Detection of broiler feeds contamination with Aflatoxins using rapid immunochromatographic test strips

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Abstract

Twenty pellet broiler feed samples (started or finished) were collected through October to December 2013, from 12 broiler flocks and 8 feed mills in Nineveh governorate, for the detection of Aflatoxins residue using total Aflatoxins rapid test strips kit. Results show that 3 (15%) of the feed samples were positive while 17 feed samples (85%) were negative to residual Aflatoxin.

Keywords: Aflatoxins, Rapid, Immunochromatographic
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الكشف عن التلوث بسموم الافلا في علائق فروج اللحم باستخدام اختبار الاشرطة المناعية السريعة

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الخلاصة

تم جمع عشرين من عينات اعلاف فروج اللحم الاصبعية (بادئة او ناهية)، في الفترة خلال أكتوبر- ديسمبر عام ٢٠١٣ (١٢) من قطعان فروج اللحم و (٨) من مصانع الأعلاف في محافظة نينوى، للكشف عن بقايا سموم الافلا باستخدام عدة شرائط اختبار سموم الافلاتوكسنات السريع. اظهرت النتائج ان ٣ (١٥%) من عينات الاعلاف كانت موجبة في حين كانت ١٧ من عينات الاعلاف (٨٥%) سلبية لبقايا سموم الافلاتوكسين.

Introduction

Aflatoxins (AF) are mycotoxins produced by Aspergillus flavus and Aspergillus parasiticus and are listed as Group I carcinogens by the International Agency for Research on Cancer (IARC), a body of the World Health Organization. The main aflatoxins are the B_1 (AFB_1), B_2, G_1 and G_2 together with their metabolites, among which the most important is the aflatoxin B_1 (1). Aflatoxins frequently contaminate cereal crops, produced by members of Aspergillus are common and widespread in nature, colonizing and contaminate grain before harvest or during storage, following prolonged exposure to a high humidity environment or damage from stressful conditions such as drought, a condition which lowers the barrier to entry (2).

For the detection of Aflatoxins contamination in feeds, a survey in Asia during 2009-2010 was carried out, in which a total of 1470 maize samples were analyzed using High performance liquid chromatography (HPLC) and Enzyme linked immunosorbent assay (ELISA), a percentage of the positive samples was ranged from 10 to 81 % with an average of 15 to 122 µg/kg (3). In Europe, and within the same period a total of 153 feedstuffs samples were analyzed for the same purpose, Aflatoxins percentages were found to be between 0 and 10 % with an average of 0 to 5 µg/kg (4). Biomin company in an attempt to provide customers insights about the contamination with Aflatoxins in feedstuffs, accomplished a survey in 3685 between 2009-2010 feedstuffs and finished feed samples all over the world, including Asian-pasfic, Europ, Middle-East, Africa and America, reviled that 31% of the feedstuffs and 44% for finished feed samples were positive respectively (5). Biomin in another survey in Asia during 2009-2011 was performed for the detection of Aflatoxin contamination on

7049 feed samples showed that 44% were positive with an average of 71 µg/kg (6).

Another worldwide Aflatoxin survey in feed materials carried out by Karin (7), of 4720 feed samples between 2010 to 2011 showed that 27% of the samples were positive with an average of 14 µg/kg. A survey in Americans (North and south) through 2009-2010 for the detection of feedstuffs contamination with Aflatoxins in 151 samples in North America and 420 samples in South America showed that 23 and 71% were positive with an average of 42 and 7 µg/kg respectively (8).

Analysis of Aflatoxin by chromatography by Traditional Aflatoxin analysis methods are based on some form of chemical chromatography. These technologies, which include thin-layer chromatography (TLC) (9), high-performance liquid chromatography (HPLC), gas chromatography (GC) (10), rapid-test kits (11), Membrane based immunoassay, Flow-through assay, Lateral flow test, Fluorometric assay (12), Immunochemical Methods (Radioimmunoassay (RIA): Enzyme-linked immunosorbent assay (ELISA), Immunoaffinity column assay (ICA) (13,14,15). Solid-phase extraction (SPE) column clean-up, Fluorescent polarization, Biosensors and nanotechnology (16). Other emerging technologies that are not yet commercially available for Aflatoxins analysis include: Evanescent wave technology, Molecular imprinted polymers, Microarray technology (17).

