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Evaluation of Some Aflatoxins in Feed Ingredients of Livestock and Poultry by HPLC Method, A Local Study in Kermanshah Province

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Abstract

BACKGROUND: Mycotoxins are secondary metabolites produced by fungi, especially *Aspergillus spp*, on grains and animal feeds. The most important mycotoxins are aflatoxins, including aflatoxin B_1, B_2, G_1, and G_2.
OBJECTIVES: The present study was conducted to determine the occurrence of aflatoxins (B_1, B_2, G_1, and G_2) in the feed ingredients of livestock and poultry and to evaluate the effect of the season and spatial variation on aflatoxin contamination.
METHODS: Ninety-three feedstock samples were collected from three major factories in Kermanshah province, Iran, during four seasons. The samples were analyzed by HPLC, and values for aflatoxins were determined. The sum of the aflatoxins was determined as the total for each sample. Data were analyzed using the SPSS software version 23 using general linear model (GLM) based on complete block design (samples and seasons).
RESULTS: Six out of 93 samples were positive for aflatoxin B_1. Positive samples were mainly related to cold seasons. Moreover, no significant difference was found between the positive samples in terms of aflatoxins (B_1, B_2, G_1, G_2, and total).
CONCLUSIONS: The aflatoxin B1 levels in animal feed were found higher during rainy seasons compared to the summer season. The aflatoxin B1, carried over from feed to livestock and poultry products in different seasons, may cause high contamination in livestock and poultry products at levels over the tolerance limit. Therefore, continuous surveillance of aflatoxins is required in animal feeds to reduce animal and consequently human exposure.
KEYWORDS: Aflatoxin, Feed ingredients, HPLC, Livestock, Poultry

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Introduction

Mycotoxins and aflatoxins contaminate more than 25% of the agricultural products and as a result a lot of economic losses occur. These toxins cause damage to agricultural products worldwide each year (de Oliveira *et al*., 2014; Gummadidala *et al*., 2019). Mycotoxins and aflatoxins are responsible for many different toxic effects on both animal and human species and are considered the most dangerous problem for feed and food (Faghihi *et al*., 2019; Serdar *et al*., 2020). Aflatoxins are toxic secondary metabolites produced by two species of the *Aspergillus genus,* including *Aspergillus flavus,* and *Aspergillus parasiticus* (Escrivá *et al*., 2015). There is a growing concern regarding the recurrent observation of aflatoxins in the milk and animal products (Khadivi *et al*., 2019). Aflatoxins are metabolites produced by the growth of fungi on foodstuffs, which may occur during the food storage and food processing (Chiewchan *et al*., 2015; Karlovsky *et al*., 2016). They contaminate a wide variety of foods and mixed foods. Studies show that mycotoxins and aflatoxins are mostly found in cereals (Hernandez-Martinez *et al*., 2010). Aflatoxins have adverse effects on growth performance, blood profiles, hepatic histopathology, intestinal morphology, relative weight of digestive organs, activity of digestive enzymes, and biochemical index of intestinal development in animals (He *et al*., 2013; Feng *et al*., 2017). Chronic aflatoxin poisoning in farm animals seems to weaken the immune system, impairing the metabolism of proteins and vital substances in the body. These compounds have toxic, carcinogenic, and mutagenic properties (Sirajudeen *et al*., 2011; Kumar *et al*., 2017; Theumer *et al*., 2018). Intake of feeds contaminated with aflatoxins in the long-term exerts adverse effects on the organs, including liver tissue injury and immune disorders (Fan *et al*., 2015). The consumption of aflatoxin-contaminated foodstuffs by humans and animals in unauthorized or continuous amounts can cause acute or chronic poisoning and cancer (Authority 2013; Ostry *et al*., 2017). One of the most important and predisposing factors for the contamination of feedstuffs of farm animals and poultry is their moisture content (Kana *et al*., 2013; Abdallah *et al*., 2015). Although the presence of molds in foods does not always imply the harmful levels of aflatoxin, it may account for a significant risk. *Aspergillus* can colonize and contaminate food during the storage, especially following prolonged exposure to high humidity environment conditions (Mannaa *et al*., 2017; Reed *et al*., 2018). The risk of aflatoxin production is also higher in severe droughts, where soil moisture is below normal and temperature is high (Ostry *et al*., 2017). Codex Alimentarius Commission (CAC), the central part of the Joint FAO/WHO Food Standards Programme has determined the maximum levels of aflatoxins (B_1, B_2, G_1, G_2, and M1) in foodstuffs (Kotinagu *et al*., 2015). Recently, this commission has announced the maximum accepted/residue levels of aflatoxins in animal feeds as 0.02 mg/kg, that is 20 ppb in all feed materials and complementary feedstuffs for cattle, sheep, goats, pigs, and poultry; while it is 0.005 mg/kg in feeding stuffs for dairy animals and 0.01 mg/kg in complete feeding stuffs for calves and lambs (Bakırdere *et al*., 2012; EFSA Panel on Food Additives and Nutrient Sources added to Food, 2014; Hamed *et al*., 2017). High-performance liquid chromatography (HPLC) is a technique in analytical chemistry, which is used to separate, identify, and quantify aflatoxin in a mixture (Wacoo *et al*., 2014). Considering the dangerous effects of aflatoxins on the health of livestock, poultry, and subsequently, humans, it is important to measure the levels of aflatoxins B_1, B_2, G_1, and G_2, as well as total aflatoxin as critical health indicators in feedstuffs (Wu *et al*., 2008; Wu 2014). Accordingly, the main purposes of this study were to measure the aflatoxin levels using the HPLC in feedstuffs of three factories of Kermanshah province and to evaluate the effect of season on feed ingredients of livestock and poultry.

Materials and Methods

A: Sampling and Analytical Instrument

Sampling was done according to the method of Iranian national standards for the accurate determination of mycotoxin level in foods and agricultural products [INSO, No 12004] (Mazaheri *et al*., 2018). Ninety-three samples of barley, wheat, soybean, rice, and maize were taken from the factories of Kermanshah province (three major factories in four seasons) and analyzed using the method of high-performance

liquid chromatography (HPLC) in the central laboratory of Provincial Veterinary Office (Campos *et al*., 2017). The HPLC system (Agilent Technologies - 1260 Infinity, USA) was equipped with a photochemical reaction device (PHRED, Aura industries, NY, USA). Based on the Food Analysis Performance Assessment Scheme (FAPAS), in the cereal-based animal feed sample using aflatoxin standard, all testing methods include three steps: 1: extraction, 2: purification, and 3: determination of toxin content (Sykes *et al.*, 2017).

