



MINIMIZING RISK OF MYCOTOXINS TO SUPPORT PIG IMMUNITY AND DISEASE PREVENTION

KIM SALVADOR | CAMDEN INDUSTRIES, INC.
22ND PORK CHALLENGE 2018
OCTOBER 4, 2018

ew | **nutrition**



- Mycotoxins
 - Mycotoxin effects in swine
- Mycotoxins and gut health
 - Intestinal functions – absorption and barrier
 - Mycotoxins MOA – consequences in the intestine
 - Aflatoxin – effects in gut health
 - Fusarium toxins – effects in gut health
 - Endotoxins – importance in swine health
- Alteration of immune function in swine health mediated by mycotoxins
 - Susceptibility to disease
 - Vaccination efficacy
- Mycotoxin analysis, risk evaluation and prevention tools

GENERAL CHARACTERISTICS OF MYCOTOXINS

Mycotoxins...

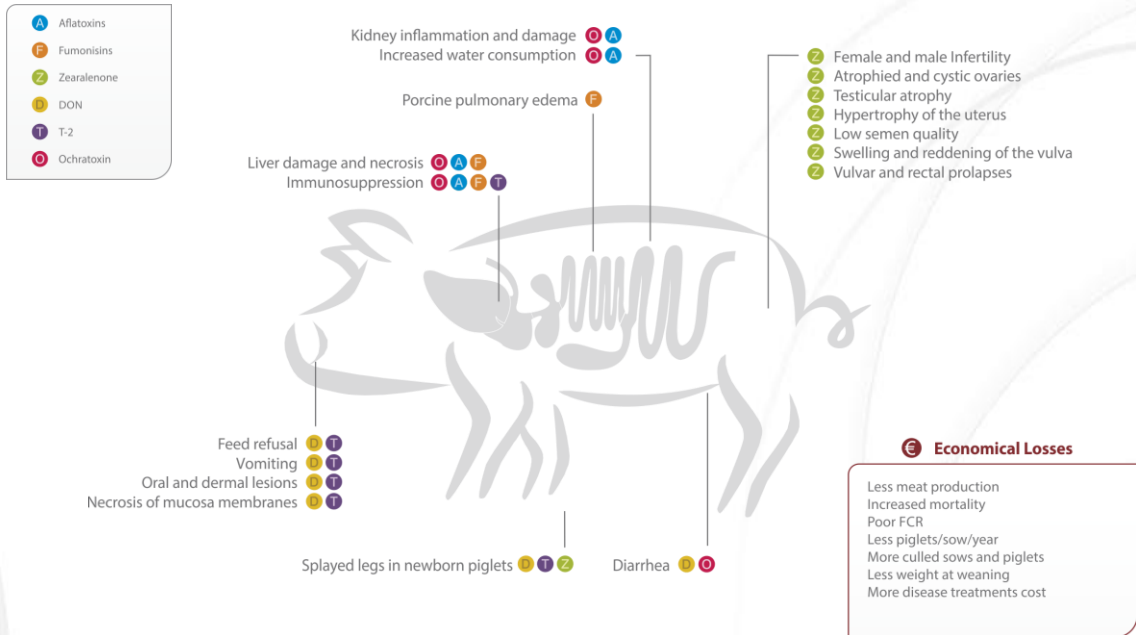
- are secondary metabolites produced by filamentous fungi.
- are found in grains, cereals and forages.
- cause a toxic response when ingested. They are colorless, odorless and tasteless.
- 6 mycotoxins of greatest importance worldwide



GENERAL CHARACTERISTICS OF MYCOTOXINS

- **Aflatoxin:** Carcinogenic, liver damage
- **Deoxynivalenol:** Feed refusal, immunosuppressive activity
- **Fumonisin:** Carcinogenic
- **Ochratoxin:** Kidney damage, reduced feed intake
- **T2/HT2:** Feed refusal, immunosuppressive activity
- **Zearalenone:** Estrogenic toxin

