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NATURAL OCCURRENCE OF T-2 TOXIN IN BROILER'S FEED COMMODITIES DETERMINED WITH ELISA

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ABSTRACT

Two hundred and twenty six broilers feed commodities were collected from different broiler flocks in Ninevah governorate (Iraq), through the period from January 2004 to December 2005. Enzyme linked immunosorbent assay method was used for T-2 toxin estimation. Results showed that, 81.5% of the total examined feed samples were positive to T-2 toxin contamination. Individually these percentages were as follows; 95% for wheat; 87% for corn; 85% for soybean; 74% for barley; and 70% for mixed feed. The range for T-2 contamination was between 8-1200 ppb. Most of the samples (58.4%) had low T-2 toxin natural contamination levels. Thirty two percent of positive samples had medium contamination level, while only 18.6% experienced high natural contamination levels with T-2 toxin.

الكشف عن التلوث الطبيعي لأعلاف فروج اللحم ومكوناته بسم T-2 بطريقة الامتصاص المناعي المرتبط بالأنزيم

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الخلاصة

تم الكشف عن سم T-2 في أعلاف فروج اللحم ومكوناته من خلال فحص 226 عينه جمعت من حقول فروج اللحم المختلفة في محافظه نينوى للفترة ما بين كانون الثاني 2004وحتى كانون الأول 2005 استخدمت طريقه الامتصاص المناعي المرتبط بالأنزيم لتحديد مستوى سم T-2 أوضحت النتائج إن 81.5 % من العينات كانت ملوثه بسم T-2 وان نسبه تلوث مكونات الأعلاف كانت كالأتي 95 % للحنطة 87 % للذرة 85% للصويا 74 % للشعير للعلف المركب 70 % وتراوحت نسب التلوث من 8 الى 1200 جزء بالبيون . كانت أكثر من نصف العينات الموجبة4. 85 % كانت ملوثه بمستويات سم T-2 المنخفضة وان 32

% كانت ملوثه بالحدود الوسطى للتلوث بينما لم يكن سوئ 18.6 % ملوثا بالحدود العليا للتلوث بسم T-2.

INTRODUCTION

Trichothecene are mycotoxins produced by common soil and plant fungi found world wide, including *Fusarium* and its perithecial stages, Calonectria and Gibberella; and the genera *Myrothecium, Stachybotrys, Cephalosporium, Trichoderma, Trichothecium,Cylindrocarpon, Veriticimonosporium,* and *Phomopsis* (1). About one-half of the more than 100 trichothecenes are produced by*Fusarium* (2). The greatest toxin production occurs with high humidity and temperatures of 6—24°C (3). Trichothecenes have a tetracyclic sesquiterpene nucleus with a characteristic epoxide ring, and toxicity resides in the epoxide ring, which is stable during prolonged storage or normal cooking temperatures (4). Poultry exposure to trichothecenes is likely to occur with the nonmacrocyclic group, which includes type A trichothecenes (T-2 toxin, neosolanial, diacetoxyscirpenol, and others) and type B (nivalenol, deoxynivalenol, fusarenone-X, and others), reviewed by Leeson *et al.* (5). In broilers, T-2 toxin produced by *Fusarium tricinctum* contaminated feed and litter caused reduced growth, severe depression skin lesions on the feet and legs, and ulceration and crusting of the oral mucosa, digestive disturbance, reddening of the gastrointestinal mucosa, mottling of the liver, gallbladder distention, atrophy of the spleen, and visceral hemorrhages, rickets, nervous disorders, abnormal feathering, pigmentation defects, leucopenia, and hemorrhages occurred and bloody diarrhea (6, 7, 8, 9, 10). In general, trichothecenes damage structural lipids and inhibit the synthesis of protein and DNA (11). Many are caustic irritants, a feature used in detection bioassays. T-2 toxin, diacetoxyscirpenol (DAS), deoxynivalenol (DON, vomitoxin), and nivalenol occur in feedstuffs worldwide, including corn, wheat, barley, oats, rice, rye, sorghum, safflower seed, mixed feed, and brewer's grains (12).

In this trail, we tried to estimate one of the trichothecene mycotoxins, namely T-2 toxin, in broiler feed commodities to elucidate the suspected causes of some characteristic field fusariotoxicities in broiler flocks.

MATERIALS AND METHODS

Feed sampling: Two hundred and twenty six samples of ground feed commodities (corn, soya beans, wheat, barley and mixed feeds) in approximately 1kg were collected from broiler flocks grain stores in Ninevah governorate during the period 2004-2005, for detection of natural T-2 toxin contamination of these feed commodities.

