

Comparison of commercial available complex mycotoxin binders on *in vitro* mycotoxin binding properties

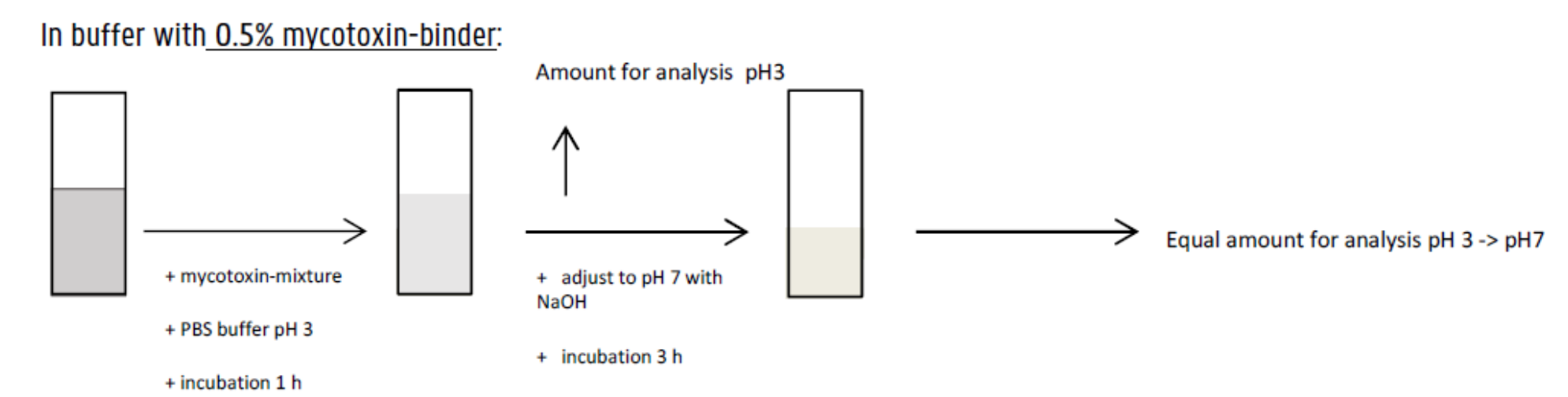
INTRODUCTION

Complexed mycotoxin binders play an important role in the prevention of mycotoxicosis in livestock. A variety of clays, yeast-based organic components, other organic components and chemical substances can form ingredients for a mixture to relieve the animal of mycotoxic stress, by binding or otherwise.

Multiple *in vitro* trials were performed to assess the binding capacity of single ingredients and formed the basis for the development of a commercial preparation (Excential Toxin Plus, Orffa Additives) consisting of 5 ingredients.

This composition was benchmarked against 19 commercial available preparations. The *in vitro* experiments were designed in close collaboration with MYTOX (Ghent University, Belgium), and executed by the Laboratory of Food Analysis (Ghent University, Belgium).

MATERIALS & METHODS



- The *in vitro* experiments comprise the following protocol:
 - Ingredients/products were mixed with buffered solutions at pH 3
 - Solutions were mixed with mycotoxins and internal standard
 - Mixes were incubated at 37°C for 1 hour (pH 3)
 - Analysis of mycotoxin binding at pH 3 by LC-MS/MS
 - Increase to pH 7 and keep stable pH for 3 hours
 - Analysis of mycotoxin binding at pH 7 by LC-MS/MS

