in spleen and the bursa of Fabricius.	Effects at 5 mg/kg *	Yu et al., 2018, (Corrigendum, 2021)

[$]: Including the number of poultry in the control group.

FUM – EFSA Update July 13th, 2022

New evidence has become available since previous opinion allowed to revise an RP* for adverse animal **health effects in poultry** from 20 mg/kg to **1 mg/kg**** in feed

Based on a LOAEL*** of 2.5 mg/kg feed for *reduced intestinal crypt depth*



SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 13 July 2022

doi: 10.2903/j.efsa.2022.7534

Assessment of information as regards the toxicity of fumonisins for pigs, poultry and horses

EFSA Panel on Contaminants in the Food Chain (CONTAM), Dieter Schrenk, Margherita Bignami, Laurent Bodin, James Kevin Chipman, Jesús del Mazo, Bettina Grasl-Kraupp, Christer Hogstrand, Jean-Charles Leblanc, Elsa Nielsen, Evangelia Ntzani, Annette Petersen, Salomon Sand, Tanja Schwerdtle, Christiane Vleminckx, Heather Wallace, Sven Daenicke, Carlo Stefano Nebbia, Isabelle P Oswald, Elena Rovesti, Hans Steinkellner and Laurentius (Ron) Hoogenboom

Abstract

In 2018, the EFSA Panel on Contaminants in the Food Chain (CONTAM) adopted a Scientific Opinion on the risks for animal health related to the presence of fumonisins, their modified forms and hidden forms in feed. A no observed adverse effect level (NOAEL) of 1 mg/kg feed was established for pigs. In poultry a NOAEL of 20 mg/kg feed and in horses a reference point for adverse animal health effect of 8.8 mg/kg feed was established, referred to as NOAEL. The European Commission (EC) requested EFSA to review the information regarding the toxicity of fumonisins for pigs, poultry and horses and to revise, if necessary, the established NOAELs. The EFSA CONTAM Panel considered that the term reference point (RP) for adverse animal health effects better reflects the uncertainties in the available studies. New evidence which had become available since the previous opinion allowed to revise an RP for adverse animal health effects for poultry from 20 mg/kg to 1 mg/kg feed (based on a LOAEL of 2.5 mg/kg feed for reduced intestinal crypt depth) and for horses from 8.8 to 1.0 mg/kg feed (based on case studies on equine leukoencephalomalacia (ELEM)). For pigs, the previously established NOAEL was confirmed as no further studies suitable for deriving an RP for adverse animal health effects could be identified. Based on exposure estimates performed in the previous opinion, the risk of adverse health effects of feeds containing FB1–3 was considered a concern for poultry, when taking into account the RP of 1 mg/kg feed for intestinal effects. For horses and other solipeds, the risk is considered low, although a large uncertainty associated with exposure was identified. The same conclusions apply to the sum of FB1–3 and their hidden forms.

Keywords: fumonisins, exposure, toxicity, animal health risk assessment, horses, poultry, pigs

Requestor: European Commission

Question number: EFSA-Q-2021-00696

Correspondence: feedco@efsa.europa.eu

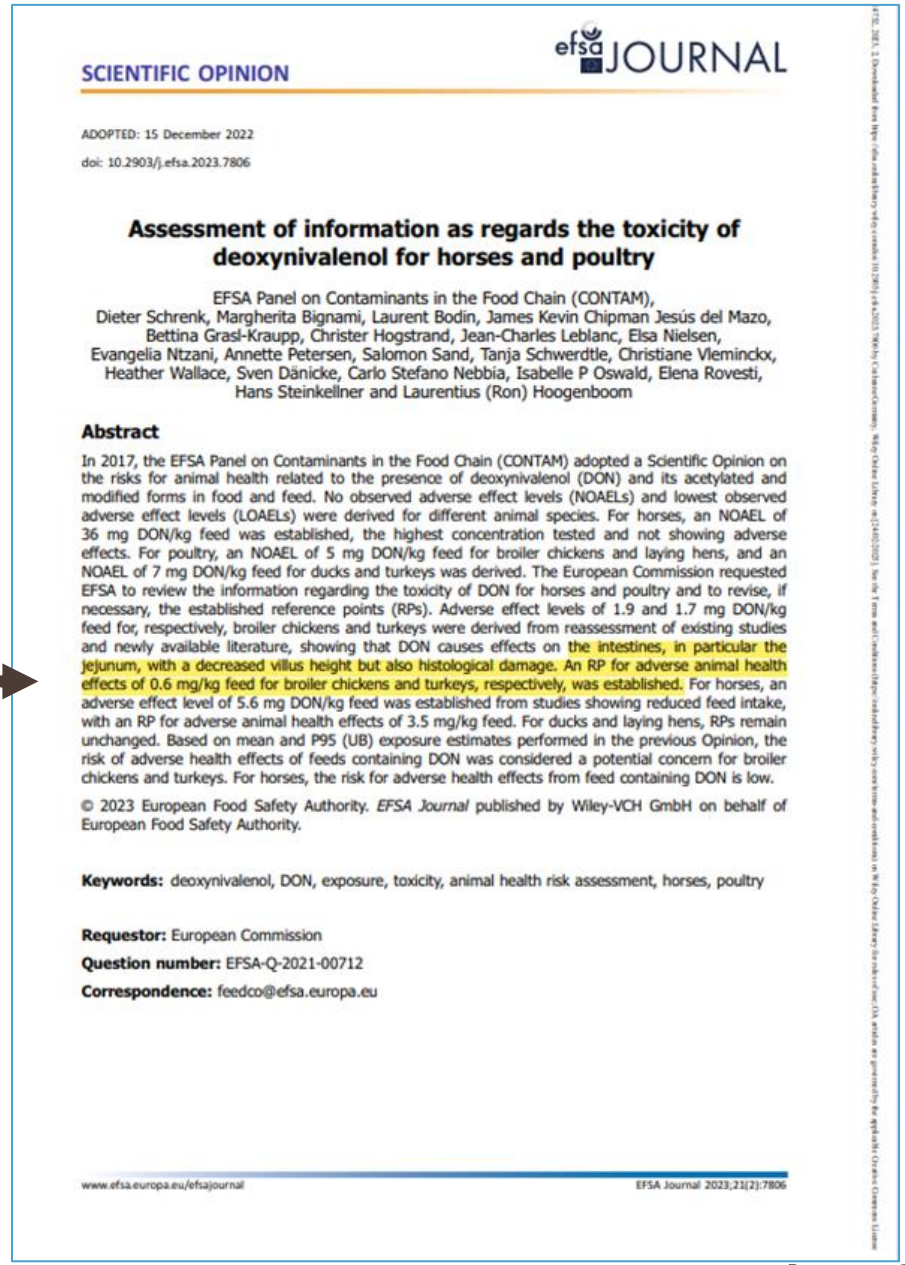
*Reference Point
**1 mg/kg: is 1 ppm
***LOAEL: Lowest Observed Adverse Effect Level

dsm-firmenich

DON – EFSA Update Dec 15th, 2022

New evidence has become available since the previous opinion allowing to revise the RP* for adverse animal **health effects in broiler chickens** and **turkeys** of resp. 5 and 7 mg/kg to **0.6 mg/kg**** in feed

Based on a LOAEL*** of resp 1.7 and 1.9 mg/kg for *decreased villus height and histological damage*



SCIENTIFIC OPINION

efsa JOURNAL

ADOPTED: 15 December 2022

doi: 10.2903/j.efsa.2023.7806

Assessment of information as regards the toxicity of deoxynivalenol for horses and poultry

EFSA Panel on Contaminants in the Food Chain (CONTAM),
Dieter Schrenk, Margherita Bignami, Laurent Bodin, James Kevin Chipman Jesús del Mazo, Bettina Grasl-Kraupp, Christer Hogstrand, Jean-Charles Leblanc, Elsa Nielsen, Evangelia Ntzani, Annette Petersen, Salomon Sand, Tanja Schwerdtle, Christiane Vleminckx, Heather Wallace, Sven Dänicke, Carlo Stefano Nebbia, Isabelle P Oswald, Elena Rovesti, Hans Steinkellner and Laurentius (Ron) Hoogenboom

Abstract

In 2017, the EFSA Panel on Contaminants in the Food Chain (CONTAM) adopted a Scientific Opinion on the risks for animal health related to the presence of deoxynivalenol (DON) and its acetylated and modified forms in food and feed. No observed adverse effect levels (NOAELs) and lowest observed adverse effect levels (LOAELs) were derived for different animal species. For horses, an NOAEL of 36 mg DON/kg feed was established, the highest concentration tested and not showing adverse effects. For poultry, an NOAEL of 5 mg DON/kg feed for broiler chickens and laying hens, and an NOAEL of 7 mg DON/kg feed for ducks and turkeys was derived. The European Commission requested EFSA to review the information regarding the toxicity of DON for horses and poultry and to revise, if necessary, the established reference points (RPs). Adverse effect levels of 1.9 and 1.7 mg DON/kg feed for, respectively, broiler chickens and turkeys were derived from reassessment of existing studies and newly available literature, showing that DON causes effects on the intestines, in particular the jejunum, with a decreased villus height but also histological damage. An RP for adverse animal health effects of 0.6 mg/kg feed for broiler chickens and turkeys, respectively, was established. For horses, an adverse effect level of 5.6 mg DON/kg feed was established from studies showing reduced feed intake, with an RP for adverse animal health effects of 3.5 mg/kg feed. For ducks and laying hens, RPs remain unchanged. Based on mean and P95 (UB) exposure estimates performed in the previous Opinion, the risk of adverse health effects of feeds containing DON was considered a potential concern for broiler chickens and turkeys. For horses, the risk for adverse health effects from feed containing DON is low.

