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Mycotoxin limits in feed

Significant changes in recent decades

Large number of measurements

Analytical methods, multitoxin determinations

Rapid tests

New generation, toxin and species-specific toxin binding and toxin neutralizing preparations

Numerous new research findings on the effects of mycotoxins in animals and humans

Introduction of different mycotoxin risk assessment systems in feed production

World Overview 2023

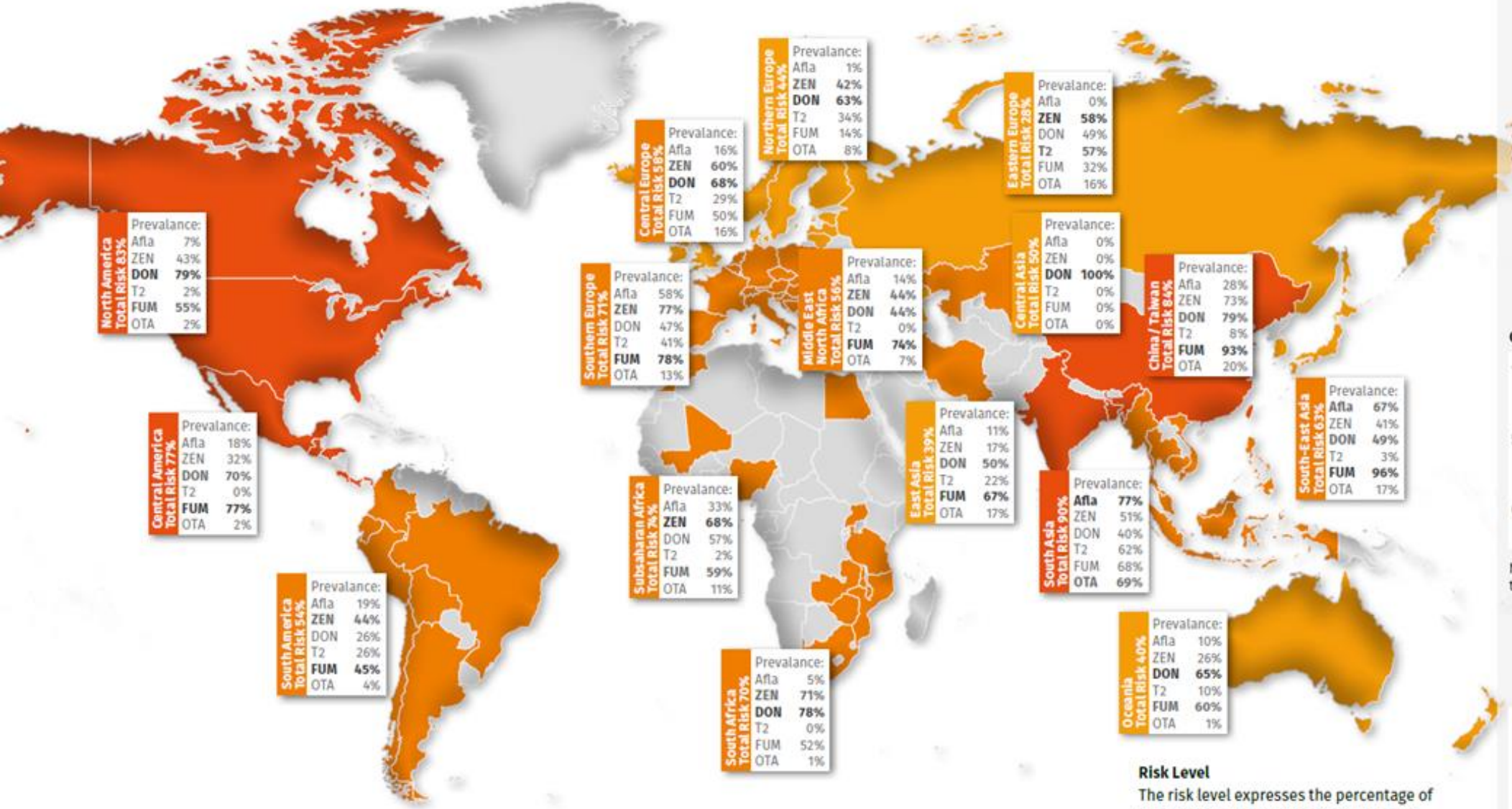


Figure 1. Global map of mycotoxin prevalence and risk in different regions.

0 – 25% | 26 – 50% | 51 – 75% | 76 – 100%

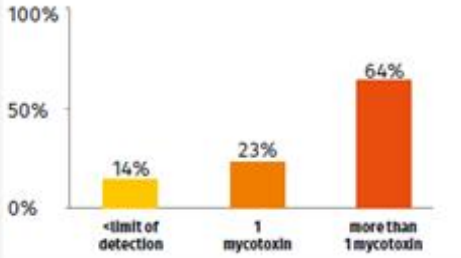
of samples above risk threshold

No samples tested

Moderate risk ⟷ Extreme risk

10 199 Samples | 50 407 Analyses | 78 Countries

Co-contamination



Number of mycotoxins per sample based on samples tested for 3 or more mycotoxins.

Risk Level

The risk level expresses the percentage of samples testing positive for at least one mycotoxin above the threshold level in parts per billion (ppb).

Recommended risk threshold of major mycotoxins in ppb

Afla	ZEN	DON	T-2	FUM	OTA
2	50	150	50	500	10

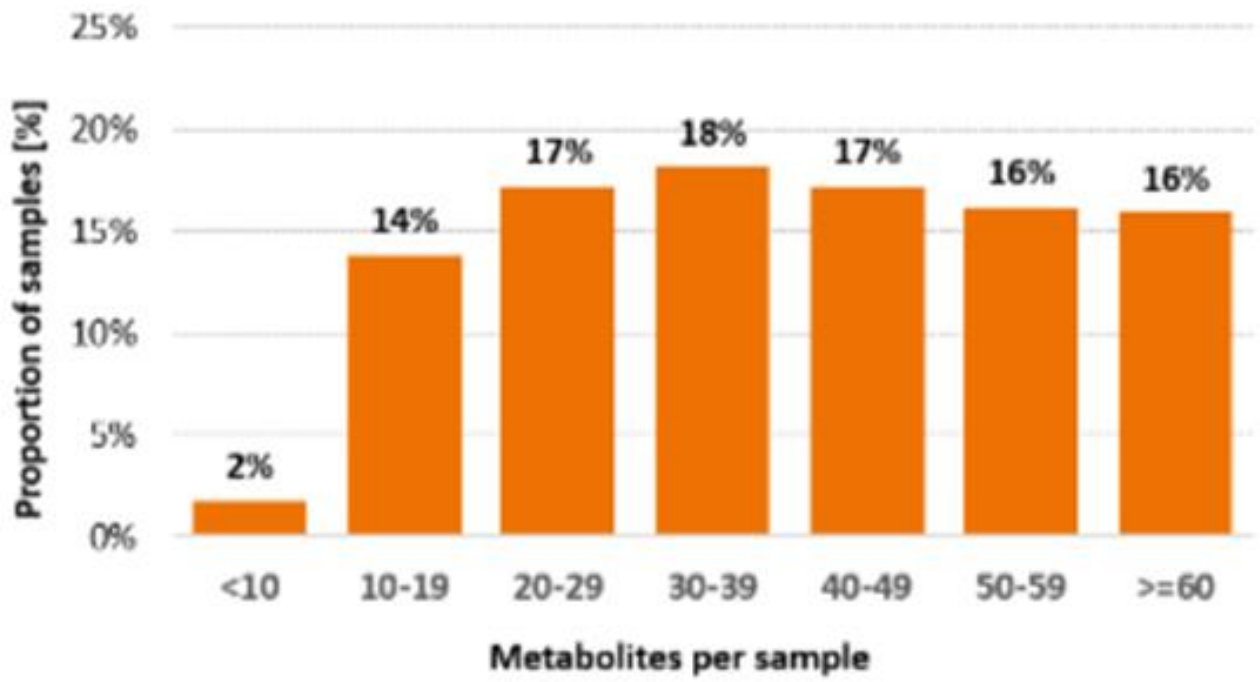
ACKNOWLEDGEMENTS
Special thanks go to Biofarma Feedlab Argentina and Anita Mengyan, Tiergesundheitsdienst Bayern e.V. for sharing their mycotoxin analysis results as part of this survey. Mycotoxin Report is published by DSM Austria GmbH, Erber Campus, 3131 Getzersdorf, Austria, Tel: +43 2782 8030, www.dsm.com/anh

