Mycotoxin challenge in aquaculture feed

Maarten Jay van Schoonhoven, Aqua Care Manager Olmix
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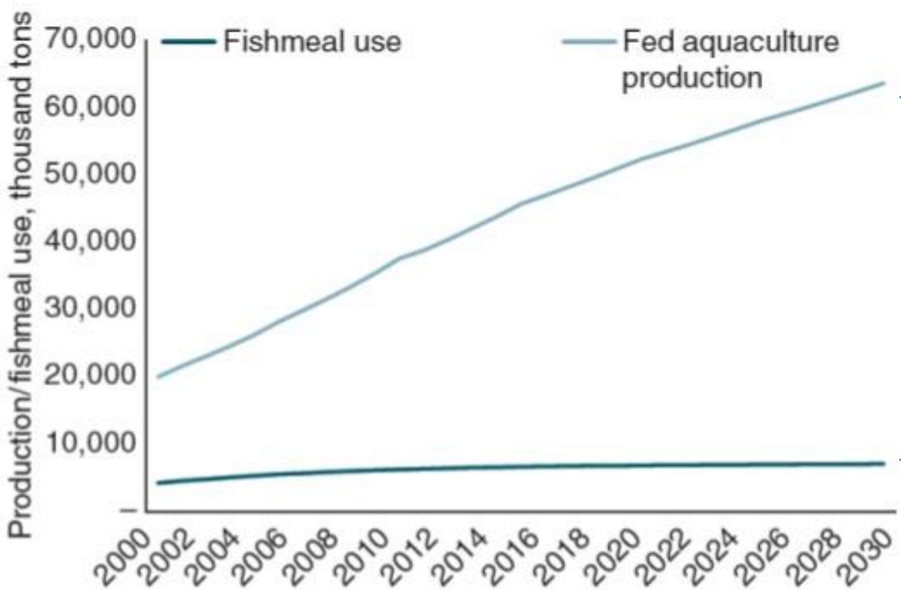
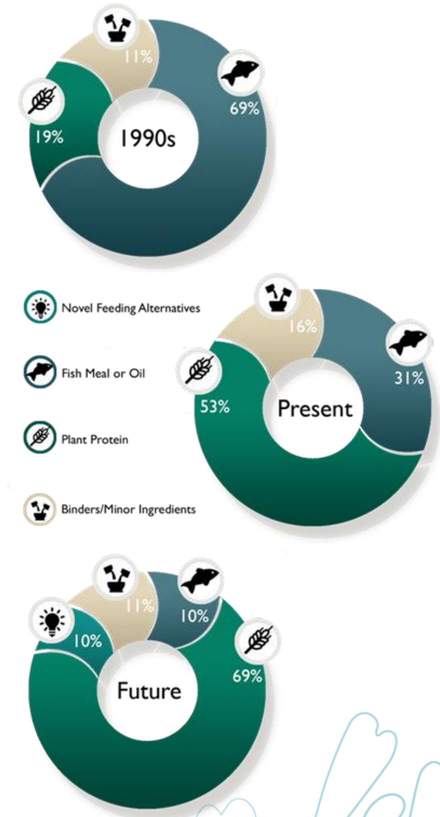
THE MYCOTOXIN CHALLENGE

FIGURE 3.7: Projected Production and Fishmeal Use in Global Fed Aquaculture



Source: IMPACT model projections.



Challenges of using plant ingredients

Proteins of plant origin:

i. Nutritional imbalance (e.g. methionine)
ii. Phosphorus availability (phytate)
iii. Higher carbohydrate fraction
iv. Palatability/attractability
v. Anti Nutritional Factors
    i. Mycotoxins

THE MYCOTOXIN CHALLENGE

EFFECT OF MYCOTOXINS IN AQUACULTURE

FIELD MYCOTOXINS

Fumonisins
Damage to hepatopancreas, immune depression, lower weight gain, lower blood hemoglobin value, lesions of liver, pancreas and kidney

Trichothecenes (DON, T2-HT2)
Feed refusal, lower weight gain, poor feed efficiency, increased FCR, decreased feed intake, lower blood hemoglobin value

STORAGE MYCOTOXINS

Aflatoxins
Liver carcinogenesis/necrosis, increased mortality, hepatopancreas damage, decreased growth, impaired blood clotting, anemia, pale to yellow kidney lesions

Ochratoxins
Poor feed efficiency, higher FCR , immune depression, lower survival, liver necrosis, lower growth rate and SGR

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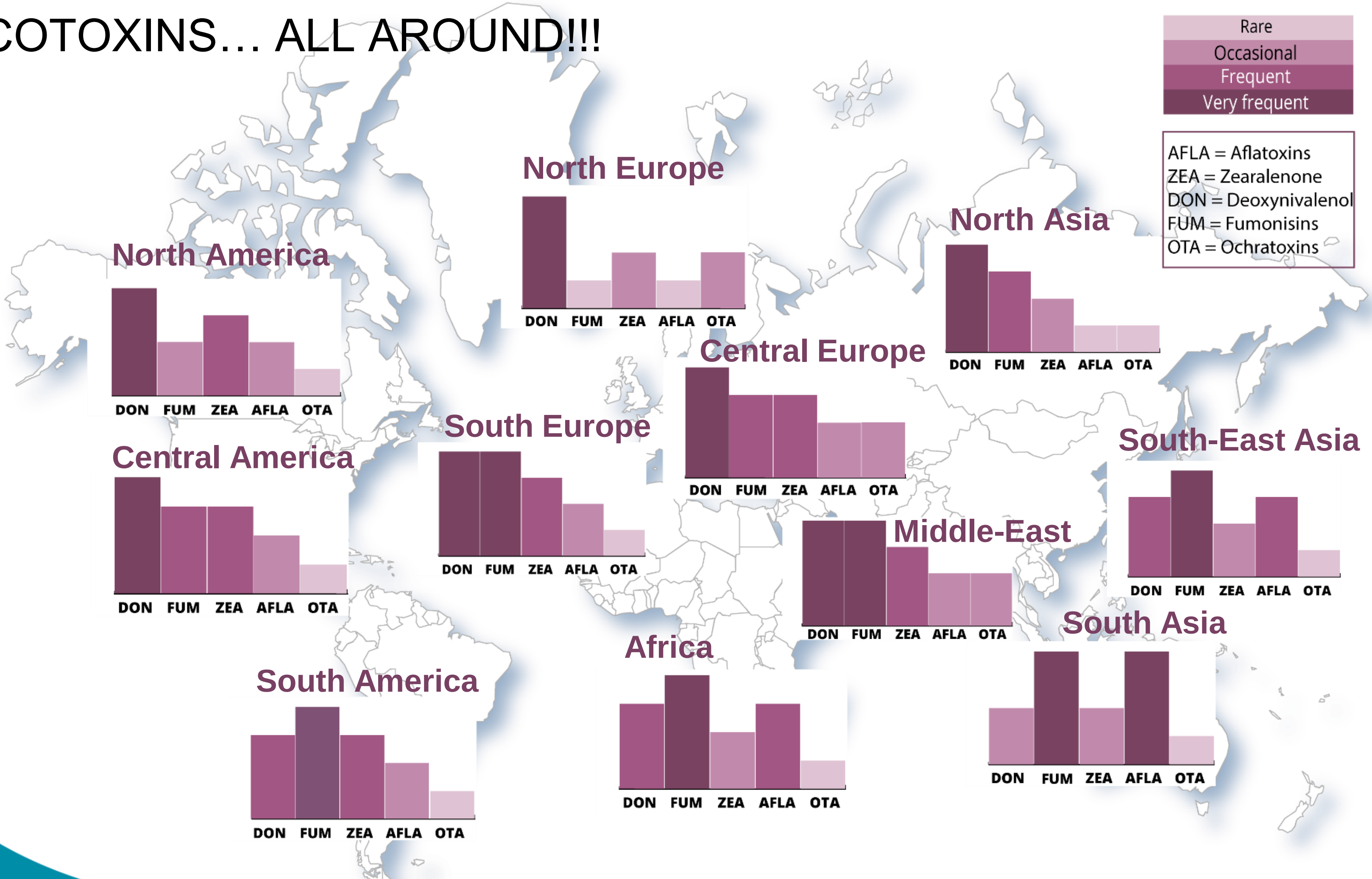
THE MYCOTOXIN CHALLENGE

MYCOTOXINS… ALL AROUND!!!