B: Chemicals and Method Validation

All the reagents were of HPLC standard grade. Standard aflatoxins were purchased from Sigma Chemical Company (Germany). They were diluted with methanol to different concentrations and used as a calibration standard. The stock standard solution of aflatoxins (a concentration of 10 mg/mL in methanol of each aflatoxin species) was prepared and wrapped in aluminum foil at -20°C. Then the aflatoxin standard mixture was prepared in methanol at 0.4 ng/mL for aflatoxins G1 and B1 and 0.08 ng/mL for aflatoxins G2 and B2. The analytical grade solvents were obtained from Merck (Sigma-Aldrich, Germany). The pure water used in the analysis was prepared by Micro Siemens water purification system using millipore filters (0.45 µ) (TDS, Chemistry Danshvar Co; Iran). PBS: Phosphate Buffer Saline (0.2 g potassium chloride, 0.2 g potassium dihydrogen phosphate, 1.16 g hydrogen; 8 g sodium chloride phosphate, and 900 mL deionized water at pH 7.4) was also used. Fifty grams of samples were weighted. Acetonitrile and methanol were at HPLC grade (Sigma-Aldrich,) and other solvents were up to analytical purity grade. The working solutions of aflatoxins were diluted in the same solvent and stored in glass-stoppered tubes at 0°C. The CH0-3817 column (5 µm C18 UG 120 A, LC column 250 x 4.6 mm) was used (Figure 1). Immunoaffinity columns (IAC) were also used for the purification of samples (China: Huaan Magnech Bio-Tech Co., Ltd.).

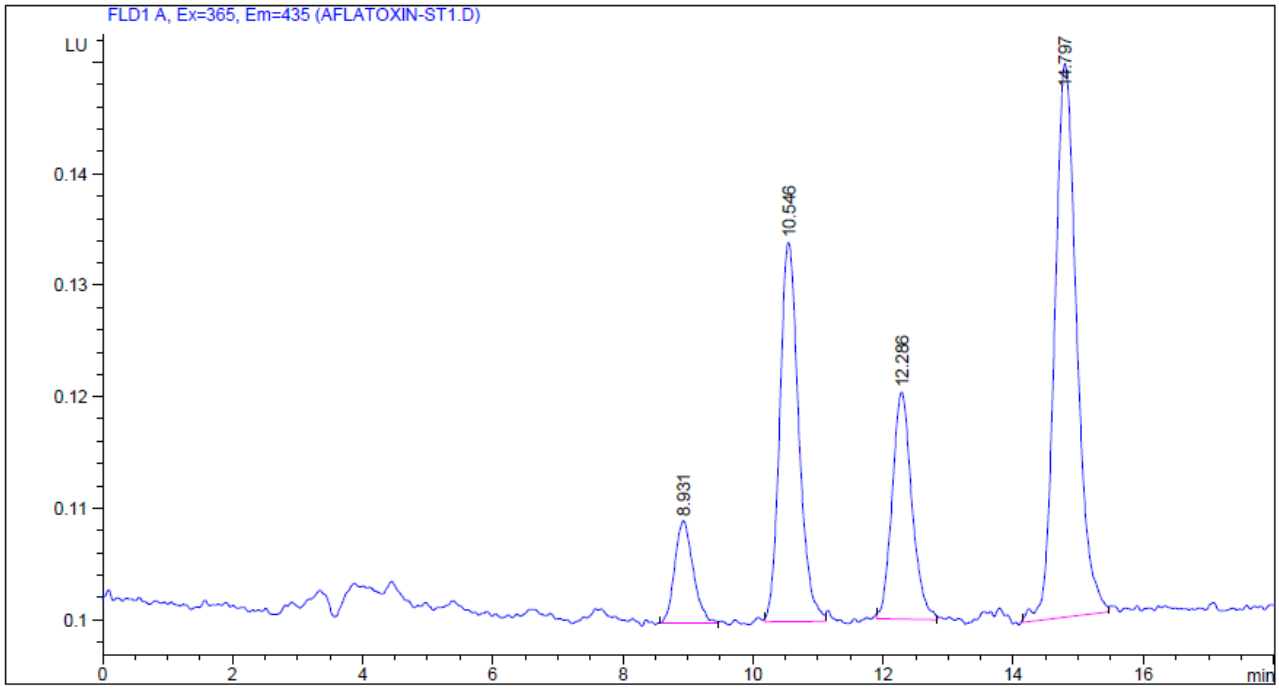

Figure 1. HPLC chromatogram of Aflatoxin standard mixture: AFG_2 and AFB_2 at 0.08 ng/mL; AFB_2 AFG_1 and AFB_1 0.4 ng/mL; Chromatographic conditions: CH0-3817; C18 UG 120 A column (5 µM; 250 × 4.6 mm); mobile phase water methanol and acetonitrile solution (60:30:20, v/v/v); flow rate; mL/min; fluorescence detector (λ ex = 365 nm and λ em = 435nm). **Note:** Retention time (min) for G_2, G_1, B_2 & B_1 peaks are seen left to right, respectively.

The method validation in terms of sensitivity and precision was performed according to the guidelines set by the International Union of Pure and Applied Chemistry (Yakubu *et al*., 2020).

