

Intervening Strategies for Aflatoxin B₁ in Cotton Seed Cake

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ABSTRACT

Intake of low-quality feed specially aflatoxin contaminated cottonseed cake by dairy animals carry over the aflatoxins through milk and causing health problems (aflatoxicosis) to consumers. The present study was conducted with the objective to check the suitable binder through *in-vitro* experiments as aflatoxin level may be controlled in cottonseed cake. All the collected samples were transported aseptically to the Department of Animal Products Technology, Faculty of Animal Husbandry and Veterinary Science, Sindh Agriculture University Tandojam for analysis. In *in-vitro* study thirty (n=30) cottonseed cake samples with known level of aflatoxin B₁ was extracted and treated with three binders Milbond, China Clay and Novasil. at three doses (T₀ = recommended dose; T₁ = < recommended dose; T₂ = > recommended dose) to compare the adsorption rate. The China Clay and Novasil showed significant (p<0.05) effect as binders on adsorption ability of aflatoxin B₁. Results revealed that the adsorption percent of T₁ of Milbond (54.81%) was higher (p<0.05) than China Clay (47.09%) followed by Novasil (12.69%). Moreover, in case of the recommended doses (T₀) by manufacturer the adsorption percent was 19.70, 67.88 and 12.41 % respectively for Milbond, China Clay and Novasil, respectively. Whereas higher dose treatments (T₂) showed that adsorption percent was high (p<0.05) in Milbond (57.41%) followed by China Clay (47.45%) and Novasil (5.10%). It was concluded that China clay shown better adsorption at recommended dose than rest of the binders. Whereas, on lower and higher doses Milbond shown higher adsorption percent of AFB₁ in cotton seed cake and in addition adsorption rate of Novasil increased (12.69%) with the dose reduction.

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Authors' Contribution

DKB conducted experiments and manuscript write up. AHS supervised the research. GBK and ZAL helped in write up and data analysis. MFH and QK proof read the manuscript.

Key words

Adsorption, Aflatoxin, China Clay, Milbond, Novasil

INTRODUCTION

The feed supply chain plays a crucial role in livestock systems, feed supply and ensuring feed and food safety. Various factors, including feed origin, processing, handling, and storage, can impact the quality and safety of feed at different stages (Jallow *et al.*, 2021). Among the safety risks in the feed industry and supply chain, mycotoxins hold significant importance (Ferrari *et al.*, 2022).

Aflatoxins (AFs) are highly toxic fungal metabolites that commonly contaminate food and feed, posing substantial health risks to both humans and animals (Ajmal *et al.*, 2022; Iqbal *et al.*, 2022). While more than 20 types of aflatoxins have been identified, aflatoxin B₁ (AFB₁), B₂ (AFB₂), G₁ (AFG₁), G₂ (AFG₂), and M₁ (AFM₁) are the most prevalent (Ajmal *et al.*, 2022; Iqbal *et al.*, 2022). Aflatoxins B₁, B₂, G₁, and G₂ are among the most common and significant types of aflatoxins (Shabeer *et al.*, 2022; Ajmal *et al.*, 2022).

Proper harvesting and storage capacity are the safest approaches to eradicate the occurrence of aflatoxins in food commodities (Ahmad *et al.*, 2023). Measures to retard the toxin levels comprise of chemical treatment and dilutions, but are generally useless, costly or these may adversely impact the nutritious properties of the food (Van Kessel and Hiang-Chek, 2004; Krska *et al.*, 2022). Nowadays the most applied technique to encounter aflatoxins in infected feedstuffs is to admix a suitable toxin binder in feed (Jaime-Garcia and Cotty, 2003; Owino, 2022; Rehagel, 2022).

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Numerous studies have highlighted the prevalence of aflatoxin contamination in various agricultural commodities, including cottonseed and its by products (Shar *et al.*, 2020; Smith *et al.*, 2020). However, limited research has specifically focused on examining the efficacy of toxin binders in reducing aflatoxin levels in cottonseed cake.