PROBLEMS CAUSED BY MYCOTOXINS



HEALTH IMPLICATIONS



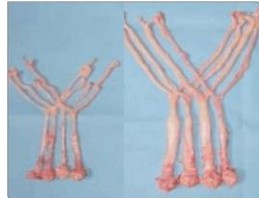
DON/Ochratoxin:
Diarrhea



Fumonisin:
Pulmonary edema



Aflatoxin:
Fatty liver



Zearalenone:
Uterus hypertrophy



Zearalenone:
Swollen/reddened vulva



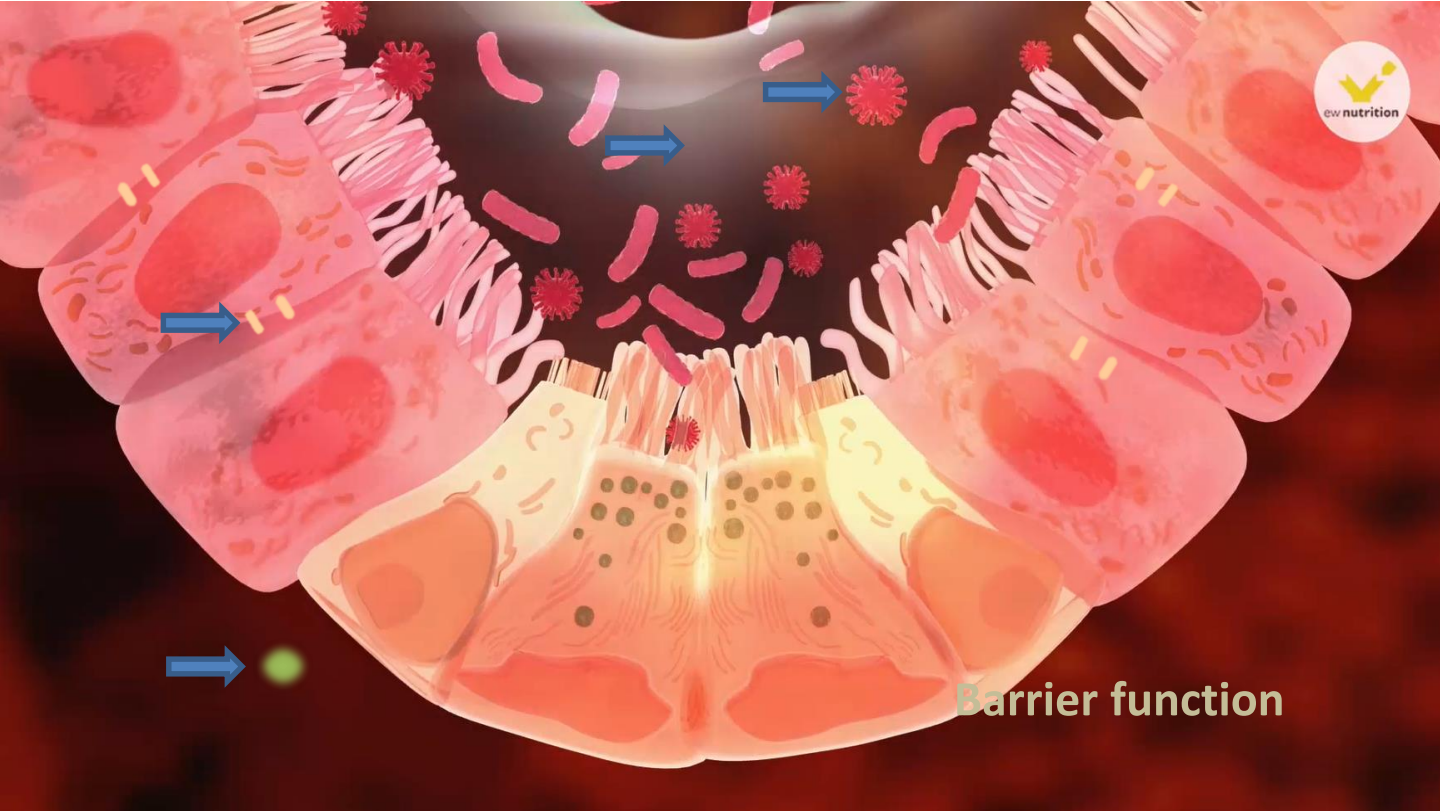
Zearalenone/trichothecenes:
Splayed legs newborn

Sources: Malone 2007; Zaviezo; University of Georgia College of Veterinary Medicine

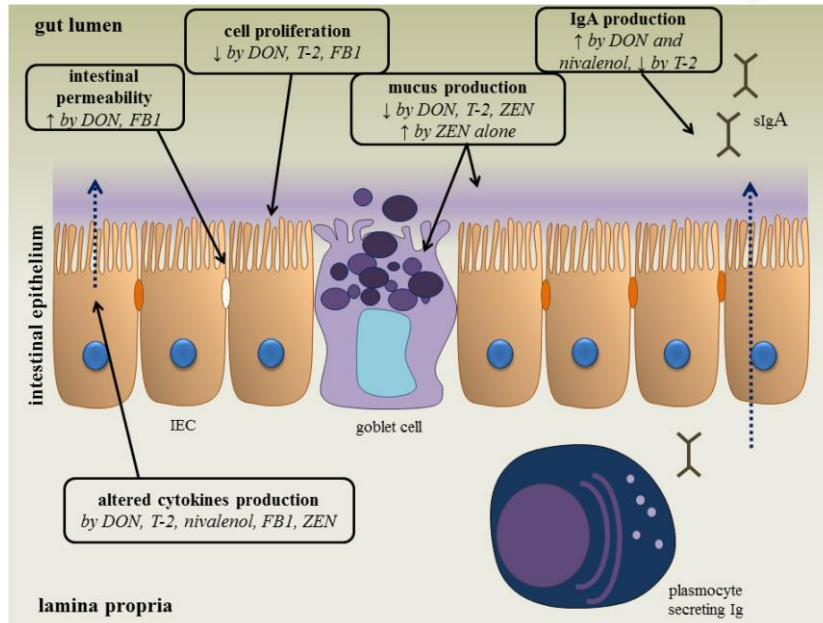
MYCOTOXIN ABSORPTION

Mycotoxin	Absorption site	Absorption rate	Biotransformation in GIT
Aflatoxin	Duodenum/jejunum entero-hepatic recirculation	80%	-
DON	Duodenum/jejunum	up to 90%	Large Intestine (LI) to DOM-1
FUM	Duodenum/jejunum	Below 5%	-
Ochratoxin	Jejunum	Up to 65%	LI to Ochratoxin- α
Zearalenone	Small Intestine (SI)/LI entero-hepatic recirculation	80-85%	-