C: Samples Preparation and Analysis

The samples were ground evenly and kept in the refrigerator. The samples were kept away from other materials to avoid secondary contamination. The extraction of the samples was done as per the standard methods of analysis that ensure the safety and integrity of foods and other products impacting public health (AOAC international method) and was quantified with the reference standard of High-performance thin-layer chromatography (HPTLC). Aflatoxin analyses were performed using 50 g of each sample. For extractions, 200 mL of methanol 80% was used and the mixture was stirred for 3 min at high speed. Then each aliquot was passed through a filter paper, diluted in water, and filtered again using a glass microfiber filter. IACs were used to clean up the aflatoxin sample (AflaOchra). First, 10 mL 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 10 mL of water and dried by applying mild vacuum. Finally, the aflatoxins were eluted with 1.5 mL methanol and then with 1.5 mL pure water and were analyzed by HPLC. To do it, the samples (100 µL) were injected into the HPLC column and heated to 40ºC. The mobile phase was a mixture of water, methanol, and acetonitrile (60:30:20, v/v/v). For the mobile phase preparation, 120 mg of potassium bromide and 350 µL of 4 M nitric acid were added to one liter of the mixture. The aflatoxins were detected with an excitation wavelength of 365 nm and emission wavelength of 435 nm fluorescence intensity. In addition, analysis at the maximum permitted limits in feeds was done based on the guidelines of Iranian National Standard (maximum tolerated levels of mycotoxins in food and feed, ICS: 67.020. ISIRI. 5925, Amendment No. 1). The limit of detection (LOD) of aflatoxin mass fraction was 0.4 for B_1 and G_1, and 0.08 for B_2 and G_2. The quantification of separated aflatoxins was performed by comparing it with standard aflatoxins using the formula:

Concentration of aflatoxins in ppm: Standard peak height× Sample peak height / Standard peak height ×Final volume of sample

D: Statistical Analysis

Data were analyzed according to the statistical method of a complete randomized block design (samples and seasons as blocks). Data were analyzed using the SPSS software version 23 (SPSS Inc., Chicago, IL., USA) with the general linear model (GLM) procedure. The statistical model of data analysis is as follows: $Yijk = \mu + Ti + Sj + eijk$. Yijk: the values of each observation; µ: total average; Ti: the effect of treating (barley, corn, etc.); Sj: the season of the year; and eijk: residual effects. Comparisons between mean values were performed using Duncan's multiple range test (at 5% and 1% levels).

Results

The aflatoxin levels for 93 samples and the significance level of interactions between the positive samples and the season are shown in Table 1. As seen, there was no significant relationship between the total aflatoxin values of the samples (0.862) and the total of the seasons (0.919). Measurement and evaluation of B1, B2, G1, G2, and total aflatoxin values in different samples did not show significant differences in impermissible levels (Figure 2, Table 2). The positive samples were related to aflatoxin B1 and were seen mainly in winter. In total, out of 93 samples, six samples (6.45%) were positive for B1 aflatoxin. Out of a total of 27 wheat samples, one sample was positive and related to the winter sampling. Moreover, out of the total 23 soybean samples, one positive sample was related to winter and the other one sample was related to autumn. Based on the evaluation of the total 26 samples of corn, two samples were positive, one sample related to spring, another to winter. In addition, out of the total 10 barley samples, one sample was positive and related to summer. In rice samples evaluated, no positive cases were observed in terms of impermissible levels of aflatoxin (Table 2).

Table 1. Amount of aflatoxin B_1, B_2, G_1& G_2 by HPLC in raw material of livestock and poultry factories based on seasons and type of samples

Samples & Seasons	Aflatoxin				
	B1	B2	G1	G2	Total
Samples					
Wheat	1.231	0.652	0.505	0.452	2.866
Rice	0.579	0.508	0.500	0.477	2.071
Soy	1.153	0.859	0.629	0.648	3.267
Corn	1.540	0.855	0.697	0.635	3.699
Barley	1.688	0.632	0.606	0.476	3.425
Season					
Spring	1.183	0.700	0.435	0.392	2.755
Summer	1.408	0.524	0.515	0.506	2.893
Autumn	1.134	0.676	0.655	0.511	3.054
Winter	1.228	0.905	0.744	0.503	3.561
The significance level					
Samples	0.781	0.828	0.856	0.892	0.862
Season	0.978	0.620	0.523	0.545	0.919
Interaction between treatment and season					
	0.552	0.927	0.966	0.984	0.948

*Comparisons between mean treatments were performed using Duncan's comparison (at 5% and 1% levels). There was no significant difference between positive and negative samples in terms of aflatoxins (B_1, B_2, G_1, G_2 and Total)

** 6 of 93 samples were positive for aflatoxin B_1.

*** Based on ppb, the positive samples were mainly in cold seasons

Table 2. Number of tested samples and positive samples of raw materials of livestock and poultry factories of Kermanshah province based on seasons and type of samples

Samples/ Seasons	Barley	Corn	Soy	Rice	Wheat
Spring	1	3(1+)	3	1	4
Summer	2(1+)	5	5	2	5
Autumn	3	9	7(1+)	2	9
Winter	4	9(1+)	8(1+)	2	9(1+)
Total positive samples= 6(6/45%)	(1+)	(2+)	(2+)	-	(1+)
Total samples=93	10	26	23	7	27

*Comparisons between mean treatments were performed using Duncan's comparison (at 5% and 1% levels). There was no significant difference between positive and negative samples in terms of aflatoxins (B_1, B_2, G_1, G_2 and Total)

