ew | nutrition

Functional Innovations backed by Science

Myco and bacterial toxin in poultry: Beyond the visible symptoms

mastersorb®

Mycotoxins - general introduction

Mycotoxins - interactions

Toxins in the GIT

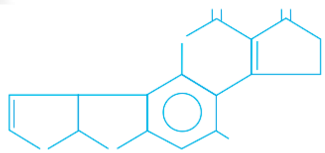
- Coccidiosis
- Necrotic enteritis
- E. coli
- Aflatoxin

Risk assesment ad economic impact

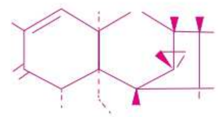
Mycotoxins

Mycotoxins are secondary metabolites produced by filamentous fungi found in grains, cereals and forages that cause a toxic response (mycotoxicosis) when ingested.

Nutrient losses in raw materials



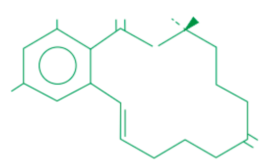
Aflatoxin



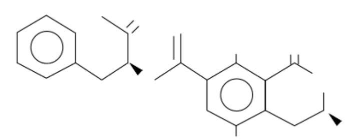
T-2

Deoxynivalenol

Fumonisin



Zearalenone



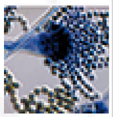
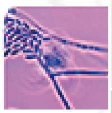
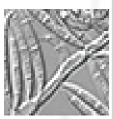
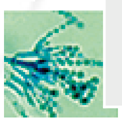
Ochratoxin

Mycotoxins

Mycotoxins are colorless, odorless and tasteless.

Lack of visible appearance of fungus does not negate presence of mycotoxins. Toxins can remain after fungus has been removed

Mycotoxins

Genus	Commonly affected commodities	Growth conditions	Mycotoxins produced	Remarks
 *Aspergillus*	Corn, peanuts, cottonseed, palm kernels	High moisture (>14%) Warm temperature (>24°C) Enhanced by drought, insects and kernel damage	Aflatoxin Ochratoxin	Field and storage
 *Fusarium verticilloides* *Fusarium proliferatum*	Corn, rice, sorghum	Drought stress, warm weather during flowering, kernel damage	Fumonisinis	Field
 *Fusarium graminarum* *Fusarium culmurom* *Fusarium sporotrichiodies*	Corn, wheat, barley, oats, sorghum, rice, rye	Cool and moist weather (6-24°C) Survives in residues form previous crops	Deoxynivalenol, T-2, Zearalenone	Field and storage
 *Penicillium*	Wheat, barley, rice, rye	High moisture (>14%) Warm temperature (>20°C) Cracked broken kernels	Ochratoxin	Storage

ew | nutrition

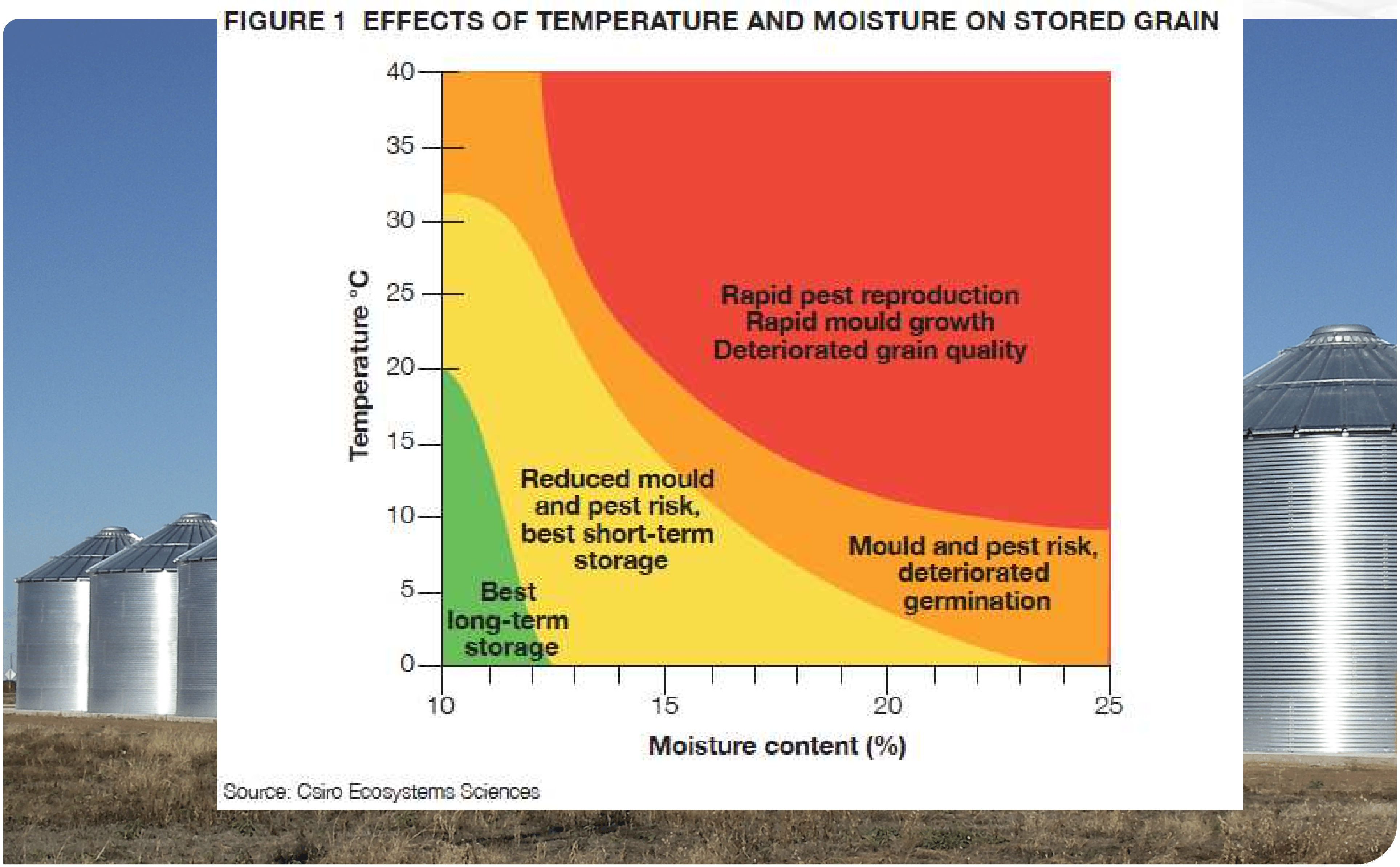
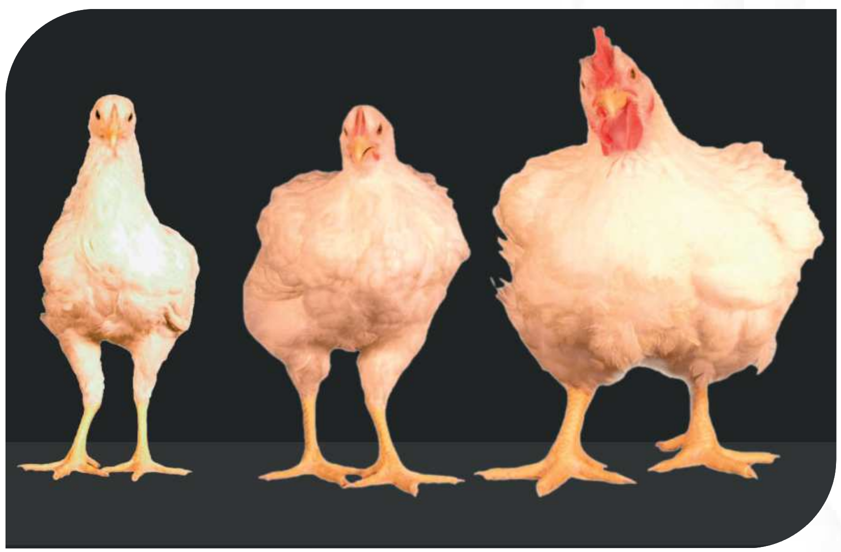


FIGURE 1 EFFECTS OF TEMPERATURE AND MOISTURE ON STORED GRAIN

Source: Csiro Ecosystems Sciences

The modern broiler has a faster growth rate that can affect their sensitivity to mycotoxins.