Mycotoxins and gut health

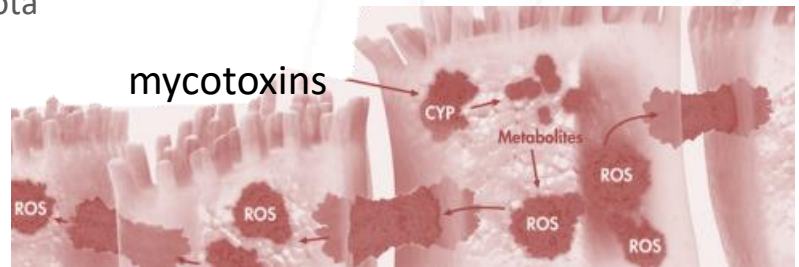
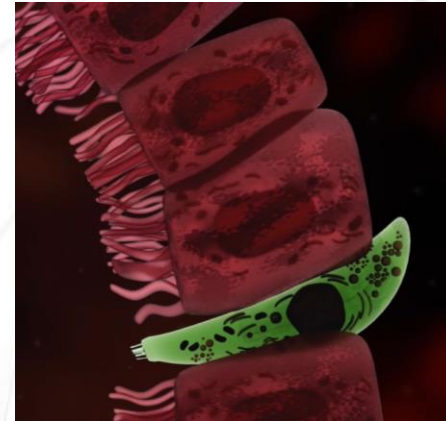


MYCOTOXINS AND GUT HEALTH



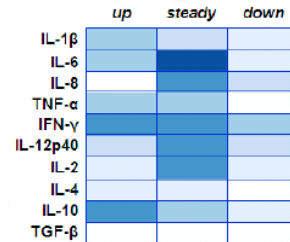
MYCOTOXINS - MECHANISMS OF ACTION

1. Decrease in protein synthesis
 - ↓ cell proliferation
 - ↓ intestinal microvilli height and regeneration
 - ↓ differentiation of intestine epithelial cells
2. Increased oxidative stress
 - ↑ damage to cell membranes
 - ↓ intestinal microbiota



MYCOTOXINS - MECHANISMS OF ACTION

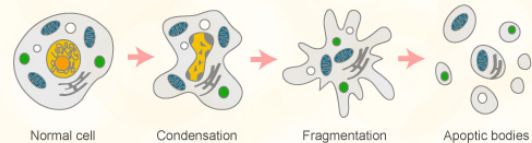
- Changes in gene expression and production of chemical messengers
 - ↓ immune function
 - ↓ cell proliferation
- Induction of apoptosis
 - ↑ permeability of the intestinal barrier
 - ↓ immune response
 - ↓ mucus production
 - Δ balance of microbiota



More than 1 week of exposure

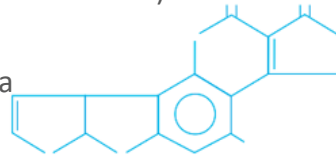
Grenier & Appelgate, 2013.

