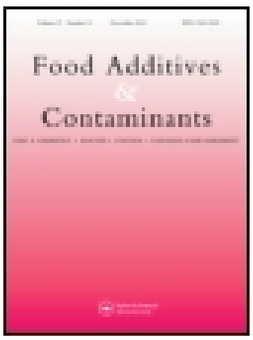

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Activated carbons as potentially useful non-nutritive additives to prevent the effect of fumonisin B_1 on the sodium bentonite activity against chronic aflatoxicosis

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Abstract

Aflatoxin B_1 (AFB_1) and Fumonisin B_1 (FB_1) are mycotoxins that often co-occur in feedstuffs. The ingestion of AFB_1 causes aflatoxicosis in humans and animals. Sodium bentonite (NaB), a cheap non-nutritive unselective sequestering agent incorporated in animal diets, can effectively prevent aflatoxicosis. Fumonisins are responsible for equine leukoencephalomalacia and porcine pulmonary oedema, and often have subclinical toxic effects in poultries. Fumonisin B_1 and aflatoxin B_1 are both strongly adsorbed *in vitro* on sodium bentonite. Co-adsorption studies, carried out with a weight ratio FB_1 to AFB_1 that mimics the natural occurrence (200:1), showed that FB_1 greatly decreases the *in vitro* ability of NaB to adsorb AFB_1. The ability of two activated carbons (AC) to adsorb FB_1 was also investigated. Both carbons showed high affinity for FB_1. A complex behaviour of the FB_1 adsorption isotherms with pH was observed. *In vitro* results suggest that under natural contamination levels of AFB_1 and FB_1, a mixture of activated carbon and sodium bentonite might be potentially useful for prevention of sub-acute aflatoxicosis.

Key words: aflatoxin B_1; fumonisin B_1, sodium bentonite; activated carbons; adsorption.

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Introduction

Aflatoxins (AFs) are among the most studied group of mycotoxins and their production is attributed to *Aspergillus flavus* and *A. parasiticus* species. Aflatoxin B_1 (AFB_1) is the most potent natural carcinogen known and is classified by the International Agency of Research on Cancer as a Group 1 carcinogen (IARC, 1993). Human and animals are severely affected by AFB_1, although in a dose dependent way. Thus, if AFB_1 is consumed in large doses it may be lethal while sub-lethal doses produce a chronic aflatoxicosis (CAST, 2003). In particular, aflatoxins produce hepatic and kidney disorders, suppress immune function and decrease productive parameters in poultry. Pale, enlarged, friable, and fatty livers are characteristic of acute aflatoxicosis (Yunus et al., 2011). As a result of the widespread occurrence of AFB_1 in feedstuffs and their deleterious effects on animal health, production and toxin carryover to human food chain, detoxification efforts have included a variety of methods (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 most useful approaches in poultry production is the incorporation of dietary non-nutritive sequestering substances that effective prevent mycotoxicosis. Several authors have demonstrated the effectiveness of different aluminosilicates (Phillips et al. 1988; Kubena et al. 1990a; 1990b; Huff et al. 1992; Scheideler 1993) and activated carbon (AC) (Galvano et al. 1996; Edrington et al. 1996; Avantaggiato 2007; Diaz et al. 2004) to adsorb AFB_1. In particular, different bentonites showed high affinity for this toxin both *in vitro* (Magnoli et al., 2008a) and *in vivo* (Rosa et al., 2001; Miazzo et al., 2005; Magnoli et al., 2008b, 2011a;b). In livestock production bentonite feed additives are suitable because of their effectiveness, availability and low cost. However, given the non-selective nature of these adsorbents, other components present

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in the diets can also be absorbed decreasing their bioavailability and/or affecting the capacity of the adsorbent to prevent aflatoxicosis (Magnoli et al. 2011a;b).

Several studies from many countries have shown both the prevalence of toxigenic mycobiota in poultry feeds and the incidence of AFs and FBs as the most frequently found mycotoxins (Oliveira et al. 2006, , Monge et al., 2012, 2013, Streit 2012).

Fumonisins (FBs) are mycotoxins produced mainly by *Fusarium verticillioides* and *F. proliferatum*. These toxins are responsible for two diseases of livestock, equine leukoencephalomalacia and porcine pulmonary oedema (CAST 2003). Among the naturally occurring FBs, fumonisin B_1 (FB_1) is usually the most abundant and represents about 70% of the total concentration in corn and feeds. Poultry are much less sensitive to FB_1 than pigs and horses, and most of the toxic effects are subclinical and often remain hidden (Voss et al., 2007).

Preliminary *in vitro* studies showed that fumonisin and AFB_1 were competitively adsorbed on a synthetic sodium zeolite type A at pH 2, although a subsequent pH increase leads to FB_1 release from the adsorbent (Kikot et al. 2002). Additionally, *in vivo* studies carried out with poultry using 0.3% of a cheaper but less selective sodium bentonite as toxin sequestering agent, have shown amelioration of the effects of acute induced aflatoxicosis but was not able to return the body weight gains to control values when the birds were fed diets containing both AFB_1 and FB_1 (Miazzo et al., 2005). It has to be emphasized that FB_1 alone did not produce any noticeable change compared to control either in productive and biochemical parameters or in liver histopathology of birds. However, this earlier study showed that FB_1 reduces the clay ability to ameliorate acute aflatoxicosis. These facts stresses the need to search potential candidates that can either selectively adsorb AFB_1 or be combined with the aluminosilicates to adsorb FB_1 in

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order to prevent the potential interference of this toxin on the clay-mediated AFB_1 decontamination process.

On the other hand, it has been shown that activated carbons (AC) effectively adsorb FB_1 *in vitro* although their *in vivo* efficacies are still quite controversial (Galvano et al. 1996, 1999; Avantaggiato et al. 2005). It has been suggested that the adsorptive properties are mainly related to both surface chemical functional groups and porous texture (Piva et al., 2007, Galvano et al., 2001). Therefore, the ability of a given activated carbon depends on the source of the raw materials as well as the conditions of synthesis and activation.