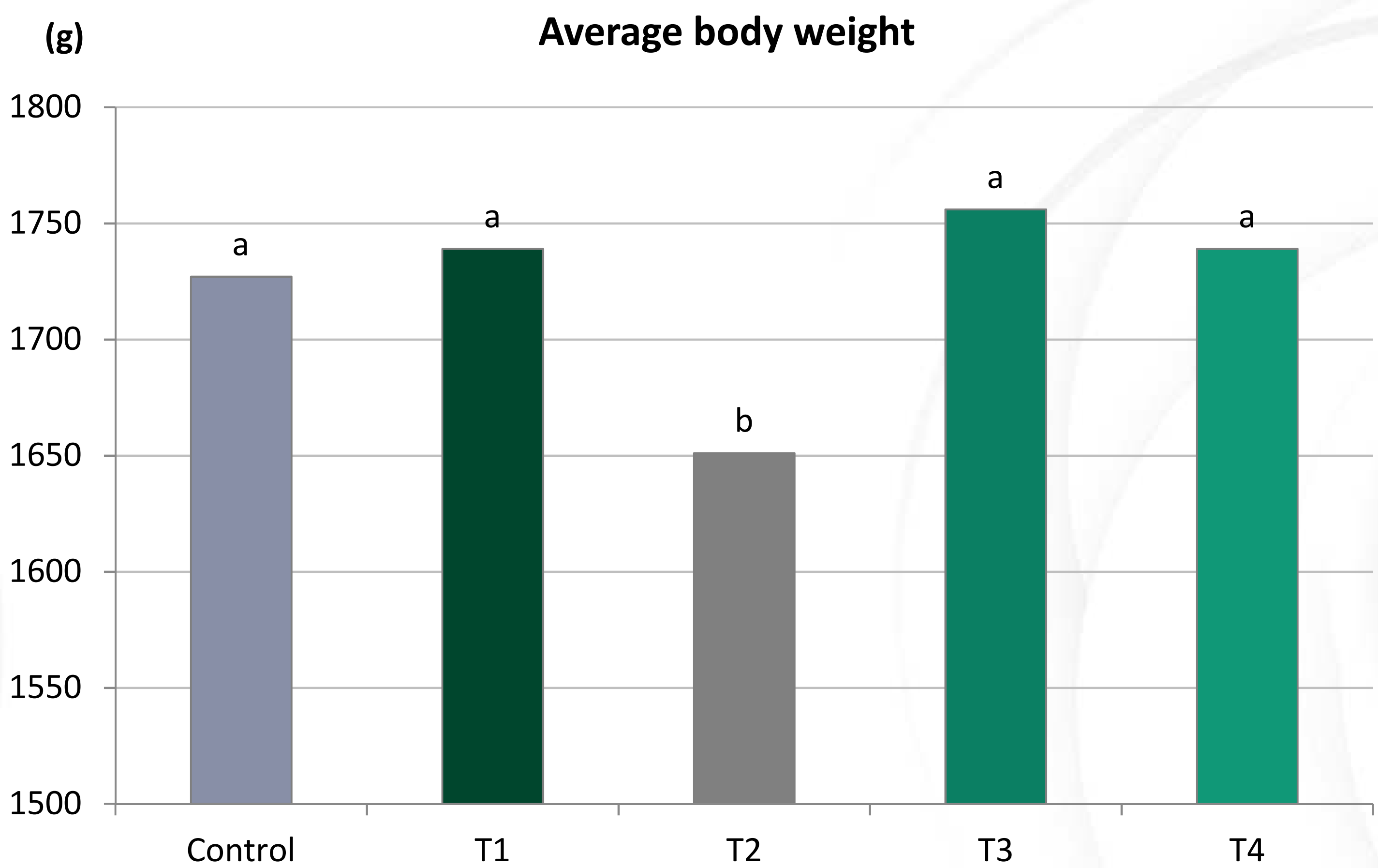
e.g. Recent reports show a toxic effect of DON in chickens at doses below the recommended limits and close to field conditions (1 to 5pm)



Mycotoxins

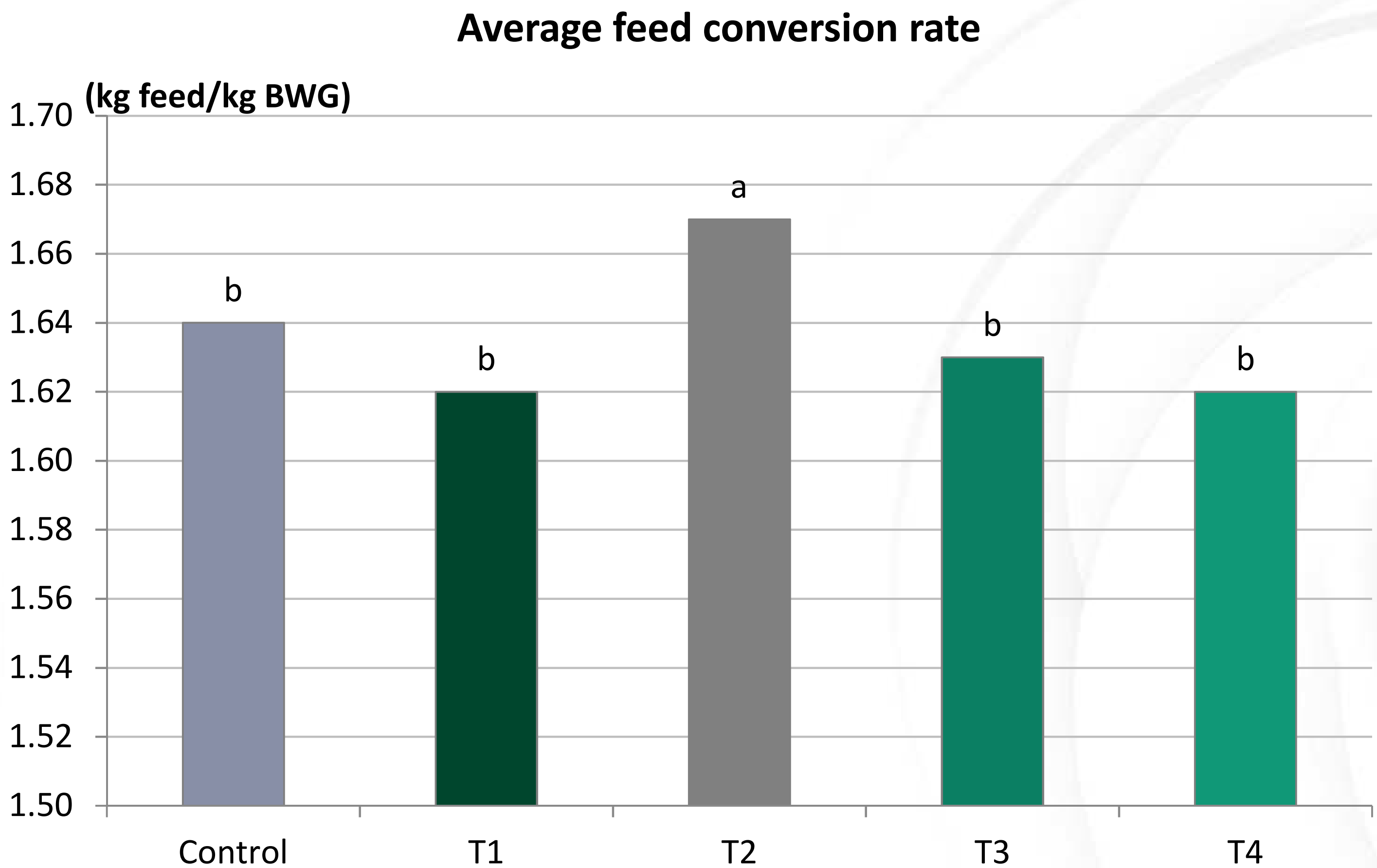
Trial facility:	Field trial
Trial Duration:	33 days
Animals:	Broilers, Ross 308
Sex:	Male
Treatments:	Control: Standard feed
	T1: Feed + anti-toxin agent- 2Kg/ton
	T2: Feed + Mycotoxins (ZEA ~300 ppb and DON ~6000 ppb)
	T3: Feed + Mycotoxins + 1Kg anti-toxin agent T4: Feed + Mycotoxins + 2Kg anti-toxin agent
Challenge:	ZEA ~300 ppb and DON ~6000 ppb
N° of animals	
Total:	480 Broilers
Per group:	12 (10 replicas per treatment)

Mycotoxins



(g)
Average body weight
1800
1750
1700
1650
1600
1550
1500
a
a
b
a
a
Control
T1
T2
T3
T4

Mycotoxins


Average feed conversion rate
(kg feed/kg BWG)
1.70
1.68
1.66
1.64
1.62
1.60
1.58
1.56
1.54
1.52
1.50
b
b
a
b
b
Control
T1
T2
T3
T4

Current risk assessment is very specific in terms of animal species, age and production stage, but in the majority of cases the assessment is done for a contamination with only one (single) mycotoxin.