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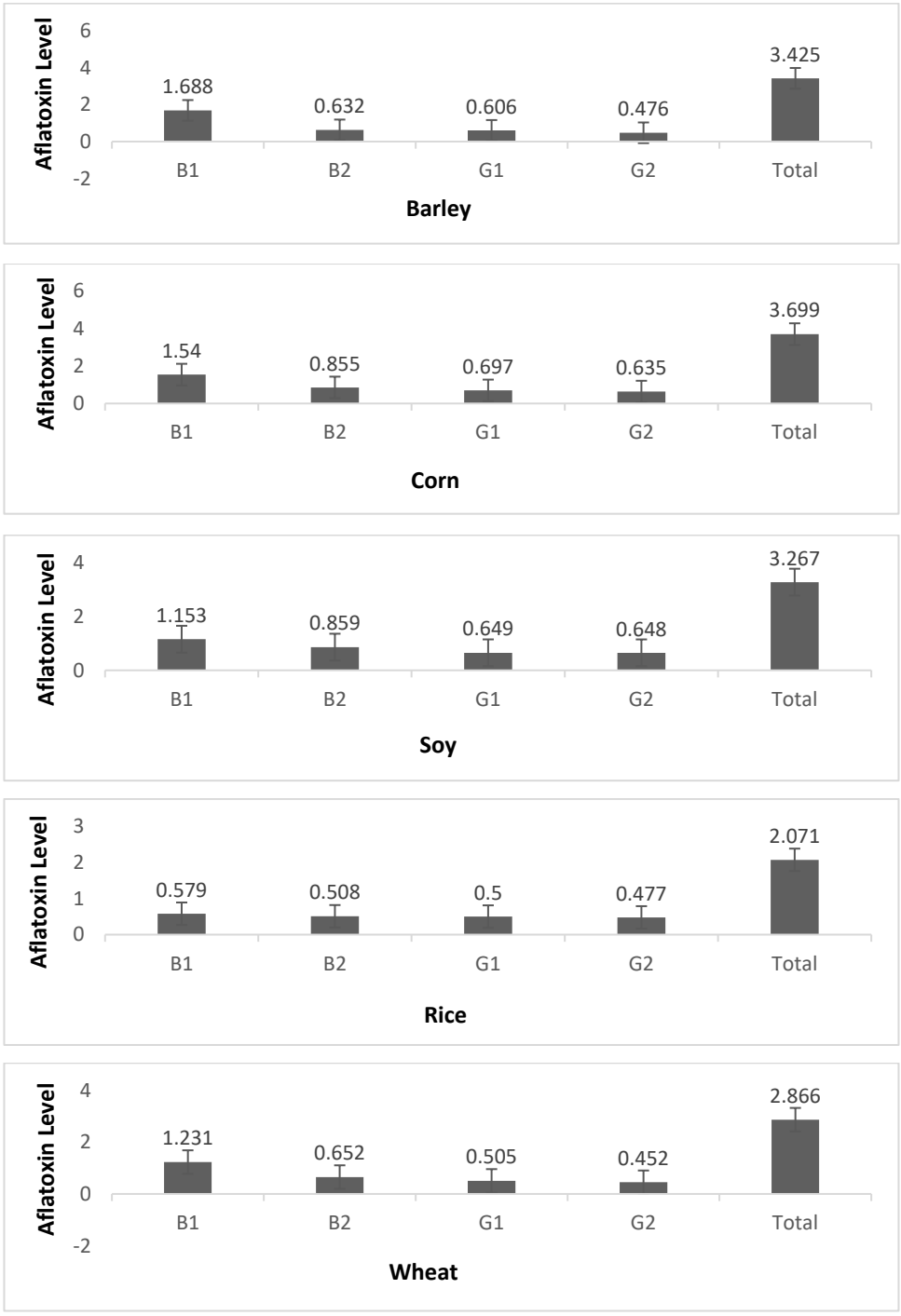

Figures 2. Measurement and evaluation Aflatoxin B_1, B_2, G_1& G_2 in different samples by HPLC

*Comparisons between mean treatments were performed using Duncan's comparison (at 5% and 1% levels). No was significant difference between positive and negative samples in terms of aflatoxins (B_1, B_2, G_1, G_2 and Total)

** Y axis based on ppb

Discussion

The presence of mycotoxins in feeds pose serious health problems (He *et al*., 2013; Feng *et al*., 2017). Among mycotoxins, aflatoxins have immense toxicity and have been associated with various health and disease risks in livestock (Joint *et al*., 2017; Serdar *et al*., 2020). Aflatoxins are the secondary metabolites produced by certain species of fungi on grains and animal feeds. The mycotoxin poisoning increases losses of animal productivity, immunosuppression, damage to vital organs, and animal death (Kumar *et al*., 2017). The infestation of toxigenic fungi can occur before or after harvesting the food crops, grains, and seeds, resulting in serious human and animal health consequences (Al-Faragi *et al*., 2014; Escrivá *et al*., 2015). Aflatoxins have been widely studied compared to other mycotoxins because of their acute toxicity (Chiewchan *et al*., 2015; Karlovsky *et al*., 2016). Despite controlling production methods, aflatoxins are considered unavoidable contaminants in feeds. Therefore, regulatory agencies have developed specific guidelines on acceptable levels of aflatoxins in human and animal feeds. Studies have shown contamination with different types of aflatoxin in field conditions. The US Food and Drug Administration (FDA) has set an aflatoxin exposure limit of 20 ppb at the lowest possible and acceptable level for animal feed. In European Union (EU), aflatoxin B1 is the only toxin with a legal limit for presence in animal feeds (Sirajudeen *et al*., 2011; Theumer *et al*., 2018). One of the most important and predisposing factors for the contamination of feedstuffs of farm animals and poultry is their moisture content (Kana *et al*., 2013; Abdallah *et al*., 2015). The results of various studies indicate that *Aspergillus* and aflatoxin contamination are directly related to the moisture content in stored feedstuffs (Qazi *et al*., 2006; Torres *et al*., 2014; Hussain *et al*., 2015). Products with high humidity are not suitable for storage, as they lead to contamination during harvesting or storing (Waliyar *et al*., 2015). Therefore, climatic conditions and storage quality are two important determinants of mycotoxin contamination (Mannaa *et al*., 2017). According to the WHO estimates, a quarter of the products are affected by fungal toxins annually (Amanloo *et al*., 2014; Adeyeye *et al*., 2016). Aflatoxins and mycotoxins have adverse effects on livestock products and increase food risk factors (Wu et al., 2008; Adeyeye et al., 2016). Furthermore, the milk, eggs, and meat of these animals can contain residues of fungal toxins (Ren *et al*., 2007; Markov *et al*., 2013). On the other hand, aflatoxin contamination is identified as a pressing issue in food hygiene and public health (Richard *et al*., 2007; Frazzoli *et al*., 2017). The amount of aflatoxin and its duration of use determine the clinical consequences of poisoning (Niu *et al.*, 2021). According to the Iran National Standards Organization, the maximum tolerance of aflatoxin B1 is 5 ng/g, and the total aflatoxin is 20 ng/g or ppb (Hedayati *et al*., 2016). The results of the present study showed that aflatoxin contamination was not significantly different in all five feedstuffs and the aflatoxin ranges were 0.452 to 0.648, 0.500 to 0.697, 0.579 to 1.688, 0.859 to 0.797, and 2.071 to 3.699 ppb for G2, G1, B1, B2, and total, respectively. Moreover, the effect of different seasons on these types of aflatoxins was investigated, which showed that although the positive cases were related to cold seasons, statistically no significant difference was observed in various seasons. Accordingly, changes in moisture levels and the ambient temperature had no effect on the contamination rate with this type of toxin in the study area. These results may be related to the lack of long-term storage of materials in factory warehouses and the timely consumption of feed. Our findings are in agreement with the previous studies where aflatoxins (especially B1) were found in most analyzed samples. This variation of percentage of the contamination may be due to differences in the types of substrates and handling processes from the time of harvesting to the time of consumption. In a study by Pourlemi *et al* (2013) on determining the rate of aflatoxin contamination in feed, industrial and local eggs in the western regions of Mazandaran in Iran, 40 industrial eggs from seven different poultry feed areas with 5 repetitions (corn, barley, chopped diet, and soybean meal) were contaminated with aflatoxin B1 in ELISA sampling; their results showed that the rate of aflatoxin contamination in local eggs was higher than that in industrial samples (Pourelmi *et al*., 2013). In the study by Mayahi (2001) on thin-layer chromatography (TLC) on silica gel coated plates of 100 poultry feed samples (25 samples of each item of corn, wheat, soybean,