Keywords: deoxynivalenol, DON, exposure, toxicity, animal health risk assessment, horses, poultry

Requestor: European Commission

Question number: EFSA-Q-2021-00712

Correspondence: feedco@efsa.europa.eu

*Reference Point
**1 mg/kg: is 1 ppm
***LOAEL: Lowest Observed Adverse Effect Level

dsm-firmenich

Take Home Messages

- High abundance of mycotoxins in **European poultry Finished Feed (pFF)**
- Levels **exceeding EFSA RP*** for **FUM** and/or **DON** in **at least 25%** of all **EU pFF samples****

Natural Mycotoxins Levels Cause:

- **(Sub)clinical effects** (predisposing factor)
- **Negatively affect intestinal homeostasis**
  - ↑ inflammatory signalling, leaky gut
  - ↑ animal susceptibility to enteric infectious diseases
- **Impair vaccine efficacy** in poultry (ROI?)
- **Impair intestinal** and **immune-function**
- **Transfer via egg:**
  - Egg safety ↓
  - Hatchability ↓
  - Quality DOC ↓

'Energy' waste!

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*Reference Points of 2022 by EFSA Scientific Opinion
**Global mycotoxin survey (2022 until march 2023) dsm-firmenich

3 Prevention

Mycotoxins are coming from the field and unproper storage

Initially 40% of material is rejected at the farm level

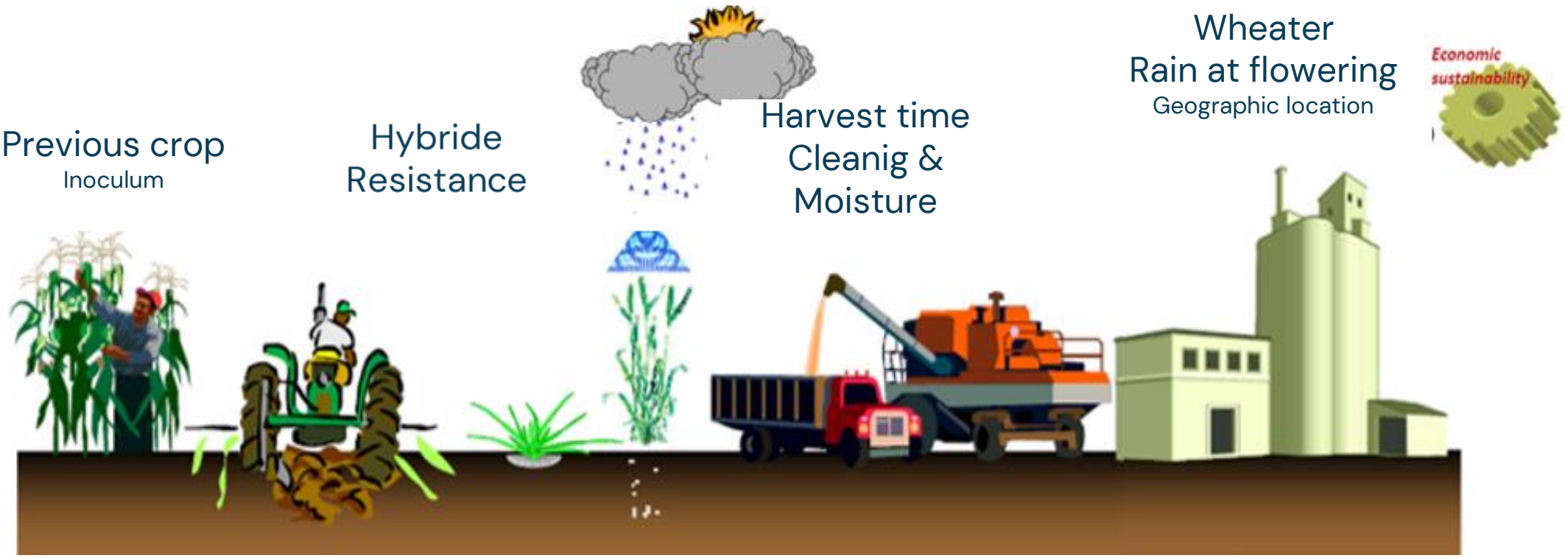
Previous crop
Inoculum

Hybride
Resistance

Harvest time
Cleanig &
Moisture

Wheater
Rain at flowering
Geographic location

Economic sustainability



Soil
Inoculum
Agro Techn.
Tilling

Fungiside
Protection
Triazoles

Storage
Adequate storage condition
Tem. C, H2O, cleanest

Jöhr (2010) presented at STDF/LNV/World Bank Workshop, Den Haag

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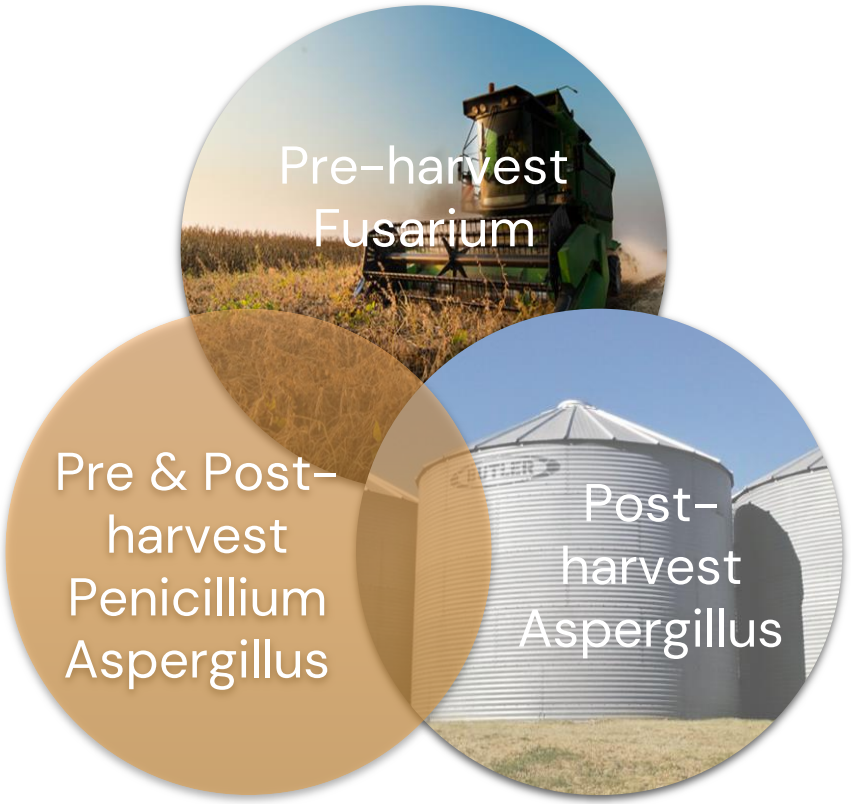
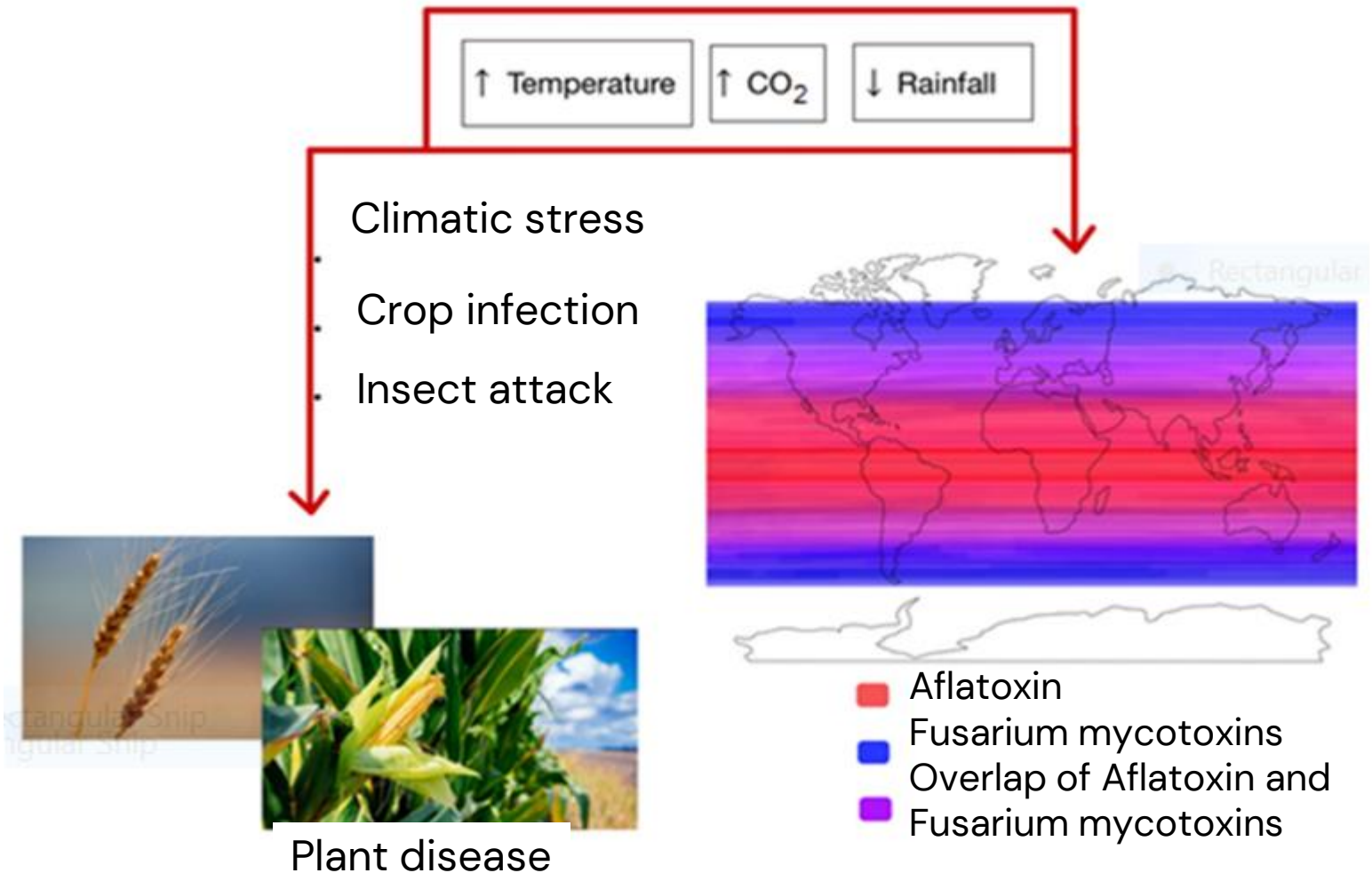
Conditions Facilitating Mycotoxin Production





