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Short Communication

Assessment of Aflatoxin in Dairy Concentrate Feeds, Total Mixed Rations, Silage and Various Feed Ingredients in Pakistan

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ABSTRACT

This study examined the aflatoxin levels in various dairy feed concentrates, silage, total mixed rations and different feed ingredients. Of the total 215 samples, 50 samples of dairy concentrate feed, roughages were i.e. silage (n=23), TMR (n=9), Hay (n=8) and 115 samples of other feed ingredients were analyzed. After Extraction and filtration, aflatoxin samples were screened by using commercially available ELISA kit. Among the feed ingredients, highest infected samples were of cottonseed cake (68%) and the lowest infected samples were of soya bean meal (16.67%). Among the dairy feed, dairy concentrates 64% were found highly infected, while the least infected (12.50%) were hay samples. It was concluded that there was high level of aflatoxin prevalence in dairy feedstuffs. Toxin binders and other feed ingredients could be utilized to minimize the risk of aflatoxicosis.

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Mycotoxins are secondary metabolites produced by a wide range of filamentous fungi present in many animal feedstuffs including roughages and concentrates (Iheshiulor *et al.*, 2011; Yiannikouris and Jonany, 2002). 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). Among all, aflatoxins (AF) the most important are further classified into B1, B2, G1 and G2. AFs are secondary metabolites produced by *Aspergillus flavus and Aspergillus parasiticus* (Mostrom and Jacobsen, 2011). The most abundant AF in naturally contaminated dairy ration is Aflatoxin B1 (AFB1) and is most toxic and carcinogenic for human and animals (Kaleibar and Helan, 2013). 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 (Akande *et al.*, 2006). Occurrence of AF in feed and feed commodities is important where temperature and humidity is higher due to optimum conditions for molds to grow. When ruminants ingest AF-contaminated feed, it is metabolically bio-transformed into hydroxylated form AF M1 by an enzyme cytochrome P and is excreted in the milk of lactating animals (Anjum *et al.*, 2012; Yiannikouris and Jonany, 2002). AF M_1 is considered in group 1 human carcinogen by International Agency for Research on Cancer (IARC). Both AF B_1 and M_1 are carcinogenic to animals and human (Rashid *et al.*, 2012).

In Pakistan, different studies have been conducted that explained the conversion and excretion pattern of AF into milk and milk products (Iqbal and Asi, 2013; Muhammad *et al.*, 2010) but little is known about the AF levels in various dairy feeds. This study examined the AF levels in various dairy feed concentrates, silage, total mixed rations and different feed ingredients.

Materials and methods

For the estimation of AFB1, a total of 215 samples of dairy concentrate feed (n=50), roughages (n=40) *i.e.*, silage (n=23), TMR (n=9), hay (n=8) and other feed ingredients (n=115) were received at Nutrition Analysis Section, Vigilant Laboratory, Lahore from commercial dairy farmers all over the country over a period of 12 months from September, 2013 to August, 2014. A total of 11 different dairy feed ingredients were included for the AFB1 analysis.

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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, Affini Tech Ltd., Bentonville, AR) (Rai *et al.*, 2011). The cut off value of ELISA for the positive samples of AFB1 samples was 20 ng/g as recommended by FDA (Rashid *et al.*, 2012).

Results

The presence of AFB1 in different dairy feed ingredients and dairy compound feeds are presented in Table I. Current study reveals that out of 11 different dairy feed ingredients incidence of AFB1 was 48% while the mean and maximum AFB1 levels were 31.80 and 210.07 ppb, respectively. Among all the feed ingredients and protein sources as well, highest percentage of AFB1 positive samples belonged to cotton seed cake (68%) and the lowest belonged to soya bean meal (16.67%). On the other hand, among the carbohydrate sources, corn and corn gluten feed has the highest percentage (60%) of positive samples and the lowest percentage (25%) belonged to beet pulp and rice broken. The highest value (210.07 ppb) of AFB1 level was observed in corn gluten meal, while the lowest value (1.02 ppb) was found in the samples of soya bean meal and rice (Table I).

As far as the dairy feeds are concerned *i.e.* dairy concentrates, total mixed rations (TMR), silage and hay, the incidence of AFB1 level was 45.56%, while the mean and maximum levels of AFB1 were 11.79 and 42.39 ppb, respectively. Among the dairy feeds, the highest AFB1 contamination (64%) was found in dairy concentrates followed by TMR (33.3%), silage (21.74%) and hay (12.50%). The highest AFB1 value (42.39 ppb) was found in dairy concentrates while the lowest value (0.56 ppb) was found in hay (Table I).

This data was also analyzed on the basis of the months in which samples were received. The highest incident of AFB1 positive samples were found in the month of August *i.e.,* 65% while the lowest incidence was found in the month of April *i.e.,* 9.09 % (Table II).

Discussion

The dairy industry being an economically important sector endures great losses in terms of decreased milk production, abortion and death due to AF in feeds (Iqbal and Asi, 2013; Kaleibar and Helan, 2013; Mostrom and Jacobsen, 2011; Muhammad *et al.*, 2010). This study observed the mean levels of AFB1 in dairy feedstuffs (except soyabean meal, rapeseed meal, canola meal, rice) and dairy feeds (except silage, hay) that were higher than the safe limit recommended by FDA *i.e.* 20ng/g. Such higher levels of AFB1 have been reported by many previous workers in the dairy around the world (Yiannikouris and Jonany, 2002).

