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Effect of monogastric and ruminant gastrointestinal conditions on *in vitro* aflatoxin B_1 adsorption ability by a montmorillonite

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The main objective of this study was to evaluate the interference of environment components on the *in vitro* evaluation of aflatoxin B_1 adsorption capacity of sodium bentonite under simulated gastrointestinal conditions of monogastric and ruminant animals. Sodium bentonite showed a high aflatoxin B_1 affinity with all of the assays. Langmuir or sigmoid isotherms were found in different assays. Both the affinities and the surface excesses at monolayer saturation were affected by the buffer components. The specific influence of ions in each buffer solution was investigated. A significant decrease in the surface excess at monolayer saturation was observed under ionic strength control. A change in the isotherm shape from sigmoidal to Langmuir was observed with the increase in the sodium chloride concentration. This was attributed to the decrease in the importance of lateral interaction between adsorbed toxin molecules compared with surface-molecules interactions under a high salt coverage. The presence of rumen fluid components in the adsorption environment decreased the aflatoxin B_1 maximum adsorption capacity of sodium bentonite. Despite the high affinity of this adsorbent to capture aflatoxin B_1, different substances present in the environment could affect the adsorption capacity, at least at low toxin concentrations that mimic chronic exposure. The environment of the gastrointestinal tract, in either monogastric or ruminant animals, affect *in vivo* aflatoxin B_1 adsorption by sodium bentonite and should be taken into account when an *in vitro* performance evaluation is done.

Keywords: aflatoxin B_1; sodium bentonite; adsorption; HPLC

Introduction

Aflatoxins (AFs) are secondary metabolites of some strains of *Aspergillus flavus*, *A. parasiticus* and *A. nominus*, molds that grow on food and feed crops. Twenty AFs have already been identified, with aflatoxin B_1 (AFB_1) being one of the most common and toxic compounds present in avian feed (Hussein & Brasel 2001). The toxicity of AFs in broilers has been widely investigated for their carcinogenic, mutagenic, teratogenic and growth inhibitory effects (Oğuz et al. 2000; Sur & Celik 2003). Animals that consume AF-contaminated feed develop various health problems, including growth retardation, reduction in feed efficiency, and liver and kidney damage (Bintvihok 2002). Humans are exposed to AFs directly by the consumption of contaminated food or indirectly by the consumption of products derived from animals that have consumed AF-contaminated feed (Bennett & Klich 2003). The presence of AFB_1 in rations leads to the appearance of aflatoxin M_1 in milk as a consequence of a biotransformation process by enzymes associated with cytochrome P450 in liver (Richard et al. 2003). Also, the filtering and pasteurisation processes do not remove or inactivate the toxin (Galvano et al. 1996).

A variety of physical, chemical and biological approaches used to counteract the mycotoxin problem have been reported in the literature (Zaki et al. 2012). However, large-scale, practical and cost-effective methods for a complete detoxification of mycotoxin-containing feedstuffs are currently not available. One of the resources used in the prevention of aflatoxicosis is the incorporation of dietary non-nutritive substances that effectively prevent aflatoxicosis in birds (Phillips et al. 1988; Kubena, Harvey, Huff, et al. 1990; Kubena, Harvey, Phillips, et al. 1990; Huff et al. 1992; Scheideler 1993; Magnoli, Monge, et al. 2011; Magnoli, Texeira, et al. 2011) and cows (Thieu & Pettersson 2008). The effectiveness of these additives seems to depend on their ability to bind aflatoxin in the gastrointestinal tract.

Particularly, several authors have demonstrated the effectiveness of sodium bentonite (NaB) to adsorb AFB_1 (Deng et al. 2010, and reference therein). Bentonites are smectite clays with a 2:1 layered structure with an inner aluminium octahedral layer that shares oxygen atoms with two outer silicon tetrahedral sheets. The substitution of Si^{4+} by Al^{3+} in the tetrahedral sheets and the substitution of trivalent by divalent cations in the octahedral sheets give rise to a negative charge in the clay framework. This charge is counterbalanced by monovalent and divalent cations, such as Na^+, K^+, Ca^{2+} and Mg^{2+}, located in the interlamellar space. These ions are easily

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exchangeable and are responsible for the cation exchange ability of the clays. Organic substances can be adsorbed in the clay, not only in the external basal surfaces and edges but also in the interlayer spaces (Dakovic et al. 2008).