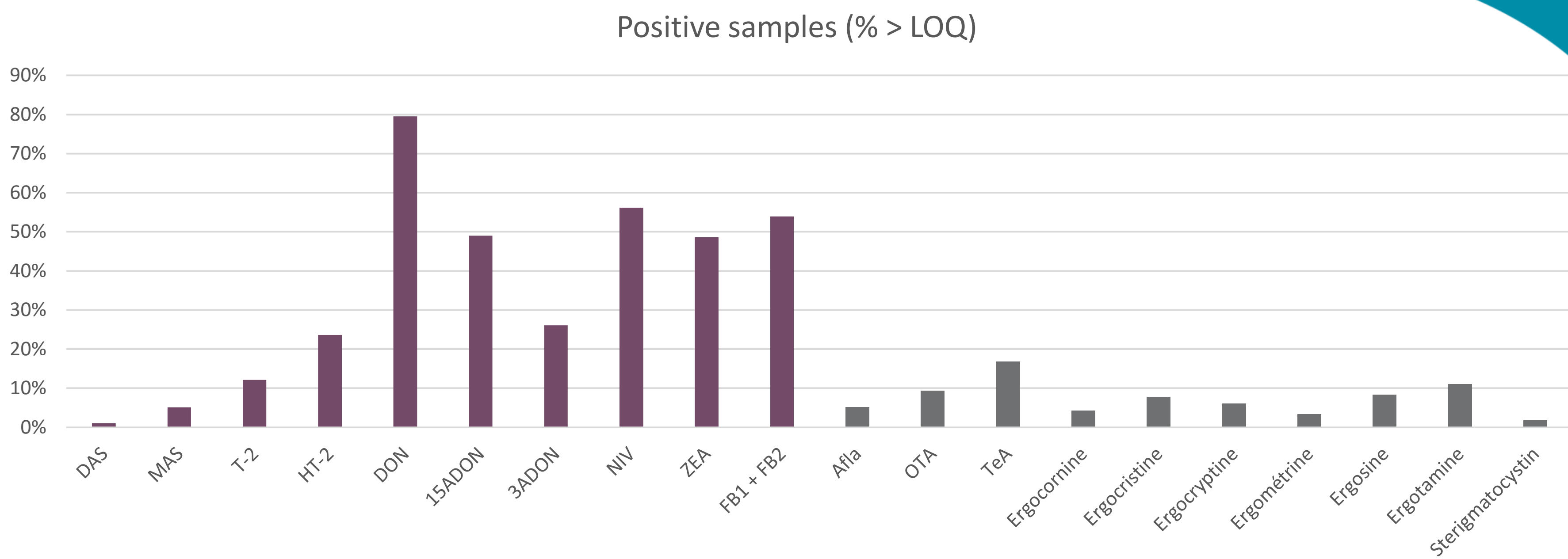
THE MYCOTOXIN CHALLENGE

MYCOTOXINS CONTAMINATION IN AQUAFEED INGREDIENTS

HPLC-MS/MS results from Olmix data base
(between 01/2013 to 31/2016)

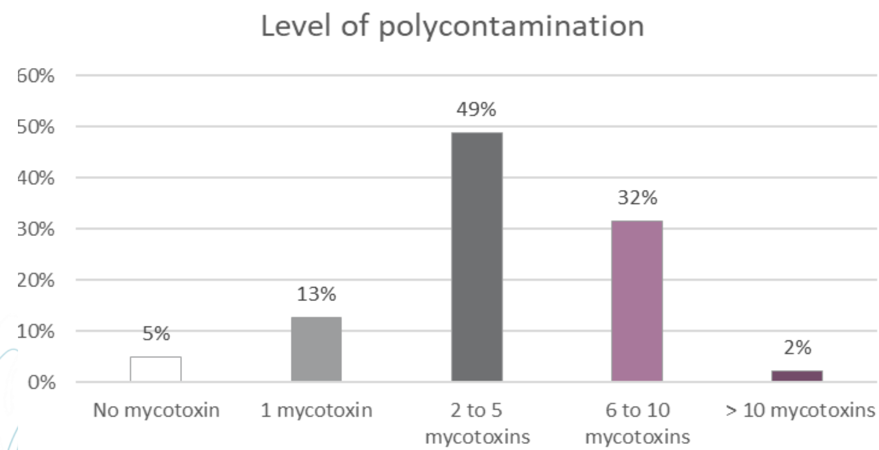
The ingredients were sampled in 42 countries
(China, Brazil, Ukraine, France, Spain, Turkey, etc.)

	n
Corn	784
Corn by-products	46
Wheat	503
Wheat by-products	81
Soya and SBM	110
Sunflower	16
Rapeseed	10
Cotton meal	2
Rice bran	1
TOTAL	**1553**



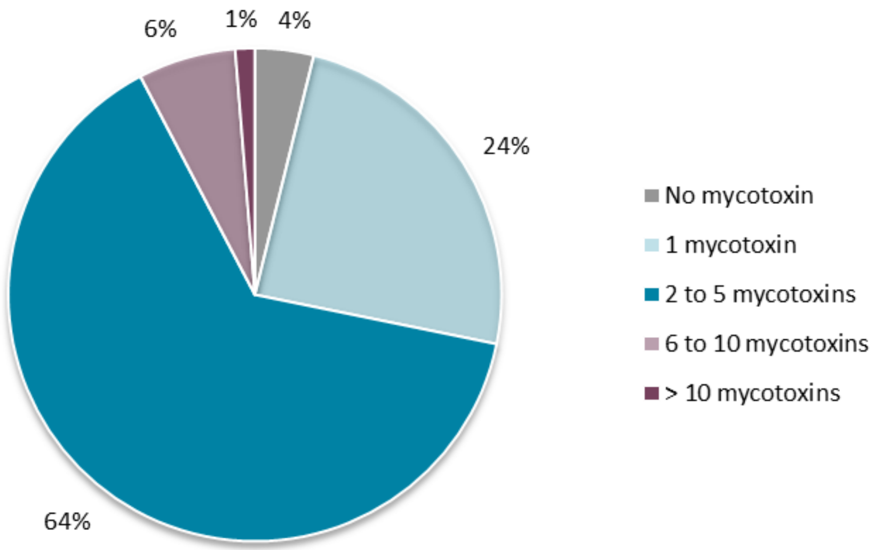
''Food processing affects mycotoxin distribution and concentration. Cereal processes concentrate mycotoxins into fractions that are commonly used as animal feed.''