RESULTS

In vitro testing of ingredients to select a best binding mix

	Inorganic Binders				Organic Binders (non yeast)						Yeast Based Binders			
	IB 1	IB 3	IB 4	IB 6	ONY 1	ONY 2	ONY 3	ONY 4	ONY 5	ONY 6	OY 1	OY 2	OY 3	OY 4
DON pH 3	0	0	0	0	0	0	n.d.	0	0	+	+	+	+	+
DON pH 3-7	0	0	0	0	0	0	0	0	0	+	+	+	+	+
ZEN pH 3	+	++	0	++	0	+++	n.d.	+	+	++	++	++	0	+
ZEN pH 3-7	0	0	0	0	0	+++	0	0	0	++	+	+	0	+
AFB1 pH 3	+++	+++	+++	+++	0	+	n.d.	0	+	+	++	+	0	+
AFB1 pH 3-7	+++	+++	+++	+++	0	+	+	0	+	0	++	+	0	+
AFB2 pH 3	+++	+++	+++	+++	0	+	n.d.	0	+	+	++	+	0	+
AFB2 pH 3-7	+++	+++	+++	+++	0	+	+	0	+	0	+	+	0	+
AFG1 pH 3	+++	+++	+++	+++	0	0	n.d.	0	++	+	++	0	0	0
AFG1 pH 3-7	+++	+++	+++	+++	0	0	+	0	+	0	0	0	0	0
AFG2 pH 3	++	+++	+++	+++	0	+	n.d.	0	++	0	++	0	0	+
AFG2 pH 3-7	+++	+++	+++	+++	0	0	0	0	+	0	0	0	0	0
OTA pH 3	++	++	+	+	0	+++	n.d.	+	0	++	++	+	0	+
OTA pH 3-7	0	0	0	0	0	+	0	0	+	0	0	0	0	0
HT-2 pH 3	0	0	0	0	0	0	n.d.	+	0	+	+	0	+	0
HT-2 pH 3-7	0	0	0	0	0	0	0	0	0	++	+	0	+	0
T-2 pH 3	0	0	0	+	0	+	n.d.	0	0	+	0	0	0	0
T-2 pH 3-7	0	0	0	0	0	+	0	0	+	0	0	0	0	0
ENN B pH 3	+++	+++	+++	+++	0	+++	n.d.	0	+	0	++	++	+	++
ENN B pH 3-7	+++	+++	+++	+++	0	+++	+	0	0	++	++	+	+	++
FUM B1 pH 3	++	+++	++	++	0	0	n.d.	0	0	0	+	0	0	+
FUM B1 pH 3-7	+	+	++	++	0	+	+	0	++	0	0	0	0	0
FUM B2 pH 3	+++	+++	+++	+++	0	+	n.d.	0	0	0	++	++	0	+++
FUM B2 pH 3-7	++	+	+	+++	0	+	+	0	+++	0	0	0	0	0

- Clay-based binders possess high binding properties towards the tested aflatoxins and ENN B.
- OTA and the tested trichothecenes (DON, T-2 and HT-2) are hardly bound by the majority of the tested binders, and there was a large variety between pH 3 and pH 7. For ZEN, a large variety could be observed between different binders.
- Yeast-based binders show the highest binding capacity towards ZEN.
- For the tested fumonisins (FUMB1, FUMB2), many binders have a very high binding capacity at pH 3 (as high as 100%), but poorly bind at pH 7 (as low as 0%).

In vitro analysis of commercial binders

	A	B	C	D	E	F	G	H	I	J	K	XC Toxin Plus
DON pH 3	+	+	+	0	0	+	0	0	0	0	0	0
DON pH 3-7	+	+	0	0	0	0	0	0	0	0	0	+
ZEN pH 3	++	++	+	+	+	++	+	+++	0	+++	+	+++
ZEN pH 3-7	++	+	+	0	+	+	+	+++	+	0	+	++
AFB1 pH 3	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
AFB1 pH 3-7	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
AFB2 pH 3	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
AFB2 pH 3-7	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
AFG1 pH 3	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
AFG1 pH 3-7	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
AFG2 pH 3	++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++
AFG2 pH 3-7	+	+++	++	++	++	++	++	+++	++	+++	+++	+++
OTA pH 3	++	++	0	+	+	++	+	+++	+	0	++	+++
OTA pH 3-7	0	0	0	0	0	0	+	+++	0	0	0	+
HT-2 pH 3	+	0	0	0	0	0	0	+	0	+	+	+
HT-2 pH 3-7	++	+	0	0	0	0	0	0	+	0	0	+
T-2 pH 3	+	0	0	0	0	+	0	0	+	0	0	++
T-2 pH 3-7	+	+	0	0	0	0	0	+	0	0	0	+
ENN B pH 3	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	++
ENN B pH 3-7	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++
FUM B 1 pH 3	0	++	+	++	+	+++	+++	+++	0	0	+++	+++
FUM B 1 pH 3-7	0	0	0	0	0	0	0	+++	0	++	+	+
FUM B 2 pH 3	+++	+++	+	+++	++	+++	+++	+++	+	0	+++	+++
FUM B 2 pH 3-7	0	0	++	0	0	0	0	+++	0	+++	+	++

