Journal of Novel Applied Sciences
Available online at www.jnasci.org
©2014 JNAS Journal-2014-3-11/1312-1316
ISSN 2322-5149 ©2014 JNAS

Aflatoxin B_1 in feed stuffs of dairy farms in Shahrekord, Iran.

Toktam Bagheri*, Hamdollah Moshtaghi and Mojtaba Boniadian

Dept. of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran

***Corresponding author*:** Toktam Bagheri

ABSTRACT: Because of contamination of feeds with mycotoxins, assessment and detection of mycotoxins, specially aflatoxins in feed stuff and comparison them to standards for prevention of aflatoxicosis will be necessary. A total of 80 samples from four different feeds (straw, alfalfa, corn and concentrate) were randomly collected over two periods, from 10 traditional-dairy farms (TDF) in shahr-e-kord region and were examined in order to determine their aflatoxin B_1 contamination with ELIZA. Aflatoxin B_1 was found above measurable level (0.125ng/g) in 100% (20), 95% (19), 100 (20) and 80% (16) of straw, alfalfa, corn and concentrate, respectively. However the toxin was detected in 75 feedstuff samples corresponding to 93.75% of total samples examined, by average of 1.9631ng/g. contamination did not significantly differ between season and dairy farms of survey. considering the European commission limit, all of feedstuff samples dident have AFB_1 in concentrations in excess of the maximum tolerance limit. Although then average of AFB_1 contamination in feedstuff samples were lower than government standard level but toxin can has a chronic effect if intake along time, thus planning for control and prevention of contamination must be an important part of any plan that is for food and feed security.

***Keywords*:** Aflatoxin, Dairy farm, Feed Pollutants.

INTRODUCTION

Mycotoxins are structurally diverse fungal metabolites produced by fungi, not essential to fungal growth and produced periodically under fungal stress (10). Aflatoxins are from this group of toxins. They were discovered in the late 1950s and early 1960s. The name of “aflatoxin” (A. flavus toxin) was assigned to toxic agents produced by fungi *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin B_1 (AFB_1) and several aflatoxin analogues occur during storage of grains and in fields. Four aflatoxins (B_1, B_2, G_1 and G_2), are ubiquitous pollutants of many foods and can arise simultaneously or independently. The AFB1 is the most potent toxin of the group and can be highly toxic and carcinogenic to many animal species (12). It has been reported that 0.3–6.2% of AFB_1 in animal feed is transformed to AFM_1 and excreted in milk (14). The human health impact of AFB_1 exposure is widespread in developing countries. It is known that AFB_1 causes teratogenicity, immunotoxicity, hepatotoxicity and even death in farm animals and humans (12). They belong to the Group I type of most carcinogenic mycotoxins. Contamination with them has been reported mostly in peanuts, cotton seed, corn, pea, sorghum, rice, pistachio, maize, oilseed rape, spices, meat and meat products, fig, fruit juices (1). Besides the presence of nutrients, the most important factors for growth and mycotoxin production are temperature, water activity and oxygen. The most commonly used methods for detection of aflatoxins are: high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC) and enzyme-linked immunosorbent assay (ELISA) and fluorometeric method . The enzyme-linked immunosorbent assay is most widely used test to detect aflatoxins, due to its simplicity, sensitivity and adaptability (3). Contamination of feeds with mycotoxins accounts for significant economic losses in animal husbandry, as well as in undesirable trade barriers for raw materials and consumable products (4). Thus assessment and detection of mycotoxins, specially aflatoxins in food and feedstuff and comparison them to standards for prevention of aflatoxicosis seem to be necessary.

MATERIALS AND METHODS

Sampling:

A total of 80 samples from four different feeds (straw, alfalfa, corn and concentrate) were randomly collected over two periods, in summer of 2013 and winter of 2014, from 10 traditional-dairy farms (TDF) in shahrekord region and were examined in order to determine their aflatoxin B_1 contamination. All samples were transported to the Food hygiene and quality control laboratory of Shahrekord University within 24 h, stored at 4°C and protected against light until the day of analysis, which was never more than 7 days after collection. All of the samples were intended for animal consumption and did not contain any visible signs of mold contamination.

Sample preparations:

Representative sample were thoroughly mixed prior to extraction. Ten grams of each sample was weighed and then 50 ml of 33% methanol was added and the mixture shaked vigorously for two minutes. After that the mixture kept in room temperature for 15 minutes. Then the extract filterd throught a whatman No.1 filter paper, centrifuged and diluted 1:2 the clear supernatant with 33% methanol(i.e. 1ml+1ml). The finally solution kept in refrigerator until the time of analyzing.