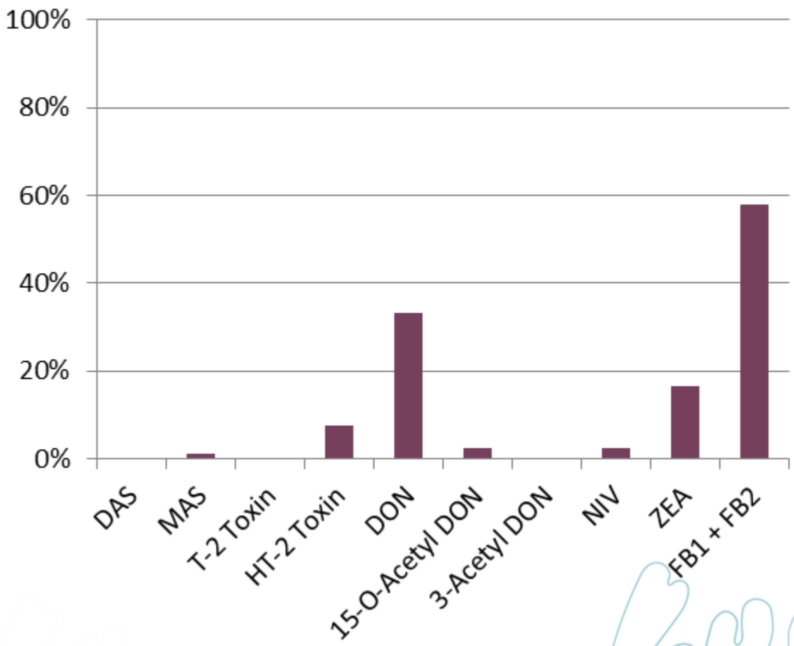
Pinotti et al. 2016



Plant ingredient mycotoxin contamination

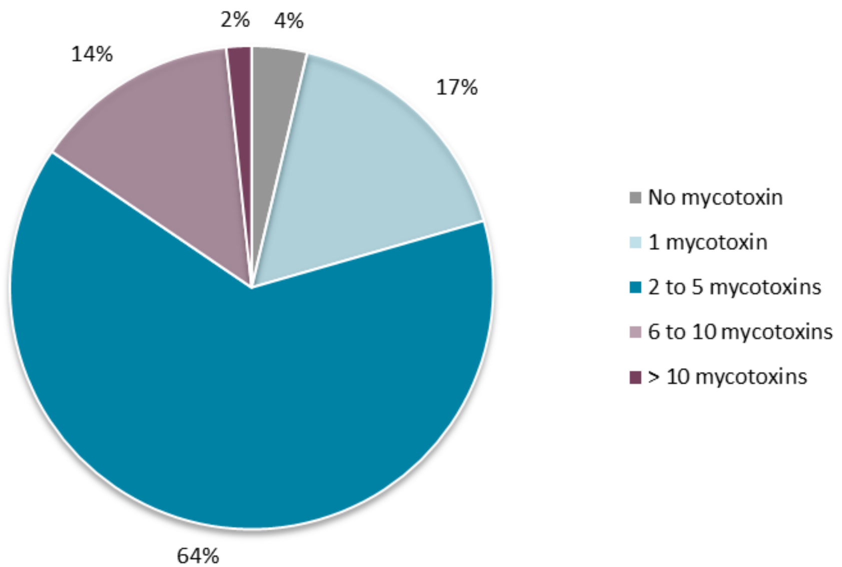
Soybean

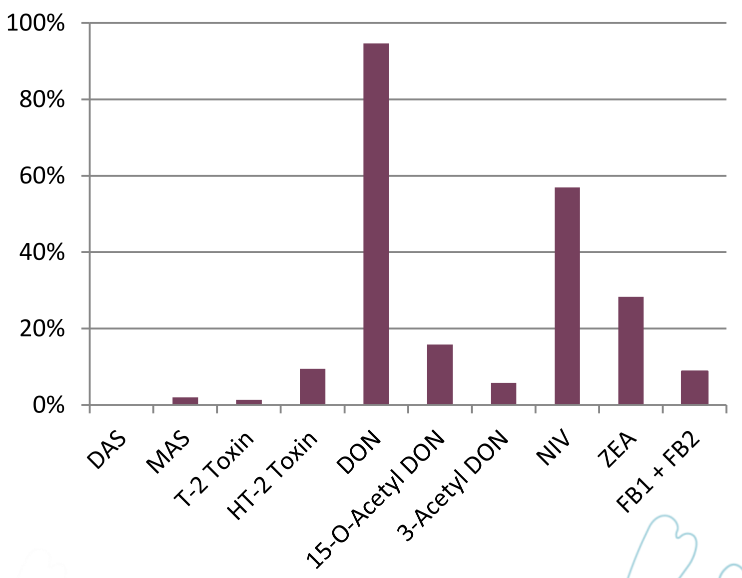




Plant ingredient mycotoxin contamination

Wheat





THE MYCOTOXIN CHALLENGE

REFLECTIONS

- Mycotoxins effects on aquaculture species are poorly studied.
  - Large variety species cultured, each with varying sensitivity
    - Cannot extrapolate from one to anothe
  - Lab vs field
  - Fish size variation
  - Synthetic vs natural

Species diversification in global aquaculture (2014)

Source: Junning Cai, 2017. FAO

Subacute vs acute mycotoxicosis!

Toxicity, biochemical effects and residue of aflatoxin B_1 in marine water-reared sea bass (*Dicentrarchus labrax* L.)

Yasser Said El-Sayed [a,*], Riad Hassan Khalil [b]

[a] *Department of Veterinary Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Alexandria University, Edfina, Rossetta-line, Behera Province 22758, Egypt*
[b] *Department of Avian and Aquatic Diseases, Faculty of Veterinary Medicine, Alexandria University, Egypt*

D. labrax
0.18mg/kg of bwt

Danio rerio
0.51mg/kg of bwt

O. mykiss
0.5 - 1mg/kg of bwt

O. niloticus
1 – 1.3mg/kg of bwt

I. punctatus
11.5mg/kg of bwt

There is variety of sensitivity between fish species.

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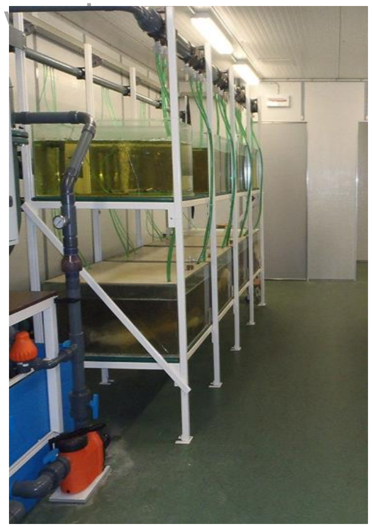

Subacute vs acute mycotoxicosis!

Growth and hepatic lesions of Nile tilapia (*Oreochromis niloticus*) fed diets containing aflatoxin B_1

Nguyen Anh Tuan [a,1], John M. Grizzle [a,*], Richard T. Lovell [a], Bruce B. Manning [a,2], George E. Rottinghaus [b]

Table 3

Final weight, aflatoxin B_1 (AFB) consumption, feed conversion ratio (FCR), hematocrit, and survival of Nile tilapia fed diets containing different concentrations of AFB for 8 weeks

AFB in diet (mg/kg)	Final weight (g)	AFB consumption (mg/kg body weight)	FCR	Hematocrit (%)	Survival (%)
0	41.0[a]	0	1.31[a]	38[a]	100[a]
0.25	40.3[a]	0.3[a]	1.34[a]	35[a]	100[a]
2.5	21.4[b]	5.3[b]	2.43[b]	26[b]	97[a]
10	5.9[c]	17.3[c]	3.24[c]	20[c]	97[a]
100	2.7[d]	59.4[d]		12[d]	40[b]

Initial weight of fish averaged 2.7 g. Values in the same column with different letters are significantly different ($P < 0.05$).

THE MYCOTOXIN CHALLENGE
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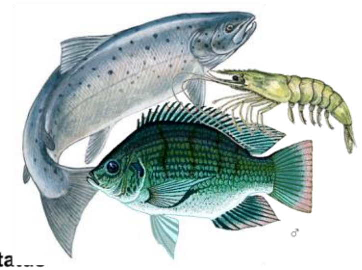

Subacute vs acute mycotoxicosis!

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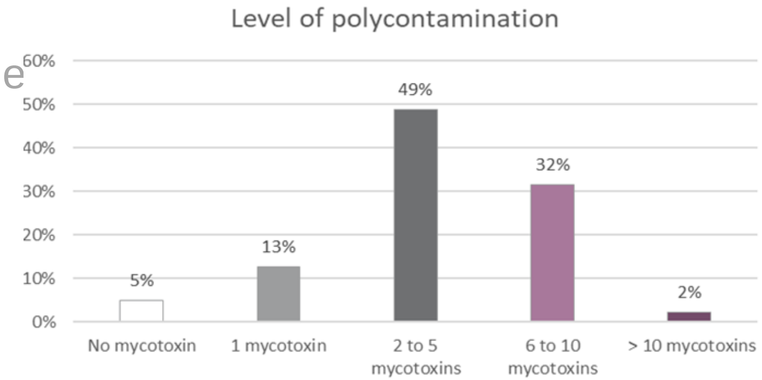
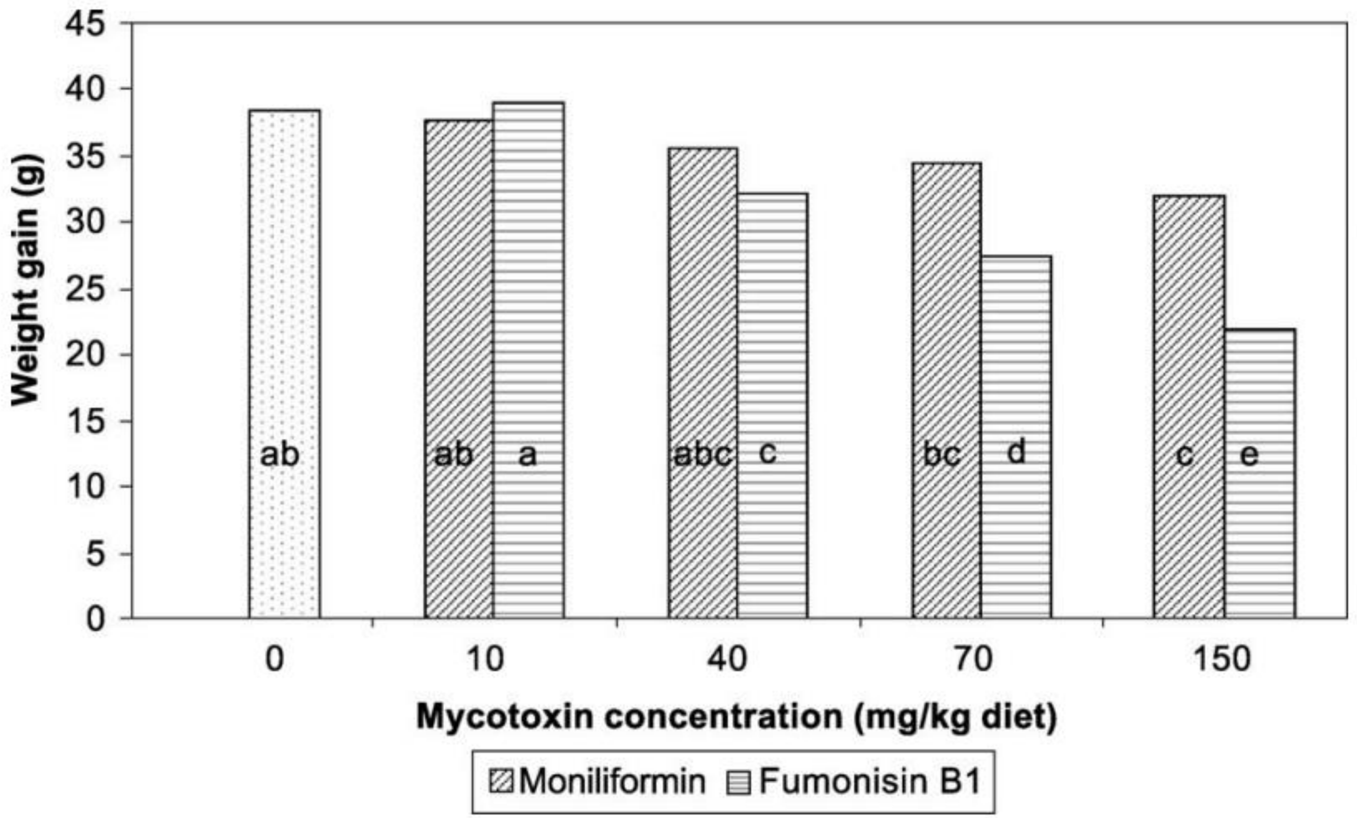


Subacute vs acute mycotoxicosis!

