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Occurrence of Aflatoxins in poultry feed and feed ingredients from northwestern Iran

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ABSTRACT

Eighty samples of poultry feed and feed ingredients were tested for total aflatoxin B (AFBs). The studied samples were included three broiler starter, broiler grower and broiler finisher feed and four feed ingredients including maize, wheat grain, and wheat bran and soybean meal. All samples were collected from poultry farms and poultry feed plants from northwestern region of Iran, during the years 2011 and 2012. Enzyme-Linked Immuno Sorbent Assay (ELISA) was used for determination of AFBs in the samples. AFBs were detected in all of tested feed and feed ingredients and ranged from 1.0 to 40 µg/kg as average value of 5.5 µg/kg. Average concentrations of AFBs were 5.65, 11.09 and 20.72 µg/kg in broiler starter, broiler grower and broiler finisher feed, respectively. The mean value of AFBs in maize, wheat grain, and wheat bran and soybean meal was 2.35, 1.54, 3.05 and 6.01 µg/kg, respectively. In 27 percent of positive sample, the aflatoxin contamination level was higher than the legal limit imposed in many countries and European Union. The results of this study showed the high incidence of aflatoxin contamination in all feed and feed ingredient samples and high contamination level of AFBs in poultry feed. The results warrants the need for surveillance and constant monitoring programs for the prevention of the sanitary impacts of these substances and reveal consumption of contaminated feed in poultry farms poses a risk of aflatoxicosis and may put human at risk of aflatoxin exposure

Key words: Aflatoxin, Feed, Enzyme linked immuno sorbent assay

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INTRODUCTION

Mycotoxins are natural food and feed toxic contaminants, secondary metabolites produced by fungi on agricultural commodities. More than 500 different mycotoxins are known. Aflatoxins, a class of mycotoxins produced by the fungi *Aspergillus parasiticus* and *Aspergillus flavus*, are major contaminants of common feed ingredients used in poultry rations [1]. These cosmopolitan fungi are found in many countries, especially in tropical and subtropical regions, where the temperature and humidity conditions are optimal for the growth of moulds and the production of toxins [2]. Contamination by Aflatoxin can take place at any point along the food/feed chain from the field, harvest, handling, shipment and storage under a wide range of climatic conditions [3]. Aflatoxins have been found to contaminate a wide variety of important agricultural products world-wide, e.g., maize, wheat, oil seeds meal, rice, spices, dried fruits, and nuts. Ingestion of Aflatoxin in contaminated feeds affects animal health and production, and is potentially dangerous to humans as the toxic metabolites are excreted in animal meat, milk and eggs [4].When aflatoxin-contaminated feed is consumed by poultry, important production parameters including weight gain, feed intake, feed conversion efficiency, and reproductive performance are compromised [5]. Aflatoxicosis in poultry and

animal also causes changes in biochemical and hematological parameters liver and kidney abnormalities, and impaired immunity, which may enhance susceptibility to infectious diseases [6].

Mycotoxins are not only dangerous for the health of consumers, they also deteriorate the marketing quality of the contaminated products; thus, involving strong economic losses. Mycotoxicological control of feed is a procedure aiming to protect human and animal health, avoid the adverse effects of these undesirable substances. Different countries have imposed different legal limits on various food items and animal feeds. The aflatoxin level in animal feeds is generally higher than for human consumption [7]. US- Food and Drug Administration (FDA) and Institute of Standard and Industrial Research of Iran (ISIRI) have set the maximum tolerable levels (MTL) for AFB1 and AFBs in cereals for human consumption as 2 and 4 µg/kg, respectively and recommend 20 µg/kg AFBs as the worldwide range of MTL /permissible levels for poultry [8, 9].

In Iran, there has been no comprehensive study regarding contamination of foods and feeds with mycotoxins [10]. Only a few studies have been conducted on the contamination of feed commodities with aflatoxin. For example in one of the studies which conducted in south of Iran and on the raw materials of poultry feed, the obtained results showed all samples of fish meal, soybean meal, wheat and maize were contaminated with aflatoxin B_1 100%, 92%, 88%, and 16%, respectively. It was concluded that the aflatoxin B_1 level found in some poultry feed ingredients were above the maximum permissible levels [11].These researchers conducted another study on 75 poultry feed samples and showed that all of the samples were below the action level for AFB1 [12]. In this regard, unpublished clinical data according to veterinary practices indicate relatively high occurrence of mycotocsicoses in broiler production farms located in East Azarbaijan province of Iran. Based on uncertainties which have been shown in previous reports and the huge concerns of practitioners in animals and poultry, further investigations on feed ingredients and products are required to keep our food and feed fairly safe. Thus, the current study aimed to survey the contamination of poultry feed and feed ingredients with aflatoxin in the North West of Iran.