Efficient multi-mycotoxin analytical procedures

Spectrum 380®

> 500 different mycotoxins and metabolites, bacterial and plant toxins/metabolites

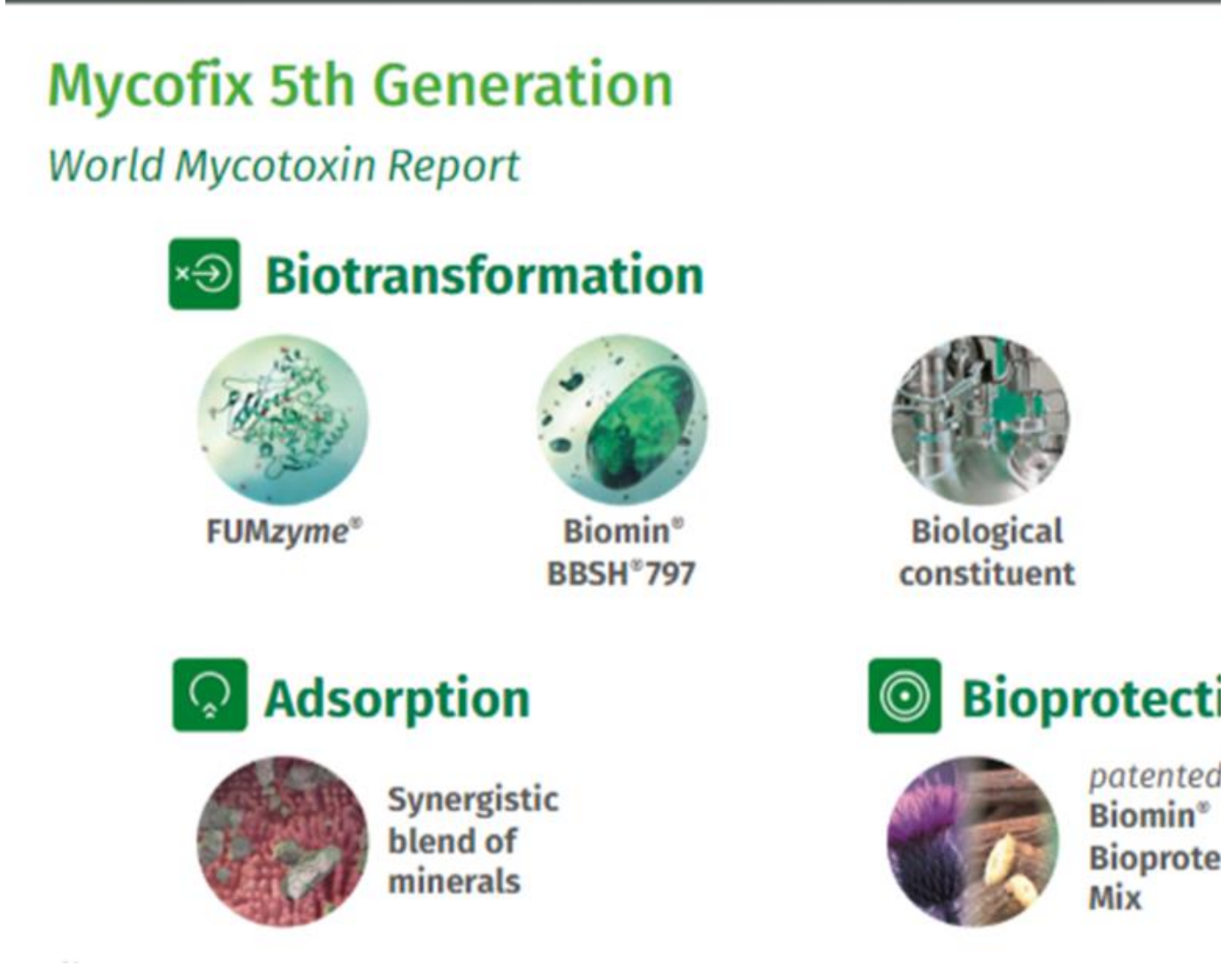
on request:
pesticide residues, veterinary drug residues



Efficient multi-mycotoxin analytical procedures

MycoFossTM

Multicomponent toxin binder/ neutralising preparations



New and continuing challanges

- effects of climate change (appearance of aflatoxin in Hungary, significant seasonal differences)
- carbon footprint, sustainability aspects in the feed industry (restructing, soy?)
- global food crisis (more grains are used as food, more by-products end up in feed)
- Russian-Ukraine war
- age specialties of farm animals
- racial specialties
- changes in the genotype of animals (selection for production parameters, immune system)

New and continuing challanges

- reduction of antibiotic use (stabilization of intestinal flora, mycotoxin - bacteriotic interactions)
- animal product safety
- multitoxin effects
- detection of matrix-bound "masked" mycotoxins
- presence of other bacterial, plant active substances, residues
- we still do not fully understand the metabolic derivatives produced in the animal and their toxicity
- there is no uniform methodology for determining the effectiveness of toxin-binding and neutralizing preparations
- the long-term negative physiological effects of subclinical toxin doses are not fully understood

Substantial differences in sensitivity between species and age groups

Production stock - breeding animals

Younger - older animal

Pig - poultry - ruminants

Hen - duck

Fumonisines - horse

Aflatoxin B1 - turkey

Zearalenone - pig

Food safety risk - ruminants, milk

Typical digestive tract pH and microbial count values in pigs

pH	digestive tract	microbial count
1,5-5	stomach	10^{2-3}
5-7	duodenum	10^{3-4}
7-9	jejunum	10^{4-5}
7-8	ileum	10^{8}
5-7	colon	10^{11-12}

Typical digestive tract pH and microbial count values in birds

pH	digestive tract	microbial count
4-6	craw	10^{8-9}
3-4	glandular stomach	
2-4	crushing stomach	10^{1-3}
6-7	duodenum	10^{3}
6-7	jejunum	10^{4-7}
6-7	ileum	10^{8-9}
5-7	cecum	10^{11-12}

Complex effects of mycotoxins

immune system

antioxidant system - inflammatory processes

organ damage

liver and kidney function

gut function, gut microbiota

....

