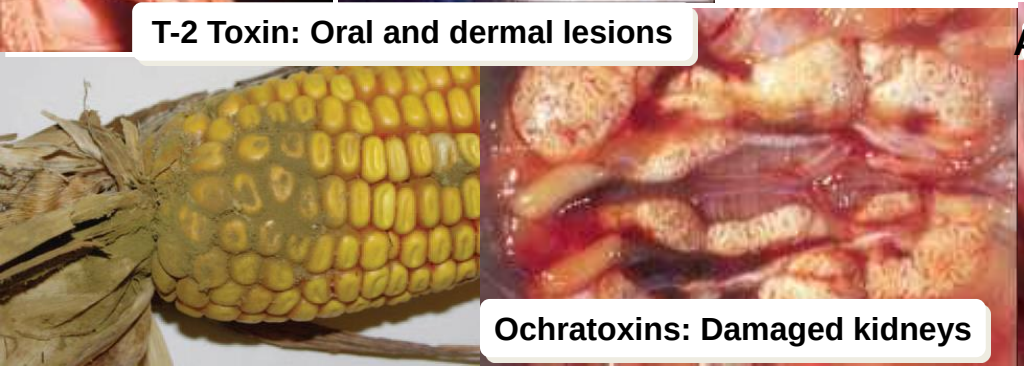
Can Mycotoxins Effect Gastro-intestinal Tract (GIT) Function?

Todd J. Applegate

T-2 Toxin: Oral and dermal lesions



Ochratoxins: Damaged kidneys

Aflatoxin B_1: Fatty liver

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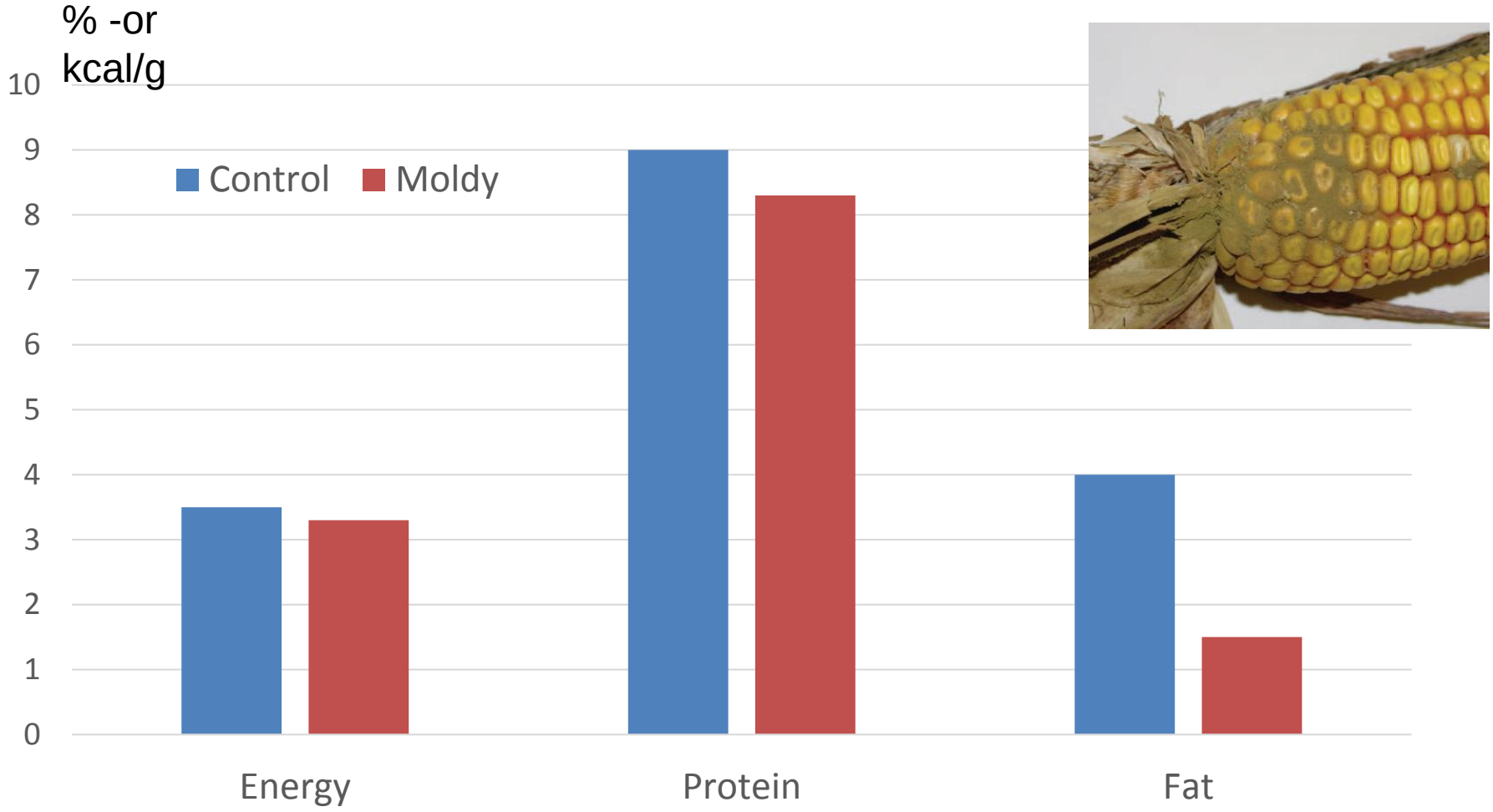
Do Mycotoxins affect the Gastro-intestinal Tract (GIT)?

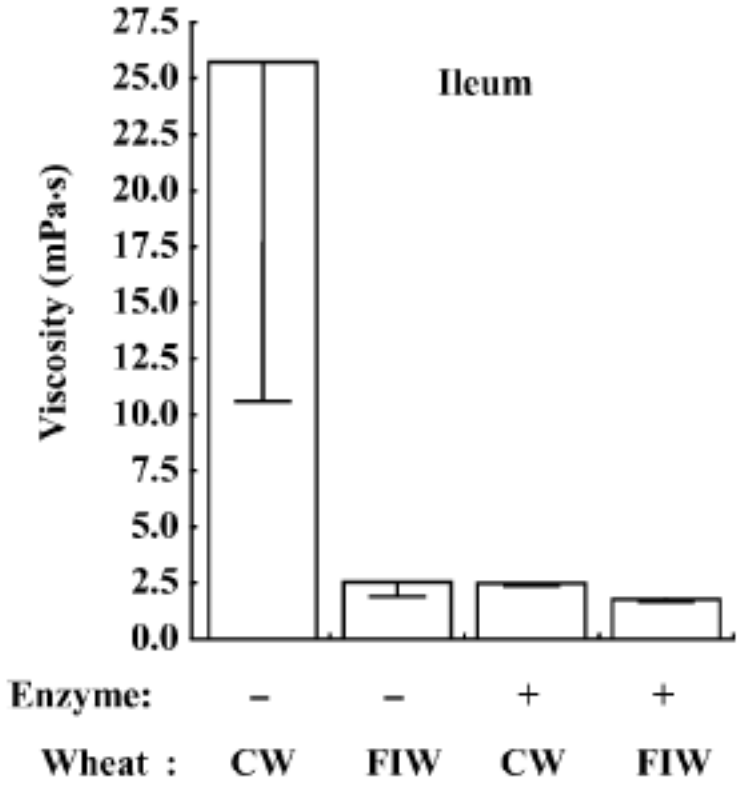
- Plausible change in nutrient content of feedstuffs contaminated with mold
- Feed intake responses to growth vs physiology
- Differentiation between “post-GIT” metabolic inefficiencies vs physiological responses
- Differences in absorption (some rapid vs virtually non-absorbed)

Reduction in nutritional quality of corn



Tindall (1983)

Change in nutritional quality of wheat



CW = control wheat diet*
FIW = *Fusarium* infected diet
(~ 1.4 mg/kg DON)

+/- endo-1,4-*β*-xylanase

**Control wheat 1% less CP*

Differences in non-starch polysaccharide content???

Dänicke et al. (2007)

Toxins **2013**, *5*, 396-430; doi:10.3390/toxins5020396

OPEN ACCESS

toxins

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Review

Modulation of Intestinal Functions Following Mycotoxin Ingestion: Meta-Analysis of Published Experiments in Animals

Bertrand Grenier [1,2] and Todd J. Applegate [1,*]

T-2 Toxin: Oral and dermal lesions

Aflatoxin B_1: Fatty liver

Ochratoxins: Damaged kidneys

GIT &
Functional “Upkeep”

- GIT consumes approximately 20% of dietary energy
- **Protein turn-over rate of 50 to 75% per day (Cant et al., 1996)**
- **~25% of daily protein synthesis can be secreted into the gut (Simeon et al., 1983)**

STUDIES

Table 1. Intestinal processes investigated—number of experiments per process and per mycotoxin in the meta-analysis.

	Nutrient digestibility	Enzyme activities	Nutrient uptake [1]	Digestive microflora	Barrier integrity	Mucosal immunity [2]	Pathogen clearance	Total [3]
Experiments	13	5	17	5	16	13	14	83
in vitro/ex vivo/in vivo [4]	0/0/13	0/0/5	1/10/12	1/2/4	13/2/5	7/1/10	1/1/13	23/16/62
Aflatoxin (AF)	5	4	1	0	2	1	1	14
Ochratoxin A (OTA)	0	0	0	0	3	0	3	6
Deoxynivalenol (DON)	1	0	11	3	8	7	2	32
T-2 toxin (T-2)	0	0	1	1	0	0	3	5
Zearalenone (ZEA) [5]	0	0	0	0	0	0	0	0
Fumonisin (FB)	2	1	2	1	2	4	2	14
Multi-contamination	5	0	2	0	1	1	3	12

NUTRITION

DEFENSE

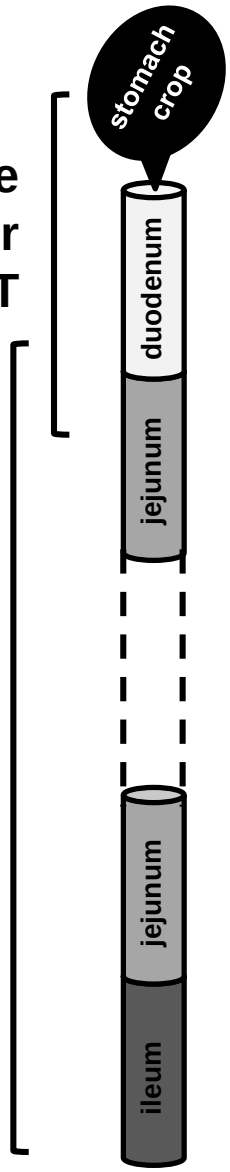
Grenier and Applegate, **2013**

Most of the absorbed dose (80-90%) occur in the upper part of the GIT

- ✓ DON
- ✓ ZEA
- ✓ AF
- ✓ OTA

Enterohepatic circulation may increase the exposure all along the GIT

- ✓ DON
- ✓ T-2 toxin
- ✓ ZEA
- ✓ FB
- ✓ OTA



Mycotoxin Absorption rate

	Pig	Poultry
AF	>80%	>80%
OTA	65%	40%
DON	55%	5-20%
FB	3-6 %	1%

Grenier and Applegate, 2013

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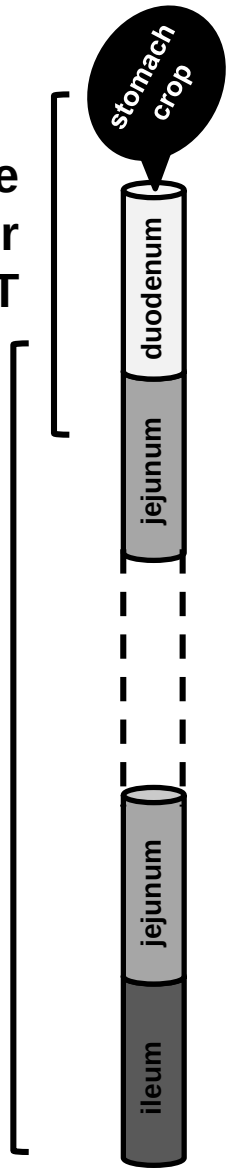

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Grenier and Applegate, 2013

Table 2. Method used to categorize the experimental doses.

