Research article

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Evaluation of Aflatoxins contamination in barley feeds distributed in Mashhad city, Iran

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Abstract

Background and objective: Aflatoxins (AFs) are a group of toxic agents, which are predominantly produced by three species of Aspergillus. Current study was conducted to determine quantity of AFs in barley feed.

Materials and methods: A total of 97 samples were analyzed with high-performance liquid chromatography (HPLC). The method was based on extraction of finely ground samples by methanol as solvent. An aliquot of 20 µl of extract was injected to HPLC after filtration. Aflatoxins were detected by ZORBAX Eclipse XDB C18 column (150 × 4.6 × 5 µm). Mobile phase of water:methanol:acetonitrile (60:20:20 v/v/v) at linear gradient mode and flow rate of 1 ml min^{-1} was used. Experiments were done at excitation and emission wavelengths of 365 and 445 nm, respectively, after post-column UV derivatisation.

Results and conclusion: The obtained results showed that AFB_1, AFB_2, AFG_1 and AFG_2 were detected in 19, 9, 4 and 1 samples, respectively. Relatively low LOD and LOQ were achieved in our analytical approach that approved a desirable goodness of fit for the analysis. Trueness was evaluated by calculation of relative recoveries of AFs that were quite good. This survey provides reliable information about aflatoxin contamination in barely feed products marketed in Mashhad city.

Keywords: Aflatoxin, barley feed, high performance liquid chromatography, Mashhad

1. Introduction

Barley is a valuable cereal grain with nutlike flavor. It has a long history of use, which backs to 7000 B.C. Egyptians and Greeks in ancient times consumed grain for a nourishing food source as well as medicinal purposes. Pearl barley is a human food made of grain by using abrasive disks to grind the hulls and bran of kernels. Half or more of the grown grain is used for livestock feed. Nutritive value of corn kernel for feed is nearly equal to barely. It is especially valuable as hog feed [1].

Fungi are widespread microorganisms in the environment. When food preservation is not suitable, inappropriate level of moisture and heat will result in growth and propagation of fungi. In

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addition, inadequate harvesting practices and inappropriate further handling leads to mycotoxins' contamination [2]. Of the most essential contaminant fungi which play critical role in toxin production are *Aspergillus flavus* and *A. parasiticus* and to lesser extent *A. noxious* which contaminate the plants and products [3]. Studies have shown that different seasons can also effect on contamination level [4]. High moisture and heat in autumn and summer compared to other seasons, if are not controlled in storage time, are the reason of higher AF contamination [5]. Contamination of animal feed to fungus, especially those of Aspergillus, can produce AF and transfer it to dairies [6]. AFB_1 is of the most important and toxic metabolite in all animal species and liver is the main target organ of toxicity [7]. When animals eat AFB_1 contaminated feed, the toxin metabolizes to AFM_1 in their body [3]. AFM_1 and AFM_2, are heterocyclic metabolites of AFB_1 and AFB_2, respectively, and may be found in dairy products. AFM_1 resists against thermal process such as pasteurization and sterilization [8]. The main harm of mycotoxicosis is their adverse effects on liver and kidney. However, some mycotoxins interfere initially in synthesis of amino acid in body and consequently causing skin sensation, necrosis or immune system impairment [9]. International agency for research on cancer (IARC) reported AFB_1 and AFM_1 as primary group of carcinogenic compounds [10]. Since AFs accumulate in body, it is necessary to pay particular attention to reducing their entrance and formation in foodstuff [11]. Following to documentation of approved health concern to human, regulatory levels have been determined by international organizations including World Health Organization (WHO), U.S. Food and Drug Administration (US FDA) and Food and Agriculture Organization (FAO). In accordance, limit of AFB_1 and total aflatoxins set on 0-5 ng g^{-1} and 0-20 ng g^{-1}, respectively [3]. In 2002, Iranian National Standards Organization (INSO) assigned a maximum tolerated limit (MTL) for mycotoxins in foods and feeds. The regulatory limit for AFB_1 were 5.0 ng g^{-1} in animal feeds [12]. Today, some measures have been taken by ministry of health and other regulatory authorities; however, the results show that AFs contamination in feeds and foods is still substantial and a matter of concern for consumers. Some of the most common and sensitive techniques like thin layer chromatography (TLC) and liquid chromatography (LC) were used for AFs analysis in food and feed samples [1,13,14]. Currently, sensitive approaches such as those developed by HPLC with fluorescence detection (HPLC–FLD) coupled with pre- or post-column derivatization, and immune-affinity clean-up are of widely used methods for AFs determination in complex matrices.

