RUFORUM Working Document Series (ISSN 1607-9345), 2021, No. 19 (1):903-911.
Available from http://repository.ruforum.org

Research Application Summary

Local solutions for global challenges: A case of clay and afatoxin in Uganda

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Abstract

To inclusively and sustainably reduce food contamination by mycotoxin, the potential of local clays to impound aflatoxins in contaminated maize bran was evaluated. Twenty five (25) samples of maize bran contaminated with 44.82±1.59µgkg^{-1} total aflatoxin (TAFL); 14.63±1.45µgkg^{-1} B1 (AFLB1), 3.49±0.22 µgkg^{-1} B2 (AFLB2), 18.35±0.16 µgkg-^{1} G1 (AFLG1) and 8.34±0.29 µgkg^{-1} G2 (AFLG2) were incorporated with 0.5, 1, 1.5 and 2 kg/ton^{-1} of potter's clay (CP), bentonitic clay (BC) and a commercial binders (CB) in five replicates. The concentration of aflatoxin (µgkg^{-1}) in each was determined using an Agilent Liquid Chromatography Tandem Mass Spectrometer (LC-MS/MS). The data were analyzed by two way analysis of variance (ANOVA) using R software. Results showed that the concentration of aflatoxin (TAFL, AFLB1, AFLB2 and AFLG1) differed ($p<0.05$) among all samples/ binders types ($p< 0.05$) except for AFLG2 ($p>0.05$). Significant interactions were recorded between all binder types and binder inclusion levels except for AFLB1. Average TAFL (23.35±0.61 µg/kg), AFLB2 (1.51±0.05µg/kg) and AFLG1 (10.97±0.17 µgkg^{-1}) were lowest in bran samples incorporated with 2 µgkg^{-1} bentonitic clays while AFLB1 (5.87± 0.12µgkg^{-1}) and AFLG2 (2.8±0.07µgkg^{-1}) were lowest in samples with 2 and 0.5kg/ton^{-1} of potters clay, respectively. Results suggested that local clays have a potential to reduce aflatoxin in contaminated maize-based feed resources thus improving food and nutrition security.

Keywords: Aflatoxin, clays, maize -based feed resources, Uganda

Résumé

Afin de réduire de manière inclusive et durable la contamination des aliments par les mycotoxines, le potentiel des argiles locales à retenir les aflatoxines dans le son de maïs contaminé a été évalué. Vingt-cinq (25) échantillons de son de maïs contaminés par 44,82±1,59µgkg-1 d'aflatoxine totale (AFLT) ; 14,63±1,45µgkg-1 B1 (AFLB1), 3,49±0,22 µgkg-1 B2 (AFLB2), 18,35±0,16 µgkg^{-1} G1 (AFLG1) et 8,34±0,29 µgkg-1 G2 (AFLG2) ont été incorporés avec 0,5, 1, 1,5 et 2 kg/tonne-1 d'argile de poterie (AP), d'argile bentonitique (AB) et d'un liant commercial (LC) en cinq répétitions. La concentration d'aflatoxine (µgkg^{-1}) dans chacun a été déterminée à l'aide d'un spectromètre de masse en tandem pour chromatographie en phase liquide Agilent (LC-MS/MS).

Les données ont été analysées par analyse de variance à deux voies (ANOVA) à l'aide du logiciel R. Les résultats ont montré que la concentration d'aflatoxine (TAFL, AFLB1, AFLB2 et AFLG1) différait (p<0,05) parmi tous les types d'échantillons/liants (p<0,05) sauf pour AFLG2 (p>0,05). Des interactions significatives ont été enregistrées entre tous les types de liants et les niveaux d'inclusion de liant à l'exception de AFLB1. L'AFLT moyen (23,35 ± 0,61 µg/kg), l'AFLB2 (1,51 ± 0,05 µg/kg) et l'AFLG1 (10,97 ± 0,17 µgkg^{-1}) étaient les plus faibles dans les échantillons de son incorporés à 2 µgkg^{-1} d'argiles bentonitiques tandis que l'AFLB1 (5,87 ± 0,12 µgkg^{-1}) et AFLG2 (2,8±0,07µgkg^{-1}) étaient les plus faibles dans les échantillons avec 2 et 0,5kg/tonne-1 d'argile de potier, respectivement. Les résultats suggèrent que les argiles locales ont le potentiel de réduire l'aflatoxine dans les ressources alimentaires à base de maïs contaminées, améliorant ainsi la sécurité alimentaire et nutritionnelle.