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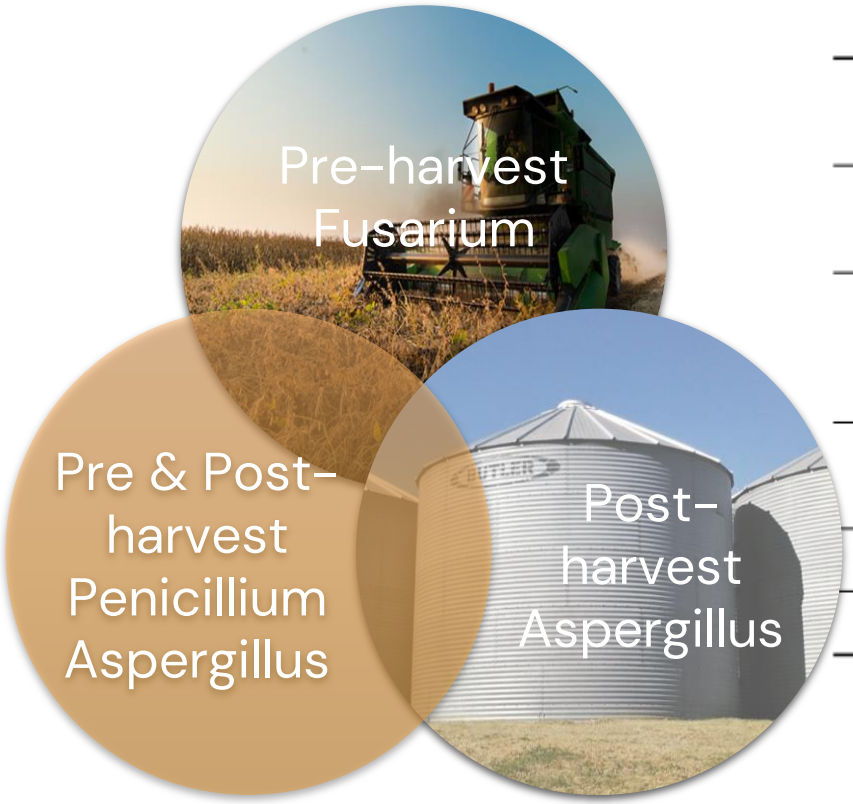
Mycotoxin-producing mould species and their optimal growth conditions



Mycotoxin	Mould	Temperature Range (°C)	Optimal Temperature (°C)	Water Activity (a_w)	pH
AFs	*A. flavus*	10–48	33	0.80–0.99	2–10
	A. parasiticus	12–42	32	0.80–0.99	3–8
OTA	*A. ochraceus*	10–40	37	0.80	3–10
	P. verrucosum	0–31	20	0.86	6–7
	A. niger	6–47	36	0.77–0.92	2–6.5
FUM	*F. verticilloides*	2.5–37	25	0.90–0.99	2.4–3
	F. proliferatum	5–37			
ZEN	*F. culmorum*	0–31	21	0.96	3–9
DON	*F. graminearum*	5–37	25	0.99	2.4–3

Pitt, J.I.; Hocking, A.D. Fungi and Food Spoilage; Springer: New York, NY, USA, 2009

Prevention of mycotoxin contamination field and manufacture

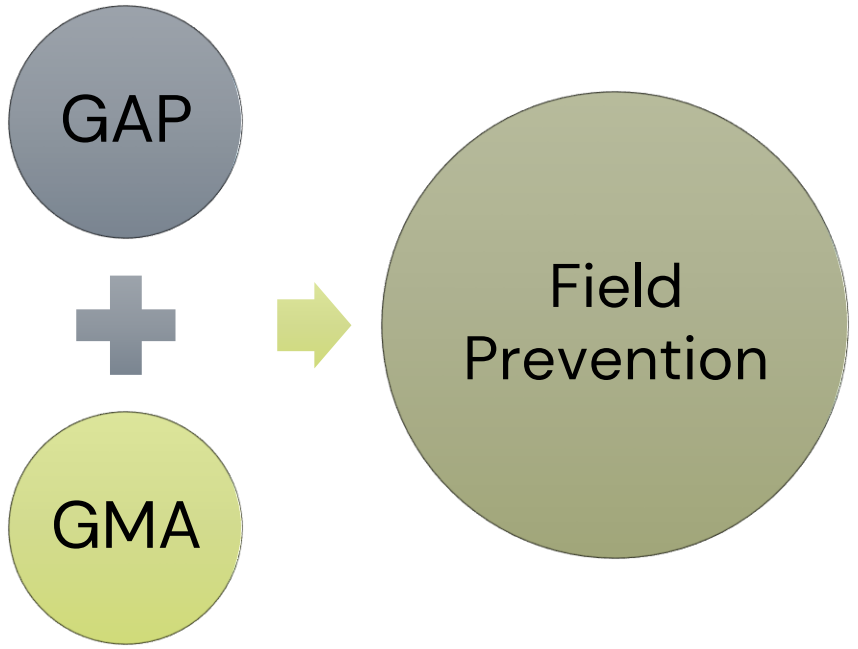
GAP &GMA

GAP Good Agriculture Practices

1. Hybrid selection, resistant to toxigenic moulds, insects, and drought;
2. Biological control, anti-pest and anti-fungal treatments;
3. Irrigation;
4. Crop rotation;
5. Herbicide application; etc.

GMP Good Manufacture Practices

1. Drying and storage practices both raw materials and final products
2. Water activity of the cereals less than 0.65, corresponds to a moisture level of 15 percent
3. Reducing levels of mycotoxins in the feed supply chain
4. Sorting, segregation, restoration, withdrawal
5. Hygiene, harvested crops are piled before drying and cleaning
6. Key parts of GMP include hazard analysis at critical control points



3 Why do you need Mycofix®

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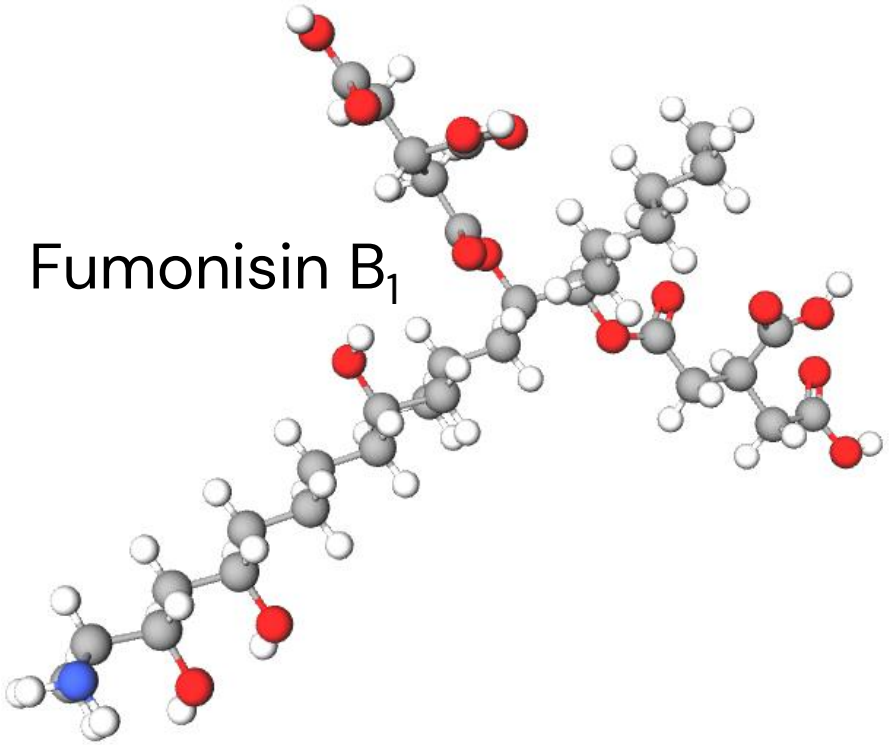
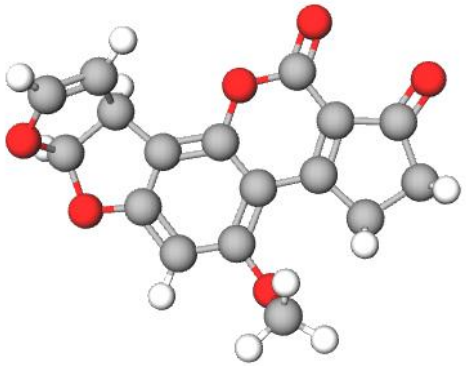
Agriculturally relevant mycotoxins – diversity in structures

