

BG-MAX Evaluation *in vitro* Research

Introduction: Research was conducted by Dr. Rene Marquez at the Mycotoxin Lab of INIFAP (Institute of National Investigations for Agriculture) Research Center in Palo Alto, Mexico comparing the *in vitro* mycotoxin binding effects.

Objective: Comparative evaluation *in vitro* of the absorption efficiency of mycotoxins with 4 different commercially available mycotoxin binders.

Material and Methods: Experimentally contaminated batches of corn and sorghum with high mycotoxins concentrations were used. The mycotoxins concentrations were adjusted to the following test levels:

- Aflatoxin B1 (AFB1) 200 micrograms/kg (ppb)
- T-2 Toxin (TT-2) 300 micrograms/kg (ppb)
- Ochratoxin A (OA) 200 micrograms/kg (ppb)
- Deoxynivalenol (DON) 2000 micrograms/kg (ppb)
- Fumonisin B1 (FB1) 2000 micrograms/kg (ppb)
- Zearalenone (ZON) 1200 micrograms/kg (ppb)

A factorial design was performed 6 x 1 x 4 x 2 x 3, where the factors were: 6 mycotoxins, 1 dose (2.5 Kg/Ton), 4 mycotoxin binder products; 2 determinations for mycotoxin (absorption and desorption) and 3 replicates per test.

Treatments: BG-MAX,[™] a mycotoxin binder product, was compared against 3 commercial leading brands. One was an organic type product, one was silica (HCSA) and one was a yeast-based product.

Ten grams of the contaminated ground grain mixture were placed in 250 ml Erlenmeyer flasks, with 3 replicates for each one of the treatments and 50 ml of pepsin acid (1,250 UI de pepsin + 150 mEq of HCl) was added. The samples were incubated in a warm bath with a temperature of 37° C with continuous agitation for 3 hours. For the desorption assay, the same described conditions were used, only an additional incubation time of 3 hours was used to quantify the de-absorbed mycotoxin.

After the first incubation period in the warm bath with continuous agitation, aliquots were taken and centrifuged for 10 minutes at 3000 rpm. Then the supernatant (upper part) was adjusted to a pH 7 with 2N NAOH and the mycotoxin was measured by enzyme-linked immunoenzymatic assay (ELISA) from Biopharm. The percent absorption was a function of the initial concentration of the mycotoxin minus the residual toxin found in the supernatant. The remaining of the original sample, was incubated at 37°C for 3 more hours to allow the possible desorption of the mycotoxins. The sample was centrifuged at 3000 rpm for 10 minutes and the supernatant was extracted to test the mycotoxin levels using the same test procedure as before. The percent desorption was the amount of increase in concentration of mycotoxin found in the supernatant as compared to the original amount of mycotoxin concentration in the supernatant.

Conclusions: BG-MAX performed equal to or superior to the 3 commercial leading brands tested in this trial. Complete results are shown in Table 1-3 attached.

Results:

Table 1: Percentage of Absorption of Mycotoxins: Binding capacity or attraction of toxin into the material.

Product	Dose Kg/ton	AFB1 200 ppb	TT-2 300 ppb	OA 200 ppb	DON 2000 ppb	FB1 2000 ppb	ZON 1200 ppb
BG-MAX™	2.5	90.4	74.5	93.0	89.3	75.0	94.0
Organic	2.5	90.3	75.5	75.0	83.5	83.2	88.5
Silica	2.5	92.2	65.8	64.3	55.5	82.5	69.7
Yeast	2.5	91.0	67.5	73.2	74.0	78.5	88.0

Table 2: Percentage of Desorption of Mycotoxins: Effect of loose bonded toxin from the specific material.

Product	AFB1 200 ppb	TT-2 300 ppb	OA 200 ppb	DON 2000 ppb	FB1 2000 ppb	ZON 1200 ppb
BG-MAX	5.3	1.3	3.5	7.3	1.0	5.0
Organic	1.8	5.5	13.0	13.1	10.4	7.2
Silica	2.7	7.3	13.3	21.9	14.2	8.2
Yeast	9.3	5.2	8.2	5.5	9.1	12.7

Table 3: Percentage of Efficiency of Absorption of Mycotoxins.

Product	AFB1 200 ppb	TT-2 300 ppb	OA 200 ppb	DON 2000 ppb	FB1 2000 ppb	ZON 1200 ppb
BG-MAX	85.1	73.2	89.5	82.0	74.0	89.0
Organic	88.5	70.0	62.0	70.4	72.8	81.3
Silica	89.5	58.5	51.0	33.6	68.3	61.5
Yeast	81.7	62.3	65.0	68.5	69.4	75.3

Efficiency of Absorption (%)