Mots clés: Aflatoxine, argiles, ressources alimentaires à base de maïs, Ouganda

Introduction

Aflatoxins are secondary metabolites produced mainly by some species of filamentous moulds (fungi); *Aspergillus flavus* and *Aspergillus parasiticu*s (Sugiharto, 2019). The four most studied types of aflatoxin that are isolated in contaminated animal feeds resources are B1, B2, G1, and G2 (Obonyo and Salano, 2018). The AFLB1 is the most common and natural potent cancer causing toxin (Lukwago *et al.,* 2019a). It is prevalent in most staple food/food resources such as maize in Sub-Saharan Africa (including Uganda) where it is sustained by warm and moist climatic conditions, poor pre and post harvest handling as well as inadequate control technologies (Williams *et al.,* 2004; Omara *et al.,* 2020). Maize based feed resources comprise 60-95% of livestock diets (including fish) mainly in form of maize bran in Uganda (Kaaya *et al.,* 2005) and are contaminated with aflatoxin above allowable thresh holds (Nakavuma *et al.,* 2020). Consumption of feed contaminated with aflatoxin is associated with poor growth and productivity due to reduced feed intake immunity, suppression of immunity, reproductive performance and inducing mortalities (Munkvold *et al.,* 2018). Residual aflatoxins are also transferred to animal by-products especially milk, meat and eggs and negatively impact agribusiness.

In 2019, Aflatoxin contamination reduced Uganda's economic growth by 0.26% due to loss of agricultural productivity worth 577 million US dollars, incidence of about 700 aflatoxin-induced liver cancer cases and decline in exports worth 7.48 million US dollars (Lukwago *et al.,* 2019). This makes control of aflatoxin in staple crops like maize key to attainment of nutrition, food and income security among other sustainable development goals (Granados-Chinchilla *et al.,* 2017; Ayo *et al.,* 2018; Sserumaga *et al.,* 2020). Techniques such as thermal inactivation, irradiation; acidification, alkalinisation, ozonation, ammonification and microbial detoxification significantly reduce aflatoxins (Kolosova and Stroka, 2017). However, use of such techniques is limited by high costs, need for sophisticated facilities, reduction of dietary palatability and nutritional values and the danger of unsafe chemical residuals (Benkerroum, 2019). Instead use of imported clays (aluminums silicate) especially bentonite is gaining adoption to reduce aflatoxin /mold toxin in Africa. Since bentonite toxin binders are imported in Sub Saharan Africa, they are expensive and less accessible to small scale feed value chain players especially in rural communities. As Uganda is endowment with a lot of natural clay minerals, this study determined the potential of local clays to bind toxins in a common feed resource as a basis for standardization and use in food quality preservation basing on 0.5 kg ton^{-1} inclusion level as often recommended for commercial binders (Ayo *et al.,* 2018).

Materials and Methods

The efficacy of three binders to sequester Aflatoxin in maize bran was studied under laboratory conditions at four inclusion levels (0.1, 0.5, 1 and 2 kg/ton). The binders included local crude potter's clay (PC) that was obtained from a wetland at Mbarara Zonal Agricultural Research and Development Institute adjacent to River Rwizi. Clay wastes from petroleum exploration activities in Hoima district locally known as "bentonite" and a commercial binder composed of yeast and aluminum silicate purchased from NutriNova, a feed additive store in Kampala City (Table 1).