	Deoxynivalenol (DON; mg/kg)	T-2 Toxin (T-2; mg/kg)	Zearalenone (ZEA; mg/kg)	Fumonisins (FB; mg/kg)	Aflatoxin (AF; mg/kg)	Ochratoxin A (OTA; mg/kg)
Realistic doses (RD) [1]						
Representative of field conditions	<5	<0.5	<1	<10	<0.3	<0.3
Occasional doses (OD) [1]	>5	>0.5	>1	>10	>0.3	>0.3
Unfavorable weather conditions	<25	<2	<5	<40	<2	<2
Unrealistic doses (UD) [1]						
Unlikely to occur in nature	>25	>2	>5	>40	>2	>2

Grenier and Applegate, **2013**

FAT DIGESTION DURING AFLATOXICOSIS IN BROILER CHICKENS

D. J. OSBORNE, Department of Poultry Science, North Carolina State University, Raleigh, NC 27607, R. D. WYATT, Department of Poultry Science, University of Georgia, Athens, GA 30602, and P. B. HAMILTON, Department of Poultry Science, North Carolina State University, Raleigh, NC 27607

Previous work has demonstrated that aflatoxin inhibits lipid synthesis and transport in chickens, but its effect on fat digestion has not been studied. The effects of graded levels of dietary aflatoxin (0, 0.625, 1.25, 2.5, 5.0, and 10 μg./g. of commercial starter diet) on gall bladder size, bile, pancreatic lipase, and fecal fat were determined. Gall bladder weight was increased significantly ($P < 0.05$) at 2.5 μg./g. and above while the bile acid content of the bile was decreased at all doses. Pancreatic lipase which is thought to be the major fat digestive enzyme was decreased at all doses. Fecal fat was increased at 5.0 and 10.0 μg./g. These data suggest that aflatoxin inhibits fat digestion with a consequent steatorrhea by decreasing the enzymes and bile acids required for fat digestion. Stated another way, aflatoxin appears to cause a malabsorption syndrome.

PSA meeting (1975)

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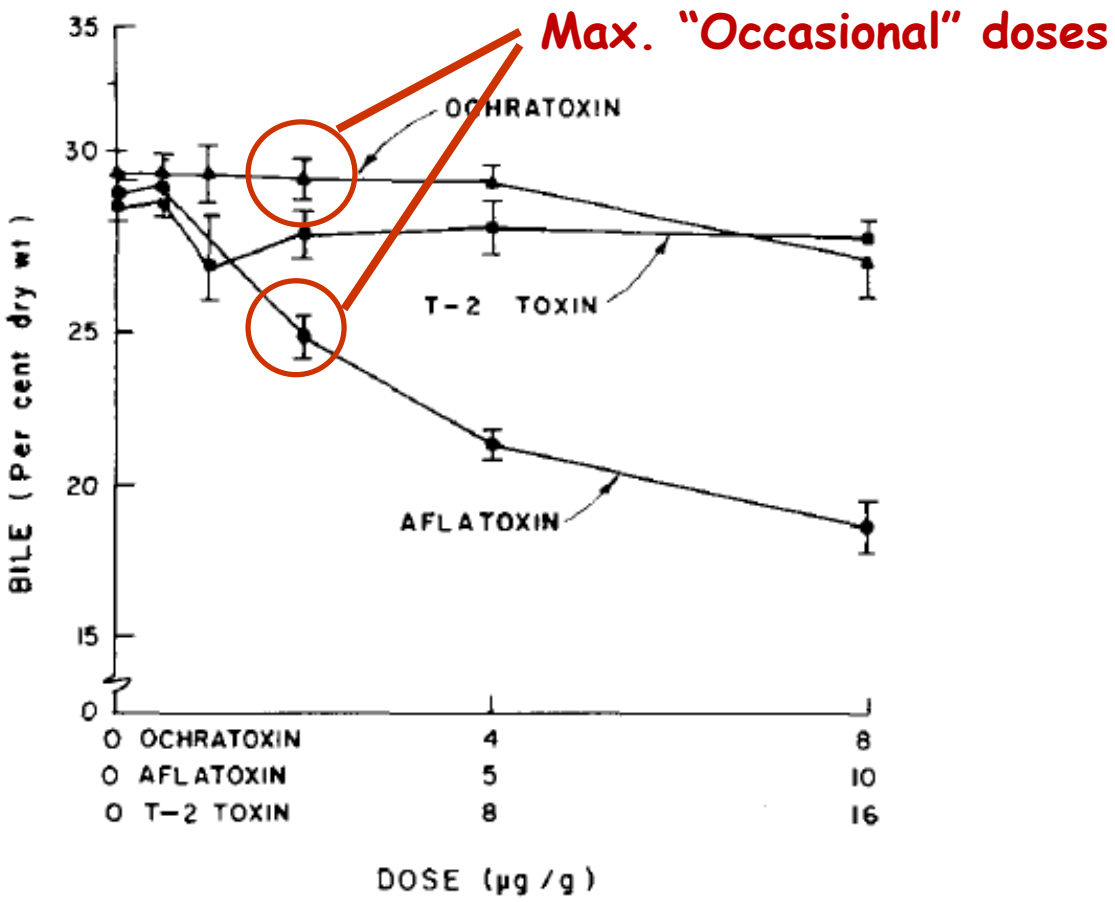
Realistic AFLA = < 0.3 mg/kg
Occasional AFLA = > 0.3 to 2 mg/kg
Unrealistic AFLA = > 2 mg/kg

Are results applicable from "Unrealistic doses" to "realistic / occasional doses"

PSA meeting (1975)

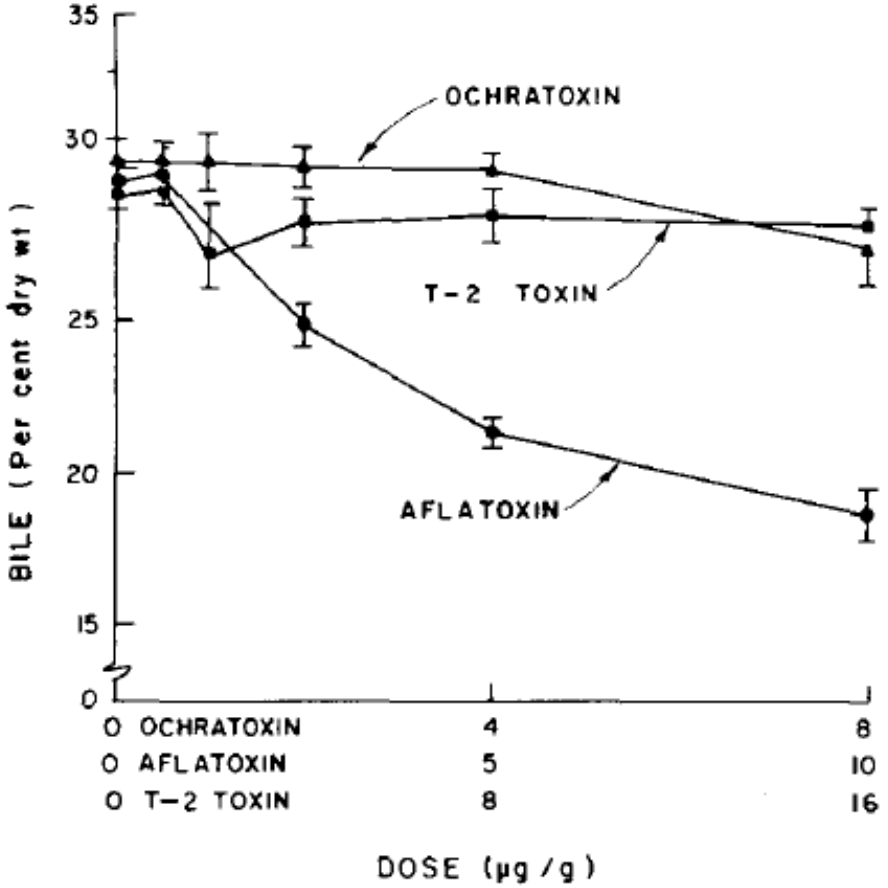
Aflatoxin = Malabsorption???



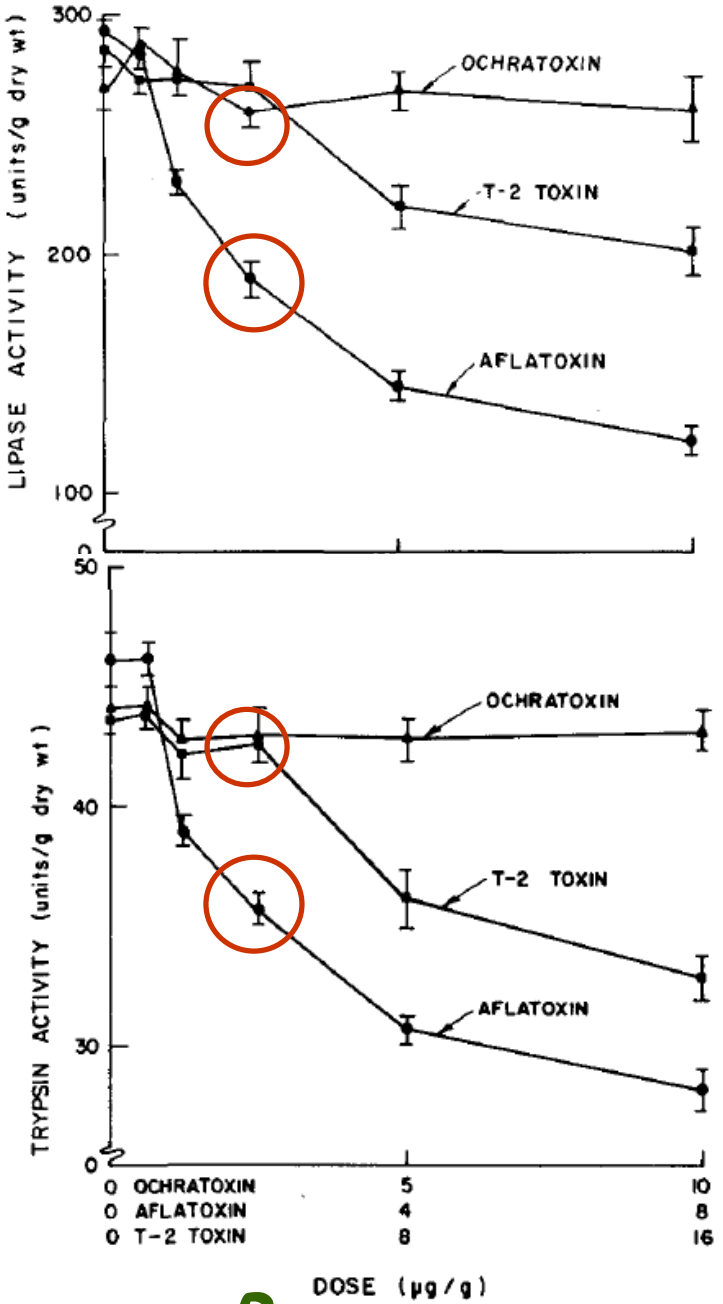
Liver

Osborne et al. (1982)

Aflatoxin = Malabsorption???