Therefore, the aims of the present work were: (1) to evaluate the *in vitro* effect of FB_1 on the AFB_1 binding of a sodium bentonite with a proven ability to prevent aflatoxicosis; (2) to evaluate the potential ability of two activated carbons, from a common precursor (eucalyptus wood) and different methods of preparation, to adsorb both FB_1 and AFB_1 under simulated gastrointestinal conditions; and (3) to study the reversibility and the environmental pH on the adsorption process.

Materials and Methods

Reagents

Aflatoxin B_1 and FB_1 were obtained from Sigma-Aldrich Chemical (Dorset, UK). Sodium bentonite was characterized as described in Magnoli et al. (2008) and activated at 110°C in a vacuum oven (Vacuum over Yamato ADP-31) for 24 h. The activated carbons were synthesized and characterized as described in Milich et al. (2002). Assays were carried out in 0.15 M NaCl in order to control the ionic strength. The pH values of the solutions were brought to pH 2, 4 or 6 by adding an appropriate amount of 6 M HCl or NaOH.

Analytical methods for adsorbates quantification

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The analysis of the toxins were carried out by High Performance Liquid chromatography (HPLC). Aflatoxin B_1 quantification was performed in a Waters e2695 separations Module (Waters Corporation, Milford, MA, USA), equipped with an autosampler and controller with dual pump, coupled to a photodiode array detector (Waters 2998) and a reverse phase column (Luna™ 5 mm C18(2) 100A, 150 x 4.6mm, Phenomenex Inc.) with a guard column (C18, 4 x 3.0 mm, Phenomenex Inc.). Samples were run at room temperature under isocratic mode, using acetonitrile/methanol/water (1:1:4 v/v) as mobile phase at a flow rate of 1.5 mL/min. Samples were injected without derivatization. Retention time of underivatized AFB_1 was 4.6 min.

Fumonisin B_1 detection was performed with a Waters 2695 separation module (Waters Corporation, Milford, MA, USA) equipped with a 2695 autosampler and interfaced to a Micromass®-Quattro Ultima™ Platinum tandem quadrupole mass spectrometer with electrospray ionization (ESI) source. A XBridge™ C18 (3.5 µm, 2.1 x 150 mm) column with a XBridge BEH C18 Sentry Guard Cartridge (130Å, 3.5 µm, 2.1 x 10 mm). An isocratic chromatographic procedure was performed with 1% formic acid methanol: water (60:40 v/v) as mobile phase. The flow rate was 0.1 mL/min and the temperature of column was kept at 22°C. Data acquisition was performed in multiple reaction monitoring mode (MRM) for quantitative analysis. The precursor peak [M + H] and two product peaks (m/z 334 and 352) were monitored to accomplish both quantification and qualification criteria. The most abundant transition (722 > 352) was used for quantitative analysis of FB_1. The interface was operated in a positive ion mode. Nitrogen heated to 150^{o}C and 200^{o}C was used as nebulization and desolvation gases, respectively. The optimized nitrogen flow rates for nebulization and desolvation were 678 and 104 L/h, respectively. The capillary and cone voltages were 3.00 kV and 91 V, respectively. The collision energies were 55 and 57 eV for m/z 334 and 352, respectively. Dwell time was set at 0.1 ms for all transitions. **S**amples were injected without derivatization and the retention time of fumonisin was 14.5 min.

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Adsorption experiments of aflatoxin B_1 on sodium bentonite

Different AFB_1 working solutions within a concentrations range from 0.24-4.3 µM were prepared using a solution 0.15 M of NaCl at the working pH as solvent. Aliquots of 20 µl of a pH stabilized NaB suspension (1 mg/ml) were added to 400 ml of each AFB_1 working solution. The solutions were incubated in an orbital shaker for 1h at 39.5 ± 1ºC to simulate, at least partially, the gastrointestinal tract conditions. After incubation, the solutions were centrifuged 15 min at 16,000 × g, and supernatants were carefully decanted into clean tubes. Controls of toxins, and blanks with the adsorbent were performed in each assay. The adsorbed AFB_1 was measured by the toxin depletion in the supernatant after incubation. Adsorptions at each toxin concentration were performed in duplicate. The AFB_1 concentration in each supernatant was determined by HPLC (Trucksess et al. 1994).

Co-adsorption experiments the aflatoxin B_1-fumonisin B_1 with sodium bentonite

Co-adsorption assays were performed following the methodology described above but using a solution 0.28 10^{-3}M of FB_1 in 0.15 M NaCl at pH 2 as solvent.

Adsorption experiments of FB_1 with sodium bentonite and activated carbon.

Working solutions with FB_1 concentrations ranging from 1.4 x 10^{-4} – 14 x 10^{-4} mM were prepared in 0.15 M NaCl at the corresponding pH. Aliquots of 40 µl of stabilized NaB suspension (1 mg/ml) or 30 µl of activated carbon suspension (0.006 mg/ml) were added to 400 µl of each FB_1 working solution following the same procedure described for AFB_1.The FB_1 concentration in each supernatant was determined by LC-MS/MS as described above.

Desorption experiments

Pellets left over from the adsorption experiments were used to carry out desorption assays. Thus, a 400 µl aliquot of the 0.15 M NaCl solution brought to the corresponding pH was

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thoroughly mixed with each pellet in microtubes, ultra-sonicated for 5 min, and then centrifuged at 16000 x g for 20 min. The adsorbed toxin was calculated taking into account the mass balance between the initial total amount of toxin and the concentration of toxin remaining in the supernatant after incubation.

Curve fitting and data processing

Curves representing the amount of bounded toxins as the free toxin concentration in equilibrium after adsorption were plotted. Different theoretical models were used to fit experimental data. Langmuir (L) and Frumkin-Fowler-Guggenheim (FFG) (Hinz 2001) and Guggenheim–Anderson–de Boer multilayer (GAB) isotherms (Guggenheim, 1966) were selected to fit the isotherms according to the observed curve shapes. Mathematical expressions and parameters of each model are shown in Table 1. The adsorbed toxins were determined by the following equation:

$$Q = \frac{\left[\left([\text{Toxin}]_0 - [\text{Toxin}]_{eq}\right) \text{ x } V\right]}{m}$$

where: Q is the amount of toxin retained on the adsorbent phase (mmol g^{-1} of dry adsorbent), $[\text{toxins}]_0$ and $[\text{toxins}]_{eq}$ are the initial and the equilibrium toxin concentrations in solutions (mmol L^{-1}), respectively. V is the volume of solution (L) and m is the mass of the adsorbent (g). A nonlinear least squares method with a tolerance limit of 0.05 was used for curve fitting.