and fish meal), 86% of the samples were contaminated with aflatoxin (Mayahi *et al*., 2001). Mayahi (2007) also showed *Aspergillus* and aflatoxin contamination in major constituents of 75 samples in bird feed (soybean, corn, and fish powder); and that 25 samples with the highest rate of contamination were from fish powder (aflatoxin B1 was 15 µg/kg). These researchers also found that soybeans, corn, and fish powder were contaminated during the storage. Therefore, controlling and training proper food storage can reduce or eliminate aflatoxin contamination in these materials (Mayahi *et al*., 2007). In a similar study, the levels of aflatoxins B1, B2, G1, and G2 were measured in farms, in domestic animal feed. The results showed that two of the 19 tested samples had aflatoxin G2 higher than the standard level; however, other aflatoxins were negligible (López Grío *et al*., 2010). Hashemi *et al* (2016) also studied feed of 144 dairy cattle in Fars province. They found that in 36 cases, the level of aflatoxin B1 was higher than its standard level (Hashemi *et al*., 2016). The aflatoxin-contaminated food consumed by livestock is absorbed into the muscles and other organs and subsequently into their products (Dashti *et al*., 2009; Magnussen *et al*., 2013). Various studies have shown that consumption of aflatoxin-contaminated food by poultry has caused aflatoxin toxication with disorders of weight gain and food intake, reduced egg production, reduced food efficiency, damage to the digestive tract, blood disorders, liver lesions, and weakened immune system (He *et al*., 2013; da Rocha *et al*., 2014). Aflatoxin-contaminated foods also increase susceptibility to environmental stresses, microbes, neurological abnormalities, and mortality (Ogodo *et al*., 2016). The permitted limit for aflatoxin B1 by FAO is 1-20 ppb. This value is 20 ppb in Iran (Sani *et al*., 2014; Mahfouz *et al*., 2015). Most of the food samples (87 samples) measured in this study had not aflatoxin higher than these values. In our study, the aflatoxin B1 levels were lower than the limit in most samples. In a similar study conducted in Turkey to measure the aflatoxin levels in 41 wheat samples, 59% of the samples contained aflatoxin. In positive samples, the share of each of the aflatoxins B2, B1, G1, and G2 was 42, 12, 37, and 12%, respectively. In this study, the total aflatoxin in the tested samples was between 10.4 and 643.5 ng/g. The results of this study differ from our results. As abovementioned, there is a significant difference between the amounts of aflatoxins. Because aflatoxin-producing fungi respond differently to several conditions, therefore, these differences may be due to the storage conditions, sampling area, geographical area, ambient temperature, and weather conditions. Different concentrations of fungal toxins have been reported in different geographical regions following seasonal climate change. As a result, the differences observed in the values of the present study can be ascribed to sampling in several seasons and specific climatic conditions of the study area.

Conclusion

Aflatoxins are dangerous toxins in livestock and poultry feed and can occur in different climatic conditions. HPLC assures good recovery and precision in the quantitative determination of aflatoxin extracted from livestock compound feed and feed ingredients. Our study showed that aflatoxin B_1 contamination was present in the feed ingredients. No significant difference was observed for aflatoxins in the samples of animal feed in the factories of Kermanshah province, Iran. However, the aflatoxin B_1 levels in animal concentrate feed were found higher during rainy seasons compared to the summer season. Therefore, continuous surveillance of aflatoxins is required in animal feeds to reduce animal and consequently human exposure. On the other hand, due to the various factors that affect the amount of aflatoxins in food, further studies are needed for identifying the source of contamination and executing control measures.

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Ethical Approval

The ethical code approved with *NO:* 2547723, 12.10. 2019 by the research council of Razi University.

Informed Consent

Only the authors are responsible for the content of the paper.

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

Abdallah, M. F., Girgin, G. Ö. Z. D. E., & Baydar, T. (2015). Occurrence, prevention and limitation of mycotoxins in feeds. *Animal Nutrition and Feed Technology*, *15*(3), 471-490. [DOI:10.5958/0974-181X.2015.00048.7]

Adeyeye, S. A. (2016). Fungal mycotoxins in foods: A review. *Cogent Food & Agriculture*, *2*(1), 1213127. [DOI:10.1080/23311932.2016.1213127]

Al-Faragi, J. K. (2014). The Efficacy of Prebiotic ([Beta]-Glucan) as a Feed Additive against Toxicity of Aflatoxin B1 in Common Carp, Cyprinus Carpio L. *Journal of Aquaculture Research & Development*, *5*(4), 1. [DOI:10.4172/2155-9546.1000240]

Amanloo, S., Rezaei Kahhka, M. R., Ramezani, A. A., & Mir, L. (2014). The Mycotoxin contamination of the imported consumer rice and its producing fungi in Zabol. *Journal of Jahrom University of Medical Sciences*, *12*(1), 17-25. [DOI:10.29252/jmj.12.1.17]

Bakırdere, S., Bora, S., Bakırdere, E. G., Aydın, F., Arslan, Y., Komesli, O. T., ... & Yıldırım, E. (2012). Aflatoxin species: their health effects and determination methods in different foodstuffs. *Central European Journal of Chemistry*, *10*(3), 675-685. [DOI:10.2478/s11532-012-0009-2]

