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Intervening Strategies for Aflatoxin B_1 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 B_1 was extracted and treated with three binders Milbond, China Clay and Novasil. at three doses (T_0 = recommended dose; T_1 = < recommended dose; T_2 = > 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 B_1. Results revealed that the adsorption percent of T_1 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 (T_0) 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 (T_2) 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 AFB_1 in cotton seed cake and in addition adsorption rate of Novasil increased (12.69%) with the dose reduction.

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).

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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 B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1), G_2 (AFG_2), and M_1 (AFM_1) are the most prevalent (Ajmal *et al*., 2022; Iqbal *et al*., 2022). Aflatoxins B_1, B_2, G_1, and G_2 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).

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 kalonite, 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 whatsman 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 B_1 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 (T_0= 0.032, T_1= 0.016 and T_2= 0.048 g), China Clay (T_0= 0.036, T_1= 0.018 and T_2= 0.054 g) and Novasil (T_0= 0.02, T_1= 0.01 and T_2= 0.04 g) and kept at 39°C for 02 h and than sample were being centrifuged to get clear supernatant for assessment of suitable binder for adsorbing AFB_1 on cottonseed cake samples. For the detection/quantification of Aflatoxin B_1 in cottonseed cake Bio-Shield total extra sensitive were used for further processing (ProGnosis Biotech, 2015).

A 200 µl of the matrix diluent with 50 µl of the binder treated sample filtrate and standards in the dilution microwells than transferred 100 µl 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 µl of detection solution was added and again incubated for 5 min at room temperature. After that 100 µl 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 µl 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 B_1 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 AFB_1. 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 AFB_1 was significantly varied ($p<0.05$) among all the cottonseed cake extracts mixed with toxin binders (Fig. 1A).

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 AFB_1 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%).

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 AFB_1 in cottonseed cake extract. Adsorption percent of aflatoxin B_1 was 57.41%, 47.45% and 5.10%, respectively for the Mibond, 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

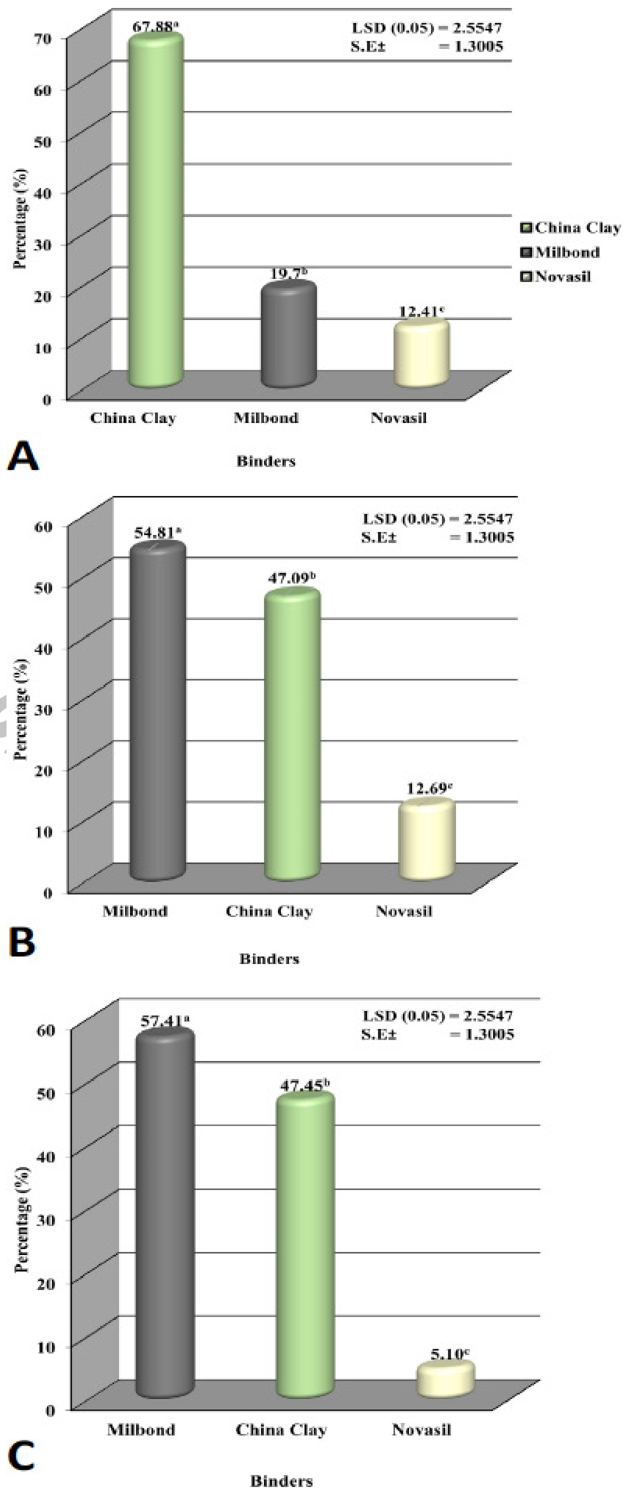

Fig. 1. Level of AFB_1 (% adsorptions) at recommended dose (A), lower dose (B) and higher dose (C) of various toxin binders.

