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Survey of Poultry Feeds for Aflatoxins from the Riyadh Region

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ABSTRACT. Since aflatoxins are formed in all the feed ingredients (Maize, Barley, Soybean) utilized in manufacturing poultry feeds, our objectives of this study were to determine the occurrence and the level of aflatoxins in the poultry feeds manufactured locally. The feed samples were collected from eighteen farms around the Riyadh region of Saudi Arabia. At least three or four different sample lots were collected from each farm. In a survey of 71 samples analyzed, the results obtained revealed aflatoxins in 55 samples (77%). Of these positive aflatoxin samples about 38% were above the present FDA guidelines of 20 ppb. The levels of total aflatoxins detected in the poultry feeds ranged between 5.4-121.2 ppb. Aflatoxins B1 and B2 were the only toxins detected in the feed samples and the concentrations of these aflatoxins varied greatly among samples. In general, results showed a high incidence of aflatoxin contamination in chicken feed samples tested.

Aflatoxins are considered, at present, to be one of the world's most dangerous contaminants in Foods and Feeds. They are a group of naturally occurring metabolites produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* (Allcroft and Carnagan 1963 and Sargeant *et al.* 1961) where growth is favoured by a warm and humid climate (Schindler *et al.* 1967). The primary metabolites of *A. flavus* and *A. parasiticus* are aflatoxins B1, B2, G1 and G2, which can contaminate a variety of agricultural food and feed products (Diener and Davis 1969). Investigation of aflatoxin in food and feedstuffs began in 1960 when aflatoxins were discovered as the cause of Turkey X disease that killed 100,000 turkeys in Britain (CAST 1979). Several investigators found that aflatoxins were acutely toxic, carcinogenic, teratogenic, and mutagenic (Adamson *et al.* 1973, Butler and Barnes 1968, Keyl and Booth 1971, Goplain *et al.* 1972, Lee *et al.* 1981, and Newberne and Buttler 1969). Aflatoxin B1 is the most potent toxic and carcinogenic metabolite recognized and can be found naturally in large amounts in agricultural crops (Scott

1978, Neberne & Buttler 1969, and Wogan and Neberne 1967). It is suspected that aflatoxins are a primary source of human cancer in some population (Neberne and Rogers 1973). It has been reported that aflatoxins in the feed of lactating animals can result in the secretion of the toxic, carcinogenic and mutagenic aflatoxin M in the milk (Masri *et al.* 1969, Allcroft *et al.* 1966, and Patterson *et al.* 1980). Several studies indicated that aflatoxins could occur in most poultry feed ingredients such as corn, soybean, barley and other common feed ingredients (Shotwell 1977, Shreeve *et al.* 1975, and Bean *et al.* 1972). In addition, the incidence of aflatoxins in corn has increased (Shotwell *et al.* 1973) and this crop represents a major feed ingredient of livestock and poultry destined for slaughter or egg production. Feeding chickens rations containing aflatoxin may causes growth retardation, an increase of liver/body weight ratio, biochemical alterations and pathological changes (Carnaghan *et al.* 1966). In addition, recent studies have indicated the presence of conjugated aflatoxins in the muscle of chickens fed diets containing crude aflatoxins (Van Zytveld *et al.* 1970 and Mabee and Chipley 1973). This study was initiated to obtain information concerning the incidence of aflatoxins in poultry feeds in the Riyadh region.

Materials and Methods

Collection of Samples

Samples of broiler and layeration feeds were collected from several farms of the Riyadh region from June-August 1986. The samples, each of about 2kg, were collected directly from the chicken feed manufacturing plant inside each farm on at least three different dates to obtain different production lots. The samples were transferred to our laboratory and analyzed for aflatoxin on arrival or they were stored at refrigerated temperature (2°C) to prevent any aflatoxin formation before analysis. About 300 g from each well mixed sample were ground in a stainless steel mill to pass 20 mesh screen to obtain 100 g subsamples for analysis.