ELISA test procedure:

According of producer corporation detection limit of the kit for aflatoxin B_1 was 125ng kg^{-1}. The euroclone total aflatoxin ELIZA kit is a competitive enzyme immunoassay for the quantitative of aflatoxin B_1, B_2, G_1, G_2 in grains, nuts, cotton seeds, cereal and animal feed. The assay was performed in ELISA microwell plate, which had been precoated with goat anti-rabbit IgG. Standard or sample, aflatoxin enzyme conjugate and rabbit anti-aflatoxin antibodies were added into the microwell. During incubation, anti-aflatoxin antibodies were bound by the immobilized IgG; free and aflatoxin enzyme conjugate compete for anti-aflatoxin antibody binding in a washing step. The bound enzyme activity was determined by adding a fixed amount of chromogenic substrate. Bound enzyme conjugate converts the colourless substrate into a blue product. The substrate reaction was stopped by the addiction of sulphuric acid, which lead to a colour change from blue to yellow. Absorbance was then measured spectrophotometrically(450 nm) and colour intensity results were inversely proportions were then calculated on the basis of a calibration curve derived from standards of known total aflatoxin concentration.

Data analysis

Data were summarized and analyzed using SPSS (version 16.0),ANOVA and Duncan's multiple range was used to determine differences in the means among samples obtained from the different AEZs ($P < 0.05$).

RESULTS AND DISCUSSION

The occurrence and levels of AFB_1 in samples of feedstuffs are presented in table 1. Aflatoxin B_1 was found above measurable level (0.125ng/g) in 100% (20), 95% (19), 100 (20) and 80% (16) of straw, alfalfa, corn and concentrate, respectively. Altoghether, the toxin was detected in 75 feedstuff samples corresponding to 93.75% of total samples examined,by average of 1.9631 ng/g . Considering the European commission limit, all of feedstuff samples didnot have AFB_1 in concentrations in excess of the maximum tolerance limit.

Table 1. Level of AFB_1 in samples

Feed type	N	mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Straw	20	2.1985	1.50920	.33747	1.4922	2.9048	.71	6.56
Alfalfa	20	2.7080	1.54193	.34479	1.9864	3.4296	.00	4.00
Corn	20	1.7215	1.09058	.24386	1.2111	2.2319	.54	4.08
Concenter	20	1.2245	1.06134	.23732	.7278	1.7212	.00	4.06
Total	80	1.9631	1.40853	.15748	1.6497	2.2766	.00	6.56

Contamination did not significantly differ between seasons and dairy farms of survey (figures 2 and 3). In table 2 level of aflatoxin describe in feed type, season of survey and dairy farm

Table 2. Level of aflatoxin in all of season, food type and dairy farmes

season	summer	1.37 ± 1.87
Feed type	winter	1.45 ± 2.04
	straw	1.50 ± 2.19
	alfalfa	1.54 ± 2.70
	corn	1.09 ± 1.72
	concentrate	1.06 ± 1.22
Dairy farm	NO.1	1.6 ± 2.69
	NO.2	2.01 ± 3.27
	NO.3	0.54 ± 2.41
	NO.4	1.82 ±1.37
	NO.5	1.27 ± 0.53
	NO.6	1.17 ± 1.68
	NO.7	1.4 ±1.8
	NO.8	0.9 ± 2.19
	NO.9	1.58 ±2.11
	NO.10	1.35 ± 1.57

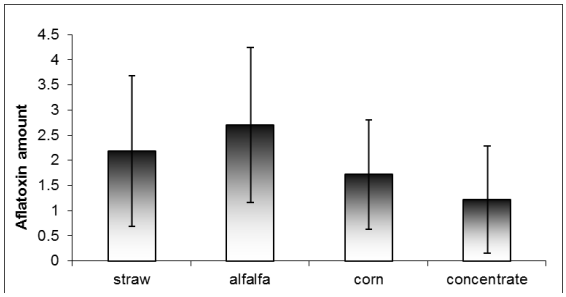

Figure1. Aflatoxin accumulation in all of feedstuffs in total season of summer and winter in shahrekord, Iran

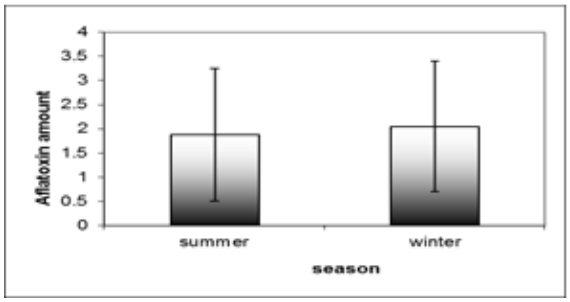

Figure 2. Aflatoxin accumulation in two season of survey (winter and summer) in shahr-e-kord, Iran