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 B_1 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 B_1 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 Almuno Silicate) and activated charcoal, mixed to AFB_1 infected feed with a proportion of inclusion at 2%, condensed AFB_1 carry-over as AFM_1 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 Almuno 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, adsoption 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 Almuno Silicate (HSCAS), and the efficiency of any absorbent 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.

REFERENCES

Ahmad, M., Han, Z. and Kong, Q., 2023. Aflatoxin in peanuts and maize: An overview on occurrence, regulations, prevention, and control methods. *World Mycotoxin J.*, **16**: 99-114. https://doi.org/10.3920/WMJ2022.2786

Ajmal, M., Bedale, W., Akram, A. and Yu, J.H., 2022. Comprehensive review of aflatoxin contamination, impact on health and food security, and management strategies in Pakistan. *Toxins*, **14**: 845. https://doi.org/10.3390/toxins14120845

Awuchi, C.G., Amagwula, I.O., Priya, P., Kumar, R., Yezdani, U. and Khan, M.G., 2020. Aflatoxins in foods and feeds: A review on health implications, detection, and control. *Bull. Environ. Pharmacol. Life Sci.,* **9**: 149-155.

Čolović, R., Puvača, N., Cheli, F., Avantaggiato, G., Greco, D., Đuragić, O. and Pinotti, L., 2019. Decontamination of mycotoxin contaminated feedstuffs and compound feed. *Toxins*, **11**: 617. https://doi.org/10.3390/toxins11110617

Daou, R., Joubrane, K., Khabbaz, L.R., Maroun, R.G., Ismail, A. and El-Khoury, A., 2021. Aflatoxin B_1 and ochratoxin A in imported and Lebanese wheat and products. *Fd. Addit. Contam. B Surveill.,* **14**: 227-235. https://doi.org/10.1080/19393210.2021.1933203

De Mil, T., Devreese, M., Baere, S., Ranst, E., Eeckhout, M., Backer, P. and Croubels, S., 2015. Characterization of 27 mycotoxin binders and the relation with in vitro zearalenone adsorption at a single concentration. *Toxins,* **7**: 21-33. https://doi.org/10.3390/toxins7010021

Diaz, D.E., Hagler, W.M., Blackwelder, J.T., Eve, J.A., Hopkins, B.A., Anderson, K.L., Jones, F.T. and Whitlow, L.W., 2004. Aflatoxin binders ii: Reduction of aflatoxin M1 in milk by sequestering agents of cows consuming aflatoxin in feed. *Mycopathologia,* **157**: 233–241. https://doi.org/10.1023/B:MYCO.0000020587.93872.59

Ferrari, L., Fumagalli, F., Rizzi, N., Grandi, E., Vailati, S., Manoni, M. and Pinotti, L., 2022. An eight-year survey on aflatoxin B1 indicates high feed safety in animal feed and forages in Northern Italy. *Toxins,* **14**: 763. https://doi.org/10.3390/toxins14110763

Galvano, F., Pietri, A., Bertuzzi, T., Fusconi, G., Galvano, M., Piva, A. and Piva, G., 1996. Reduction of carryover of aflatoxin from cow feed to milk by addition of activated carbons. *J. Fd. Prot.*, **59**: 551–554. https://doi.org/10.4315/0362-028X-59.5.551

Harvey, R.B., Phillips, T.D., Ellis, J.A., Kubena, L.F., Huff, W.E. and Petersen, H.D., 1991. Effects on aflatoxin M_1 residues in milk by addition of hydrated sodium calcium aluminosilicate to aflatoxin-contaminated diets of dairy cows. *Am. J. Vet. Res.*, **52**: 1556–1559.

Hojati, M., Norouzian, M.A., Alamouti, A.A. and Afzalzadeh, A., 2021. *In vitro* evaluation of binding capacity of different binders to adsorb aflatoxin. *Vet. Res. Forum,* **12**: 211.

Iqbal, S.Z., Waqas, M., Razis, A.F.A., Usman, S., Ali, N.B. and Asi, M.R., 2022. Variation of aflatoxin levels in stored edible seed and oil samples and risk assessment in the local population. *Toxins,* **14**: 642. https://doi.org/10.3390/toxins14090642

Ismail, A., Levin, R.E., Riaz, M., Akhtar, S., Gong, Y.Y. and de Oliveira, S.A.F., 2017. Effect of different microbial concentrations on binding of aflatoxin M1 and stability testing. *Fd. Contr.,* **73**: 492–496. https://doi.org/10.1016/j.foodcont.2016.08.040

Jaime-Garcia, R. and Cotty, P.J., 2003. Aflatoxin contamination of commercial cottonseed in South Texas. *Phytopathology*, **93**: 1190-1200. https://doi.org/10.1094/PHYTO.2003.93.9.1190