Does it make sense?

YES - mycotoxins affect for example poultry in a different way than cattle, and broilers in a different way than breeders or laying hens

NO - in the practice of farming and feeding animals having individual mycotoxin challenges is not a reality.

Co-occurence of mycotoxins

- Fungi produce more than one mycotoxin in the same time
- Feed/Food could be contaminated by many fungi
- The feed is composed by a combination of raw materials

The co-contamination by many mycotoxins is the RULE, not the exception.

Most studies analyzed the effects of individual mycotoxins.

Mycotoxins - interactions

Combined mycotoxins have additive, synergic or (rarely) antagonistic effects in the animal.

Additive effect

Synergic effect

Mycotoxins - interactions

The interactions are complex, depend on:

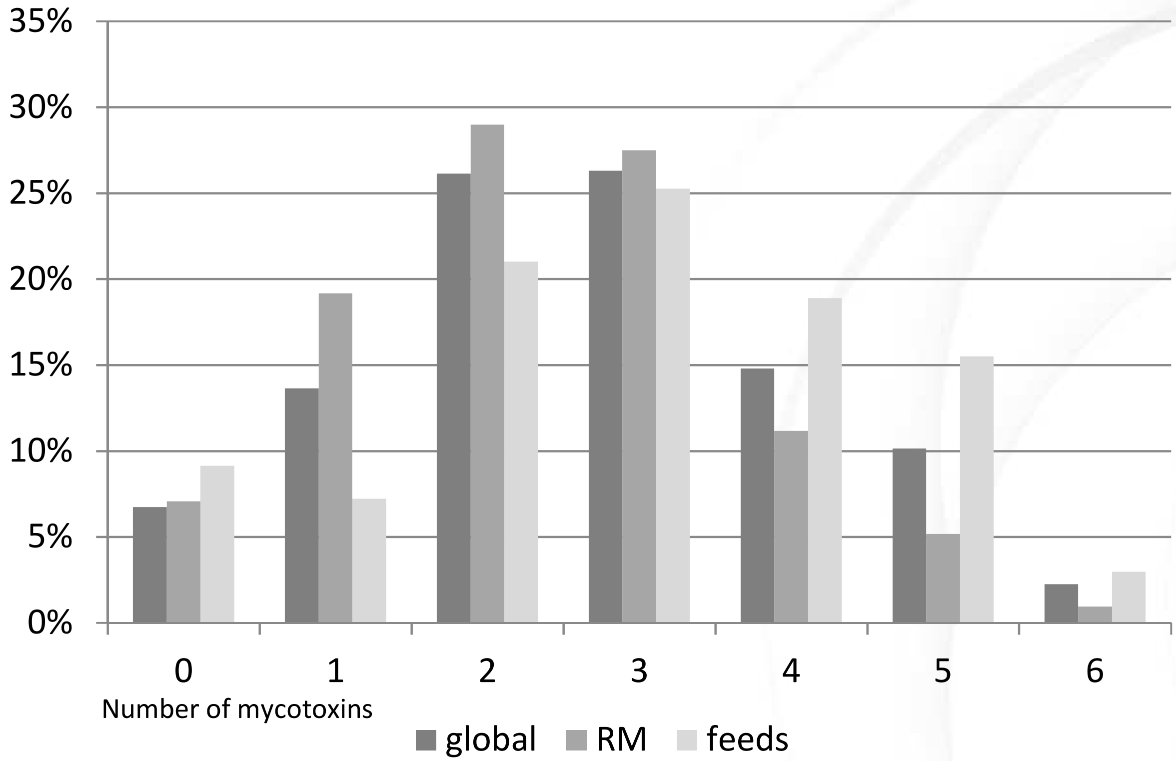
- species
- age
- sex
- nutritional status
- dose and duration of exposure
- environmental factors.

Synergistic	Additive
Aflatoxin-T-2	Aflatoxin-DON
DON -T-2	Fumonisin-T-2
Aflatoxin-Ochratoxin	Ochratoxin-T2
Fumonisin-Ochratoxin	

The effects and types of interactions can vary according to the measured parameters.

Mycotoxins	Effect	Affected parameters	Possible mode of action
AB1+DON	Additive	Relative liver weight, BWG, AST	Inhibition of protein synthesis, apoptosis
AF+FUM	Additive	Immune response	
AB1+OTA	Synergy	FCR, BWG, mortality, relative kidney and liver weights, albumin, GGT and CK, abnormalities in embryos	Production of reactive oxygen species, inhibition of protein synthesis
AB1+OTA	Additive	Egg production, embryo mortality	
DON+ZEA	Additive	Egg production, eggshell thickness, embryonic mortality	Inhibition of protein synthesis, interference with estrogen receptors
DON+T2	Synergy	Reduction in villi height in both the duodenum and jejunum	Inhibition of protein synthesis
OTA+T2	Additive	Decreased body weight, food intake, bone ash content and serum GGT activity	Production of reactive oxygen species, inhibition of protein synthesis

Mycotoxins - interactions



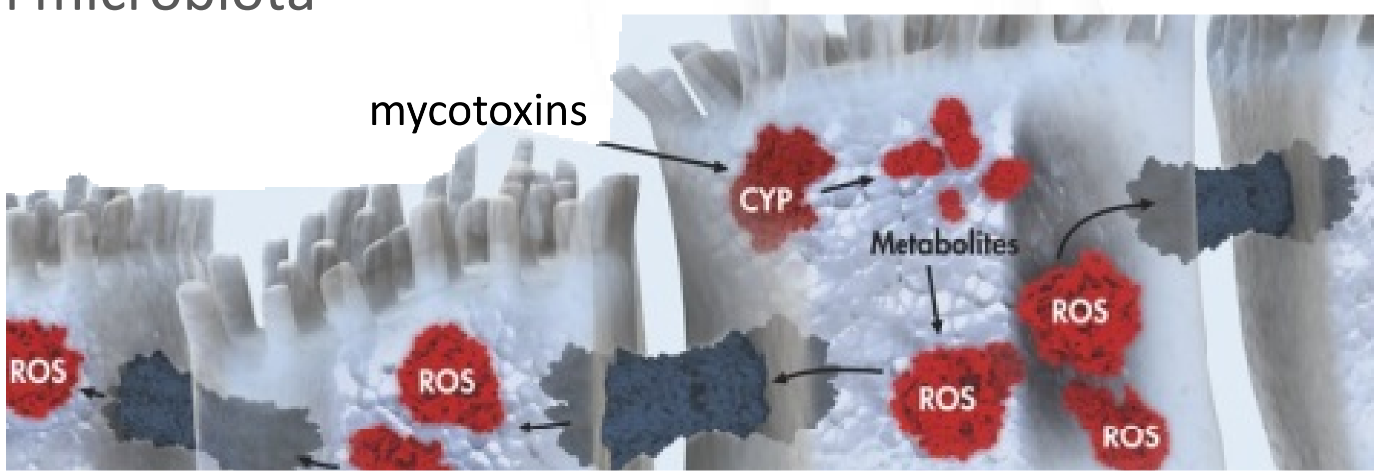
% of samples with none, single and multi-mycotoxin contamination worldwide 2015-2016
n= 1750 – samples were tested for aflatoxin, DON, fumonisin, ochratoxin, T-2 and zearalenone

EW Nutriton 2015-2016.

