Research Article

Aflatoxin B1 Contamination Status of Concentrate Feeds of Dairy Goats in Lahore, Pakistan

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Abstract | Aflatoxins are the secondary metabolites produced by molds particularly by *Aspergillus flavus* and *Aspergillus parasiticus.* Aflatoxin B1 is the most common metabolite highly toxic for humans and animals. In the present study contamination status of aflatoxin B1 in cotton seed cake, wanda, wheat bran and homemade concentrate mixture of dairy goats was investigated in district Lahore of Pakistan. A total of Twenty 20 goat farms were randomly selected and out of that 40 feed samples 2 from each farm were collected in the month of April. All the samples were analysed for the determination of aflatoxin B1 by using reverse phase High Performance Liquid Chromatography (HPLC. Out of total samples Aflatoxin B1 was detected in 33 feed samples, with a percent contamination rate of 83%. The level of aflatoxin B1 ranged from 0 to 225.736 ppb in different feed samples. The maximum level of aflatoxin B1 was detected in cotton seed cake (mean level 137.059 ± 22.293 ppb) followed by wanda, homemade concentrate mixture and wheat bran.. Amongst the positive concentrate feed samples, 28 samples (85% of the positive) were having the concentration of aflatoxin B1 higher than the permissible level which is 5 ppb for concentrates as recommended by European Communities. It is concluded that the concentrate available for goat feeding is having aflatoxin B1 and its concentration is higher than the permissible level.

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Introduction

Pakistan is an agricultural country and produces an abundant quantity of milk mainly from five species of animals, including cow, buffalo, sheep, goat, and camel. Pakistan has 41.2 million cows, 35.6 million buffaloes, 68.4 million goats, 29.4 million sheep, and 1.0 million camels (Pakistan Economic Survey, 2015). Pakistan is the home tract of high milk yielding breeds of dairy animals especially Sahiwal cow, Nilli-Ravi buffalo, Beetal goat, and Kajli sheep (Tipu et al., 2007). But these high producing animals are facing great challenges in the farm of harsh climatic condition, poor management and infectious and non-infectious diseases. Among non-infectious diseases the metabolic disease like contamination of Mycotoxin possess serious threat to the animal health as well as public health.

The mycotoxin are secondary metabolites produced by fungi (moulds). Among the common fungi the Fusarium, Aspergillus and Penicillium are the most frequently occurring that contaminate human and animals feed sources particularly during pre-harvest and

storage period (Binder, 2007). Environmental conditions related to storage are the factors that affect the production of mycotoxins in feed by fungi and these factors can be controlled (Hussein and Jeffrey, 2001). Ruminants are less susceptible to the toxic effects of mycotoxins as compared to mono gastric animals because the mycotoxins are inactivated and degraded by the microorganisms in the rumen. However many mycotoxins are able to resist ruminal degradation and cause toxicity. Cows in transition period which are already in negative energy balance are especially sensitive to mycotoxins exposure (Gremmels, 2007). The most toxic aflatoxin is aflatoxin B1 that is hepatocarcinogenic. (Etzel, 2002; Creppy, 2002). After the ingestion of aflatoxin, the dairy animals secrete aflatoxin M1 series as carcinogenic metabolites in their milk (Yiannikouris and Jouany, 2002). Ruminants excrete aflatoxins M1 and M2 as hydroxylated metabolites in their milk when they consume feed contaminated with aflatoxin B1 and B2 (Var and Kabak, 2009). Human may ingest mycotoxins through contaminated cereals or indirectly when they consume animal products such as milk and eggs from animals that were already exposed to contaminated feed sources (Capriotti et al., 2012). Many human diseases are related with intake of mycotoxins. Mycotoxin toxicity in humans and animals depends upon various factors like species of animal, mode of action, biotransformation and host immune system. In earlier research the species specific toxicities of aflatoxin were investigated. The LD_{50} reported were 500 mg/kg, 1, and 0.4 for sheep, rats and ducklings respectively (Wogan and Newberne, 1967).