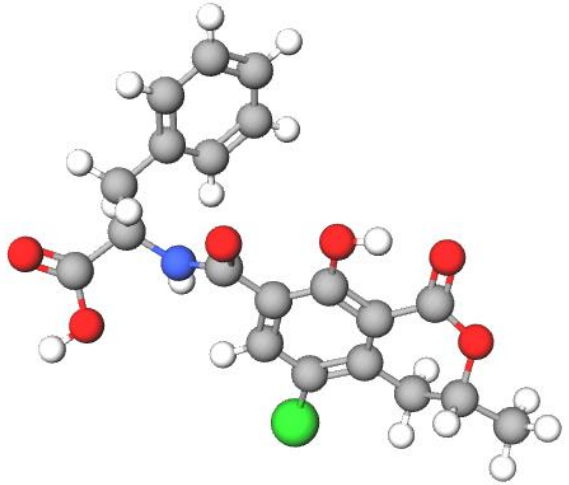
Need for a toolbox of different detoxification strategies



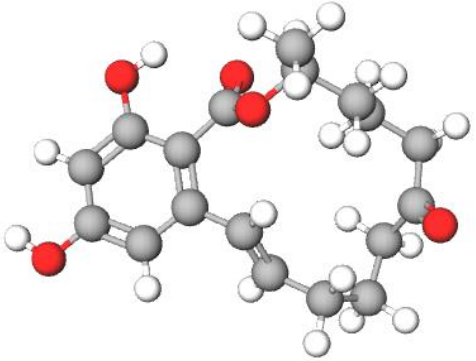
Fumonisin B_1



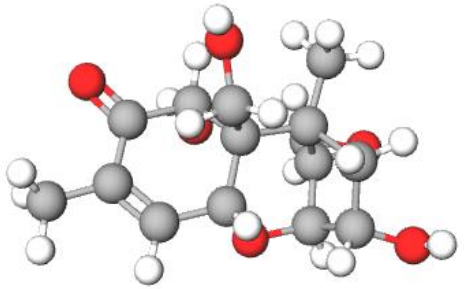
Aflatoxin



Ochratoxin A



Zearalenone



Deoxynivalenol

http://molview.org

Scientifically described approaches

Binding / adsorption of mycotoxins

- Products referred to as “binder”, “adsorbents”, “enterosorbents”, etc.)
- Organic (microbial) or inorganic (mostly clay)

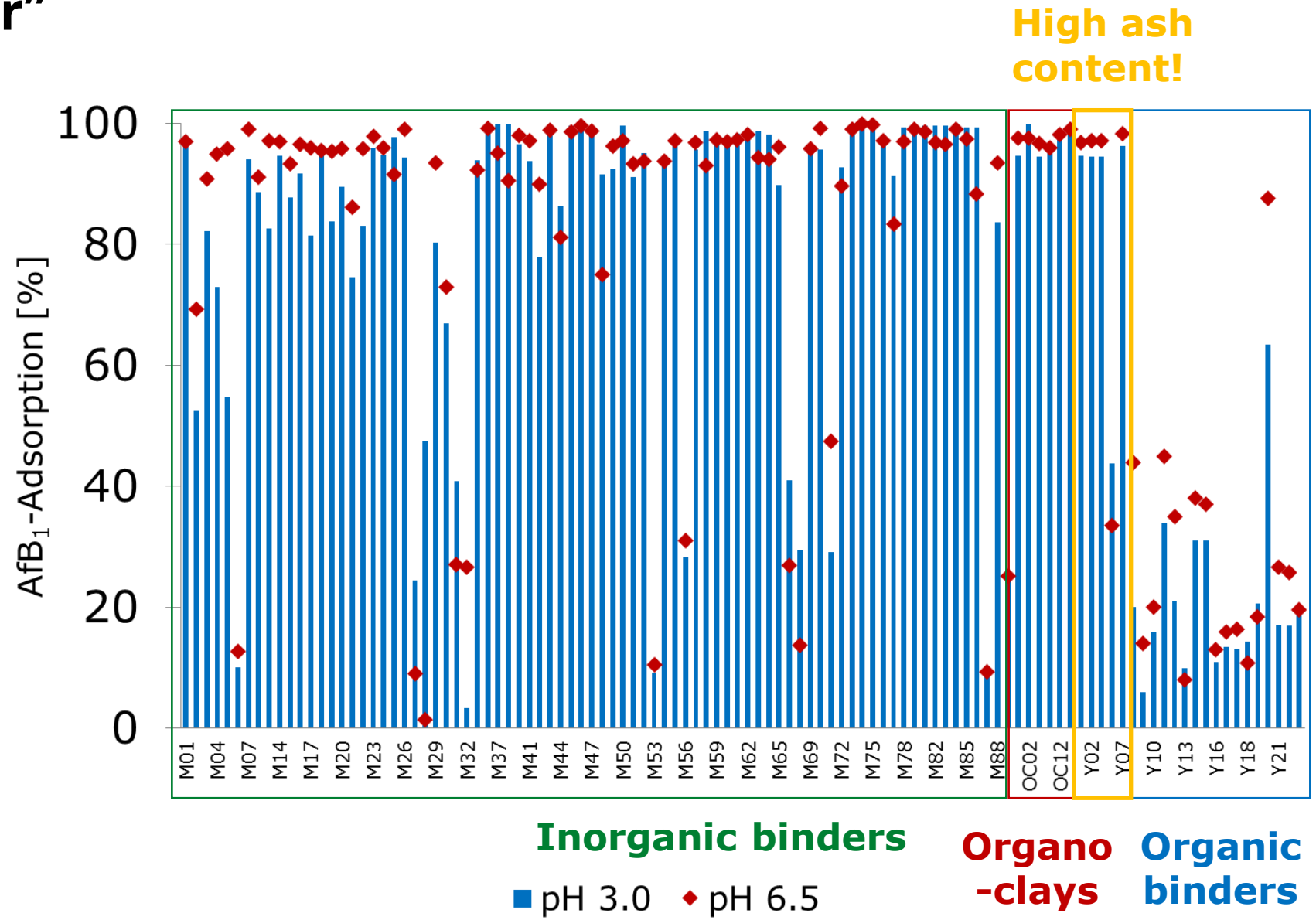
Enzymatic detoxification (biotransformation)

- Microorganisms/enzymes which transform or degrade mycotoxins to less- or non-toxic metabolites

Bioprotection

- Protection of vulnerable organs or strengthening of immune system

Conventional Binding Test: 200 ppb AFB_1 and 2 kg/tonne "binder"



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Stringent evaluation of binding – testing scheme

efsa
European Food Safety Authority

- **Conventional Binding Test** — 200 ppb AfB1, 0.2% binder, pH 3 and pH 6.5
- **Testing under "intensified" conditions** — 4 ppm AfB1, 0.02% binder, pH 5
- **Determination of chemisorption index** — Extraction with methanol and chloroform
- **Binding of nutrients (vitamins, trace elements)** — Binder mixed with nutrients, analyzed with and without binder

- **Adsorption Isotherms** — High AfB1 and low binder concentration
- **Efficacy in gastro-intestinal tract *(in vitro)*** — Binding study in gastric juices
- **Check for risk contaminants** — Heavy metals, dioxins
- ***In vivo* evaluation**