Liver Vs. Pancreas



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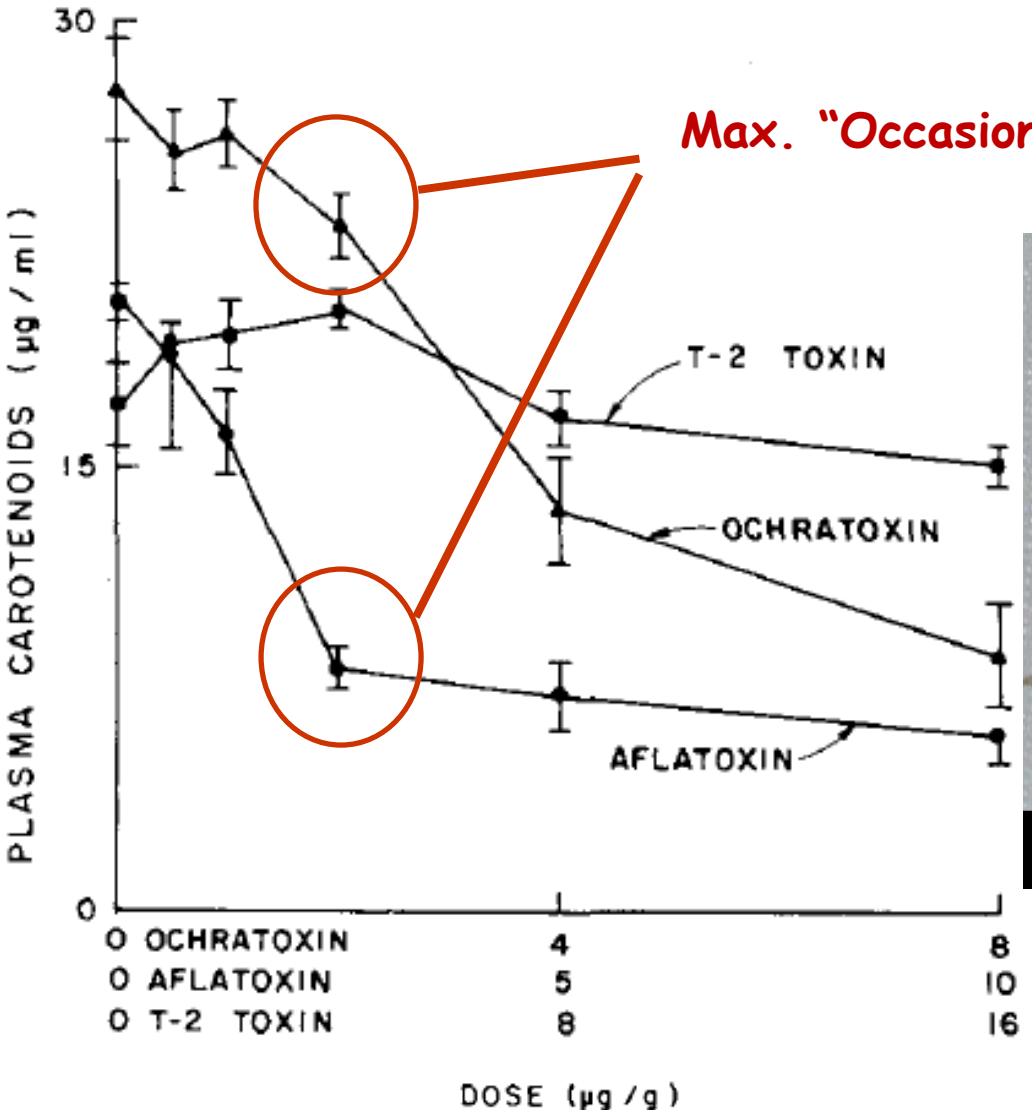
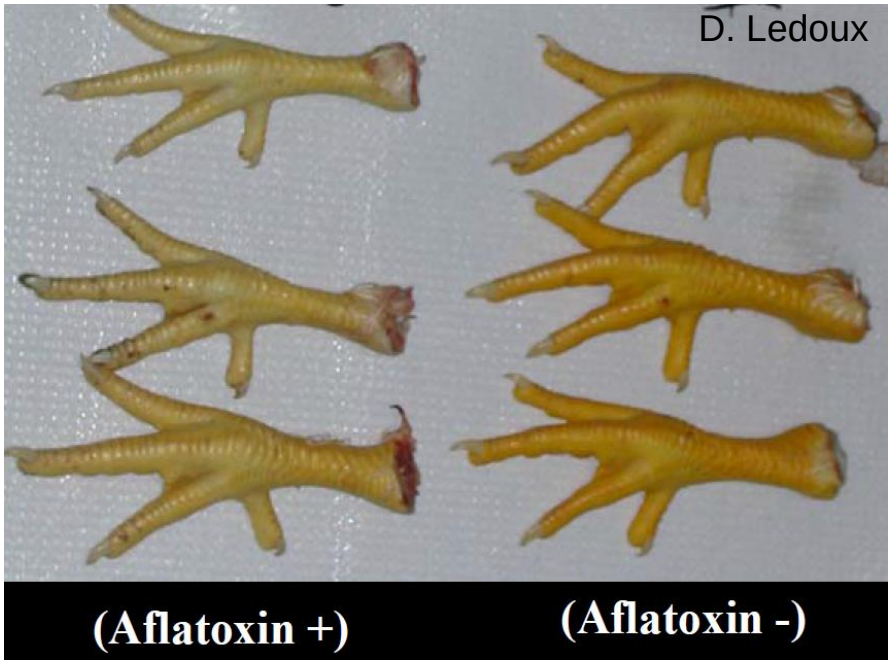




Osborne et al. (1982)

Mycotoxins

Clinical signs are only the visible part of a (much bigger) problem !

Clinical Mycotoxicosis:
Lesions

Sub -Clinical Mycotoxicosis:
Impaired immune function, performance loss, FCR reduction

UNKNOWNS.....
MYCOTOXIN INTERACTIONS???

Mycotoxins

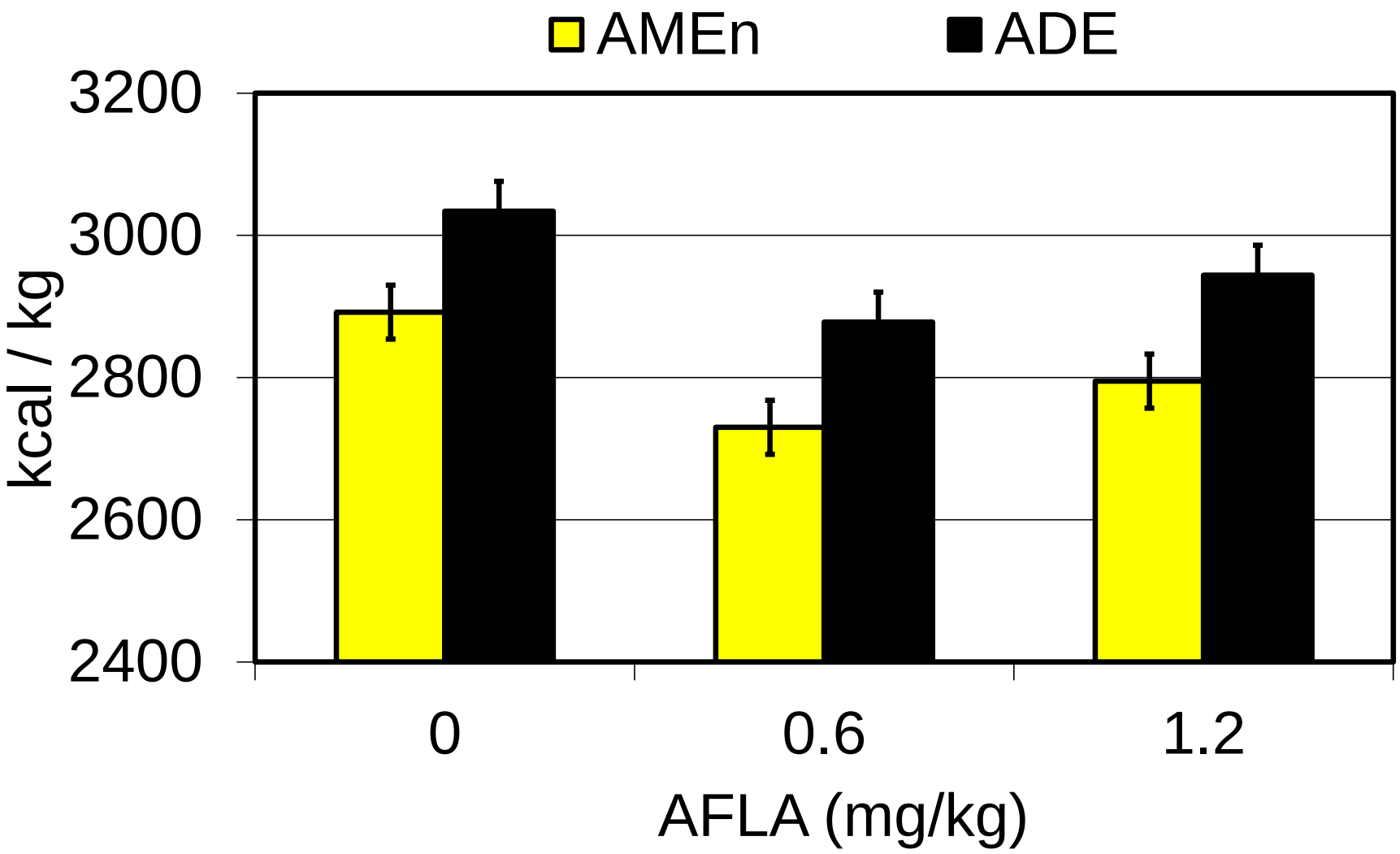
Performance is only the visible part of a (much bigger) problem !

Performance

Biomarkers (physiology, toxicology, immunology, nutritional consequences…)

UNKNOWNS…..
MYCOTOXIN INTERACTIONS???