Different techniques are used to measure the level of mycotoxins in animal feeds . High performance liquid chromatography, thin layer chromatography (TLC) and ELISA are currently used for detection of aflatoxin B1 in feed samples. High performance liquid chromatography is the most sensitive technique and it gives qualitative and quantitative analysis of feed material simultaneously. The main objectives of the study were to investigate the aflatoxin B1 (AFB1) contamination status of different commonly fed concentrate feed of dairy goats under field conditions. The findings of the study will prove helpful in the control of mycotoxin in animal feeds.

Materials and Methods

Study design

The study was conducted in District Lahore of Punjab province to find out the contamination of Aflatoxin B1 in concentrate feeds of goats. A total of 20 goat farms in District Lahore were randomly selected and 40 concentrate feed samples 2 from each farm were collected. The concentrate feed comprised of 10 samples of each of Cotton seed cake, Wheat brans and Homemade concentrate mixture. The samples were analysed for the determination of Aflatoxin B1 through HPLC in quality operations laboratory, UVAS, Lahore.

Feed sampling

The feed samples were collected in the month of April (summer season). In order to obtain a representative sample, each feed sample was collected from 10 different spots in feed bags and was thoroughly mixed. The samples were shifted to quality operations laboratory, UVAS, Lahore in zip locked plastic bags for Aflatoxin B1 (AFB1) determination. Concentrate feeds were stored in plastic bags in a separate dry room at all dairy goats farms, thus providing almost same storage conditions at experimental farms.

Determination of Aflatoxin B1 (AFB1) in feed

Chemicals used

The aflatoxin B1 (AFB1) analysis was performed with acetonitrile and methanol (HPLC grade) of Merck (Germany). The double distilled water was used in the analysis.

Extraction of Aflatoxin B1 (AFB1)

The concentration of AFB1 in feed samples was determined by High Performance Liquid Chromatography (HPLC) technique as used by Masoero et al. (2007), with some modifications. Twenty-five gram of feed sample was taken and grinded in automatic grinder (Panasonic, MJ-w176P, Japan). After grinding, the sample was added in 84 ml acetonitrile then 5g sodium chloride dissolved in 16 ml distill water was added. The mixture was shaked in automatic shaker for 1 hr. The mixture was filtered through Whatmann filter paper (No.4) and final volume of filtrate was recorded. Then 70µl of acetic acid were added to 9 ml of the filtrate and the mixture was vortexed for 30 seconds (Barnstead International Company, M37610-33, USA). The mixture was eluted through immuno- affinity column (mycosep®, 226 aflazone + multifunctional columns, Romer Labs, USA). The 2ml of the supernatant was taken in a glass tube and made dried under nitrogen gas (Nitrogen Peak Scien-

tific, N118LA, Germany).

Passing filtrate through HPLC apparatus

After evaporation n-hexane and triflouro-acetic acid were added with a rate of 200µl and 50µl respectively. The volume 1.95 ml of the solution acetonitrile: water (1:9) was added in the tube and was vortexed (Barnstead International Company, M37610-33, USA). It was filtered with filter paper (polyamide, Sartorius Stedim Biotech GmbH, Germany) before HPLC analysis. One ml of the filtrate was taken in HPLC vial and was analysed through HPLC apparatus (Agilent 1100 series, USA), having an auto sampler and fluorescent detector (FLD G1321A) with excitation wave length of 365 nm and emission wavelength of 435 nm. HPLC column used was of the specifications (Lichrospher® 100, RP-18, end capped 5µm, Germany).

Calculations

The AFB1 concentration in feed was calculated by the following formula:

AFB1 = (area of sample/area of standard) × concentration of standard

Statistical analysis

The data obtained from the study were statistically analysed using One-way analysis of variance (one-way ANOVA) with SPSS-18. Least significant difference test (LSD) was used to determine differences in means of different feed types.