Jallow, A., Xie, H., Tang, X., Qi, Z. and Li, P., 2021. Worldwide aflatoxin contamination of agricultural products and foods: From occurrence to control. *Compr. Rev. Fd. Sci. Fd. Saf.*, **20**: 2332-2381. https://doi.org/10.1111/1541-4337.12734

Kabak, B., Dobson, A.D. and Var, I., 2006. Strategies to prevent mycotoxin contamination of food and animal feed: A review. *Crit. Rev. Fd. Sci. Nutr.,* **46**: 593–619. https://doi.org/10.1080/10408390500436185

Kihal, A., Rodríguez-Prado, M. and Calsamiglia, S., 2022. The efficacy of mycotoxin binders to control mycotoxins in feeds and the potential risk of interactions with nutrient: A review. *J. Anim. Sci.*, **100**: 328. https://doi.org/10.1093/jas/skac328

Krska, R., Leslie, J.F., Haughey, S., Dean, M., Bless, Y., McNerney, O. and Elliott, C., 2022. Effective approaches for early identification and proactive mitigation of aflatoxins in peanuts: An EU–China perspective. *Comp. Rev. Fd. Sci. Fd. Saf.*, **21**: 3227-3243. https://doi.org/10.1111/1541-4337.12973

Murugesan, G.R., Ledoux, D.R., Naehrer, K., Berthiller, F., Applegate, T.J., Grenier, B., Phillips, T.D. and Schatzmayr, G., 2015. Prevalence and effects of mycotoxins on poultry health and performance and recent development in mycotoxin counteracting strategies. *Poult. Sci.,* **94**: 1298-1315. https://doi.org/10.3382/ps/pev075

Oladeji, O.M., 2022. *Occurrence of mycotoxins, pesticide residues and heavy metals in rooibos teas and other consumed teas in South Africa*. Doctoral dissertation, University of Johannesburg.

Owino, G.A., 2022. *Effectiveness of a milk safety intervention by smallholder dairy farmers on bacterial and aflatoxin M1 contamination in milk in Kisumu County, Kenya*. Doctoral dissertation, Maseno University.

ProGnosis Biotech, S.A., 2015. *Bio-shield M1 milk and youghurt and bio-shield aflatoxin total extra sensitive, immunoassay method for determination of the Aflatoxin M1 in milk and yoghurt and total aflatoxin in grains, nuts, spices, cereals and other commodities including animal feed, respectively.* Available at. https//www.prognosis-biotech.com.

Raju, M.V.L.N. and Devegowda, G., 2002. Esterified glucomannan in broiler chicken diets contaminated with aflatoxin, ochratoxin and T-2 toxin: Evaluation of its binding ability (*in vitro*) and efficacy as immunomodulator. *Asian-Aust. J. Anim. Sci.,* **15**: 1051. https://doi.org/10.5713/ajas.2002.1051

Rehagel, C., 2022. *Analysis of mycotoxins in the food chain by enzyme immunoassay: Possibilities and limitations.*

Shabeer, S., Asad, S., Jamal, A. and Ali, A., 2022. Aflatoxin contamination, its impact and management strategies: An updated review. *Toxins*, **14**: 307. https://doi.org/10.3390/toxins14050307

Shar, Z.H., Pirkash, O., Shar, H.H., Sherazi, S.T.H. and Mahesar, S.A., 2020. Aflatoxins in cotton seeds and cotton seed cake from Pakistan. *Fd. Addit. Contamin.,* **13**: 72-76.

Smith, D.L., Atungulu, G.G., Wilson, S.A. and Mohammad-Shad, Z., 2020. Deterrence of Aspergillus flavus regrowth and aflatoxin accumulation on shelled corn using infrared heat treatments. *Appl. Engineer. Agric.,* **36**: 151-158.

Taheur, F.B., Fedhila, K., Chaieb, K., Kouidhi, B., Bakhrouf, A. and Abrunhosa, L., 2017. Adsorption of aflatoxin B1, zearalenone and ochratoxin A by microorganisms isolated from kefir grains. *Int. J. Fd. Microbiol.,* **251**: 1–7. https://doi.org/10.1016/j.ijfoodmicro.2017.03.021

Van Kessel, T.F.M. and Hiang-Chek, N., 2004. Aflatoxin binders-how to get the best value for money. *Int. Poult. Prod.,* **12**: 33-35.

Vieira, S.L., 2003. Nutritional implication of mould development in feed stuffs and alternatives to reduce the mycotoxin problem in poultry feeds. *World Poult. Sci. J.,* **59**: 111. https://doi.org/10.1079/WPS20030007

Xu, R., Yiannikouris, A., Shandilya, U.K. and Karrow, N.A., 2023. Comparative assessment of different yeast cell wall-based mycotoxin adsorbents using a model-and bioassay-based *in vitro* approach. *Toxins*, **15**: 104. https://doi.org/10.3390/toxins15020104

Yiannikouris, A., Apajalahti, J., Siikanen, O., Dillon, G.P. and Moran, C.A., 2021. *Saccharomyces cerevisiae* cell wall-based adsorbent reduces aflatoxin B1 absorption in rats. *Toxins*, **13**: 209. https://doi.org/10.3390/toxins13030209