Apoptosis

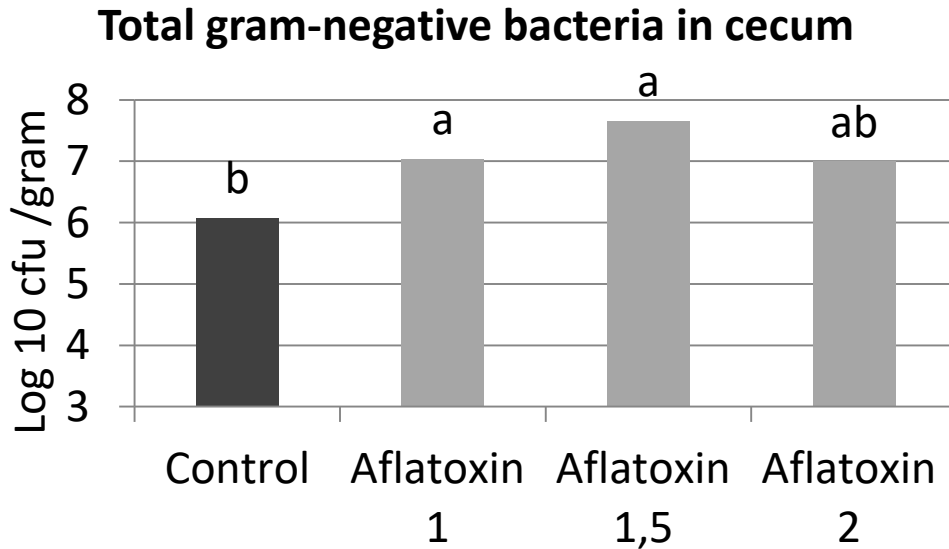


AFLATOXIN – EFFECTS IN THE INTESTINE

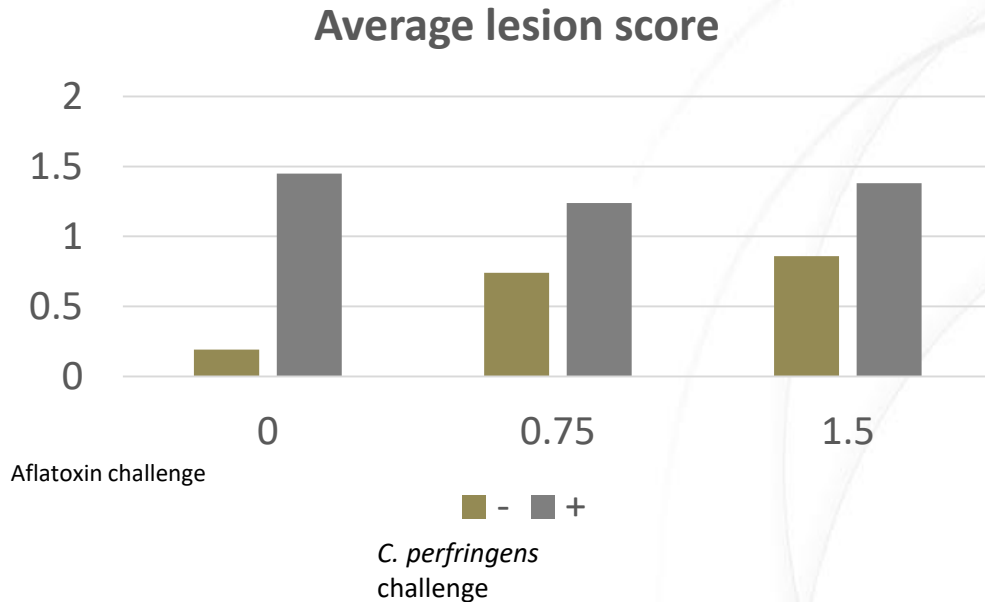
- AFB1 can cause histopathological changes in small intestine:
- Duodenum is more affected
 - Intestinal Hemorrhages due to disruption of the coagulation cascade
 - reduction of thromboplastin, prothrombin, fibrinogen and factors V, VII and X
 - Damage in Lieberkühn glands
 - Reduced enzyme (saccharose and maltose) secretion
 - Alteration in microbiota
 - Favours gram-negative bacteria
 - Increases sub-clinical NE
- **Double exposure:** The digestive tract is the main route of excretion of AFB metabolites through the bile.



AFLATOXINS – INCREASE GRAM-NEGATIVE BACTERIA

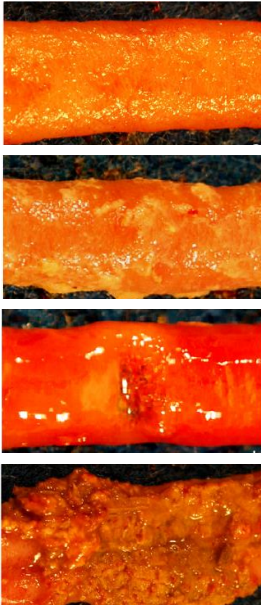


AFLATOXINS — INCREASE NECROTIC ENTERITIS LESIONS



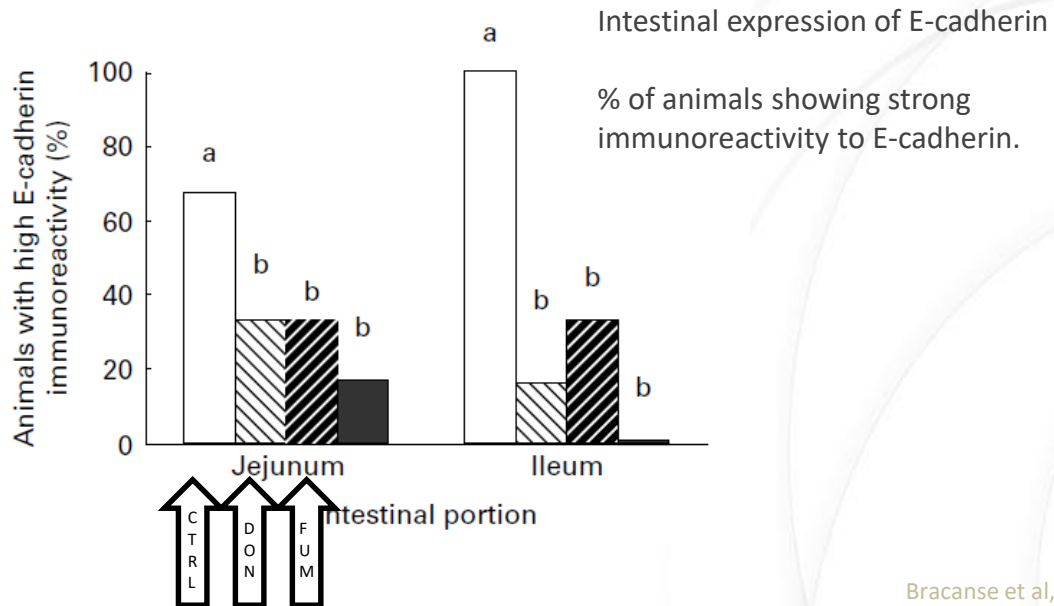
Cravens et al, 2013.

SCORING NECROTIC ENTERITIS

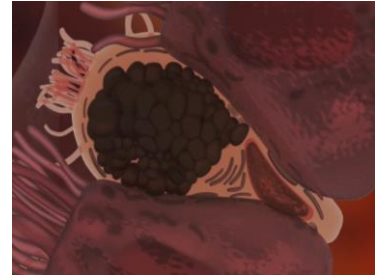
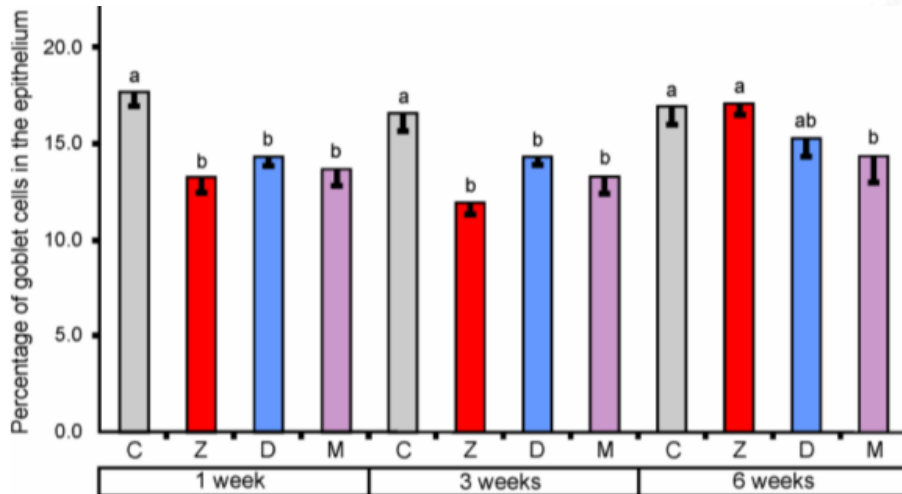