In agreement with the previous works done, among the carbohydrate sources, corn is found to be the most contaminated dairy feedstuff (Anjum *et al.*, 2012; Bhatti *et al.*, 2001). Similarly among the protein sources, cotton seed cake and sunflower meal are dominant susceptible ingredients. The possible factors for the fungal contamination are related to their post-harvest storage conditions with high moisture level (Saleemi *et al.*, 2012). These results are helpful in indicating the buying and storage of only those ingredients that are less vulnerable to develop AFB1 contamination according to local environmental conditions.

As far as dairy feeds are concerned, in present study, commercial compound feed is found to be most susceptible for AF contamination followed by TMR rations, whereas silage and hay have low percentage of positive samples. The present study revealed that incidence of AFB1 contaminations is higher in feed as compared to silage. These results are in agreement with previous studies (Anjum *et al.,* 2012; Bhatti *et al.,* 2001). This study also reflects the possibility of faults in pre-manufacturing quality based negligence and post-manufacturing storage of commercial compound feed. Silage could have higher AFB1 contamination because during ensiling process, several factors such as insufficient drying, condensation, moisture content, heat, insects and other conditions could lead to undesirable growth of fungi which subsequently lead to mycotoxin production (Sultana *et al.*, 2013).

Different parameters related to season *i.e.*, humidity and temperature play crucial role in fungal growth and AFB1 incidence (Sultana *et al.*, 2013). In current study, high levels of AFB 1 were observed in the months from June to November. On the other hand, low concentrations of AFB1 were observed during December to May elaborates the evident effect of rainy season. Monsoon rainy season starts in Pakistan from June to September (Anjum *et al.*, 2012; Rashid *et al.*, 2012). During this span, hot season with heavy rain fall that produces high relative humidity and temperature. These parameters provide the ideal conditions for the growth of fungus and higher AFB1 levels. This higher AFB1 incidence during rainy season reflects the lack of proper technical and storage facilities.

The high levels of mycotoxins in the dairy feed samples and limited data on factual contamination of feed with mycotoxin imply that more emphasis should be given to the routine inspection of dairy feed and milk for mycotoxins. There is a need to improve storage practices and adopt effective strategies for mycotoxin decontamination and detoxification.

Table I.- AFB1 levels in different dairy feed ingredients and dairy feeds.

	AFB1 level (ng/g)	Minimum- Maximum	Positive samples
Feed ingredients			
Maize (n=37)	28.18 ± 1.72*	2.56-59.96	12 (32%)
Beet pulp (n=4)	15.89 ± 0.14	9.52-25.01	1 (25%)
Cotton seed cake (n=25)	111.94 ± 9.36	6.92-185.97	17 (68%)
Soya bean meal (n=12)	19.20 ± 1.85	3.56-38.45	2 (16.7%)
Rice (n=12)	16.42 ± 1.88	1.02-30.98	6 (50%)
Corn gluten meal (n=10)	116.77 ± 17.55	17.14-210.07	6 (60%)
Poppy seed meal (n=4)	31.68 ± 12.82	8.23-57.51	2 (50%)
Rice polish (n=8)	22.07 ± 2.85	15.19-40.15	4 (50%)
Sunflower meal (n=5)	23.86 ± 2.59	12.39-39.21	2 (40%)
Canola meal (n=4)	7.08 ± 1.66	3.26-21.33	1 (25%)
Rape seed meal (n=4)	8.78 ± 3.77	1.58-28.59	1 (25%)
Total (n=125)			54 (43%)
Dairy feed			
Vanda (n=50)	29.30 ± 1.14	5.31-42.39	32 (64%)
Silage (n=23)	9.98 ± 0.78	2.51-21.56	5 (21.74%)
TMR (n=9)	21.97 ± 1.76	3.02-55.17	3 (33.33%)
Hay (n=8)	4.91 ± 1.53	0.56-22.31	1 (12.50%)
Total (n=90)			41 (45.56%)

*Mean ± S.E.

Table II.- AFB1 levels in total feed samples around the year.

Months	AFB1 level (ng/g)	Minimum- Maximum	Positive samples
January (n=8)	48.31 ± 28.75*	0.99-185.97	2 (25%)
February (n=16)	69.68 ± 19.56	0.9-210.07	3 (18.8%)
March (n=9)	52.50 ± 21.28	1.02-176.39	3 (33.3%)
April (n=11)	8.89 ± 2.27	1.36-23.49	1 (9.1%)
May (n=4)	1.94 ± 0.08	1.69-2.06	1 (25.0%)
June (n=30)	29.58 ± 4.59	1.02-97.89	18 (60.0%)
July (n=36)	21.26 ± 4.76	2.16-156.37	19 (52.8%)
August (n=32)	30.12 ± 5.76	1.52-132.85	21 (65.6%)
September (n=18)	20.99 ± 3.25	3.26-42.39	8 (44.4%)
October (n=13)	32.13 ± 9.95	2.56-119.73	6 (46.2%)
November (n=24)	30.28 ± 8.30	0.56-156.79	10 (41.7%)
December (n=14)	48.58 ± 12.53	1-129.82	3 (21.1%)
Total (n=215)			95 (44.2%)

*Mean ± S.E.

Conflict of interest statement

The authors have no conflict of interest to declare.

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