In the past few years there has been a growing interest in grain mycotoxins, particularly in zearalenone (ZEA). This is the 6-(10-hydroxy-6-oxo-trans-1-undecenyl)-β-resorcyclic acid lactone produced by strains of Fusarium, especially Fusarium graminearum (17). ZEA is a natural contaminant of corn, wheat, barley, oat, sorghum, and hay. It was first isolated as an anabolic and uterotrophic compound (21) from corn infected with Gibberella zeae. Frequently, ZEA is found in contaminated foods mixed with the mycotoxin tricothecenes (14). Despite their structural dissimilarity to steroidal estrogens, ZEA and several of its derivatives possess estrogenic activity and might be of importance in carcinogenesis (12, 22, 24).

In 1968, Mirocha et al. (13) detected ZEA in hay and associated it with infertility in dairy cattle. In recent years, corn crops were severely damaged by contamination with ZEA, causing important economic losses; therefore, reliable means of control and detoxification are needed. Livestock producers, as well as food and feed processors, are concerned with the presence of this toxin in corn because their profitability depends on ZEA control in animal diets. Because mold-damaged corn is often used in animal feed, the risk of ZEA intoxication is higher for farm animals (5).

Because of its high biological activity and its frequent occurrence in cereals, particularly wheat and maize, nine countries have specific regulations for ZEA, with tolerance limits ranging from 0 to 1 mg/kg in foods (5). It is difficult to detoxify corn contaminated with ZEA, and no commercial or practical treatments exist to destroy ZEA efficiently. Addition of potential binding agents in the diet is one of the methods to reduce ZEA concentration in feed. In fact, alfalfa, zeolite, cholestyramine, yeast cultures, and yeast cell wall constituents reduce the absorption of ZEA in the gastrointestinal tract (4, 11, 20, 23). Because further studies were needed to evaluate the importance of adsorbents to trap ZEA, we conducted the series of experiments described in this paper.

Activated carbons (ACs) are an important group of sorbents. Although the adsorption abilities of ACs vary widely, depending on the typology of carbonaceous substances and activation processes (8), different studies showed that ACs can adsorb aflatoxin B1 (AFB1), deoxynivalenol, ochratoxin A, and fumonisin B1 in aqueous solutions. These results might suggest that ACs could be used as multimycotoxin-sequestering agents (6, 8, 9). On the other hand, bentonite and talc are clay minerals (silicates) belonging to the Montmorillonite/Smectite Group (3) and might possess interchangeable cations Na+, K+, Ca2+, and Mg2+ (16). Their adsorption properties mainly depend on the composition and the preparation of the material surface. This study was conducted to evaluate the adsorbing power of five adsorbents—AC, bentonite, talc, sandstone, and calcium sulfate—to trap ZEA in vitro.

**MATERIALS AND METHODS**

**Adsorbents.** Five adsorbents were tested in aqueous suspensions of different adsorbent proportions: AC (Sigma Chemical, St. Louis, Mo.), bentonite (La Elcha, Luján, Mendoza, Argentina), talc (Parafarm, China), calcium sulfate (Mallinkrodt Chemical Works, St. Louis, Mo.), and sandstone made of illite 36%, montmorillonite 16%, beidellite 36%, chlorite 8%, kaolin 4%, and traces of feldspar and quartz. The adsorbent system is a suspension of the solid adsorbent in a phosphate (pH 7.3) or citrate (pH 3.0) buffer, varying the amount of solid in the aqueous suspension.
TABLE 1. ZEA bound by different adsorbents