Score	Lesions/Number of lesions
0	No gross lesions
1	Thin or friable walls or diffuse, superficial and removable fibrin
2	Focal necrosis or ulcerations or non removable fibrin/ 1-5
3	Focal necrosis or ulcerations or non removable fibrin/ 6-15
4	Focal necrosis or ulcerations or non removable fibrin/ >16
5	Necrotic patches 2-3 cm/variable
6	Diffuse necrosis/extensive

FUSARIUM TOXINS – NEGATIVE EFFECT ON TIGHT JUNCTIONS

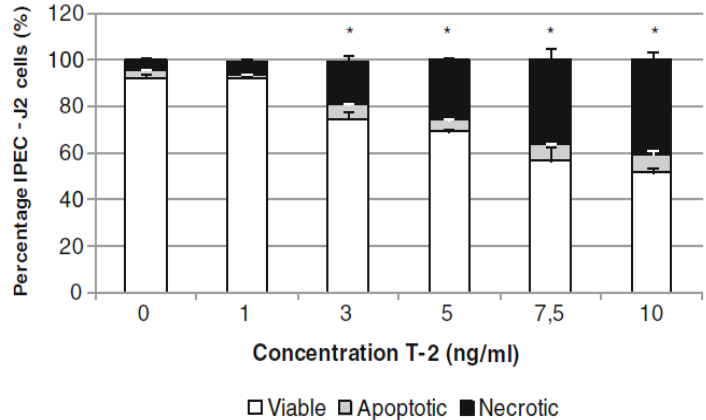
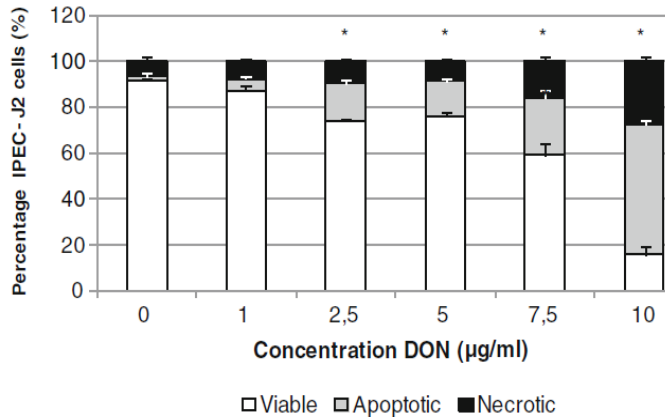


FUSARIUM TOXINS – REDUCE NUMBER OF GOBLET CELLS



Effects of Zearalenone (Z – 40 $\mu\text{g/kg BW}$) Deoxynivalenol (D – 12 $\mu\text{g/kg BW}$) and their combination in the number of Goblet cells in the large intestine epithelium

FUSARIUM TOXINS – INDUCE APOPTOSIS AND NECROSIS

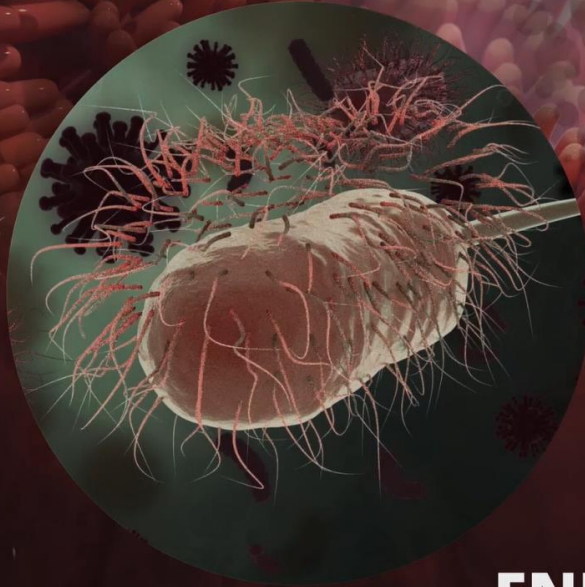


Percentage viable, apoptotic and necrotic cells after incubation of undifferentiated IPEC-J2 cells for 72 h with different concentrations of DON and T-2



Microbiota is altered

- Increase in gram-negative bacteria (*E.coli*) - endotoxins
- Increase in *Clostridium perfringens* – exotoxins and NE