Binder	Silicate	Aluminum	Sodium	Calcium	Magnesium
Bentonite	87.24	9.4	0.15	0.05	0.2
Potters Clay	86.67	0.4	0.05	0.01	0.02
Commercial binder	77.61	1.64	1	1.01	0.87
Average	83.84	3.81	0.40	0.36	0.36

Preparation of maize bran samples. Sacks **(**100kg) of wet de- hulled maize bran were picked from 10 points/locations within the maize bran storage house at Mbarara ZARDI maize mill. They were packed in polyethene bags and stored for one week without drying as usually practiced by local maize bran vendors. Out of the stored 100 kg of maize bran, 25 samples of 250g each were weighed and to each was added (spiked with) 25µg of a standard aflatoxin (Sigma Chemical Co., St. Louis, MO) stored in aceto-nitrile to enhance the concentration of aflatoxin to detectable levels (Figure 1).

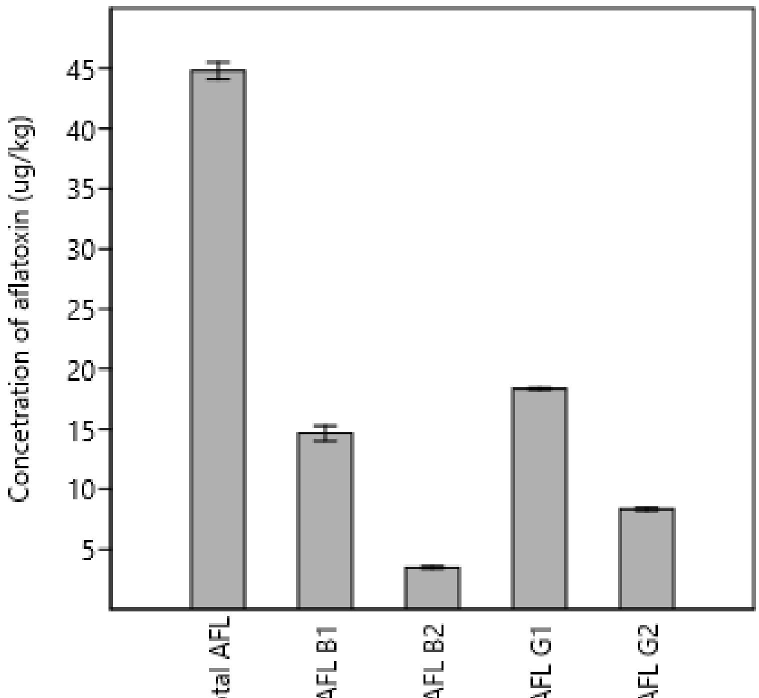

Figure 1. The mean concentration of aflatoxin in the 20 samples of maize bran studied

Out of the 25 spiked bran samples, 20 samples samples (250g each) were randomly mixed with 0.5, 1 1.5 and 2 kg ton^{-1} of potters clay (PC), Bentonite (B) and imported commercial binder (CB) (i.e. 0.125g, 0.25g, 0.375 and 0.5g in each 250g of maize bran) using a blender in five replicates The treated/spiked maize bran samples were packed in air tight zip lock bags and kept under room conditions for one week before laboratory analysis was conducted.

Laboratory analysis. Aflatoxins were extracted from 25g of each sample using 60/40 methanol/ water mixture. The extracts were then diluted with PBS until the acetonitrile content was lower than 10% v/v. The diluted extracts were then applied and allowed to pass through AflaStarTM R-Immunoaffinity Columns completely and then after rinsed with 2x10ml of de-ionized water. The available aflatoxins were then recorded using HPLC.

Statistical analysis. Data were analysed for two-way analysis of variance (ANOVA) using the general linear model (GLM) procedure of R software. Statistical difference was declared at 95% confidence level.

Results

The concentration of the aflatoxin types TAFL, AFL B1, B2 differed among binder types ($p<0.05$) except for AFLG2. The concentration of the five different aflatoxin types differed among all binder inclusion levels and interaction existed between binder type and inclusion level ($p< 0.05$) except for aflatoxin B1 (Table 2).