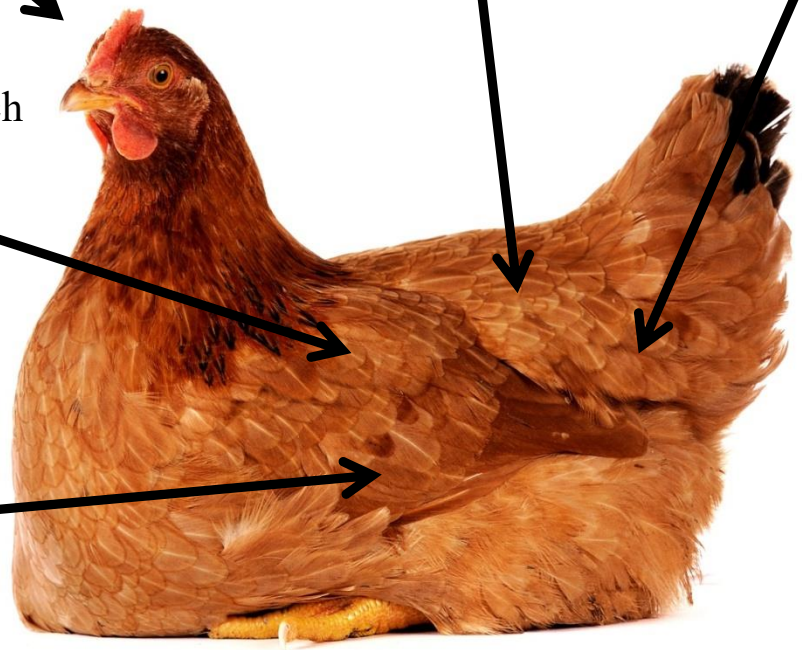
Effects of mycotoxins in poultry species

T-2, DON, AFB1, NIV, DAS
Injuries on the beak and skin
Inflammation of the oral mucosa
Respiratory problems

OTA
Renal degeneration
Increased water absorption

DON, T-2, DAS, ZEN
Decreased egg production and hatchability
Ovarian cysts
Breeding maturation delay
Embryonecrosis

T-2, DON
Injuries on the gizzard stomach
Feed refusal
Diarrhea
Aortic stenosis

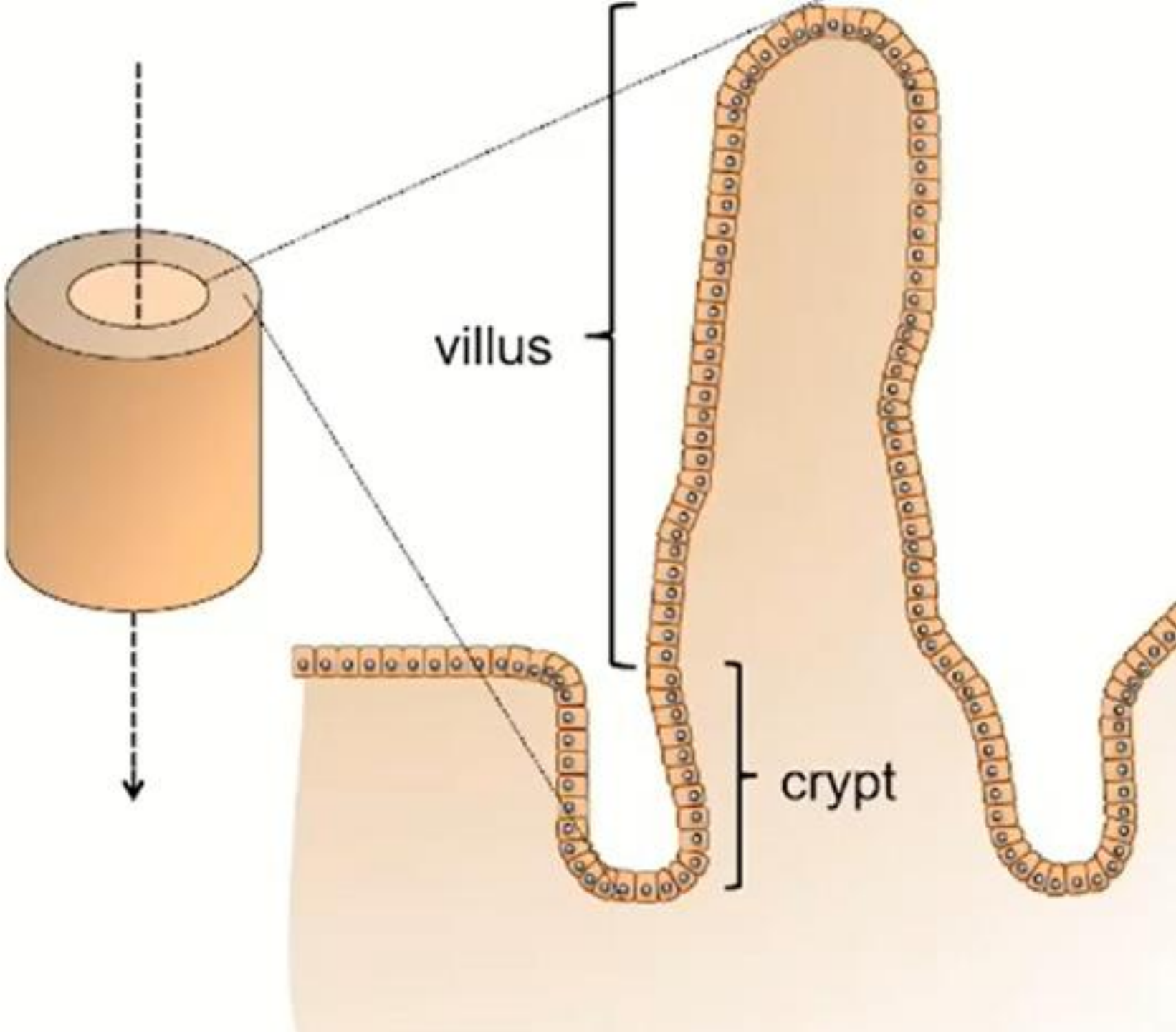


AFB1, OTA, T-2, DON, ZEN
Toxin residue in eggs Blood- and meat stained eggs
Weak eggshell

