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Deoxynivalenol and *Zearalenone* adsorption by two products obtained from Jadis Additiva

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INTRODUCTION

Two mycotoxins produced by the genus *Fusarium*, deoxynivalenol (DON) and zearealenone (ZEN) appear to be an increasing threat to animal husbandry, especially swine production, in temperate climates such as Europe (Fink-Gremmels, 1999; Krska et al., 2001; Josephs et al., 2001). DON leads to symptoms like vomiting, diarrhoea, lower weight gain and feed intake and causes immunodepression (for a review see Rotter et al., 1996). ZEN exerts remarkable estrogenic effects and affects fertility and reproduction in farm animals (for a review see Gajecki et al 2001).

Different physical and chemical methods have been recommended for detoxification of mycotoxin-contaminated feed (Galvano et al., 2001), but most of them have not been accepted for practical use. Therefore, the use of nutritionally inert mycotoxin sorbents as feed supplements is one of the most promising approaches to combat mycotoxicoses in farm animals, and to prevent carry-over of mycotoxins from contaminated feeds into animal products. Successful adsorption of aflatoxins, the most thoroughly studied and understood mycotoxin, by different minerals clays has been demonstrated *in vitro* and *in vivo* (Ramos et al, 1996). However, adsorption of mycotoxins other's than aflatoxins has been less investigated.

The aim of the present study was to evaluate the ZEN and DON adsorption by several mineral products provided by **Jadis Additiva**. For this purpose, a practical *in vitro* method was applied, intending to reproduce the conditions of a monogastric gastro-intestinal tract, as swine is the most sensitive species for ZEA and DON.

MATERIALS AND METHODS

Materials

Two mineral products (**Myco Ad A-Z and Myco Ad w.s.**) were obtained from Jadis Additiva (Schiedam, The Netherlands). Zearalenone and deoxynivalenol were supplied by SIGMA (St. Louis, MO, U.S.A). All solvents used in chromatography were of HPLC grade; all other reagents were of analytical grade.

Methods

Gastro-intestinal tract simulation: The tested products were suspended in a saline solution (CaCl x $2H_2O$, 1.2 mM; KCl, 2.7 mM; $KHPO_4$, 1.5 mM; $MgCl_2$ x $6H_2O$, 1.1 mM; NaCl, 138 mM; Na_2HPO_4 x $2H_2O$, 8.1 nM) containing 1000 ppb of the mycotoxin, either DON or ZEN, to reach final concentrations of 2.5 mg of product/ml. Negative controls, mycotoxin suspension without adsorbent, were included. The obtained suspensions were set to a pH of 2.5 and incubated at 37°C for 1 hour under constant agitation to simulate the gastric conditions of a monogastric system. After taking an aliquot, the pH of the suspensions was raised to 8 and the incubation at 37°C prolonged 3 hours more under constant agitation to simulate the intestinal conditions of a monogastric system. After this time, a second aliquot was taken. Both aliquots were immediately filtered (Minisart-GF; Sartorious, Gottingen, Germany) to separate the binder and stored at -20°C until analysis.

DON HPLC analysis: 1 ml of each filtrated aliquot was cleaned-up using DON inmunoafinity columns (DONtest™ HPLC; VICAM, Watertown, USA). The final methanol eluent was evaporated under a nitrogen stream and the obtained residue re-dissolved in mobile phase. HPLC analyses were performed using a AS300 Thermo Separation Products HPLC system (Spectra-Physics, USA). A 2 glass C18 ChromSpher, stainless steel, 5 µm column (100 x 3.0 mm, Chrompack, The Netherlands) was connected to SpectraSeries P100 Isocratic pumps set at a flow rate of 0.2 ml/min. Mobile phase consisted of acetonitril

/ water (10:90 v:v). UV detection was performed by a UV150 detector at 218 nm communicated with a Data Jet Integrator.

Zearalenone HPLC analysis: 1.5 ml of each filtrated aliquot was extracted with 7.5 ml of chloroform. The water phase was discarded and the chloroform evaporated under a nitrogen stream. The obtained residue was re-dissolved in mobile phase. A C18 Luna II, stainless steel, 5 µm column (150 x 4.6 mm; Phnomenex, The Netherlands) was connected to two high precision pumps (Gynkotek model 300) set at a flow rate of 0.7 ml/min and controlled by a Chromeleon-Gynkotec HPLC software (Softron). Fluorescence detection was performed with a FP 920 fluorescence detector (Jasco, Japan) set at 236 nm excitation wavelength and 418 nm emission wavelength. Mobile phase consisted of methanol / water (70:30 v:v).

Calculation of the DON and ZEN adsorption capacity per substance: Two figures are given to express the DON and ZEN adsorption capacity of each absorbent. The first figure, called gastric adsorption (GA = ADS pH 2.5), is based on the analyses of the aliquot taken after the first incubation and it is considered to be equivalent to the binding capacity of the substance after the stomach passage. The second figure, called intestinal adsorption (IA = ADS pH 8), is based on the analyses of the aliquot taken after the second incubation and it is considered to be equivalent to the binding capacity of the substance after the intestinal passage. Both, GA and IA, are calculated in percentage to the negative control (mycotoxin only).

Mycotoxin binders are usually added to the feed in a concentration from 1 to 5 kg/t. Higher concentrations are avoided to minimize the risk of non-specific nutrient adsorption, as well as to minimize the caloric/nutritional losses. Thus, comparable concentrations of the binders were used in the *in-vitro* system. Finally, moderate (DON-1000 ppb) to high (ZEN-1000 pppb) toxin levels were applied in the assay.

RESULTS

DON adsorption: Mycofix Ad A-Z showed a considerable binding capacity for DON as shown in Table 1, whereas the binding of Myco Ad w.s. was negligible.

Table 1. DON adsorption by different mineral products[1]

Products (2.5 mg/ml)	GA (ADS pH 2.5)	IA (ADS pH 8)
Myco Ad A-Z	62.49	65.91
Myco Ad w.s.	0.0	8.23

[1] *Values are means of three independent experiments*

ZEN adsorption: The ZEN adsorption by the investigated products at 2.5 mg/ml is presented in Table 2, showing corresponding results, as ZEN was bound very effectively by Myco AD A-Z, whereas only minimal amounts of the toxin were absorbed by Myco-Ad w.s..

Table 2. ZEN adsorption by different mineral products[1]

Products (2.5 mg/ml)	GA (ADS pH 2.5)	IA (ADS pH 8)
Myco Ad A-Z	96.11	98.96
Myco Ad w.s.	0.0	7.65

[1] *Values are means of three independent experiments*

DISCUSSION

The aim of the conducted experiments was to demonstrate whether or not a stable bound between the adsorbent and the mycotoxins DON and ZEN can be formed under conditions of pH, temperature and time comparable to those of a the gastrointestinal tract of pigs. According to the results, Myco Ad A-Z showed a relevant DON and ZEN adsorption. However, Myco Ad w.s. did not exhibit any measurable binding of DON and ZEN at the gastric pH of 2.5 and a marginal binding under the conditions of the intestines.

In conclusion, Myco Ad A-Z is a very promising mycotoxin binding agent as it exerted a remarkable and significant binding activity towards the mycotoxins DON and ZEN. It has to be emphasized, however, that the applied *in vitro* methodology has to be regarded as screening method. For the ultimate evaluation of the product, *in vivo* studies in the target animal species (pigs) are essential.

Literature

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