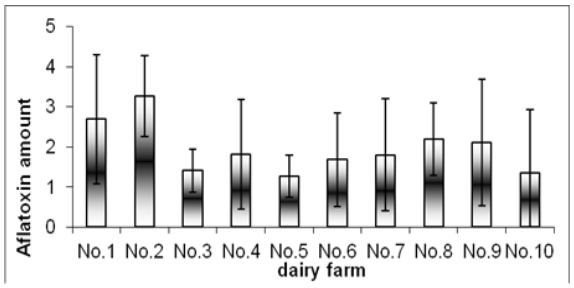

Figure 3. Aflatoxin accumulation in ten dairy farms of survey in Shahrekord, Iran

In comparision of various feedstuffs in survey it was shown that there was a significantly difference between kind of feed and concentration level of AFB_1 thus the most and the least contamination level were for alfalfa and concentrate, respectively (P<0.596) (Figure 1).

Aflatoxin contamination and A. flavus infection are often associated with drought and temperature(9) .shahr-e-kord town is located in mountainous and dry climate and has a cool day in winter and dry day in summer.the extremely low level of aflatoxin contamination in feedstuffs might be due , in particular, to this condition of climate.

According to matrix of feed we expected the most level contamination in corn silage but it didn't happen. It might be for good condition of storage or because of fresh using without remaining in dairy farm or it was for using some preservative substances in this type of feed and another feeds for prevention of fungi growing and thus toxin production.

Though aflatoxin content in these samples was below the governmental regulated level, but because of this subject that feedstuffs of survey are the main diet of dairy cattle, it become important and serious problem even at low level, So improvement in storage conditions to prevent grain spoilage and reduce aflatoxin contamination is recommended.

Dutta . (5) characterized the percentage positive of AFB_1 contamination of samples in three season, winter, monsoon and summer.all of samples are positive.the average AFB_1 content was 0.412± o.154 ppm which is sufficiently higher than the permissible safe level in in season of survey highest AFB1 accumulation was for monsoon.

Pereyra . (11) conducted a survey about mycotoxin contamination of some kind of feed for sows and detected no positive sample for AFB_1.

Almeida . (3) survey mycobiota and AFB_1 of feed for farmed sea bass.from 87 samples (40.2%) 35 samples were positive for fungi. Aspergillus flavus was the most frequently found mold in the 35 samples (40.2%), presenting a mean value of 2.7 log10 CFU·g−1, ranging between 2.0 and 3.2 log10 CFU·g−1. The presence of A. flavus in some samples has been pointed to as a potential risk factor to Aflatoxins produced in the feed during storage.

Alam . (2) survey the complex interaction of feed type, agroecological zones, seasons and their effects on B1 production.it was shown that minimum AFB_1 contents were examined in rice (13.71 ng/g) wheat (13.37ng/g) and rice (6.96 ng/g) Samples collected in winter and maximum were in finisher ration in summer season in different zone of pakhtunkhwa, Pakistan.

Kana . (7) examined occurrence of aflatoxin in some maize, peanut meal, broiler feed and layers feed.it was shown that from 201 samples, 110 feeds (54.72%) were contaminaton.

Liu . (9) in their survey determined level of aflatoxins in stored maize and rice grains.they found that between 110 samples of all food included maize, whole grain rice and brown rice (dehusked), 107 samples were aflatoxin positive.

In 2004 asimilar study performed by rahimi . (13) In 73 (67.6%) of the 108 samples the presence of aflatoxin B_1 was detected in concentration ranging between 0.80 µg/kg and 155.18 µg/kg. Aflatoxin B_1 level in 19 (17.6%) of these samples were higher than the maximum tolerance limit (30). Results showed that there were statistical difference between aflatoxin B_1 contents in cottonseed meal with wheat bran (p=0.014), barley (p=0.032) and alfalfa meal (p=0.027). Statistical evaluation showed that mean contamination level of aflatocxin B_1 in Winter samples were significantly higher than those of Summer (p=0.008).

Often contamination of food by fungi may vary due to different origins of contamination, especially storage buildings, bins or underground pits. Often, fungi invade only a minor fraction of feed particles with appropriate condition for a growth such as enough water content, aeration. Substrates differ in their ability to support fungal growth due to differences in their physical and chemical characteristics, which include water activity, oxygen availability and surface area, while chemical characteristic include carbohydrates, fat, protein, trace elements and amino acid composition. While some substrates are susceptible to colonization, other environmental conditions increase the vulnerability of the fungi to the substrate. The conditions include temperature, water activity, pH and atmospheric air (oxygen) (2). The tolerance levels currently set by the regulatory bodies worldwide are typically 0.05 íg/kg for AFM_1 in milk, 10 íg/kg for AFB_1 and 20 íg/kg for total aflatoxins in food intended for human consumption and 20-300 íg/kg for total aflatoxins in animal feeds. The European Commission is finalizing a proposal to set new tolerance levels at 2íg/kg for AFB_1 and 4 íg/kg for total aflatoxins in certain species (8).

