

IN VITRO EVALUATION OF DIFFERENT CHEMICAL DETOXIFICANTS AGAINST AFLATOXIN (B1&B2) IN COTTON SEED CAKE

M. A. Tipu^{*1}, M. Ilyas², M. A. Khan³, M. Afzal³ and D. Hussain¹

^{1*}Barani Livestock Production Research Institute Kherimurat, District Attock, ²Glasgow Caledonian University, Glasgow, United Kingdom, ³Buffalo Research Institute Pattoki, District Kasur

^{*}Correspondence Author's Email: murtaza_76@yahoo.com

ABSTRACT: Four different types of chemical detoxificants namely, Copper sulphate, Benzoic acid, Calcium propionate and Sodium bisulfide were used against 100ug/kg aflatoxin B₁(AFB₁) and 10ug/kgB₂ (AFB₂) in cotton seed cake. Four different levels (0.25%, 0.5%, 0.75% and 1.0%) of each of above mentioned chemicals were tested in ELISA technique for detoxification of AFB₁ and AFB₂infected cotton seed cake. Calcium propionate 0.5% level decreased AFB₁from 100ug/kg to 16ug/kg followed by Benzoic acid 0.25% level, which limited AFB₁ to 23.3ug/kg. Copper sulphate (0.25%) reduced AFB₁ to 25 ug/kg. Other treatments did not remarkably reduce AFB₁. Meanwhile Calcium propionate 0.5% level also decreased AFB₂.The decrease was from 10 ug/kg to 1.5 ug/kg. Calcium propionate (0.5% level) is quite effective to reduce AFB₁andAFB₂in cotton seed cake at laboratorial level.

Key words: Mycotoxin, oil seed, toxin binder, calcium propionate, detoxificants.

(Received 17.09.2024

Accepted 02.12.2024)

INTRODUCTION

In livestock farming there is a problem of feed shortage prevailing in Pakistan. For food crops a struggle lies among animals and humans. Animal feed is an important indicator which affects meat, milk production and its constituents. Feed is the biggest cost factor in dairy farming (Ribeiro *et al.*2011).

Cotton is emerged as a leading cash crop in South Asia. The cotton production was 9917 thousand bales in the year 2015–2016 in Pakistan (Anonymous, 2017). CSC is a byproduct of cotton seed and is the most commonly used in feeding of dairy animals, its availability in specific season, contamination with, low quality materials and pesticides are some issues in its usage (Zahid *et al.*2003). Cotton seed cake also contains high level of aflatoxin (Weidenner, 2012).

In recent past, the quality of CSC is emerged a big issue as most of its supply was thought to pose health problems in small as well as large ruminants. The inadequate processing and storage cause the toxin problem in this ingredient (Yunus *et al.*2015).

Mycotoxins are produced by some fungal species of Claviceps, Fusarium, Penicillium, Aspergillus and Alternaria. It has been estimated that at least 300 of these fungal metabolites are highly toxic to animals and humans (Huwig *et al.* 2001). Improper storage can cause aflatoxin, ochratoxin and cyclopiazonic acid in CSC. There is an increased awareness for human and animal health hazards due to the presence of fungi in feed and food (Huwiget *et al.* 2001). Animal food products like milk and meat may contain aflatoxins although other health

issues to animals also prevail (Visconti, 1998). These mycotoxins are harmful to human beings as well, as these are transmitted through infected milk. Although only a few have real practical application, many approaches have already been adopted to control this menace.

Physical treatment is the first option which is used without involving any chemical like washing, polishing, mechanical separation, flotation and autoclaving (Jouany, 2007). The chemical treatment includes use of chemicals like calcium hydroxide, ammoniation, copper sulphate, sodium bisulfide and various acids to reduce this menace (Giovati *et al.* 2015)

The research in the past does not tell us the optimum levels of certain chemicals to minimize aflatoxin in cattle feed. However, some levels are being used on the basis of earlier studies to assess their efficacy to detoxify aflatoxin.

MATERIALS AND METHODS

Laboratory Evaluation of chemical toxin binders for treatment of cotton seed cake: The first step was the evaluation of various chemical toxin binders. This experiment was conducted in Nutrition Division at Buffalo Research Institute, Pattoki, District Kasur. Based on the review, some levels are selected. In the first step, about 80 kg cotton seed cake having aflatoxin around 100 ug/kg was searched and collected from different cotton expellers and market. This was then grounded. The aflatoxin level B₁ was tested by using Elisa Kit method. At the end, cotton seed cake was treated with four concentrations of each of four chemical toxin binders.

