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Occurrence of Aflatoxin B1 in Poultry Feed and Feed Ingredients in Jordan Using ELISA and HPLC

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Abstract: A total of 157 samples representing 105 samples of regular poultry feed ingredients and 52 samples of feed composites were collected from various parts of Jordan and analyzed for aflatoxins B1 (AFB1). Incidences of contamination with AFB1 of feed samples were 40% and 23.07% for Enzyme-Linked Iimmunosorbent Assay (ELISA) and High-Performance Liquid Chromatography (HPLC), respectively. The minimum and maximum were 3.23 and 39.41 ppb, by ELISA test. The HPLC result was 1.10 for the minimum and 14.05 ppb for the maximum. Storage temperature and humidity were the key factors in corn and fish meal contamination with AFB1. HPLC and ELISA results of both techniques were almost similar in indicating that poultry feed contaminated with aflatoxins. The HPLC results were lower of about 0.3%.

Key words: Aflatoxins B1 · Poultry Feed · ELISA · HPLC.

INTRODUCTION

Aflatoxins are a group of toxic metabolites produced mainly by some common fungi; such as *Aspergillus flavus* and *Aspergillus parasiticus* species. [1, 2] at optimal environmental temperature and humidity [3, 4]. These microorganisms produce aflatoxins B1, B2, G1 and G2 the four naturally occurring forms of aflatoxins with AFB1 being the most potent carcinogenic [5]. AFB1 is the widespread contaminants occurred in food and poultry feed with particular spread in the tropical and subtropical countries.[6]. Poultry is considered as one of the most susceptible animal species to aflatoxins [7, 8]. This susceptibility coupled with its feed, being an important source of aflatoxins, makes its study very vital and important, [9]. Furthermore, it was reported to cause human liver damage and carcinogenicity [10]. Aflatoxins detrimental effects are due to its binding to nucleic acids, which impair protein formation in the body [11]. Thus, it may cause organ damage and / or cancer as a result of prolonged exposure that leads to adverse effects on animal health and productivity, particularly on animals reared under intensive production systems; such as poultry [12]. Hassan *et al*. [13] observed severe depression in growth rate and immune suppression in broiler chickens due to aflatoxicosis. Natural toxins produced by fungus have threatened the quality and safety of food and have caused severe losses to poultry industry in recent years [14].Prevalence of aflatoxins in poultry feeds is regularly and frequently reported in many countries including Brazil [15], India [16], Malaysia [17], Pakistan [18]. Also a number of aflatoxins have been detected in commercial poultry feed and feed ingredients in Jordan, Annual Report [19]. Therefore, the European Union [20] has imposed a 20 ug/Kg as a legal limit for aflatoxin B1 in animal feeds. Traditionally, Thin-Layer Chromatography (TLC) and High-Performance Liquid Chromatography (HPLC) methods have been used for assessment of aflatoxins in feedstuff, [21]. Presently simple, rapid and specific immunoassays methods are more used, such as ELISA [22]. ELISA tests are based on the affinities of the monoclonal or polyclonal antibodies for aflatoxins and it is possible to detect aflatoxin B1 in foods and feedstuffs, specially and quickly with very little sample preparation, [23, 24].

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In Jordan, research concerning aflatoxins in poultry feed and feed ingredients is insufficient and needs further assessments. Additionally, the number of new cancer cases reported in Jordan was 6820 compared to 4341 cases in 2007 [25]. Therefore, this investigation was planned to evaluate the occurrence of aflatoxins in poultry feed and feed ingredients using ELISA as well as comparing the results obtained with HPLC and ELISA techniques.

MATERIALS AND METHODS

Sample Collection: A total of 157 feed samples representing 105 samples of feed ingredients and 52 samples of compound feeds collected from commercial sources (starter, grower, finisher of broiler and layers) were collected from different regions representing Jordan governorate. A duplicate of 1 Kg samples stored under cool and dry conditions were used for detection of aflatoxins.

Sample Preparation for ELISA Analysis: 100-200 g from poultry feeds were ground and sieved through a sieve of 1-mm opening using Poltron miller (Kinematica, Lucerne, Switzerland).. The prepared samples were tested using microplate enzyme-linked immunosorbent assay (ELISA) quantitative test kits (Ridascreen, r-biofarm, Germany) and ELISA reader (Expert plus, Switzerland). The quantitative analyses for aflatoxin AFB1 (Sigma Chemical. St. Louis, MO, USA).