	L	M	N	O	P	Q	R	S	XC Toxin Plus
DON pH 3	+	+	+	+	+	+	+	+	0
DON pH 3-7	0	0	0	+	0	+	+	0	+
ZEN pH 3	+	++	++	+	+	++	++	+	+++
ZEN pH 3-7	+	++	++	+	+	+	++	0	++
AFB1 pH 3	+++	+++	+++	+++	+++	+++	+++	+++	+++
AFB1 pH 3-7	+++	+++	+++	+++	+++	+++	+++	++	+++
AFB2 pH 3	+++	+++	+++	+++	+++	+++	+++	++	+++
AFB2 pH 3-7	+++	+++	+++	+++	+++	+++	+++	++	+++
AFG1 pH 3	+++	+++	+++	+++	+++	+++	+++	+++	+++
AFG1 pH 3-7	+++	+++	+++	++	+++	+++	+	++	+++
AFG2 pH 3	+++	+++	+++	+++	+++	+++	++	++	+++
AFG2 pH 3-7	++	+++	+++	++	++	++	0	++	+++
OTA pH 3	++	++	++	0	+	++	++	+	+++
OTA pH 3-7	0	+	+	0	0	0	0	+	+
HT-2 pH 3	0	0	0	+	0	+	++	++	+
HT-2 pH 3-7	0	0	+	+	0	++	++	0	+
T-2 pH 3	0	+	+	0	0	+	++	++	++
T-2 pH 3-7	0	+	+	+	0	+	++	+	+
ENN B pH 3	+++	+++	+++	+++	+++	+++	+++	+++	+++
ENN B pH 3-7	+++	+++	+++	+++	+++	+++	+++	+++	+++
FUM B 1 pH 3	+++	++	+++	0	+++	++	0	+++	+++
FUM B 1 pH 3-7	+	+	++	++	0	0	0	+++	+
FUM B 2 pH 3	+++	++	+++	0	+++	+++	++	+++	+++
FUM B 2 pH 3-7	+	++	++	+++	0	0	0	+++	++

% binding: 0 < 10% | + < 50% | ++ > 50% | +++ > 90% | A, C, F, G, K, M | Top premium product

- All commercial products show very high binding towards aflatoxins and ENN B.
- Very large variety in binding ZEN, a pH effect can also be observed.
- Tricothecenes: Difficult to bind at any pH, only one product shows overall binding (DON excluded).
- High recovery of the tricothecenes (DON in particular) in the supernatans. This suggest biotransformation by any binder into less toxic metabolites is minimal.
- Fumonisin and ochratoxins are difficult to bind, especially at pH 7. There is a clear pH effect.

CONCLUSION

Clay based binders can effectively bind aflatoxins and ENN B. The best binding properties for ZEA are found in yeast based binders and some other organic binders. Tricothecenes are difficult to bind for both pH 3 and 7, while fumonisins and ochratoxins are difficult to bind at pH 7

There are differences in mycotoxin binding efficiencies *in vitro* between commercial products, although some commercial binders have a higher binding efficiency towards specific mycotoxins.