ENDOTOXINS

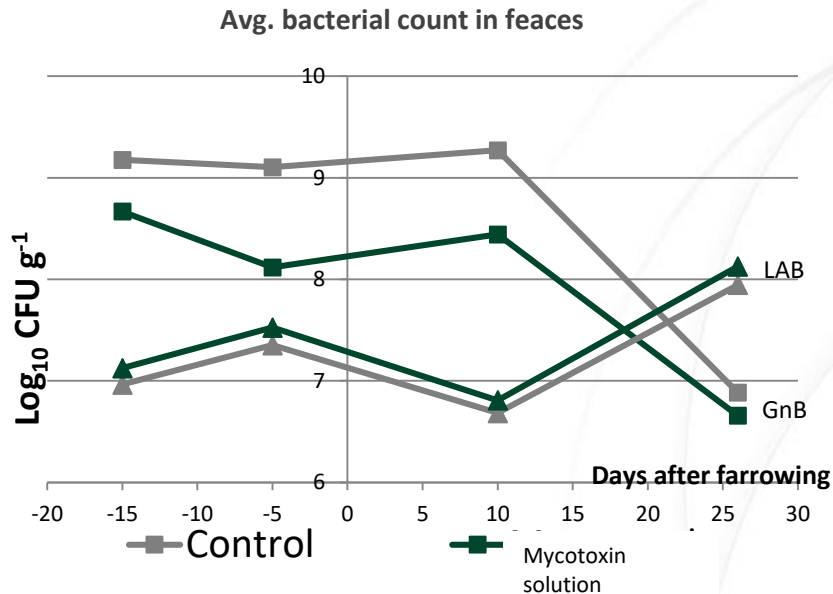
BACTERIAL TOXINS - ENDOTOXINS

- Endotoxins are one of the strongest bioactive substances
- Depending on sensitivity, dose, origin (bacterial species) as well as specific situation health status they trigger immune reactions – even at low concentrations
 - Endotoxic shock (Pain, fever, loss of blood pressure)
 - Coli diarrhea
 - MMA in sows
 - Ear necrosis
 - Weakened immune system



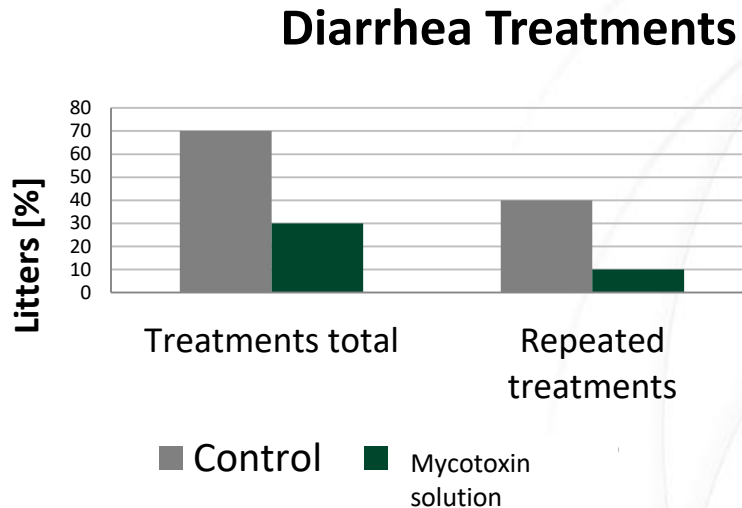
M. SOLUTION - LOWERS GRAM-NEGATIVE BACTERIA IN SOW FECES

Sows with high
blood
endotoxin



M. SOLUTION - LOWERS DIARRHEA TREATMENTS

Sows with high
blood
endotoxin



M. SOLUTION – IMPROVES PERFORMANCE

Sows with high
blood
endotoxin

Performance parameters		
	Control	Anti-mycotoxin product
Piglets born alive, avg.	16.8	16.9
Piglets born dead, avg.	2.2	1.8
Weaned piglets, avg.	11.2	11.9
Birth weight, avg. [kg]	1.35	1.32
Weaning weight, avg. [kg]	7.05	7.47
Mortality, excluding crushed [%]	6.55	5.92



CONSEQUENCES OF ALTERATIONS OF IMMUNITY IN PIG HEALTH

- **Susceptibility to infectious diseases**
- **Reactivation of chronic infection**
- **Vaccination efficacy**



SUSCEPTIBILITY TO INFECTIOUS DISEASES

- Aflatoxin reduces incubation time of *Brachyspira hydysenteriae*, and increased severity of diarrhea (Joens et al., 1981)
- In presence of PCV2 virus, DON increases severity of viral infection
- In presence of PRRSV, DON increases infection with more tissue lesions induced (Savard et al., 2014, 2015b).

SUSCEPTIBILITY TO INFECTIOUS DISEASES

- Fumonisin B1 increases susceptibility to pulmonary infection (Devriendt et al., 2009; Halloy et al., 2005; Oswald et al., 2003; Posa et. al., 2011, 2013; Ramos et al., 2010)
- Ochratoxin-contaminated feed increases salmonellosis (Stoev et al., 2000)
- In PCV2, ochratoxin increases viremia in sera and tissues (Gan et al., 2015)

VACCINATION EFFICACY

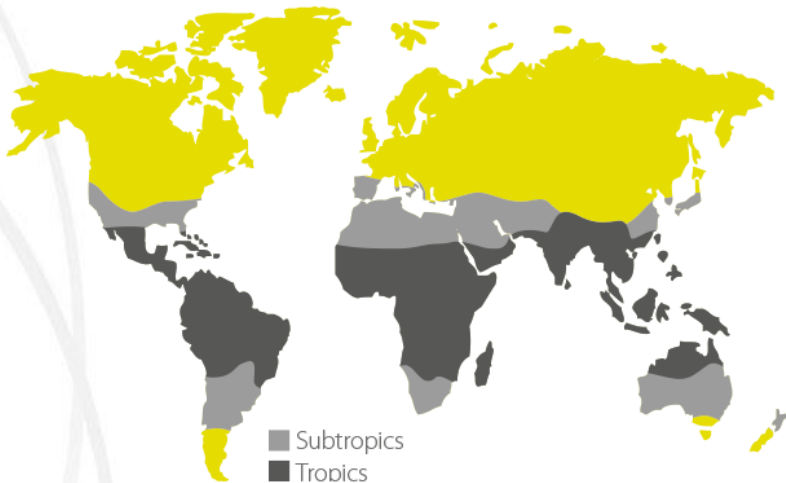
- Aflatoxin B1 interferes with development of acquired immunity in swine following erysipelas vaccination with bacterin preparation (Cysewski et al., 1978).
- Aflatoxin B1 or T-2 toxin contaminated feed reduced vaccine response to ovalbumin, acting on cellular and humoral response, respectively (Meissonnier et al., 2008a, 2008b).