MATERIAL AND METHODS

Sample collection

A total of 80 samples of poultry feed and feed ingredients were collected from local poultry farms and 15 poultry feed factories in East Azarbaijan, West Azarbaijan and Ardabil provinces of Iran during the years 2011 and 2012. The samples included of 20 maize and 13 wheat grain, 8 wheat bran, 19 soybean meal and 20 poultry feed designed for broiler feeding. The feed samples represented the following three poultry feed categories: broilers-starter feed: 7 samples, broilers-grower feed: 8 samples and broilers-finisher feed: 5 samples. In order to achieve reasonably representative samples, primary large samples of approximately 10 kg were composed of several samples collected from different parts of storage lots. The samples were homogenized and quartered to obtain a 1 kg of laboratory sample. All collected samples were stored at 4°C prior analysis and thoroughly ground for analysis.

Aflatoxin determination

The AFBs was analysed using the Enzyme Linked Immunesorbent Assay (ELISA) method. A Euroclone® competitive enzyme immunoassay kit, based on antigen–antibody reaction, was used for the quantitative analysis of total aflatoxin. Aflatoxin extraction and determination were performed according to the Euroclone manufacturer's procedure. All feed samples were ground in a laboratory mill (MS10Basic IKA®, Germany) to pass 1mm sieve. About 5 g of the ground sample was extracted by 25 mL of methanol 33%. The extract was filtered through a Whatman filter (No 1), and 1 mL from the filtered sample was then diluted by 1 mL of methanol 33%. About 50 µL of diluted filtrate per well was used for the ELISA test. The optical density was measured at 450 nm using an ELISA 96-well plate reader. Absorbance percentages were taken to the calibration curve performed with standards at different concentrations. The evaluation of ELISA data and the aflatoxin concentrations for samples were performed using the software program R-biopharm (Ridasoft win, version 1.78, R-biopharm, Germany). Total Aflatoxin kit called Euroclone® with LOD = 0.125 ppb, Intrassay CV<10%, and Interassay CV<20% was used to measure total aflatoxins.

RESULTS

The present study has provided information about the AFBs contamination of poultry feeds and feed ingredients in North West part of Iran. The unknown values of AFBs for concentration in samples are determined from a calibration curve. The plot of correlation of % absorbance (%ABS) and concentration of AFBs showed polynomial correlation (%ABS =-15.94 X + 111.24 with R2 = 0.98) (Fig. 1).Table 1 shows the contamination of the commodities tested, the number of samples, the number and percentage of positives, the mean of positives and the contamination values range concentrations per commodity. Of the 80 feed samples that were tested with the competitive ELISA technique, all of them were found to be contaminated with AFBs at range of 1.0 - 40 µg/kg. The

levels of AFBs in some of these samples were exceeded from the legal limits of 20 µg/kg (Table 2). In the present study, the incidence of AFBs contamination in wheat, wheat bran and yellow maize was very low, with mean concentrations of 1.54, 3.05, and 2.35 µg/kg, respectively. Whereas, in soybean meal samples; aflatoxin contamination was 6.01 µg/kg as average. Of a total of 60 feed stuffs samples analysed, only one was contaminated with AFBs at 20.23 µg/kg. Generally, high incidences of aflatoxins were found in poultry feed stuffs, but the mean concentrations were low and less than 5µg/kg.

The highest concentration of AFBs was detected in broiler feed samples with a mean level of 11.6 µg/kg. Five of 20 feed samples (25%) were contaminated with AFBs above the acceptable limits for poultry consumption. The range of contamination in these samples was between 1.28 and 40 µg/kg. AFBs contamination for broiler starter, broiler grower and broiler finisher diet were determined with the average values of 5.65, 11.09 and 20.72 µg/kg, respectively.

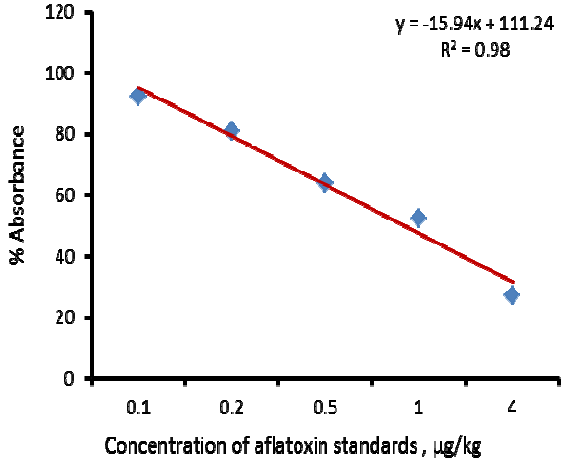

Figure 1: Typical calibration curve of AFBs by competitive ELISA at 450 nm.