Results and Discussion

All the samples were analysed for AFB1 contamination status through HPLC. Aflatoxin B1 (AFB1) was detected in 33 feed samples out of 40 thus with a percent contamination rate of 83%. The concentration of aflatoxin B1 was variable in different concentrate feed samples ranging from 0 to 225.736 ppb (Table 1). In positive feed samples, the highest level of AFB1 was detected in cotton seed cake (mean level 137.059 ppb) and minimum level was detected in wheat bran (mean level 5.676 ppb) as reflected in Figure 1. All the samples of cotton seed cake were positive for aflatoxin B1 while the feed samples of commercial wanda, wheat brans and homemade concentrate mixture were negative for aflatoxin B1 (Figure 2). The concentration of aflatoxin B1 in cotton seed cake was significantly higher ($p<0.05$) then in Wanda, Wheat brans and Homemade concentrate mixture (Table 1 and 2).

Table 1: *Aflatoxin B1 (AFB1) concentration in cotton seed cake, wanda, wheat bran and homemade concentrate mixture (Mean ± S.E)*

Type of feed	AFB1 (ppb)
Cotton seed cake	137.059 ± 22.293[a]
wanda	12.759 ± 4.705[b]
Wheat bran	5.676 ± 1.047[b]
Home made	10.932 ± 3.644[b]

** Values in column with different superscript are significantly different at p<0.05;* **S.E***: standard error;* **ppb***: parts per billion;* **AFB1***: aflatoxin B1*

Table 2: *Analysis of variance (ANOVA) of aflatoxin B1 (AFB1) in cotton seed cake, wanda, wheat bran and homemade concentrate mixture*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	121752.97	3	40584.32	30.427	0.000
Within Groups	48017.361	36	1333.816		
Total	169770.33	39			

AfB1 (ppb)

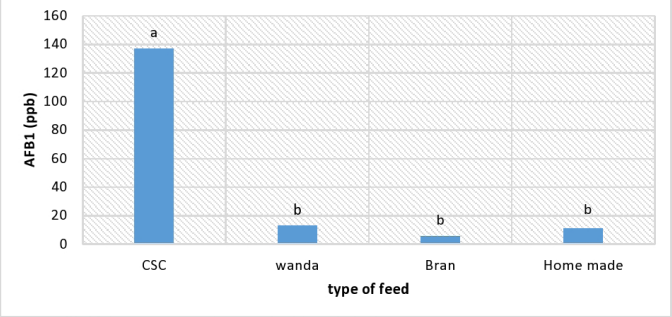

Figure 1: *Concentration of aflatoxin B1 in different concentrate feeds*
CSC*: cotton seed cake;* **wanda***: commercial wanda;* **Bran***: wheat bran;* **Homemade***: homemade concentrate mixture;* **AFB1***: aflatoxin B1;* **ppb***: parts per billion*

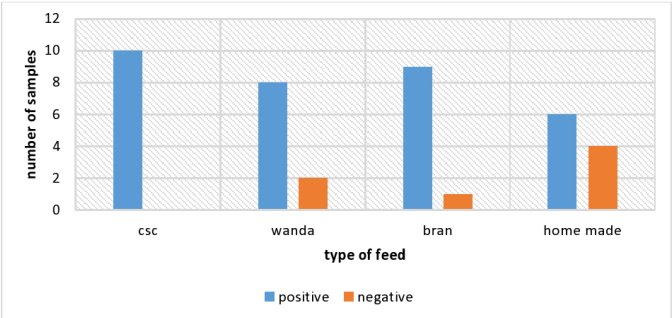

Figure 2: *Number of positive and negative samples for aflatoxin B1*
CSC*: cotton seed cake;* **wanda***: commercial wanda;* **Bran***: wheat bran;* **Homemade***: homemade concentrate mixture*