An inert, stable and insoluble complex between NaB and AFB_1 was assumed to be responsible for preventing toxin absorption in the intestine (Chiacchiera et al. 2000; Miazzo et al. 2000; Rosa et al. 2001; Miazzo et al. 2005). Different mechanisms have been proposed to explain the adsorption. Grant and Phillips (1998) proposed for a hydrate of sodium aluminium silicate a mechanism involving electron donor-acceptor interactions between AFB_1 molecules with optimal planar orientations and the interlayer surface. These authors demonstrated a good correlation between the magnitude of the partial positive charges on carbons C_1 and C_{11} of the β-dicarbonyl system and the strength of adsorption of planar analogues and derivatives of AFB_1. This evidence supported the electron donor-acceptor-binding mechanism assumption that involves the sharing of electrons from the negative surface of the clay with atoms in the absorbed molecule that are electron deficient. Other potential mechanisms may involve the chelation of transition metal counterions at the interlayer, or interaction of AFB_1 with positive-edge metal sites in the clay structure (Deng et al. 2010). The knowledge of the binding interactions and the way they are affected by the experimental conditions allow improving the efficiency of the adsorbent utilisation.

In vitro assays are the best and cheaper way to perform an easy pre-selection of adsorbents. Although expensive, laborious and time-consuming, *in vivo* trials with potentially useful adsorbents have to be performed prior to the ultimate adsorbent proposal. Different factors, such as pH, feed composition and additives, can affect the mycotoxin binding during digestion. In fact, recent studies have shown the influence of the coccidiostat monensin upon the detoxification potential of an Argentinean NaB (Magnoli, Monge, et al. 2011; Magnoli, Texeira, et al. 2011).

The awareness of potential interference in the detoxification procedure allows us to take decisions about the prevention protocol and also about the need to develop new adsorbents. To elucidate the intrinsic nature of the adsorption and the way it is affected by experimental conditions, a deep systematic study of the adsorption process has to be conducted. Therefore, the main objective of this study was to evaluate the influence of pH, ionic strength and ruminal fluid on the adsorption capacity of AFB_1 by NaB under simulated gastrointestinal conditions of monogastric and ruminant animals.

Materials and methods

Reagents

A previously characterised NaB from a mine in the province of Mendoza-Argentina, mainly composed of sodium montmorillonites, was used for the assays (Magnoli et al. 2008). The adsorbent was activated for 24 h at 110°C in a vacuum oven (Vacuum over Yamato ADP-31).

Production and purification of AFB_1

AFs for *in vitro* assays were produced via the fermentation of milled corn by *A. parasiticus* NRRL 3000. The sterile substrate placed in Erlenmeyer flasks was inoculated with 2 mL of the mold's aqueous suspension containing 10^6 spores/mL. Cultures were allowed to grow for 7 days at 25°C in darkness. At the seventh day, Erlenmeyer flasks were autoclaved and culture material dried at 40°C in a forced-air oven for 48 h. AFs were extracted with chloroform and purified by flash chromatography following the procedure described in AOAC (1994). Spectrophotometric determinations of the content of total AFs in the purified extract of the culture were carried out by UV-Vis spectroscopy, assuming that the molar absorptivities of AFB_1 and AFG_1 are not very different, which was corroborated by HPLC according to Trucksess et al. (1994) and AOAC (1994).

Detection and quantification of AFB_1

Detection and quantification of AFB_1 were performed on a diode array spectrophotometer (Hewlett Packard model 8453, Waldbronn, Germany) and an HPLC equipment with a Gilson pump (Model 302) and detector of fluorescence (fluorometer Gilson model 121). The excitation range and emission wavelength ranges were 305–395 and 430–470 nm, respectively. A C_{18} Luna Phenomenex column (150 mm × 4.6 mm, 5 μm) with the corresponding pre-column was used. The mobile phase was methanol/acetonitrile/water (1:1:4 v/v) at a flow rate of 1.0 mL/min and precolumn derivatisation. For derivatisation, aliquots (200 μL) were allowed to react with 700 μL of acetic acid/trifluoroacetic acid/water (20:10:70) solution. The tube was allowed to stand for 9 min at 65°C in the dark (AOAC 1994). The AFs in the extract were mainly AFB_1 and AFG_1. The calibration curve was made with a mixture of solutions of AFB_1, AFG_1, AFG_2 and AFB_2 (purity >99%, Sigma Chemical Co., Louis, MO, USA), with concentrations 2.06, 1.99, 0.520 and 0.508 μg/mL, respectively. The concentrations of chromatographic standards were 5, 10 and 15 ng/mL of AFB_1. Standard solutions for the calibration curves were prepared daily.