To best of our knowledge, current data on AFs in barely feed is rare and more information is needed to find out the potent problems. Therefore, the objective of present study was to study the occurrence and distribution of AFs in barely feed.

2. Materials and methods

2.1. Sampling

All of the feeding barely samples were purchased from wholesalers in Mashhad city, Iran. Average weight of 2-3 kg of samples was mixed and homogenized. Then, they were transferred to the laboratory at room temperature and stored at 4°C up to preparation of the sample for analysis. The prepared analytes were stored at -20°C until analysis. In day of analysis, samples were defrosted at ambient temperature.

2.2. Chemicals

All chemicals used for analysis were HPLC grade and supplied by Merck Company (Darmstadt, Germany). AF standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). Standard solutions including B_1, B_2, G_1 and G_2 were prepared and used for calibration curves and trueness experiments. Neogene immune-affinity column

(Neogene Europe, Ltd, Scotland, UK) was used for analysis.

2.3. Apparatus

Analysis was performed by HPLC, Agilent Technologies SL 1200 Series (Waldbronn, Germany) composed of fluorescence detector, a ZORBAX Eclipse XDB C_{18} column (150 × 4.6 mm × 5 µm, Agilent Technologies), thermos-stated auto-sampler and binary pump equipped with 152 micro vacuum degasser. On-line photochemical derivatization with a commercially available system UVE™ LCTech GmbH (Dorfen, Germany) was used. UV spectrophotometer was used for determination of AF stock solution concentration (10 mg l^{-1}) [15]. A Waring blender (PB25E, USA) was used for mixing the samples.

2.4. HPLC condition

The analysis was done by a mixture of water:methanol:acetonitrile (60:20:20, v/v/v) as mobile phase at flow rate of 1.0 ml min^{-1} in isocratic elution mode. The column temperature and injection volume were 40°C and 20 µl, respectively. The fluorescence detection was carried out at 365 and 445 nm as the excitation and emission wavelengths, respectively [16].

2.5. Sample preparation

Five g of sodium chloride and 50 g of ground barley sample were weighted, blended for 5 min at high speed with 200 ml methanol 80% and then filtered. Twenty ml of filtrate was mixed with 130 ml of phosphate buffer solution. The mixture was passed through a glass microfiber filter (Whatman, Inc., Clifton, NJ). One hundred ml of final solution was transferred to a reservoir and passed through immune-affinity column at a flow rate of 1.0 ml min^{-1}. For further purification and clean-up, 10 ml of distilled deionized water was passed through immune-affinity column, at a flow rate of 5.0 ml min^{-1}. AF was eluted with methanol and water according to the following procedure. Firstly, 2 ml of HPLC grade methanol was passed through the column and then 2 ml of HPLC grade water was given and collected to a glass vial [16]. For AFs determination in the samples, standard curve was plotted by using the absorbance against concentration of external standards of AFs.

2.6. Statistical analysis

The experimental analysis was done in three replicates. Statistical analysis was conducted by Minitab 16 and Excel 2010 software. Differences of data were significant at $p \leq 0.05$.