Toxin binders, such as activated charcoal and clay minerals, have shown promise in mitigating aflatoxin contamination in different feed ingredients (Shar *et al.*, 2020; Awuchi *et al.*, 2020). These binders have the ability to adsorb aflatoxins, preventing their absorption and reducing their bioavailability in the gastrointestinal tract of animals (Shar *et al.*, 2020). Aflatoxin binders are those substances which when added to animal feed trap mycotoxins, thus prevent their entrance into the blood stream and avoid causing harmful results to animals (Oladeji, 2022). A good toxin binder can reinstate the nutritious standards of feedstuffs infected with aflatoxin. As, most toxin binders are mineral clays those avert aflatoxins from being absorbed by the intestine (De Mil *et al.*, 2015; Čolović *et al.*, 2019; Kihal *et al.*, 2022). Toxin binder combinations incorporated in diet or feed or ingested individually throughout mealtimes, to decrease aflatoxin adsorption are called as adsorbing agent. Adsorbing agents in gastric tract, as a result decreasing additional phases of toxin dispersal and breakdown in organs and tissues (Kabak and Dobson, 2006; Diaz *et al.*, 2004; Yiannikouris *et al.*, 2021; Xu *et al.*, 2023). Activated charcoal, bentonite, zeolite, and hydrated sodium calcium aluminosilicate (clay materials) presented variable capabilities to bind AFs *in-vitro*. Four limits are being used differently for expressing the quality of a toxin binder i.e., capacity of binding, efficiency in adsorption, time for activation and rate of inclusion (Hojati *et al.*, 2021). Looking at the adsorption ability of aflatoxins by the toxin binders in the intestine with restoring the nutritional standards of aflatoxin contaminated feedstuffs following binders selected because of their effectiveness.

Given the lack of comprehensive studies investigating the use of toxin binders in cottonseed cake, conducting a thorough analysis is crucial to determine their potential as a preventive measure against aflatoxin contamination. The objective of this manuscript is to address the crucial issue of aflatoxin contamination in cotton seed cake, a commonly used feed ingredient, and to investigate the effectiveness of using a toxin binder to control aflatoxin levels in Pakistan. Besides this, this research also aims to fill this knowledge gap and provide valuable insights into the efficacy of toxin binders in controlling aflatoxin levels in cottonseed cake, ultimately contributing to the enhancement of food safety and animal health.

MATERIALS AND METHODS

Materials used for controlling aflatoxin in feed

The following materials were used in the present study.

1. Cottonseed cake sample obtained from commercial dairy farm from Southern, Central and Northern zone of Sindh province.
2. Novasil® plus rich in calcium bentonite (calcium montmorillonite), a light brown powder (TM-1474-03/03-KNKL, Trouw Nutrition LLC, origin of USA) used for controlling aflatoxin in feed.
3. Milbond TX an aluminosilicate with over 80% montmorillonite (MTX A Milwhite Incl., 5487 South Padry Island Hwy, Brownsville, TX 78521, USA) used for controlling aflatoxin in feed.
4. China clay rich in kaolinite, known as kaolin or China Clay, white to cream in color (China) and also most commonly mined in Pakistan naturally used for controlling aflatoxin in feed.

Extraction and quantification of aflatoxin

The cottonseed extraction was done using the Bio-Shield Total Extra Sensitive kit extraction method. A representative sample of cotton seed cake was blended to a fine instant coffee particle size (50 percent passes through a 20-mesh screen). Then after, the extract was produced by blending 20 g of powdered material with 80 ml of 70% methanol Honeywell Co, CAT24229, Germany, UN1230; Daejung Co, HPLC solvent 555-2304, China. The mixture was mixed for another 10-15 min. The sample-to-extraction-solvent ratio was kept fixed at 1:5 (w/v). After letting the solution mixture to settle for 2 to 3 min, 100 ml of phosphate buffer solution (PBS OmnipurCo 6501, calbiochem, USA) was poured to it. To form a homogenate, the mixture was vortexed for a further 30 min and then filter the extract with whatsmann filter paper # 01 (ProGnosis Biotech, 2015).

