In vitro evaluation of binding capacity of different binders to adsorb aflatoxin

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Abstract

This study was conducted to compare the efficacy of different feed additives as mycotoxin binders in vitro. Four prevalent aflatoxin-sequestering agents (SAs) including two bentonite clays (common and acid activated bentonite), a yeast cell wall product and an activated charcoal product were evaluated in vitro to verify their capacity for binding aflatoxin B1 (AFB1). The SAs were individually mixed at two different ratios with AFB1 (1:70,000, 1:120,000) and their binding capacity indices were determined. Experimental bentonites showed high adsorption abilities, binding more than 70.00% of the available AFB1. At the 1:70,000 and 1:120,000 aflatoxin binder (AF:B) ratios, acid activated bentonite were sequestered over 87.00 and 99.00% of the AFB1, respectively. Yeast cell wall showed moderate adsorption ability at the 1:120,000 AF:B ratio, adsorbing 47.00% of AFB1. The adsorption ability of activated carbon at two AF:B ratio and yeast cell wall at 1:70,000 AF:B ratio were significantly lower than other binders. The ratio of chemisorption and binding equivalency factor were higher for acid activated bentonite compared to other sequestering agents. Based on the result of this study, it seems that acid activated bentonite could be considered efficient at sequestering the available AFB1, resulting as promising agents for use in animals diet.

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Materials and Methods

Four aflatoxin-sequestering agents including two bentonite clays (common and acid activated bentonite were obtained from a local mine in South Khorasan province, Iran), a yeast cell wall product and an activated charcoal product were evaluated in vitro. The standard solution of AFB1 was prepared by dissolving 5.00 mg of pure toxin (Sigma-Aldrich, Munich, Germany) in 3.00 mL of acetonitrile (Ava Gostar, Tehran, Iran) for delivery into 500 mL of distilled water (approximately 10.00 mg mL⁻¹). Each sequestering agent (1.00 g) was added to a 125 mL Erlenmeyer flask containing 100 mL of 10.00% methanol.
and mixed for 30 min with a magnetic stirrer.

Binding capacity for the test sorbent was determined by adding 5.00 mL of aliquots of stock solution to 0.50% suspension of each sequestering agent. Then each sample was incubated for 2 hr in shaking incubator at 39.00 °C. The incubation of samples was conducted in triplicates for each sequestering agent sample. After 2-hr incubation, the mixture was centrifuged and the supernatant was obtained for analysis of residual unbound AFB1 using High-performance liquid chromatography (HPLC). The HPLC system (Perkin Elmer, Boston, USA) was equipped with ODS 5.00 µm column and fluorescence detector set (RF-551; Shimadzu, Columbia, USA). The percent adsorption of AFB1 by sequestering agents was calculated using the following equation:

\[
\text{Percent adsorption} = (IA-RA / IA) \times 100
\]

where, \( IA \) (ng mL\(^{-1}\)) is the initial amount of AFB1 in the digestion conical tube; \( RA \) (ng mL\(^{-1}\)) is the residual amount of unbound AFB1 in the conical tube after digestion procedure.

The HPLC adsorption data were used to calculate the AFB1 binding parameters. Linear regression analysis was conducted for each sorbent. Aflatoxin binding capacity (BMax) for each sorbent was calculated from the inverse of y-intercept for the linear regression. The ratio of chemisorption (rc) was calculated by determining the amount of toxin bound (Cb) to the pellet during the capacity studies, and the amount of toxin desorbed (Cd).

\[
rc = (Cb – Cd) / Cb
\]

Binding equivalency factor (BEF) was determined by the following equation:

\[
\text{BEF} = (BMax \times rc) / Ci
\]

where, \( Ci \) is the amount of toxin (ng) added at the theoretical point.

Statistical analysis. Data were analyzed by GLM procedure of SAS (version 9.2; SAS Institute Inc., Cary, USA). Differences among groups mean were determined using the following equation:

\[
\text{Final AFB1 concentration} = \frac{\text{Initial AFB1 concentration}}{1 + \text{Adsorption capacity}}
\]

Results

The in vitro AFB1 adsorption capacity of bentonite used at different AF:B ratios are listed in Table 1. A higher binding capacity was observed at the 120,000 ratio. At the 1:70,000 and 1:120,000 AF:B ratios, bentonite was sequestered over 87.00 and 99.00% of the AFB1, respectively. No toxin was found in a ratio higher than 1:120,000. Adsorption capacity of AFB1 by different binders is presented in Table 2. A higher sequestering capacity was observed for acid activated bentonite at the 1:120,000 AF:B ratio. Common and acid activated bentonite showed high adsorption abilities, binding more than 70.00% of the available AFB1. Yeast cell wall showed moderate adsorption ability at the 1:120,000 AF:B ratio, adsorbing 47.00% of AFB1. The adsorption ability of activated carbon and yeast cell wall was significantly lower than other binders (\( p < 0.05 \)). Also, adsorption capacity of acid activated was higher than common bentonite at different time after incubation (Table 3). The effect of incubation time on aflatoxin adsorption, BMax, rc and BEF of different bentonites are presented in Tables 3 and 4. Acidified bentonite had higher adsorption efficiency indices versus common bentonite (\( p < 0.05 \)). Adsorption capacity parameters (rc and BEF) were decreased (\( p < 0.05 \)) over the time.