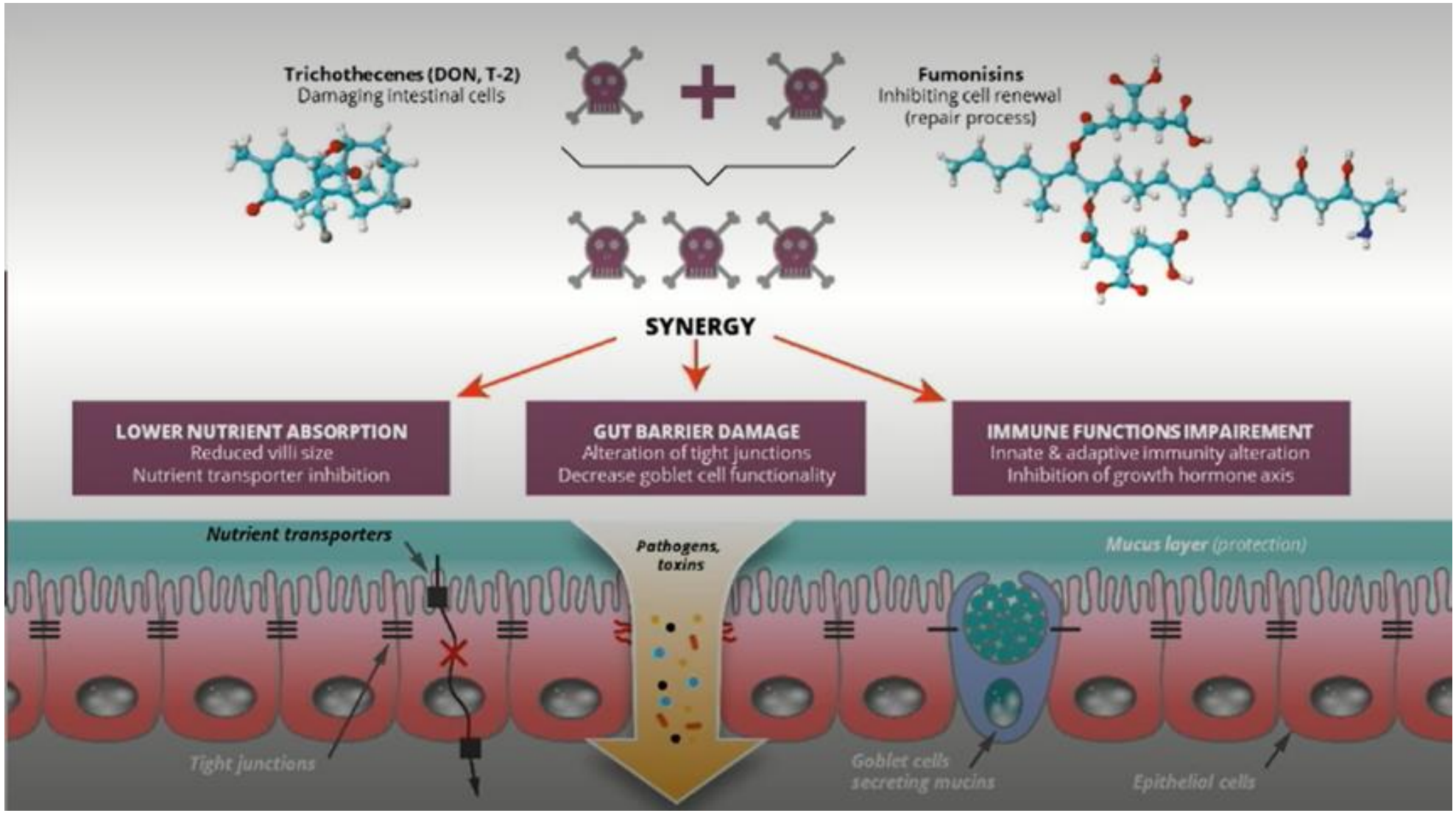
DON, DAS, T-2, OTA, AFB1, NIV
Fatty liver
Abnormal plumage
Heterogeneous stock

DAS – Diacetoxyscirpenol
OTA – Ochratoxin A
DON – Deoxynivalenol
ZEN – Zearalenon
AFB1 – Aflatoxin B1
T-2 – T-2 toxin
NIV – Nivalenol

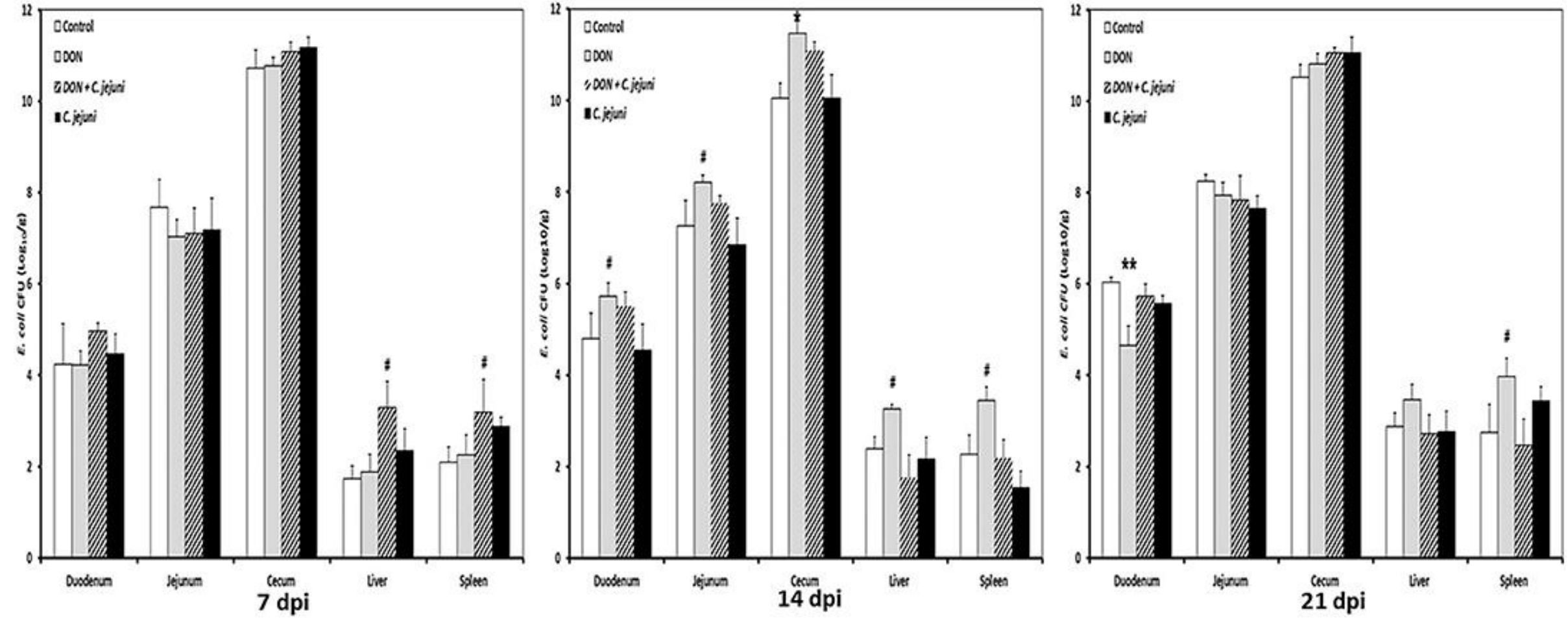
Effects of mycotoxins on the intestinal tract