Table 2. P-values for concentration of aflatoxin among binder types, inclusion levels and the interaction between binder types and inclusion levels (binder type x inclusion level)

	TAFL	AFL B1	AFLB2	AFL G1	AFL G2
Binder type	8.52-8	2.34-9	1.62-13	< 2-16	0.25
Inclusion level	16-2	1.41-10	5.54-12	< 2-16	1.90-5
Interaction binder type and inclusion level	3.99-8	0.18	1.12-7	< 2-16	5.29-3

Total aflatoxin (TAFL) was lower in maize bran samples incorporated with 2 kg/ton of bentonite than 0.5 kg ton^{-1} of potter’s clay and 0.5bentonite and higher in maize bran samples with 1 kg/ton of potter’s clay than 1kg ton^{-1} commercial binder (Figure 2).

The concentration of AFL B1 (15µg/kg) in the control was reduced to the lowest (5.87±0.12µg/ kg) by 2kg/ton of crude potters clay. However this concentration did not differ among all bran with varying levels of potters clay and 2kg/ton of bentonite but differed from the highest concentration (12.62±0.14µg/kg) of AFLB1 recorded in bran samples incorporated with 0.5kg/ton of the imported commercial binder (Figure 3).

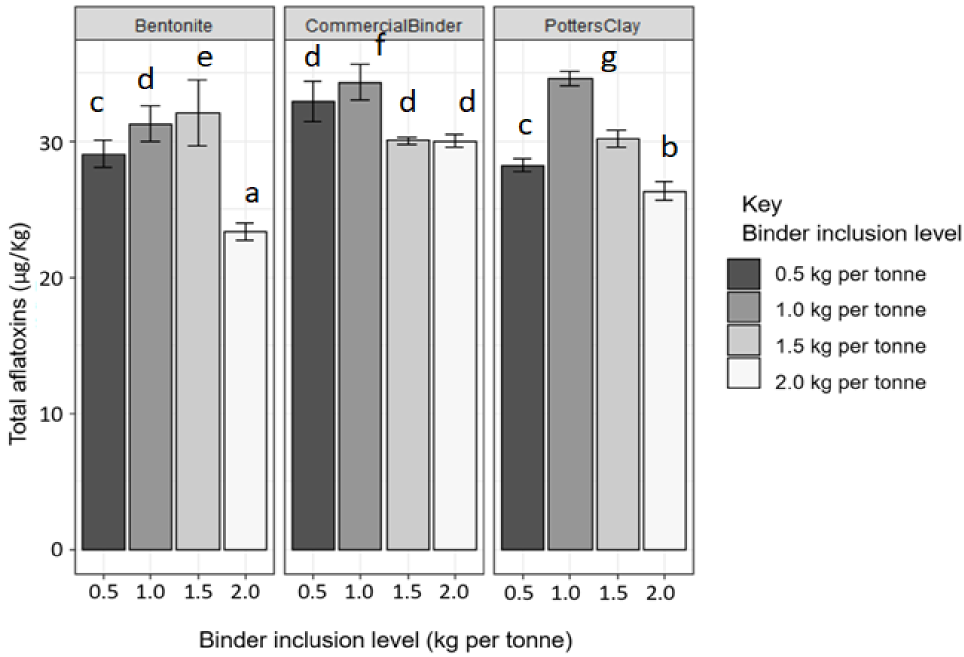

Figure 2. Concentration of aflatoxin B1 in maize bran samples

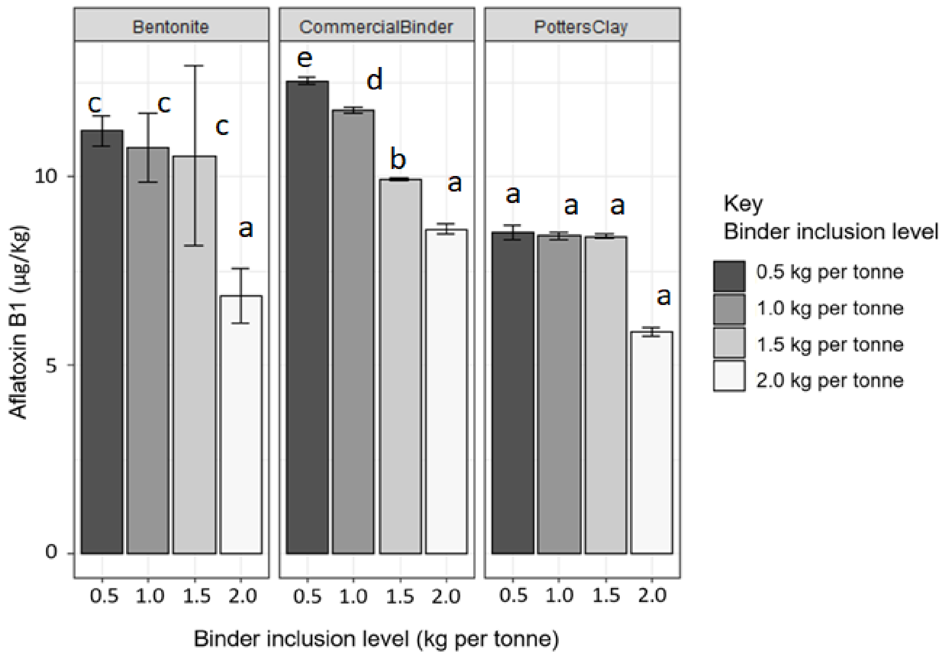

Figure 3. Concentration of aflatoxin B2 in maize bran samples incorporated with binders

The concentration of AFL B2 was lowest in maize bran incorporated with 1 and 2 kg/ton of bentonite and this concentration did not differ from that in bran samples incorporated with 1.5 and 2kg/ton of potter's clay (Figure 4).

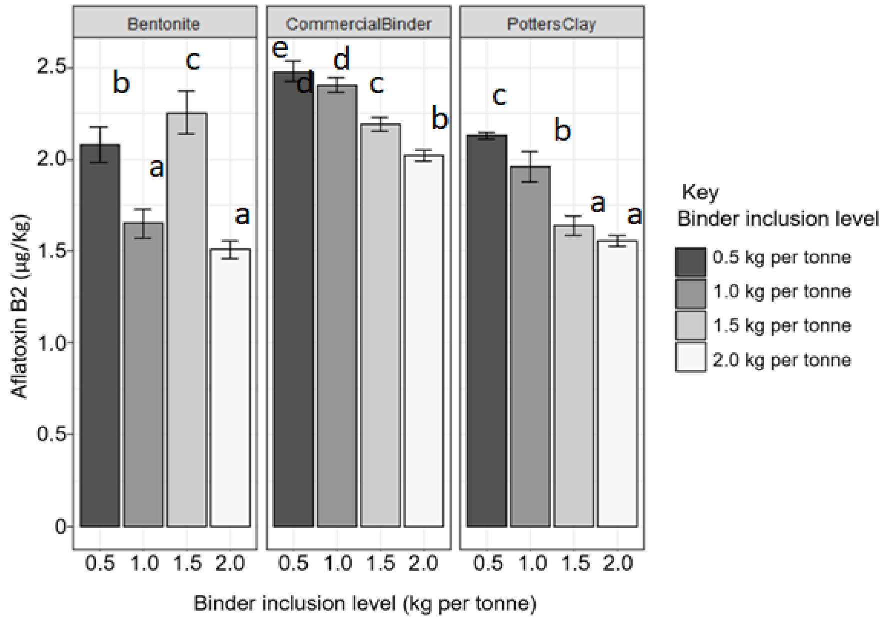

Figure 4. Concentration of aflatoxin G1 in maize bran samples incorporated with binders

The concentration of aflatoxin G1, the second most toxic aflatoxin after B1, was least in maize bran samples incorporated with 2 kg/ton of bentonite and highest in samples incorporated with 1 kg/ton of potter's clay (Figure 5).