IFA TULLN

Christian Doppler Forschungsgesellschaft

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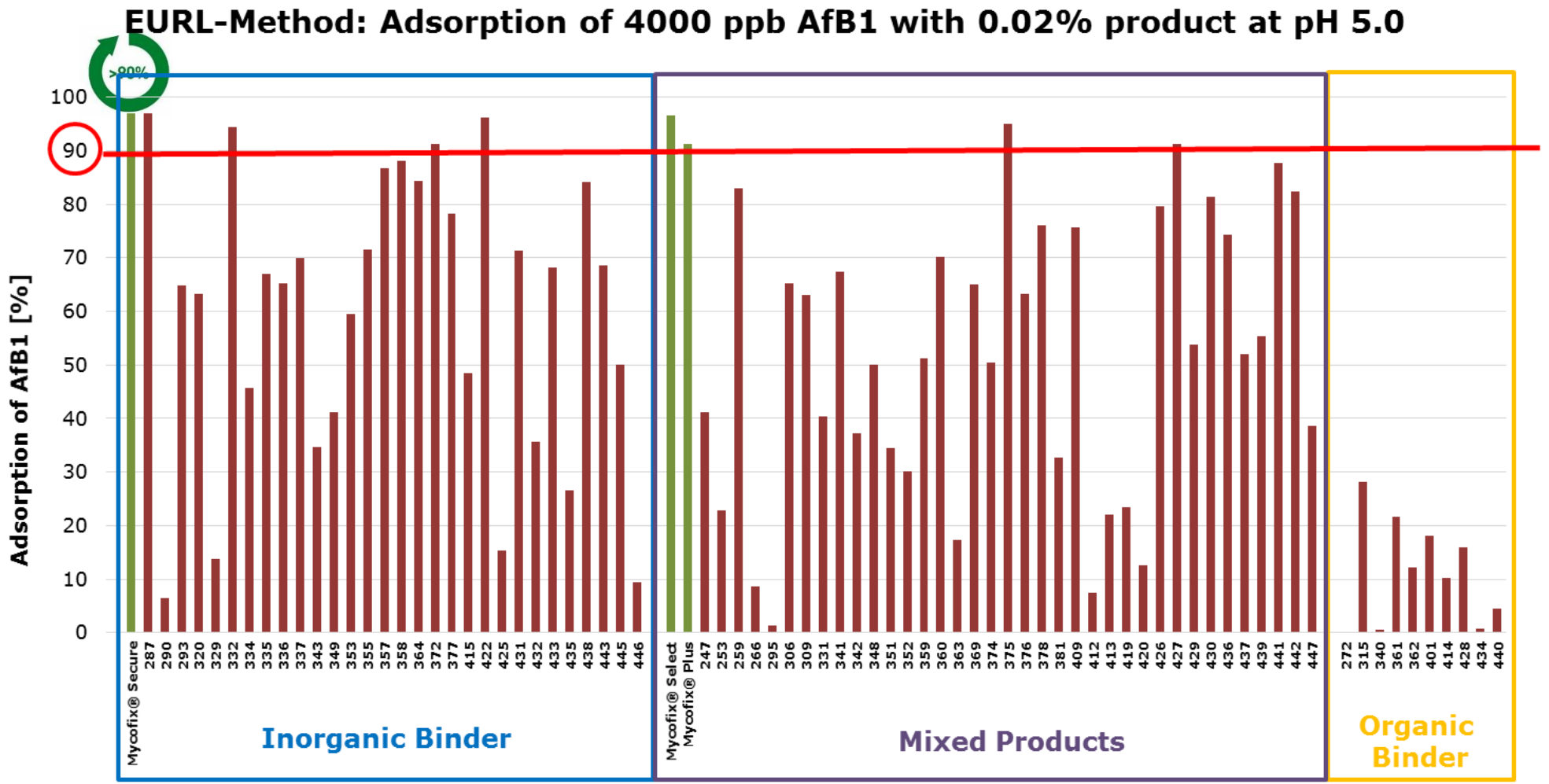
Stringent EURL *in vitro* method better reflects *in vivo* situation



EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements (Geel)
Standards for Food Bioscience Unit
European Union Reference Laboratory for Feed Additives - Authorisation

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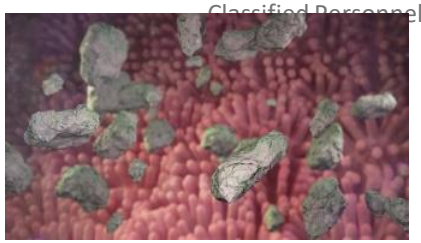
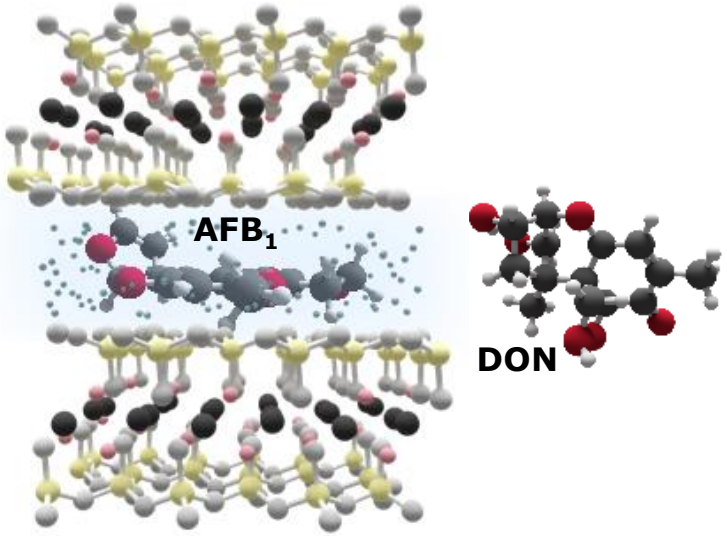
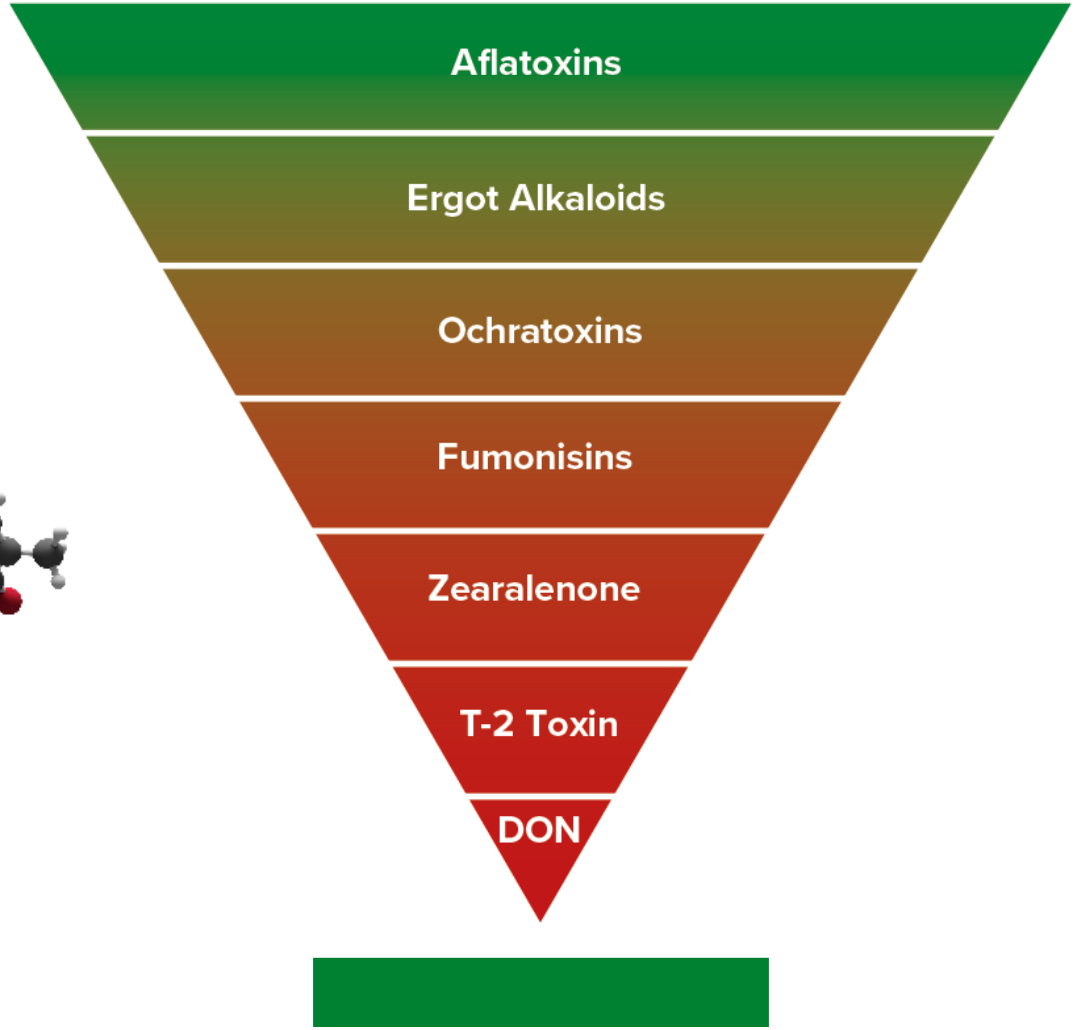
Adsorption (elimination of toxins)



EU authorized bentonite enables the elimination of the toxin by adsorption

COMMISSION IMPLEMENTING REGULATION (EU) No 1060/2013
of 29 October 2013
concerning the authorisation of bentonite as a feed additive for all animal species





1 polarity
2 functional groups
3 planarity

Adsorption efficacy

Hahn *et al.* 2015; Vekiru *et al.* 2014; EU Regulation 1060/2013; Frühauf *et al.* 2012; Vekiru *et al.* 2010; Deng *et al.* 2010; Friend *et al.* 1984; Kubena *et al.* 1990, 1991, 1993; Bursian *et al.* 1992; Williams *et al.* 1994; Phillips *et al.* 1995; Ramos *et al.* 1996; Scott *et al.* 1998