Mechanisms of action

- Decrease in protein synthesis
  - ↓ cell proliferation
  - ↓ intestinal microvilli height and regeneration
  - ↓ differentiation of intestine epithelial cells

- Increased oxidative stress
  - ↑ damage to cell membranes
  - ↓ intestinal microbiota



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

Mycotoxins – interactions with the GIT

Mycotoxins – interactions with the GIT

The gastro-intestinal epithelial cell layer is first exposed to mycotoxins

- The mucosal immunity -innate and adaptive- can be affected mycotoxins
- Mycotoxins exacerbate infections with parasites, bacteria and viruses
  - coccidiosis
  - necrotic enteritis
  - colibacillosis
  - Salmonellosis

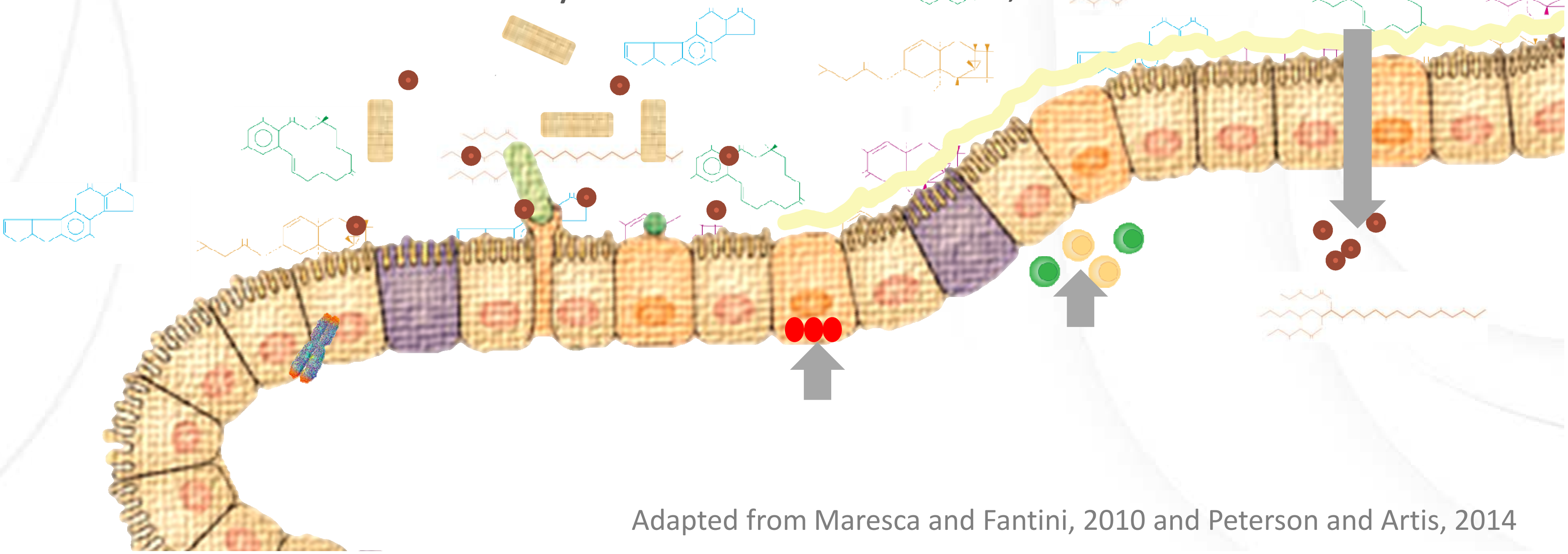
Mycotoxins play an important role in the balance of intestinal health in animal production.

Coccidiosis

Fusarium mycotoxins decrease CD4+ and CD8+ T-cells in jejunal mucosa, interfere with other chemical messengers that regulate immunity

Mycotoxins affect intestinal morphology (villus height and area)

- Impair immune response against *Eimeria*
- Interfere with recovery from coccidial infection
- Increase of the trans-epithelial passage of antigens
- Interfere with recovery from coccidial infection,



Adapted from Maresca and Fantini, 2010 and Peterson and Artis, 2014

Necrotic enteritis

- Any agent capable of causing a disruption of the gastrointestinal epithelium -among them mycotoxins and coccidiosis- promotes the development of necrotic enteritis.

- The inhibition of the intestinal immune system potentially caused by mycotoxins such also promotes the development of necrotic enteritis.

- This decreases absorption of nutrients and leaves higher intestinal protein amounts available for clostridia proliferation.

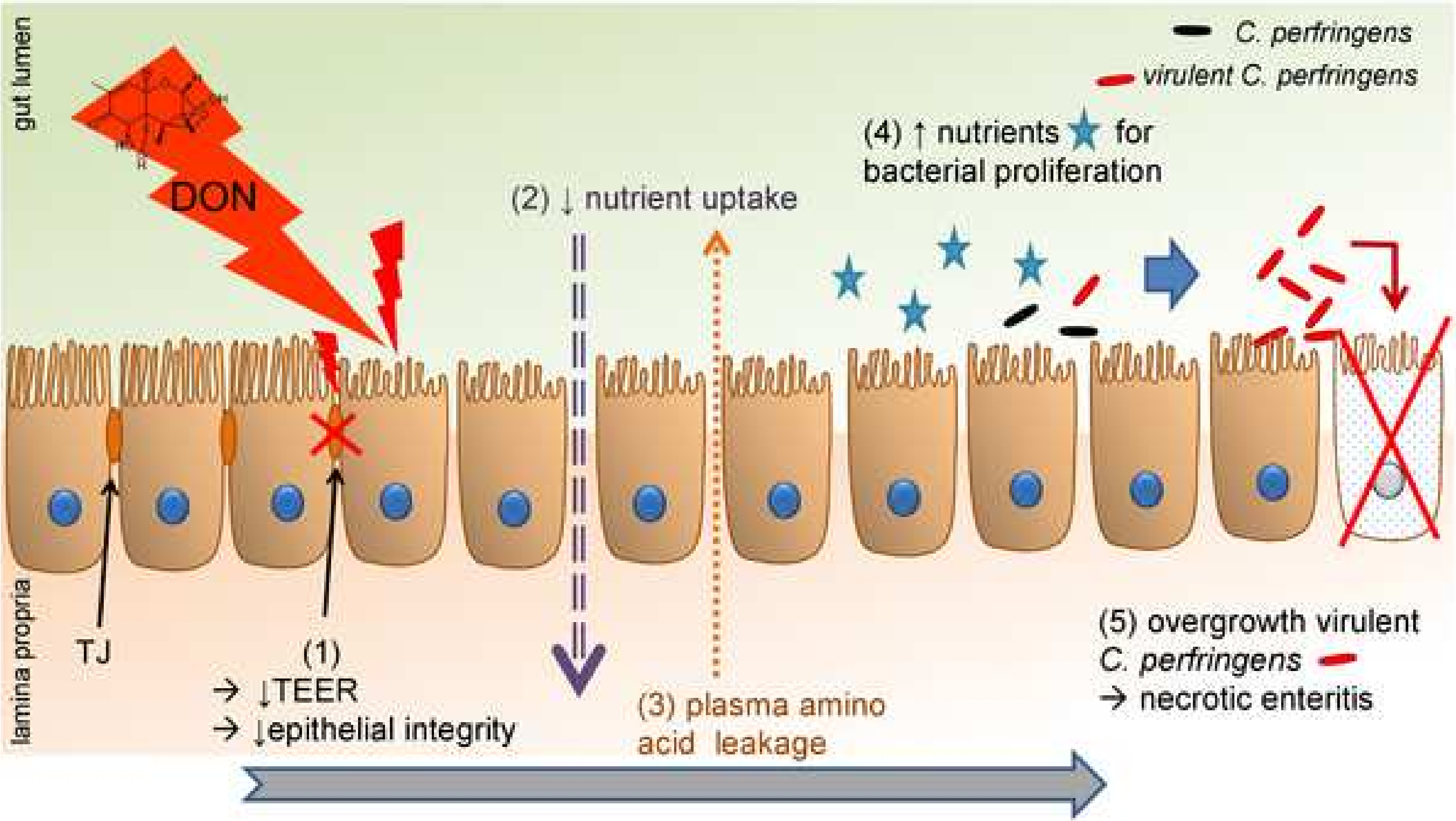
Necrotic enteritis

- Today its subclinical form is prevalent.
  - No clinical signs
  - No peak mortality.