A total of two hundred and seventy cottonseed samples 10 ml extract (thirty samples per group/treatment) with known level of aflatoxin B₁ were treated with three binders i.e., Milbond, China Clay and Novasil. Each binder was added to cottonseed cake at three doses i.e., Milbond (T₀ = 0.032, T₁ = 0.016 and T₂ = 0.048 g), China Clay (T₀ = 0.036, T₁ = 0.018 and T₂ = 0.054 g) and Novasil (T₀ = 0.02, T₁ = 0.01 and T₂ = 0.04 g) and kept at 39°C for 02 h and then sample were being centrifuged to get clear supernatant for assessment of suitable binder for adsorbing AFB₁ on cottonseed cake samples. For the detection/quantification of Aflatoxin B₁ in cottonseed cake Bio-Shield total extra sensitive were used for further processing (ProGnosis Biotech, 2015).

A 200 μ l of the matrix diluent with 50 μ l of the binder treated sample filtrate and standards in the dilution microwells than transferred 100 μ l from each dilution microwells into the antibody coated microwells and incubated for 10 min at room temperature after that washed for four times and 100 μ l of detection solution was added and again incubated for 5 min at room temperature. After that 100 μ l of TMB Microwell Substrate (3, 3', 5, 5' tetramethylbenzidine) was added and left for 5 min in the dark at room temperature for the development of color and then 100 μ l of stop solution was added and in the last absorbance was noted at 450 nm within 60 min (ProGnosis Biotech, 2015).

Statistical analysis

The statistical analyses were performed by using analysis of variance in a computer-based application called Student Edition of Statistics (SXW), Version 8.1 (Analytical software-USA) (ANOVA). The data were expressed as means SD, as well as the least significant difference (LSD) test ($P < 0.05$) has been used to determine significance.

RESULTS

Aflatoxin B₁ level in CSC

Milbond, China Clay and Novasil at recommended dose ($T_0 = 0.032, 0.036$ and 0.02 g, respectively) were admixed with cottonseed cake extract for intake and adsorption of AFB₁. The results revealed statistically higher ($p < 0.05$) adsorption percent of China clay (67.88%) compared to that of Milbond (19.70%) and Novasil (12.41%) binders. Furthermore, statistical analysis proved that average adsorption % of the AFB₁ was significantly varied ($p < 0.05$) among all the cottonseed cake extracts mixed with toxin binders (Fig. 1A).

Effect of lower dose of binders (T_1) on aflatoxin B₁ level in CSC

Milbond, China Clay and Novasil at lower than recommended dose (T_1) at the rate of 0.016g, 0.018g and 0.01g were used to treat the CSC extract for adsorption of AFB₁ in *in-vitro* experiment. The outcomes revealed (Fig. 1B) that the adsorption percent of Milbond (54.81%) was significantly higher ($p < 0.05$) than that of China Clay (47.09%) followed by Novasil (12.69%).

Effect of higher dose (T_2) of binder to control aflatoxin B₁ level in CSC

In this experiment higher dose (T_2) of Milbond (0.048g) China Clay (0.054g) and Novasil (0.04g) were applied for the purpose to assess the adsorption rate of

