DETECTION OF AFLATOXIN IN COMPOUND FEEDS
OF BROILER FLOCKS SUFFERED FROM FIELD
AFLATOXICOSIS

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ABSTRACT
One hundred and fifty eight broiler compound feed samples were
delivered from broiler flocks in Ninevah governorates. These flocks were clamed
to be affected with field aflatoxicosis. All the examined samples had aflatoxin
levels higher than of the permissible limit (20 ppb). The range of AF levels was
22-2263 ppb. The mean value of AF concentration was 592.7 ppb. The percentage
of AF concentrations from 0-500 ppb was 48.7%, and from 500-1000 ppb was
41.2%, while that from 1000-2500 was 10.2%.

INTRODUCTION
Aflatoxins are difuranocoumarin derivatives produced by a polyketide
pathway by certain strains of Aspergillus flavus and A. parasiticus; in particular,
Aspergillus flavus is a common contaminant in agricultural commodities.
Aspergillus bombycis, Aspergillus ochraceoroseus, Aspergillus nomius, and
Aspergillus seudotamari are also aflatoxin-producing species, but they are
encountered less frequently (1). Aflatoxins are a family of extremely toxic,
mutagenic, and carcinogenic compounds (2). Toxigenic A. flavus isolates produce aflatoxins B1, and B2 and toxigenic A. parasiticus isolates produce aflatoxins B1, B2, G1, and G2 (3). Many substrates support growth and aflatoxin production by aflatoxigenic molds. Natural contamination of cereals, oilseeds, nuts, and a long list of other commodities is a continuing worldwide problem (4). Crops could be contaminated with aflatoxin in the field before harvest, where it is usually associated with drought stress (5); even more problematic is the fate of crops stored under conditions that favor mold growth. In storage, usually the most important variables are the moisture content of the substrate and the relative humidity of the surroundings (6). Aspergillus flavus is the predominant fungus in aflatoxin – contaminated corn, and with A. parasiticus are temperature-tolerant fungi and can be selectively isolated on a high salt culture medium incubated at 37°C (7). Aflatoxin contamination has been linked to lowering resistance to diseases and interfering with vaccine-induced immunity and increased mortality in poultry, and also significantly lowers the value of grains as animal feed, (8). Few surveys on the occurrence of aflatoxins in poultry feeds have been conducted. Jindal et al., (1993) (9) analyzed 240 poultry feeds from India. All samples were positive for aflatoxins with levels ranging from 7 to 11,600 µg/kg (ppb). Levels higher than 30 ppb were detected in 76% of the samples. On the other hand, aflatoxin levels of 30-1610 ppb were found in 19% of 31 samples of compound poultry feed in Nigeria (10), while 91% of 34 samples of poultry feed in Indonesia contained aflatoxin levels ranging from 22 to 6171 ppb (11). Hegazy et al., (1991) (12) reported that 30.7% of 1175 poultry feed samples collected from Egyptian farms were contaminated with aflatoxin. The concentration of aflatoxin in the positive samples ranged from 1 to 2000 ppb. In Mosul province (Iraq), it was found that out of 450 broiler mixed feed samples, 66% were positive to one or more of aflatoxins B1, B2, G1, and G2 during four years of study 1999-2003 (13). The present study was aimed to estimate AF levels in compound feeds of broiler flocks claimed to be suffering from natural outbreaks of field aflatoxicosis in the northern governorates of Iraq.

MATERIALS AND METHODS

Feed sampling: One hundred and fifty eight samples of ground broiler compound feed samples in approximately 1kg were delivered from different broiler farms located in Ninevah governorate, showing signs and post-mortem changes of aflatoxicosis, during the period 2003-2005.