- Chronic intestinal mucosal damage
  - Poor digestion and absorption
  - Production losses
  - Reduced weight gain
  - Increased feed-conversion

.

Necrotic enteritis



Antonissen G, Van Immerseel F, Pasmans F, Ducatelle R, Haesebrouck F, et al. (2014) The Mycotoxin Deoxynivalenol Predisposes for the Development of Clostridium perfringens-Induced Necrotic Enteritis in Broiler Chickens. PLOS ONE 9(9): e108775. doi:10.1371/journal.pone.0108775
http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0108775

The intestinal damage allow bacteria to reach the bile duct and portal blood stream.

- Colonization of the liver *C. perfringens*
- Cholangiohepatitis.

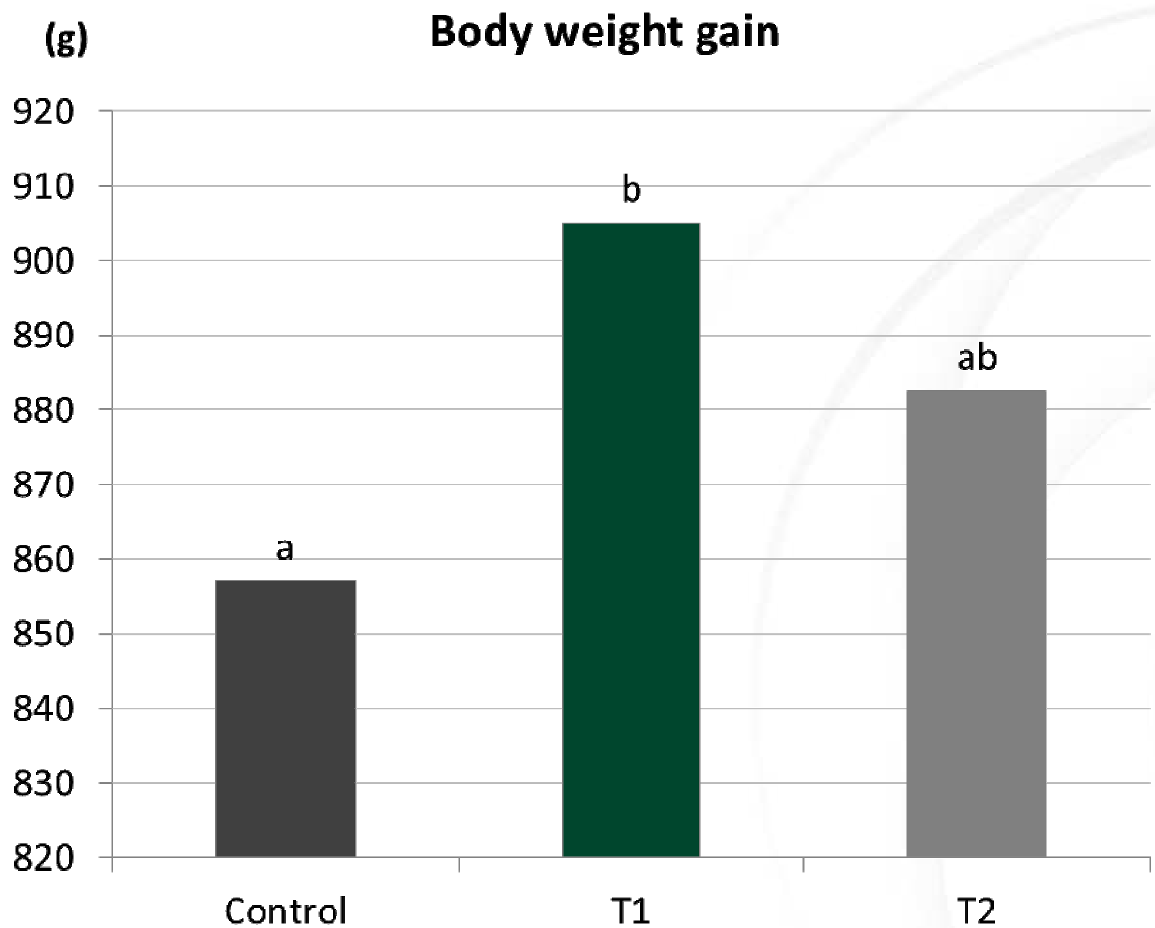
Liver lesions found at slaughter

No sign of clinical disease in the flock.

Subclinical necrosis causes great economic losses in the poultry industry
-undetected disease
-untreated animals

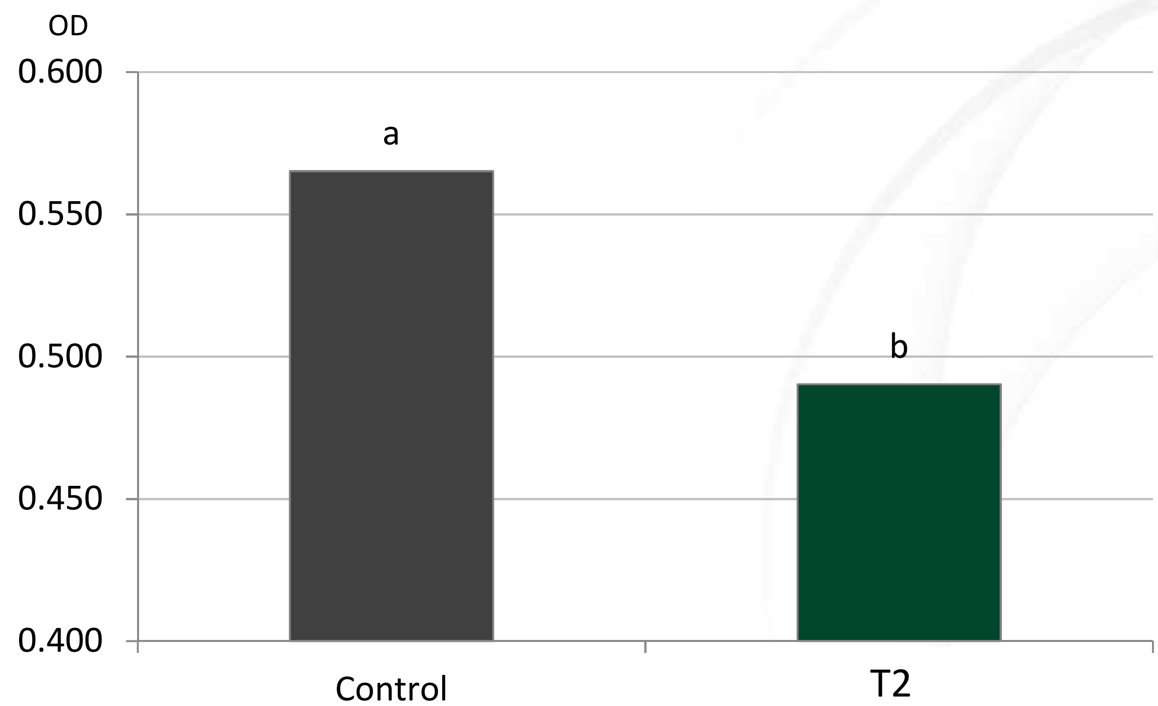
Necrotic enteritis

Trial facility:	**EW-Nutrition, Japan**
Trial Duration:	21 days
Animals:	Cobb 500
Sex:	male
Treatments:	Control: Standard feed + Challenge
	T1: Feed + Challenge + anti-toxin 1– 1 Kg/ton
	T2: Feed + Challenge + anti-toxin 2– 1 Kg/ton
Challenge model:	*C. perfringens* type A culture broth at 3 and 4 days 10^8 CFU/1mL/broiler
N˚ of animals	
Total:	30 broilers
Per group:	15 (1 replica per treatment)