After one week of the treatment, the aflatoxin estimation and proximate analysis were performed.

Sample collection and chemical analysis: Cotton seed cake samples were collected with sample size of 2 grams. Oven dried at the temperature of 55°C. Ground to the particle size of 2 mm using Wiley mill. Sample analysis was done by using the methods of AOAC (1990).

Chemical treatments with copper sulphate, benzoic acid and calcium propionate: Weight of cotton seed cake was done and added to conical flask of 500ml. to make the moisture level 180g/kg 9ml distil water was added to each flask. Samples were selected and added to flasks (except to control flasks) in triplicate keeping in view the results taken after preliminary screening. Autoclaving of flasks was done at temperature of 121°C for period of 15mins inoculated with 0.1mm spore suspension (25×10⁷ CFU/ml) of *Aspergillus parasiticus*

strain NRRL. Incubation of each was done at temperature of 27-30°C for 7days in darkness. For spore counting 1g of sample was taken, aflatoxin estimation of remaining sample at day 7 was done after drying and autoclaving. Benzoic acid will directly be added to contaminated samples according to the respective doses.

Chemical treatments with sodium bisulfide: Sodium bisulfide was certified ACS grade. 1 kg portions of naturally contaminated cotton seed cake were treated by placing into 1-liter flasks and chemical treatment was done according to their respective levels on the dry matter basis. Distilled water was also added to bring the moisture content of samples to approximately 20% on a wet basis. Flasks was sealed, mixed by hand for a few minutes and stored at ambient temperature (22°C) in the dark for 24 h and then aflatoxin level was determined.

Table 1 Scheme of use of different levels of different chemicals

Acidic salts		Acid	
Calcium propionate	Copper sulphate	Sodium Bisulfide	Benzoic acid
0.25%, 0.50% 0.75% and 1.0% of cotton seed cake	0.25%, 0.50% 0.75% and 1.0% of cotton seed cake	0.25%, 0.50% 0.75% and 1.0% of cotton seed cake	0.25%, 0.50% 0.75% and 1.0% of cotton seed cake

RESULTS AND DISCUSSION

The results are summarized in table: Calcium propionate 0.5% level decreased AFB₁ from 100ug/kg to 16ug/kg followed by Benzoic acid 0.25% level which limited AFB₁ to 23ug/ kg. Copper sulfate (0.25%) level stood third by decreasing AFB₁ to 25 ug/kg. In the case

of AFB₂, Calcium propionate 0.5% level reduced the AFB₂ from 10ug/kg to 1.5ug/kg followed by 2ug/kg which was shown by Calcium propionate 0.25% level, Copper sulphate 0.25% and Benzoic acid 0.25% level. The proximate analysis of cotton seed cake was done before and after treatment with chemical detoxificants and there was no change in nutrient profile (table 3).

Table 2. Treatment of cotton seed cake with different chemical detoxificants.

Level of Aflatoxin (B1)&(B2) in Cotton Seed Cake before treatment	Treatments	Level	Aflatoxin (B1) Level ug/kg	Aflatoxin (B2) Level ug/kg
103 ug/kg (AFB1) 10 ug/kg (AFB2)	Copper Sulphate	0.25%	25	2
		0.50%	50	4
		0.75%	70	7.5
		1.00%	88	8
	Benzoic Acid	0.25%	23.3	2
		0.50%	39.9	3
		0.75%	89.3	8.5
		1.00%	98.4	8
	Calcium Propionate	0.25%	27.5	2
		0.50%	16	1.5
		0.75%	49.1	5
		1.00%	80	8
	Sodium Bisulfite	0.25%	100	9
		0.50%	95	9
		0.75%	93	9.5
		1.00%	90	9.5

The results of this study coincide with the (Mukendi *et al.*, 1991). They used different chemicals i.e. sodium sulfite, sodium hydroxide and hydrogen peroxide to detoxify aflatoxin. The above mentioned chemicals performed well. This study is also in agreement with (Bintvihok and Kosit, 2006). These scientists used different levels of calcium propionate in feed and 0.5% level was proved to be effective to control aflatoxin B₁. Moreover, our experiment is in partially line with (Arshad *et al.*, 2012). They used chemicals in the culture of *Aspergillus*. The chemicals were only used for the *Aspergillus* species. Calcium propionate was also used to

increase the storage time of feed (Alam *et al.*, 2014). The level of 0.5 g of calcium propionate for 1 kg of finished feed was used. The level used in that study was lower as compared to this study. The difference of dose was due to different forms of feed. They used broiler finisher feed, so less level was used. In this study, best results were shown for 0.5 % level because cattle feed has normally higher level of aflatoxin (B₁&B₂). Meanwhile, a study concluded that reduction of aflatoxin (B₁) is 85-90% through chemical detoxificants (Ismail *et al.*, 2014). In this experiment best reduction was 84% by calcium propionate

Table 3 Proximate analysis of cotton seed cake before and after treatment.

