TRENDS OF AFLATOXIN IN LIVESTOCK FEED AND VARIOUS FEED INGREDIENTS IN PAKISTAN

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ABSTRACT: Objective of current study was to determine aflatoxin B1 level in cotton see cake (CSL) rape seed cake (RSC), wheat bran (WB) and compound feed (CF) in 10 districts of Punjab, Pakistan namely Bahawalnagar, Bhakkar, Jhang, Kasur, Khanewal, Layyah, Multan, Muzaffargarh, Rahim Yar Khan and Rajanpur. For this purpose samples of CSC (n=375), RSC (n=100), WB (n=75), CF (n=100) were analyzed at Nutrition Division of Buffalo Research Institute, Pattoki District Kasur. Different samples were extracted, filtered and further screened by using commercially available ELISA Romer kit. The data were analyzed by one-way analysis of variance technique using SPSS software. Highest value of aflatoxin in CSC, RSC, WB and CF was found in districts; Bahawalnagar, Sialkot, Muzaffargarh and Bahawalnagar respectively, while its lowest value in CSC, RSC, WB and CF was found in Muzaffargarh, Multan, Bahawalnagar and Rahim Yar Khan, respectively. Highest infected samples were found in compound feed (21%) followed by cotton seed cake (11.93%), wheat bran (11.71%) and rape seed cake (11.11%) by percentage formula. Based upon the result of recent study toxin binders should be added in processed feed and storage condition of feed ingredients must be improved to minimize the risk of aflatoxicosis.

Key words: Aflatoxin, cotton seed cake, rape seed cake, wheat bran, compound feed.

(Received 03.10.2020 Accepted 11.11.2020)

INTRODUCTION

Mycotoxins which are of greatest concern for dairy animals include aflatoxin, ochratoxin, Vomitoxin, deoxynivalenol, zearalenone, T-2 toxin, fumonisin and PR toxin (Sultana *et al*., 2013). Aflatoxin contamination in foods and animal feeds is of global concern due to their potential toxicity, mutagenicity, carcinogenicity, teratogenicity, neurotoxicity and immunosuppressiveness (Lee and Rachmawat, 2006). Aflatoxins are cancer causing secondary metabolites produced primarily by Aspergillus flavus and Aspergillus parasiticus in agricultural foodstuff such as peanuts, maize grains, cereals, and animal feeds. Aflatoxins are basically difuranocoumarin molecules which are synthesized through the polyketide pathway. Total 18 type of aflatoxins present of which six have been identified and considered most important , designated as B_1, B_2, G_1, G_2, M_1, and M_2, respectively. These aflatoxin groups have different moleculer structure. For example, the B-group aflatoxins ($B_1$and B_2) have a cyclopentane ring while the G-group (G_1 and G_2) contains the lactone ring. Whereas the B-group aflatoxins exhibit blue fluorescence, the G-group exhibits yellow-green fluorescence under ultraviolet (UV) light, thus making the use of fluorescence important in identifying and differentiating between the B and G groups. Aflatoxin B_1 is the most common (khayoon *et al* 2010) and the most widespread (Mostrom and Jacobsen, 2011), in the world and accounts for 75% of all aflatoxins contamination of food and feeds. Aflatoxins M_1 and M_2 are hydroxylated products of aflatoxins B_1 and B_2, respectively, and are associated with cow milk upon ingestion of B_1 and B_2 aflatoxins' contaminated feed. Moreover, once formed from B_1 and B_2 forms, aflatoxins M_1 and M_2 remain stable during milk processing.

The most abundant AF in naturally contaminated dairy ration is Aflatoxin B1 (AFB1) and is most toxic and carcinogenic for human and animals. Toxic effects of AF-contaminated ration are due to liver damage and it decreases growth rate, milk production, milk quality and decreased resistance to infectious diseases. Aflatoxins detrimental effects are due to its binding to nucleic acids, which impair protein formation in the body.

ELISA tests are based on the affinities of monoclonal or polyclonal antibodies for aflatoxins and it is possible to detect aflatoxin B1 in foods and feedstuffs, specially and quickly with very little sample preparation. In Pakistan, different studies have been conducted that explained the conversion and excretion pattern of Aflatoxin into milk and milk products but little research about the Aflatoxin levels in various dairy feeds. This study examined the Aflatoxin levels in various dairy feed concentrates, silage, total mixed rations and different feed ingredients.