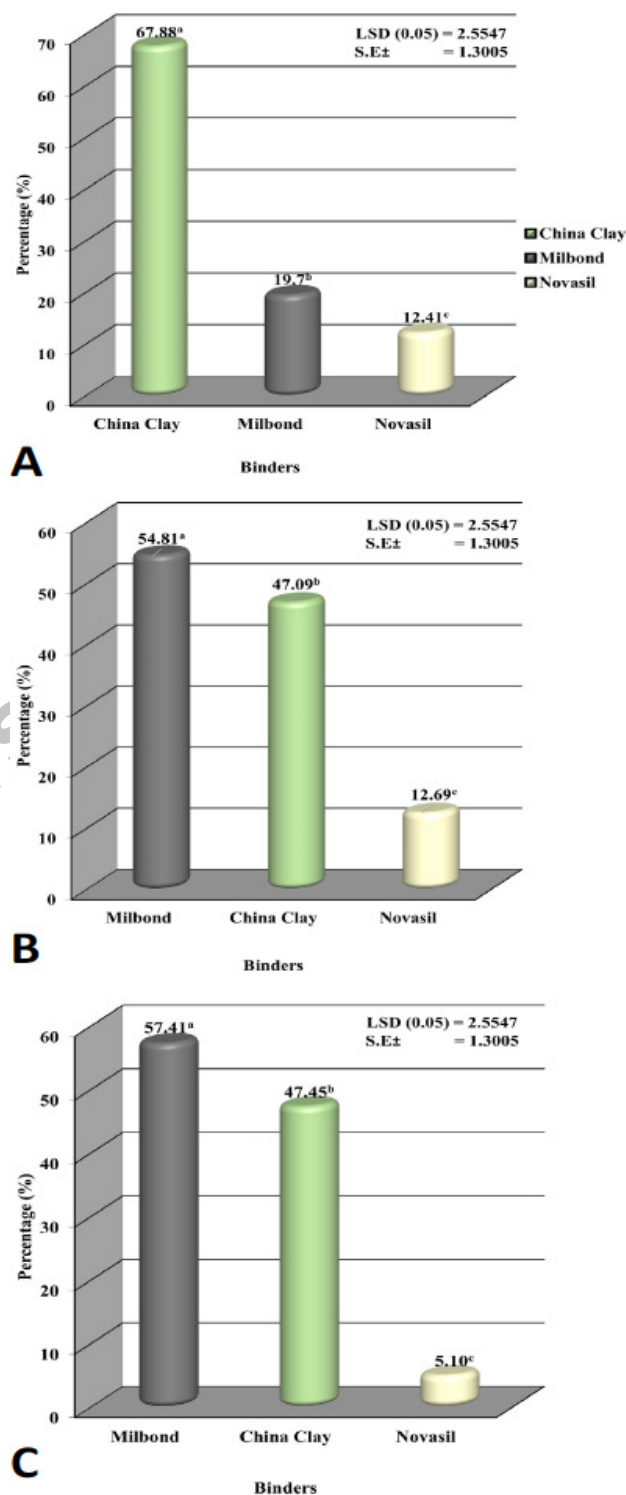


Fig. 1. Level of AFB₁ (% adsorptions) at recommended dose (A), lower dose (B) and higher dose (C) of various toxin binders.

AFB₁ in cottonseed cake extract. Adsorption percent of aflatoxin B₁ was 57.41%, 47.45% and 5.10%, respectively for the Milbond, China Clay and Novasil, respectively (Fig. 1C). It was observed from the results that with the increase dose of Milbond, adsorption (%) was found to be statistically high ($P < 0.05$) to that of China Clay and Novasil binders mixed with cottonseed cake extracts. However, on the contrary adsorption rate of Novasil increased (12.69%) with the dose reduction.

The results of the *in-vitro* experiments demonstrated that the adsorption efficiency of aflatoxin B₁ varied significantly among the three binders tested. China Clay exhibited the highest adsorption percentage (67.88%) at the recommended dose (T_0), indicating its effectiveness in reducing aflatoxin levels in cottonseed cake. Milbond showed increased adsorbent efficiency (from 19.70% to 57.41%) when the dose was increased (T_2), while Novasil exhibited increased adsorbent efficiency (12.69%) when the dose was reduced (T_1).

These findings suggest that the efficacy of an adsorbent depends on various factors such as its adsorbing capacity, molecular arrangement, activation time, and inclusion rate. The binding capacity of the adsorbent may be influenced by pH, geographic region of origin, and the interaction between aflatoxins and the binder. Additionally, the number of adsorbents can also affect the binding ability.