Levels were determined in the prepared samples. The samples were analyzed using the AFB1 test procedure (Art. No.; R4701), which is described by r-biofarm test procedure kit producing company (Enzayme Immunoassay for the quantitative Analysis of Aflatoxins [26].

Aflatoxins Working Standards: Six working standard dilutions of 0, 0.5, 1.5, 4.5, 13.5 and 40.5 µg/L (ppb) were provided with ELISA kit. Aflatoxin was used and immunoassayed in triplicate. Also, a zero standard was methanol diluted in sample buffer (1+9) and assayed in the presence of the enzyme conjugate. Blank wells were the same as zero standards, but assayed without the enzyme conjugate.

Test Protocol: A 2-g of the ground powdered feed samples was mixed in a screw cap glass vial with 10 ml methanol/distilled water (70/30) mixture and mixed for 30 min at room temperature (20-25 ºC/ 68-77 ºF) using a shaker (IKA, Germany). The mixture was filtered using a filter paper (Whatman No. 1, Germany). A 100- µl of the eluate was diluted with 600- µl (1+6) of the sample dilution Buffer (phosphate buffer solution, pH 7.2).

Recovery of Aflatoxins by ELISA: The standard curves were linear with correlation coefficient of 0.998. Mean coefficient of variation within-day and between-day analysis was 1.56% for AFB1 aflatoxin kit. Further calculations for quantitative determinations were followed according to the instruction given in the kit manual [27].

Aflatoxins Recoveries and HPLC Determination: The HPLC analysis was carried out using Water HPLC equipped with Water 1525 binary HPLC pump, column oven 5CH (Waters Co., MA, USA). The column used was 250 x 4.6 mm Thermo LC-Si. The recoveries of aflatoxins were prepared and analyzed as indicated by Herzallah [28] and found linear with 0.998 correlations.

Statistical Analysis: The data obtained with ELISA and HPLC method were processed and compared using LSD for comparing differences among different feed ingredient samples using PC-SAS software version 9.0 [29] with significant differences when $P < 0.05$.

RESULTS AND DISCUSSION

The results of aflatoxins contamination of feed ingredients and finisher are presented in Table 1 stating the total number of each commodity tested and the number of feed samples contaminated with aflatoxins. A total of 105 different feed samples were received and analyzed. Overall, incidence of AFB1 in feed ingredients samples was 19.04 % (20 samples) with an average concentration of 5.84 ppb in rice and 31.26 ppb in fish meal tested by ELISA compared to HPLC values of AFB1 were 3.18 ppb in rice and bone meal and 17.06 ppb in fish meal.

A total of 25 broiler starter feed samples of unknown compositions were analyzed with 24% (6 samples) detected as positive in ELISA and HPLC. The minimum and maximum concentration observed were 4.79 and 19.7 ppb, respectively using ELISA. While in HPLC the minimum and maximum concentration observed were 2.48 and 12.7 ppb, respectively (Table 2).

Table 1: ELISA and HPLC results for different feed ingredients*

Feed ingredient	No of Positive	No of Negative	Total Number of sample tested	ELISA**(ppb)	Minimum (ppb)	Maximum (ppb)	HPLC (ppb)	Minimum (ppb)	Maximum (ppb)
Barely	2	13	15	9.74±3.03b	4.24	15.23	5.58±2.68bc	3.11	8.05
Bone meal	1	14	15	7.25±4.29b	7.25	7.25	3.18±3.79bc	3.18	3.18
Fish meal	5	10	15	31.26±1.92a	24.17	39.41	17.06±1.69a	11.22	14.05
Rice	2	13	15	5.84±3.03b	3.23	8.45	3.18±2.68c	1.10	5.25
Soya bean	1	14	15	9.42±4.29b	9.42	9.42	5.55±3.79bc	5.55	5.55
Wheat bran	3	12	15	13.88±2.48b	12.80	15.71	11.87±2.19b	10.25	13.17
Corn	6	9	15	9.30±1.75b	7.14	11.45	5.35±1.55c	4.22	7.05
Total	20	85	105						

*Values are means (± SD) of four replicates

** Means in a column with different superscript letters indicate a significant difference between feed samples

Table 2: ELISA and HPLC results for different ration types*

Broiler Ration type	No of Positive	No of Negative	Total Number of sample tested	ELISA**(ppb)	Minimum (ppb)	Maximum (ppb)	HPLC (ppb)	Minimum (ppb)	Maximum (ppb)
Starter	6	19	25	19.65±6.71a	4.79	19.70	12.84±5.42a	2.48	12.70
Grower	2	12	14	14.80±11.62c	6.42	13.17	10.15±9.39a	5.20	10.10
Finisher	4	9	13	16.38±8.22b	4.02	15.12	11.30±6.64a	3.14	11.80
Total	12	40	52						

*Values are means (± SD) of four replicates.