3. Results and discussion

In the current work, 97 barley samples were analyzed to assess the concentration of AFs B_1, B_2, G_1 and G_2. According to Table 1, AFs B_1, B_2, G_1 and G_2 were detected in 19.59%, 9.28%, 4.12% and 1.03% of the samples with a mean value of 3.53, 1.73, 1.46 and 0.29 ng g^{-1}, respectively. In similar research carried out by Beheshti and Asadi in 2014 in Iran, 60 barley samples were examined. In their study, AFB_1 was detected in only five samples with a mean value of 0.48 ng g^{-1} and AFs B_2, G_1 and G_2 were not detected [17]. Another research was done by Eskandari and Pakfetrat in same year in Iran. They studied occurrence of AFs and heavy metal in forty brands of animal feed that produced from samples of southwest of Iran. The results showed that all samples were contaminated with aflatoxins. Importantly, AFB_1 and total aflatoxins were higher than maximum permitted levels in the all [18]. In comparison, lower contamination in our samples may be due to the fact that our sampling was done in summer and autumn of 2018 that weather was not humid.

Table 1- Concentration of mycotoxins (ng g^{-1}) in barley feed samples

Toxin	Average	Number of negative samples (<LOD)	Numbers of samples in the range	
			0.0-5.0	5.1-8.5
AFB_1	3.53	78	14	5
AFB_2	1.73	88	7	2
AFG_1	1.46	93	3	1
AFG_2	0.29	96	1	-

Wu et al. determined AFB_1 in corn and corn germ meal. AFB_1 was detected in 176 out of 220 (80%) corn and 25 out of 34 (74%) corn germ meal samples. The mean concentration of AFB_1 in corn and corn germ meal was 3.9 and 7.4 ng g^{-1}, respectively. In their study, AFB_1 was also analyzed in cattle feed and it was detected in all samples with mean of 4.5 ng g^{-1} [7]. A total of 97 samples containing 48 livestock feeds and 49 feed ingredients from different livestock farms and farmers were analyzed by high-performance thin layer chromatography (HPTLC). In total, 16 contaminated feeds and 13 contaminated feed ingredients were found. The results of that research showed that HPTLC had good recovery, precision and linearity in quantitative determination of aflatoxin B_1 [19]. Mateo et al., determined AFs and ochratoxin A in 105 barely grain in Spain from 2008 to 2010. Twenty-nine out of 105 samples were contaminated by at least one of the mycotoxins. Species-specific PCR assays were used for detection of *A. flavus*, *A. parasiticus*, *A. ochraceus*, *A. steynii*, *A. westerdijkiae*, *A. carbonarius* and *A. niger* in mycotoxin-positive samples. The obtained results indicated the presence of all fungi species, except *A. westerdijkiae* [20]. Analytical data are summarized in Table 2. For repeatability measurement, standard solutions containing 3.6 ng ml^{-1} of AFB_1 and AFG_1 and 0.72 ng ml^{-1} of AFB_2 and AFG_2 were used. The calibration curve was drawn by seven concentrations of aflatoxins resulted in correlation coefficient (r^2) of AFB_1=0.9970, AFB_2=0.9973, AFG_1=0.9967, and AFG_2=0.9942. The calibration curves exhibited good linear regression. Limit of detections (LODs) for AFB_1, AFB_2, AFG_1 and AFG_2 were 0.008, 0.017, 0.017 and 0.025 and limit of quantifications (LOQs) were 0.025, 0.05, 0.05 and 0.075 ng g^{-1}, respectively. Accuracy was calculated by determination of relative recoveries of AFs. The relative recovery was calculated by comparing the AF concentration in spiked samples (containing a known amount of added analyte) to the concentration in native samples. Recovery of spiked samples at 10 ng g^{-1} for AFB_1 and AFG_1 and 2 ng g^{-1} for AFB_2 and AFG_2 are shown in Table 2. In mix standard solution of AFs, concentration of B_1 and G_1 was five times of B_2 and G_2. The recovery range was within the guideline of acceptable recovery limits of AOAC (Association of Official Analytical Chemists) and Codex Alimentarius. AOAC acceptable recovery at concentration of 10 ng g^{-1} is 70-125% and the Codex acceptable recovery range is 70-110% for concentration of 10-100 ng g^{-1} and 60-120% for concentration of 1-10 ng g^{-1} [21].

