Original Article

Investigation of Aflatoxins in Imported Animal Feeds in Iran

Mansooreh Mazaheri[1], Masoumeh Mahmoudi Maymand[2]*

Received: 12.08.2018 *Accepted: 23.10.2018*

ABSTRACT

Background: Aflatoxins (AF's) are secondary metabolites produced by certain species of fungi on grains and animal feeds. Because of serious impact of AF's on health, they should be monitored closely in potentially hazardous foods and feeds.

Method: In this study, AF's in imported animal feeds including cereals, corn gluten and meals (pastes derived from the cotton, colza and sunflower seeds) from several Iranian ports were investigated. To determine AF's contamination in feeds, the HPLC method coupled with immunoaffinity column clean up and post column derivatization were used.

Results: The results showed that 44.2% of samples were positive for AF's at levels of 0.5 to 103.8µg/Kg. Nine of the positive samples (3.7%) showed total AF's concentrations (B_1, B_2, G_1 and G_2) higher than the limit established by the regulated Iranian standards.

Conclusions: The results of the present investigation showed that the level of aflatoxin contamination in these feeds samples was variable. Among the samples, corn was more susceptible to various AF species than other tested substrates.

Keywords: Aflatoxins, Aflatoxin B_1, Animal Feeds, Immunoaffinity HPLC.

IJT 2018 (6): 33-38

INTRODUCTION

Mycotoxins are secondary metabolites produced by molds and have adverse effects in humans, animals, and crops, and often result in illnesses. They are a structurally and functionally diverse group of organic compounds that affect humans and animals alike [1]. Mold contamination has challenged the safety of feeds production and processing because of its undeniable role in the spoilage and the possible consequent toxic impact on human health and healthcare cost [2]. Reportedly, molds are highly toxic compounds and can cause both acute and chronic conditions in humans and many animals. Because of the serious impact on health, the presence of molds should be monitored closely in foods and feeds [3]. Mold may form grains and may continue to grow if grain is stored under high moisture and temperature conditions. Aflatoxin contamination can occur before harvesting when the crops undergo drought stress due to elevated temperatures during storage and when humidity rises during harvesting. Contamination also occurs when there is insect damage, delayed harvesting and high moisture levels during storage and transportation [4].

Among major species of AF's (aflatoxin B_1, B_2, G_1, and G_2), aflatoxin B_1 (AFB_1) is the most prevalent and poisonous molecule, and it is generally the main contributor to total AF's adverse effects. Also, aflatoxin M_1 (hydroxylated from AFB_1) can appear in meat, milk, and eggs of animals that have consumed aflatoxin-contaminated feeds. Maximum limit at part per billion (ppb) level for AF's has been determined for foods and feeds in many countries and organizations such as European Union (EU) [5] and Codex Alimentarius Commission [6]. Also the Iranian National Standardization Organization (INSO) has set the national limits at 15µg/mL for AFB_1 and 50µg/mL for total AFs in cotton seed paste and 5µg/mL for AFB_1 and 20µg/mL for total AFs in other animal feeds [7]. Determination of AF's level is carried out to ensure that the limits are not exceeded. Since animal feeds may contain different species of AF's, animal products, such as eggs, meat, milk, etc., may be contaminated. Therefore, it can be concluded that the aflatoxin inside the feeds can contaminate a wide range of food derived from animals and live stocks. Seed pastes, especially cottonseeds are popular feeds for animals used in dairy industry.

Aflatoxin contamination in commercial cottonseeds in some areas is a perennial problem. In 2012, Feizy *et al*., reported the presence of AF's in cottonseed samples. Among 140 cottonseed samples from wholesalers in Iran, AFB_1 was detected in 129 samples. The highest concentration of AFB_1 was reported to be 14.4ng/g. Also, 13 samples were contaminated above the regulatory limits offset by the European Union (5ng/g), but no sample was above the Iranian regulatory limits (50ng/g) for total AFs [8]. In another research conducted in 2014, Fareed *et al.* analyzed 186 samples comprising of poultry feed ingredients and the finished feeds and

