Isolation of Fungi and Detection of Aflatoxin in Poultry Feeds using ELISA and Fluorometry in Khartoum State

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ملخص البحث

أجريت هذه الدراسة في ولاية الخرطوم للكشف عن مدى تلوث أعلاف الدواجن بالفطريات بما فى ذلك الرشاشية الصفراء وقياس نسبة الأفلاتوكسين في الأعلاف الملوثة بإستخدام تقنية مقايسة الإمتصاص المناعي المرتبط بالإنزيم ومقياس التألق (اللّصف الإستشعاعى؛ الفلوروميتر). جُمعت مائة وستون عينة من أعلاف الدواجن ، 80 عينة من علف الدجاج البياض و80 أخرى من علف الدجاج اللاحم. أُستزرعت العينات في وسط السابرود المزود بالكورامفينكول. الفطريات المعزولة تم التعرف عليها علي أساس شكل المستعمرة في وسط دكستروز أجار سابرود بإستخدام صبغة اللاكتوفينول ومن ثم قياس تركيز مادة الأفلاتوكسين. أثبتت الدراسة وجود التلوث بالفطريات في 70 عينة من أعلاف الدجاج البياض بنسبة 87.5 % وفى 62 عينة من أعلاف الدجاج اللاحم بنسبة 77.5%. أظهر التحليل أن نسبة 25% من هذه العينات تجاوزت الحد المسموح به (20جزء من البليون) حسب ما أوصت به منظمة الأغذية والزراعة العالمية وإدارة الأغذية والعقاقير. أوضحت الدراسة أيضاً أن التحليل بواسطة مقياس التألق أكثر حساسية من التحليل بواسطة مقايسة الإمتصاص المناعي المرتبط بالإنزيم وأن علائق الدجاج اللاحم أكثر تلوثاً من علائق الدجاج البياض.

Summary

This study was carried out in Khartoum State to determine contamination of poultry rations with fungi including *Aspergillus flavus* and measurement of aflatoxin concentration using ELISA and fluorometry techniques. A total of 160 poultry rations (80 samples of layer and broiler rations each) were collected from poultry farms. Poultry rations were cultured on Sabouraud's Dextrose Agar, supplemented with chloramphenicol and observed periodically for development of fungal growth. Fungal colonies were identified on the basis of their colonial morphology and cultural characteristics. Seventy samples (87.5%) of layer feed and 62 samples (77.5%) from broiler ration showed contamination with fungi. Analysis of aflatoxin in contaminated rations revealed that 25% of the rations had aflatoxin concentration above the permissible level (20ppb) according to FAO and FDA regulations. The study also, revealed that fluorometric analysis was more sensitive than ELISA and that broiler feed was more contaminated compared to layer feed.

Introduction

Many agricultural commodities are vulnerable to attack by a group of fungi which is able to produce toxic metabolites called mycotoxins. Among various mycotoxins, aflatoxins have assumed significance due to their deleterious effects on humans, poultry and livestock (Qazi and Fayyaz, 2006). Aflatoxin problem was first recognized in 1960 when there were mass deaths of turkeys from a disease referred to as "Turkey X" in UK. The disease was caused by toxins in peanuts meal infected with *Aspergillus flavus* (Blount, 1961).

Feed contaminated with fungi and mycotoxins is considered to be a serious problem that faces poultry industry in most countries especially in hot zones (Ahmed, 1997). Groundnut meal infected with *A. flavus* was the cause of poisoning in cattle and this mould was reported as a primary producer of aflatoxin (Govrama and Bullerman, 1995). Different methods were used to detect aflatoxin

contamination, of which Thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), immunoassays and ELISA were used to determine aflatoxin concentration in various feeds and food (Chu, 1984; Dorner *et al,* 1989; Dutta, 2004). This study was conducted to investigate the contamination of poultry feed with aflatoxins using ELIZA and affinity column.

Materials and Methods

Sample collection

One hundred and sixty feed samples (80 samples of layer and broiler feeds each) were collected in sterile plastic bags from Shambat, Kuku, Omdurman, Abu Hamama, El kalakla and El Halfaia extensive poultry farms as well as from Veterinary Service Centres.

Direct examination of samples

A drop of 20% potassium hydroxide solution was placed on a slide then small amount of poultry ration was loaded, covered with a thin cover glass and microscopically examined under 10x and 40x objectives (Hungerford *et al,* 1998).