Adsorption experiments

Different AFB_1 working solutions with concentrations ranging from 0.18×10^{-5} to 3.5×10^{-5} M were prepared at the corresponding experimental conditions. Aliquots of 40 μL of a pH-stabilised NaB suspension (1 mg/mL) were added to 4 mL of each AFB_1 working solution. The solutions were incubated in an orbital shaker for 1 h at 39.5 ± 0.5°C to simulate the gastrointestinal tract

conditions. After incubation, the solutions were centrifuged for 15 min at 16,000 g and the supernatant was carefully decanted into a clean tube. Controls of AFB_1 and blanks with the NaB were also included for comparison in each isothermal assay. The adsorbed AFB_1 was calculated from the depletion of the toxin in the supernatant after incubation. Adsorptions experiments at each toxin concentration were performed in triplicate.

Buffer at pH 2 was prepared by mixing 62.5 mL of 0.2 M sodium chloride with 16.25 mL of 0.2 M hydrochloric acid. The final pH value was adjusted to make up the volume to 250 mL. Buffer at pH 4 was prepared following the same general procedure but mixing 125 mL of potassium hydrogen phthalate (0.1 M) with 0.1 mL solution of hydrochloric acid (0.1 M). Buffer at pH 6 was prepared by mixing 125 mL of potassium dibasic phosphate (0.1 M) with 14 mL of sodium hydroxide (0.1 M) following the procedure described above.

Assays carried in water brought to pH 2, 4 and 6 were performed by using as solvent distilled water carried to corresponding pHs by the addition of a proper amount of a HCl and NaOH solution. In the experiment carried under controlled ionic strength, a NaCl (0.15 M) solution in buffer at pH 2 was used as solvent.

The ruminal fluid was collected from a good health female adult cow from experimental fields (Facultad de Agronomia y Veterinaria, Universidad Nacional de Rio Cuarto). The extraction was performed by trained personnel following strict hygienic procedures. The material remained for 1 h at room temperature, was filtered three times through sterile gauze and further centrifuged for 5 min at 16,000 g to discard solids. Fresh supernatant was used for the isotherm assay.

Curve fitting and data processing

Curves representing the amount of bounded AFB_1 as a function of the concentration of the free toxin in equilibrium after adsorption were plotted. Two theoretical models – Langmuir (L) and Frumkin-Fowler-Guggenheim (FFG) – were selected from the literature to fit the isotherms (Giles, D'Silva, et al. 1974; Giles, Smith, et al.1974; Hans Jürgen et al. 2003). The selection was made following the criteria suggested by Hinz (2001). Mathematical expressions and parameters of each model are shown in Table 1. The surface excess of AFB_1 (Γ_{AFB_1}) in moles of AFB_1/kg of adsorbent was determined as follows:

$$\Gamma_{AFB_1} = \frac{\left[\left([AFB_1]_0 - [AFB_1]_{,eq}\right) \times V\right]}{m}$$

where $[AFB_1]_0$ and $[AFB_1]_{eq}$ are the initial and the equilibrium concentrations (mol/L), respectively, V is the volume of solution (L) and "m" is the amount of adsorbent (kg). A nonlinear least squares method, with a tolerance limit of 0.05, was used for curve fitting.

Table 1. Theoretical adsorption models, mathematical equations and adjusting parameters.

Models	Mathematical expression	Parameters
Langmuir	$\beta = \frac{\Gamma}{(\Gamma\max - \Gamma)[AFB_1]}$	Γ_{max}, β
FFG	$\beta = \left[\frac{\Gamma}{(\Gamma\max - \Gamma)[AFB_1]}\right] \exp(-2a\Gamma/\Gamma_{max})$	Γ_{max}, β, a

Notes. Γ is AFB_1 surface excess per kilogram of NaB, $[AFB_1]$ is the residual toxin at equilibrium, Γ_{max} is the surface excess at saturation per kilogram of adsorbent, β is the Langmuir adsorption constant (L) or the extrapolated adsorption constant at low coverage in the case of FFG and a is the FFG parameter that measures the interaction between adsorbed AFB_1 molecules.
AFB_1, aflatoxin B_1; FFG, Frumkin-Fowler-Guggenheim.

Results and discussion

Previous adsorption studies carried out with NaB from different geological sources demonstrated that the adsorption capacity of AFB_1 seemed to be related to both the isomorphic substitution and the surface charges of the montmorillonite component of the mineral (Magnoli et al. 2008). The results of the comparative adsorption study showed that the particular NaB used in the present assay had the best performance as AFB_1 binder and therefore was selected to conduct further *in vitro* studies.