1. PhD of Biophysics, Standard Research Institute (SRI), Karaj, Iran.
2. MSc of Toxicology, Standard Research Institute (SRI), Karaj, Iran.
* Corresponding Author: Masoumeh Mahmoudi Maymand E-mail: mah_maymand@standard.ac.ir

detected various levels of total AFs. This study demonstrated that all of the corn, cotton seed meals, sunflower meals, and cotton gluten meals were contaminated with AFs [9]. Another study conducted by Anjum *et al*. in 2012, demonstrated that among 487 feed samples analyzed for AFB_1, the mean AFB_1 contamination was 26 ppb, with maximum level being39 ppb in corn gluten meals. Further, the contamination levels for corn were 25 ppb and 56 ppb, and for rice samples were 21 ppb and 39 ppb [10].

Due to the health concerns on AF's in foods, this study investigated the incidence of AF's contamination in imported animal feeds, including cereals corn gluten and meals, such as cotton seed paste, colza seed paste and sunflower seed paste, collected from several ports in Iran.

MATERIALS AND METHODS

Chemicals

Standard AFs were purchased from Sigma Chemical Company (Germany) and the immunoaffinity columns (IACs) for AF's were supplied by VICAM (USA). The analytical grade solvents were obtained from Merk (Germany), and the pure water for assays was produced by Millipore filters (specify filtration). For stock standard solution of AFs, a concentration of 10000µg/mL in methanol of each aflatoxin species was prepared and wrapped in aluminium foil at -20°C; and the AF's mixture was prepared in methanol at 1000µg/mL for AFB_1 and AFG_1 and 200µg/mL for AFB_2 and AFG_2. The working solutions of AFs were diluted in the same solvent and stored in glass-stoppered tubes at 0°C. High performance liquid chromatography (HPLC; Waters, USA) columns equipped with quaternary pump, auto injector and fluorescence detectors were used with a stainless steel reverse phase column (Shanghai, China) C18-WP, 100A, 4.6mm ×250mm, 5µm used for the determination of AF species.

Sampling and Sample Preparation

Samples of different feeds were collected from ports in Hormozgaan and Khoozestaan, two major ports in southern Iran. Animal feed samples were collected from March 2013 to January 2015. Sampling was done according to methods of Iranian National Standards for accurate determination of mycotoxins' levels in foods and agricultural products [11]. Samples were transferred to mycotoxins labs at Standard Research Institute, in Karaj, Iran. For decreasing the sub-sampling error in AF's analysis, all samples were grinded and the powders collected. AF's analyses were performed, using 50g of each sample.

Sample Analysis

Sample analysis was performed using HPLC method according to the Iranian National Standard guidelines [12]. Based on this method, there are three steps: extraction of the AFs from samples, clean up, and purification, and HPLC analysis. For extraction, 200ml of methanol 80% was used and the mixture was stirred for 3 min at high speed. After extraction, each aliquot was passed through a filter paper, and was diluted in water and filtered again, using a glass microfiber filter. We used IACs to clean up the AFs samples (Aflatest). First, 10mL phosphate buffer saline (PBS) was passed through each IAC. Then 70 mL of the filtrate was passed through the IAC at a flow rate of 1 drop/sec. The IAC was washed with 10mL water and dried by applying mild vacuum. Finally, the AF's were eluted with 1.5 mL methanol and 1.5mL pure water, and then they were analyzed by HPLC. Samples (100µL) were injected into the HPLC column and heated to 40 ºC. The mobile phase was water, methanol and acetonitril solution (60:30:20, v/v). For the mobile phase preparation, 120 mg of potassium bromide and 350µL of 4M nitric acid were added to one liter of mixture. We used Kobra Cell (city, country) for derivatization at a flow rate of one mL/min. For fluorescent detection of AFs, the excitation wavelength was 365 nm, and the emission wavelength was 435nm. Also by sample fortification, the recovery of analysis at maximum permitted limits in feeds was determined, based on the guidelines of Iranian National Standard (ISIRI 5925). For daily quantification of AFs in samples, a calibration curve with five points was built for individual AFs, including AFB_1, AFB_2, AFG_1 and AFG_2, and the linearity of curves was checked. The LOD was 0.4 for AFB_1 and AFG_1, and 0.08 for AFB_2 and AFG_2. The recovery was determined by using a blank feed samples spiked with standard solution of total AF The resultant contamination levels were 5 ppb for AFB_1 and AFG_1, and 1 ppb for AFB_2 and AFG_2. The AF recoveries ranged between 75 to 89%.