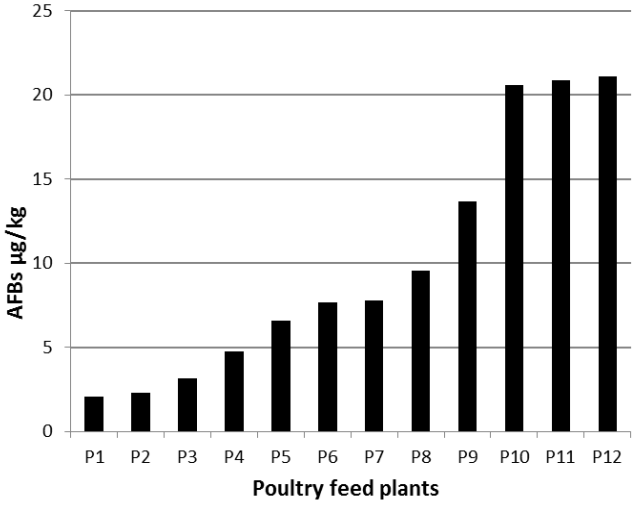

Figure 2: The AFBs contamination of poultry feed in different feed factory.

The mean of AFBs contamination levels in commercial poultry feed produced by 12 different poultry feed plants located in East Azarbaijan, Ardabil and West Azarbaijan provinces were 2.08, 2.31, 3.165, 4.77, 6.58, 7.68, 7.79, 9.55, 13.68, 20.85,20.87 and 21.1 µg/kg, respectively (Figure 1). In general, commercial feed produced by poultry feed plants, three of them were beyond the limit of ISIRI regulations. Also, results showed that in 8 feed factories (67%) the AFBs concentrations were less than 10 µg/kg and in 4 factories AFBs concentration was found more than 10 µg/kg.

Table 1. Occurrence of aflatoxins in poultry feed and feed ingredients from North West of Iran sampled in 2011-2012.

Commodity	Total samples analyzed (n)	Percent of positive samples	Aflatoxin(µg/kg)	
			Contamination values range	Mean level
all commodities	80	100	1.0-40.0	4.90
Feed ingredients	60	100	1.0-20.32	3.24
Feeds	20	100	1.28-40	11.60
Feed ingredients:				
Wheat	13	100	1.0-2.86	1.54
Wheat bran	8	100	1.0-4.61	3.05
Yellow maize	20	100	1.0-8.77	2.35
Soybean meal	19	100	3.25-20.32	6.01
Feeds:				
Broiler starter	7	100	1.28-19.32	5.65
Broiler grower	8	100	1.74-40.0	11.09
Broiler finisher	5	100	2.12-40.0	20.72

Table 2. Distribution of total aflatoxin B in feed and feed ingredients samples.

Range of AFBs concentration (µgkg^{-1})	Number of samples	Percentage
all commodities		
10-20	4	5.00
<20	75	93.75
>20	6	7.50
Feeds		
10-20	2	10.00
<20	15	75.00
>20	5	25.00
Feed ingredients		
10-20	1	1.67
<20	59	98.33
>20	1	1.67