Effects of mycotoxins on intestinal function

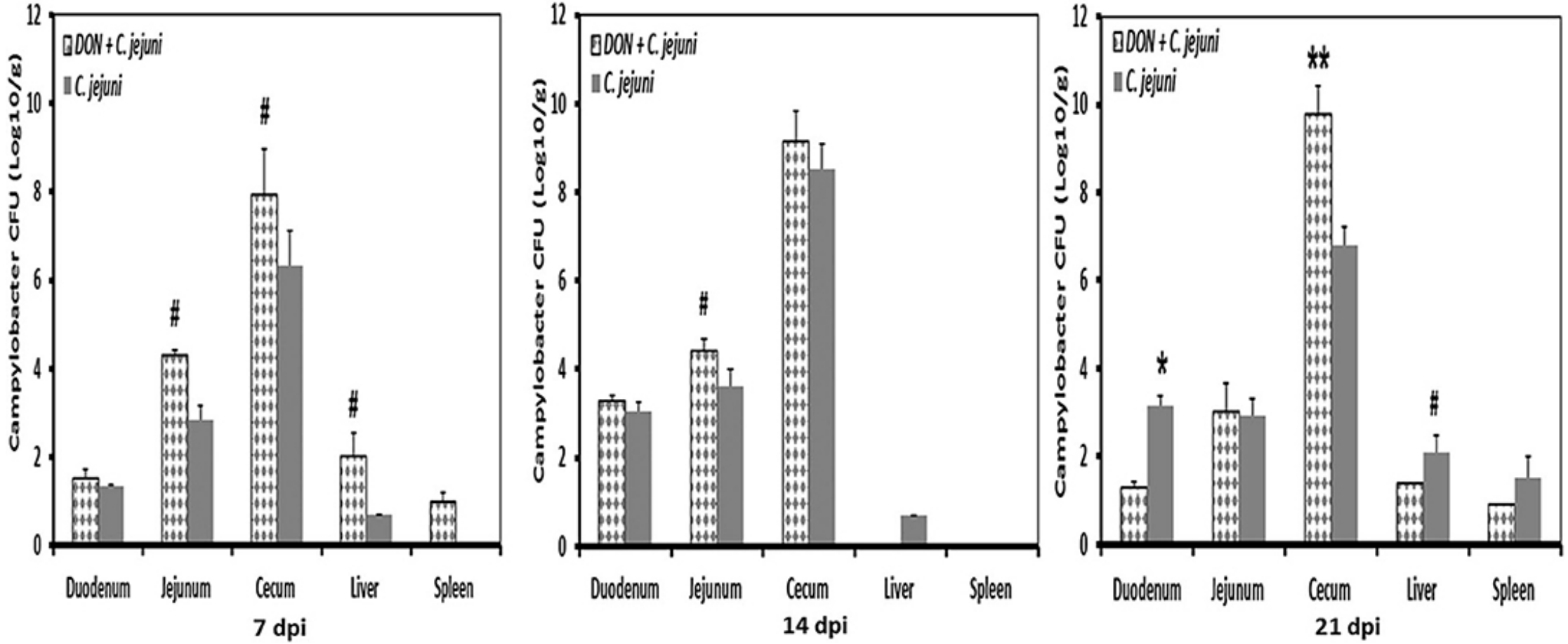


Antonissen, 2022



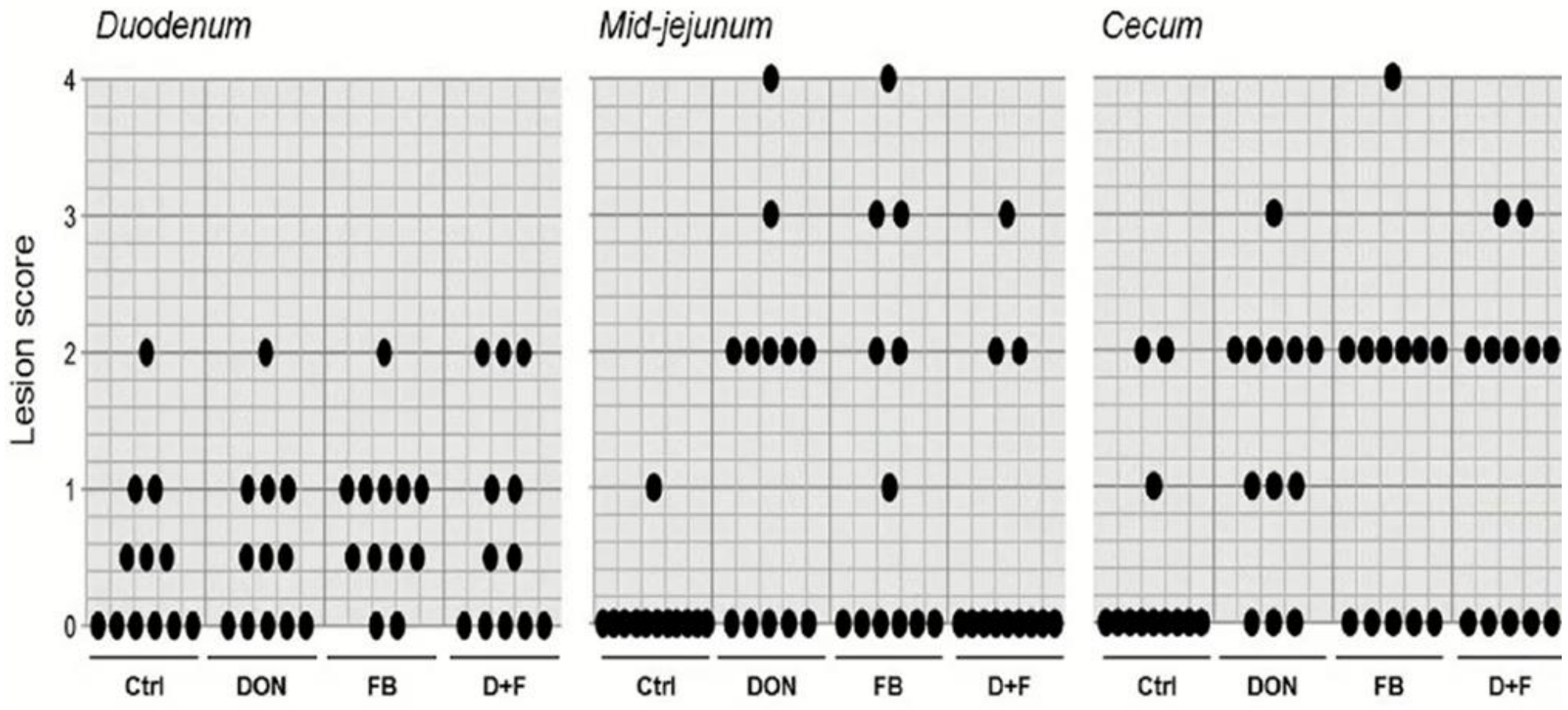
Ruhnau et al., 2020

Effects of 5mg/kg DON toxin on tissue translocation of *Escherichia coli*



Ruhnau et al., 2020

Effects of 5mg/kg DON toxin on tissue translocation of *Campylobacter jejuni*



Greiner et al., 2016

Mycotoxins - coccidiosis

25 X Eimeria vaccine treatment, DON: 1,5 mg/kg
FB: 20 mg/kg

Effects of mycotoxins on the gut microbiota

DEOXYNIVALENOL

2.5 – 10 mg DON/kg feed (age 0-5 wk)
(Lucke *et al.*, 2018)

- ↑ *Clostridialis*
- ↓ *Ruminococcaceae, Clostridiaceae, Oscillospira, Enterobacteriaceae*

3.3-3.7 mg DON/kg feed (age 0-1 wk)
(Antonissen *et al.*, 2017)

- ↑ *Ruminococcaceae / Lachnospiraceae*
  a.o. genus *Faecalibacterium* / genus *Incertae Sedis*
- ↓ *Lachnospiraceae* genus *Coprococcus*

FUMONISINS

15.5-18.3 mg FB_1+FB_2/kg feed (age 0-1 wk)
(Antonissen *et al.*, 2017)

- ↑ *Ruminococcaceae / Lachnospiraceae*
  a.o. genus *Faecalibacterium* / genus *Incertae Sedis*
- ↓ *Lachnospiraceae* genus *Coprococcus*

Effects of mycotoxins on intestinal tract function

- induction of inflammatory processes
- reduced digestibility
- greater intestinal permeability
- dysbiosis
- increased chance of developing infectious diseases

(*Clostridium perfringens* – necrotic enteritis; coccidiosis, *Campylobacter, E. coli, Salmonella* …)

Mycotoxin limit values

- Definition criteria
  - Initially
    - toxicological signs
    - food safety risks
  - today
    - production parameters
    - the animal's antioxidant system
    - immune system
    - special enzymes for their activity
    - the digestive system, intestinal morphology, intestinal integrity
    - effect on the composition of intestinal microflora

Limit values do not mean safety, but less risk, guidance for practice!!