RESULTS

The results, as presented in Figures 1-14, revealed high levels of AF's in cereal samples of animal feeds collected from the two port cities in Iran. The highest total AF's level (48.9µg/Kg) was recorded for the cereal samples (Fig.14). This level was more than twice as much higher than the accepted level (20µg/kg) in cereal feeds for animals [7]. Generally, the percent contamination of animal feeds varied. Thirteen gluten corn samples were analyzed for AFB_1 and total AF. The mean for AB_1 and total AF were 1.96 and 2.19 ppb, and the maximum was 7 and 7.65 ppb for AB_1 and total AF, respectively (Figurs1 and 4). Among 20 meal samples, AFB_1 and total AF were detected in 13 samples, with the means being 1.9 ppb for AFB_1 and 2.32 ppb for AF_{total}. The Maximum for AFB_1 and total AF were 12.04 ppb and 13.41, respectively (Figures 5 and 9). AFB_1 was detected in 40 out of 100 cereal samples. The mean for AFB_1 was 2.85 ppb with the maximum being 42.5 ppb (Fig.10). The mean for total AF was 3.45 ppb with the maximum being 48.9 ppb (Fig. 14).

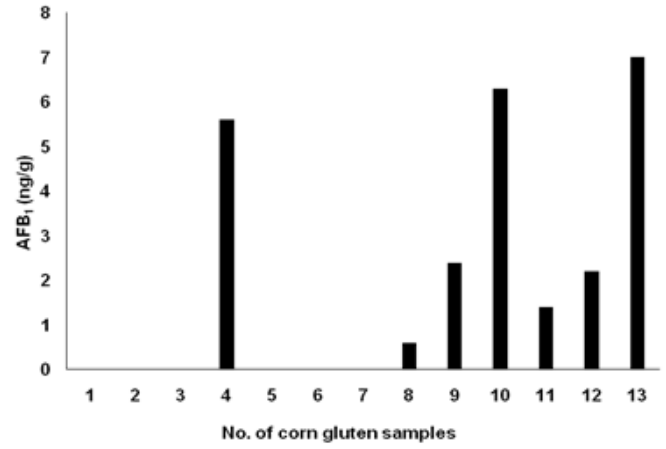

Figure 1. Concentration of AFB_1 in corn gluten samples.

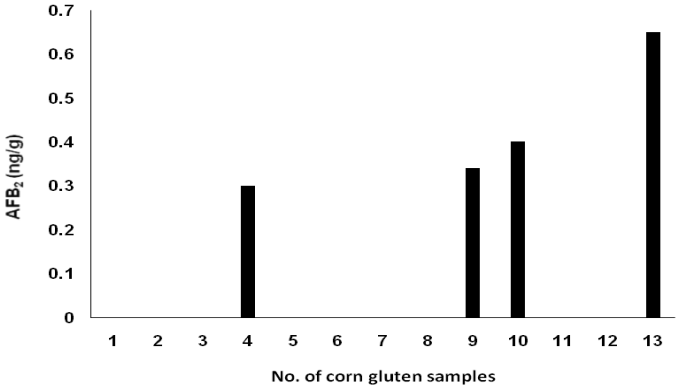

Figure 2. Concentration of AFB_2 in corn gluten samples.

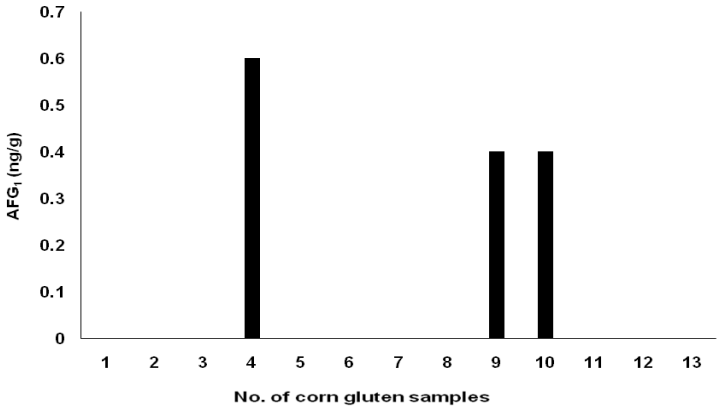

Figure 3. Concentration of AFG_1 in corn gluten samples.