Hen Energy Utilization

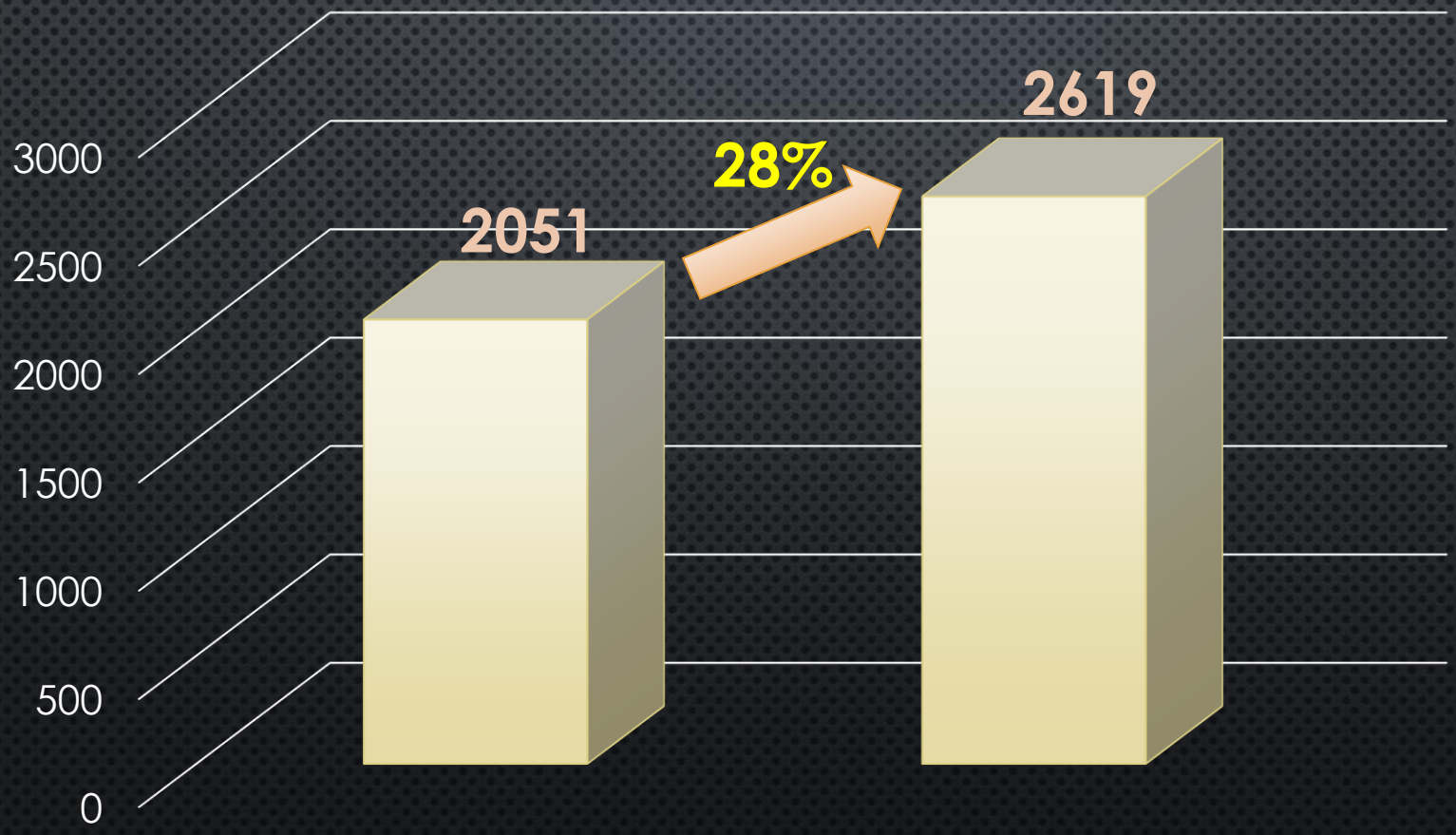


Applegate et al., 2009

0.6 and 1.2 mg/kg ↓ ADE (10%) and AME_n (4%)

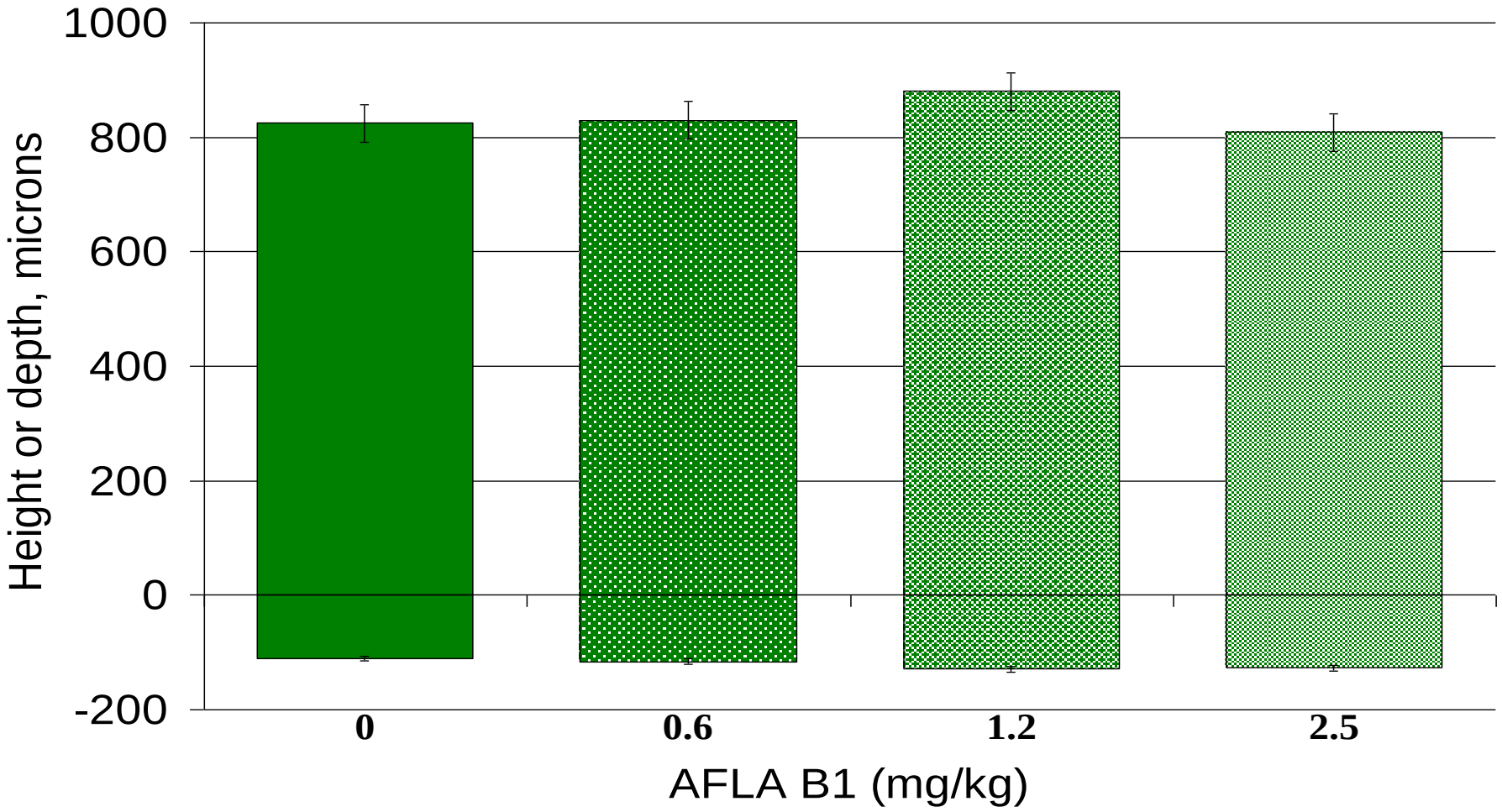
Chen et al., 2015

Dietary Protein & Aflatoxin



P-value	**0.09**

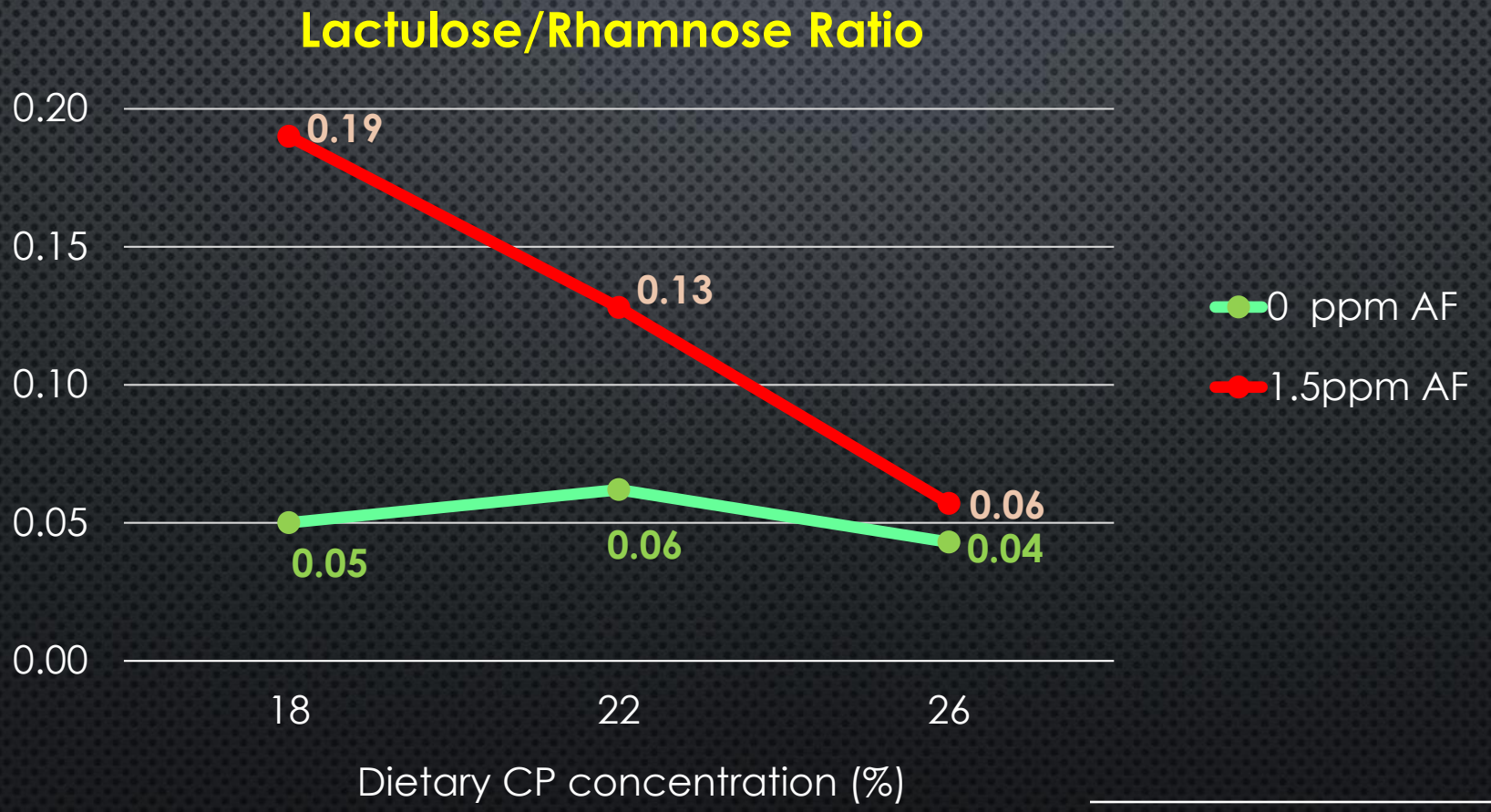
Small intestinal Morphology



Villus length; no effect
Crypt depth; linear increase ($P < 0.016$)
Goblet cells; no effect (number or number / villus length)