Mikotoxin	Depresszív koncentráció	Toxikus koncentráció
	mg/kg	
Zearalenon és származékai		
Szarvasmarha	0,15	0,30
Borjú (preruminális kor)	0,25	-
Tenyészsertés (felnőtt)	0,15	0,25
Tenyészsüldő (ivarérés előtt)	0,05	-
Süldő- és hízósertés	0,20	0,40
Brojler (baromfi)	0,50	-
Tenyésztojó (házityúk)	0,50	-
Tenyésztojó (lúd, kacsa, pulyka)	0,20	-
Egyéb takarmánykeverékek	0,50	1,00
T-2 toxin		
Szarvasmarha	1,00	2,00
Sertés	0,25	0,60
Brojler (baromfi)	0,30	0,60
Tojó (tyúk, pulyka, viziszárnyas)	0,25	0,80
Egyéb takarmánykeverékek	1,00	2,00
DON		
Szarvasmarha	5,00	-
Borjú (preruminális kor)	0,20	-
Sertés	0,40	1,00
Tyúkfélék (tojó és brojler)	2,00	-
Lúd, kacsa, pulyka	0,50	-
Trichotecén toxinok együttesen (T-2, DAS, HT-2, NIV)		
Szarvasmarha	2,00	4,00
Sertés	0,50	1,20
Brojler (baromfi)	0,60	1,20
Tojó (tyúk, pulyka, viziszárnyas)	0,30	1,60
Egyéb takarmánykeverékek	2,00	4,00
Fumonizin B1		
Ló	5,00	-
Szarvasmarha	50,00	-
Sertés	5,00	10,00
Baromfi	30,00	-
Egyéb takarmánykeverékek	30,00	-
Ochratoxin-A (OTA)		
Sertés és baromfi	0,20	-
Egyéb takarmánykeverékek	0,20	-
Aflatoxin B1		
Minden állatfaj	0,05	0,05

Mycotoxin limit values in compound feed (Committee of Veterinary Medicine of the Hungarian Academy of Sciences (2003))

2006/576/EK és 2013/165/EU ajánlások

Mikotoxin	Takarmány alapanyag/takarmány	Javasolt maximális mennyiség (mg/kg takarmány)
T-2 és HT-2 toxin	Gabona és gabona termékek Keveréktakarmányok	0,5 0,25
Dezoxinivalenol (+ 3AcDON + 15AcDON)	Gabonafélék és gabona-készítmények, kivéve kukorica melléktermékek Kukorica melléktermékek Kiegészítő és teljes értékű takarmányok Sertéstakarmányok Borjú (<4 hónap), bárány és gida takarmányok	8 12 5 0,9 2
Zearalenon	Gabonafélék és gabona-készítmények, kivéve a kukorica melléktermékeket Kukorica melléktermékek Malac és kocasüldő takarmányok Tenyészkoca, kan és hízósertés takarmányok Borjú, tejelő tehén, bárány, juh, gida és kecske takarmányok	2 3 0,1 0,25 0,25
Fumonizin B1+B2	Kukorica és kukorica készítmények Sertés, ló és nyúl takarmányok Haltakarmányok Baromfi, borjú, bárány és gida takarmányok Felnőtt kérődző takarmányok	60 5 10 20 50
Ochratoxin A	Gabonafélék és gabonakészítmények Sertéstakarmányok Baromfi takarmányok	0,25 0,05 0,10

Regulation at EU level

Mikotoxin	Takarmány alapanyag/takarmány	Javasolt maximális mennyiség
Aflatoxin B1	Gazdasági állatok takarmányai Tejelő tehén takarmányok	20 µg/kg 5 µg/kg

574/2011/EU rendelet

Critical toxicological values of certain mycotoxins in farm animal species

NOEL: No Observed Effect Level;
LOAEL: Low Observed Adverse Effect Level

Mikotoxin	Kritikus érték / gazdasági állatfaj
Zearalenon	NOEL: 10 μg/kg ttm./nap / sertés NOEL: ≅10 μg/kg ttm./nap / nyúl
Nivalenol	LOAEL: 53 μg/kg ttm./nap /baromfi LOAEL: 100 μg/kg ttm./nap /sertés
T-2 / HT-2 toxin	LOAEL: 40 μg/kg ttm./nap / baromfi LOAEL: 29 μg/kg ttm./nap / sertés LOAEL: 100 μg/kg ttm./nap/ nyúl LOAEL: 300 μg/kg ttm./nap / kérődző LOAEL: 13 μg/kg ttm./nap / hal
Fumonizin B1	LOAEL: 2000 μg/kg ttm./nap / baromfi LOAEL: 200 μg/kg ttm./nap / sertés LOAEL: 200 μg/kg ttm./nap / ló LOAEL: 600 μg/kg ttm./nap / kérődző LOAEL: 10 mg/kg ttm./nap / hal

PROPOSAL ON MYCOTOXIN CONTAMINATION OF COMPOUND FEED FOR FARM ANIMALS

(µg/kg takarmány; 88% szárazanyag tartalom)

Mikotoxin	Alacsony kockázat	Közepes kockázat	Nagy kockázat
T-2 + HT-2 toxin			
Sertés (malac)	<250	500-1000	>1000
Sertés (növendék, hízó)	<250	500-1000	>1000
Sertés (koca, kan)	<250	500-1000	>1000
Baromfi (brojler, tojó)	<250	500-1000	>1000
Baromfi (kacsa, pulyka)	<250	300-800	>800
Borjú. bárány, gida	<250	500-1000	>1000
Húsmarha, tejelő tehén	<250	500-1000	>1000
DON + 3-acetil DON + 15-acetil DON			
Sertés (malac)	<1000	2000-4000	>4000
Sertés (növendék, hízó)	<1500	3000-6000	>6000
Sertés (koca, kan)	<900	1500-2000	>2000
Baromfi (brojler, tojó)	<4000	8000-10000	>10000
Baromfi (kacsa, pulyka)	<4000	8000-10000	>10000
Borjú, bárány és gida	<2000	400-6000	>6000
Húsmarha, tejelő tehén	<5000	10000-20000	>20000
Ló	<1000	2000-4000	>4000
Zearalenon			
Sertés (malac, süldő)	<100	200-400	>400
Sertés (növendék, hízó, koca)	<250	500-1000	>1000
Baromfi (jérce, tojó)	<1000	2000-4000	>4000
Baromfi (brojler)	<1000	2000-4000	>4000
Baromfi (kacsa, pulyka)	<1000	2000-4000	>4000
Borjú, bárány, gida	<1000	2000-4000	>4000
Húsmarha, tejelő tehén	<1000	2000-4000	>4000
Ló	<1000	2000-4000	>4000