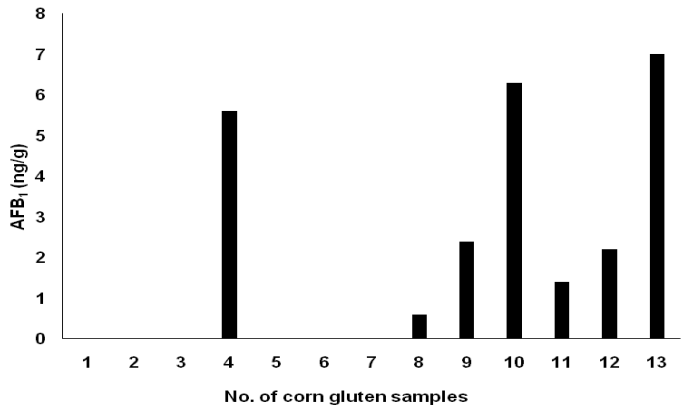

Figure 4. Concentration of total AF in corn gluten samples.

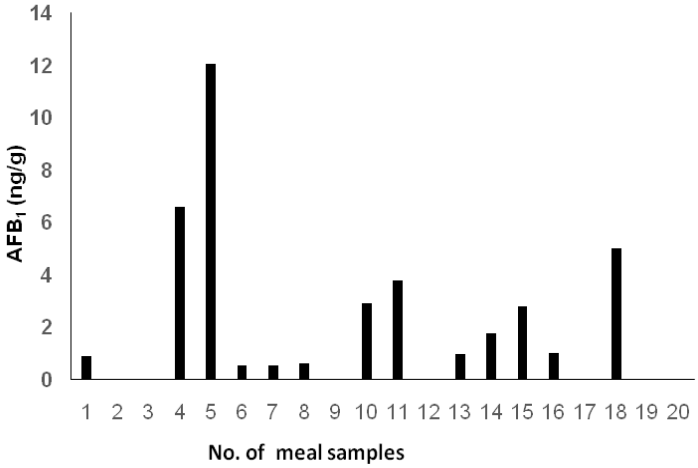

Figure 5. Concentration of AFB_1 in meal samples.

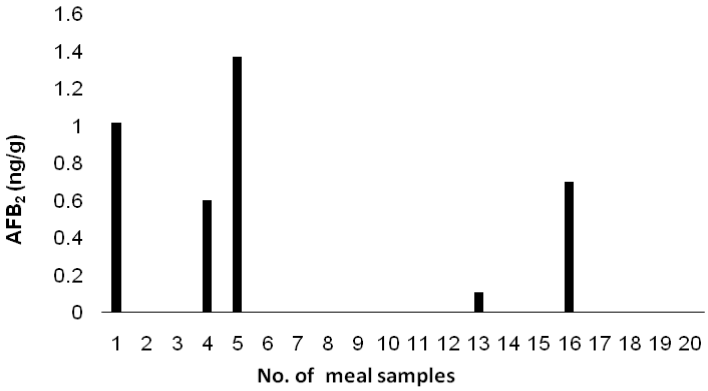

Figure 6. Concentration of AFB_2 in meal samples.

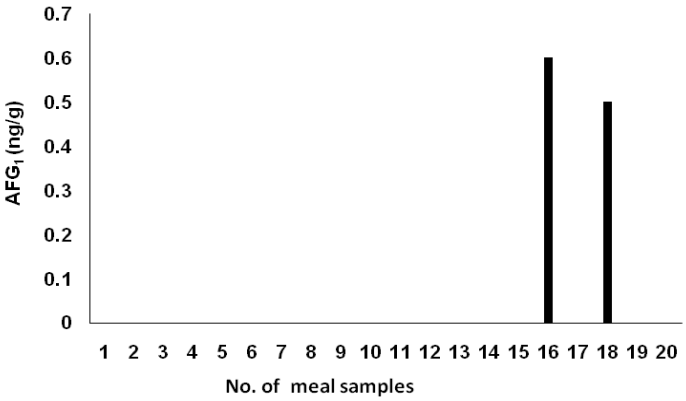

Figure 7. Concentration of AFG_1 in meal samples.