Figure 1 shows the effect of buffer (pH 2, 4 and 6) on AFB_1 adsorption isotherms on NaB. The isotherms at pH 2 and pH 6 were both S type while at pH 4 a Langmuir behaviour was observed. Langmuir and FFG models are appropriate to explain L- and S-type isotherms, respectively. The mathematical expressions and the fitting parameters for each model are shown in Table 1. The fitting curves are shown as solid lines superposed to the corresponding isotherm graphs, and the fitting parameters are collected in

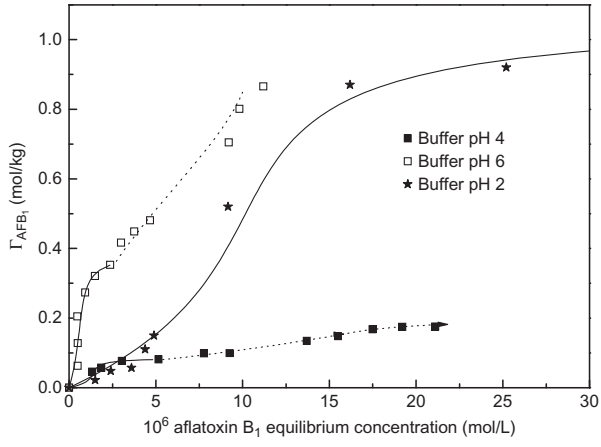

Figure 1. AFB_1 adsorption isotherms obtained at different pHs. Note: AFB_1, aflatoxin B_1.

Table 2. Adjustment parameters.

	$10^{-4}\ \beta\ (M^{-1})$	Γ_{max} (mol/kg)	a	R^2	N
Buffer pH 2	2.2 ± 0.4	1.07 ± 0.02	1.5 ± 0.2	0.98	10
Buffer pH 4	93 ± 11	0.17 ± 0.01	0	0.99	6
Buffer pH 6	43 ± 16	0.39 ± 0.01	1.3 ± 0.3	0.97	13
Water brought to pH 4	48 ± 6	0.12 ± 0.01	0	0.99	7
Water brought to pH 6	13 ± 2	0.14 ± 0.01	0.6 ± 0.2	0.98	20
NaCl 0.15 M, buffer pH 2	13 ± 4	0.37 ± 0.04	0	0.97	7
Rumen fluid	13 ± 3	0.051 ± 0.001	1.04 ± 0.2	0.95	8

Table 2. The high values of the adsorption constant (β) showed an elevated *in vitro* affinity between the adsorbent and the toxin at all of the assayed pHs. Higher AFB_1 affinities were observed at pH 4 and 6. Positive values of the FFG parameters a at pH 2 and 6 (1.5 ± 0.2 and 1.3 ± 0.3, respectively) suggest a cooperative adsorption mechanism. A value of FFG parameter a close to zero was obtained at pH 4, demonstrating the equivalence of the adsorption sites on the adsorbent surface and the suitability of the Langmuir model to perform the data fitting.

As can be observed in Table 2, the highest surface excess at monolayer coverage (Γ_{max}) was observed in buffer at pH 2 (1.07 ± 0.02 mol/kg) while the lowest value was observed in buffer at pH 4. An intermediate value for the monolayer saturation was observed at pH 6, although an increase after the plateau was observed in this case. The completion of a second layer adsorbed on top of the first was not achieved at least within the assayed concentration range. Summarising, the surface excess was affected by different buffers. A similar behaviour was reported by Thieu and Pettersson (2008), although the reports in the literature are controversial because Dakovic et al. (2008) and Diaz et al. (2003) reported that the adsorption capacity of AFB_1 did not change with the pH. Phillips et al. (1988) found no differences in AFB_1 binding at pH 2, 7, and 10 with a related hydrated sodium calcium aluminosilicate.

Desheng et al. (2005) demonstrated that the maximum amount of adsorbed AFB_1 was obtained from aqueous solution at pH 2 using a calcium montmorillonite as adsorbent. Komadel (2003) suggested that at pH ≤3.0, the hydroxyl groups of the bentonite octahedral layer were attacked by protons' penetration in the phase and the layer started to redissolve. This fact could explain at least partially the observed results.

The higher adsorption capacity (Γ_{max}) was obtained at a pH close to the zeta potential of the NaB, that is, 6.2 (Magnoli et al. 2008). The significant decrease in the number of sites in the monolayer at pH 4 could hardly be attributed to a unique effect of pH. The competitive adsorption of the buffers ions, whose concentration was three orders of magnitude higher than that of AFB_1, could be affecting the toxin-adsorbent interaction. Therefore, as both the pH and the buffer ions could be responsible for modifying the bentonite surface charges, the sites availability and/or the aggregation state of the adsorbent, the influence of specific buffer ions was investigated.