Campos, W. E., Rosas, L. B., Neto, A. P., Mello, R. A., & Vasconcelos, A. A. (2017). Extended validation of a senstive and robust method for simultaneous quantification of aflatoxins B1, B2, G1 and G2 in Brazil nuts by HPLC-FLD. *Journal of Food Composition and Analysis*, *60*, 90-96. [DOI:10.1016/j.jfca.2017.03.014]

Chiewchan, N., Mujumdar, A. S., & Devahastin, S. (2015). Application of drying technology to control aflatoxins in foods and feeds: a review. *Drying Technology*, *33*(14), 1700-1707. [DOI:10.1080/07373937.2015.1068795]

da Rocha, E. B. M., Freire, FCO, Maia, FEF, Guedes, MIF, and Rondina, D. 2014. *Mycotoxins and Their Effects on Human and Animal Health. Food Control*, *36*, 159-165. [DOI:10.1016/j.foodcont.2013.08.021]

Dashti, B., Al-Hamli, S., Alomirah, H., Al-Zenki, S., Abbas, A. B., & Sawaya, W. (2009). Levels of aflatoxin M1 in milk, cheese consumed in Kuwait and occurrence of total aflatoxin in local and imported animal feed. *Food Control*, *20*(7), 686-690. [DOI:10.1016/j.foodcont.2009.01.001]

de Oliveira, C. A., & Corassin, C. H. (2014). Aflatoxins. Future Science Ltd. [DOI:10.4155/ebo.13.468] [PMID]

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS). (2014). Scientific Opinion on the reconsideration of the ADI and a refined exposure assessment of β-apo-8′-carotenal (E 160e). *EFSA Journal*, *12*(1), 3492. [DOI:10.2903/j.efsa.2014.3492]

Escrivá, L., Font, G., & Manyes, L. (2015). In vivo toxicity studies of fusarium mycotoxins in the last decade: A review. *Food and Chemical Toxicology*, *78*, 185-206. [DOI:10.1016/j.fct.2015.02.005] [PMID]

European Food Safety Authority. (2013). Refined exposure assessment of ethyl lauroyl arginate based on revised proposed uses as a food additive. *EFSA Journal*, *11*(6), 3294. [DOI:10.2903/j.efsa.2013.3294]

Faghihi Shahrestani, F., Tajabadi Ebrahimi, M., Bayat, M., Hashemi, J., & Razavilar, V. (2021). Identification of

Dairy Fungal Contamination and Reduction of Aflatoxin M1 Amount by Three Acid and Bile Resistant Probiotic Bacteria. *Archives of Razi Institute*, *76*(1), 119.

Fan, Y., Zhao, L., Ji, C., Li, X., Jia, R., Xi, L., ... & Ma, Q. (2015). Protective effects of Bacillus subtilis ANSB060 on serum biochemistry, histopathological changes and antioxidant enzyme activities of broilers fed moldy peanut meal naturally contaminated with aflatoxins. *Toxins*, *7*(8), 3330-3343. [DOI:10.3390/toxins7083330] [PMID] [PMCID]

Feng, G. D., He, J., Ao, X., & Chen, D. W. (2017). Effects of maize naturally contaminated with aflatoxin B1 on growth performance, intestinal morphology, and digestive physiology in ducks. *Poultry Science*, *96*(6), 1948-1955. [DOI:10.3382/ps/pew420] [PMID]

Frazzoli, C., Gherardi, P., Saxena, N., Belluzzi, G., & Mantovani, A. (2017). The hotspot for (global) one health in primary food production: Aflatoxin M1 in dairy products. *Frontiers in Public Health*, *4*, 294. [DOI:10.3389/fpubh.2016.00294] [PMID] [PMCID]

Gummadidala, P. M., Omebeyinje, M. H., Burch, J. A., Chakraborty, P., Biswas, P. K., Banerjee, K., ... & Chanda, A. (2019). Complementary feeding may pose a risk of simultaneous exposures to aflatoxin M1 and deoxynivalenol in Indian infants and toddlers: Lessons from a mini-survey of food samples obtained from Kolkata, India. *Food and chemical toxicology*, *123*, 9-15. [DOI:10.1016/j.fct.2018.10.006] [PMID]

Hamed, A. M., Moreno-González, D., García-Campaña, A. M., & Gámiz-Gracia, L. (2017). Determination of aflatoxins in yogurt by dispersive liquid–liquid microextraction and HPLC with photo-induced fluorescence detection. *Food Analytical Methods*, *10*(2), 516-521. [DOI:10.1007/s12161-016-0611-6]

Hashemi, M. (2016). Aflatoxin B1 levels in feedstuffs from dairy cow farms in south of Iran. *Food and Agricultural Immunology*, *27*(2), 251-258. [DOI:10.1080/09540105.2015.1086319]

He, J., Zhang, K. Y., Chen, D. W., Ding, X. M., Feng, G. D., & Ao, X. (2013). Effects of maize naturally contaminated with aflatoxin B1 on growth performance, blood profiles and hepatic histopathology in ducks. *Livestock Science*, *152*(2-3), 192-199. [DOI:10.1016/j.livsci.2012.12.019]

Hedayati, M. T., Omran, S. M., Soleymani, A., & Armaki, M. T. (2016). Aflatoxins in food products in Iran: A review of the literature. *Jundishapur Journal of Microbiology*, *9*(7). [DOI:10.5812/jjm.33235] [PMID] [PMCID]

Hernandez-Martinez, R., & Navarro-Blasco, I. (2010). Aflatoxin levels and exposure assessment of Spanish infant cereals. *Food Additives and Contaminants*, *3*(4), 275-288. [DOI:10.1080/19393210.2010.531402] [PMID]

Hu, Y. Y., Zheng, P., Zhang, Z. X., & He, Y. Z. (2006). Determination of aflatoxins in high-pigment content samples by matrix solid-phase dispersion and high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry*, *54*(12), 4126-4130. [DOI:10.1021/jf0601564] [PMID]

Hussain, A., Afzal, A., Irfan, M., & Malik, K. A. (2015). Molecular detection of aflatoxin producing strains of Aspergillus flavus from peanut (Arachis hypogaea). *Turkish Journal of Agriculture-Food Science and Technology*, *3*(5), 335-341. [DOI:10.24925/turjaf.v3i5.335-341.123]

Joint, F. A. O., World Health Organization, & WHO Expert Committee on Food Additives. (2017). *Evaluation of certain contaminants in food: eighty-third report of the Joint FAO/WHO Expert Committee on Food Additives*. World Health Organization.