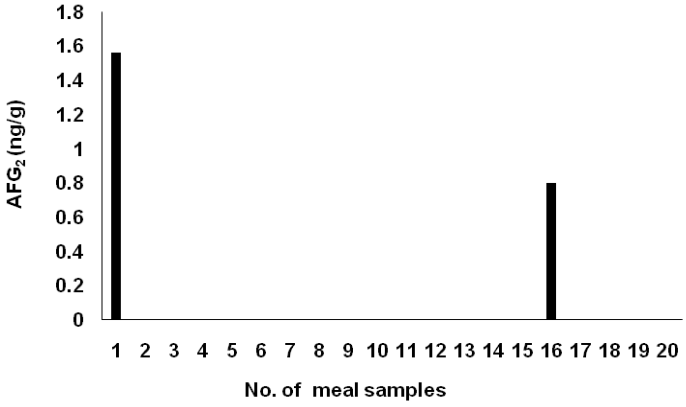

Figure 8. Concentration of AFG_2 in meal samples.

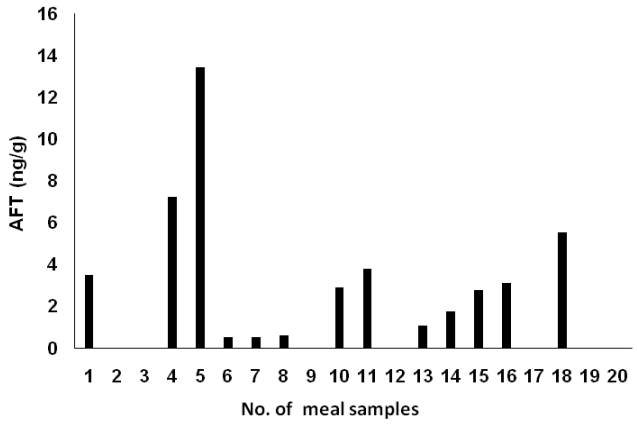

Figure 9. Concentration of total AF in meal samples.

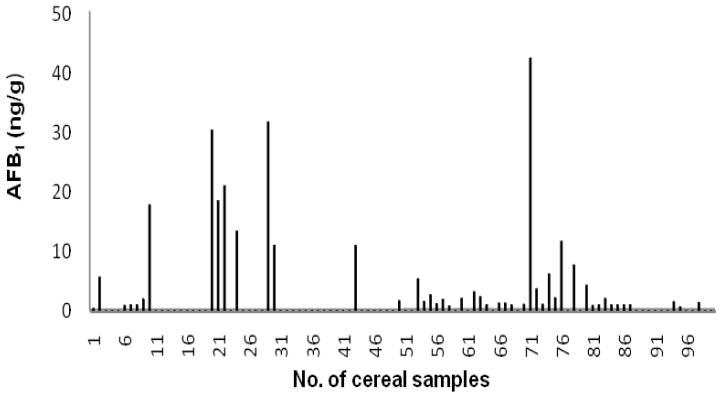

Figure 10. Concentration of AFB_1 in cereal samples.

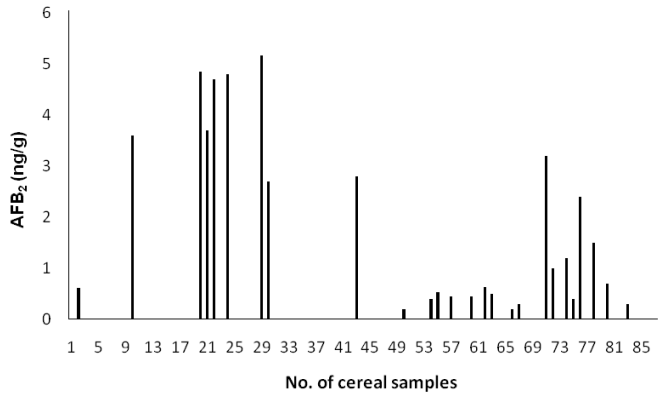

Figure 11. Concentration of AFB_2 in cereal samples.