<table>
<thead>
<tr>
<th>% of adsorbent</th>
<th>Activated carbon, pH 7.3 and 3</th>
<th>Bentonite pH 7.3</th>
<th>Bentonite pH 3</th>
<th>Talc</th>
<th>Calcium sulfate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>100 ± 0 A</td>
<td>16.4 ± 5.2 A</td>
<td>7.3 ± 1.0 A</td>
<td>36.2 ± 0.1 A</td>
<td>9.7 ± 2.7 A</td>
</tr>
<tr>
<td>0.25</td>
<td>100 ± 0 A</td>
<td>53.6 ± 6.0 B</td>
<td>12.1 ± 0.1 A</td>
<td>34.4 ± 3.6 A</td>
<td>21.2 ± 0.3 B</td>
</tr>
<tr>
<td>0.5</td>
<td>100 ± 0 A</td>
<td>61.4 ± 4.7 B</td>
<td>44.8 ± 5.9 B</td>
<td>30.7 ± 13.2 A</td>
<td>24.0 ± 3.6 B</td>
</tr>
<tr>
<td>1.0</td>
<td>100 ± 0 A</td>
<td>51.5 ± 10.8 B</td>
<td>31.3 ± 1.5 B</td>
<td>77.3 ± 10.0 B</td>
<td>31.8 ± 4.8 B</td>
</tr>
</tbody>
</table>

Each value is a mean ± standard error of the mean for three samples. Means within columns with no common letters differ significantly (P < 0.05).

from 0.1 to 1%. The pH was measured on a Orion Research model 201 digital pH meter (Cambridge, Mass.).

**Heat activation.** Bentonite, talc, and calcium sulfate were activated by heating to 120°C for 6 h. After cooling to room temperature in vacuo, they were further stored at −20°C until use.

**ZEA binding assay.** Portions of 1.5, 3.75, 7.5, and 15 mg of each adsorbent were placed into glass tubes. An aqueous solution of phosphate buffer (1.5 ml, pH 7.3) and a dimethyl sulfoxide (DMSO) solution of ZEA (20.5 μg in 5 μl) were then added to the adsorbents. ZEA and DMSO were purchased from Sigma (purity > 99%). Experiments at pH 3.0 (only with AC and bentonite) employed citrate buffer. After a reaction time of 1 h at 37°C, with shaking at 15-min intervals, all the tubes (1.5 ml) were centrifuged in a tabletop centrifuge for 15 min at 800 × g to give supernatants and pellets. Supernatants containing unbound ZEA were collected, filtered through a 0.22-μm pore-size membrane and stored at −20°C for quantitative analysis by high-performance liquid chromatography (HPLC). In all cases, positive and negative controls were included: positive: buffer plus ZEA; negative: buffer plus sorbents, and experiments were conducted in three replicates. Pellets obtained from the centrifugation procedure were suspended in 0.5 ml of acetone, and the suspensions were further shaken and centrifuged at 800 × g for 15 min. The supernatants containing released ZEA were collected, and the process was repeated three times. Acetone was evaporated, and ZEA was detected by gas chromatography–mass spectrometry (GC-MS) or HPLC to check for further degradation of the adsorbed toxin.

**Quantification of ZEA by HPLC.** Reversed-phase HPLC was used to quantify ZEA. ISCO HPLC equipment (ISCO, Inc., Lincoln, Neb.) with a UV detector (250 nm) and a Luna C 18 column (250 mm, 4.6 mm inside diameter [i.d.], 5 μm, Phenomenex Corporation, Torrance, Calif.) were used. A mixture of acetonitrile–methanol–water (ratio 1:6:1:1) was used as the mobile phase, with a flow rate of 0.5 ml/min. Retention time was 16 min for ZEA. ZEA percentage in samples was calculated with the following equation.

\[
\left[\frac{1 - \text{(peak area of supernatant)}}{\text{peak area of ZEA in the positive control)}}\right] \times 100
\]

**Detection of ZEA by GC-MS.** Mass spectrometry was carried out by electron impact at 70 eV. An HP 6890 Series II chromatograph (Hewlett-Packard Co., Wilmington, Del.) linked to an HP 5972 mass selective detector with an HP-5MS 5% phenyl methyl siloxane column (30 m by 0.25 mm i.d.) was employed. Temperature program was from 50 to 100°C at a rate of 1.5°C/min, from 100 to 160°C at a rate of 3°C/min, and from 160 to 280°C at a rate of 10°C/min.