Results and Discussion

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Figure 1 shows the AFB_1 adsorption and desorption isotherms on NaB carried out in 0.15 M NaCl solution brought to pH 2 with addition of the proper amount of HCl in order to mimic, at least partially, the gastrointestinal conditions. The pH of the solutions did not change during the adsorption process. A Langmuir type adsorption isotherm was obtained. The mathematical expression of the Langmuir model is shown in Table 1. The desorption isotherm showed that the process was partially reversible. The solid line shows the Langmuir fit of experimental data points and the fitting parameters are shown in Table 2. On the other hand, the adsorption isotherm of FB_1 on NaB, shown in Figure 2, exhibits the same behaviour as AFB_1. The Langmuir fit is displayed as a solid line in the plot and the adjusting parameters are also shown in Table 2. A totally irreversible process was observed as shown by the desorption isotherm in Figure 2.

Although NaB showed the highest affinity for FB_1 (see ^{2} values in Table 2), the order of magnitude of the adsorption constants indicate a high adsorbent affinity for both toxins. The binding efficiency of mycotoxins with adsorbents depends on their chemical and physical properties (Dakovic et al., 2008). Previous studies have shown that the amount of AFB_1 retained on sodium bentonites seemed to be related to both the isomorphic substitution and the surface charges of the montmorillonite, which corresponds to 70% of the mineralogical composition of the assayed bentonite (Magnoli et al. 2008). A combination of different adsorption mechanisms could probably be responsible for the AFB_1 binding to montmorillonite. Among them, a donor acceptor interaction between carbonyl groups of the aflatoxin molecules planar to the surface, as the acceptor moieties, and the negative charges of the clay surface acting as donor, have been proposed by Grant and Phillips (1998). The chelation of charge compensation cations by AFB_1 molecules in the montmorillonite interlayer, and the interaction of the toxin carbonyl groups with the positive-edge metal sites in the clay structure were also proposed as alternative mechanisms (Deng et al., 2010). On the other hand FB_1, a water-soluble polar toxin, can exist in cationic,

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anionic or zwitterionic forms depending on pH. At pH < 3 the protonated form may be electrostatically bounded to negatively charged surfaces of the clay. Such cationic exchange mechanism was already proposed for a dioctahedral smectite clay commercialized as NovaSil (Robinson et al., 2012).

Previous adsorption isotherm studies carried out using the same NaB and AFB_1 have shown a β value of (1.3 ± 0.4) x 10^5 M^{-1}, i.e. seven times lower than the one obtained in the present study (Magnoli et al., 2013). Temperature differences between experiments (39.5 ± 0.5)^oC in the former and room temperature in the present study) together with an exothermic adsorption process could explain the observed results. Pimpukdee et al. (2004) reported a high and negative adsorption enthalpy for AFB_1 adsorption on calcium montmorillonite.

Previous studies have shown S-shaped isotherms for AFB_1 adsorption on NaB from the toxin aqueous solutions (Magnoli et al., 2013). However, as previously showed, an excess of NaCl in the adsorption medium promotes L-shaped isotherms. A homogeneous ion saturated surface was assumed to be the responsible for the observed behaviour. Moreover, the amount of toxin retained on the NaB at saturation (Qmax = 2.38 ± 0.04 mmol g^{-1}) under the present experimental conditions was more than six times greater than the observed in the abovementioned work (0.37 ± 0.04 mmol g^{-1}). In colloidal clay dispersions of NaB, particles tend to interact, aggregate, and form volume-filling networks under various conditions (Christidis, 2011). Therefore, the lower amount of toxin retained in the former assay may be explained by changes in interlayer accessibility as clay aggregation takes place. A light scattering study of NaB suspensions, performed in our laboratory, showed that below 5% w/v the mean diameter of clay particles diminishes as the concentration of NaB is decreased (results not shown). Thus, the difference between the percentage of NaB among experiments (0.05 x 10^{-3} and 1 x 10^{-3} w/v% in

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the present and the former study, respectively), could explain the observed amounts of toxin retained on the adsorbent phase at saturation. Moreover, previous isotherms were performed in buffer at pH 2, and therefore, further differences could be expected (Magnoli, 2013). It is already known that operative variables as temperature, pH and adsorbent dose define the equilibrium systems with their adsorption isotherms and the corresponding thermodynamic parameters (Cotoruelo et al., 2011).

Besides, amount of toxins retained on NaB at saturation at pH 2 were 2.38 ± 0.04 and 25.4 ± 0.1 mmol g^{-1} for AFB_1 y FB_1, respectively. The value, ten times higher for FB_1 compared with AFB_1, can be explained due to favourable electrostatic interactions between the cationic form of FB_1 and the fixed anionic charges of the montmorillonite surface occurring at pH 2.

In order to investigate the effect of FB_1 on AFB_1 adsorption under competitive conditions were performed. Results are shown in Figure 3. In the isotherm plot the fraction of the covered surface ($\Theta = Q/Q_{max}$) was used instead of the amount of toxin retained on the adsorbent phase (Q). It was assumed that the presence of FB_1 does not affect the total number of AFB_1 sites (Q_{max}) on the NaB surface. An S-type AFB_1 isotherm was obtained in the presence of FB_1. The mathematical expressions of the FFG are shown in Table 1. The FFG fitting curve is shown as solid lines in the corresponding isotherm graphs, and the fitting parameters are shown in Table 2. In the presence of FB_1, lateral interactions between adsorbed AFB_1 molecules become important and the adsorption sites are no longer equivalents (Butt et al., 2006). The presence of FB_1 greatly decreases (ca 87%) the *in vitro* ability of NaB to adsorb AFB_1 (value estimated at 4 mM AFB_1 in the equilibrium). A competition between AFB_1 and FB_1 for adsorption sites on NaB might explain such behaviour.