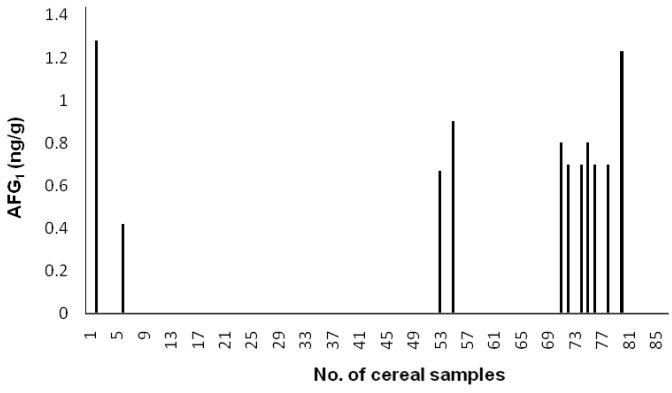

Figure 12. Concentration of AFG_1 in cereal samples.

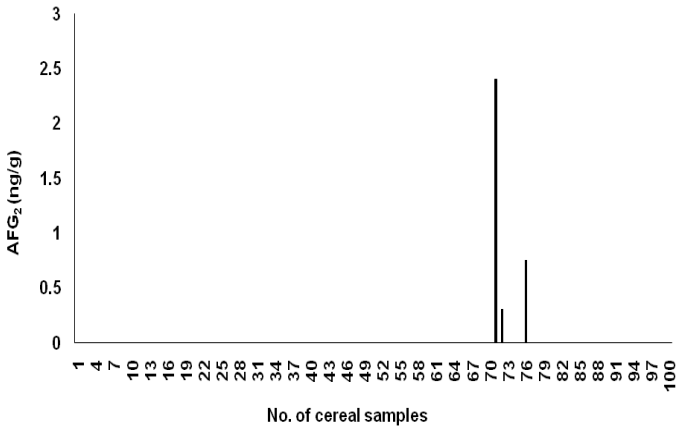

Figure 13. Concentration of AFG_2 in cereal samples.

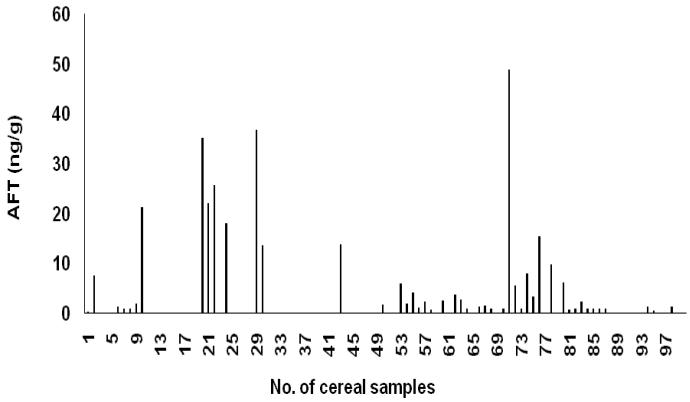

Figure 14. Concentration of total AF in cereal samples.

DISCUSSION

Aflatoxins are a family of related bisfuranocoumarin compounds. They are the secondary metabolites produced by certain species of fungi on grains and animal feeds [13]. Presence of mycotoxins in foods and feeds pose serious health problems for the respective industry. Significant economic losses and suffering of animals are the consequence of mycotoxins in agricultural products. Among mycotoxins, AF's have immense toxicity and have been associated with various health risks in livestock and domestic animals. The economic losses of animal productivity, increased incidence of disease due to immunosuppression, damage to vital organs, and interference with the reproductive capacity of animal are many times greater than the impact caused by animal death due to mycotoxin poisoning. The infestation of toxigenic fungi can occur before or after harvest of food crops and grains, oilseeds, fruits, and vegetables, resulting in serious human and animal health consequences. Aflatoxins have been studied to a greater degree than most other mycotoxins, because of their acute toxicity in humans [14]. Aflatoxins are considered unavoidable contaminants in foods and feeds, even where good manufacturing practices are in place, so many regulatory bodies around the word, have established specific guidelines on acceptable levels of AF's in human foods and animal