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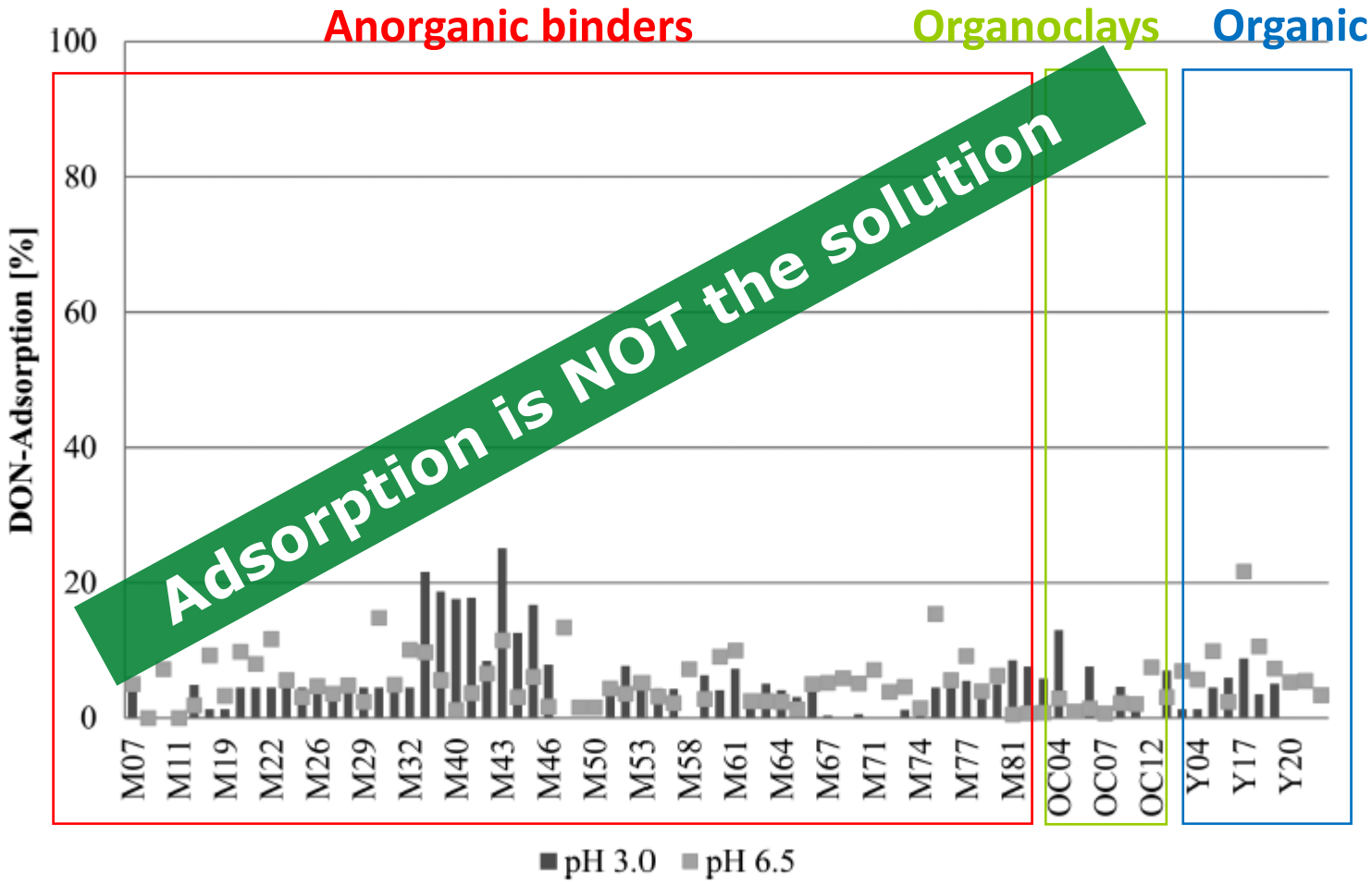
Adsorption of deoxynivalenol – commercially available products on the market (1000 ppb – by 2kg/ton binder)



DON

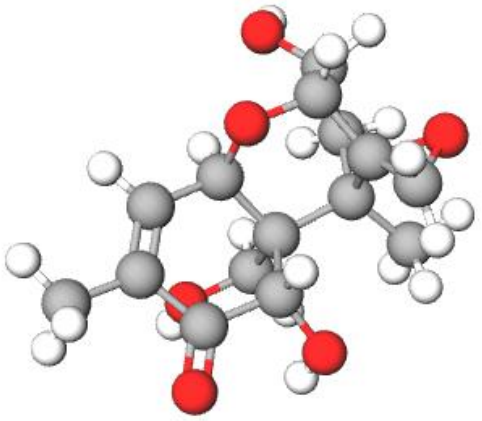


Figure 3. Adsorption capacity of mycotoxin binder products of different origins at pH 3.0 and pH 6.5 on deoxynivalenol (DON). M: Mineral; OC: Organoclay; Y: Yeast.

Murugesan, et al 2015, Poultry Science

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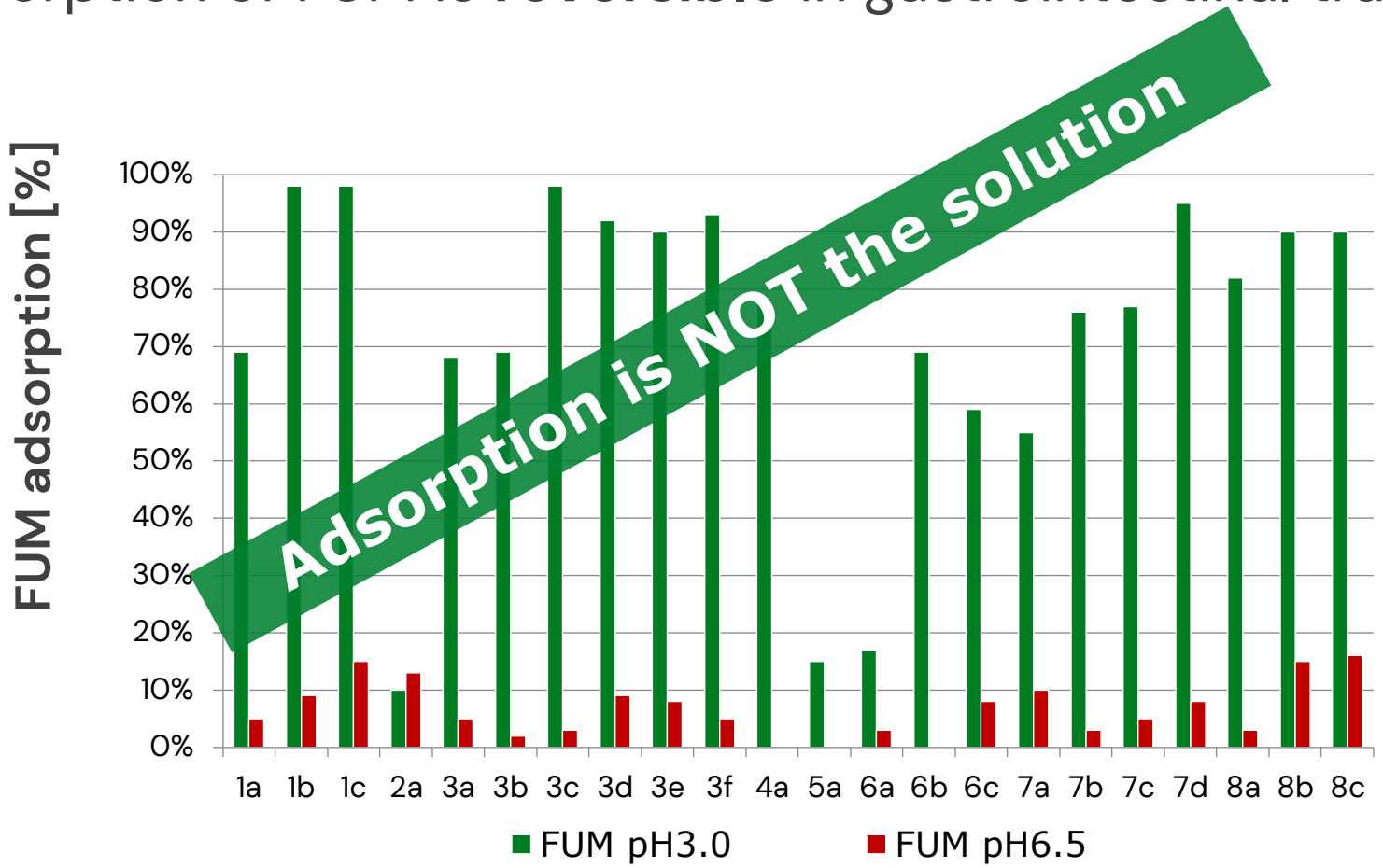
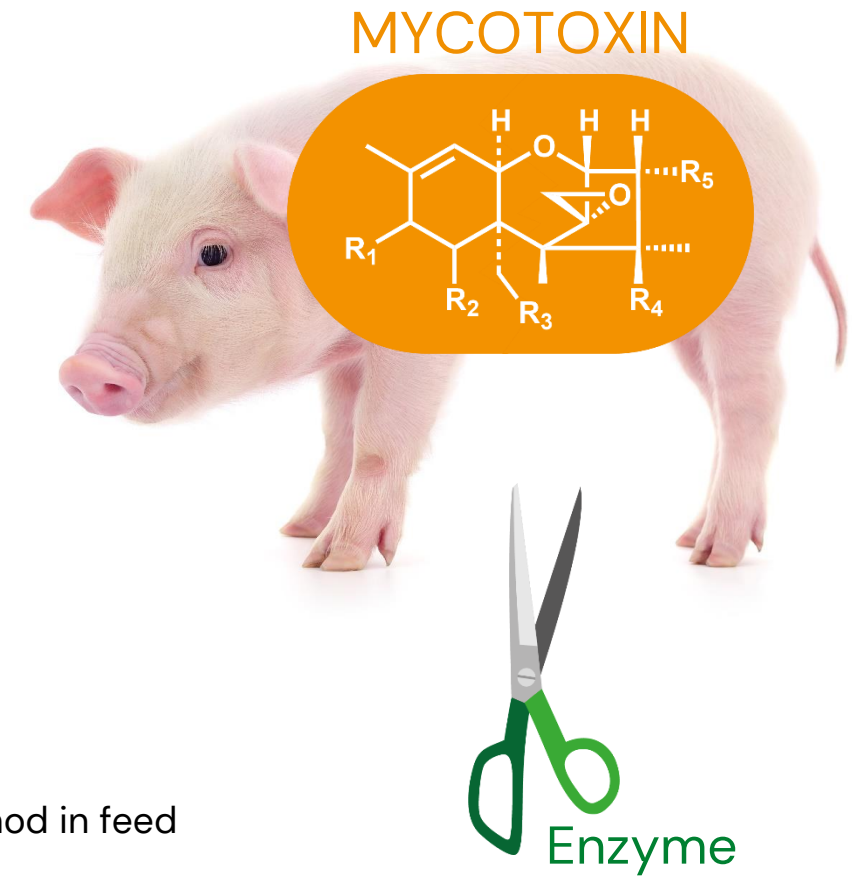
Adsorption of FUM: depends on pH

Adsorption of FUM is **reversible** in gastrointestinal tract!