Dietary Protein & Aflatoxin: Gut Permeability (dual sugar test)

Chen et al., 2015



AFB	P=0.04

↑ L: R ratio = Impaired barrier integrity

INTESTINE: FIRST TARGET

NUTRITION

DIGESTIVE PROCESSES

MYCOTOXIN	DOSE	SPECIES	OUTCOMES
Aflatoxin	Realistic	Duck & Hen	Modulation of activity of digestive enzymes (protease, amylase, trypsin and chymotrypsin)
Aflatoxin	Realistic	Duck	Reduced apparent digestibility of crude protein
Aflatoxin	Moderate	Chicken & Hen	Reduced apparent digestibility, digestible & metabolizable energy

Grenier and Applegate, **2013**

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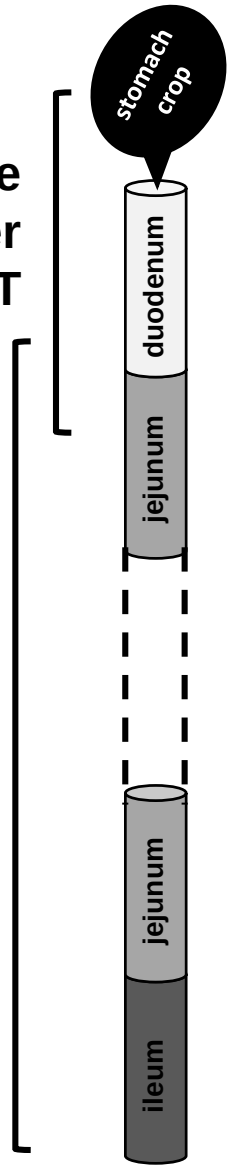

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	Pig	Poultry
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IMPACT OF THE NON-ABSORBED PART ??

Grenier and Applegate, 2013

INTESTINE: FIRST TARGET

NUTRITION

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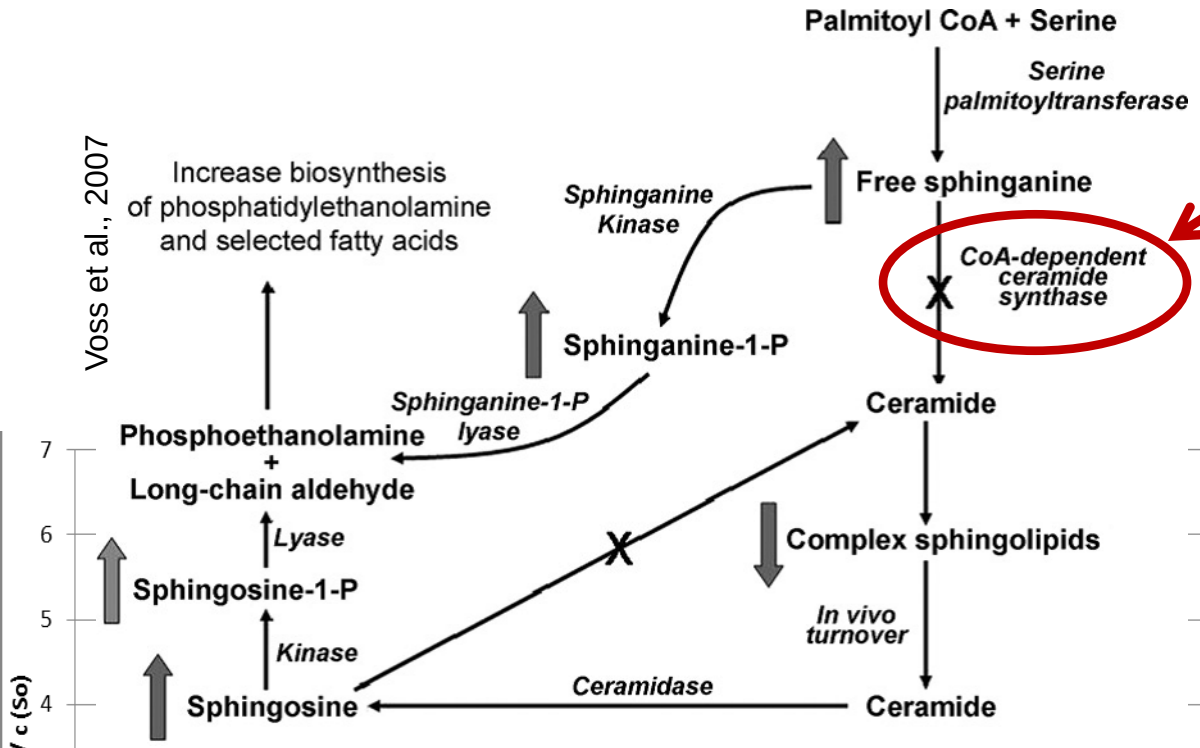
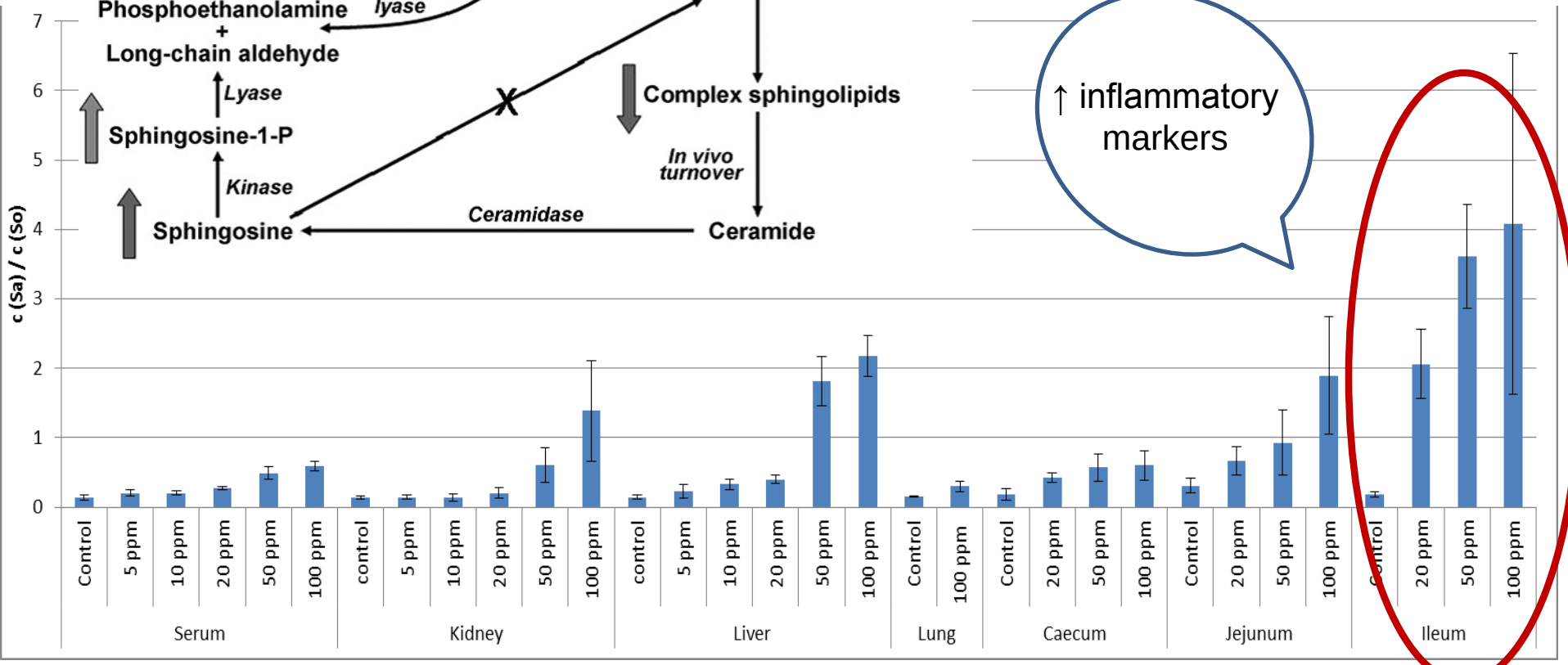
Aflatoxin	Realistic	Duck	Reduced apparent digestibility of crude protein
Aflatoxin	Moderate	Chicken & Hen	Reduced apparent digestibility, digestible & metabolizable energy
Fusariotoxins	Moderate	Chicken	Increased protein digestibility & net protein utilization
Fumonisin	Moderate	Rat & Pig	Reduced nutrient digestibility

Grenier and Applegate, **2013**

Spinganine (Sa): Sphingosine (So) ratio – Fumonisin Effect in Broilers



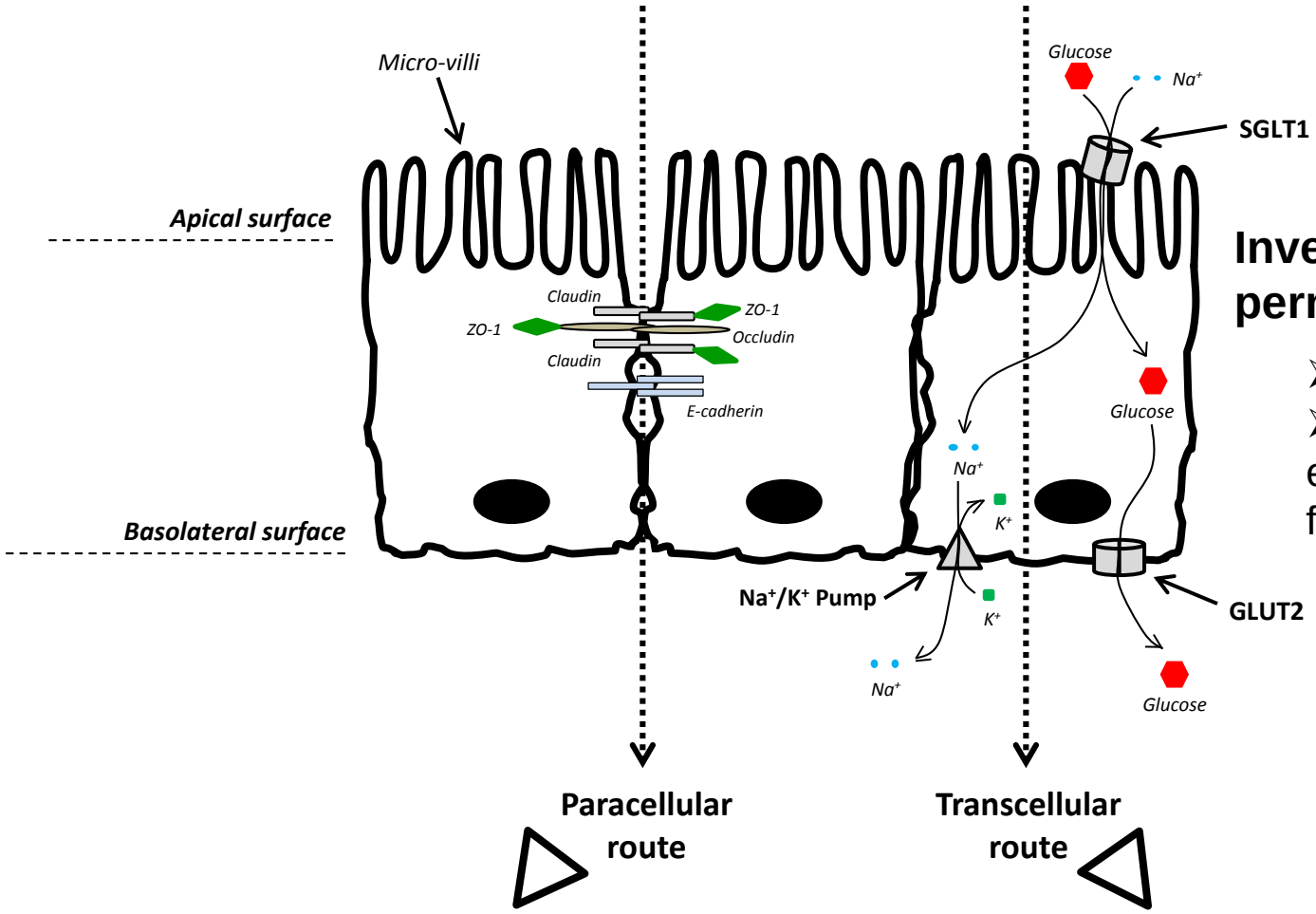


↑ inflammatory markers

Grenier et al. 2015

INTESTINE: FIRST TARGET

PERMEABILITY



Investigations on permeability:

- mostly with DON
- reduced TEER, expression of claudin family and ZO-1

IF IMPAIRMENT:

Higher translocation of luminal antigens
- commensal flora
- pathogens
- food antigens
- toxins and mycotoxins

IF IMPAIRMENT:

Lower uptake of nutrients, such as glucose
Malabsorption of water

Grenier and Applegate, **2013**

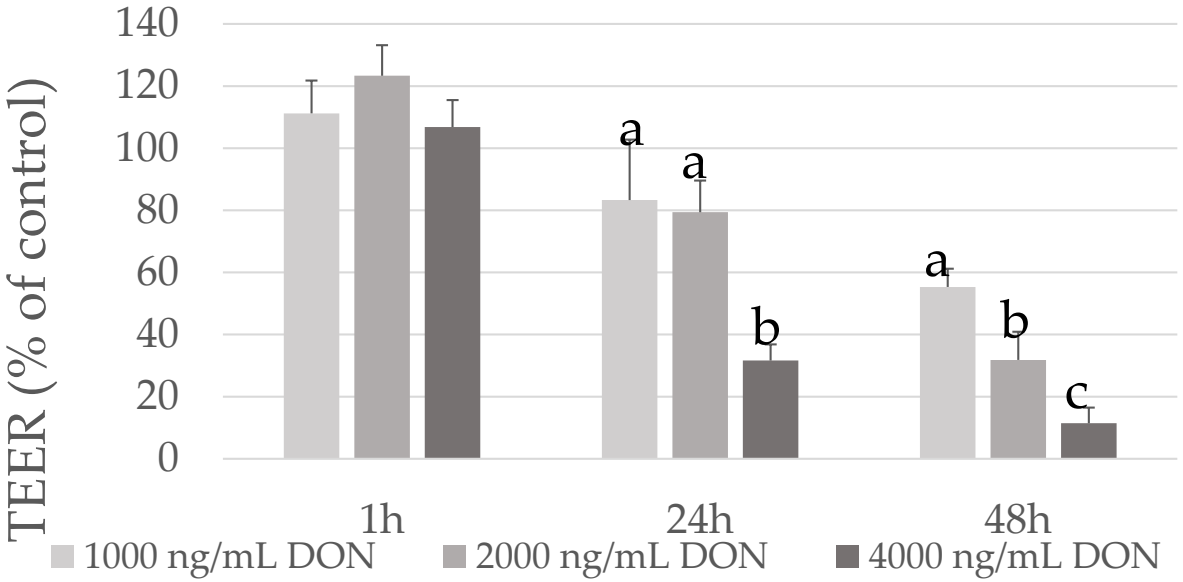
Effects of DON and FUM in broilers challenged with Coccidiosis vaccine

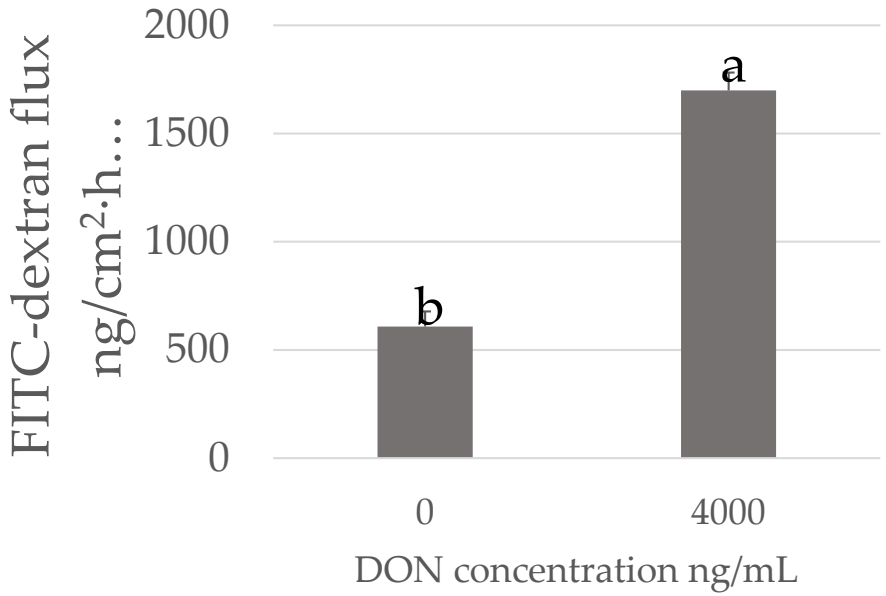
- Treatments (4 diets x cocci challenge/no challenge):
  - Control feed,
  - DON (2 mg/kg),
  - FUM (20 mg/kg),
  - DON+FUM (2 and 20 mg/kg)

- **Challenge:** Coccivac-B 25X (mix of 4 strains of *Eimeria*) at day 14

Grenier et al., 2015

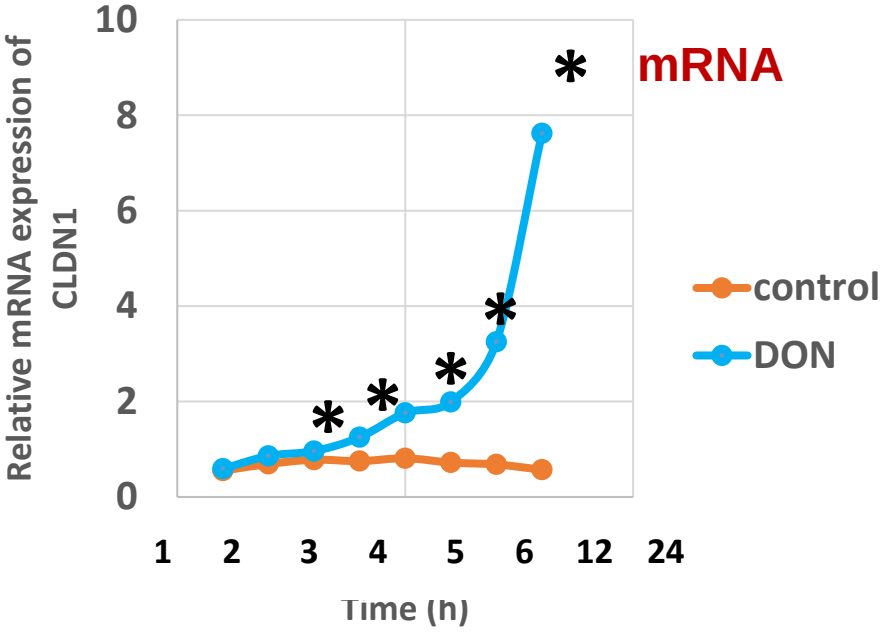
DON & cellular barrier integrity in IPEC-J2 cells



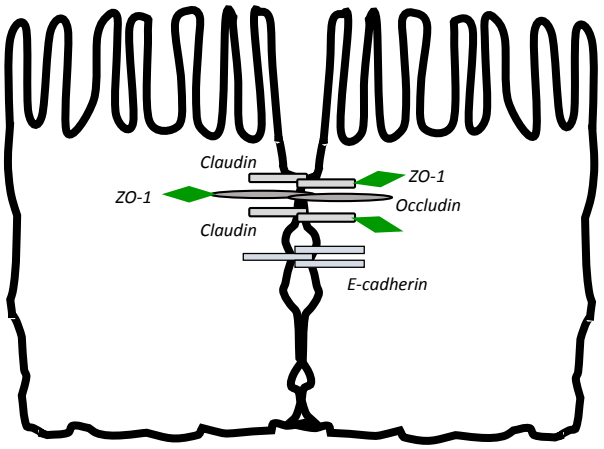


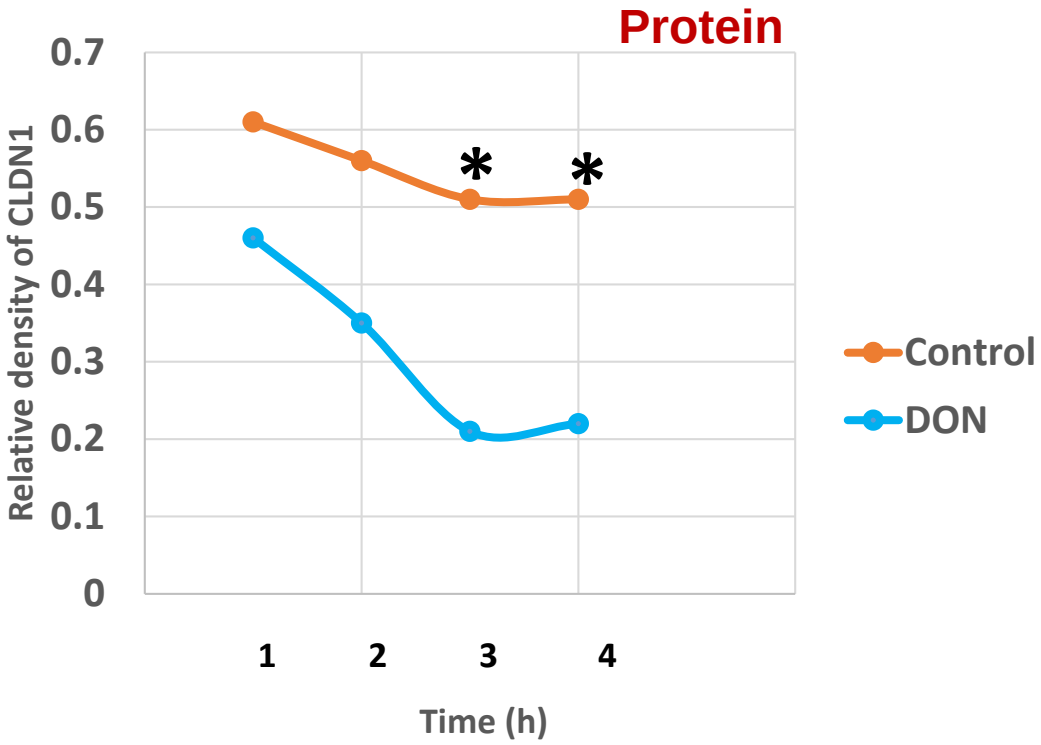
- **DON impaired cellular barrier integrity in IPEC-J2 by decreasing TEER value and increasing FITC-dextran passage.**

Zhang & Applegate, unpublished



DON & Claudin1 (CLDN1)





DON ↓ CLDN1 Protein (3h) prior to ↑of mRNA (4h) in IPEC-J2 cells.