DISCUSSION

Occurrence of aflatoxin in poultry feed and feed ingredients are a worldwide concern since they reduce poultry performance and could be important vehicles for introducing aflatoxins residues into the human diet [13]. Consequently, many countries have regulated the maximum permissible levels of AFBs in food and feed products to reduce this hazard. These regulations vary in different countries by the fact of economic considerations [14] and ISIRI regulate 20 µg/kg as the range of AFBs for poultry feed MTL [8, 9]. According to the results of present study, the AFBs mean level in maize, wheat grain and wheat bran was lesser and in soybean meal higher than the legal limits of 20 µg/kg as imposed in many countries (e.g., USA, Iran, Austria, India, Argentina, the Netherlands and Brazil) [15]. Similar to our results, Mayahi and Sohrabi (2001) reported low levels of AFB1 at level 4, 2 and 3 µg/kg respectively from the poultry feed ingredients samples comprising wheat grain, maize and soybean meal in Ahvaz, Iran [11]. However, contrary to our study Yazdanpanah et al. [10] in 2001 in their analysis of AFB1 in fourteen barley and nine corn samples as feed stuffs from the Golestan and Mazandaran provinces in the north of Iran reported that 89% samples of maize were contaminated at a mean level of 15.83 ng/g above the Iranian MTL of AFB1 in corn for animal feed (5 µg/kg) [9]. In another study on animal feed stuffs samples collected from the Fars province Iran, 43.36% of the feed stuff samples were found to be contaminated with aflatoxin B1, which exceed the permissible concentration of AFB1 [16]. Several factors such as climatic conditions [17] and storage conditions [15], like high temperature and humidity are optimal for the growth of moulds and affect fungal colonization and mycotoxin production in grains and compound feeds [2]. Therefore relatively warm and humid conditions in Golestan and Mazandaran provinces and high temperature in Fars province may provide suitable conditions for the fungal growth ultimately resulting in increased production of aflatoxin in feed stuff.

Based on our results the highest level of AFBs with range 1 to 40 µg/kg was found in the poultry feed particularly in finished broiler feed. These data are remarkably similar to those reported previously [18]. High levels of AFB1 with a range 10 to 166 µg/kg and mean 47.64 µg/kg has been reported in poultry finished feed samples from Pakistan. Also Anjum et al., [19] reported high levels of AFB1 in commercial poultry feed ingredients collected from Pakistan. In the present study, 27 percent of the positive samples contained higher mounts of aflatoxin compare to the legal limit imposed for aflatoxin in many countries. However, in another study low levels of aflatoxin have been detected in 75 Iranian poultry feed samples subjected to toxicological evaluation of aflatoxin [12]. In Brazil, aflatoxins were detected in 88.2% of the 34 broiler feed samples by indirect competitive ELISA and HPLC at means of 10.48 µg/kg and 8.41 µg/kg, respectively, while 92% of laying hen feed samples (n = 36) showed aflatoxin contamination at means of 20.83 and 19.75 µg/kg [20]. Zinedine et al. [21] studied a few poultry feed samples (n = 21) from Morocco and observed that the percentage of contamination by aflatoxin is about 66.6%, while the contamination levels of poultry feeds samples ranged between 0.05 and 5.38 µg/kg for AFB1.

The presence of fungi and mycotoxin in feeds, result from the feed ingredients and raw materials used in their production [22]. Mycological surveys on the presence of the toxigenic and non-toxigenic fungi in Iranian animal feeds have shown that [23] several toxigenic (67 %) and non-toxigenic fungal groups (33%) were present with , *Aspergillus* (56%) as the predominant genus followed by Mucor (17%), *Penicillium* (15%), *Fusarium* (6%), *Cladosporium* (2%) and yeast (4%). In Spain, samples of poultry feeds were reported to be contaminated with *A. flavus* and *A. parasiticus* that produce AFB1 and AFB2 [24]. The high level of aflatoxin contamination in feed samples was not unexpected as this rations are manufactured using ingredients that are stored in bulk and for long times under conditions that are favour for development of toxigenic fungi.

As previously reported, the level of mycotoxin contamination in feed and food grains varies among commodities, years and regions [25, 26]. The current prevalence of AFBs mean level contamination in the feed reported in the

present investigation is lower than that reported in Middle East and African countries (42%) at means of 24 µg/kg [27] and Thailand (92%) with an average concentration of 7.56 µg/kg. In 2007, Binder et al. [28] reported the occurrence of mycotoxins in 30% of samples from Asian-Pacific countries and 50% in European and Mediterranean samples including Iran and concluded that the incidence of mycotoxins relevant for animal production is quite high in animal feed. Surveys from other countries have reported the occurrence of aflatoxins from feed in Vietnam [29], Kuwait [30], Greece, Turkey [31, 32].

CONCLUSION

In conclusion, the present study provides information about the natural occurrence of aflatoxins in feed and feed ingredients in North West region of Iran. Eighty animal feed and feed ingredients, comprising maize, wheat, wheat bran, soybean meal and broiler feed were analysed for the content of AFBs. Twenty seven percent of samples were found to contain aflatoxin higher than 20 µg/kg, exceeding the legal limit imposed in many countries. The results warrants the need for surveillance and constant monitoring programs for the prevention of the sanitary impacts of these substances and reveal consumption of contaminated feed in poultry farms poses a risk of aflatoxicosis and may put human at risk of aflatoxin exposure.

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