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Slighter reductions (5% less AFB_1 and 16% less FB_1) were reported for a uniform particle calcium montmorillonite commercialized as NovaSil (UPSN) even when both toxins were present at the same contamination level (Brown et al., 2014). Mitchell et al. (2014) reported a reduction to half adsorbent capacity in decreasing the bioavailability of both toxins in the gastrointestinal tract of rats. Such difference in behaviour could probably be mainly due to the higher swelling capacity and lower selectivity of sodium over calcium montmorillonites. Differences in the clay isomorphic substitution could also severely influence adsorbate binding.

A previous *in vivo* experiment with poultry showed that FB_1 decreased the decontaminating capacity by 0.3% of a sodium bentonite from southern Argentina (Miazzo et al., 2005). The adsorbent was able to counteract the effect of an induced acute aflatoxicosis (2.5 mg kg^{-1}). On the other hand, productive (Body weight and feed gain ratio) and biochemical parameters (albumin, globulin, and total protein) were not affected in birds fed diets contaminated with 200 mg kg^{-1} of FB_1. The adsorbent was unable to return these parameters to control values in birds fed a diet contaminated with 2.5 mg kg^{-1} of AFB_1 + 200 mg kg^{-1} of FB_1. The observed interference could be even higher under natural levels of AFB_1 contamination, because they are usually much lower than 2.5 ppm and the competitive adsorption of FB_1 for the adsorbent could be favoured.

A similar behaviour was observed with monensin, a prophylactic coccidiostatic agent, frequently added to poultry diets (100 mg Kg^{-1}). The presence of monensin in the diets decreased the ability of NaB (0.3%) to prevent sub-acute induced aflatoxicosis (0.050 mg kg^{-1}) (Magnoli et al., 2011a;b).

Therefore, taking into account that FB_1 co-adsorption seriously decreases the adsorption of AFB_1 by sodium bentonite, the potential use of two activated carbons as FB_1 absorbers, was investigated.

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Activated carbon I (AC1) was activated under controlled O_2 atmosphere, while activated carbon 2 (AC2) was activated under controlled CO_2 atmosphere. The porous structures of the ACs were characterized by N_2 adsorption at 77K to calculate the specific surface area (A_{BET}). Dubinin-Raduschkevich (DR) and Horvath Kawazoe (HK) methods were utilized to determine the volume and micropore (> 2 nm) size distribution. The Pierce method was used for determining the mesopore (2 -50 nm) volume (Milich et al., 2002 and references therein). The properties of both activated carbons are shown in Table 3 and 4.

Typical adsorption-desorption isotherms are shown in Figure 4. The isotherms can be classified as type L3 (Giles et al., 1960). These isotherms are commonly in physical adsorption with multilayer formation. A multilayer adsorption can be formed by a first adsorption layer followed by subsequent adsorbed layers. In such cases, there is not a strong competition between the solvent and the solute for the adsorbent surface sites (Cotoruelo et al., 2011). Experimental data were fitted using the Guggenheim–Anderson–de Boer (GAB) multilayer model (Guggenheim, 1966). The mathematical expression for GAB model is shown in Table 1.

Since in multilayer adsorption the amount of adsorbate adsorbed in a subsequent layer must be smaller than that in the previous one, the term $[1 - (K_2\ C_e)^n]$ would be very close to unity and the simplified version of the GAB expression showed in Table 1 can be obtained. All isotherms were adjusted using the simplified GAB equation, making no hypothesis. On the other hand, irreversible desorption isotherms were observed in all cases.

The influence of the pH of the adsorption media was investigated in the pH range that covers that observed in the gastrointestinal tract of birds (González-Alvarado et al., 2008, Morgan et al., 2014). The experiments were performed under controlled ionic strength in order to partially mimic physiological conditions. To accomplish the equilibrium treatment, the simplified GAB expression was utilized to estimate M, K_1 and K_2. Experimental data at different pH and

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the corresponding GAB fittings by non-linear least squares regression for AC1 and AC2 are shown in Figures 5 and 6. The adjusting GAB parameters are shown in Table 5. As expected, the K_1 values are greater than K_2 values, indicating the higher affinity of the surfaces to the first layer compared to the second one.

All isotherms showed high affinity for FB_1 at all the assayed pH values, as denoted by the high values of the adsorption constants. A complex behaviour of the adsorption isotherms with pH is observed, evidencing differences in in the nature of acidic groups between both carbons. The highest affinity for the adsorbent (K_1) coupled with a multilayer adsorption reached at rather low FB_1 concentration was observed for AC2 at pH 2 as shown in Figure 6 and Table 5. At pH 2, the formation of a second layer at quite low FB_1 equilibrium concentration could probably be the result of a greater accessibility of the inner surface throughout mesoporous channels. On the other hand, AC1 showed a L1 type isotherm in a wider range of equilibrium concentration.

For both ACs the highest maximum adsorption capacity on the first layer (M) were found at PH 4, when favourable interactions between the AC surface and FB_1 seems to be occurring. The maximum adsorption capacity on the first layer is slightly higher for AC1 than for AC2, in spite of BET areas of 681 and 906 m^2/g, respectively.

Molecular axes of 1.5 and 2.6 nm were estimated for FB_1 in gas phase after AM1 geometry optimization (Hyperchem^{TM} Release 8.0.8). Thus, the solvated toxin is too voluminous to access narrow pores. Therefore, not all the surface area is accessible to FB_1 and the toxin should be mainly confined to mesopores that can act as channels leading to internal surfaces (Yang et al., 2007; Gupta et al., 2011).

At pH 6 the lowest amount of toxin retained on the adsorbent phase was observed for AC2. A complex behaviour of the isotherms was observed with changes in the pH of aqueous media. It is known that textural properties and surface functional groups as well as the adsorbate chemical

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nature play an important role in adsorption processes. Among adsorbate properties solubility, molecular size, and dissociation grade are important factors to be considered (Al Bahri et al., 2015, Bandosz and Ania, 2007, Cotoruelo et al., 2011). Therefore, differences in the surface density of acidic functional groups between ACs, and mesoporous accessibility, could probably explain the observed behaviours. Oxygen activation used in the preparation of AC1 increases oxidized functions that can interact with FB_1, as can be seen from the %O in Table 4 which are 16.95% and 5.95% for AC1 and AC2, respectively. On the other hand, at pH 2, protonated FB_1 probably interact by hydrogen bonding with the non-ionized oxidized groups on AC surfaces. At pH 4 favourable interactions seems to be occurring with both adsorbents. On the other hand at PH 6, repulsive interaction between the negative form of FB_1 and the surface with the highest density of carboxylate anions might be occurring. In order to check this hypothesis a Boehm titration of activated carbon with NaOH to neutralize carboxylic, lactonic and phenolic groups was performed (Boehm et al., 1964). The results gave 0.748 and 0.12 mmol g^{-1} for AC1 and AC2, respectively, i.e. 6.2 times higher for AC1. Taking into account that the percentage of oxygen in AC1 is only 3 times higher than the observed in AC2, the latter should have higher proportion of carboxylic groups than AC1. Therefore, at pH 6 a more negative surface might be responsible of the low retention of toxin in the anionic form on the adsorbent phase.