User-friendly, very rapid, have long-term stability over a wide range of climates, and are particularly suitable for testing for mycotoxins in the field is immunochromatographic test. However, the technology can only provide semi-quantitative results; for any positive samples, the exact mycotoxin concentration would require confirmation by a reference method such as HPLC. With the purpose of increasing knowledge of the worldwide presence and concentration of Aflatoxins on a variety of commodities and finished feed, a survey has been initiated**.**

Materials and methods

Sampling and Sample Preparation for Aflatoxin analysis

Twenty pellet feed samples were collected through October to December 2013, from 12 broiler flocks and 8 feed mills in Nineveh governorate. Samples were collected from the moving stream of feed in broiler farms or feed mills (4.5 kg) for each sample. Either started or finished feed samples were mixed well to form a composite sample and tested for residual Aflatoxin using total Aflatoxins rapid immunochromatographic strip test kit (Shenzhen Lvshiyuan Biotechnology Co., Ltd.Version:2012-2,China).

Immunochromatography strip test

A typical immunochromatography strip test is composed of a sample pad, a conjugate pad, a membrane, an absorbent pad and an adhesive backing. The competitive reaction scheme is used most often when testing for small molecules with single antigenic determinants such as mycotoxins. A sample extract is added onto the sample pad. Any mycotoxin present binds to the anti-mycotoxin antibody gold particle complex in the conjugate pad and they migrate together with the anti-2nd antibody gold particle complex along the membrane. The membrane contains a test zone and a control zone, onto which a mycotoxin-protein conjugate and a 2nd antibody are dried, respectively. The mycotoxin protein conjugate in the test zone can capture any free anti-mycotoxin antibody gold particle complex, allowing color particles to concentrate and form a visible line. Hence, a positive sample with a mycotoxin concentration greater than or equal to the assay cut-off level will result in no visible line in the test zone. Conversely, a negative sample with a mycotoxin concentration less than the cut-off level will form a visible line in the test zone. The control zone will always be visible regardless of the presence or absence of mycotoxin because the 2nd antibody always captures the anti-2nd antibody gold particle complex indicating the validity of the performed test (18).

Feed samples preparation

After grinding of pellet feed samples, a representative 3 gms were taken in centrifuge tube, adding to it 10 ml Acetonitrile. Samples were violently shacked using vortex for 2 minutes, then centrifuged at 4000r/min at room temperature for 10 minutes. Six ml of the supernatant were transferred into another centrifuge tube and dried at 56°C. After drying, 0.3 ml double distilled water and 1ml n-hexane were added to dissolve dry residue, mixed for 30 seconds and centrifuged at 4000 r/min at room temperature for 5 minutes. After the up-layer solution was removed, then the down-layer solution was used for test.

Aflatoxin analysis

Twenty strips were taken flatly, 6o µl using micropipettes were used. The results were viewed and red after 3-5 min.

Test result interpretation

Negative: Red T line appears, which means there is no Aflatoxins residue in sample or the content is lower than 3ppb. Positive: Red T line is invisible, which means the content of Aflatoxins in sample is higher than 3ppb. Invalidation: C line isn't seen wine red, which means the test card is invalid, out of date or operating error (Figure 1 and 2).

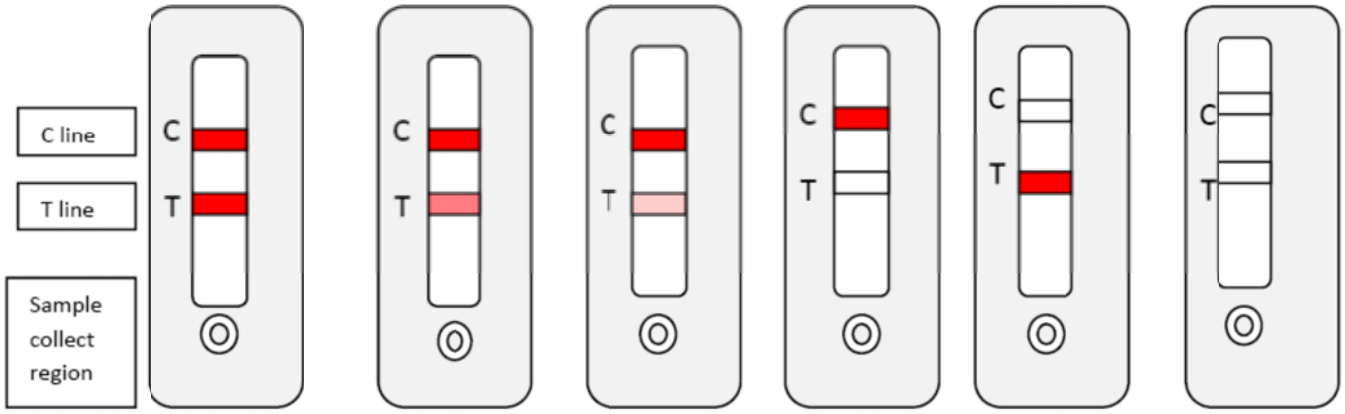

Figure 1: Result interpretation of the total Aflatoxin rapid strip test.