Table 2- Method validation of aflatoxin analysis by high performance chromatography in barley samples

Toxin	Retention time (min)	Repeatability (%RSD, n=6)	Limit of detection (ng g^{-1})	Limit of quantification (ng g^{-1})	Relative recovery (%)	Linear dynamic range (ng ml^{-1})
		Peak area				
AFB_1	9.98	1.3	0.008	0.024	96.08	0.40-10
AFB_2	8.25	2.4	0.017	0.051	99.20	0.08-2
AFG_1	7.48	1.5	0.017	0.051	93.90	0.40-10
AFG_2	6.32	3.2	0.025	0.075	98.70	0.08-2

4. Conclusion

Our results showed that the current analytical method was able to determine aflatoxin amount in feeding samples with high recovery percent. In addition, 19 out of 97 barley samples contained AFB_1. Among them, AFB_1 in five contaminated samples were higher than the level of Iranian National Standard and European regulation (5 ng g^{-1}). However, total aflatoxins were within the acceptable range of 15 ng g^{-1} in all samples. Due

to lack of fresh forage, elimination of AFs in animal feed is not applicable but proper monitoring and storage practices could decrease their concentration. In this regard, barley feed should be checked regularly and storage condition must be under strict control. At the same time, surveillance programs must be continuous and widespread in the market. What is important is that now animal being is at risk of daily exposure to fungal toxins mainly AFs. Feedings whose levels of toxin are higher than acceptable limit should not be consumed by animals because there is a risk of their transfer to human body through consumption of animal foods. Therefore, consideration of standards and regulations that highlight livestock-based food products to assess toxins and other contaminants is recommended.

5. Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Bauer M, Lardy G, Swanson K, Zwinger S. Barley grain and forage for beef cattle. In: University NDS, editor. 2017.

2. Xue Z, Zhang Y, Wancong Y, Juncai Z, Wang J, Wan F, Kim Y, Liu Y, Kou X. Recent advances in aflatoxin B_1 detection based on nanotech-nology and nanomaterials-A review. Analytica Chimica Acta. 2019; 1069: 1-27. https://doi.org/10.1016/j.aca.2019.04.032.

3. Feizy J, Jahani M, Beigbabaei A. Graphene adsorbent-based solid-phase extraction for aflatoxins clean-up in food samples. Chromato-graphia. 2019; 82: 917-926. https://doi.org/10.1007/s10337-019-03725-w

4. Akbar N, Nasir M, Naeem N, Ahmad MD, Iqbal S, Rashid A, Imran M, Aslam GT, Atif M, Salehi B, Sharifi-Rad J, Martorell M, Cho WC. Occurrence and Seasonal Variations of Aflatoxin M_1 in Milk from Punjab, Pakistan. Toxins. 2019; 11(10): 574-589. https://doi.org/10.3390/toxins11100574.

5. Negash D. A Review of Aflatoxin: Occ-urrence, Prevention, and Gaps in Both Food and Feed Safety. Journal of Applied Microbiological Research. 2018; 1(1): 35-43.

6. Omeiza GK, Kabir J, Kwaga JKP, Kwanashie CN, Mwanza M, Ngoma L. A risk assessment study of the occurrence and distribution of aflatoxigenic *Aspergillus flavus* and aflatoxin B_1 in dairy cattle feeds in a central northern state, Nigeria. Toxicology Reports. 2018;5: 846-856. https://doi.org/10.1016/j.toxrep.2018.08.011

7. Wu L, Li J, Li Y, Li T, He Q, Tang Y, Liu H, Su Y, Yin Y, Liao P. Aflatoxin B_1, zearalenone and deoxynivalenol in feed ingredients and complete feed from different Province in China. Journal of Animal Science and Biotechnology. 2016; 7(1): 63. https://doi.org/ 10.1186/s40104-016-0122-8

8. Zakaria AM, Amin YA, Fawzy Khalil OS, Abdelhiee EY, Elkamshishi MM. Rapid detection of aflatoxin M_1 residues in market milk in Aswan Province, Egypt and effect of probiotics on its residues concentration. Journal of Advanced Veterinary and Animal Research. 2019; 6(2): 197-201. https://doi.org/10.5455/javar.2019.f332.9.