Kana, J. R., Gnonlonfin, B. G. J., Harvey, J., Wainaina, J., Wanjuki, I., Skilton, R. A., & Teguia, A. (2013). Assessment of aflatoxin contamination of maize, peanut meal and poultry feed mixtures from different agroecological zones in Cameroon. *Toxins*, *5*(5), 884-894. [DOI:10.3390/toxins5050884] [PMID] [PMCID]

Karlovsky, P., Suman, M., Berthiller, F., De Meester, J., Eisenbrand, G., Perrin, I., ... & Dussort, P. (2016). Impact of food processing and detoxification treatments on mycotoxin contamination. *Mycotoxin Research*, *32*(4), 179-205. [DOI:10.1007/s12550-016-0257-7] [PMID] [PMCID]

Khadivi, R., Razavilar, V., Anvar, S. A. A., & Akbari-Adergani, B. (2020). Aflatoxin M1-binding ability of selected lactic acid bacteria strains and Saccharomyces boulardii in the experimentally contaminated milk treated with some biophysical factors. *Archives of Razi Institute*, *75*(1), 63.

Kotinagu, K., Mohanamba, T., & Kumari, L. R. (2015). Assessment of aflatoxin B1 in livestock feed and feed ingredients by high-performance thin layer chromatography. *Veterinary World*, *8*(12), 1396. [DOI:10.14202/vetworld.2015.1396-1399] [PMID] [PMCID]

Kumar, P., Mahato, D. K., Kamle, M., Mohanta, T. K., & Kang, S. G. (2017). Aflatoxins: A global concern for food safety, human health and their management. *Frontiers in Microbiology*, *7*, 2170. [DOI:10.3389/fmicb.2016.02170]

López Grío, S. J., Garrido Frenich, A., Martínez Vidal, J. L., & Romero-González, R. (2010). Determination of aflatoxins B1, B2, G1, G2 and ochratoxin A in animal feed by ultra high-performance liquid chromatography–tandem mass spectrometry. *Journal of Separation Science*, *33*(4-5), 502-508. [DOI:10.1002/jssc.200900663] [PMID]

Magnussen, A., & Parsi, M. A. (2013). Aflatoxins, hepatocellular carcinoma and public health. *World Journal of Gastroenterology: WJG*, *19*(10), 1508. [DOI:10.3748/wjg.v19.i10.1508] [PMID] [PMCID]

Mahfouz, M. E., & Sherif, A. H. (2015). A multiparameter investigation into adverse effects of aflatoxin on Oreochromis niloticus health status. *The Journal of Basic & Applied Zoology*, *71*, 48-59. [DOI:10.1016/j.jobaz.2015.04.008]

Mannaa, M., & Kim, K. D. (2017). Influence of temperature and water activity on deleterious fungi and mycotoxin production during grain storage. *Mycobiology*, *45*(4), 240-254. [DOI:10.5941/MYCO.2017.45.4.240] [PMID] [PMCID]

Markov, K., Pleadin, J., Bevardi, M., Vahčić, N., Sokolić-Mihalak, D., & Frece, J. (2013). Natural occurrence of aflatoxin B1, ochratoxin A and citrinin in Croatian fermented meat products. *Food Control*, *34*(2), 312-317. [DOI:10.1016/j.foodcont.2013.05.002]

MAYAHI, M., & SOHRABI, G. R. (2001). Determination of aflatoxin in raw materials of poultry feed in Ahwaz. *Scientific-Research Iranian Veterinary Journal, 4* (7), 37-45.

Mayahi, M., Razi, J. M., & Salamat, N. (2007). Isolation of Aspergillus spp and determination of aflatoxin level in fish meal, maize and soya meal. *Shahid Chamran University Journal of Science, 17*, 95-105.

Mazaheri, M., & Mahmoudi Maymand, M. (2018). Investigation of Aflatoxins in Imported Animal Feeds in Iran. *Iranian Journal of Toxicology*, *12*(6), 33-38. [DOI:10.32598/IJT.12.6.514.1]

Niu, Y., Fan, S., Luo, Q., Chen, L., Huang, D., Chang, W., ... & Shi, G. (2021). Interaction of Hepatitis B Virus X Protein with the Pregnane X Receptor enhances the synergistic effects of Aflatoxin B1 and Hepatitis B Virus on promoting hepatocarcinogenesis. *Journal of Clinical and Translational Hepatology*, *9*(4), 466. [DOI:10.14218/JCTH.2021.00036] [PMID] [PMCID]

Ogodo, A. C., & Ugbogu, O. C. (2016). Public health significance of aflatoxin in food industry—A review. *European Journal of Clinical and Biomedical Sciences*, *2*(5), 51-58.

Ostry, V., Malir, F., Toman, J., & Grosse, Y. (2017). Mycotoxins as human carcinogens—the IARC Monographs classification. *Mycotoxin Research*, *33*(1), 65-73. [DOI:10.1007/s12550-016-0265-7] [PMID]

Pourelmi, M. R., Palizdar, M. H., Shirali, S., & Barami, A. R. (2013). Aflatoxin B1 contamination in local and industrial eggs measured by ELISA technique in Mazandaran. *European Journal of Zoological Research*, *2*, 89-92.

Qazi, J. I., & Fayyaz, Z. (2006). Aflatoxin contaminated foods and health risk perspective for Pakistani population. *Mycopathol*, *4*(2), 27-34.