Body weight gain of broilers at 21 days of age under a *C. perfringens* challenge and diets without anti-toxin additives and two anti-toxin products
P>0,05

Necrotic enteritis



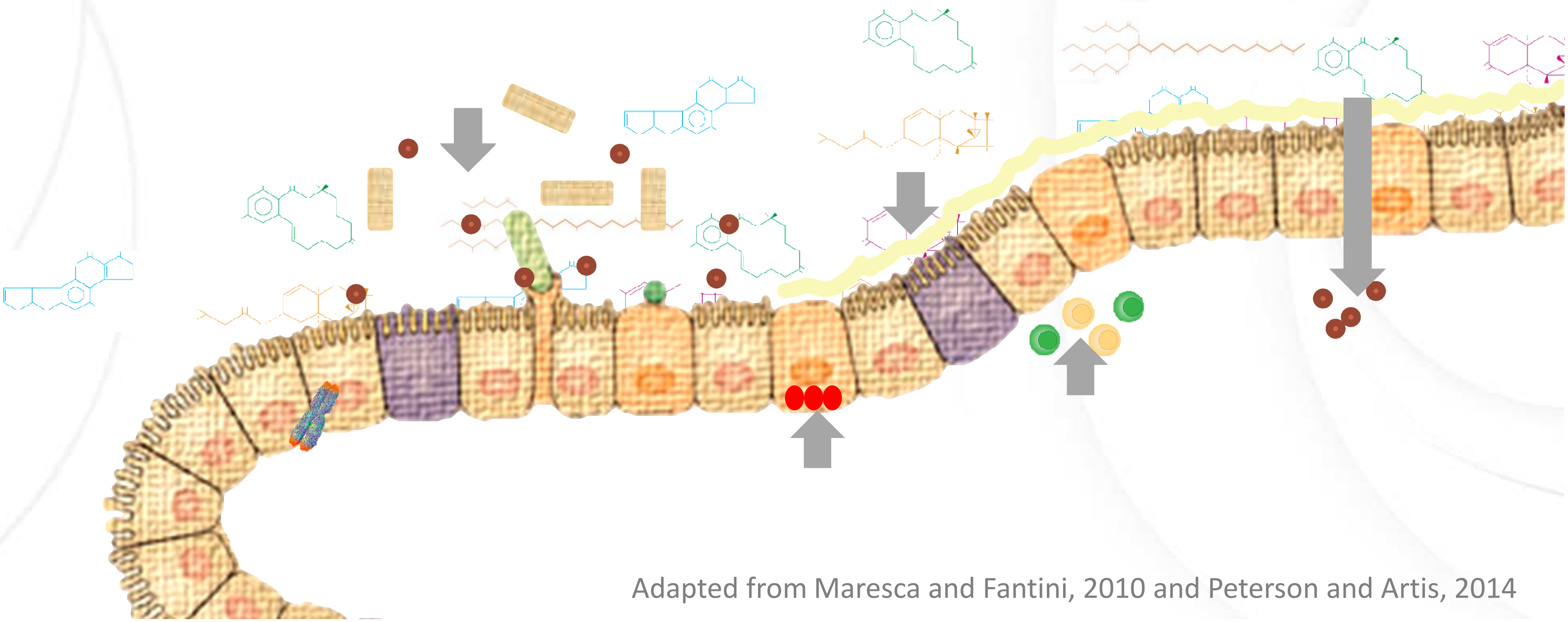
C. Perfringens alpha toxin antibody (IgG) in the serum of broilers at 21 days of age under a *C. perfringens* challenge and diets with and without anti-toxin products.
P>0,05

Aflatoxins have immune-inhibitory activity in the intestinal barrier

Predispose animals to various infectious diseases

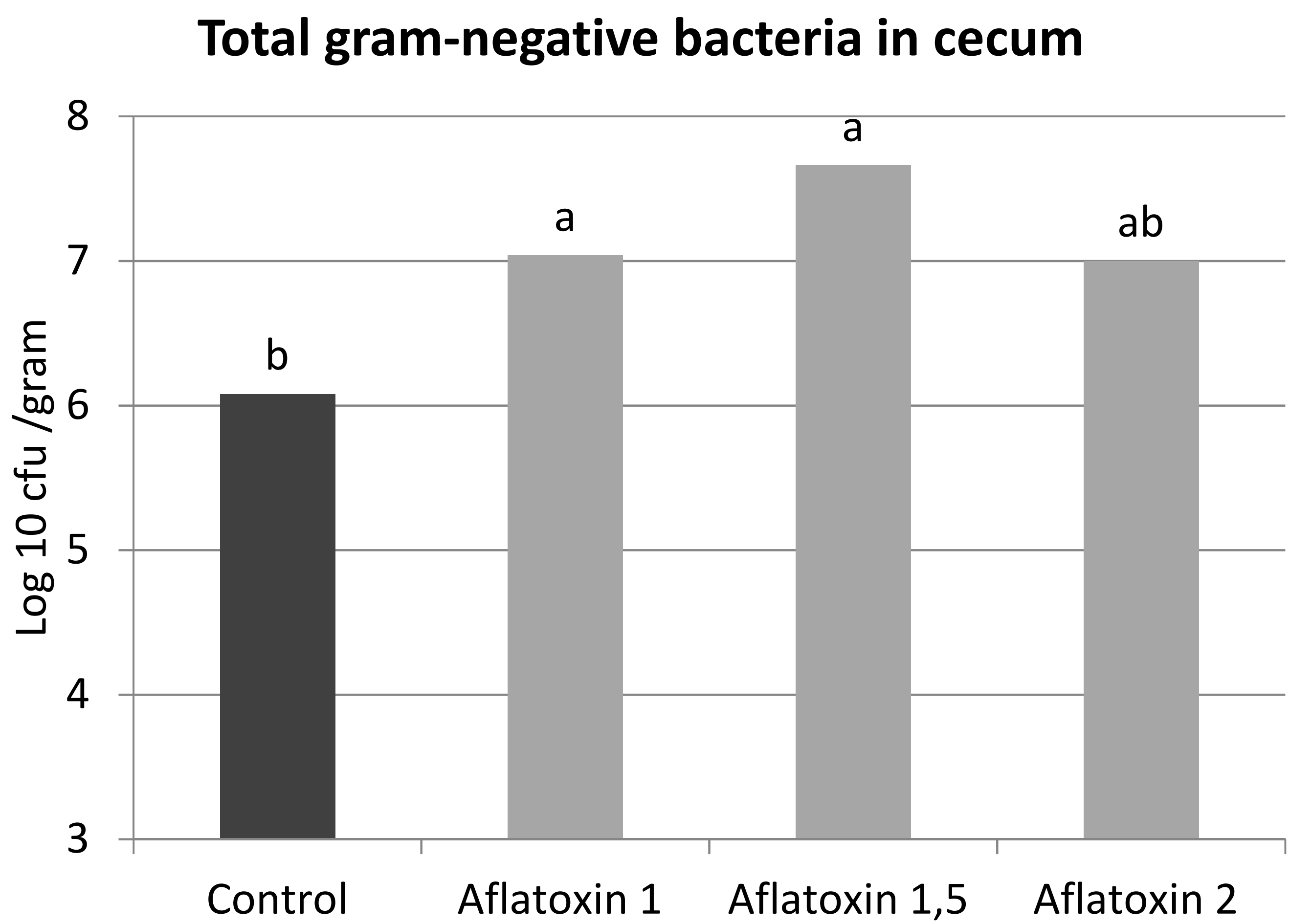
Damage the gut epithelium/barrier

Increase populations of *E. coli*, *Salmonella*, and total gram-negative bacteria