Conclusion

Although then average of AFB_1 contamination in feedstuff samples were lower than government standard level but toxin can has a chronic effect if intake along time, thus planning for control and prevention of contamination must be an important part of any plan that is for food and feed security.destroy contaminant food(feed) and regard standard in all stage include implant, harvest and storage can be effective for reduce contamination.

Acknowledgments

The authors thank the faculty of veterinary for the supply of research material. This study was supported by the Shahre Kord University.

REFERENCES

Ahmad MM, Ahmad M, Ali A, hamid R, javad S and abdin MZ. 2014. Detection of Aspergillus flavus and Aspergillus parasiticus from aflatoxin-contaminated peanuts and their differentiation using PCR-RFLP. Ann Microbiol. 2014, 10.1007/s13213-014, 0803-5.

Alam S, shah HN, khan H, Magen N. 2012. the effect of substrate, season, and agroecological zone on mycoflora and aflatoxin contamination of poultry feed from Khyber pakhtunkhwa, Pakistan. Mycopathologia. 2012, 174, 341-349.

Almeida IFM, martins HML, santos SMO, feritas MS, de costa JMGN and bernardo FMD. 2011. mycobiota and aflatoxin B1 in feed for farmed sea bass (dicentrarchus labrax). Toxins. 2011, 3, 163-171.

Code of practice for the reduction ofaflatoxin B1 in raw materials and supplemental feedingsruffs for milk-producing animals.CAC/RCP 45-1997.

Dutta TK and das P. 2000. Isolation of aflatoxigenic strains of aspergillus and detection of aflatoxin B_1 from feeds in india. indian veterinary research institute. 2000, 151, 29-33.

Imtiaz H. 2011. Aflatoxin Measurement and Analysis, Aflatoxins - Detection, Measurement and Control: Dr Irineo Torres-Pacheco (Ed.), ISBN: 978-953-307-711-6, InTech, Available from,: http://www.intechopen.com/books/aflatoxins-detection-measurement-and-control/aflatoxin-measurement-andanalysis1

Kana JR, gnonlonfin BGJ, Harvey J, wainaina J, wanjuki I, skilton RA and teguia A. 2013. Assessment of Aflatoxin Contamination of Maize, Peanut Meal and Poultry Feed Mixtures from Different Agroecological Zones in Cameroon. Toxins. 2013, 5, 884-894.

LEE NA, WANG S, ALLAN RD and KENNEDY IR. 2004. A Rapid Aflatoxin B1 ELISA: Development and Validation with Reduced Matrix Effects for Peanuts, Corn, Pistachio, and Soybeans. Journal of agricultural and food chemistry. 2004, 52, 2746-2755.

liu Z, gao j and yu j. 2006. aflatoxins in stored maize and rice grains in Liaoning province,china.journal of stored products research,2006; 42: 468-479.

Martins HM, marques M, almeida I, Guerra MM and bernardo F. 2008. mycotoxins in feedstuffs in Portugal: an overview. Mycotoxin research. 2008, 24(1), 19-23.

Pereyra CM, caraglieri lR, chiacchiera SM and Dalcero AM. 2010. fungi and mycotoxins in feed intended for sows at different reproductine stages in argentina. sage-hindawi access to research veterinary medicine international. 2010, 7, 1-7.

Quintana RV, Alarcon JS, tenorio MG, deng Y, waliszewski SM and Valera MA. 2012. Preventive Strategies Aimed at Reducing the Health Risks of Aflatoxin B1. Toxicology of inviroment and health science. 2012, 4(2), 71-79.

Rahimi E, kargar A and zamani F. 2004. Assessment of aflatoxin B1 levels in animal feed of dairy farms in Chaharmahal & Bakhtiari. 2004, Pajouhsh & Sazandegi, 79, 66-71.

Shouman BO, Doaa El Morsi DE, Shabaan S, Abdel-Hamid AH and Mehrim A. 2012. Aflatoxin B1 Level in Relation to Child's Feeding and Growth. Indian journal pediatr. 2012, 79(1), 56-61.

Zaki MM, El-Midany SA, Shaheen HM and Rizzi L. 2012. Mycotoxins in animals: Occurrence, effects, prevention and management. Journal of Toxicology and Environmental Health Sciences. 2012, 4(1), 13-28.