Responses of Nile tilapia (*Oreochromis niloticus*) fed diets containing different concentrations of moniliformin or fumonisin B_1

Nguyen A. Tuan [a], Bruce B. Manning [a,*], Richard T. Lovell [a], George E. Rottinghaus [b]



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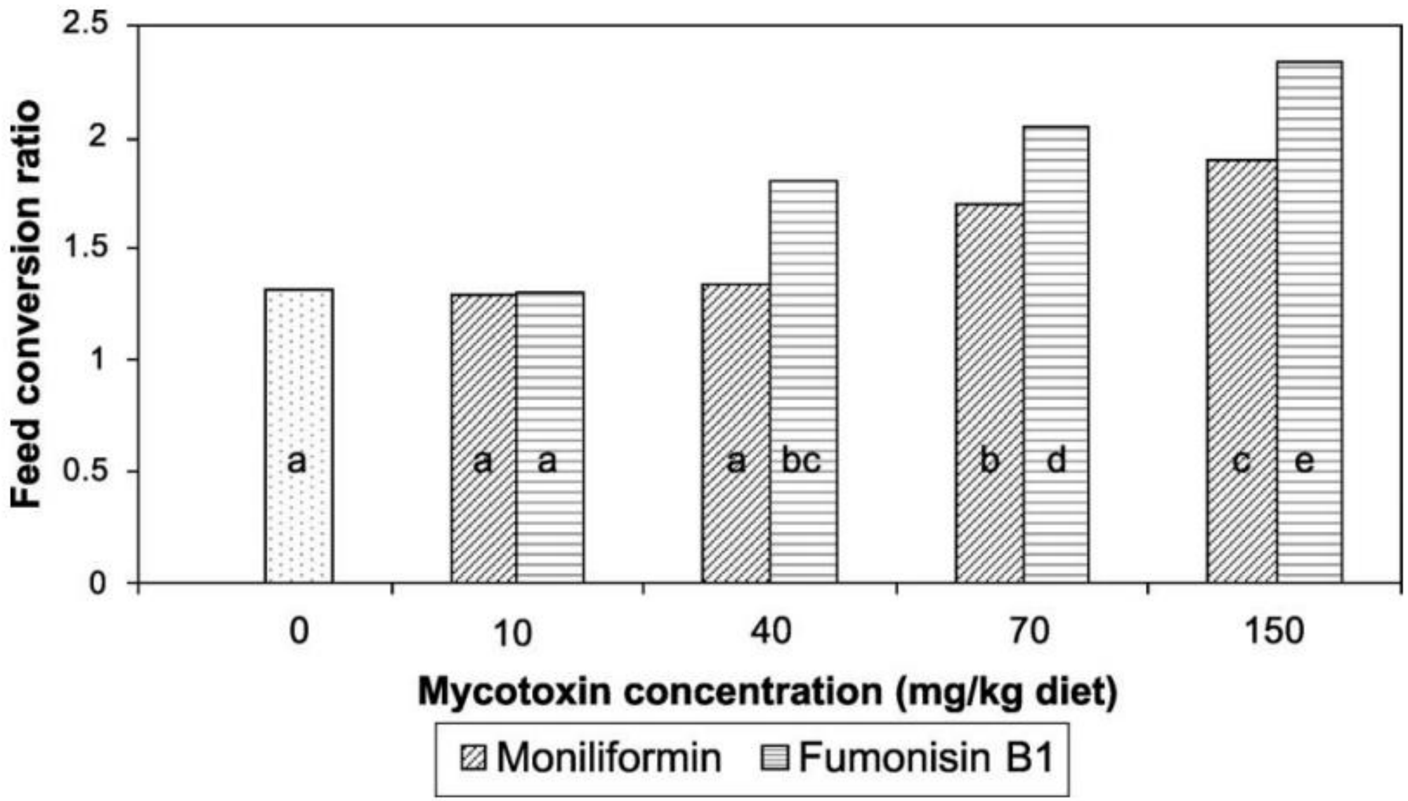
Nguyen A. Tuan [a], Bruce B. Manning [a,*], Richard T. Lovell [a], George E. Rottinghaus [b]



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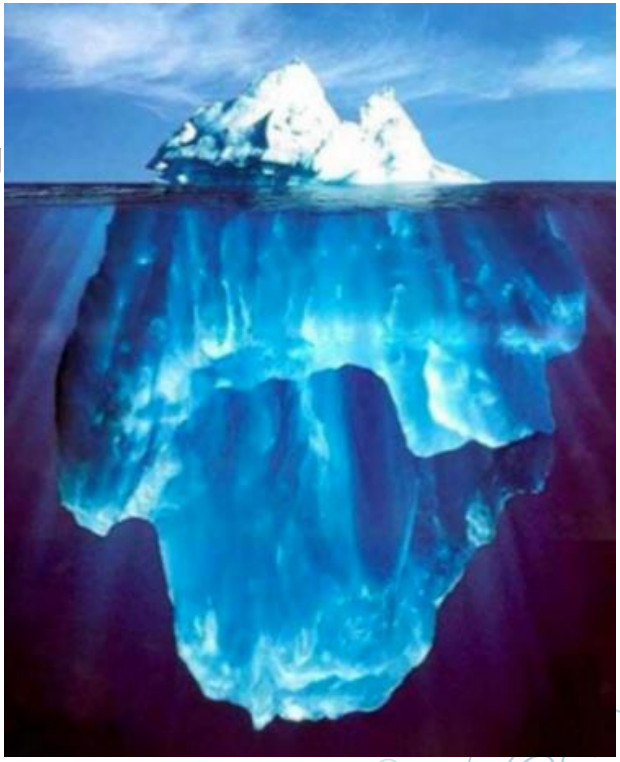

Subacute mycotoxicosis is now widely considered to be the most important impact of mycotoxins, particularly in developing countries. (FAO, 2001)

Subacute vs acute mycotoxicosis!

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THE MYCOTOXIN CHALLENGE

Acute toxicity of ochratoxin-A in marine water-reared sea bass (*Dicentrarchus labrax* L.)

Yasser Said El-Sayed [a,*], Riad Hassan Khalil [b], Talaat Talaat Saad [b]

[a] Department of Veterinary Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Alexandria University, Edfina, Rossetta-line, Behera Province, Egypt
[b] Department of Avian and Aquatic Diseases, Faculty of Veterinary Medicine, Alexandria University, Egypt

In this article, Dr El-Sayed shows this figure:

- The results indicate fast absorption and rapid onset of the toxic effect of OTA.
- Bellow 0.15ppm, behavioural changed and lesions were observed due to the potent acute neurotoxic and oxidative damaging effects of OTA.
- **At a farm level where fish are exposed to the open sea, these symptoms,** sluggish movements, fin erosions and internal lesions, **are not specific to mycotoxins which can make mycotoxicosis difficult to diagnose.**

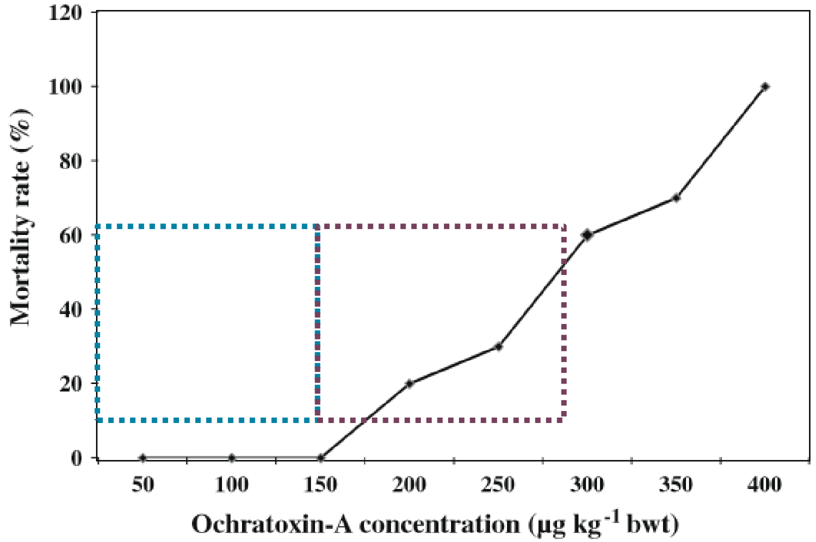
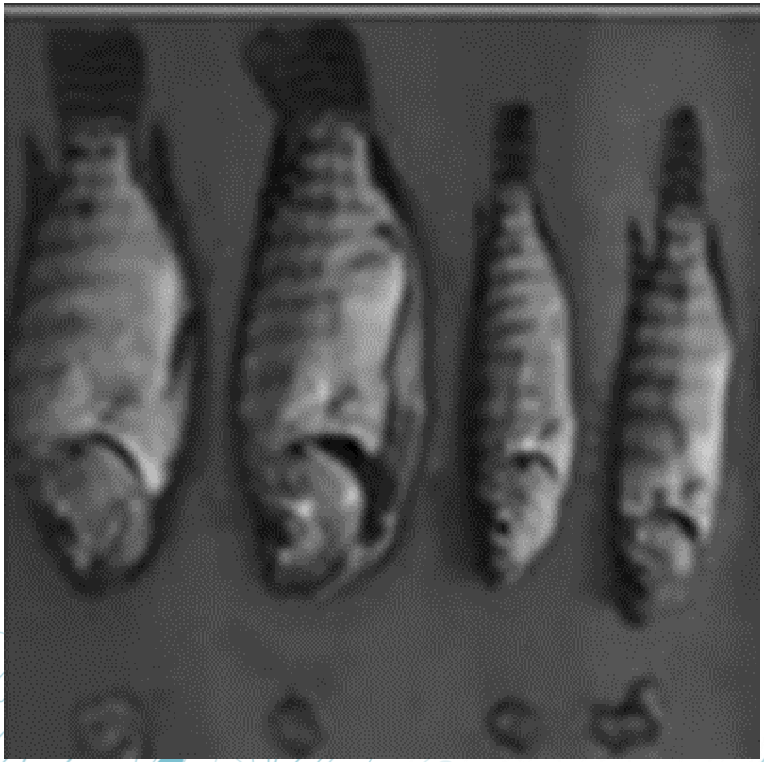