	Dry Matters%	Protein%	Fat%	Fiber%	Ash%
Before treatment with chemical detoxificants	90	21.05	8.76	23.19	10.29
After Treatment	90	21.05	8.65	23.18	10.21

Conclusion: Contamination of cotton seed cake with aflatoxin is one of the most important challenges which are being faced by the feed industry and common livestock owners. We used a variety of chemical detoxificants to mitigate this problem. In vitro Calcium propionate 0.5% gave good results to reduce AFB₁&AFB₂.

REFERENCES

- Alam, S., H. Shah, N.A. Khan, A. Zeb, A.S. Shah and N. Magan. 2014. Water availability and calcium propionate effect on fungal population and aflatoxin production in broiler finisher feed during storage. *Feed Add. & Cont. Part A*, 1-8.
- Anonymous, Economic Survey., 2016-17. Ministry of Finance, GOP. Islamabad, Pakistan.
- AOAC, 1990. Official Methods of Analysis, 17th edition. Association of Analytical Chemists, Arlington, Virginia., USA.
- Arshad, H., Shafqatullah, A. Javed and Z. Rehman. 2012. Inhibition of aflatoxin producing Fungus growth using chemical, herbal compounds/ species and plants. *Pure Appl Bio.* 1 (1), 8-13.
- Bintvihok, A. & S. Kositcharoenkul. 2006. Effect of dietary calcium propionate on performance, hepatic enzyme activities and aflatoxin residues in broilers fed a diet containing low levels of aflatoxin B₁. *Toxicon.* 47 (1), 41-6.
- Giovati, L., M. Walter, C. Tecla, S. Claudia, C. Stefania and P. Luciano. 2015. AFM1 in Milk: Physical, biological, and prophylactic methods to mitigate contamination. *Toxins.* (7), 4330-4349.
- Huwig, A., S. Freimund, O. Kappeli and H. Dutler. 2001. Mycotoxin detoxification of animal feed by different adsorbents. *Toxicol Lett.* 122, 179-188.
- Ismail, A., L. Bruna, V. Goncalves, Diane, P. de Neefi, Barbara, F.S.C.C. Carolina, H. Henning, M. Sajid, G.C. Adrono, H.C. Carols, and A.F.O. Carols. 2018. Aflatoxin in foodstuff: Occurrence and recent advances in decontamination. *Food Res. Intl.* 113,74-85.
- Jouany, J.P., 2007. Methods for preventing, decontaminating and minimizing the toxicity of mycotoxin in feeds. *Anim. Feed Sci. Technol.* 137, 342-362.
- Mukendi, N., B.D. Dollmann and C. Meester. 1991. Detoxification of aflatoxin B₁ by different chemical methods and evaluation of the effectiveness of the treatment applied. *J. Pharm Belg.* 46 (3), 1828.
- Ribeiro, R.D.X., R.L. Oliveira, F.M. Macome, A.R. Bagaldo, M.C.A. Silva, C.V.D.M. Ribeiro, Carvalho and D.P.D. Lanna. 2011. Meat quality of lambs fed on palm kernel meal, a by-product of biodiesel production. *Asian-Aust J. Anim Sci.* 10, 1399-1406.
- Visconti, A., J. Zuxun, L. Quan, L. Yongsheng, T. Zianchang and G. Lianghua. 1998. Problems associated with *Fusarium* mycotoxins in cereals in Stored product protection. Chengdu: Sichuan. 173-186.
- Weidenner, M., 2012. Mycotoxins in Feed Stuffs. (2nd Edition). Springer Science Media. 2(5), 190.
- Yunus, A.W., M. Sulyok and J. Bohm. 2015. Mycotoxin cocktail in the samples of oilseed cake from early maturing cotton varieties associated with cattle feeding problems. *Toxin.* 7, 2188-2197.
- Zahid, I.A., L.A. Lodhi, N. Ahmad, N. Rehman and M.S. Akhtar. 2003. Effect of cottonseed cake (Gossypol) on live weight of Teddy male goats. *Pak Vet J.* 23, 27-30.