The present research project is one of the novel studies in Pakistan. Most of the previous studies have been focusing on the aflatoxin M1 (AFM1) concentration in milk and other dairy products in Pakistan. In this study high number of concentrate feed samples were found positive (83%) for aflatoxin B1. The reasons behind this high number of aflatoxin B1 contaminated feed samples could be the favorable environmental conditions and poor storage conditions of the feed in the country (Pakistan). Similar reports about climatic conditions of the region have been presented by earlier researchers (Gowda et al., 2003; Singhal and Kaur, 2005). The moisture content and storage duration of feed also affected the aflatoxins level (Gowda et al., 2003). Cotton seed cake was found to have highest level of aflatoxin B1 in all positive samples. This finding is in agreement with earlier works that because of the certain components present in cotton seed cake like protein and lipids favor the growth and multiplication of fungi. These components act as nutrient source for fungi (Bewley and Black, 1978; Jones and King, 1990).

Wheat bran was found to have lowest level of aflatoxin B1. Similar results have been narrated by other researchers. In a study wheat bran, rice bran showed very minute quantity of aflatoxin B1 (Gowda et al., 2003). The low level of aflatoxin B1 in wheat bran may be due to low energy profile that may not favour the growth and multiplication of toxigenic fungi. However the production of aflatoxins in animal feeds depends on many factors like storage conditions and feeding management etc. (Signorini et al., 2012). Therefore the aflatoxin contamination of concentrate feed varies with the location, season of the year and nature of sample. From the findings of the study it can be concluded that cotton seed cake is the most contaminated concentrate feed source of AFB1 for dairy goats. As aflatoxin B1 contaminated feed consumption by dairy goats will result in excretion of its carcinogenic metabolite aflatoxin M1 in milk, thus the milk from goats consuming aflatoxin B1 contaminated concentrate feed can be a potential hazard for public health.

Conclusions

Aflatoxins contamination of animal concentrate feeds is the major issue in Pakistan. Cotton seed cake is usually contaminated with high level of aflatoxins. Aflatoxin B1 is excreted in animals' milk as metabolite aflatoxin M1 which is carcinogenic, thus the milk from dairy goats in district Lahore can be a potential hazard for human health.

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Authors' Contribution

All the authors in the list, contributed significantly in conduction of this study. The study was designed by Haq Aman Ullah (HAU), Aneela Zameer Durrani (AZD), Muhammad Ijaz (MI) and Aqeel Javeed (AJ). HAU performed sample collection, farmers interviewing and laboratory work. HAU, AZD, MI and AJ analysed the data and wrote the article.

References

Pakistan Economic Survey. 2015: Annual report for 2014-15. Ministry of Finance Government of Pakistan Livestock population. p. 29.

Bewley, J.D., and M. Black. 1978. Structure of seeds and their food reserves. Physiology and Biochemistry of Seeds in Relation to Germination. Chapter 2. p. 7-37. Vol. 1, Development, Germination and Growth; Springer-Verlag: Berlin. http://dx.doi.org/10.1007/978-3-642-66668-1_2

Berg, T. 2003. How to establish international limits for mycotoxins in food and feed. Food Control. 14:219–224. http://dx.doi.org/10.1016/S0956-7135(02)00021-X

Binder, E.M. 2007. Managing the risk of mycotoxins in modern feed production. Anim. Feed Sc. Technol. 133(1-2):149-166. http://dx.doi.org/10.1016/j.anifeedsci.2006.08.008

Creppy, E.E. 2002. Update of survey, regulation and toxic effects of mycotoxins in Europe. Toxicol. Let. 127:19–28. http://dx.doi.org/10.1016/S0378-4274(01)00479-9

Capriotti, A.L., G. Caruso, C. Cavaliere, P. Foglia, R. Samperi and A. Lagana. 2012. Multiclass mycotoxin analysis in food, environmental and

biological matrices with chromatography/mass spectrometry. Mass Spectrometry Rev. 31(4): 466-503. http://dx.doi.org/10.1002/mas.20351

Etzel, R.A. 2002. Mycotoxins. J. Am. Med. Assoc. 287(4):425. http://dx.doi.org/10.1001/jama.287.4.425

EC. 2003a. Commission Directive 2003/100/EC of 31 October 2003 amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed (Text with EEA relevance). Off. J. Euro. Union. 285:33–37.