Culturing of samples

This was carried out as described by Raper and Fennell (1973). Subculture of positive samples was made using slide culture as described by Cheesbrough (1984).

Identification of the isolates

Isolated fungi were identified on the basis of their gross morphology and cultural characteristics according to monograph of Rhodes and Kwon-Chung (1989).

Determination of aflatoxin concentration

Concentration of aflatoxin in poultry feed was measured using ELISA and fluorometry techniques.

ELISA kits (Veratox Quantitative Aflatoxin Test, Neogen Corporation, USA) were used. Twenty samples, 10 from each feed, were tested according to kits manufacturer's instructions.

The fluorometre (VICAM series 4/ 4EX) was calibrated and used according to manufacturer's instructions using mycotoxin calibration standards which are provided with the kits.

Statistical analysis

Data were analyzed using SPSS version 6. Chi-square test was used to test the differences between layer and broiler rations. *P* value of ≤ 0.05 was considered significant.

Results

Mycological findings

A. flavus was isolated from 48 samples (Fig. 1a). The prevalence of *A. flavus* in contaminated feeds was 34.6%. Other fungi isolated are shown in Table 1.

Table 1: Prevalence of fungi isolated from layer and broiler rations.

Isolated fungus	Layer isolates (%)	Broiler isolates (%)	Total isolates (%)
A. flavus	16 (22.9)	32 (51.6)	**48 (36.4)**
A. fumigatus	2 (2.9)	16 (25.8)	**18 (13.6)**
A. niger	13 (18.6)	8 (12.9)	**21 (15.9)**
Rhizopus spp	36 (51.4)	6 (9.7)	**42 (31.8)**
Penicillium spp	3 (4.3)	0 (0)	**3 (2.3)**
Total	**70**	**62**	**132**

Colonies of *A. flavus* on Sabouraud Dextrose Agar were granular flat, with radial grooves, yellow at first but quickly turned to bright and dark yellow green (Fig. 1a). Hyphae were septated with rough conidiophores. Conidia were elliptical to spherical that became echinulated with age (Fig. 1b). Fungi were detected in 70 (87.5%) of layer feeds samples and from 62 (77.5%) of broiler samples. However, broiler feed was more contaminated with *A. flavus* compared with layer feed (Table 1).

Aflatoxin concentration

Analysis of poultry feed using ELISA method revealed contamination with aflatoxin in one sample of layer feed with a concentration of 36.6 ppb (Table 2). More samples were found contaminated with aflatoxin when analyzed by fluorometre; three samples from layer feed and nine samples from broiler feeds showed concentrations above the permissible level (20ppb). Thus, fluorometry analysis was more sensitive than ELISA (Table 2). Both techniques showed high aflatoxin contamination of broiler rations compared to layer rations. The difference is highly significant ($P< (0.01)$.

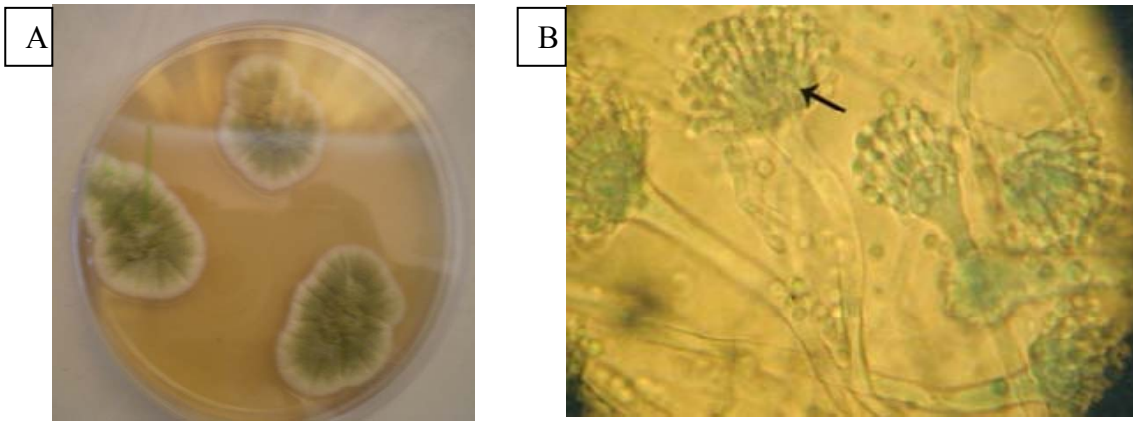

Fig. 1: Colonies of *A. flavus* (A) and hyphae on slide culture (B).