To check the influence of buffer ions on the toxin adsorption at pH 4, the isotherm obtained in buffer solution was compared with one obtained under external pH control (Figure 2). Langmuir isotherms were observed in both cases; solid lines on top of the experimental data show the fitting curves. The adjusting parameters are collected in Table 2. The Langmuir behaviour indicates a finite number of equivalent adsorption sites in a monolayer arrangement on the absorbent surface. A decrease in both β and Γ_{max} was observed in the presence of buffer ions. This can be attributed to the competition between the phthalate ions, mainly hydrogen phthalate, and AFB_1 molecules for the surface adsorption sites. Phthalate ions are planar and have two carbonyls that are able to interact through the electron donor acceptor complex with the

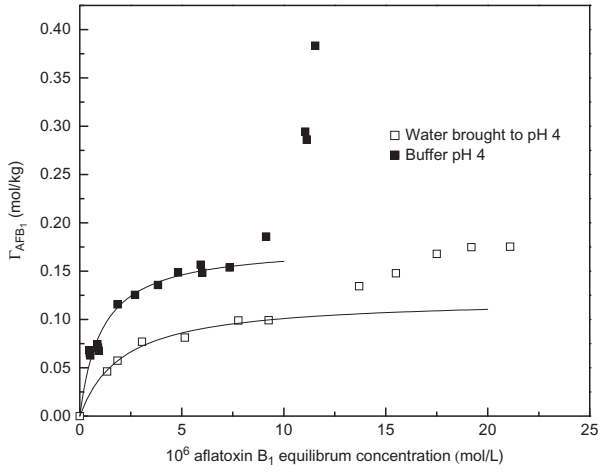

Figure 2. Effect of phthalate ions on the adsorption of AFB_1 on NaB.
Note: AFB_1, aflatoxin B_1.

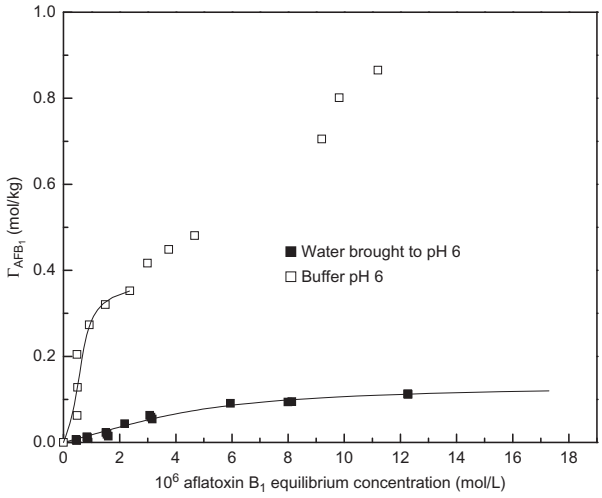

Figure 3. Effect of phosphate ions on the adsorption of AFB_1 on NaB.
Note: AFB_1, aflatoxin B_1.

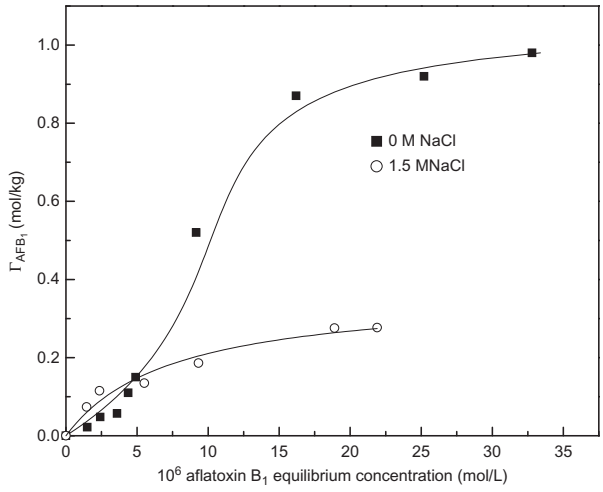

Figure 4. Adsorption of AFB_1 at pH 2 under ionic strength control.
Note: AFB_1, aflatoxin B_1.

surface in a similar way as proposed by Grant and Phillips (1998) for AFB_1 adsorption.

In a similar way, Figure 3 shows the effect of phosphate ions present in pH 6 buffer solution on AFB_1 adsorption isotherms. The isotherms are both S shaped and were fitted with the FFG equation. The fitting parameters are shown in Table 2. Both the surface excess at the saturation (Γ_{max}) and the adsorption constant (β) were higher in the presence of phosphate ions. Taking into account the positive values of the FFG parameter a (1.3 ± 0.3 and 0.6 ± 0.2, respectively), a cooperative adsorption mechanism might be operating. The species in the buffer solution at pH 6 are mainly hydrogen phosphate and dihydrogen phosphate ions. It is known that phosphate ions strongly interact with montmorillonites, yielding an increase in the edge charge density that affects the coagulation (Lagaly & Ziesmer 2003). Therefore, the presence of these ions could decrease the clay aggregation, making it more accessible for toxin adsorption at the interlayer surface.