Aflatoxin assay:
The levels of aflatoxin contamination of feed samples were determined by the method of direct competitive enzyme-linked immunoassay using Neogen extraction kit (Neogen Corporation) as follows:
1-Sample preparation and extraction:
Twenty five gram- samples were collected for analysis. These samples were finely ground, so that at least 75% of them pass through a 20 mesh. After grinding, 5 gram samples were blended with 25 ml of 70% v/v methanol/water solution (7 parts methanol/3 parts deionized water) for 3 minutes. Extracts were filtered through a Whattman no.1 filter paper. The filtrates were then collected.
2-Test procedure:
All Neogen extraction reagents were allowed to warm at room temperature (18-30°C) before use. Red marked mixing wells were prepared, one for each
sample plus four red wells for controls 0.5, 15 and 50 ppb. All red-marked wells prepared were placed in the well holder. An equal number of antibody-coated (AB) white wells to those red-marked wells were also prepared. Hundred µl of conjugate were transferred to each red-marked mixing well. To those red wells containing the conjugate another (with new pipette tips) 100µl of controls and samples were added by using a 12-cannel pipettor liquid in wells were mixed by pipetting it up and down for 3 minutes. After mixing 100µl of the (conjugate+ samples, or conjugate+ controls) were transferred to AB-coated wells. These wells were moved back and forth for well mixing the contents in each well for 10-20 seconds without splashing reagents from the wells. Antibody-coated wells were then incubated at room temperature (18-30°C). The contents in AB-coated wells were shacked out, by filling the wells with deionized water and dumping them out. This step was repeated 5 times. Turning the wells upside down and tamping them out on a paper was carried out until the remaining water has been removed. Substrate was then added to AB-coated wells, by using the 12-channel pipettor through pipetting 100µl of substrate to these wells. Mixing was done by sliding the well holder back and forth for 10-20 seconds, followed by incubation for 3 minutes. Stop solution was poured to these wells (100µl) to each, mixing was done by sliding well holder back and forth on a flat surface. Within 20 minutes after the addition of stop solution. Results were read, using a micro well reader (Elx800) with a 650 nm filter. More blue color means less aflatoxin. Results of the yield optical densities of the controls and samples were obtained by using computerized Neogen Verotex Software program version 2.0.16 (Neogen Corporation).

**Statistical analysis:** The data were analyzed using computerized statistical program (SPSS, 2005 (14)).

**RESULTS**

**Post-mortem findings:**
Necropsy findings of some broilers delivered with mixed feeds they consumed, show enlarged pale liver and kidneys(Figure 1, 2 and 3). Discolored livers were ranged from clay to yellow color, owing to fat accumulation in hepatocytes. Livers also show sub capsular hemorrhages, focal areas of necrosis, and many of them were friable(Figure 4). Gall bladders were full, and the intestines were filled with catarrhal contents. Many necropsed birds were also exhibited varying degrees of skeletal myopathy.

**Aflatoxin levels:**
The entire broiler compound feed samples, which delivered from farmers claimed from mycotoxicosis in their broiler flocks, attempting to clarify the presence of mycotoxins in their delivered feed samples for detection of mycotoxins (here aflatoxin) show that there was surprisingly high levels of aflatoxin contamination (Table 1).

Levels were ranged from 22 ppb to 2263 ppb, with a mean value of 592.7 ppb, with a median value of 522(Table 2). Most of the obtained AF concentrations were scattered between 22 to 1000 ppb

It could be collectively said that higher number of AF contaminated samples(77 samples) had AF values from 0-500 ppb, and 65 samples had AF levels of 500-1000 ppb, while only 16 samples had the remaining AF levels of 1000-1500 ppb
(6 samples), 1500-2000 ppb (5 samples), and 2000-2500 ppb (5 samples) respectively.

To explore these numbers in percentages, it is evident from Figure 5, that the highest percentage (17%) was obtained in samples with AF concentration ranged from 100-200 ppb. From 3-10% were all the samples with each AF concentrations between 0-1000 ppb (except those of levels from 100-200 ppb). Two and lower percentages were experienced in the remaining AF levels (1000-2500 ppb).

Figures; 1, 2, 3 and 4, shows Lethal aflatoxicosis in broiler chicken causing liver and kidney discoloration, from clay to yellow liver, owing to fat accumulation in hepatocytes, with sub capsular hemorrhage and focal areas of necrosis. Aflatoxin (2218 ppb) was detected in the mixed feed offered to these broilers.

Figure 1: Enlarged pale liver of broiler chick with aflatoxicosis

Figure 2: Enlarged pale liver and kidneys of broiler chick with aflatoxicosis

Figure 3: Enlarged pale liver and kidneys of broiler chick with aflatoxicosis
Figure 4: Livers also show sub capsular hemorrhages, focal areas of necrosis of broiler chick with aflatoxicosis.

The cumulative percentage of AF contaminated samples with levels between 0-500 ppb was 48.7%, and those with levels ranged from 500-1000 was 41.2, while only 10% were the cumulative percentages of the remaining AF levels 1000-1500ppb (3.8%), 1500-2000ppb (3.1%), and 2000-2500ppb (3.2%) respectively (Figure 6).

The entire tested broiler mixed feed samples had AF levels higher than the permissible limit for broilers of 20 ppb.