Analysis of Aflatoxins

Aflatoxins were analyzed in feed samples by the "BF" method given in the Official Methods of Analysis of AOAC (1980, Secc 26.032). With slight modification: 100 g of the ground sample were weighed into a blender and mixed at high speed for 1 min with methanol-water (500 ml 55:45 v/v;) with the addition of 5 g of sodium chloride and 5 g of celite to speed the filtration process. 20 ml of lead acetate solution (20%) were added and blended again for a further 30 sec. The mixture was left to stand undisturbed until separation occurred (in our modified procedure separation occurred in less than 15 min). The mixture was filtered and 25 ml of filtrate was recovered. This was placed in a 500 ml separating funnel and was partitioned with two 25 ml aliquots of hexane. The hexane layers were discarded and the aqueous layer extracted with chloroform to obtain the toxin.

Quantitation was conducted by thin layer chromatography according to the AOAC procedure (1980). The pre-coated aluminium TLC plates of Silica gel 60, 200 Mm (Art 5554, E. Merck, Darmstadt), which were spotted both by the feed extracts and the aflatoxin standards, were developed in chloroform-acetone (9:1, v:v) solvent. They were then viewed in a cabinet under UV of 365 μm wave length light (CAMAG UV-cabinet 11). The quantity of aflatoxin was determined by comparing the spotted feed extracts (dissolved in 200 μl benezene-acetonitrile, 98:2, v:v) with a known concentration of aflatoxin standards (cofirmed according to procedure described by Jones 1972) obtained from the Sigma Chemical Company (Lot 54F-4003).

Results and Discussions

This survey was conducted to obtain information concerning the incidence and the levels of aflatoxins in poultry feed since aflatoxins are one of the world's most dangerous contaminants in feed. Tables 1 and 2 show the aflatoxin concentration in broiler and layer rations respectively collected from various farms in the Riyadh region of Saudi Arabia. In the survey of a total of 71 feed samples assayed, 77% were found to be contaminated with aflatoxins at a concentration range between 5.4-121.2 ppb. Furthermore, of the aflatoxin positive samples (55), about 38% were above the present Food and Drug Administration (FDA) guidelines of 20 ppb. Aflatoxin B1 and B2 were the only toxins found. Aflatoxin G1 and G2 were not detected in any of the samples. Of the 71 broiler and layer ration samples analyzed, B1 was found in the majority of samples, while aflatoxin B2 was detected in only eleven. However, aflatoxin B2 was never present in the absence of B1. The highest total aflatoxin concentration was detected in the broiler feed samples and were 121.2, 112 and 91.8 ppb. In the survey of total 71 samples analyzed, 16 gave aflatoxin negative results. The concentration of aflatoxins detected in the positive samples are presented in Tables 1 and 2.

Conclusion

The results obtained reflect a high incidence of aflatoxin contamination in the chicken feed samples tested. This result could be due to the presence of a high proportion of low quality maize (40-60%), which is internationally known to be one of the highest aflatoxin contaminated crop, in chicken feed. More consideration should be given to the analysis of aflatoxin in imported chicken feed ingredients, and to purchasing feed ingredients of high quality standards. In addition factors affecting the formation of aflatoxin in feed ingredients during storage should be effectively controlled. These data are not intended to be conclusive, but the survey on aflatoxins in feed ingredients should be continued in order to determine the main source of aflatoxin contamination in poultry feeds.