Table 1. The in vitro AFB1 adsorption capacity of acidified bentonite used at different Aflatoxin:Binder ratios.

| Initial AFB1 concentration (ng) | Binder concentration (mg) | Aflatoxin: Binder ratios | Adsorption capacity (%) |
|--------------------------------|---------------------------|--------------------------|------------------------|
| 50                            | Control (0)               | -                        | 0.00                   |
| 50                            | 0.25                      | 1:5,000                  | 7.00                   |
| 50                            | 0.75                      | 1:15,000                 | 9.00                   |
| 50                            | 2.00                      | 1:40,000                 | 22.50                  |
| 50                            | 3.50                      | 1:70,000                 | 87.00                  |
| 50                            | 6.00                      | 1:120,000                | 99.10                  |
| 50                            | 10.00                     | 1:200,000                | ND                     |
| 50                            | 37.50                     | 1:750,000                | ND                     |
| 50                            | 500                       | 1:1,000,000              | ND                     |

ND = Not detected.

Discussion

A sizable proportion of the animal feeds are contaminated with mycotoxins that adversely affect health and performance of animals. The best strategy to prevent the mycotoxins contamination is to avoid the mycotoxin production at the time of cultivation and storage of the feed crops.\(^9\) In many countries it is difficult to achieve this goal.\(^10\) Therefore, in order to prevent mycotoxins poisoning, several approaches have been reported.\(^8\) In recent years, organic and inorganic sorbent materials are used to reduce mycotoxin bioavailability in animal feeds, thus, it is necessary to evaluate the adsorption capacity of these adsorbent products. An in vitro practical method was used to compare the aflatoxin binding capacity of prevalent mycoadsorbents in the current experiment. According to our results, experimental common or acidified bentonites was appeared to bind AFB1 efficiently rather than other organic binders. It is well established that swelling day especially bentonite are composed of interlayer spacing and have the external basal surfaces and edges and lead to high degree of adsorption and aflatoxins reacting at these sites.\(^11,12\) Recent studies indicated that binding of AFB1 on interlayer surfaces of bentonite involved chemical bonding mechanisms\(^13\) resulting into more pronounced ability to adsorb aflatoxins in the range of 90.00 - 95.00%.\(^10\)
In comparison with the clay sorbent, it was reported that the mechanism of AFB1 binding to organic binders such as glucosaminan products was shown to be Van der Waals and hydrogen bonds and these attractions were reversible and depended largely on the orientation of the molecules. In agreement with our results, Moschini et al. reported that yeast cell wall products had a very low in vitro efficiency in all of tested conditions. Low capacity of β-D-glucans, a major component of the inner layer of yeast cell wall, to interact with aflatoxins indicated the involvement of non-covalent bonds (adsorption) rather than real binding. In contrast, the binding of AFB1 on interlayer surfaces of bentonite is chemisorption bonding mechanisms and are stronger than Van der Waals and hydrogen bonding interactions.

Among the many factors that affect the absorbent capability, interaction of organic molecules with clay mineral surface chiefly depends upon the concentration of the organic molecule and clay mineral (mycotoxin: binder ratio), pH, and incubation time. Based on the results, the in vitro efficiency of the different mineral and organic binders tested were found to be related to the AF:B ratio. The amount of the sequestering agents used were ranged between the practical dose (Table 1) and the level indicated in the in vitro studies (1:5,000). A higher binding capacity was observed at the 1:120,000 ratio. At the 1:70,000 and 1:120,000 AF:B ratio bentonite was sequestered over 0.87 and 0.99 of AFB1.

Moschini et al. studied the effect of AF:B ratio (i.e., 1:5,000; 1:50,000 and 1:500,000) on adsorption efficacy of SAs and reported a higher sequestering capacity at the 1:500,000 AF:B ratio. They found over 0.87 and 0.98 of the AFB1 adsorption capacity by sequestering agents, respectively, in water and rumen solutions.

In the present study acid activated bentonite showed higher adsorption efficiency during the incubation time (Tables 3, 4) which indicated stronger connections in acid activated bentonite. Vekiru et al. showed that adsorption ability of a bentonite was influenced by the pH of the incubation media. Consistent with our results in study of Chansiripornchais and Fink-Gremmels six out of seven tested sequestering agents showed higher binding efficiency (98.97 - 100%) of AFB1 at the of pH 2.50. However, Gallo and Masoero reported that acid condition (pH 2.00) did not influence the amount of AFB1 recovered. These authors demonstrated that this effect could be related to the limited incubation time. Desheng et al. showed that the maximum amount of adsorbed AFB1 was obtained from aqueous solution at pH 2.00 using a calcium montmorillonite as adsorbent. Komadel suggested that at pH ≤ 3.00, the hydroxyl groups of the bentonite octahedral layer were attacked by protons' penetration in the phase and the layer started to redissolve. Also, ion exchange is an important factor in
clay mineral-organic interaction. Thus, protonated clay increases the exchange reactions with the organic cations normally occupying exchange sites on the surface of the clay mineral. Some organic molecules may become cationic after adsorption at clay surface by protonation that increase adsorption capacity of clay. This act depends upon the Bronsted acidity of the clay surface. Organic molecules have the possibility of accepting protons from the clay surface. The ability of the clay surface to donate protons is determined by the nature of the mineral. Acidification of mineral could be resulted in increasing the available proton that increases protonated organic molecules.

In conclusion, our results indicated that the in vitro efficiency of the sequestering tested agents was related to the AF:B ratio. Our in vitro results ranked the adsorbents as good (bentonites), average (yeast cell wall) or poor (activated carbon). Moreover, acid activated bentonite was more effective than the tested common bentonite.

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Conflict of interest

Authors disclose no conflict of interest.

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