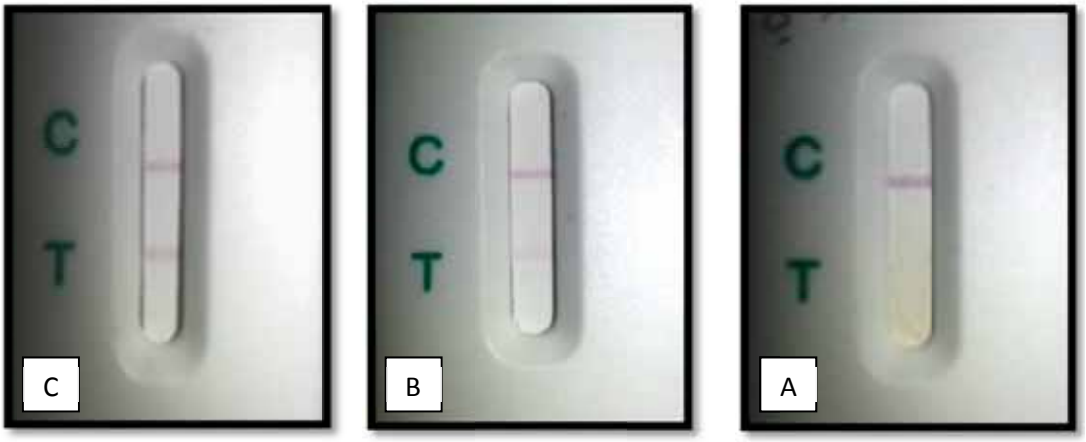

Figure 2: Illustration of the Positive and negative feed samples to residual Aflatoxins using total Aflatoxins rapid test strips. A: Positive for Aflatoxines 3ppb and more: Red T line is invisible, which means the content of Aflatoxins in sample is higher than 3ppb. B: Negative for Aflatoxines 0-3ppb, and C: Negative for Aflatoxines 0ppb: Red T line appears, which means there is no Aflatoxins residue in sample (C) or the content is lower than 3ppb (B).

Results

Feed samples from broiler farms

Samples of starter feeds and finished feeds showed that three (one starter (8.33%) and two finishers (16.66%), totally 25%) of these samples were positive (containing 3ppb Aflatoxin and more), while the remaining 9 feed samples (five starter (41.66%) and four finisher (33.33%), totally 75%) were negative to Aflatoxin residual contamination either by containing less than 3ppb Aflatoxin or free from it (33.33% and 41.66% respectively) (Table 1).

Feed samples from feed mills

Feed samples (4 starters and 4 finishers) (Table 2), collected from two feed mills show no positive feed samples to contamination with residual Aflatoxins (0%). All the tested feed samples (100%) of both feed types taken from feed mills were negative to residual Aflatoxins, either by containing less than 3ppb Aflatoxin or free from it (37.5% and 62.5%).

The summarized results of all feed samples collected from broiler farms and feed mills are illustrated in Figure 3, which shows that 3 (15%) of the feed samples were positive while 17 feed samples (85%) were negative to residual Aflatoxin.

Table 1: Residual Aflatoxins contamination of feeds collected from broiler farms

Facilities	No.	Feed type		Residual Aflatoxins ppb					
				positive		Negative			
		Starter	Finisher	3 and more		< than 3		0	
				Starter	Finisher	Starter	Finisher	Starter	Finisher
Broiler farms	1	+		+					
	2		+		+				
	3		+		+				
	4		+				+		
	5	+				+			
	6		+				+		
	7	+						+	
	8		+						+
	9	+				+			
	10		+						+
	11	+						+	
	12	+						+	
Total		6	6	1(8.33%)	2(16.66%)	2(16.66%)	2(16.66%)	3(25%)	2(16.66%)

Table 2: Residual Aflatoxins contamination of feeds collected from feed mills

Facilities	No.	Feed type		Residual Aflatoxins ppb					
				positive		Negative			
		Starter	Finisher	3 and more		< than 3		0	
				Starter	Finisher	Starter	Finisher	Starter	Finisher
Feed mills	1	+						+	
			+						+
		+				+			
			+				+		
		+				+			
	2		+						+
		+						+	
			+						+
Total		4	4	-	-	2(25)	1(12.5%)	2(25)	3(37.5%)