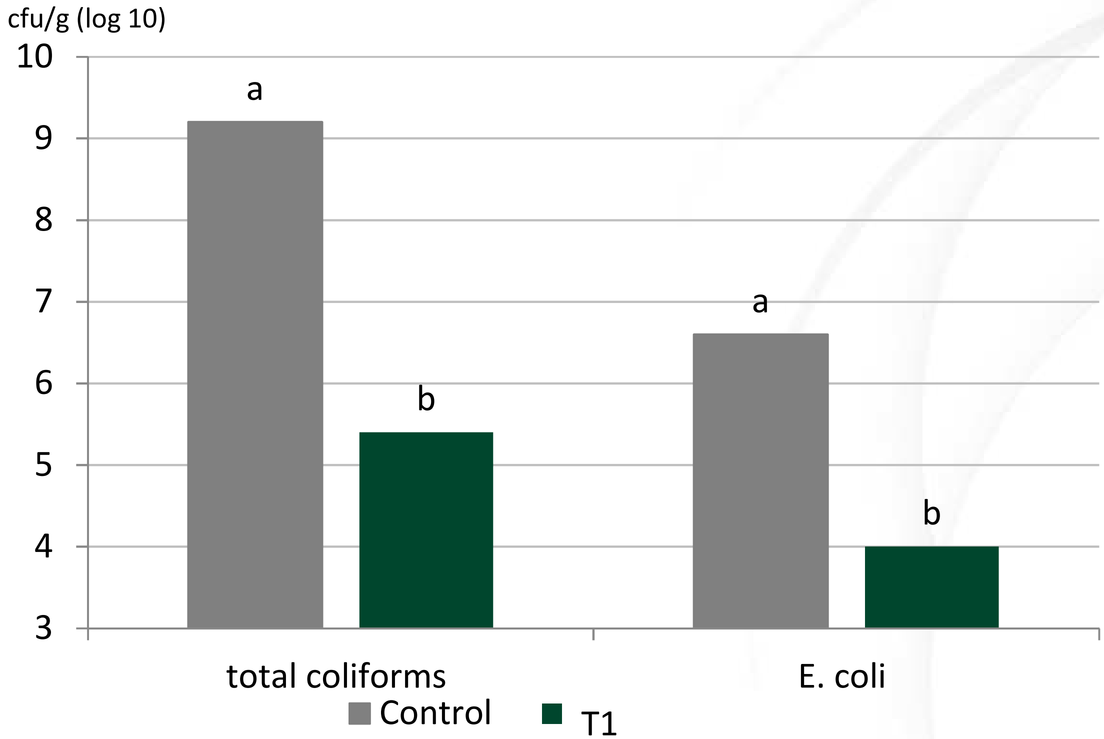
Adapted from Maresca and Fantini, 2010 and Peterson and Artis, 2014

E. coli



Galaraza et al 2016

E. coli



E. coli and total coliform count of cecal content of broilers at 42 days of age fed an AFB1 and AFB2 contaminated diet with and without anti-toxin agent.
P>0,05

EW Nutriton 2013.

- AFB1 can cause histopathological changes in small intestine

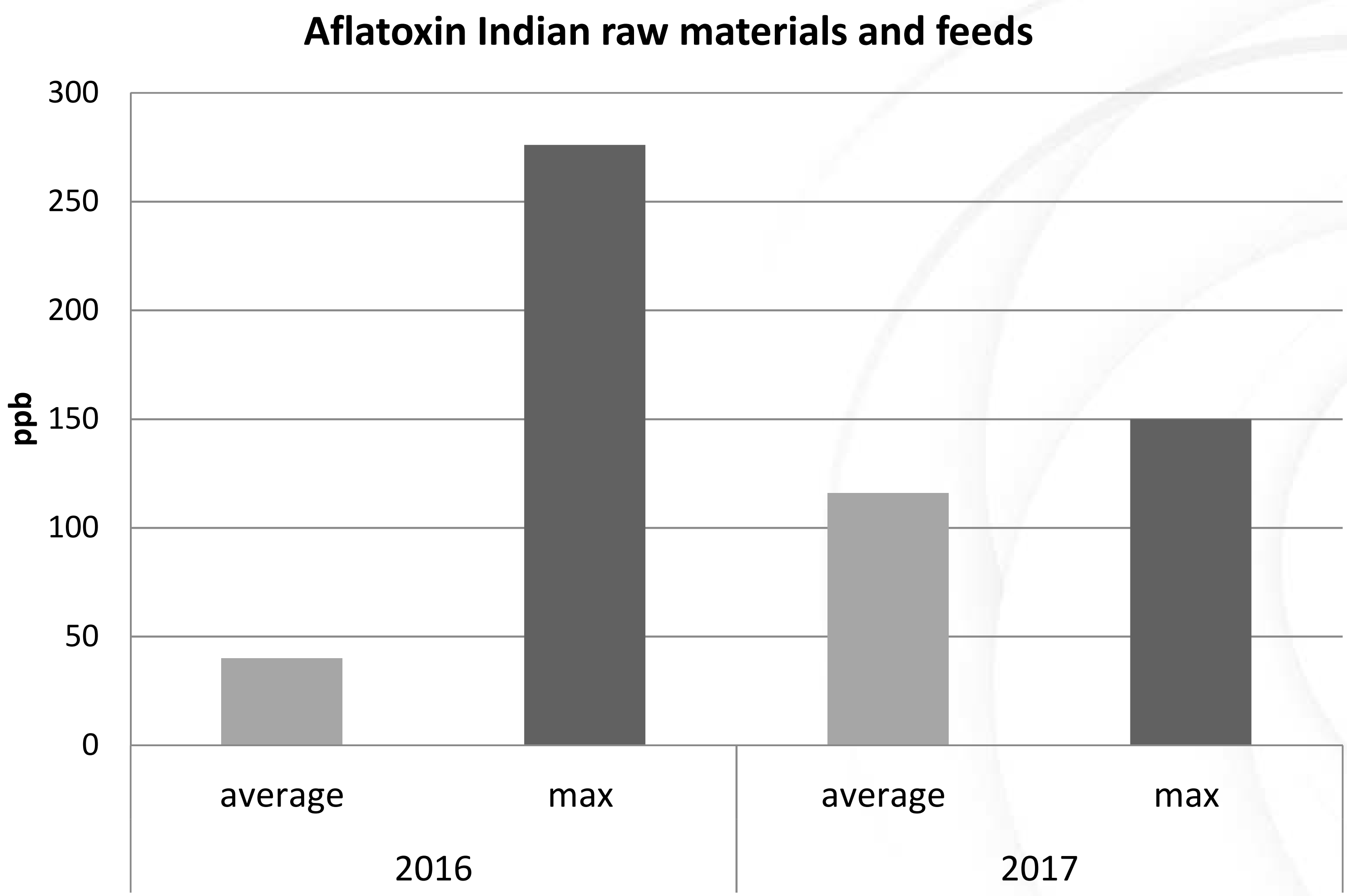
the duodenum is more affected

- Hemorrhages due to disturbances of the coagulation cascade
  - reduction of thromboplastin, prothrombin, fibrinogen and factors V, VII and X

- Damage in Lieberkühn glands cause reduced enzyme (saccharose and maltose) secretion.

- **Double exposure:** The digestive tract is the main route of excretion of AFB metabolites through the bile.