feeds. In 1969, the Food and Drug Administration (FDA) in the US set an acceptable level of AF's at 20 ppb for all foods, including animal feeds, based on its analytical capability and the agency's aim of limiting aflatoxin exposure to the lowest possible level. In European Union (EU), Aflaxtoxin B1 is the only toxin with a legal limit for presence in animal feeds. A number of studies have demonstrated that AF's occur simultaneously in field situations.

A study in 2016 [15] examined 74 animal feeds samples for determination of Aflatoxin B1 (AFB1) and Deoxynivalenol (DON), using ELIZA method. Their result demonstrated that Aflatoxin B1 contamination levels in 41 out of 47 (56 %) of the animal feeds that exceeded the EU regulation for the presence of AF's in feeds (5µg/kg) ranging from zero to147.86µg/kg. Another study in 2010 (16), determined the levels of AF's B1, B2, G1 and G2 in 42 animal feeds, comprising corn [16], soya bean meal [8], mixed meal [13], sunflower, wheat, canola, palm kernel, copra meals (1 each), using a reversed-phase HPLC method with fluorescence detection. Their results showed that eight samples (19%) were contaminated with AF's, with the concentration ranging from 6.5 to 101.9ng/g. Total aflatoxin levels in three samples exceeded the legal limits of many countries, i.e., 20ng/g. Another study in 2010 [17], examined the levels of AF's B1, B2, G1, G2 and ochratoxin A in 19 samples of animal feeds by ultra HPLC and tandem mass spectrometry. They detected aflatoxin G2 in two samples at 13 and 17µg/Kg respectively, whereas the other mycotoxins were detected at trace levels (<LOQ) in five samples.

One study [18] examined the prevalence of Aflatoxin B1 and B2 in 65 poultry feed samples using TLC, and found that 75.4% of the samples were positive, 85.71% of which were positive for both AFB1 and B2. Only 10.2% of samples were positive with AFB1 and 4.1% were positive with AFB2. Among these samples, 20% were found positive with aflatoxin above permissible levels. In another study in 2004 [19], the distribution of aflatoxin contamination was examined among 22 maize samples, with 20 samples showing a detectable level of AFs contamination. Jaime-Garcia and Cotty [20] studied AF contamination in commercial cottonseeds in south Texas, USA. Between 4,472 and 9,949 truckloads of cottonseeds were analyzed for aflatoxin content each year from 1997 to 2001. The highest levels of contamination occurred in 1999, with an average AF content of 112ng/g and 66% of the cottonseed truckloads exceeded 20ng/g. Years 1997 and 2000 had the lowest AF levels, averaging 24ng/g, with the lowest incidence (16%) of the truckloads exceeding 20ng/g in 1997. In general, aflatoxin contamination increased as the gin production season progressed.

CONLUSION

The results obtained from the present investigation showed that the level of AF's contamination varied in the Iranian feed samples. Among the samples, corn was more susceptible to AF's contamination. Livestock producers often use corn as a base for animal feeds, because these protein-rich grains help raise animal weights faster for marketing and are cheaper than other feed options. Ripe corns that remain on the ground in farms or stored without proper ventilation and dehumidification, are subject to *Aspergillus* growth and AF production. Evidently, this poses a serious problem to the poultry and livestock industry and to the human consumers. Using GAP, GHP and GSP, specially controlling the moisture and temperature levels during storage, could control the mold growth and toxin production at any stage of the production of animal feeds.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the support of Biology Department, Standard Research Institute, Karaj, Iran. We also thank our colleague, Mr. Kazemi, for his collaboration in performing the laboratory investigations.

CONFLICT OF INTEREST

The authors declare that there was no conflict of interest with any entity in conducting this research.