Conclusion

Natural levels of FB_1 could severely decrease the potential ability of NaB to ameliorate the effects of chronic aflatoxicosis. Surface chemical composition of the ACs plays an important role in their FB_1 sequestering ability over the pH range in the gastrointestinal tract of animals. The use of mixtures of properly formulated activated carbons and NaB as dietary additive in animal diets might to be potentially useful to counteract chronic aflatoxicosis. Further studies should be

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performed in order to test *in vivo* the ability of mixtures of NaB and activated carbons to prevent subacute aflatoxicosis.

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References

Al Bahri M, Calvoa L, Gilarranza M A, Rodriguez J. J. 2016 Diuron Multilayer Adsorption on Activated Carbon From CO_2 Activation of Grape Seeds. Chem Eng Commun 203: 103–113.

Avantaggiato G, Havenaar R, Visconti A. 2007. Assessment of the multi-mycotoxin-binding efficacy of a carbon/aluminosilicate-based product in an *in vitro* gastrointestinal model. J Food Agr Chem 55: 4810–4819.

Avantaggiato G, Solfrizzio M, Visconti A. 2005. Recent advances on the use of adsorbent materials for detoxification of *Fusarium* mycotoxins. Food Add Cont 22: 379-388.

Bandosz TJ, Ania CO. 2007. Surface chemistry of ACs and its characterization. Interface Sci Technol 7: 159-229.

Boehm HP, Diehl E, Heck W, Sappok R. 1964. Surface Oxides of Carbon. Angew Chem Int Ed 3: 10.

Brown KA, Mays T, Romoser A, Marroquin-Cardona A, Mitchell NJ, Elmore SE, Phillips TD. 2014. Modified hydra bioassay to evaluate the toxicity of multiple mycotoxins and predict the detoxification efficacy of a claybased sorbent. J. Appl Toxicol 34:40-48.

ACCEPTED MANUSCRIPT

Butt HJ, Graf K, Kappl M. 2006. Physics and Chemistry of Interfaces. 3rd Ed., Wiley-VCH Verlag GmbH & Co. KGa, Weinheim.

CAST (Council of Agricultural Science and Technology) 2003. Mycotoxins:Risks in plant, animal, and human systems. Richard JL, Payne GA. ed. Council for Agric. Sci. Tech. Task Force Report no. 139, Ames, IA.

Christidis GE. 2011. Advances in the characterization of industrial minerals. European Mineralogical Union; Mineralogical Society of Great Britain and Ireland, London.

Cotoruelo LM, Marques MD, Rodriguez-Mirasol J, Rodriguez JJ, Cordero T. 2011. Cationic dyes removal by multilayer adsorption on activated carbons from lignin. J. Porous Mater, 18, 693-702.

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

Deng Y, Barrientos Velázquez AL, Billes F, Dixon JB. 2010. Bonding mechanisms between aflatoxin B_1 and smectite. Appl. Clay Sci. 50:92–98.

Diaz DE, Hagler Jr. WM, Blackwelder JT, Eve JA, Hopkins BA, Anderson KL, Lones FT, Whitlow LW. 2004. Aflatoxin binders II: Reduction of aflatoxin M_1 in milk by sequestering agents of cows consuming aflatoxin in feed. Mycopathologia 157, 233–241.

Edrington TS, Sarr AB, Kubena LF, Harvey RB, Phillips TD. 1996. Hydrated sodium calcium aluminosilicate (HSCAS), acidic HSCAS, and activated charcoal reduce urinary excretion ofaflatoxin M, in turkey poults. Lack of effect by activated charcoal on aflatoxicosis. Toxicol Lett 89: 115–122.

Galvano F, Pietri A, Bertuzzi T, Fusconi G, Galvano M, Piva A, Piva G. 1996. Reduction of carryover of aflatoxin from cow feed to milk by addition of activated carbons. J Food Prot 59: 551–554.

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Galvano F, Pietri A, Solfrizzio M, Visconti A, Galvano M. 1999. Reduction of the toxicity of fumonisin B_1 by addition of activated carbon. Proccedings of the ASPA XIII Congress, 330-331.

Galvano F, Piva A, Ritieni A, Galvano G. 2001. Dietary strategies to counteract the effects of mycotoxins: A review. J. Food Prot 64: 120-131.

Giles H, MacEwan TH, Nakhwa SN, Smith D.1960. Studies in Adsorption. Part XI. A System of classification of solution adsorption isotherms, and its use in diagnosis of adsorption mechanisms and in measurement of Specific Surface Areas of Solids. J Chem Soc 3973-3993.

González-Alvarado JM, Jiménez-Moreno E, Valencia DG, Lázaro R, Mateos GG. 2008. Effects of Fiber Source and Heat Processing of the Cereal on the Development and pH of the Gastrointestinal Tract of Broilers Fed Diets Based on Corn or Rice. Poult Sci 87:1779–1795

Grant PG, Phillips TD. 1998. Isothermal adsorption of aflatoxin B1 on HSCAS clay. J Agric Food Chem. 46:599–605.

Gupta VK, Gupta B, Rastogi A, Agarwal S, Nayak A. 2011. A comparative investigation on adsorption performances of mesoporous activated carbon prepared from waste rubber tire and activated carbon for a hazardous azo dye—Acid Blue 113. J Hazard Mat, 186: 891–901.

Guggenheim EA. 1966, Application of statistical mechanics, Clarendon Press, Oxford.

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. Poult Sci 71:64–69.