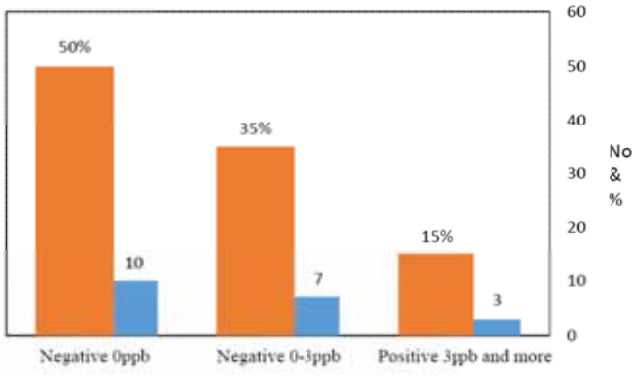

Figure 3: Summarized number and percentage of residual Aflatoxins contaminated tested feed samples collected from broiler farms and feed mills.

Discussion

The emergent sophisticated and easier access to methods for testing residues and undesirable substances led to increased safety awareness in food and feed production. Therefore, nowadays, regulations for control of mycotoxins in food and animal feed are of high importance. Various are the factors influencing the establishment of limits for certain mycotoxins and this requires monitoring commodities. Aflatoxin detection in poultry feed samples is of great importance now a days in order to ameliorate their negative effects on poultry health and production (19-23).

Aflatoxin detection in poultry feeds and other feedstuffs could be achieved through physical elimination of the contaminated any of the feed component used in poultry

feeding or by addition of adsorbents or any alternative feasible method (20).

The question is the choosing method, which is preferably rapid, accurate and sensitive one. Non expensive and not time consuming, rapid method is the ultimate request. In our study a total Aflatoxins rapid immunochromatographic strip test was used as qualitative and semi quantitative rapid method to fulfill this urgent demand. It is an easy test to perfume and to get results within a short time of 10 minutes.

In the current study, it appears that no positive sample (0%) was detected for residual Aflatoxin contamination in feed mill samples.

This result occurs within the range referred by Rodrigues (4), how found that Aflatoxin contamination percentage of 153 feedstuffs samples analyzed were between 0 and 10 % with an average of 0 to 5 µg/kg. The low incidence of Aflatoxin reported here could be due to selection of good quality graded samples by the feed manufacturers. (21), or may be due to the pellet type of feeds produced by these mills which made up of compressed mash and crumbles of broken up pellets, therefore usually it takes more time for toxins to build up in the pellets to a fatal level (22).

The results of the present study showed lower incidence and contamination level of Aflatoxin in poultry feed during Autumn dry months of October-December. The low occurrence of Aflatoxins during dry season of the year appears to be due to proper storage facilities of poultry feed ingredients and poultry feed. It was reported that variations in the levels of AFB1 in poultry feeds and ingredients were due to marked fluctuations in the environmental temperature and humidity during different seasons of the year. Similarly, (23) reported higher occurrence of Aflatoxin in the month of July with the prevalence ranging from 13.64 to 18.18 percent. Tangendjaja *et al*. (24) reported that corn harvested during the wet season had higher (66.4 mg/kg) level of Aflatoxin than those harvested in the dry season (36.5 mg/kg). A comparatively higher contamination level in maize was observed during warm and humid months (25).

A positive results of residual Aflatoxin contamination (3ppb or more) was recorded in broiler farm feed samples, which are higher than safe limit of 20µg/kg recommended by FDA (26). This observation coincides with our previous year's survey study conducted from 2002-2012 in Mosul governorate in the contamination of feeds and feed ingredients with Aflatoxins (19,27-30). All feeds in broiler farms in our study were purchased from feed mills used in this study. So the positivity of three (one starter feed sample and two finished samples) (25%) in the broiler farms feeds could be traced to the effect of storage and the time of storage especially with the case of finished feeds and a possible contamination from these farms under inappropriate environmental conditions (31). The remaining samples (75%) were negative either by contaminated with less than 3ppb or with zero contamination at all. Our results were coincides with the survey done in Kuwait for Aflatoxin contamination in the samples of poultry feed prepared for broiler starter, broiler finisher. The results revealed low average Aflatoxin concentration than the permissible levels (26). But the percentage and concentration of Aflatoxin contamination reported during 2009 to 2012 in feed stuffs all over the world by many authors were much higher than recorded in this present study. The quantum of twenty samples used here was too small compared with the highly contaminated samples appeared to have been used in the above-mentioned studies (3-8).

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