VACCINATION EFFICACY

- Fumonisin B1 decreases specific antibody response mounted during Mycoplasma vaccination in pigs (Taranu et al., 2005).
- Pigs exposed to ochratoxin or fumonisin B1 and vaccinated against Aujeszky disease (Suid Herpesvirus 1 [SuHV1]), the humoral immune response was greatly disturbed, with strong decrease in antibody observed (Stoev et al., 2012).
- DON-contaminated feed inhibited vaccination efficiency of PRRSV modified live vaccine (Savard et al., 2015a).

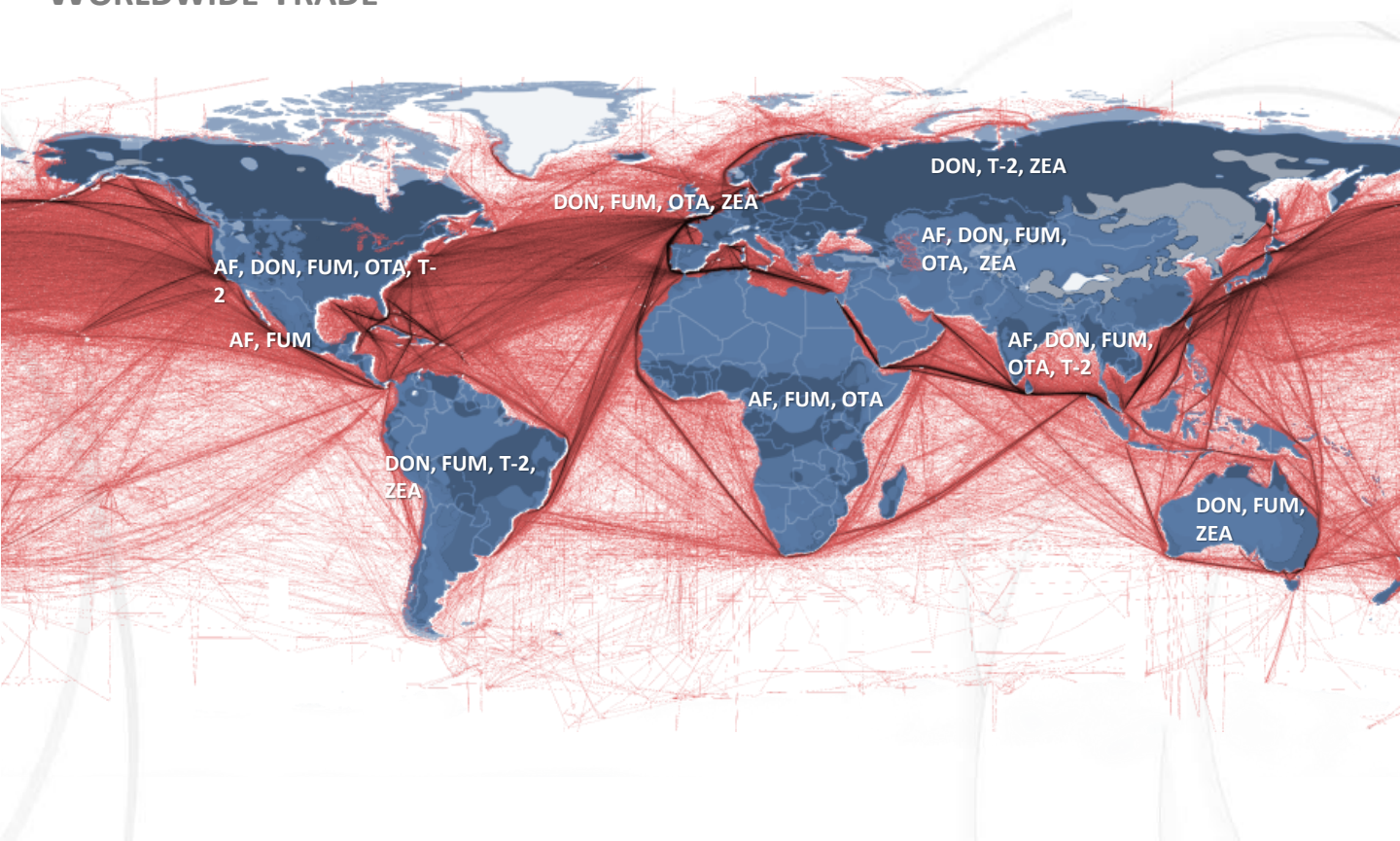
MYCOTOXIN ANALYSIS
RISK EVALUATION AND
TOOLS PREVENTION

MYCOTOXINS IN THE TROPICS AND SUBTROPICS



Climatic conditions in these regions are most conducive for mould invasion, proliferation and elaboration of mycotoxins like Aflatoxins and Fumonisin

WORLDWIDE TRADE



MYCOTOXIN ANALYSIS

Why? Knowing the mycotoxin values!