Aflatoxin


Aflatoxin Indian raw materials and feeds
300
250
200
150
100
50
0
ppb
average
max
average
max
2016
2017

Intestinal Health

ew | nutrition

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

- The maintenance of a healthy gastrointestinal tract is crucial:
  - ensures that the nutrients are absorbed at optimum rate
  - provides effective protection against pathogens through its own immune system, and
  - maintains the microflora in suitable proportions and numbers

- Risk assessment - preventive measures
  - limiting highly contaminated raw materials
  - using effective anti-mycotoxin additives

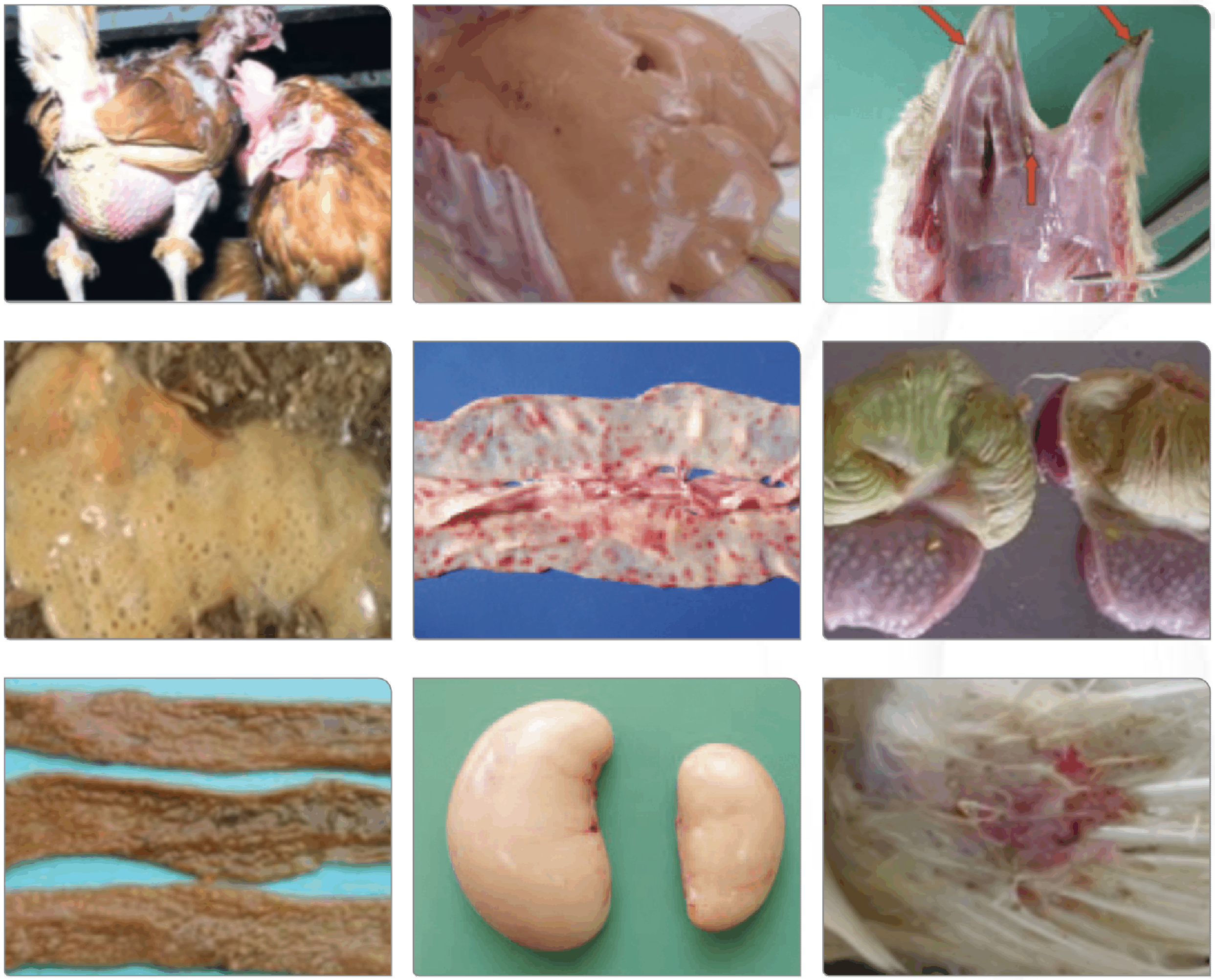
- Maintaining low levels of mycotoxins
- Promoting mycotoxin adsorption
  - avoids direct contact with the gastrointestinal epithelium
  - better health and productivity

Risk assessment – economic impact

- Direct losses related to effects on animal health
- Indirect influence of mycotoxins on animal health, by enhancing infectious diseases and secondary infections

- Impact of low to medium/moderate mycotoxin contamination levels.

Mycotoxins the past



Mycotoxins today

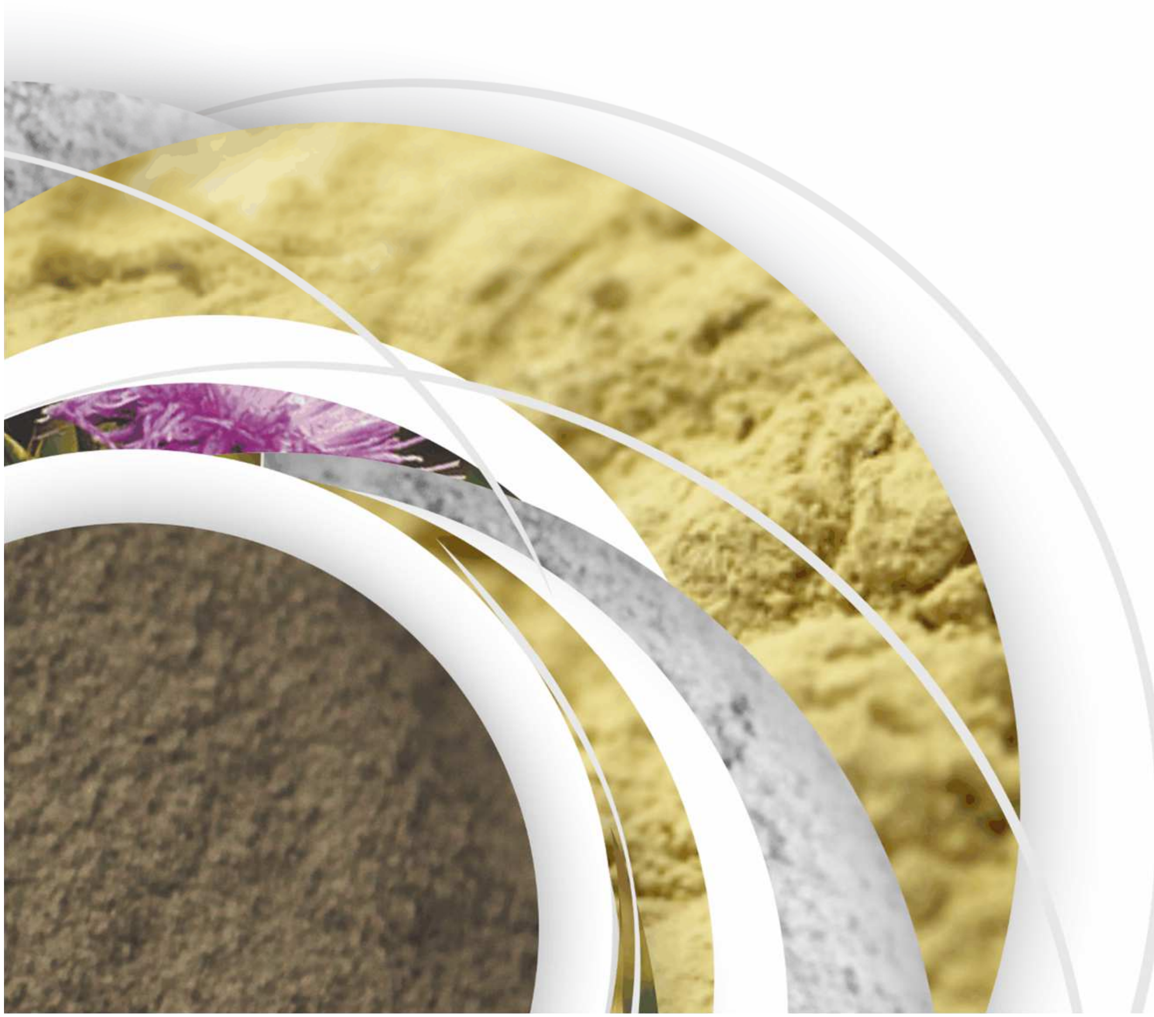
ew | nutrition

Have questions?
Want more information?
Risk assessment tool
Visit us today! A9 Hall C

For us, innovation is natural.
So are results.

GRASP®

ew | nutrition



Marisabel Caballero
Global Technical Manager Poultry
Product Manager Mastersorb®
Phone: +49 4445 9868136
Mobile: +49 173 1892897
marisabel.caballero@ew-nutrition.com

GRASP®