Fig. 2. The relation between the ochratoxin-A concentrations and the mortality rate of adult sea bass according to Finney's Probit Analysis (EPA, 1999). The axes are given in nearly a linear scale and not yield the sigmoid type relation.

THE MYCOTOXIN CHALLENGE



Control 5ppm FUM

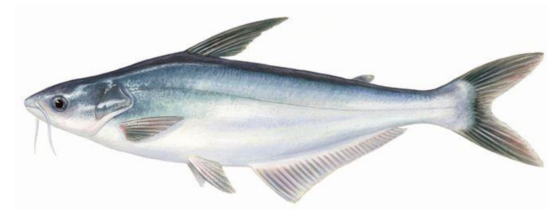
Samitec, Brasil

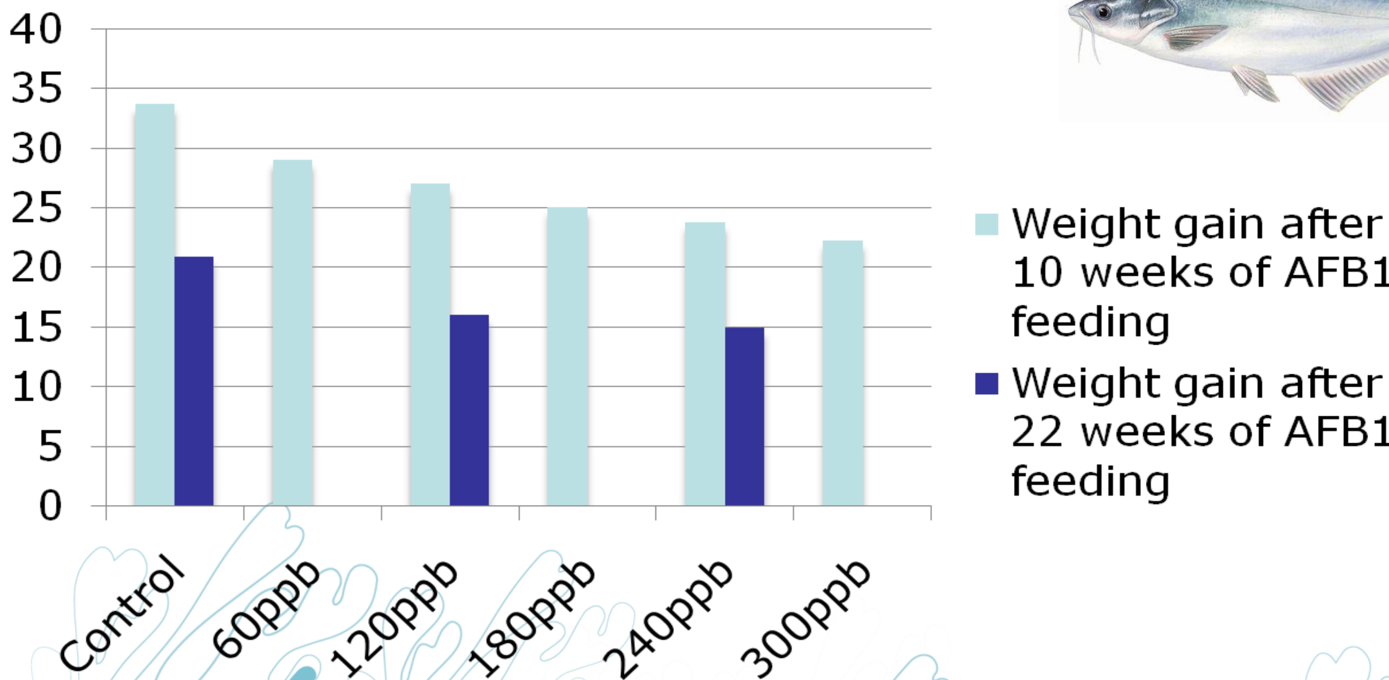
Conditions:

- 21 days
- 5ppm FUM

THE MYCOTOXIN CHALLENGE

SPECIES DURATION EXPOSURE SENSITIVITY TO SINGLE MYCOTOXIN





Nguyen Tan Phuoc, 2003

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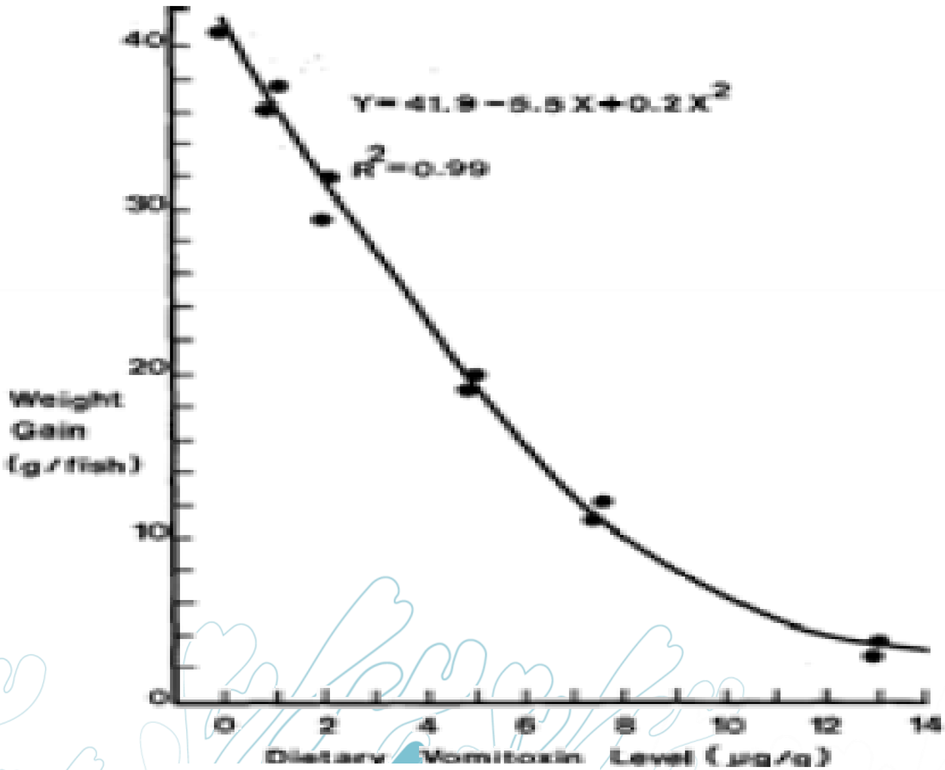
THE MYCOTOXIN CHALLENGE

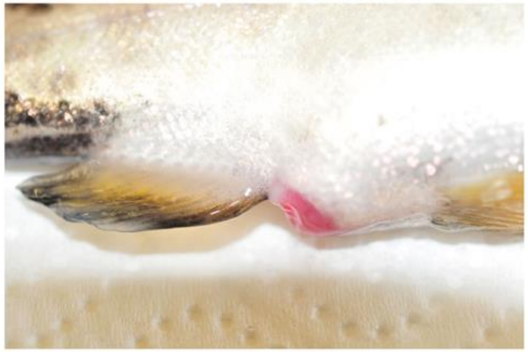
SPECIES SENSITIVITY TO A DOSE DEPENDANT SINGLE MYCOTOXIN



Dietary DON (ppb)

Figure 2. Trout fed 2.7 DON showing a slight hemorrhage in the abdominal cavity.