A javaslatot készítette: Kovács Melinda, MTA r. tagja Mesterházy Ákos, MTA r. tagja Mézes Miklós, MTA r. tagja, 2020

PROPOSAL ON MYCOTOXIN CONTAMINATION OF COMPOUND FEED FOR FARM ANIMALS

(µg/kg takarmány; 88% szárazanyag tartalom)

Mikotoxin	Alacsony kockázat	Közepes kockázat	Nagy kockázat
Ochratoxin A			
Sertés (malac, koca)	<50	100-200	>200
Sertés (hízó)	<50	100-200	>200
Baromfi (brojler)	<100	200-400	>400
Baromfi (tojó, kacsa, pulyka)	<100	200-400	>400
Borjú, bárány és gida	<200	400-800	>800
Húsmarha, tejelő tehén	<200	400-800	>800
Fumonizin B1+B2			
Sertés (malac)	<5000	10000-20000	>20000
Sertés (hízó)	<5000	10000-20000	>20000
Sertés (koca)	<3000	6000-12000	>12000
Baromfi (brojler, tojó)	<20000	40000-80000	>80000
Baromfi (jérce, kacsa, pulyka)	<20000	40000-80000	>80000
Borjú, bárány, gida	<20000	40000-80000	>80000
Húsmarha, tejelő tehén	<50000	100000-200000	>200000
Ló	<20000	40000-80000	>80000
Aflatoxin B1+B2+G1+G2			
Sertés (malac, növendék, hízó, koca)	<20	40-80	>80
Baromfi (brojler, tojó)	<20	40-80	>80
Baromfi (jérce, kacsa, pulyka)	<20	40-80	>80
Borjú, tejelő tehén	<5	10-20	>20
Húsmarha	<20	40-80	>80
Ló	<20	40-80	>80

A javaslatot készítette: Kovács Melinda, Mesterházy Ákos és Mézes Miklós, 2020

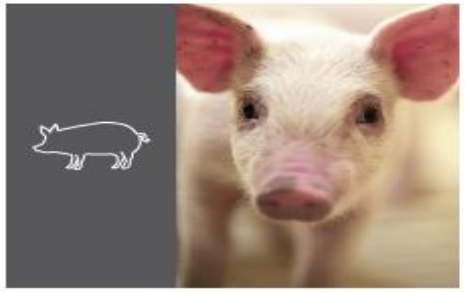
- **Cargill's recommended Mycotoxin Limits**

Global	
Aflatoxin (AFL)	10
Fumonisin (FUM)	500
Ochratoxin (OTA)	20
T2 toxin (T2)	25
Vomitoxin (DON)	200
Zearalenone (ZEN)	35

Beef	
AFL	100
FUM	5,000
OTA	150
T2	100
DON	200
ZEN	100

Calf/Heifer	
AFL	5
FUM	3,000
OTA	150
T2	100
DON	200
ZEN	70

Dairy	
AFL	3
FUM	3,000
OTA	150
T2	100
DON	250
ZEN	100



Sow	
AFL	20
FUM	3,000
OTA	25
T2	50
DON	750
ZEN	100

Hog	
AFL	20
FUM	1,000
OTA	40
T2	100
DON	500
ZEN	300

Nursery Pig	
AFL	15
FUM	750
OTA	25
T2	50
DON	200
ZEN	200

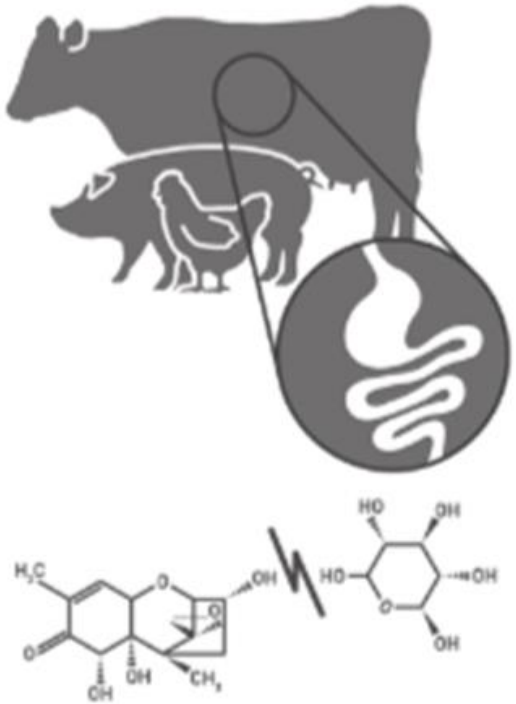
Broiler	
AFL	15
FUM	500
OTA	20
T2	25
DON	400
ZEN	50

Breeder	
AFL	15
FUM	1,000
OTA	25
T2	50
DON	400
ZEN	35

Layer	
AFL	15
FUM	1,000
OTA	25
T2	50
DON	400
ZEN	35

Masked Mycotoxins – An emerging issue for feed and food safety?

World Mycotoxin Report



Fungus produces mycotoxins e.g. DON

Defense mechanism of the plant: addition of sugar, or other substances, to the mycotoxin = masked mycotoxin

Animal ingests contaminated feed containing masked mycotoxins. Sugar is cleaved in the gut: parental mycotoxin is released

Average proportion of bound mycotoxins in certain cereal grains

Grain	Masked mycotoxin form	Proportion within a given mycotoxin
Corn	zearalenon szulfát	zearalenone - <30%
Wheat	zearalenon-4-glükozid	zearalenone - <30%
Wheat	T-2-glükozid/HT-2-glükozid	T-2/HT-2 toxin - <12%
Wheat	DON-3-glükozid	DON 8-30%, korpa:70%.
Oats	T-2-glükozid/HT-2-glükozid	T-2/HT-2 toxin - ≅2%
Corn	Physically bound fumonisin	fumonisin B1+B2 +B3 – 36%
Corn	fumonisin B1 fatty acid esters	fumonisin B1 – 5-6%

(*Lemmens et al. (2016): World Mycotoxin Journal, 9 (5): 741-754 DOI 10.3920/WMJ2015.2029*)