IARC (International Agency for Research on Cancer) 1993. Evaluation of carcinogenic risks of chemical to humans. Some naturally-occurring, Evaluation of carcinogenic risks of chemical to humans. Some naturally-occurring substances: Food Items and Constituents. Heterocyclic aromatic amines and mycotoxins. IARC Monographs 56: 359–362, Lyon.

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ACCEPTED MANUSCRIPT

Kikot A, Magnoli C, Chiacchiera S, Dalcero A, Miazzo R, Basaldella EI. 2002. Effect of fumonisin B_1 on NaA zeolite effectiveness to adsorb aflatoxin B_1. En: Khassanova, L., Collery, Ph., Maymard, Y., Khassanova, Z., Étienne, J-C. (Eds.) Metal ions in Biology and Medicine, John Libbey Eurotext, Paris, 7: 242-246.

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

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

Magnoli AP, Alonso VA, Cavaglieri LR, Dalcero AM, Chiacchiera SM. 2013 Effect of monogastric and ruminant gastrointestinal conditions on *in vitro* aflatoxin B_1 adsorption ability by a montmorillonite. Food Addit Contam Part A 30:743-9.

Magnoli AP, Monge MP, Miazzo RD, Cavaglieri LR, Magnoli CE, Merkis CI, Cristofolini AL, Dalcero AM, Chiacchiera SM. 2011b. Effect of low levels of aflatoxin B_1 on performance, biochemical parameters, and aflatoxin B_1 in broiler liver tissues in the presence of monensin and sodium bentonite. Poult 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. 2011a. Sodium bentonite and monensin under chronic aflatoxicosis in broiler chickens. Poult Sci 90:352–357.

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ACCEPTED MANUSCRIPT

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. Poult Sci 84:1–8.

Milich P, Möller F, Píriz J, Vivó G, Tancredi N. 2002. The influence of preparation methods and surface properties of activated carbons on Cr(III) adsorption from aqueous solutions, Separation Science and Technology 30: 1453-1467.

Mitchell NJ1, Xue KS, Lin S, Marroquin-Cardona A, Brown KA, Elmore SE, Tang L, Romoser A, Gelderblom WC, Wang JS, Phillips TDJ. 2014. Calcium montmorillonite clay reduces AFB_1 and FB_1 biomarkers in rats exposed to single and co-exposures of aflatoxin and fumonisin. Appl Toxicol 34:795-804.

Monge MP, Dalcero AM, Magnoli CE, Chiacchiera SM. 2013. Toxigenic fungi and natural co-occurrence of mycotoxins in poultry feeds from Entre Ríos, Argentina. Food Add and Cont, Part B, Surveillance 6:168-174.

Monge MP, Magnoli CE, Chiacchiera SM. 2012. Survey of Aspergillus and Fusarium species and their mycotoxins in raw materials and poultry feeds from Córdoba, Argentina. Mycotoxin Res 28: 111-122.

Morgan NK, Walk CL, Bedford MR, Burton EJ. 2014. The effect of dietary calcium inclusion on broiler gastrointestinal pH: Quantification and method optimization, Poult Sci 93 :354–363.

Oliveira GR, Ribeiro JM, Fraga ME, Cavaglieri LR, Direito GM, Keller KM, Dalcero AM, Rosa CA. 2006. Mycobiota in poultry feeds and natural occurrence of aflatoxins, fumonisins and zearalenone in the Rio de Janeiro State, Brazil. Mycopathologia 162:355–362.

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

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ACCEPTED MANUSCRIPT

Pimpukdee K, Kubena LF, Bailey CA, Huebner HJ, Afriyie-Gyawu E, Phillips TD. 2004. Aflatoxin-induced toxicity and depletion of hepatic vitamin A in young broiler chicks: protection of chicks in the presence of low levels of NovaSil PLUS in the diet. Poult Sci. 83:737-44.

Piva A, Galvano F. 2007. Nutritional approaches to reduce the impact of mycotoxins available on July 2015 in en.engormix.com.

Robinson A, Johnson NM, Strey A, Taylor JF, Marroquin-Cardona A, Mitchell NJ, Afriyie-Gyawu E, Ankrah NA, Williams JH, Wang JS, Jolly PE, Nachman RJ, Phillips TD. 2012. Calcium montmorillonite clay reduces urinary biomarkers of fumonisin B_1 exposure in rats and humans. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 29:809-818.

Rosa, C. A., R. Miazzo, C. Magnoli, M. Salvano, S. M. Chiacchiera, S. Ferrero, M. Saenz, E. C. Carvalho, and A. Dalcero. 2001. Evaluation of the efficacy of bentonite from the south of Argentina to ameliorate the toxic effects of aflatoxin in broilers. Poult 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. Poult Sci 72:282–288.

Streit E, Schatzmayr G, Tassis P, Tzika Eleni, Marin D, Taranu I, Tabuc C, Nicolau A, Aprodu I, Puel O, Oswald IP. 2012. Current Situation of Mycotoxin Contamination and Co-occurrence in Animal Feed Focus on Europe. Toxins 4:788-809.

Trucksess MW, Stack ME, Nesheim S, Albert RH, Romer TR. 1994. Multifunctional column coupled with liquid chromatography for determination of aflatoxins B_1, B_2, G_1, and G_2 in corn, almonds, brazil nuts, peanuts, and pistachio nuts: collaborative study. J. AOAC. Int. 77:1512–1521.

Voss KA, Smith GW, Haschek WM. 2007. Fumonisins: Toxicokinetics, mechanism of action and toxicity. Animal Feed Science and Technology 137 (2007) 299–325

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ACCEPTED MANUSCRIPT

Yang W, Wu D, Fur R. 2007. Porous structure and liquid-phase adsorption properties of AC aerogels, *Appl Polym Sci* 106:2775-2779.

Yunus AW, Razzazi-Fazeli E, Bohm J. 2011. Aflatoxin B1 in Affecting Broiler's Performance, Immunity, and Gastrointestinal Tract: A Review of History and Contemporary Issues. Toxins 3:566-590

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.