Enzymatic detoxification (“Biotransformation”) is a very specific, irreversible detoxification method

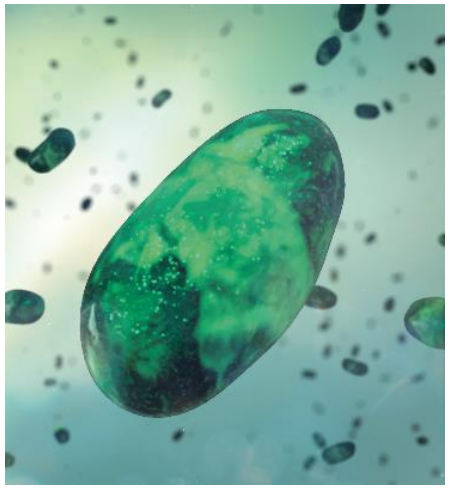
Non-toxic metabolite



Non-toxic metabolite

- ✓ Specific & direct effect
- ✓ Irreversible
- ✓ Safe
- ✓ Not interfering analytical MTXs method in feed

Biomin® BBSH® 797 – for the deactivation of trichothecenes

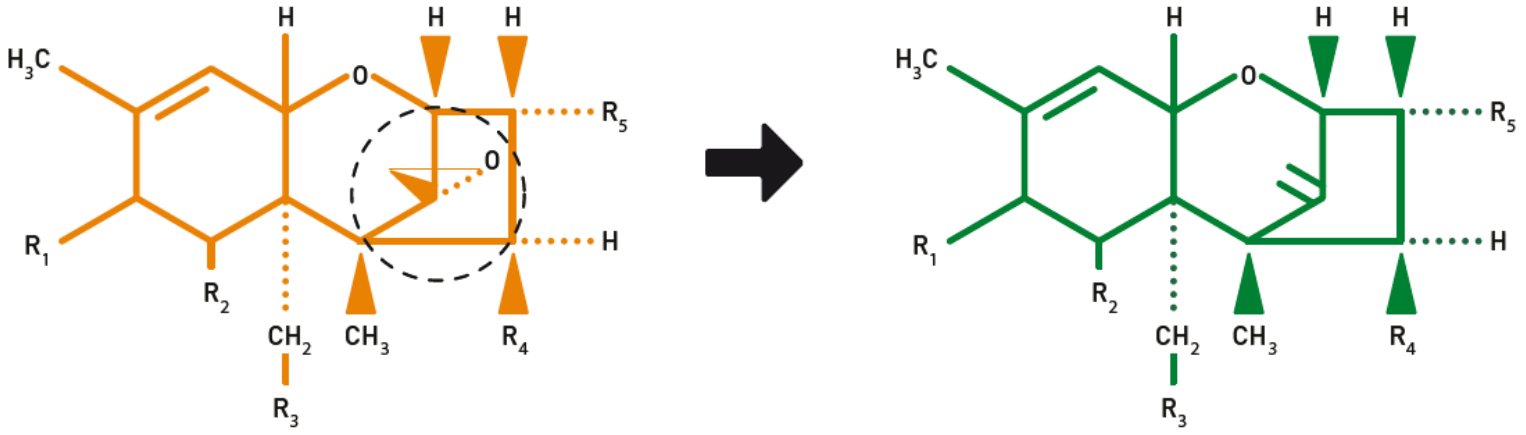


German collection of microorganisms and cell cultures

DSMZ

Biomin® BBSH® 797

- Genus nov. (formerly *Eubacterium*) sp. nov. BBSH 797
- DSM 11798
- Live organism
- Produces de-epoxidases which open the toxic epoxide ring of trichothecenes (e.g. DON)



Trichothecenes

detoxified form

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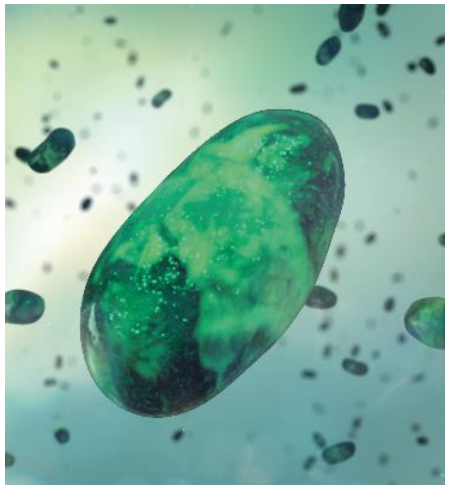
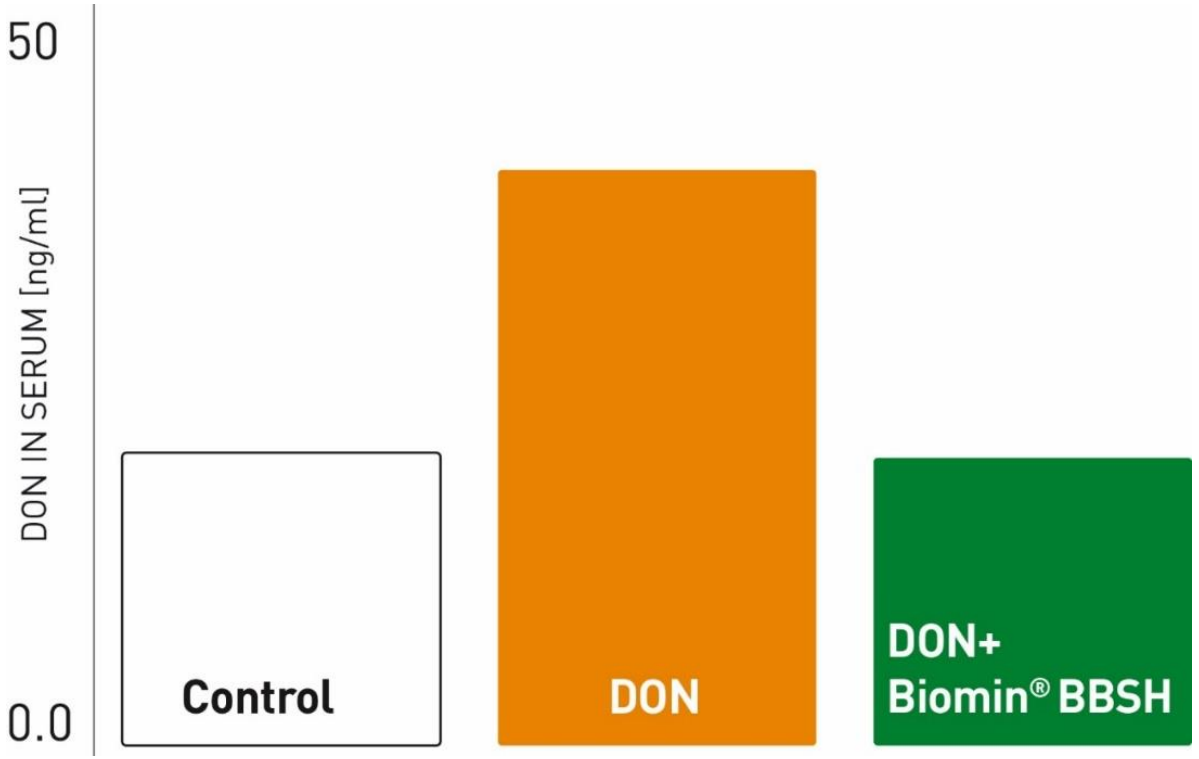
Biomin® BBSH® 797 – for the deactivation of trichothecenes



Biomin® BBSH 797

Biomin® BBSH® 797

The microorganism that biotransforms trichothecenes into harmless metabolites.



EU authorized Biomin® BBSH® 797 significantly reduces deoxynivalenol (DON) concentration in serum.