Toxin effects below limit value

toxins

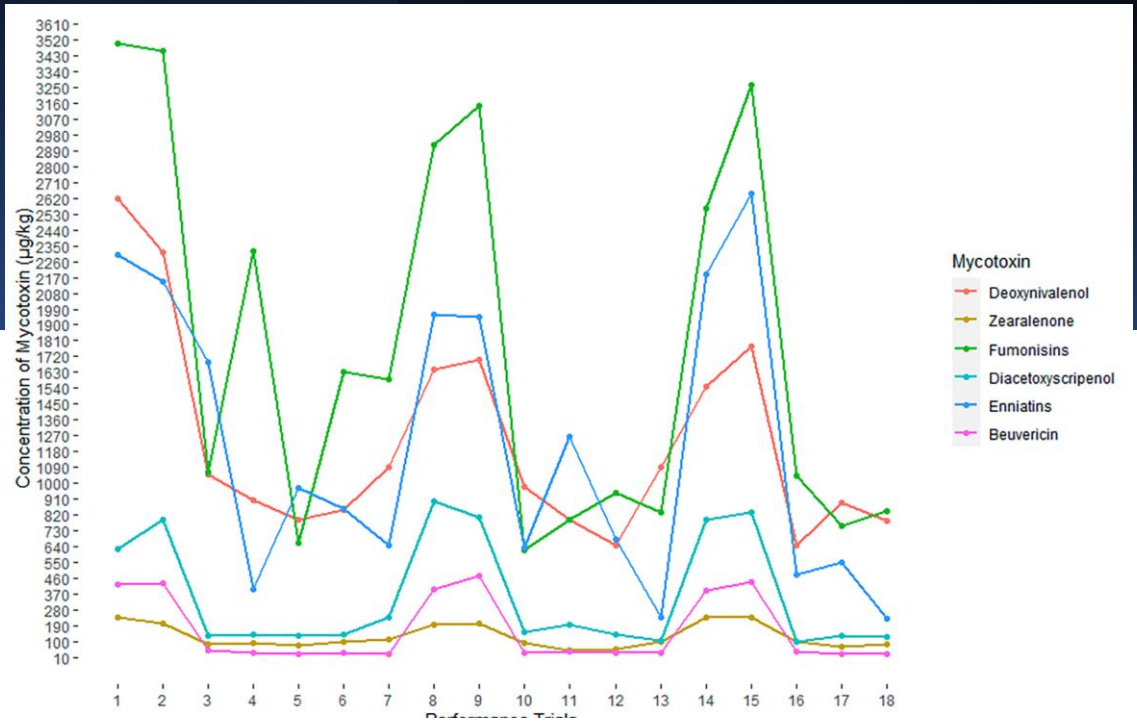
Article

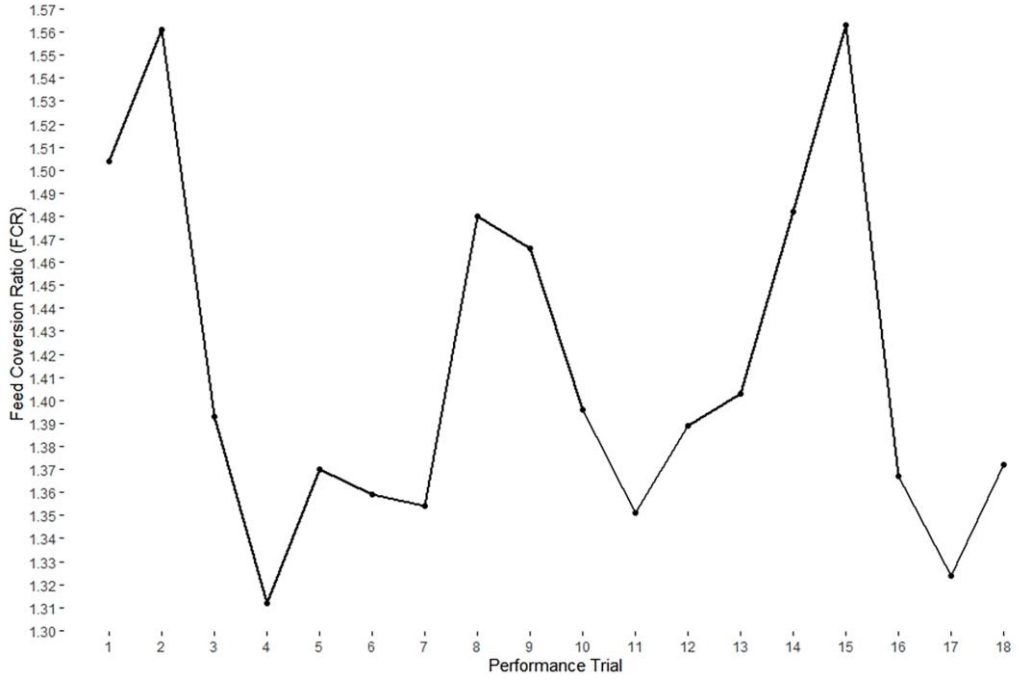
Low Doses of Mycotoxin Mixtures below EU Regulatory Limits Can Negatively Affect the Performance of Broiler Chickens: A Longitudinal Study

Oluwatobi Kolawole [1], Abigail Graham [2], Caroline Donaldson [2], Bronagh Owens [2], Wilfred A. Abia [1], Julie Meneely [1], Michael J. Alcorn [2], Lisa Connolly [1] and Christopher T. Elliott [1,*]

- 18 term
- 2200 Ross 308 chicken
- feed in different amounts, contained mycotoxins below the permissible limit

Mycotoxin (ppb)	Median	Max
DON	898	2621
ZEA	78,4	241
OTA	Nt	Nt
FB1+ FB2	814,5	4260





Toxin effects below limit value

Effects of DON on swine Alizadeh et al., 2015

Deoxynivalenol Impairs Weight Gain and Affects Markers of Gut Health after Low-Dose, Short-Term Exposure of Growing Pigs

Arash Alizadeh [1,2], Saskia Braber [1,*], Peyman Akbari [1,2], Johan Garssen [2,3] and Johanna Fink-Gremmels [1]

- Trial performed with 35 days old piglet
- Duration: 10 days
- 0,9ppm DON

Table 2. Body weight (BW), relative weight gain, average daily gain, feed intake and feed conversion ratio.

Item	Start weight (kg)		End weight (kg)		Relative weight gain (% increase)		Average daily gain (kg/day)		Feed intake (kg/day)	Feed conversion ratio
Exp. group	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	Mean
Control	8.67	0.48	10.98	0.53	27.21	1.82	0.29	0.01	0.31	1.12
DON	7.87	0.47	9.48	0.61	20.17 *	1.15	0.20 ***	0.01	0.30	1.57

** p-value <0.01; *** p-value <0.001; relative weight gain = ((end weight-start weight)/start weight) × 100% per individual animal.

On 13 OCTOBER 2021, THE MYCOTOXIN PLATFORM WAS ESTABLISHED IN HUNGARY

Goals:

- discover the occurence of mycotoxins in Hungary in order to reduce intake
- To help the domestic agricultural sector prepare for new mycotoxins related to climate change addressing challenges
- …

Toolbox

- elaboration of scientific opinions at the request of MTA, AM, NÉBIH and other institutions, in response to media requests or of one's own initiative
- Coordination of the system of own checks and official controls
- developing a scientific knowledge base for mycotoxin practitioners and society
- collecting, developing and disseminating training and awareness-raising materials to reduce mycotoxin intake
- informing actors in the food chain about the latest techniques and tools currently available to prevent or reduce mycotoxin contamination
- ...

Toolbox – database shaping

- in cooperation with the WHO/FAO CCMAS Committee on laboratory test methods
- basic and practical researches according to the results in Hungary
- control and inspection results
- correlations between meteorological data and mycotoxin results
- mycotoxin cases and incidents
- Mycotoxin intake assessment
- mycotoxin legislation and EFSA guides
- …

Workgroups

- **Plant protection, resistance breeding**
- **Analytics**
- **Molecular biology, microbiology, toxicology**
- **Decontamination possibilities, industrial relations**
- **Protection of natural communities**
- **Risk assessment, risk management**

Thank you for your attention!