Reed, K. M., Mendoza, K. M., Abrahante, J. E., & Coulombe, R. A. (2018). Comparative response of the hepatic transcriptomes of domesticated and wild turkey to aflatoxin B1. *Toxins*, *10*(1), 42. [DOI:10.3390/toxins10010042] [PMID] [PMCID]

Ren, Y., Zhang, Y., Shao, S., Cai, Z., Feng, L., Pan, H., & Wang, Z. (2007). Simultaneous determination of multi-component mycotoxin contaminants in foods and feeds by ultra-performance liquid chromatography tandem mass spectrometry. *Journal of Chromatography A*, *1143*(1-2), 48-64. [DOI:10.1016/j.chroma.2006.12.064] [PMID]

Richard, J. L. (2007). Some major mycotoxins and their mycotoxicoses—An overview. *International Journal of Food Microbiology*, *119*(1-2), 3-10. [DOI:10.1016/j.ijfoodmicro.2007.07.019] [PMID]

Sani, A. M., Azizi, E. G., Salehi, E. A., & Rahimi, K. (2014). Reduction of aflatoxin in rice by different cooking methods. *Toxicology and Industrial Health*, *30*(6), 546-550. [DOI:10.1177/0748233712462466] [PMID]

Santacroce, M. P., Conversano, M. C., Casalino, E., Lai, O., Zizzadoro, C., Centoducati, G., & Crescenzo, G. (2008). Aflatoxins in aquatic species: metabolism, toxicity and perspectives. *Reviews in Fish Biology and Fisheries*, *18*(1), 99-130. [DOI:10.1007/s11160-007-9064-8]

Serdar, S. A., El Tawila, M. M., Madkour, M. H., & Alrasheedi, A. A. (2020). Determination of aflatoxins (AFs) in different food samples: A case study from

Jeddah, Saudi Arabia. *Journal of King Abdulaziz University-Meteorology, Environment and Arid Land Agriculture Sciences*, *29*, 23-34.

Sirajudeen, M., Gopi, K., Tyagi, J. S., Moudgal, R. P., Mohan, J., & Singh, R. (2011). Protective effects of melatonin in reduction of oxidative damage and immunosuppression induced by aflatoxin B1-contaminated diets in young chicks. *Environmental Toxicology*, *26*(2), 153-160. [DOI:10.1002/tox.20539] [PMID]

Sykes, M., Czymai, T., Hektor, T., Sharman, M., & Knaggs, M. (2017). Chloramphenicol stereoisomers need to be distinguished: consequences observed from a proficiency test Part A Chemistry, analysis, control, exposure & risk assessment. [DOI:10.1080/19440049.2016.1270469] [PMID]

Theumer, M. G., Henneb, Y., Khoury, L., Snini, S. P., Tadrist, S., Canlet, C., ... & Audebert, M. (2018). Genotoxicity of aflatoxins and their precursors in human cells. *Toxicology Letters*, *287*, 100-107. [DOI:10.1016/j.toxlet.2018.02.007] [PMID]

Torres, A. M., Barros, G. G., Palacios, S. A., Chulze, S. N., & Battilani, P. (2014). Review on pre-and post-harvest management of peanuts to minimize aflatoxin contamination. *Food Research International*, *62*, 11-19. [DOI:10.1016/j.foodres.2014.02.023]

Wacoo, A. P., Wendiro, D., Vuzi, P. C., & Hawumba, J. F. (2014). Methods for detection of aflatoxins in agricultural food crops. *Journal of Applied Chemistry*, *2014*(1-15), 706291. [DOI:10.1155/2014/706291]

Waliyar, F., Osiru, M., Ntare, B. R., Kumar, K. V. K., Sudini, H., Traore, A., & Diarra, B. (2015). Post-harvest management of aflatoxin contamination in groundnut. *World Mycotoxin Journal*, *8*(2), 245-252. [DOI:10.3920/WMJ2014.1766]

Wu, F. (2015). Global impacts of aflatoxin in maize: trade and human health. *World Mycotoxin Journal*, *8*(2), 137-142. [DOI:10.3920/WMJ2014.1737]

Wu, F., Liu, Y., & Bhatnagar, D. (2008). Cost-effectiveness of aflatoxin control methods: economic incentives. *Toxin Reviews*, *27*(3-4), 203-225. [DOI:10.1080/15569540802393690].

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مجله طب دامی ایران، ۱۴۰۱، دوره ۱۶، شماره ۳، ۲۹۸-۳۱۰

ارزیابی برخی از آفلاتوکسین ها در ترکیبات خوراک دام و طیور با روش HPLC یک مطالعه محلی در استان کرمانشاه

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زمینه مطالعه: مایکوتوکسین‌ها متابولیت های ثانویه‌ای هستند که توسط قارچها به‌ویژه آسپرژیلوس روی دانه‌ها و خوراک حیوانات تولید می‌شوند. مهمترین مایکوتوکسین‌ها آفلاتوکسین‌ها هستند. چهار جزء اصلی آفلاتوکسین شامل B_1 ، B_2 ، G_1و G_2 است.

هدف: مطالعه حاضر با هدف تعیین میزان وجود آفلاتوکسین (B1 ، B2، G1و G2) در ترکیبات خوراک دام و طیور و بررسی تأثیر فصل، تغییرات مکانی آلودگی به آفلاتوکسین انجام شد.

روش کار: در این مطالعه پس از جمع‌آوری ۹۳ نمونه مواد اولیه خوراک از سه کارخانه بزرگ استان کرمانشاه در چهار فصل مختلف، مقادیر آفلاتوکسین در آنها با استفاده از HPLC تعیین شد. داده‌ها با استفاده از نرم‌افزار SPSS نسخه ۲۳ با روش مدل خطی عمومی (GLM) بر اساس طرح بلوک‌های کامل (نمونه ها و فصل) تجزیه و تحلیل شد.

نتایج: از ۹۳ نمونه، ۶ نمونه از نظر آفلاتوکسین B1 مثبت بودند. نمونه‌های مثبت عمدتاً در فصول سرد سال بود. همچنین تفاوت معنی‌داری بین نمونه‌های مثبت و منفی از نظر آفلاتوکسین(B1 ، B2، G1، G2و Total) مشاهده نشد.

نتیجه‌گیری نهایی: سطوح AFB1 در خوراک کنسانتره حیوانات در طول فصول بارانی در مقایسه با فصل تابستان بالاتر بود. انتقال AFB1 از محصولات دام و طیور در فصول مختلف ممکن است منجر به آلودگی بالای محصولات دام و طیور در سطوح بالاتر از حد تحمل شود. بنابراین، نظارت مستمر بر آفلاتوکسین در خوراک دام برای کاهش مواجهه دام و در نتیجه انسان ضروری است.

واژه‌های کلیدی: آفلاتوکسین، مواد تشکیل‌دهنده خوراک، HPLC، دام، طیور

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