

## Aflatoxin Contamination of Feed Materials in Qom Province, Iran

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### ABSTRACT

**Background:** Aflatoxins are fungal toxins which may be present in some foods and due to their negative health effects, represent a major concern for humans and food industries. In the present study, total aflatoxin contamination in products from eight feed materials production centers located in Qom City of Iran were evaluated by an ELISA technique in November 2012 .

**Methods:** A total of 40 feed samples were analyzed for total aflatoxin. The samples were collected randomly from eight feed materials production centers (C1-C8) located in Qom city. Samples were conditioned in sterile plastic container and kept at 4 °C until analyses that were carried out in same day.

**Results:** The total average of Aflatoxins concentration in samples were 1.83µg/kg. All samples demonstrated total aflatoxin levels lower than European Union standard and National Standard of Iran recommended limits .

**Conclusion:** Considering the low values of aflatoxin contamination, maintaining vigilant preventive measures is recommended. These results do not preclude the need for continuing comprehensive studies for aflatoxin contamination .

**Keywords:** Aflatoxins, Agricultural Crops, ELISA, Iran.

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### INTRODUCTION

Aflatoxins, subset of mycotoxins, consist of a large group of extremely toxic components which are produced by certain species of fungi, specifically *Aspergillus flavus* and *Aspergillus parasiticus* [1]. These fungi contaminate a wide range of agricultural products mainly cereal grains, during pre- and post-harvest stages [2, 3] and factors, such as season, humidity, temperature, and drought in the field as well as storage conditions (i.e. temperature, relative humidity and duration), have critical roles in production of aflatoxins [4-6]. These metabolites are highly carcinogenic, mutagenic, and teratogenic components which pose serious health and economic concerns to humans; hence, they should be monitored closely in potentially hazardous food [7,8].

Among four common aflatoxins available (aflatoxin B1, B2, G1, and G2), aflatoxin B1 (AFB1) is the most prevalent and poisonous molecule and it is categorized as

group 1 human carcinogen by International Agency for Research on Cancer (IARC) [1-3]. When lactating mammals ingest feeds containing aflatoxin B1, it is converted to AFM1 by hydroxylation [9]. AFM1 is secreted in milk and subsequently enters human body through consumption of milk and other dairy products [10]. Since the feed may contain different aflatoxins, animal products, such as eggs, meat, milk, etc., will be affected by the outlined contamination. Therefore, it can be concluded that the aflatoxin inside the feed can contaminate a wide range of animal-based food .

Most of the recent investigations have focused on aflatoxin B1 and its metabolite AFM1 in animal milk and urine. This study, however, measured total aflatoxins in the animal and poultry feed in major feed materials production centers of Qom, a large province of Iran with many agricultural facilities.

There are several regulations for total aflatoxin limitation in animal feeds and feed

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ingredients in many countries. Accordingly, the Institute of Standards and Industrial Research of Iran (ISIRI) has set national legal limits at 20 µg/kg for animal feed [11].

Considering the inadequacy of information on total aflatoxin levels in animal feeds of Qom City, this study investigates its occurrence in animal feeds obtained from different feed materials production centers in the vicinity of this city.

## METHODS AND MATERIALS

### *Samples collection*

A total of 40 samples were randomly acquired from 8 feed materials production centers (C1-C8) located in Qom City, in November 2012. Samples were conditioned in sterile plastic containers and kept at 4°C until analysis that was carried out in same day.

### *Extraction procedure*

Aflatoxins were analyzed in 5 g of feed samples which was shaken with 25 ml of methanol for 3 min. The extract was filtered with filter paper (Whatman No. 1. USA).

### *Method*

Total aflatoxin content in samples was analyzed by Enzyme Linked Immunosorbent Assay method. All standards and samples were tested in duplicates. The Limit of Detection (LOD) of aflatoxin for ELISA methods was 2 ppb.

### *Procedure*

100µl of each standard solution and prepared samples in separate duplicate wells were added and mixed gently by shaking the plate and were incubated at room temperature. After 30 minutes, the liquid in the wells was poured out, and the micro-well holder was tapped upside down on an absorbent paper to remove the liquid. The wells were washed 3 times with 250 ml washing buffer. Then 100µl of the cross-reactive antibody for total aflatoxin assay (B1, B2, G1 and G2) was added to each well and mixed gently by shaking the plate manually. The contents were incubated for 10 minutes at room temperature in a dark place. After incubation, the liquid in the wells was poured out and the wells were washed three times with 250 ml washing buffer and 100 ml substrate/chromogen was

added to each well and incubated for 15 minutes at room temperature in the dark. By adding 100 ml of the stop solution to each well, absorbance was measured at 650 nm in ELISA plate reader (ELX 800, Bio-Tek Inst).

## RESULTS

Aflatoxin contamination levels are presented in Table 1. The obtained results show that among 40 samples from eight centers the total aflatoxin numbers of 19 (45%) had the rate of 0.9 to 4.2 µg/kg and 21 (55%) were completely free of aflatoxin.

**Table 1.** Aflatoxin contamination levels of feed materials from 8 feed materials production centers in Qom, Iran in November 2012.

Feed materials production center	Number of samples	Mean concentration (µg/kg)	Range
Center 1	5	0.6	<LOD -1.5
Center 2	5	1.1	<LOD -1.9
Center 3	5	1.6	<LOD -2.2
Center 4	5	1.7	<LOD -2.3
Center 5	5	2.1	1.3-2.8
Center 6	5	2.1	<LOD -3.2
Center 7	5	2.5	1.8-3.3
Center 8	5	3	0.9-4.2
Total	40	1.83	<LOD -4.2

## DISCUSSION

Contamination of food and feed mycotoxins, especially aflatoxins, is one of the main concerns of food safety researchers. Since feed is a suitable milieu for the growth of aflatoxin-producing fungi, it can be easily contaminated in poor hygiene conditions. Patrick B Njobeh et al. found that feed samples in South Africa were contaminated with AFs (30% of samples) in the range of 0.2 – 71.8 µg/kg [12]. Oruç, H et al. reported 100% feed material contamination with AFB1, with an average level of 8.29±2.19 µg/kg in Turkey [13]. In a study in Poland performed by J. Grajewski et al., animal feeds were less than 7% contaminated with AFs with a maximum level of 0.61 ng/g [14]. Ahsan et al. reported that the percentage of aflatoxin contamination in maize samples were 80%, 87%, and 90% with their respective mean values of 45 µg kg<sup>-1</sup>, 54µg

kg-1 and 62 $\mu$ g kg-1 in urban, semi-urban, and rural areas, respectively [15].

In the present study, ELISA method was used for determination of total aflatoxins in animal feeds used in the Qom city. ELISA analysis is suitable for determination of contaminants in a large number of samples over a short period of time [16]. There were some differences between the obtained results and the available results in the literature and that is because of the wide variety of factors involved in animal feeds production; therefore, these differences were expected. Based on the obtained results, the production and storage of animal feeds in Qom were acceptable and maintaining careful and adequate monitoring is recommended.

### CONCLUSION

Our result showed that due to the implementation of proper maintenance procedures of animal feeds, regarding feed safety regulations and regular and meticulous inspections, aflatoxin contamination of animal feeds in Qom was present in 45% of samples with concentrations of less than the maximum acceptable level. These results do not preclude the need for continuing comprehensive studies for aflatoxin contamination.

### CONFLICT OF INTEREST

The authors declare that there were no conflicts of interest.

### ACKNOWLEDGMENTS

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