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

Models	Mathematical Expression	Parameters
Langmuir	$\beta = \frac{Q}{(Q_{max} - Q)[toxin]}$ or $\beta = \frac{\Theta}{(1-\Theta)[toxin]}$	Q_{max} or Θ, β
FFG	$\beta = \left[\frac{Q}{(Q\,max - Q)[toxin]}\right]\exp(-2aQ/Q_{max})$ or $\beta = \left[\frac{\Theta}{(1-\Theta)[toxin]}\right]\exp(-2a\Theta)$	Q_{max} or Θ, β, a
GAB	$Q = \frac{K_1 M[toxin]\left(1-(K_2[toxin])^n\right)}{(1-K_2[toxin])(1+(K_1-K_2)[Toxin])}$ or $Q = \frac{K_1 M[toxin]}{(1-K_2[toxin])(1+(K_1-K_2)[Toxin])}$	K_1, K_2, M

Q and Q_{max} are the amount of toxin retained on the adsorbent phase (mmol g^{-1} of dry adsorbent) at each equilibrium condition and at saturation, respectively, Θ (= Q/Q_{max}) is the fraction of the surface covered by the toxin at each equilibrium condition, [toxin] is the residual toxin concentration at equilibrium, β is the adsorption constant (L mol^{-1}), "a" is the FFG parameter that measures the lateral interaction between adsorbed toxin molecules. The Guggenheim–Anderson–de Boer (GAB) multilayer isotherm parameters K_1 and K_2 (e.g. L/mmol) are

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the equilibrium constants for the first and the second layers, respectively, M (e.g. mmol g^{-1}) is the maximum adsorption capacity on the first layer; and n represents the number of layers.

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Table 2. Adjusted parameters of absorption isotherms on sodium bentonite at pH 2

Toxin	FB_1 C / M	Model	$10^{-5}\beta$ (L mol^{-1})	Q_{max} (mmol g^2)	a	R^2
AFB_1	-	L	8.7 ± 0.6	2.38 ± 0.04	-	0.9985
FB_1	-	L	44 ± 3	25.4 ± 0.1	-	0.9678
AFB_1	$2.8x10^{-4}$	FFG	0.23 ± 0.02	2.38 ± 0.04 *	1.8 ± 0.4	0.9941

* assumed as the same Γ_{max} obtained for AFB_1 alone

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Table 3. Porosity parameters for carbons and raw materials, determined from N_2 adsorption at 77K

sample	A_{BET} $m^2 g^{-1}$	V_{DR} N_2 $cm^3 g^{-1}$	V_{micro} $cm^3 g^{-1}$	V_{meso} $cm^3 g^{-1}$
AC1	681	0.27	0.270	0.040
AC2	906	0.35	0.365	0.119

A_{BET} is the specific surface area; V_{DR} N_2 is the Dubinin-Raduschkevich volume; V_{micro} and V_{meso} are the micropore and mesopore volume, respectively.

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Table 4. Ultimate analysis for activated carbons (Dry Basis, Ash Free)

Sample	C (%)	N (%)	H (%)	S (%)	O[a](%)
AC1	81.07	0.09	1.23	0	16.95
AC2	93.32	0.20	0.53	0	5.95

[a] Obtained by difference

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Table 5. Guggenheim–Anderson–de Boer (GAB) parameters of the FB_1 adsorption isotherms on different activated carbons

Activated Carbon	pH	K_1 (L μmol^{-1})	K_2 (L μmol^{-1})	M mmol g^{-1}	R^2
AC1	2	2.2 ± 0.8	0.40 ± 0.07	3.5 ± 0.8	0.9882
	4	16 ± 2	0.24 ± 0.13	7.8 ± 0.6	0.9681
	6	4 ± 1	0.71 ± 0.08	4.3 ± 0.8	0.9897
AC2	2	60 ± 15	5.6 ± 0.1	3.6 ± 0.2	0.9829
	4	16 ± 2	0.87± 0.05	5.2 ± 0.3	0.9835
	6	12 ± 4	0.50 ± 0.06	0.20 ± 0.02	0.9986

K_1 and K_2 (e.g. L mmol^{-1}) are the equilibrium constants for the first and the second layers, respectively; M (e.g. mmol g^{-1}) is the maximum adsorption capacity on the first layer and R^2 is the determination coefficient.

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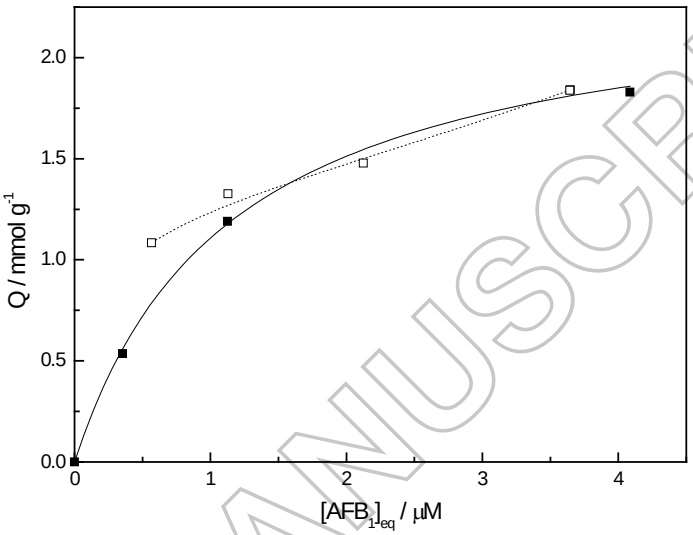

Figure 1. Aflatoxin B_1 adsorption ( ) and desorption (□) isotherms on sodium bentonite in 0.15 M NaCl brought to pH 2 with HCl

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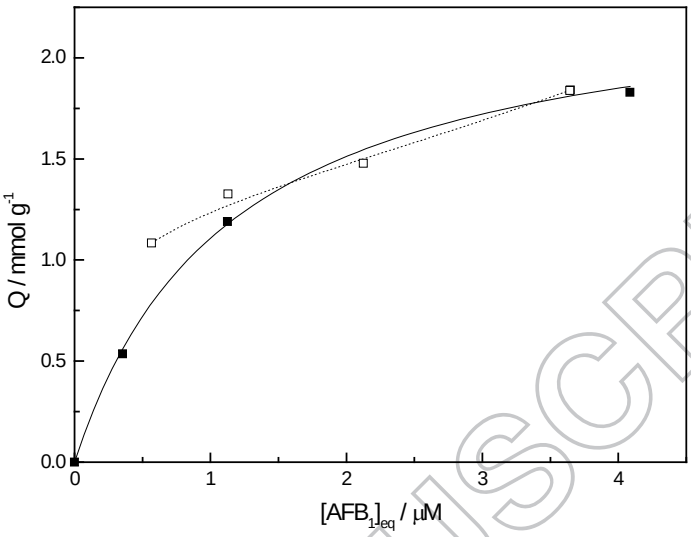