MATERIALS AND METHODS

Sample Collection: For the estimation of AFB1, a total of 500 samples of dairy compound feed i.e., compound (n=100), Cotton Seed Cake (n=375), Wheat Bran (n=75), Rapeseed Meal (n=100) were selected from five districts namely (Multan, Bahawlalnagar, Okara, Muzaffargarh, Rahim Yar khan) of Punjab which were further analyzed at Nutrition Analysis Laboratory, BRI, Pattoki, Kasur.

Sample Preparation for ELISA Analysis: Each sample (30 g) was finally ground mixed with 70% methanol (1:5 W/V) as a solvent through blending for 3 min, filtered through a Whattman filter and screened by using commercially available ELISA kit (Aflatoxin ELISA kit, by Rommer compny).

Test Protocol: Procedure was followed mention in log book of kit. Verotox kit was used.1st take 100ml conjugate in each using wells (mixing wells). Enter standard 100 ml in specific wells. Coming cell used for samples. Mix well mixture. Take 100ml out mixture shift in antibody coated well Incubate for 3 mints. After this washing of eliza plate. Dry wells and add substrate Incubate for 2 mints and then add stop solution. Last step read this plate under eliza reader

RESULTS

The recovery percentage of aflatoxin B1 was 90% in the present study. The contamination of aflatoxin B1 found in cotton seed cake was 11.93% after analysis of 375 samples. Maximum level of aflatoxin (CSC) in Bahawalnager was 200ppb and minimum in Muzafergarh, while livestock compound feed had aflatoxin 21% which is highest among all feed ingredients in district Bahawalnagar (130.52ppb) and minimum in Rahim Yar Khan.

Among the rape seed cake livestock feed samples analyzed (Table-1), 11.11% (11 of 100) were contaminated with aflatoxin B1 with maximum (20.72ppb) in district Sialkot and minimum in Bahawalnagar with (4ppb), in case of wheat bran highest value of aflatoxin was found in district Muzaffargarh with 79.68ppb and incidence of 11.71% (11 of 75).

The livestock feed ingredients analyzed for aflatoxin B1, were within the safe limit of feed compound act (2016) standards (Cotton Seed Cake=200ppb, compound feed= 50 ppb, Rape seed cake=20ppb and wheat bran = 20 ppb).

Table-1.

Sr. #	Ingredients	Total	Positive	Negative	Incidence%	Max ppb	Min ppb
1	Cotton seed cake	375	44	331	11.79[a]	200	24
2	Compound feed	100	21	79	21[b]	130	26
3	Rape seed cake	100	11	89	11.11[a]	20.72	4
4	Wheat bran	75	11	64	11.71[a]	79	3.12

*Mean with different superscript differ significantly.

DISCUSSION

The highest infected samples were found in compound feed (21%) followed by cotton seed cake (11.93%), wheat bran (11.71%) and rape seed cake (11.11%) by percentage formulas. The result of present study partially coincides with Bahram *et al* 2016 and Kabir *et al* 2016. The above mentioned studies also stated the high incidence of aflatoxin in feed samples and feed ingredients. In our study incidence was somewhat low (21%) in compound feed as compared to Bahran *et al* 2016 and Kabir *et al* 2016. It might be due to change in ecological conditions. Sultana *et al* 2012 also pointed out high incidence of aflatoxin B1 in compound feed. Our study also had high level of aflatoxin B1 in compound feed. Similar studies with respect to contamination of feed for AFIBI have been conducted, Charoenpornsook and Karusarasai 2006 and Akosy *et al* 2009. The incidence of aflatoxin B1 in cotton seed cake was (11.93%) in our study. The incidence correlates with Younas *et al* 2015. Younis *et al* 2015 also pointed out high level of aflatoxin B1, B2 in cotton seed cake samples.

Rodrigues *et al* 2017 also noted the high contamination of grain and feed ingredients. They analyzed maize grain and other feed ingredients which are being used in animal feed industry meanwhile our study also co-relates with the with the Ademse *et al* 2013 in which they concluded high level of aflatoxin B_1 in oil containing seeds and their products in our study major incidence was examined in Cotton Seed Cake and Rape Seed Cake (11%). Incidence was higher in another study which was conducted Alshowabkeh *et al*. 2015 having incidence of aflatoxin B_1 of 40% in Jordon. In our study highest incidence was 21%. The difference might be in storage conditions which seemed to be better in Pakistan as compared to Jordon. Similarly our study is in live with Facis *et al* 2018. According to that research feed ingredients, maize, maize products and oil seed products

had medium incidence of aflatoxin same was the case in our study.

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