Table 1. Aflatoxins contents in broiler feed collected from various farms

Farms	No. of different sample lots	Aflatoxin concentration* (ppb)		
		B1	B2	Total
A	I	35.5	2.4	38.9
	II	24.3	0.0	24.9
	III	48.6	9.6	58.2
	IV	10.8	0.0	10.8
B	I	15	0.0	14
	II	20	0.0	20
	III	30	0.0	30
C	I	0.0	0.0	0.0
	II	0.0	0.0	0.0
	III	0.0	0.0	0.0
	IV	0.0	0.0	0.0
D	I	27	0.0	27
	II	81	10.2	91.2
	III	108	13.2	121.2
	IV	81	10.8	91.8
E	I	37.8	Trace	37.8
	II	20	0.0	20
	III	18.9	0.0	18.9
	IV	15.3	0.0	15.3
F	I	20	0.0	20
	II	13.3	0.0	13.3
	III	100	12	112
	IV	10	0.0	10
G	I	0.0	0.0	0.0
	II	0.0	0.0	0.0
	III	0.0	0.0	0.0
	IV	0.0	0.0	0.0
H	I	5.4	0.0	5.4
	II	16.2	0.0	16.2
	III	10.8	0.0	10.8
	IV	5.5	0.0	5.5
I	I	11	0.0	11
	II	19.8	0.0	19.8
	III	19.2	0.0	19.2
J	I	12.8	0.0	12.8
	II	13.3	0.0	13.3
	III	10.5	0.0	10.5

* Aflatoxins G1 and G2 were not detected in any of these samples.

Table 2. Aflatoxins content of layer feeds collected from various farms

Sources (Farms)	No. of different sample lots	Aflatoxin concentration* (ppb)		
		B1	B2	Total
K	I	62.4	8.4	70.8
	II	63.9	6	69.9
	III	60.4	5.4	65.8
L	I	0.0	0.0	0.0
	II	0.0	0.0	0.0
	III	0.0	0.0	0.0
	IV	0.0	0.0	0.0
M	I	13.3	0.0	13.3
	II	20	0.0	20
	III	10.8	0.0	10.8
N	I	32.4	3.6	36
	II	21.6	0.0	21.6
	III	10.8	0.0	10.8
	IV	32.3	4.8	37.1
O	I	11.4	0.0	11.4
	II	13.3	0.0	13.3
	III	26.7	Trace	26.7
P	I	16.2	0.0	16.2
	II	18.9	0.0	18.9
	III	5.4	0.0	5.4
Q	I	Trace	0.0	Trace
	II	Trace	0.0	Trace
	III	Trace	0.0	Trace
	IV	Trace	0.0	Trace
R	I	44.6	4.6	49.2
	II	59.4	5.9	65.3
	III	39.6	5.1	44.7
S	I	32.4	0.0	32.4
	II	16.2	0.0	16.2
	III	16.2	0.0	16.2
T	I	20	0.0	0.0
	II	19	0.0	19
	III	20	0.0	0.0
	IV	18.3	0.0	18.3

* Aflatoxins G1 and G2 were not detected in any of these samples.

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سموم الأفلاتوكسين في علائق الدواجن في منطقة الرياض

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من المعروف أن سموم الأفلاتوكسين يمكن أن توجد في جميع المكونات الغذائية التي تستخدم في تصنيع علائق الدواجن مثل الذرة والشعير وفول الصويا.

نتيجة لذلك فقد صممت هذه الدراسة لتحديد مستوى سموم الأفلاتوكسين في علائق الدواجن التي تصنع محلياً في مزارع منطقة الرياض، باستخدام المكونات الغذائية السابق ذكرها. جمعت عينات العلائق من مزارع مختلفة، حيث أخذ من كل مزرعة ثلاث أو أربع عينات خلال فترات زمنية مختلفة.

أظهر تحليل علائق الدواجن مستوى خطيراً من التلوث بسموم الأفلاتوكسين، حيث حللت ٧١ عينة ووجدت السموم في ٥٥ عينة (٧٧٪)؛ كما وجد أن ٣٨٪ من مجموع العينات التي أظهرت نتائج إيجابية للافلاتوكسين كانت تحتوي على مستوى من سموم الأفلاتوكسين أعلى من المستوى المسموح به (٢٠ ميكروجرام/ كيلوجرام) من قبل منظمة الأدوية والأغذية (FDA). وقد تراوحت بين ٤,٥ و ١٢١,٢ ميكروجرام/ كيلوجرام. وسموم الأفلاتوكسين التي وجدت في علائق الدواجن هي من نوع أفلاتوكسين ب$_1$ و ب$_2$ فقط.