An experiment with ionic strength control was carried out in buffer at pH 2. Figure 4 shows the effect of 0.15 M of NaCl on the AFB_1 adsorption. The isotherm in the presence of NaCl was L-shaped and could be fitted according to the Langmuir model, while, as previously shown, the isotherm in buffer pH 2 was S-shaped. The adjustment parameters are included in Table 2. A significant decrease in Γ_{max} along with an increase in β was observed in the presence of an excess of NaCl. Therefore, NaCl strongly competes with the toxin for adsorption sites, and therefore decreases the probability of lateral interactions between toxin molecules that occupy neighbour sites in the surface. The competence of such lateral interactions between adsorbed neighbour molecules with the interactions between these molecules and the surface is responsible for the cooperative binding mechanism. These lateral interactions are responsible for the dependence of the desorption activation energy on the degree of coverage.

In ruminant it is known that the residence time of feed in the rumen is greater than in other digestive organs; therefore, rumen is the place where AFB_1 adsorption could take place (Adin et al. 2009; Gookin et al. 2009). Figure 5 shows the effect of rumen fluid on AFB_1 adsorption isotherm. Rumen pH is either neutral or slightly acidic (6–7), and their environment and physiology are variable according to health status, age and animal feed. The isotherm obtained in water brought to pH 6 was also included in the graph for the sake of comparison. Slightly sigmoidal isotherms were obtained in both cases and therefore were adjusted by the FFG equation. The adjustment parameters are shown in Table 2. The maximum adsorption capacity at monolayer coverage was influenced by the rumen fluid, and a decrease in the surface excess at monolayer saturation (Γ_{max} = 0.051 ± 0.001 mol/kg) was observed. However, strong adsorbent affinities for AFB_1, denoted by a high β value, were observed. Unlike what was observed in Figure 4, no changes in adsorption mechanism were promoted by rumen fluid components. The cooperative mechanism characteristic of pH 6 remains operating in a rumen environment, as shown for positive FFG parameters a. As can be observed, the presence of rumen fluid components decreased about 74% the maximum adsorption capacity at monolayer coverage NaB. Similar results were previously reported by Spotti et al. (2005). Thieu and Pettersson (2008) also found that the adsorption of AFB_1 on a bentonite changed in the presence of gastrointestinal fluid. As can be observed in Figure 5, for the study in rumen fluid, an inflection point appears on the top of the

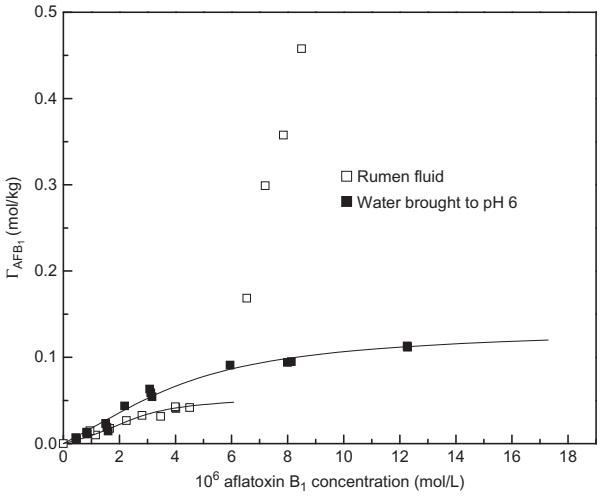

Figure 5. Effect of rumen fluid components on the AFB_1 adsorption isotherm by NaB.
Note: AFB_1, aflatoxin B_1.

first monolayer with a fast increase in the adsorption as the toxin concentration increases.

Previous *in vitro* studies have also shown that monensin, an antibiotic agent used in poultry industry as a prophylactic therapy against coccidian, competes with AFB_1 for the adsorption sites on the NaB. Liver histopathology demonstrated the interfering of monensin with the ability of NaB to prevent chronic aflatoxicosis (Magnoli, Monge, et al. 2011; Magnoli, Texeira, et al. 2011). These results suggested that the presence of different substances, such as coccidiostats, vitamins, minerals, amino acids or other dietary components, could affect the ability of the adsorbent to bind low levels of AFB_1. This behaviour is particularly important at low concentrations of the toxin because when the toxin concentration increases, the monensin displacement by the toxin was observed.