9. Liew WP, Mohd-Redzwan S. Mycotoxin: Its Impact on Gut Health and Microbiota. Frontiers in Cellular and Infection Microbiology. 2018; 8:1-17. https://doi.org/10.3389/fcimb.2018.00060

10. IARC. Monographs on the evaluation of carcinogenic risks to humans; Chemical agents and related occupations, review of human carcinogens. World Health Organization. Lyon, France. 2012; 100F: pp. 229.

11. Alshannaq A, Yu JH. Occurrence, toxicity, and analysis of major mycotoxins in food. International Journal of Environmental Research and Public Health. 2017; 14(6): 632-652. https://doi.org/10.3390/ijerph14060632.

12. Iranian National Standards Organization. Food and feed. Mycotoxins-maximum tolerated level. 2002. 1st ed. INSO No. 5925.

13. Es'haghi Z, Beheshti HR, Feizy J. Extraction of aflatoxins from food samples using graphene-based magnetic nanosorbents followed by high-performance liquid chromatography: A simple solution to overcome the problems of immunoaffinity columns. Journal of separation science. 2014; 34(18): 2566-2573. https://doi.org/10.1002/jssc.201400260.

14. Jangampalli Adi PM. Analysis of aflatoxin B_1 in contaminated feed, media, and serum samples of *Cyprinus carpio L*. by high-performance liquid chromatography. Food Quality and Safety. 2018; 2(4): 199–204.
https://doi.org/10.1093/fqsafe/fyy013.

15. Kisan B, Shruthi Nagaral K, Halepyati AS, Shankergoud I. Quantitation of AFB_1 by Spectro-photometric Method. International Journal of Current Microbiology and Applied Sciences. 2018; 7(4): 2267-2271.
https://doi.org/10.20546/ijcmas.2018.704.259.

16. Feizy J, Beheshti HR, Asadi M. A survey of aflatoxin in cotton seed in Iran by HPLC with on-line photochemical derivatisation and fluoresc-ence detection. Food Additives & Contaminants: Part B. 2012; 5(3): 200-203.
https://doi.org/10.1080/19393210.2012.696148.

17. Beheshti HR, Asadi M. Aflatoxins in animal feed in Iran. Food Additives and Contaminants: Part B. 2014; 7(1): 40-42.
https://doi.org/10.1080/19393210.2013.834485

18. Eskandari M, Pakfetrat S. Aflatoxins and heavy metals in animal feed in Iran. Food Additives and Contaminants: Part B. 2014; 7(3): 202-207.
https://doi.org/10.1080/19393210.2013.876675

19. Kotinagu K, Mohanamba T, Rathna Kumari L. Assessment of aflatoxin B_1 in livestock feed and feed ingredients by high-performance thin layer chromatography. Veterinary World. 2015; 8(12): 1396-1399.
https://doi.org/10.14202/vetw-orld.2015.1396-1399

20. Mateo EM, Gil-Serna J, Patiño B, Jiménez M. Aflatoxins and ochratoxin A in stored barley grain in Spain and impact of PCR-based strategies to assess the occurrence of afla-toxigenic and ochratoxigenic Aspergillus spp. International Journal of Food Microbiology. 2011; 149(2): 118-126.
https://doi.org/10.1016/j.ijfoodmicro.2011.06.006

21. Trucksess MW, Weaver CM, Oles CJ, Fry FS, Noonan GO, Betz JM, Rader J. Determination of aflatoxins B_1, B_2, G_1, and G_2 and ochratoxin A in ginseng and ginger by multitoxin immunoaffinity column cleanup and liquid chromatographic quantitation: collabo-rative study. Journal of AOAC International. 2008; 91(3): 511-523.