DISCUSSION

Aflatoxin remedial approaches are not simple to take rather needs an inclusive approach including food safety and economic growth to report overall good farming and food production practices. Complexes that can be incorporated in human diet or animal feed or taken individually during the period of meal-times, to lessen aflatoxin adsorption are called as adsorbing agent. The role of adsorbent (binder) is to trap the toxins from gastrointestinal tract to prevent them from entering the systemic circulation which ultimately reduce the chance of undesirable effects on health of subject. Aflatoxin adsorbents are getting much attention these days because of their cost efficiency, ease of use, and good efficacy in trace amounts (Murugesan *et al.*, 2015).

Results of the present study during *in-vitro* experiments showed significant effect of adsorbing ability on aflatoxin B₁ by the three binders. It was revealed from the present study that Milbond adsorbent efficiency increased (from 19.70% to 57.41%) by increasing the dose of adsorbent intake (T_2) through cottonseed cake. On the contrary, when the Novasil dose was reduced (T_1), its adsorbent efficiency increased compared to control (T_0). However, the recommended dose (T_0) of China Clay remained more effective with adsorbent percentage of 67.88. China Clay

was much more effective at recommended dose just because of the high adsorbing capacity of particles present is higher compared to that of other binders. Currently identified dose of Novasil adsorbent is quite cost effective since it is much more expensive compared to Milbond binder. In dairy cows, HSCAS (Hydrated Sodium Calcium Almino Silicate) and activated charcoal, mixed to AFB₁ infected feed with a proportion of inclusion at 2%, condensed AFB₁ carry-over as AFM₁ in milk of 36% and 50%, respectively (Galvano *et al.*, 1996) which is just because that charcoal has the higher adsorbing capacity than that of Hydrated Sodium Calcium Almino Silicate (HSCAS). While, Harvey *et al.* (1991) found that HSACS at 1% resulted in 24% reduction in a carry-over. The efficiency of any adsorbent depends on four factors i.e. binding capacity, adsorption efficiency, activation time and inclusion rate (Van Kessel and Hiang-Chek, 2004). The ability of toxin binder to bind mycotoxin depends on various factors such as pH, molecular arrangement and its geographic region of origin (Daou *et al.*, 2021; Vieira, 2003). The reduction in percent binding could occur because of interaction of aflatoxins (Raju and Devegowda, 2002). Binding capacity also depends on number of adsorbents. For examples as in case of Lactic acid bacteria, the binding ability increased with the number of lactic acid bacterial count increase (Ismail *et al.*, 2017; Taheur *et al.*, 2017).

The present study holds significant importance in addressing the issue of aflatoxin contamination in cottonseed cake and evaluating the efficacy of toxin binders in controlling aflatoxin levels. The significance of this study can be highlighted in several ways: food safety enhancement, novelty in research, agricultural and economic impact, implementation in Pakistan, and cost-effective approach.

Overall, this study's significance lies in its contribution to food safety, novel insights into toxin binders' efficacy, and its relevance to the agricultural sector in Pakistan. By implementing the findings, stakeholders can take proactive steps to minimize aflatoxin contamination in cottonseed cake, ensuring the well-being of animals and humans alike.

CONCLUSION

Comparisons with previous studies revealed that charcoal has a higher adsorbing capacity than Hydrated Sodium Calcium Almino Silicate (HSCAS), and the efficiency of any adsorbent depends on these factors. In conclusion, China Clay at the recommended dose exhibited the highest adsorption efficiency among the tested binders, while Milbond showed increased efficacy with increased dose and Novasil demonstrated increased adsorbent efficiency with reduced dose. These findings highlight the

potential of using adsorbents as a cost-effective approach to mitigate aflatoxin contamination in cottonseed cake, contributing to food safety and animal health.

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IRB approval

Not applicable.

Ethics statement

Not applicable.

Statement of conflict of interest

The authors have declared no conflict of interest.

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