Measuring the danger is the first step to be ahead of it

- Considering all the raw materials of the final diet
- Analysing the most important mycotoxins
- Laying a reliable foundation to take the right decisions

SAMPLING

The importance of sampling

- Sampling procedure is the main factor of variability in mycotoxin analysis
- Contaminated parts may not be evenly distributed
 - The sample should be an accumulation of many subsamples from different locations through the lot...
 - Every individual particle on the lot should have an equal chance of being chosen



QUANTIFICATION

High performance liquid chromatography

Separate, identify and quantitate the compounds being present in any sample that can be dissolved in liquid

PRO	CON
Accuracy	Time
Wide range of mycotoxins	Cost
Wide range of commodities	Mobility

QUANTIFICATION

Lateral Flow Devices

Fast, Economical and Accurate Mycotoxin Detection

- Lateral Flow Device System allows to perform tests at the point of receiving, eliminating testing in a laboratory setting.
- Real-time analysis on raw ingredients allows screening upon reception
- Food manufacturers can be assured of acceptable levels of mycotoxins.



RISK EVALUATION

Why? Knowing the values is not enough!
Understanding is the key to master the risk

- Linking the mycotoxin contamination to the risk
- Knowing possible health implications in the animals
- Take appropriate decisions and actions to lower the threat

EUROPEAN REGULATIONS

	Aflatoxin [ppb]	Fumonisin [ppb]	Ochratoxin [ppb]	Zearalenone [ppb]	DON [ppb]
Feed for poultry	20	20.000	100	-	2.000
Feed for swine	20	5.000	500	100	900
Feed for dairy cattle	5	50.000	-	500	5.000
Feed for calves	10	20.000	-	500	2.000
Feed for horses	20	5.000	-	-	5.000
Pet food	5	5.000	-	-	-
Raw materials	20	60.000	250	200	8.000

PHILIPPINE NATIONAL STANDARD

Annex 2

The No Observed Adverse Effect (NOAEL) /Lowest Observed Adverse Effect Level (LOAEL)
in Animals

Mycotoxin	Critical Effect	NOAEL¹/LOAEL² (mg/kg bw/day)	Tolerable Daily Intake³ (ug/kg bw/kg)
DON	Reduced feed intake and weight gain	0.1 (NOAEL)	1.0
T-2 + HT-2	Immunotoxicity/ haematotoxicity	0.03 (LOAEL)	0.1
Nivalenol	Reduced growth, leucopenia	0.7 (LOAEL)	0.7
Zearalenone	Oestrogenic activity	0.01 (NOAEL)	0.25

¹) NOAEL – No Observed Adverse Effect Level

²) LOAEL – Lowest Observed Adverse Effect Level

³) Tolerable Daily Intake, TDI, is a term used by EFSA, while JECFA uses the term Provisional Maximal daily Intake (PMTDI)

Source: <http://www.Vkm.no/dav/eee04d10c4.pdf>

DOES THAT MAKE SENSE?

Mycotoxin interactions

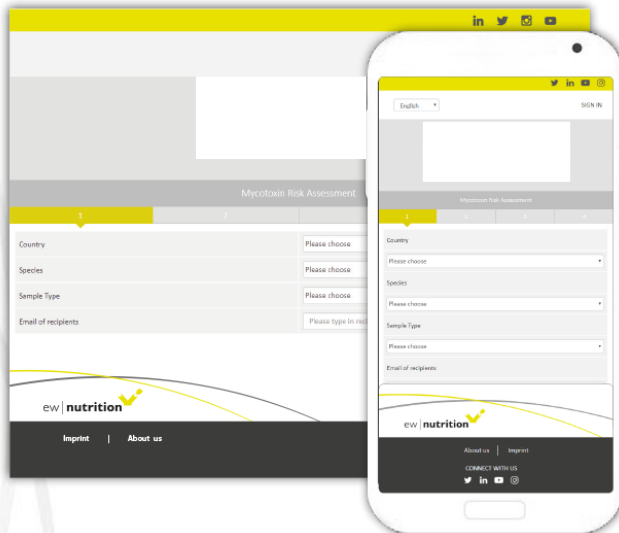
Additivity



Synergism



Risk Assessment tool with EW Nutrition



The image shows a desktop and a mobile phone displaying the 'Mycotoxin Risk Assessment' tool. The desktop version is on the left, and the mobile phone is on the right. Both screens show a form with the following fields: Country, Species, Sample Type, and Email of recipients. Each field has a 'Please choose' dropdown menu. The desktop version also has a 'Please type in recipient' field. The mobile phone version has a 'SIGN IN' button in the top right corner. The ew | nutrition logo is visible at the bottom of both screens.

- ✓ Individual effects AND their combination
- ✓ Instantly links contamination to effects
- ✓ Wide range of species
- ✓ Raw materials, finished feeds and complete diets

Key points

- Mycotoxins play an important role in the balance of intestinal health in animal production.

Taking a preventive approach

- Toxin Risk Management
- Anti-mycotoxin products
 - Avoid direct contact with the gastrointestinal epithelium
 - Better health and productivity

Toxin Risk Management Program

EW Nutrition's Toxin Risk Management program offers assessment, strategies and solutions that support animal welfare, health and productivity.



THANK YOU FOR YOUR ATTENTION

KIM SALVADOR | CAMDEN INDUSTRIES, INC.