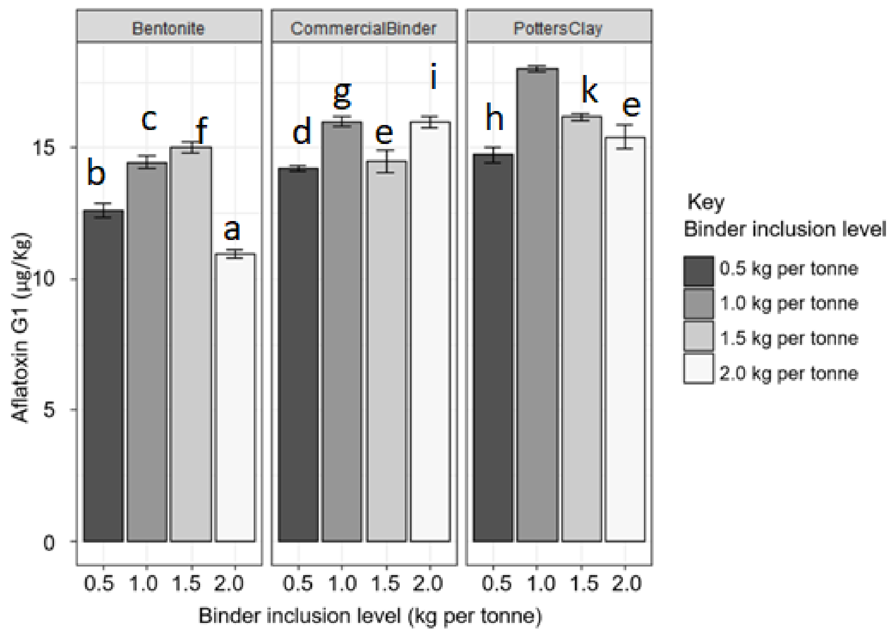

Figure 5. Concentration of aflatoxin G1 in maize bran samples incorporated with binders

The concentration of aflatoxin G2 was did not differ among binder types but was significantly reduced across all binder inclusion level except in bran containing 1 kg/ton of potter's clay (Figure 6).

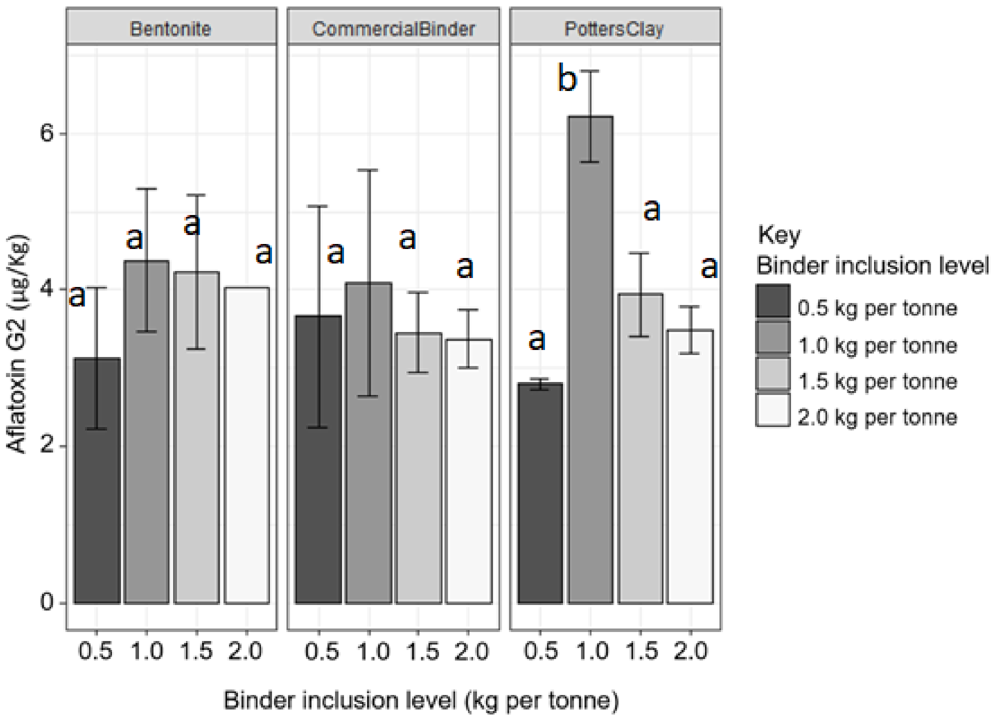

Figure 5. Concentration of aflatoxin G2 in maize bran samples incorporated with binders

Discussion

The types of aflatoxin analysed were dominated by AFLG1 (49.33 ±4.73µg/kg) and B1 (31.20±5.13 µg/kg) while G2 (12.90 ± 3.13 µg/kg) and B2 6.59±0.83 µg/kg) were the least. This was similar to observations of Matumba *et al.* (2015) who reported that the proportion of AFLB1 in maize samples within Malawi was about 50% of the total aflatoxins while that of AFLG1 was slightly higher than that of AFLB1.