Differences in the behaviour of clay adsorbents even of the same nature could be explained by chemical and structural differences that could affect the AFB_1 binding ability. The composition of the clays may vary with the mine source and within a mine with clay location (Magnoli et al. 2008). During digestion, pH, feed composition and the presence of specific ions or molecules can affect the mycotoxin binding to the bentonite.

The assayed NaB showed that no matter the high affinity of NaB to adsorb AFB_1, different substances present in the environment could affect the adsorption capacity to bind AFB_1, at least at low toxin concentration that mimics chronic exposure. The environment present in the gastrointestinal tract, either monogastric or ruminant, could affect *in vivo* AFB_1 adsorption by the bentonite. Therefore, care should be taken to select the best conditions to perform *in vitro* studies under physiological simulated conditions to perform a good *in vitro* evaluation of the adsorbent.

Author Note

Alejandra Magnoli and Veronica Alonso contributed equally to the development of assays.

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References

Adin G, Solomon R, Nikbachat M, Zenou A, Yosef E, Brosh A, Shabtay A, Mabjeesh SJ, Halachmi I, Miron J. 2009. Effect of feeding cows in early lactation with diets differing in roughage-neutral detergent fiber content on intake behavior, rumination, and milk production. J Dairy Sci. 92:3364–3373.

[AOAC] Association of Official Agricultural Chemists. 1994. Official methods of analysis 979.22 see column chromatographic procedure. Gaithersburg, MA: AOAC.

Bennett JW, Klich M. 2003. Mycotoxins. Clin Microbiol Rev. 16:497–516.

Bintvihok A. 2002. New insights to controlling mycotoxin danger in ducks. Feed Tech. 6:28–29.

Chiacchiera SM, Magnoli CE, Astorga P, Miazzo R, Combina M, Dalcero AM, Kikot E, Basaldella E. 2000. Use of synthetic zeolites to adsorb different mycotoxins, prevention of mycotoxicoses. Atual Fisicoquím Org. 12:218–236.

Dakovic A, Matijasevic S, Rottinghaus GE, Ledoux DR, Butkeraitis P, Sekulic Z. 2008. Aflatoxin B_1 adsorption by natural and copper modified montmorillonite. Colloid Surface B. 66:20–25.

Deng Y, Barrientos Velázquez AL, Billes F, Dixon JB. 2010. Bonding mechanisms between aflatoxin B1 and smectite. Appl Clay Sci. 50:92–98.

Desheng Q, Fan L, Yanhu Y, Niya Z. 2005. Adsorption of aflatoxin B1 on Montmorillonite. Poultry Sci. 84:959–961.

Diaz DE, Hagler Jr WM, Hopkins BA, Whitlow LW. 2003. Aflatoxin Binder I: *in vitro* binding assay for aflatoxin B1 by several potential sequestering agents. Mycopathologia. 156:223–226.

Galvano F, Galofaro V, Galvano G. 1996. Occurrence and stability of aflatoxin M1 in milk and milk products: a worldwide review. J Food Prot. 59:1076–1090.

Giles CH, Smith D, Huitson A. 1974. A general treatment and classification of the solute adsorption isotherm: I. Theor J Colloid Interface Sci. 47:755–765.

Giles HC, D'Silva AP, Easton IA. 1974. A general treatment and classification of the solute adsorption isotherm. Part. II. Experimental interpretation. J Colloid Interface Sci. 47: 766–778.

Gookin JL, Foster DM, Harvey AM, McWhorter D. 2009. An animated model of reticulo rumen motility. J Vet Med Educ. 36:444–447.

Grant PG, Phillips TD. 1998. Isothermal adsorption of aflatoxin B_1 on HSCAS clay. J Agric Food Chem. 46:599–605.

Hans Jürgen B, Graf K, Kappl M. 2003. Physics and chemistry of interfaces. Weinheim: Wiley VCH Verlag GmbH & Co, KGaA.

Hinz C. 2001. Description of sorption data with isotherm equations. Geoderma. 99:225–243.

Huff WE, Kubena LF, Harvey RB, Phillips TD. 1992. Efficacy of hydrated sodium calcium aluminosilicate to reduce the individual and combined toxicity of aflatoxin and ochratoxin A. Poultry Sci. 71:64–69.

Hussein HS, Brasel M. 2001. Toxicity, metabolism, and impact of mycotoxins on humans and animals. Toxicol Rev. 167: 101–134.

Komadel P. 2003. Chemically modified smectites. Clay Miner. 38:127–138.

Kubena LF, Harvey RB, Huff WE, Corrier DE, Phillips TD, Rottinghaus GE. 1990. Efficacy of a hydrated sodium calcium aluminosilicate to reduce the toxicity of aflatoxin and T-2 toxin. Poultry Sci. 69:1078–1086.