Figure 3. Trout fed 2.7 DON showing a rectal hemorrhage/irritation.



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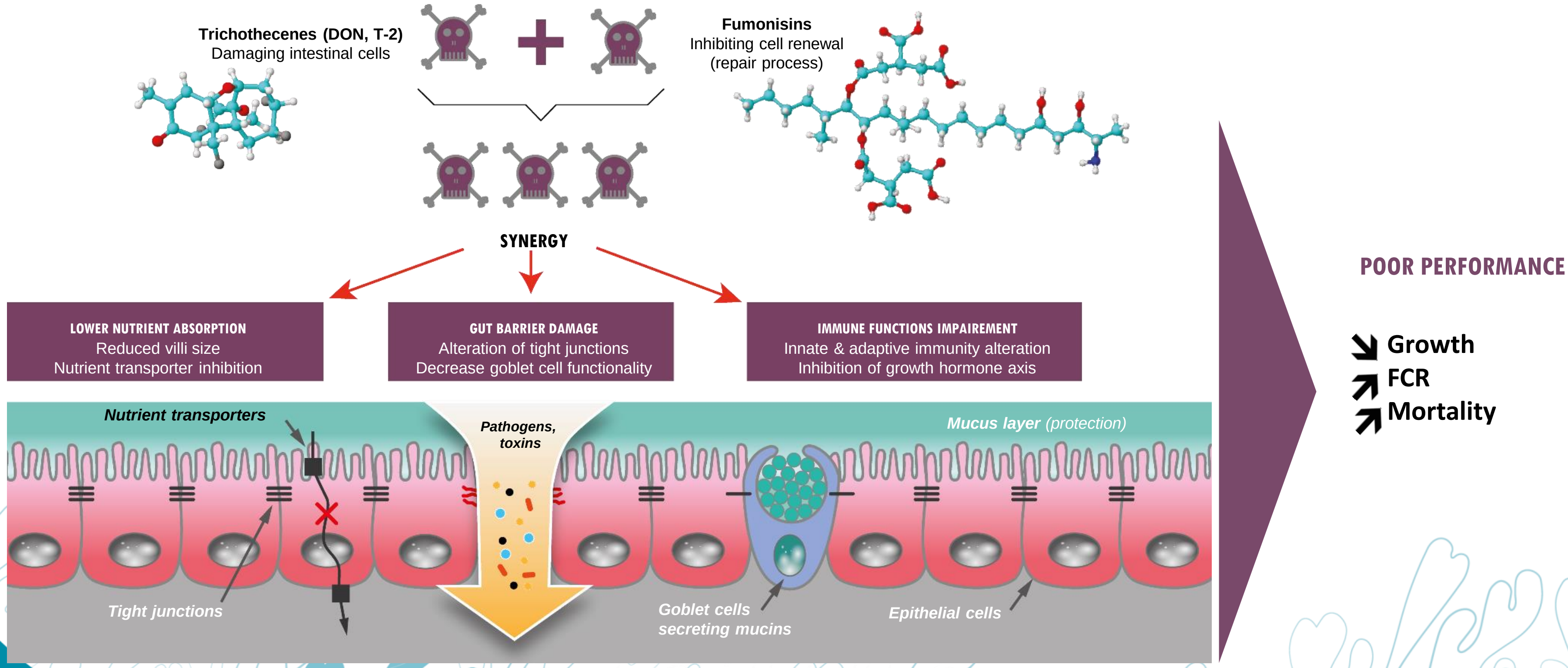
THE MYCOTOXIN CHALLENGE

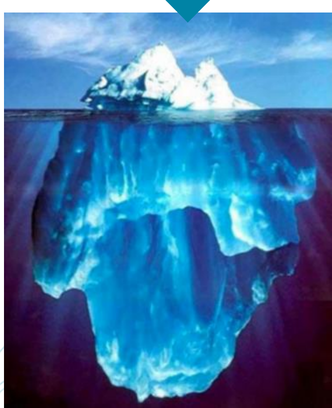
EFFECT OF SUBACUTE MYCOTOXICOSIS ON PERFORMANCE



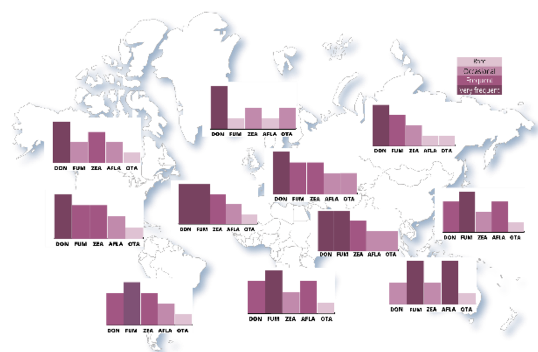
THE MYCOTOXIN CHALLENGE

SUMMARY

Plant origin raw materials and by-products are used to replace FM, but they are a high mycotoxin risk



Mycotoxins are everywhere



Sensitivity to mycotoxins

How to protect the animals?

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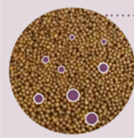
THE MYCOTOXIN CHALLENGE

PREVENTION

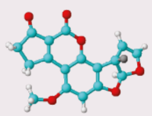
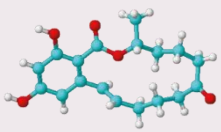
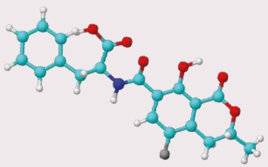
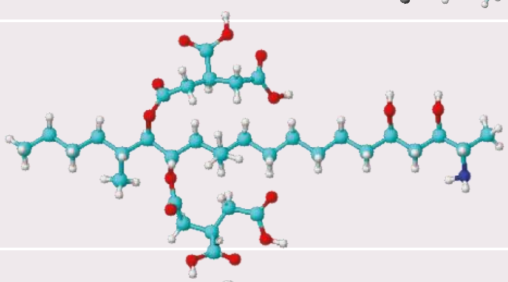
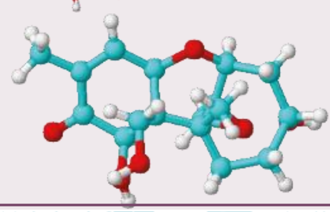
1) Implement good quality control, analyzing ingredients used for feed production

Type of sample	Adapted type of analysis	Accuracy	Caracteritics of analysis
 Complete feed	Chromatography 	Quantitative	All mycotoxins including derivates. Long delay and cost.
 Raw materials	ELISA (immunoaffinity) 	Semi-quantitative	One mycotoxin per test. Quick and cheap test.

2) Use mold inhibitors
3) Use a broad spectrum mycotoxin binder

TOXIN BINDERS EFFICACY

MYCOTOXIN VARIABILITY

	**Aflatoxins** - Planar molecules, rigid - Medium polarity	Easily adsorbed by aluminosilicates (clays), especially the Montmorillonite type.
 	**Zearalenone and ochratoxins** - Larger molecules and very flexible - Medium polarity	Not adsorbed by unmodified clays. Adsorbed by specific polysaccharides.
	**Fumonisins** - Much larger molecules, very flexible - More polar	Due to their size and structural configuration, they are the most difficult mycotoxins to adsorb.
	**Trichothecenes** - Larger volume, globular shape, epoxy ring = **VERY rigid** - Medium polarity	

Toxin binders must have the capacity to bind mycotoxins with different properties

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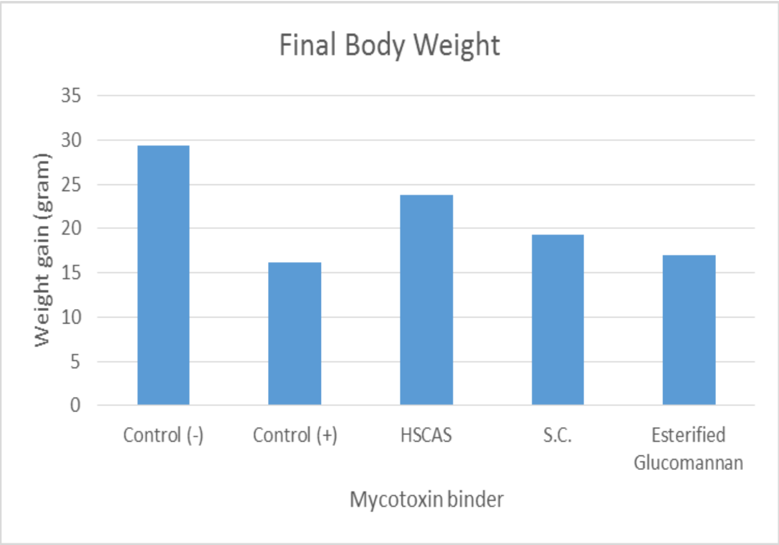
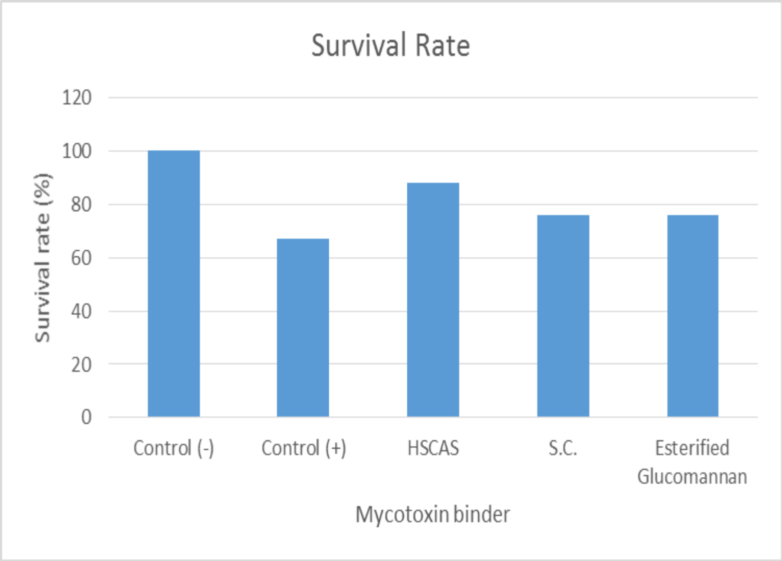
The efficacy of three mycotoxin adsorbents to alleviate aflatoxin B_1-induced toxicity in *Oreochromis niloticus*