** Means within a column with different superscript letters indicate a significant difference between broiler ration types

Fourteen samples of broiler grower were analyzed with 2 samples representing 14.28% detected as positive in both ELISA and HPLC. The minimum and maximum concentration observed were 6.42 and 13.17 ppb, respectively by ELISA, compared to the minimum and maximum concentration of 5.2 and 10.10, respectively measured by HPLC. Finisher broiler feed samples were evaluated by testing 13 samples by ELISA showed 30.76% detected as positive (4 samples) with minimum and maximum concentration of 4.02 and 15.12 ppb, respectively, compared to 3.14 and 11.8 ppb, respectively, by HPLC.

Corn was the most commonly used feed ingredient accounting incidence of AFB1 was 40% (6 out of 15 samples) of all analyzed samples with ELISA, the average contamination levels of minimum and maximum were 7.14–11.45 ppb in ELISA compared to the minimum and the maximum of 4.22 and 7.05 ppb by HPLC, respectively.

Wheat bran and fish meal showed significantly the highest concentration of aflatoxin B1 among all feed ingredients using ELISA and HPLC method. Other ingredients were not significant differences. However, using HPLC method fish meal and wheat bran significantly had the highest concentration of AFB1, whereas, no significant differences between wheat bran and barely, bone meal and soya bean. The rice, soya bean and bone meal had the lowest AFB1 concentration among all feed samples tested. The three ration types screened for AFB1 were showed insignificant deference among each other; however, starter had numerically the highest concentration value of AFB1. The occurrence of AFB1 in different poultry feed ingredient is presented in Table 1. Out of total 105 different poultry feed ingredient analyzed by ELISA an overall incidence of ~19% of AFB1 was observed with average minimum and maximum concentration levels of 3.23 and 39.41 ppb, respectively. While in HPLC results the average of minimum and maximum concentrate level 1.10 and 14.05 ppb, respectively.

However, occurrence and concentration of aflatoxin B1 was observed to be relatively low in HPLC compared to ELISA methods. A 52 different poultry feed, tested by ELISA, an incidence of aflatoxin B1 was found 23.07% (Table 2) with an average of minimum and maximum concentration levels of 4.02 -19.70 ppb, respectively. The incidence of AFB_1 measured by ELISA was found lower in feed ingredients (~ 19 %) than in feed ration samples (~ 23%). It was observed that the higher incidence was found in finisher of about 30.7% compared to about 14% in grower ration.

The total aflatoxins in feed ingredient samples measured by HPLC were ranged between 1.1 and 14.05 ppb with Some of these samples contain afflatoxins that exceed the legal limits of 20 ppb as imposed in many countries (e.g. USA, Austria, India and Brazil. Moss [30].

The incidence of aflatoxins in corn and fish meal were 40% and 33 % respectively, this clearly indicated that better storage and processing practices must be in place in the preparation of these commodities. The traditional method of drying these items on the ground in the open air in poor storage conditions promotes the growth of moulds and production of mycotoxins. Surveys from other countries have reported the occurrence of afflatoxins in corn and related products from Turkey [31,32] and the contamination of animal feeds has been reported in Greece and Turkey [33].

A total of 25 samples of broiler starter of unknown composition were analyzed with 24% detected as positive, concentration. A total of 14 samples of broiler grower were analyzed with 2 (14.28%) detected as positive, the minimum and maximum concentration was observed as 6.42 and 13.17 ppb, respectively. It has been estimated that 25% of the world's crops may be contaminated with mycotoxins and the world wide contamination of foods and feeds with mycotoxins still is a significant problem encountered in both industries [34].

In this study a survey has been carried on the natural occurrence of aflatoxins in commercially available broiler feeds. The results showed that aflatoxin B1 was measured in 157 samples, 32 samples were positive (~ 20%).

It was observed that variations in the levels of AFB1 in poultry feeds and ingredients were due to marked fluctuations in the environmental temperature and humidity conditions during sample collections. In conclusion, the use of ELISA in AFB1 determination is considered competent to HPLC and even achievable in terms of cost and speed of analysis.

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