Table 2: Aflatoxin concentration (ppb) in poultry feed measured by ELISA and fluorometry

	ELISA		Fluorometry	
Sample No.	**Layer**	**Broiler**	**Layer**	**Broiler**
1	10.6	2.1	24	32
2	15.0	9.1	15.0	17
3	18.2	49.9	15.0	42
4	9.6	64.8	11	60
5	6.3	18.2	6.3	36
6	16.4	0.0	18	31.5
7	12.1	37.8	19	28
8	14.6	98.1	24	94
9	15.1	10.4	18	34
10	36.6	10.1	33	34
Mean±SD	**15.43±8.22**	**30.05±32.21**	**18.33±7.46**	**40.85±21.63**

Discussion

Great economic losses are caused by contaminated feed stuffs in form of poisoned products or reduced production (Ahmed, 1997). Aflatoxins have gained considerable importance due to their toxicity and frequent occurrence in feed ingredients used in poultry feeds. In this study, poultry feeds were found contaminated with aflatoxin. This finding is similar to the previous findings of Fraga *et al* (2007) and Mursal and Saad (2010) who revealed occurrence of aflatoxin in cereals and poultry feeds. The climatologic conditions of the Sudan in the Savanna Zone where groundnut is cultivated are favourable for aflatoxin production if care is not taken (Jarvis, 1971; Amin *et al,* 2009). Aflatoxins contamination can occur in a wide variety of feed stuffs including corn, sorghum, wheat, groundnuts, soya bean, rice, cotton seed and various derivative products made from these primary feedstuffs (Busby and Wogan, 1979; Dutta, 2004).

In the present study, among the *Aspergillus* spp. isolated from poultry feed samples, *A. flavus* was the predominant species found in poultry feed. This finding is similar to the finding of Muhammed (2010). The presence of *A. flavus*, however, does not indicate contamination of poultry feed with aflatoxin as only certain strains of *A. flavus* have the potential for aflatoxin production (Dorner *et al,* 1989). However, the prevalence of *A.*

flavus in poultry feeds in Khartoum State is found to be 36.4%. This finding is similar to the that of Hegazy *et al* (1991). Isolation of other fungi such as *A. fumigatus, A. niger*, *Penicillium* and *Rhizopus* species were also reported by Babiker (2009).

Various analytical methods are available for aflatoxin detection in feed and biological fluids such as TLC and HPLC. Although these methods are sensitive, they need an intensive clean up of the samples and require expensive instruments and reagents. Thus immunoassay such as ELISA and fluorometric method are used in this study because they are highly sensitive and specific, require minimal sample preparation and allow high rates of sample analysis using aflatoxin standard of known concentration (Chu, 1984; Dorner *et al,* 1989).

Analysis of poultry feed using ELISA technique revealed that 25% of the samples were contaminated with aflatoxin in a range of 36.6 - 98.1 ppb. This finding is similar to that of Mursal and Saad (2010) who recorded high concentration of Aflatoxin (36-97 ppb) in broiler ration.

In this study, broiler feed was found to be more contaminated with aflatoxin than layer feed. This might be due to the difference in the components of the two rations where more protein and energy contents are used in broiler's ration. However, there is an insignificant difference ($P \geq 0.05$) in the mean aflatoxin concentration between the samples collected from poultry farms and Veterinary Service Centres. This finding is similar to that reported by Mursal and Saad (2010).

Although the majority of layer feed showed aflatoxin concentration less than 20 ppb when measured by ELISA, in fluorometric analysis, 30% showed concentrations above the permissible level according to FAO regulations (Reddy *et al,* 2002). Moreover, broiler feed is found to be prone to aflatoxin contamination more than layer feed when analyzed by fluorometric method. Thus, ELISA method is recommended as a screening method while fluorometric method is recommended for measuring aflatoxin concentration because it is highly sensitive, rapid, less expensive and easy to apply.

In general, aflatoxin levels measured in poultry feed are considered high according to the international acceptable maximum level adopted by FDA and FAO (Reddy *et al,* 2002) that growing poultry should not receive more than 20 ppb aflatoxin in the diet. Thus, the maximum level of aflatoxin for livestock which represent the level of contamination at which the feed may be injurious to their health or results in contamination of milk, meat or eggs should be determined.

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