Khaled M. Selim · Hana El-hofy · Riad H. Khalil 2014





MTx+ - testing binding capacity

Dynamic *in vitro* test

TIM-1 of TNO

Avantaggiato *et al.*, 2004	**Binder level**	**% Reduction of the bioaccessibility of DON**
TIM-1 TNO (Dynamic *in vitro*)	0,5%	29%
	2%	**45%**

- Important differences of results between type of tests, **84-95%** *in vitro* classical vs. **29-45%** *in vitro* dynamic
- Active Carbon was the best solution in 2004 **BUT**, the use of active carbon **must be limited to 0,5%** in order to avoid nutrients adsorption (NOSB, 2002; Ramos et *al*., 1996).

Is it possible to have the efficacy of activated carbon in dynamic *in vitro* test, at a lower rate of inclusion with no nutritional components capture?

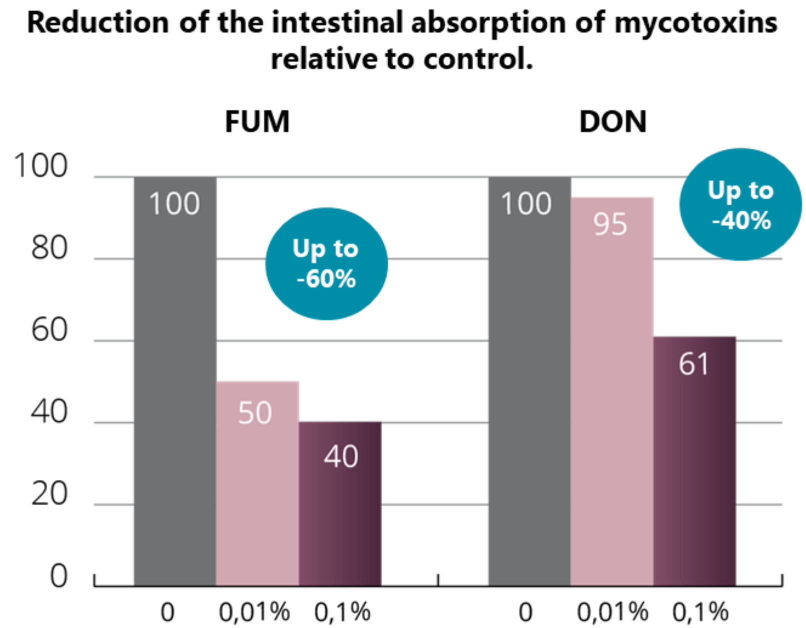
MT.X+: THE OLMIX SOLUTION

Test in TNO *in vitro* dynamic gastrointestinal model (TIM-1).

Use of complete feed contaminated with both:

- Deoxynivalenol (DON) at 1 ppm and,
- Fumonisin B1 (FB1) at 2 ppm.

Level of Interspaced MMT in feed: 0%; 0.01% and 0.1%.

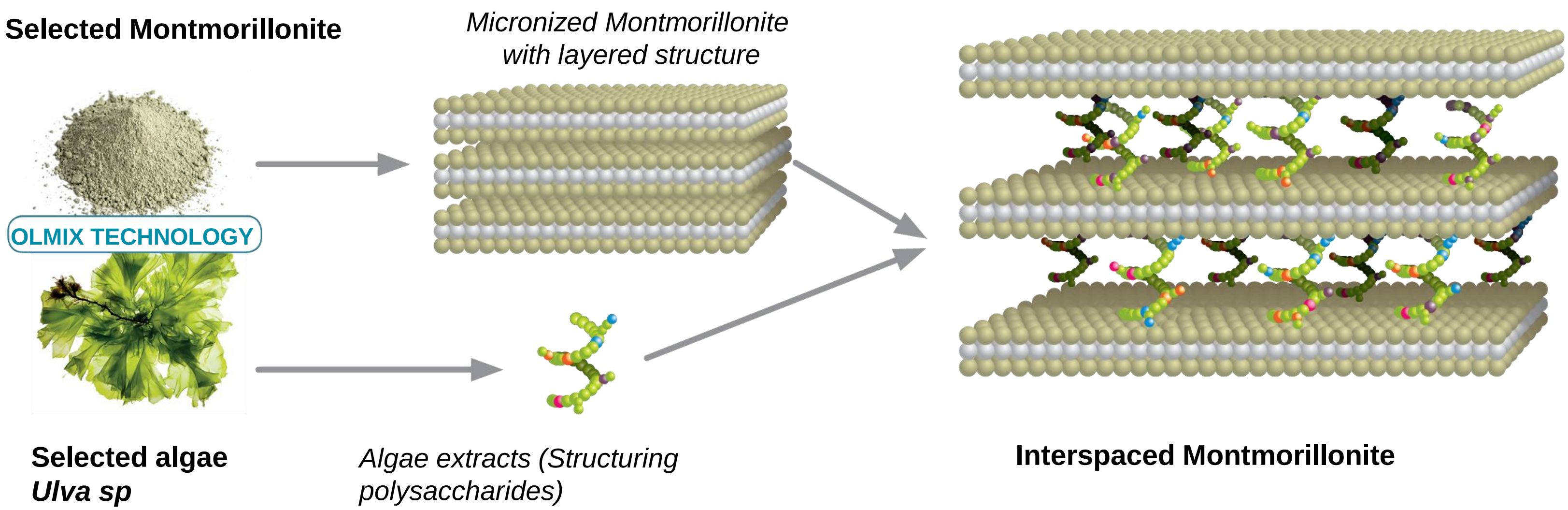


➔ **Promising alternative based on algo-clay to bind DON and fumonisins**

Extracted from Demais and Havenaar, 2006

MT.X+: THE OLMIX SOLUTION

INTERSPACED MONTMORILLONITE TECHNOLOGY



Interspacing Montmorillonite with algae extracts allows:

- ↗ Accessible adsorptive surface
- ↗ Available adsorption sites
- ↗ Types of adsorption sites
- ↗ Complexity of structure decreasing desorption

➡ **Wide adsorption spectrum**

MT.X+: THE OLMIX SOLUTION

A SINGULAR COMBINATION OF NATURAL ADSORBENTS

Interspaced Montmorillonite

Bentonite

Diatomaceous earth

Yeast cell walls

Seaweed extracts (Marine Polysaccharides)

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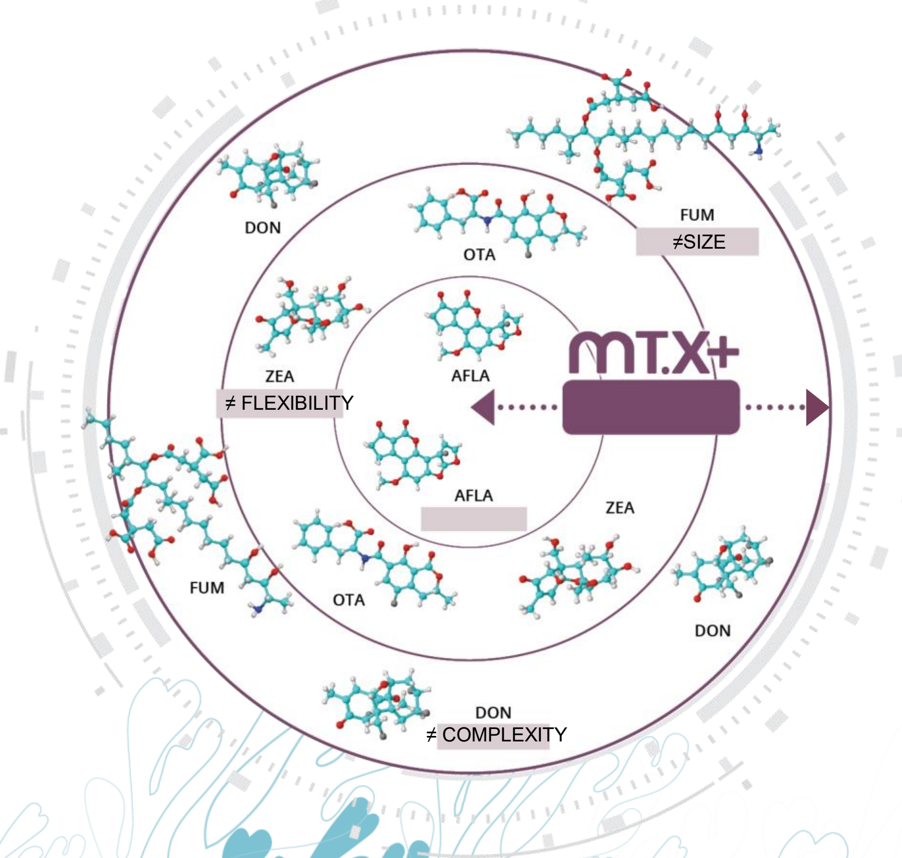
MT.X+: THE OLMIX SOLUTION