**Statistical analysis.** The results are reported as a mean ± standard error of the mean. The differences in the mean values were evaluated by analysis of variance (ANOVA). The Tukey test was used for all pairwise multiple comparisons of groups. In all statistical analyses, P > 0.05 was considered not significant (19).

**RESULTS**

Percentages of toxin trapped at pH 7.3 by different adsorbents and proportions in buffer suspensions are listed in Table 1. For comparison, AC and bentonite were tested at pH 3, and the results are also included. As shown, AC was able to remove the total ZEA content at the adsorbent concentrations tested, and it was the most efficient adsorbent, whereas sandstone did not trap ZEA at any concentration in the experimental conditions employed. The efficiency of the remaining adsorbents was between the AC and sandstone limits. Bentonite could remove half of the content of ZEA at 0.25% with no improvement when the adsorbent concentration increased. Talc and CaSO₄ removed ZEA to some extent, depending on the adsorbent concentration, with talc more efficient at lower concentrations. The adsorption capacity of bentonite and carbon were evaluated at pH 3. No differences were observed in the AC experiments, but the bentonite suspension was more efficient at a neutral pH. No chemical degradation occurred during the work-up or when the toxin remained adsorbed, as could be demonstrated by removing the toxin from the pellets, employing acetone with shaking. The acetone solution was later analyzed by GC-MS, which showed the ZEA peak and no others. Furthermore, ZEA could not be recovered when the complex was formed with AC at 1%, showing the strength of AC binding.

**DISCUSSION**

This is the first report on the in vitro binding ability of bentonite, talc, and calcium sulfate to trap ZEA at the doses evaluated. It is known the capacity of bentonite to bind other toxins. In fact, it was able to bind efficiently AFB₁ in vitro and reduce its toxic effects in broilers (18). It also reduced the toxic effects of T2 toxin in rats (2). However, in vivo studies showed that bentonite was not effective as a ZEA-sequestering agent, even at doses of 2 to 5% (15).

AC was the best adsorbent, strongly binding 100% of ZEA at 0.1, 0.25, 0.5, and 1% dose levels. It is important to point out the adsorptive power of AC independent of pH, which might be important for the use of carbon in vivo.
because the trapping of the toxin would be possible along the whole gastrointestinal tract. Galvano et al. (10) studied AC extensively in vivo and in vitro, showing that this substance is a multimycotoxin-sequestering agent, although there have been no reports regarding the adsorption of ZEA. In this study, we demonstrated that carbon is effective when adding $1/20$ of the dose previously reported by Galvano et al. (7). This is an important fact from an economic point of view, considering carbon will also adsorb essential nutrients (i.e., vitamins and minerals). The later undesired effect should be taken into account when evaluating the long-term consequences of incorporating AC into animal diets. Recently, Avantaggiato et al. (1) found a significant reduction of intestinal absorption of ZEA, in a laboratory model that mimics the gastrointestinal tract of healthy pigs, after inclusion of AC or cholestyramine at a dose of 0.25% in the feed. AC differs from other sorbent materials in the absence of polar groups in its structure, so it does not participate in hydrogen bond formation. Therefore, the adsorption capacity of AC depends on the adsorbate polarizability, which increases with the amount of aromatic groups and double or triple bonds. All of these structural features are present in ZEA.

Because ZEA is able to cross the intestinal lumen at a high rate (16) and can be easily absorbed in the gastrointestinal tract, the detoxifying agent also should form a toxin-adsorbent complex at high rate and irreversibly to be efficient. This study suggests that AC, bentonite, and talc could be good candidates for in vivo tests of detoxification of ZEA when incorporated into feeds at 0.1 and 0.25%.

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REFERENCES