The capacity of local clay and bentonite to bind/sequester and reduce aflatoxin generally increased with increase in inclusion levels with the least toxins recorded mainly at 2kg/ton of maize bran. These observations were close to those reported by Oluwaseyi (2016) when commercial bentonite was used as a feed toxin binder. The results indicated that incorporation of local clays including potters clay in maize bran reduced aflatoxin levels particularly AFLB1 which is the major toxin of concern in animal nutrition as reported by Jaynes and Zartman (2011). The percentage of the bound AFB1 by clay (60%) and bentonite was however lower than (90%) reported by Diaz *et al.* (2002).

Maize bran samples incorporated with bentonite and potters clay recorded the lowest concentration of aflatoxin. This translated to higher potential to reduce aflatoxin than the commercial binder that was used as a positive control. However basing on toxicity of AFL B1, potter's clay was the most effective although the concentration did not differ from that removed by bentonite at inclusion of 2 kg ton^{-1}.

Conclusions

The results from this study showed that local crude clays have potential to reduce aflatoxins in maize bran hence reducing their toxicity in feed resources. This study will also be conducted in-vivo with dairy cattle, tilapia fish and layer chicken to confirm consistency of the results under field conditions and how they correlate with body condition score and aflatoxin concentration in byproducts of milk, carcass and eggs, respectively. Further research is also required on the content of heavy metals in the clay samples from various locations in the country to be sure that they do not present additional risks.

Acknowledgement

This study was conducted with support from the Agricultural Research Organisation (NARO) under the recurrent grant scheme from the Government of Uganda. The team is grateful to Dr. Alice Nakiyemba and Prof. Moses Isabirye of Busitema University, Namasagali campus for their technical and support during the study. This paper is a contribution to the Seventh Africa Higher Education Week and RUFORUM Triennial Conference held 6-10 December 2021 in Cotonou, Benin.

References

Ayo, E. M., Matemu, A., Laswai, G. H. and Kimanya, M. E. 2018. Socioeconomic Characteristics Influencing Level of Awareness of Aflatoxin Contamination of Feeds among Livestock Farmers in Meru District of Tanzania. *Scientifica* Article ID 3485967, 11 pages. https://doi.org/10.1155/2018/3485967

Benkerroum, N. 2019. Retrospective and Prospective Look at Aflatoxin Research and Development from a Practical Standpoint. *International Jounal of Enviromental Research and Public Health* 16 (3633): 1–47. https://doi.org/10.3390/ijerph16193633

Diaz, D.E., Hagler, W.M., Hopkins, B.A. and Whitlow, L.W. 2003. Aflatoxin binders I: in vitro binding assay for aflatoxin B1 by several potential sequestering agents. *Mycopathologia* 156 (3): 223-226.

Granados-Chinchilla, F., Molina, A., Chavarría, G., Alfaro-Cascante, M., Bogantes-Ledezma, D. and Murillo-Williams, A. 2017. Aflatoxins occurrence through the food chain in Costa Rica: Applying the One Health approach to mycotoxin surveillance. *Food Control* 82: 217–226. https://doi.org/10.1016/j.foodcont.2017.06.023

Jaynes, W. F. and Zartman, R. E. 2011. Aflatoxin toxicity reduction in feed by enhanced binding to surface-modified clay additives. *Toxins* 3 (6): 551–565. https://doi.org/10.3390/toxins3060551

Kaaya, A. N., Warren, H. L., Kyamanywa, S. and Kyamuhangire, W. 2005. The effect of delayed harvest on moisture content, insect damage, moulds and aflatoxin contamination of maize in Mayuge district of Uganda. *Journal of the Science of Food and Agriculture* 85 (15): 2595–2599. https://doi.org/https://doi.org/10.1002/jsfa.2313