Table 1: Total broiler mixed feed samples, delivered from different broiler flocks, suffering from aflatoxicosis, in all governorates involved, and the concentrations of aflatoxin (ppb) in these samples.

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Table 2: Maximum, minimum, and median aflatoxin concentrations (ppb), in all tested compound feed samples.

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* Figure 5: Percentages of AF contaminated samples distributed according to their AF levels

1= 0-100  2=101-200  3=202-300  4=301-400  5=401-500  6=501-600  
7=601-700  8=701-800  9=801-900  10=901-1000  11=1001-1100  12=1101-1200  
13=1201-1300  14=1301-1400  15=1401-1500  16=1501-1600  
22=2101-2200  23=2201-2300
DISCUSSION

Aflatoxin contamination of broiler feed commodities, and the natural aflatoxicosis in broilers have been reported worldwide, and also here in Iraq (15,16), but the occurrence of aflatoxin in poultry feed exhibits in most instances a geographical pattern, and Aspergillus species meet optimal conditions in tropical and subtropical regions, (16). Feed commodities likely to be contaminated with aflatoxins are many feed ingredients (17). Natural contamination of poultry feeds with aflatoxin was reported in many countries, like India (18), Malaysia(19), Indonesia (20), Sudan (21), Nigeria (22), Morocco(23), Poland(24), the United Kingdom(25), Australia (26), and the United States(27), and recently here in Ninevah governorate (15).

The results showed that all broiler compound feed samples delivered from flocks with field aflatoxicosis had aflatoxin levels above 20 ppb, the regulatory levels in feeds in most countries. (Leeson, 1995).Aflatoxin levels were ranged from 22-2263 ppb, with a mean value of 592.7 ppb. The maximum AF level reported here was higher than that reported by us in the preceding work, in which mean AFB1 was 114 ppb, with a mean value of, and also higher than that reported in India of (11600ppb) (9), and in Indonesia of (6171ppb)(11), while it was higher than that reported in poultry feed in Nigeria by Shetty et al., (1987) of 1610ppb(10), and that in Egypt of 2000ppb (12). Most of the contaminated feed samples (89.9%) recovered in our study had AF concentrations between 22-1000 ppb, while the remaining AF levels of 1000-2500ppb were about 10% of all tested samples. These concentrations are very likely to induce field aflatoxicosis, since (28) reported that as little as little as 30 ppb was enough to induce field aflatoxicosis in broiler chickens. So it is very likely that AF concentration in tested mixed feed samples, could lead to many changes characteristics for aflatoxicosis in broiler flocks, which confirmed and resembled those reported by Rajion and Farrell (1976) (29), who found that feeding 1100 ppb of AF to New Hampshire chickens were resulted in enlarged livers of necropsed chickens, reduction in body weight and poor feed conversion in the surviving birds. These adverse AF changes were
also reproduced by (Reddy et al., 1984) (30), when fed broiler chickens AFB1 up to 1000 ppb for 28 days, or for 5 weeks by (Giambrone et al. 1985) (31), who stated that gross liver lesions indicative for aflatoxin toxicity, of yellow, ochre discoloration of the liver, with multifocal hemorrhages and white foci, accompanied by reduction in body weight gain and feed conversion occurred when AF was fed at concentration of 1000 ppb and more. These changes were also reported by (Doerr et al., 1983) (32) by feeding AF at the same concentration for 7 weeks.

In addition to the effect of AF on broiler performance and immunity, it could deleteriously interact with different factors under field conditions. Some of these factors include presence of other mycotoxins in the feed like aflatoxin and ochratoxin A (43), aflatoxin and deoxynivalenol (44). Interaction of aflatoxin with other fungal infections like pulmonary aspergillosis has been reported in chickens(45). Interaction of AF with several dietary nutrients, like the change in response to AF with different source and level of dietary protein (46), or the greater effect of AF in broilers fed low fat diet (47), or the increased sensitivity of broilers to too small concentration of AF when fed diets deficient in riboflavin and cholecalciferol (48) has been reported.

It could concluded that as aflatoxin contamination of feeds is virtually inevitable, particularly in tropical and subtropical areas, like here in Iraq, where temperature and humidity favor development of Aspergillus fungi and their production of mycotoxins, several strategies should be developed in order to minimize the adverse effects of aflatoxins on poultry and also to prevent human aflatoxicosis. This could be accomplished by the most recent applied strategies like dietary supplements (49), detoxification of aflatoxin-contaminated feeds by physical and chemical methods (50).

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