Figure 2. Aflatoxin B_1 adsorption ( ) and desorption (□) isotherms on sodium bentonite in 0.15 M NaCl brought to pH 2 with HCl

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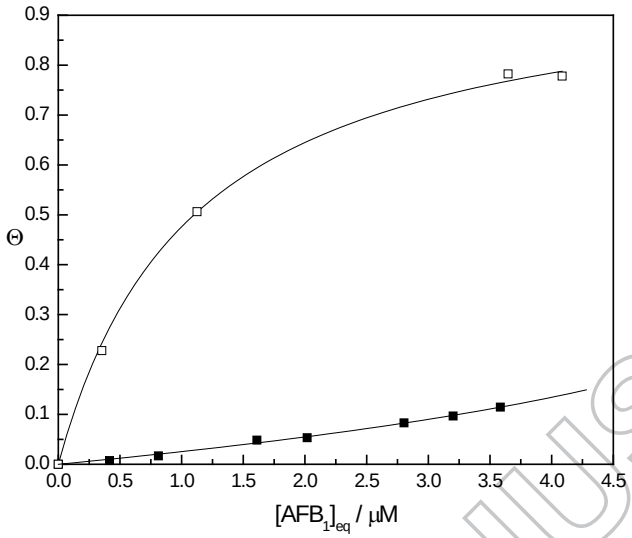

Figure 3. Influence of FB_1 upon the adsorption isotherm of AFB_1 on sodium bentonite carried down in 0.15 M NaCl brought to pH 2 with HCl. Concentrations of FB_1: 0 M (□) and ( ) $2.8x10^{-4}$ M

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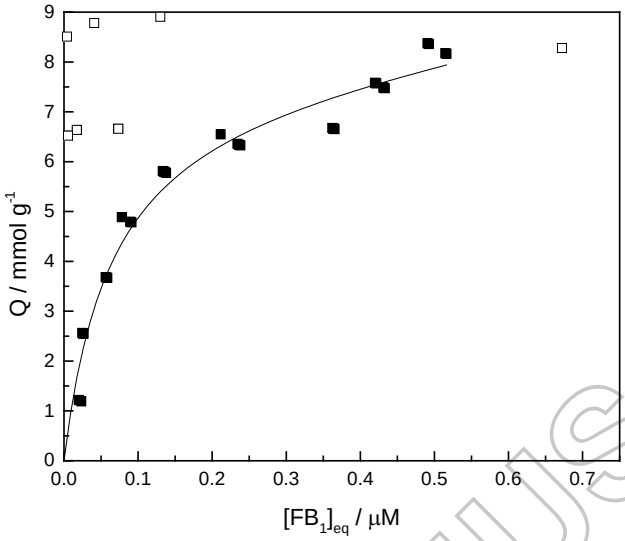

Figure 4. Fumonisin B_1 adsorption ( ) and desorption (¡) isotherms on activated carbon AC1 in 0.15 M NaCl brought to pH 4 with HCl

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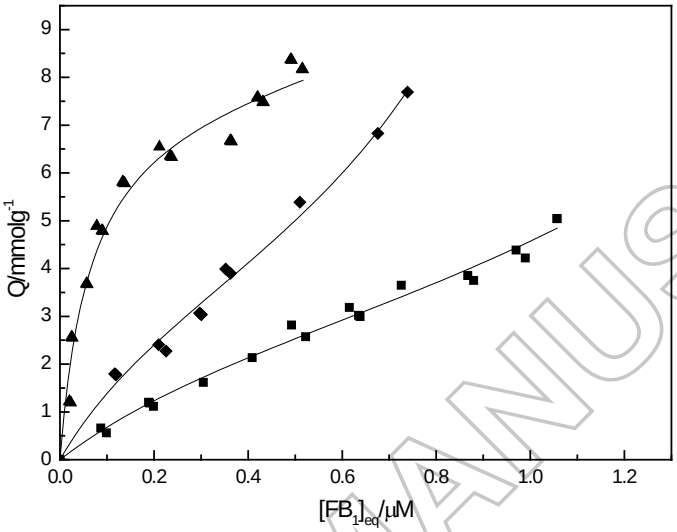

Figure 5. Effect of pH on the adsorption isotherms of FB_1 on activated carbon AC1 in 0.15 M NaCl at pH 2 ( ), pH 4 (²) and pH 6 (f).

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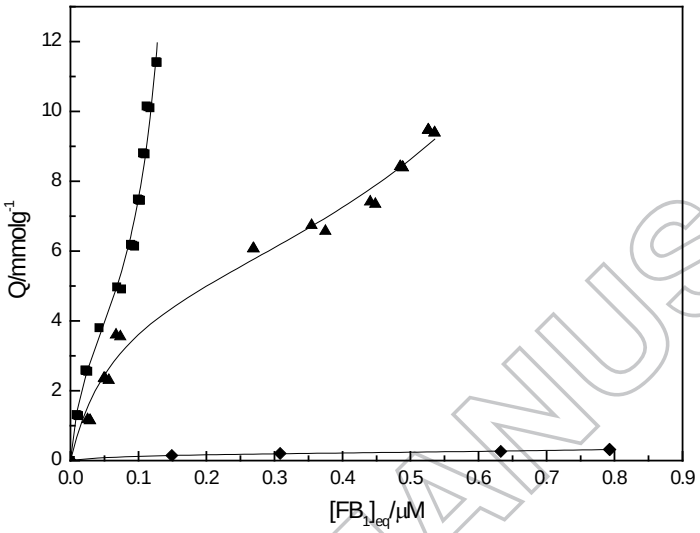

Figure 6. Effect of pH on the adsorption isotherms of FB_1 on activated carbon AC2 in 0.15 M NaCl at pH 2 ( ), pH 4 (²) and pH 6 (f).

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