T-2 toxin assay: Twenty – five grams sub samples were prepared from the original 500 gm sample of feed commodities, were placed in a bag to be used for analysis, and otherwise stored at -20C^{O} until analysis. Samples were ground so that at least 75% of them passes through a 20 mesh sieve. After grinding, samples were blended with 125 ml of 70% methanol /water solution (7 parts / 3 parts) for 2 minutes in a high

speed blender. Extract was filtered by pouring at least 5 ml through whattman no. 1 filter paper, and then filtrate was collected. The level of T-2 toxin contamination of feed commodities was determined by the method of competitive direct enzyme- linked immunosorbent assay (CD-ELISA) using Neogen's mycotoxin extraction kit (Neogen corporation). Free T-2 toxin in the samples and controls was allowed to compete with enzyme- labeled T-2 toxin (conjugate) for the antibody binding sites. After a wash step, substrate is added which reacts with the bound conjugate to produce blue color more blue color means less T-2 toxin. The test was read in a micro well reader (EL x 800) to yield optical densities. The optical densities of the controls from the slandered curve, and the sample optical densities are plotted against the curve to calculate the exact concentration of T-2 toxin.

RESULTS

1- Incidence of T-2 toxin in different feed commodities: Out of the total 226 broiler feed commodities examined 185 (81.2%) were positive. The incidence of T-2 toxin in poultry mixed feed and feed commodities are presented in table 1. From table it is evident that soybean samples show the highest average concentration value of 963 ppb, ranged from 30-12000 ppb in the 55% positive tested samples. The results of mixed feed samples were in the second order. The average concentration in these samples was 314 ppb, ranged from 10-1000 ppb, among 46 positive concentration of the toxin in samples (70%).In the third order were concentration of the toxin in wheat samples, which had an average of 291 ppb in the 95% positive samples, ranging from 8-1500 ppb. In the fourth position were these concentrations of corn samples, ranged from 5-850 ppb with an average of 218 ppb in the 45 positive samples (87%). In the last order were those results obtained from barley samples, which had an average of 120 ppb, ranging from 25-600 ppb in the 30 positive samples (74%).

2- Distribution of T-2 toxin levels into specific concentrations: The distributions of T-2 toxin concentration were arranged into three levels as shown in figure 1&2. The levels were those ranged from low level of 0-150 ppb, medium level between 150-400, and high level >400 ppb. From figure 2, it is evident that the higher percentage of positive samples (58.4%) had low toxin levels. In the second order was the percentage of those positive samples of 23% which occur in the ranges of medium T-2 toxin levels of contamination, while only 18.6% of the tested samples had high levels of T-2 toxin natural contamination. From figure 3, it is evident that 66% of soybean tested samples were occur in the low concentration level of T-2 toxin, while 10% of the samples participating in the medium level and the remaining 24% in the high T-2 toxin level. A similar pattern was repeated with mixed feed and corn contamination levels; being 44% and 70% of the positive samples respectively in the low T-2 toxin contamination level; 25% and 13% respectively in the medium level; while the remaining 30% and 17%in the high levels. The picture was differed in the remaining seeds, wheat and barley. In case of wheat, the higher T-2 toxin percentage of contamination was in the medium level (49%), while lower in the low level (34%), and lowest in the high levels (17%). With barley, the

distribution of T-2 toxin contamination at specific levels occurred in the following descending manner, from (78%) of low level samples, and 17% of the medium toxin levels, to 5% of those samples with high T-2 toxin levels.

Table 1: Incidence of T-2 toxin in poultry mixed feed commodities.

Commodities	Number of tested samples	Number of positive samples	% Of positive samples	T-2 toxin concentration ppb	
				Average	Range
Soybean	34	29	85	963	30-1200
Mixed feed	60	46	70	314	10-1000
Wheat	37	35	95	291	8-1500
Corn	52	45	87	218	50-850
Barley	43	30	74	120	25-600