Kolosova, A. and Stroka, J. 2017. 2012. Evaluation of the effect of mycotoxin binders in animal feed on the analytical performance of standardised methods for the determination of mycotoxins in feed. pp. 1959-1971. In: Food Additives and Contaminants: Part A (Food Addit). Taylor & Francis. https://doi.org/10.1080/19440049.2012.720035

Lukwago, F. B., Mukisa, I. M., Atukwase, A., Kaaya, A. N. and Tumwebaze, S. 2019a. Mycotoxins contamination in foods consumed in Uganda: A 12-year review (2006–18). *Scientific African* 3: e00054. https://doi.org/10.1016/j.sciaf.2019.e00054

Lukwago, F. B., Mukisa, I. M., Atukwase, A., Kaaya, A. N. and Tumwebaze, S. 2019b. Mycotoxins contamination in foods consumed in Uganda: A 12-year review (2006–18). *Scientific African* 3: e00054. https://doi.org/10.1016/j.sciaf.2019.e00054

Matumba, L., Sulyok, M., Njoroge, S. M. C., Njumbe Ediage, E., Van Poucke, C., De Saeger, S. and Krska, R. 2015. Uncommon occurrence ratios of aflatoxin B1, B2, G1, and G2 in maize and groundnuts from Malawi. *Mycotoxin Research* 31 (1): 57–62. https://doi.org/10.1007/s12550-014-0209-z

Munkvold, G. P., Arias, S., Taschl, I. and Gruber-Dorninger, C. 2018. Mycotoxins in corn: Occurrence, impacts, and management. pp. 235–287. In: Serna-Saldivar, S. O. (Ed.), Corn: Chemistry and Technology, 3rd Edition. Elsevier. https://doi.org/10.1016/B978-0-12-811971-6.00009-7

Nakavuma, J. L., Kirabo, A., Bogere, P., Nabulime, M. M., Kaaya, A. N. and Gnonlonfin, B. 2020. Awareness of mycotoxins and occurrence of aflatoxins in poultry feeds and feed ingredients in selected regions of Uganda. *International Journal of Food Contamination* 7 (1): 1–10. https://doi.org/10.1186/s40550-020-00079-2

Obonyo, M. A. S. O. and Salano, E. N. 2018. Perennial and seasonal contamination of maize by aflatoxins in eastern kenya. *International Journal of Food Contamination* 5 (1): 1–5. https://doi.org/10.1186/s40550-018-0069-y

Oluwaseyi, A. M. 2016. Application of dietary bentonite clay as feed additive on feed quality, water quality and production perfomance of African catfish (*Clarias gariepinus*). Doctoral Dissertation, Stellenbosch: Stellenbosch University.

Omara, T., Nassazi, W., Omute, T., Awath, A., Laker, F., Kalukusu, R. and Adupa, E. 2020. Aflatoxins in Uganda: An encyclopedic review of the etiology, epidemiology, detection, quantification, exposure assessment, reduction, and control. *International Journal of Microbiology* Article ID 4723612, 18 pages. https://doi.org/10.1155/2020/4723612

Sserumaga, J. P., Ortega-Beltran, A., Wagacha, J. M., Mutegi, C. K. and Bandyopadhyay, R. 2020. Aflatoxin-producing fungi associated with pre-harvest maize contamination in Uganda. *International Journal of Food Microbiology* 313: 108376. https://doi.org/10.1016/j.ijfoodmicro.2019.108376

Sugiharto, S. 2019. A review of filamentous fungi in broiler production. *Annals of Agricultural Sciences* 64 (1): 1–8. https://doi.org/10.1016/j.aoas.2019.05.005

Williams, J. H., Phillips, T. D., Jolly, P. E., Stiles, J. K., Jolly, C. M. and Aggarwal, D. 2004. Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. *Am J Clin Nutr* 80: 1-3. Retrieved from https://academic.oup.com/ajcn/article/80/5/1106/4690412