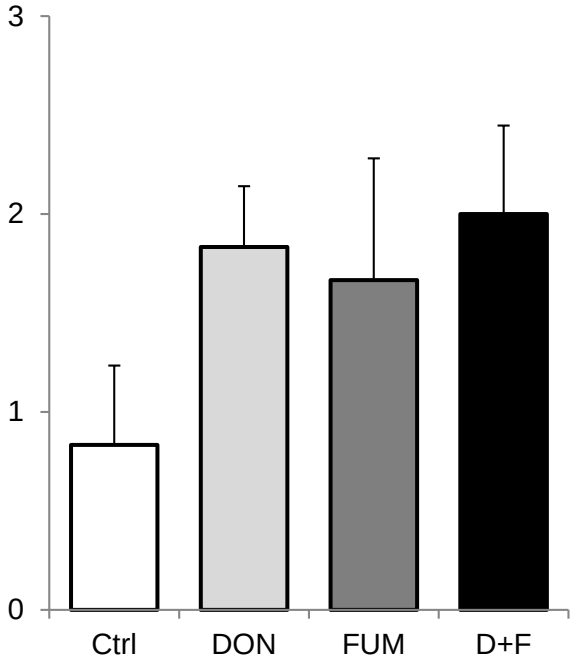
(translation of RNA can not keep pace with protein degradation)

Zhang & Applegate, unpublished

Combinded effects: FUM+DON+Eimeria

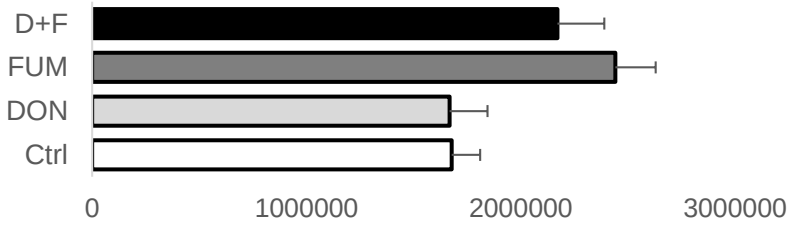
Major findings: LESION SCORE & OOCYST COUNTS

Lesion score in ceca



Number of oocysts in mucosa

Number of oocysts in excreta

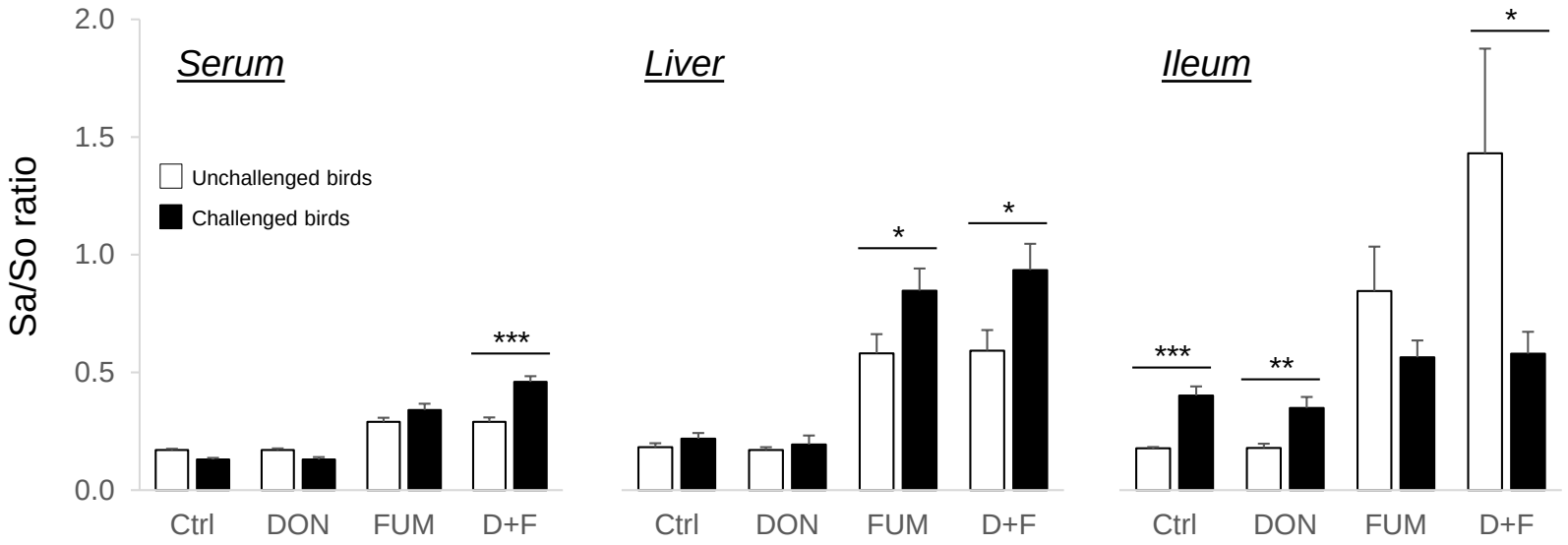


DON (2 mg/kg),
FUM (20 mg/kg),
DON+FUM (2 and 20 mg/kg)

Grenier et al., 2015

Combinded effects: FUM+DON+Eimeria

Major Findings: Spinganine (Sa): Sphingosine (So) ratio



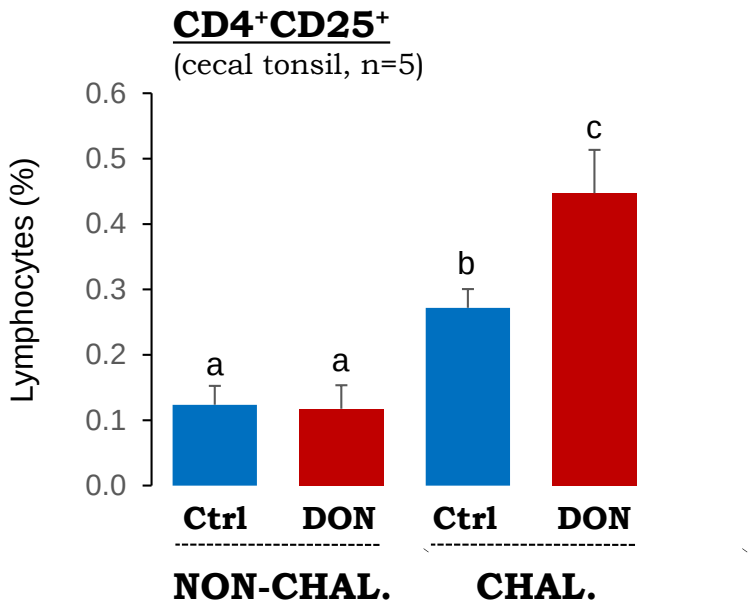
Coccidial challenge compromised the intestinal barrier (confirmed by histology with destruction of the epithelium) and facilitated the passage of FUM

Grenier et al., 2015

Combinded effects: DON+Eimeria

Major findings: T_{reg} lymphocytes

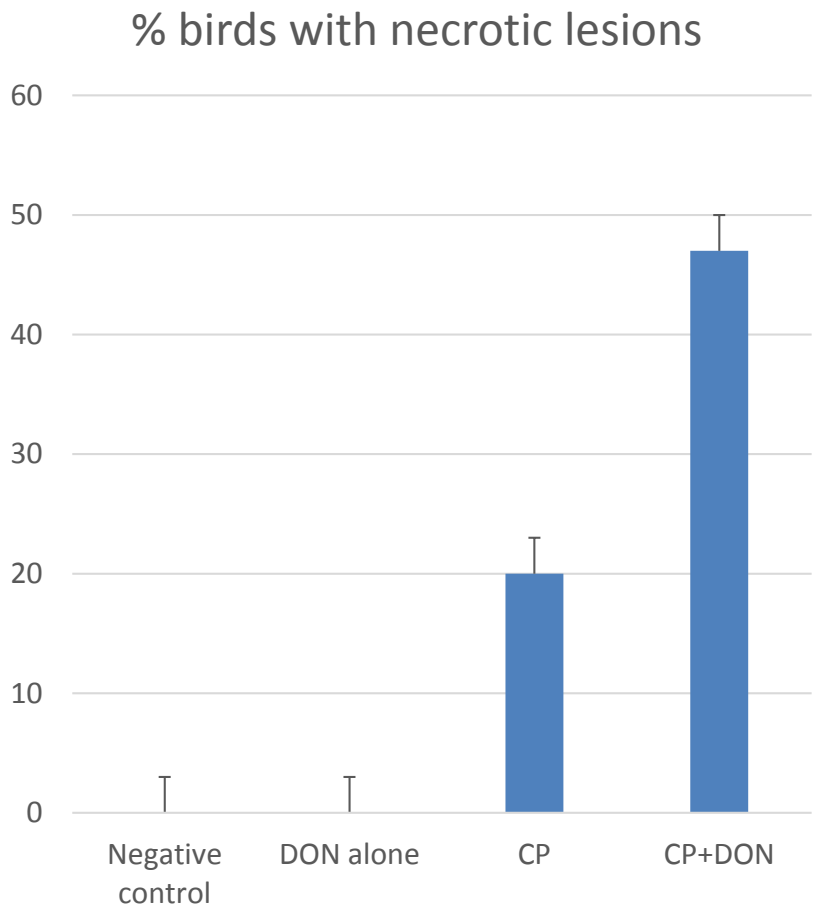
$CD4^+CD25^+$
T_{reg} lymphocyte, role in regulation of inflammation



Assumption:
challenged birds fed DON required more T_{reg} to control inflammation?