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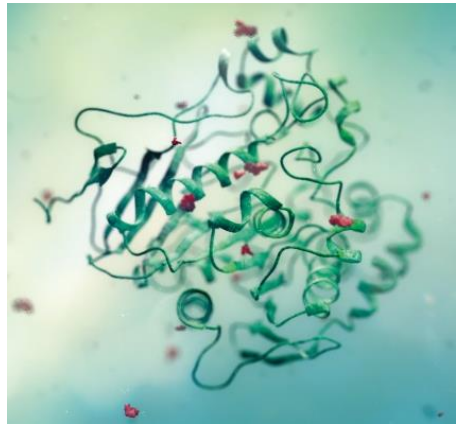
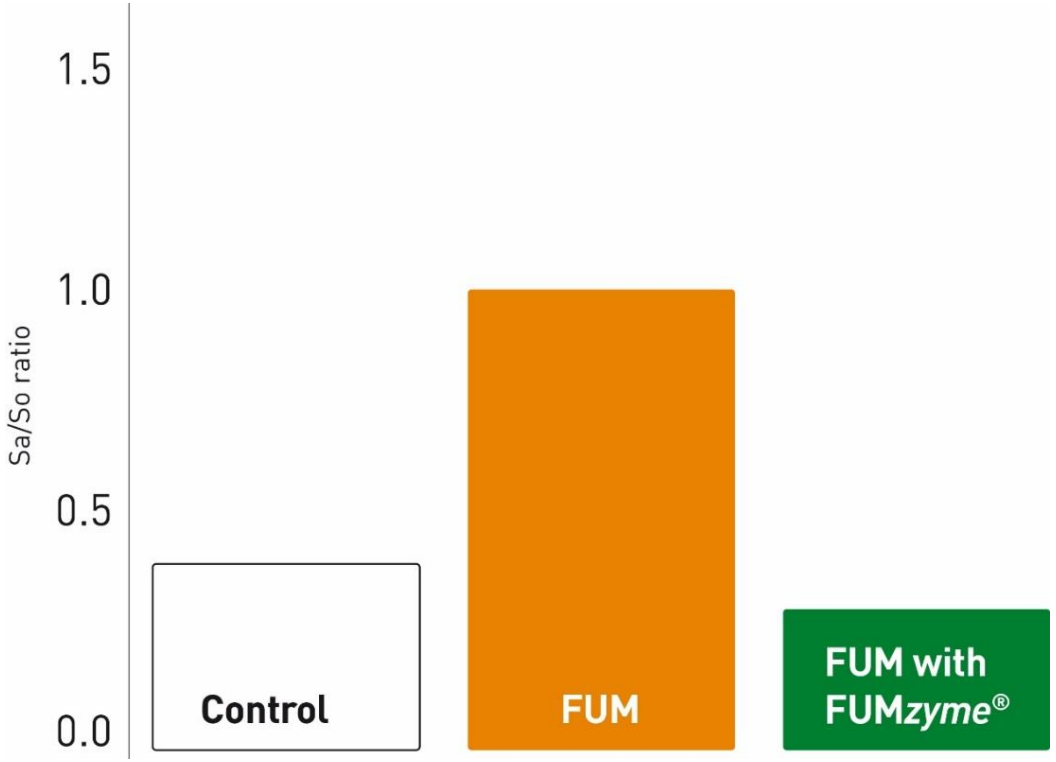
FUM*zyme*® – for the deactivation of fumonisins

FUM*zyme*®

The first-ever purified enzyme to degrade fumonisins specifically and irreversibly into nontoxic metabolites.



FUM*zyme*®

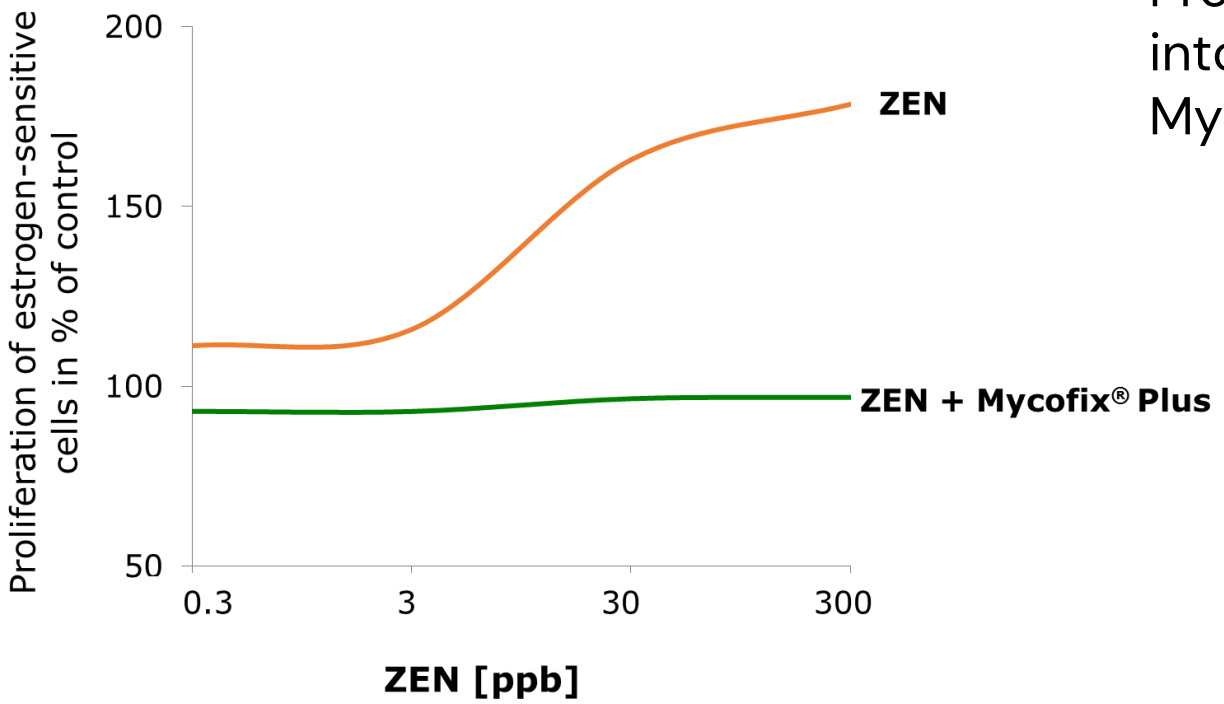


Detoxifies fumonisins (FUM), proven by the decrease of the sphinganine/sphingosine ratio (Sa/So) biomarker.

Biological constituent – for the deactivation of zearalenone

Biological constituent

The yeast strain specifically detoxifies zearalenone into nontoxic substances.



Proven zearalenone (ZEN) degradation into non-estrogenic metabolites by Mycofix® 5.E tested in the E-screen assay.

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Biomin® Bioprotection Mix

An innovative blend of natural ingredients

- Provides immune and liver support
- Protects the animal from the toxic effects caused by mycotoxins by supporting proliferation of immune cells
- Supports the intestinal barrier and the tight junctions from the negative effects of mycotoxins

Mycofix® 5.E

The all-in-one mycotoxin risk management solution for the most complete protection

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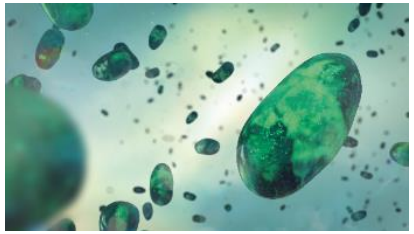
Mycofix® product line – The absolute protection against mycotoxins

Biotransformation

A unique combination of patented specific enzymes and biological components converts mycotoxins into nontoxic, environmentally-safe metabolites in the digestive tract of animals.

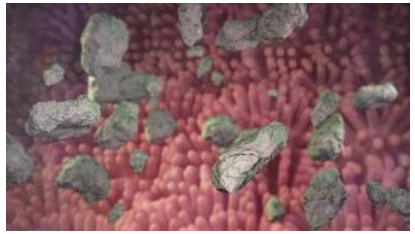
FUM*zyme*® - purified enzyme degrades FUM



Biomin® BBSH® 797 DSM 11798 degrades trichothecenes

Biological constituent deactivation of zearalenone

Adsorption



Synergistic blend of minerals

Adsorbs Aflatoxins, Ergot alkaloids and endotoxins

Bioprotection

Sjn: delete?

Biomin® Bioprotection Mix

Supports the liver, immune system and intestinal integrity

Mycofix® product line

Strategy	Mycotoxin Challenge	Mycofix Secure	Mycofix PRO-tect	Mycofix Select	Mycofix Plus
Bio trans formation	Zearalenone				■
	Trichothecenes			■	■
	Fumonisins			■	■
Bio protection	GIT integrity		■	■	■
	Immune support		■	■	■
	Protection of liver		■	■	■
Adsorption	Aflatoxins	■	■	■	■
	Ergot alkaloids	■	■	■	■
	Endotoxins	■	■	■	■
	Adsorbable Mycotoxins	■	■	■	■

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EU authorization – A benchmark for quality!

- ✓ 1st time an official authority imposes demanding and rigid requirements on **identity, safety and efficacy** of a mycotoxin-deactivation products
- ✓ The registration demonstrates the capacity of such products in a **standardized** and **fair process.**

- ✓ **Even outside of the EU**

More than **500** binders on market**, none** granted EFSA or FDA authorizations.

Just **3** ingredients have it: **Bentonite, Biomin® BBSH ® 797, FUM*zyme*®**

Take Home Messages

Mycotoxins are highly prevalent in our feed

Check MTXs in Feed* !

High synergism among mycotoxins could lead to (sub)clinical processes

Put it in your Differential Diagnosis

EU (EFSA) guidance levels of mycotoxins in animal feed are getting lower

Use an EFSA approved Mycotoxin-deactivator

Effective mycotoxin risk management program should be the base for **PROTECT**ing **production animals**

*Golden standard for MTXs (farm) risk assessment

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We bring progress to life™

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