REFERENCES

1. Wanda M. Haschek1, Kenneth A. Voss2. Haschek and Rousseaux's Handbook of Toxicologic Pathology (3rd ed), 2013.
2. Fernanda Bovo, Karina C. Ganey, Amin Mousavi Khaneghah, Jessica B. Portela, Adriano G. Cruze, Daniel Granato, et al. The occurrence and effect of unit operations for dairy products processing on the fate of aflatoxin M1: A review. J. Food Control. 2016; 68:310-329.
3. Elzupir AO, Elhussein AM. Determination of aflatoxin M_1 in dairy cattle milk in Khartoum state, Sudan. J. Food control. 2010; 21(6): 945-946.
4. Aflatoxin control in maize and peanuts projects. African Agricultural Technology Foundation. 2015.
5. EN. Setting maximum levels for certain contaminants in food stuffs. Commission regulation (EC) No, 1881. 2014.
6. Codex Alimentarius Commision. General standard for contaminants and toxins in foods and feeds. Codex Stan 193. Adopted in 1995, Amended in 2016.
7. INSO. Foods and feeds - maximum tolerated limits of mycotoxins. Iranian national standard, No, 5925. 2002.
8. Javad Feizy, Hamed Reza Beheshti, Mohammad Asadi. A survey of aflatoxin in cotton seed in Iran by HPLC with on-line photochemical derivatization and fluorescence detection. J. Food Addit Contam: Part B: Surveillance. 2012; 5(3).
9. Ghulam Fareed, Sohail Hassan Khan, Muhammad Ashraf Anjum, Naveed Ahmed. Determination of Aflatoxin and Ochratoxin in poultry feed ingredients and finished feeds in humid semi-tropical environment. J. Adv. Vet. Anim. Res. 2014; 1(4): 201-207.
10. Anjum MA, Khan SH, Sahota AW, Sardar R. Assessment of aflatoxin B1 in commercial poultry feeds and feed ingredients. Int. J. Pl. An & Env. Sci. 2010; 22(2): 268-272.

11. INSO. Methods of sampling for official control of the levels of mycotoxins in food and agricultural products. Iranian National Standard, No, 12004. 2008.
12. INSO. Foods and feeds stuff - determination of AF's B& G by HPLC method using immunoaffinity column clean up-test method. Iranian national standard, No, 6782. 2003.
13. Fallah AA. Aflatoxin M_1 contamination in dairy products marketed in Iran during winter and summer. J. Food Control. 2010; 21(11): 1478-81.
14. Dinesh Babu. Rapid and sensitive detection of aflatoxins in animal feeds and food grains using immunomagnetic bead based recovery and real-time immune quantitative PCR. 2010.
15. Caroline Mwende Makau, Josef wafula Matofari, Patrick Simiyu Muliro, Bockline Omedo Bebe. Aflatoxin B1 and Deoxynivalenol contamination of dairy feeds and presence of Aflatoxin M1 contamination in milk from smallholder dairy systems in Nakuru, Kenya. Int. j. food contam.2016; 3(6).
16. Wejdan Shakir Khayoon, Bahruddin Saad, Chew Bee Yan, Nor Hasani Hashim, Abdussalam Salhin, Mohamed Ali, et al. Determination of aflatoxins in animal feeds by HPLC with multifunctional column clean-up. Food Chem.2010; 118(3): 882-886.
17. Sergio Jose Lopez Grio, Antonia Garrido Frenich, Jose Luis Martinez Vidal, Roberto Romero-Gonzalez. Determination of aflatoxins B1, B2, G1, G2 and ochratoxin A in animal feeds by ultra high-performance liquid chromatography–tandem mass spectrometry. J. Sep. Sci. 2010; 33(4-5):502-508.
18. Sita R. Aryal, Durga Karki. Prevalence of Aflatoxin B1 and B2 in poultry feeds. Nepal Agric. Res. J. 2009;(9):109-112.
19. Eduardo Micotti da Gloria, César Francisco Ciacco, José Francisco Lopes Filho, Cynthia Ericsson, Silvio Sandoval Zocchi. Distribution of aflatoxin contamination in maize samples. J. Food Sci. Technol. 2004; vol.24.
20. Ramon Jaime-Garcia, Peter J. Cotty. Aflatoxin contamination of commercial cottonseed in South Texas. Phytopathology. 2003; 93:1190-1200.