Grenier et al., 2015

DON increases Incidence & Severity of Necrotic Enteritis



DON=3.8 to 4.4 mg/kg

CP=*Clostridium perfringenes*

UNIVERSITEIT GENT

Antonissen et al., 2014; PLOS One 9:e108775

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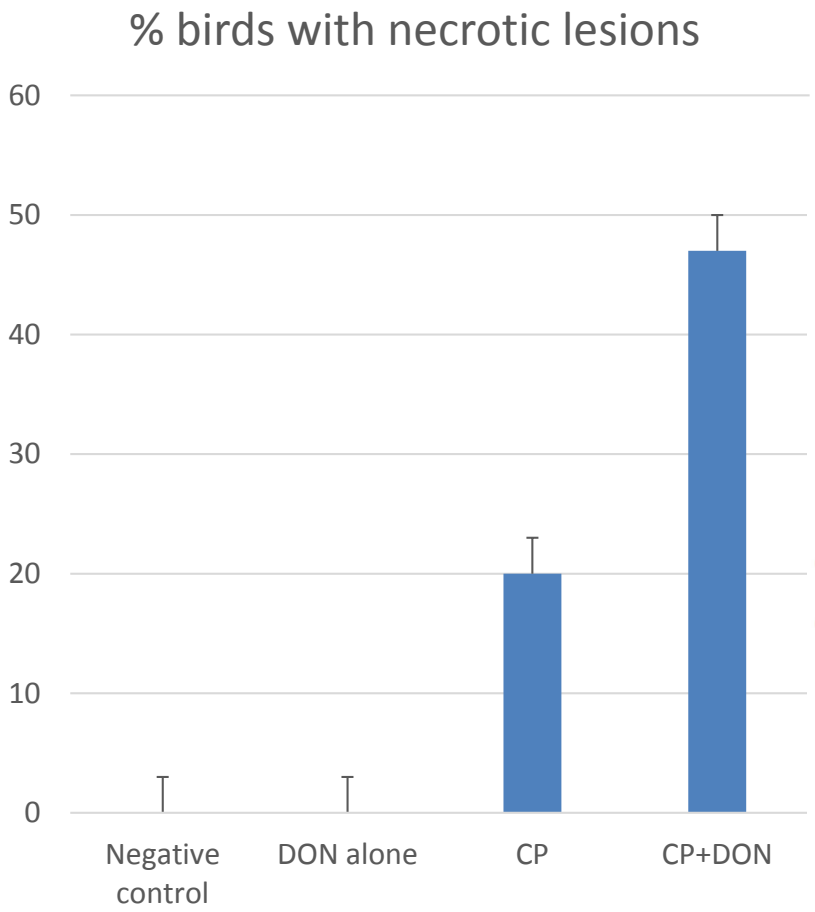

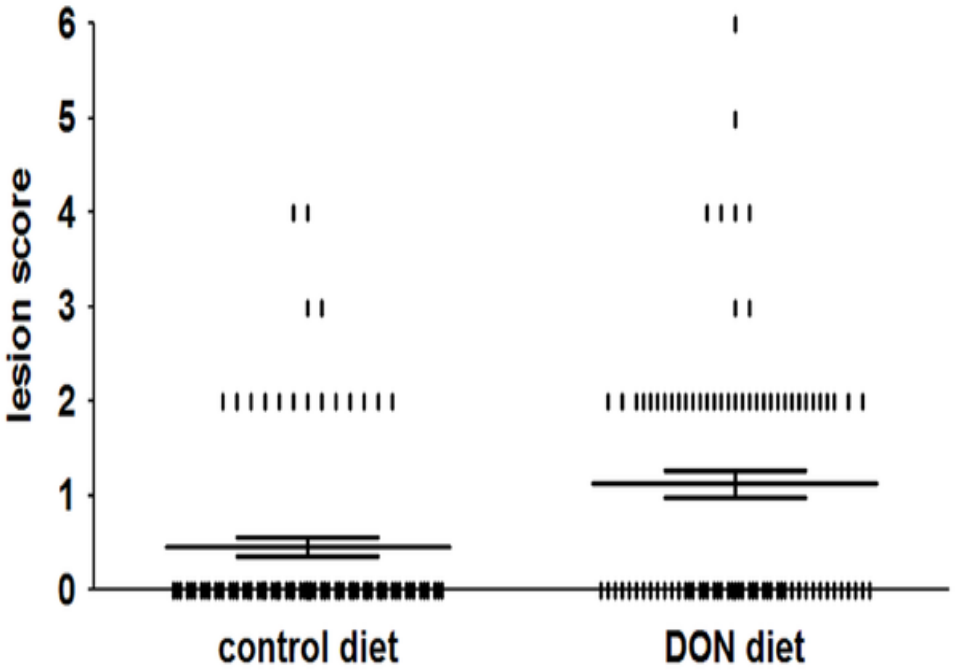

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UNIVERSITEIT GENT

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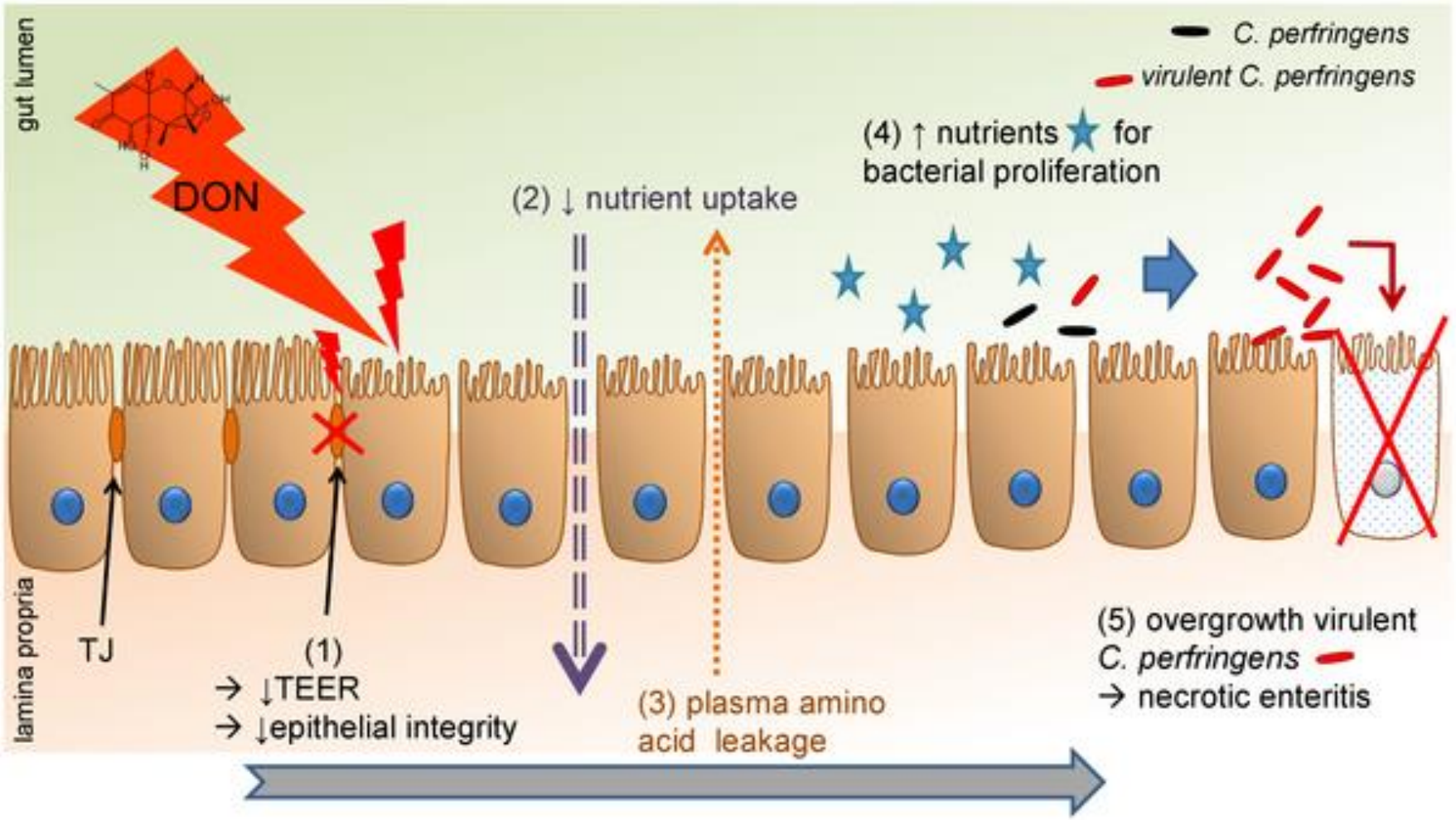
Deoxynivalenol predisposes for C. perfringens induced necrotic enteritis



Antonissen G, Van Immerseel F, Pasmans F, Ducatelle R, et al. (2014) PLoS ONE 9(9): e108775.

INTESTINE: FIRST TARGET

DEFENSE

COMBINATION WITH DIGESTIVE PATHOGENS

PARASITE

Fusariotoxins *(chicken):*
- impaired recovery of duodenal villi from coccidial lesions,
- upregulation of IFN-γ expression in CT,
- delayed recruitment of $CD4^+$ and $CD8^+$ cells in jejunum

OTA *(turkey, chicken)*:
- bloody diarrhea,
- higher lesions and oocyst in intestine,
- duodenal hemorrhages

BACTERIA

FB_1 *(pig)*:
- increased intestinal colonization by *E. coli,*
- affect APC maturation, T cell stimulatory capacity, specific Ig in PP

DON *(porcine cells & ileal loop)*:
- enhanced *S. typhimurium* invasion and translocation,

OTA *(chicken)*:
- higher number of *S. typhimurium* in duodenum & cecum, acute enteritis

HIGHER SENSITIVITY WHEN COMBINED WITH MYCOTOXINS

Grenier and Applegate, **2013**

Do Mycotoxins affect the GIT?

- Yes….but it depends on:
  - Which mycotoxin
  - Concentration (realistic, occasional, unrealistic)
  - Route of effect
- Not easy to elucidate as research is inherently confounded:
  - Changes to nutrient/energy content of feedstuffs
  - Changes to feed intake, metabolism
- Particular unknowns:
  - Interactive effects of multiple mycotoxins
  - Interactive effects of DON/FUM with intestinal pathogens

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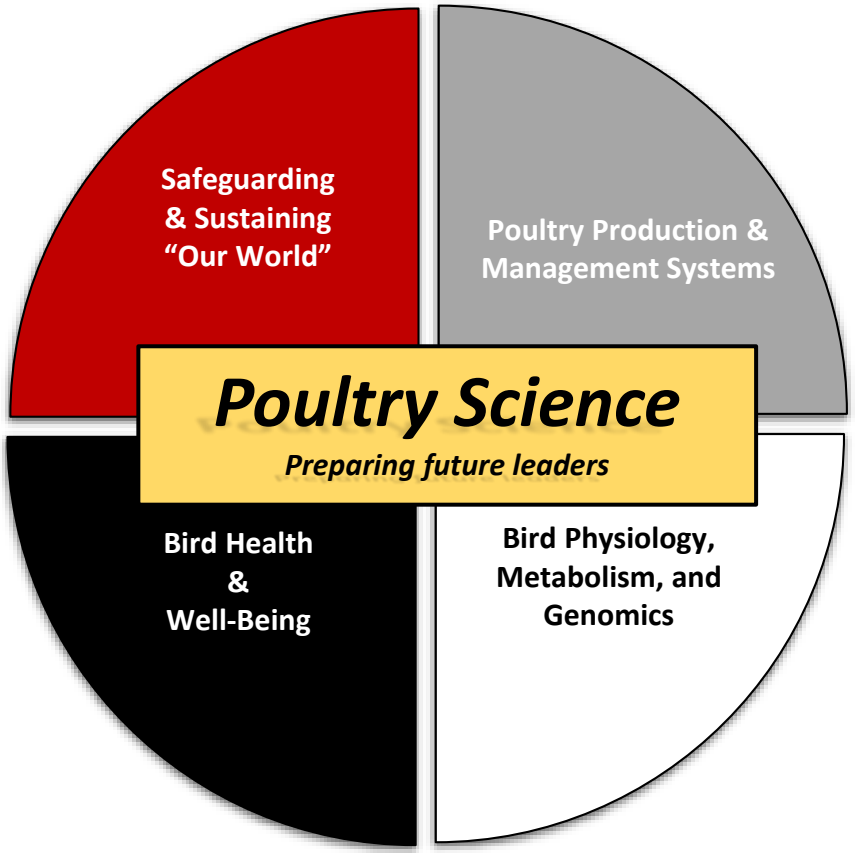
Department of Poultry Science



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