WIDE SPECTRUM TOXIN BINDER



Summary:

- Montmorillonite/bentonite
  - Aflatoxin
- Polysaccharides
  - Zearalenone and Ocratoxin
- Intercalated montmorillionite
  - Fumonisin and Trichotecene
- Diatomaceous earth
  - Endotoxins
- Seaweed extracts
  - Health improvement

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Trials with MTx+

Tilapia

MTx+ - mycotoxin binder trial on juvenile red tilapia

EXPERIMENTAL DESIGN

Trial implemented in Mekong delta 2,400 Red-Tilapia, average weight 5,5g.

Diets:

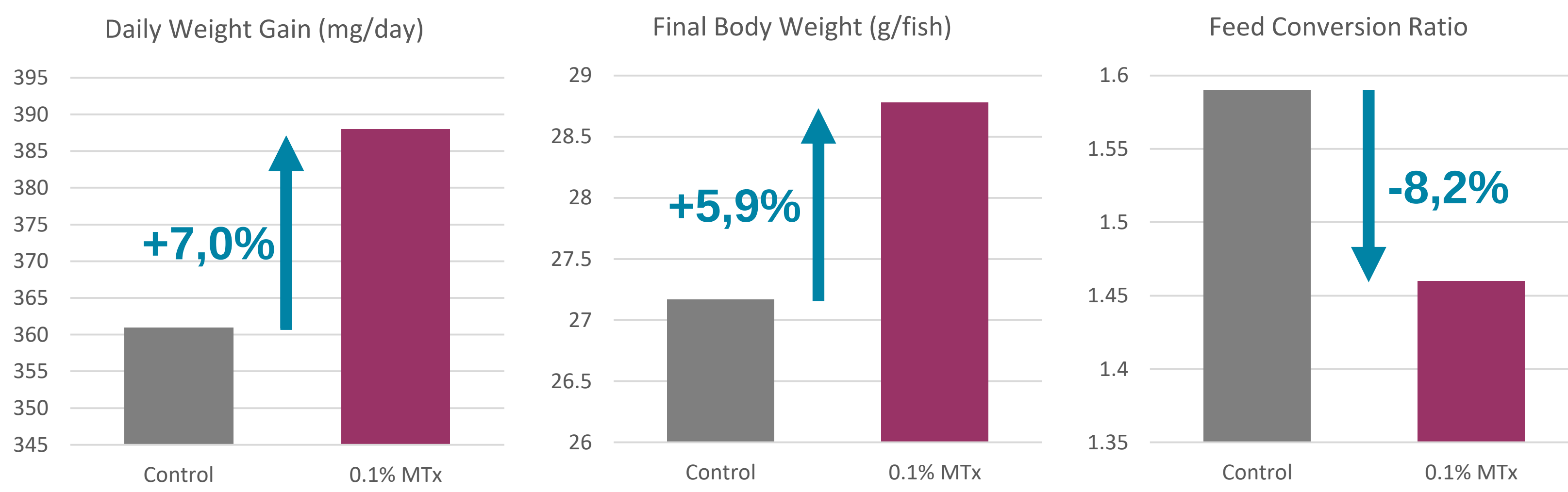
Control group: Commercial Feed

Treated group: Commercial Feed+ 0,1% MT.X+

3 replicates/ treatment
Duration 2 months

Mycotoxin contamination
- 13 different mycotoxin found in the feed by using LC-MS/MS method

MTx+ - mycotoxin binder trial on juvenile red tilapia



MTx+, the efficient and natural to for mycotoxin risk management in fish feeds

Trials with MTx+

Atlantic Salmon

MTx+ - mycotoxin binder trial on Atlantic pre-smolts Salmon

EXPERIMENTAL DESIGN

97,000 atlantic salmon pre-smolts

6 outdoor tanks (3 control, 3 treatment)

Fed with commercial salmon feed

0.2% MTx+ inclusion at feed mill

Duration from November-April

⇒**winter period**

Contamination with Ergot Alkaloids

CLAVICEPS sp. Mycotoxins (moisissures de champ et de stockage)

ERGOT ALKALOIDS

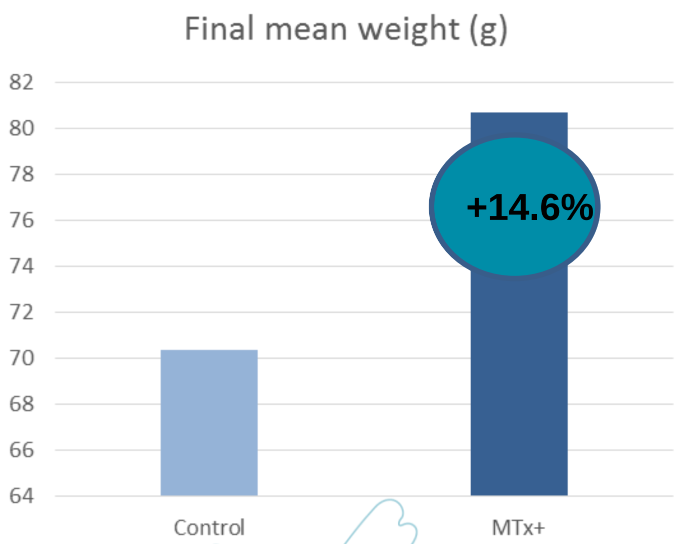
Ergocornin	0,070
Ergocristin	0,085
Ergocryptin	0,070
Ergométrin	0,030
Ergosin	0,140
Ergotamin	0,160

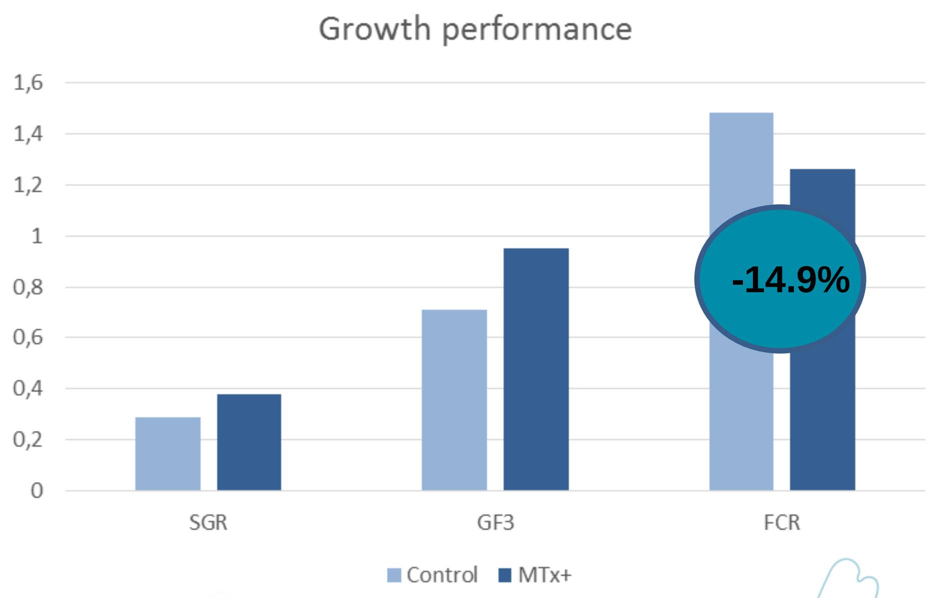
MTx+ - mycotoxin binder trial on Atlantic pre-smolts Salmon

Parameter	Control	Mtx+	Variation[1]
Initial mean weight, g	45.2	45.1	-0.1g
Final mean weight, g	70.4	80.7	+10.3g
Feed per fish, g	37.5	44.2	+6.7g
SGR	0.29	0.38	+31%*
GF3[2]	0.71	0.95	+33.8%†
FCR	1.48	1.26	-14.9%

*[1]Analysis of variance: *P<0.05, †P<0.10*
**GF3, or thermal growth coefficient, is an assessment of growth performance which accounts for the effect of temperature.*

MTx+ - mycotoxin binder trial on Atlantic pre-smolts Salmon





Thanks to algae!

Thank you

Maarten Jay van Schoonhoven, Aqua Care Manager Olmix
mjvanschoonhoven@olmix.com

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