Kubena LF, Harvey RB, Phillips TD, Corrier DE, Huff WE. 1990. Diminution of aflatoxicosis in growing chickens by the dietary addition of a hydrated, sodium calcium aluminosilicate. Poultry Sci. 69:727–735.

Lagaly G, Ziesmer S. 2003. Colloid chemistry of clay minerals: the coagulation of montmorillonite dispersions Adv. Colloid Interface Sci. 100:105–128.

Magnoli AP, Monge MP, Miazzo RD, Cavaglieri LR, Magnoli CE, Merkis CI, Cristofolini AL, Dalcero AM, Chiacchiera SM. 2011. Effect of low levels of aflatoxin B1 on performance, biochemical parameters, and aflatoxin B1 in broiler liver tissues in the presence of monensin and sodium bentonite. Poultry Sci. 90:48–58.

Magnoli AP, Tallone L, Rosa CAR, Dalcero AM, Chiacchiera SM, Torres Sanchez RM. 2008. Commercial bentonites as detoxifier of broiler feed contaminated with aflatoxin. Appl Clay Sci. 40:63–71.

Magnoli AP, Texeira M, Rosa CAR, Miazzo RD, Cavaglieri LR, Magnoli CE. 2011. Sodium bentonite and monensin under chronic aflatoxicosis in broiler chickens. Poultry Sci. 90:352–357.

Miazzo R, Peralta MF, Magnoli C, Salvano M, Ferrero S, Chiacchiera SM, Carvalho ECQ, Rosa CA, Dalcero A. 2005. Efficacy of sodium bentonite as a detoxifier of broiler feed contaminated with aflatoxin and fumonisin. Poultry Sci. 84:1–8.

Miazzo R, Rosa RCA, Carvalho De Queiroz EC, Magnoli C, Chiacchiera SM, Palacio G, Saenz M, Kikot A, Basaldella E, Dalcero A. 2000. Efficacy of synthetic zeolite to reduce the toxicity of aflatoxin in broiler chicks. Poultry Sci. 79:1–6.

Oğuz H, Kurtoglu V, Coskun B. 2000. Preventive efficacy of clinoptilolite in broiler during chronic aflatoxin (50 and 100 ppb) exposure. Res Vet Sci. 69:197–201.

Phillips TD, Kubena LF, Harvey RB, Taylor DR, Heidelbaugh ND. 1988. Hydrated sodium calcium aluminosilicate: a high affinity sorbent for aflatoxin. Poultry Sci. 67:243–247.

Richard JL, Payne GA, Desjardin AE, Maragos C, Norred WP, Pestka JJ, Phillips TD, Van Egmond HP, Vardon PJ, Whitaker TB, et al. 2003. Mycotoxins, risks in plant, animal and human systems. CAST Task Force Report 139. Ames (IA): Council for Agricultural Science and Technology.

Rosa CAR, Miazzo R, Magnoli C, Salvano M, Chiacchiera SM, Ferrero S, Saenz M, Carvalho ECQ, Dalcero A. 2001. Evaluation of the efficacy of bentonite from the south of Argentina to ameliorate the toxic effects of aflatoxin in broilers. Poultry Sci. 80:139–144.

Scheideler SE. 1993. Effects of various types of aluminosilicates and aflatoxin B_1 on aflatoxin toxicity, chick performance and mineral status. Poultry Sci. 72:282–288.

Spotti M, Fracchiolla M, Arioli F, Caloni F, Pompa G. 2005. Aflatoxin B_1 binding to sorbent in bovine ruminal fluid. Vet Res Commun. 29:507–515.

Sur E, Celik I. 2003. Effects of aflatoxin B1 on the development of the bursa of Fabricius and blood lymphocyte acid phosphatase of the chicken. Br Poultry Sci. 44:558–566.

Thieu NQ, Pettersson H. 2008. In vitro evaluation of the capacity of zeolite and bentonite to adsorb aflatoxin B1 in simulated gastrointestinal fluids. Mycotoxin Res. 24:124–129.

Trucksess MW, Stack ME, Nesheim S, Albert RH, Romer TR. 1994. Multifunctional column coupled with liquid chromatography for determination of aflatoxins B1, B2, G1, and G2 in corn, almonds, brazil nuts, peanuts, and pistachio nuts: collaborative study. J AOAC Int. 77:1512–1521.

Zaki MM, El-Midany SA, Shaheen HM, Rizzi L. 2012. Mycotoxins in animals: occurrence, effects, prevention and management. J Toxicol Environ Health Sci Rev. 4:13–28.