EC. 2006. Commission Regulation 1881/2006/EC of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs (Text with EEA relevance). Off. J. Euro. Union. L364:5–24.

Gowda, N.K.S, Malathi, V., Suganthi, R.U. 2003. Screening for aflatoxin and effect of moisture, duration of storage and form of feed on fungal growth and toxin production in livestock feeds. Anim. Nutr. Feed Technol. 3:45–51.

Gong, Y., A. Hounsa, S. Egal, P.C. Turner, A.E. Sutcliffe, A.J. Hall, K. Cardwell, C.P. Wild. 2004. Postweaning exposure to aflatoxin results in impaired child growth: a longitudinal study in Benin, West Africa. Environ. Health Perspect. 112:334–1338. http://dx.doi.org/10.1289/ehp.6954

Gremmels, F.J. 2007. The role of mycotoxins in the health and performance of dairy cows. Vet. J. 176: 84–92. http://dx.doi.org/10.1016/j.tvjl.2007.12.034

Hussein, S.H., and M.B. Jeffrey. 2001. Toxicity, metabolism, and impact of mycotoxins on Humans and animals. Toxicology. 167:101–134. http://dx.doi.org/10.1016/S0300-483X(01)00471-1

Hussain, I., A. Jamil, R.A. Muhammad, A.M. Munawar, K. Muhammad. 2010. Aflatoxin M1 contamination in milk from five dairy species in Pakistan. Food Control. 21:122–124. http://dx.doi.org/10.1016/j.foodcont.2008.12.004

Iaha, M.H., C.B. Barbosa, I.A. Okada, M.W. Trucksess. 2011. Occurrence of aflatoxin M1 in dairy products in Brazil. Food Control. 22(12):197-1974. http://dx.doi.org/10.1016/j.foodcont.2011.05.013

Jones, L.A., and C.C. King. 1990. Cottonseed oil composition. Cotton seed Oil; National Cottonseed Products Association: Memphis, TN, Chapter 4. p. 22-30.

Masoero, F., A. Gallo, M. Moschini, G. Piva, D. Diaz. 2007. Carryover of aflatoxin from feed to milk in dairy cows with low or high somatic cell counts. Animal. 1:1344-1350. http://dx.doi.org/10.1017/S1751731107000663

Singhal, K.K., and H. Kaur. 2005. Aflatoxins in livestock and poultry: A review. Indian J. Anim. Sci. 75(1):113–120. http://dx.doi.org/10.1016/j.fct.2011.09.036

Signorini, M.L., M. Gaggiotti, A. Molineri, C.A. Chiericatti, M.L. Zapata de Basílico, J.C. Basílico, M. Pisani. 2012. Exposure assessment of mycotoxins in cow's milk in Argentina. Food Chem. Toxicol. 50:250–257.

Turner, P.C., S.E. Moore, A.J. Hall, A.M. Prentice, C.P. Wild. 2003. Modification of immune exposure to dietary aflatoxin in Gambian children. Environ. Health Perspect. 111:217–220.

Tipu, M.Y., I. Altaf, M. Ashfaq, S. Siddique. 2007. Introduction. In: Proceedings of the workshop on monitoring of chemical adulterants and hygienic status of market milk. Lahore, Pakistan: WTO-Quality Control Laboratory. p. 7–39.

Var, I., and B. Kabak. 2009. Detection of aflatoxin M1 in milk and dairy products consumed in Adana, Turkey. Int. J. Dairy Technol. 62:15-18. http://dx.doi.org/10.1111/j.1471-0307.2008.00440.x

Wogan, G.N., and P.M. Newberne. 1967. Dose-response characteristics of aflatoxin B1 carcinogenesis in the rat. Cancer Res. 27:2370–2376.

Yiannikouris, A., and P. Jouany. 2002. Mycotoxins in feed and their fate in animals: A review. Anim. Res. 51:81–89. http://dx.doi.org/10.1051/animres:2002012