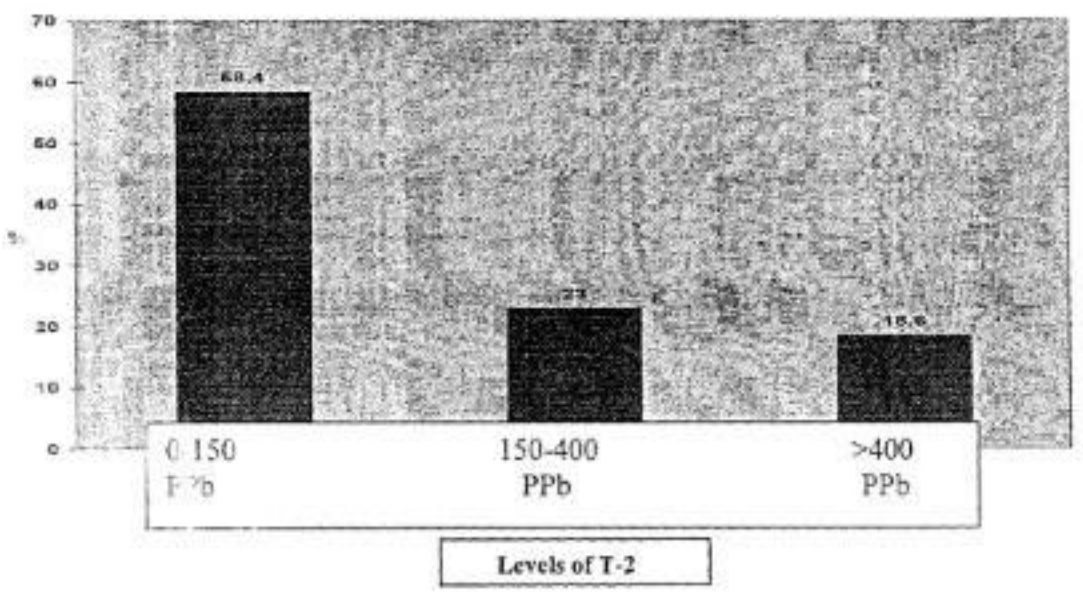

Figure 2: Total distribution of low, medium and high T-2 toxin concentrations of the tested feed commodities.

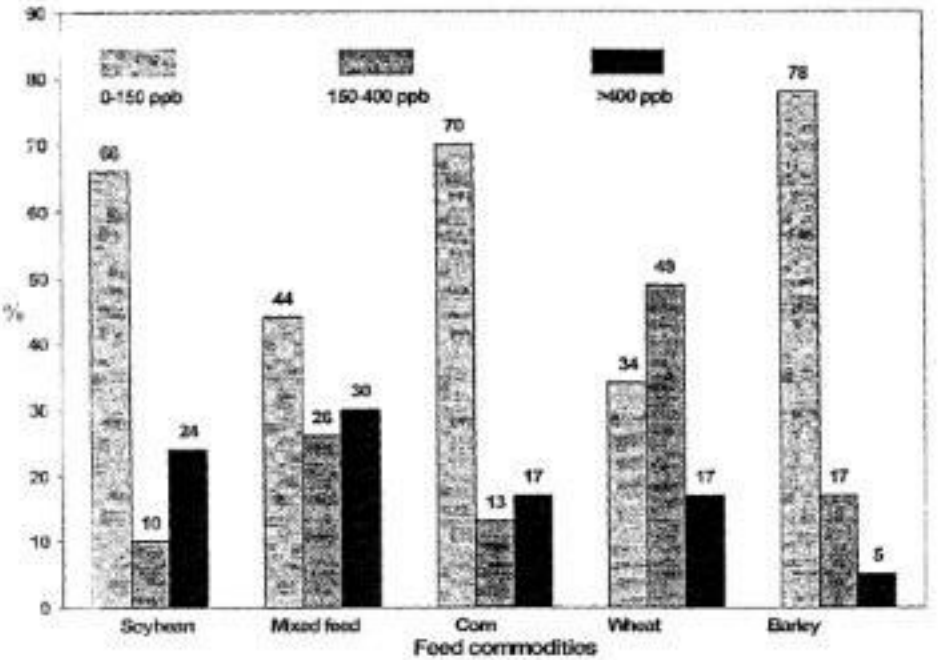

Figure 3: Distribution of low, medium and high T-2 toxin concentrations of the tested feed commodities.

DISCUSSION

No attempt was made in this study to isolate Fusarium fungi from feed commodities, because the presence of the fungi doesn't necessarily mean presence of the trichothecene mycotoxins (5). Although high contamination rate of feed commodities was reported in our study with T-2 toxin, being (70%) for mixed feed; (74%) for barley; (85%) for soybean; (87%) for corn; and (95%) for wheat, but in the same time it is not surprising to see that more than half of the positive samples were contaminated with low T-2 toxin levels (0-150 ppb). This may be due to, that high production of T-2 toxin almost occurs in temperate regions with high humidity and temperature of 6-24 C^0 (3). Our results were in agreement with Whitlow *et. al.*,1998)(13), who found that the concentration of T-2 toxin >500 ppb were 6% for corn and 7% for all feeds submitted by North Carolina farmers over a nine-year period, likewise, in our results they were not exceed 7.45%. T-2 toxin has been identified in feedstuffs worldwide, including corn, wheat, barley, oats, rice, rye, sorghum, safflower seed, and mixed feed (12), as here in our studied samples. Natural occurrence of trichothecenes has been reported in Asia, Africa, South America, Europe, and North America (3). In our trial, T-2 toxin was also reported here in Iraq (one of the subtropical Asian countries) with a range of 5-1200 ppb. This range occurred within NRC ranges of that from zero to 10000ppb; with few exception of 15000-40000 ppb (14).The primary effect of T-2 toxin in chickens is an inflammatory response in the mouth that progresses to necrosis and invasion by normal microbial flora (15). Other adverse effects of dietary T-2 toxin exposure, at levels ranging from 1 to 16 ppm, include decreased

growth rate, feed consumption, conversion, and negative effects on relative weight of internal organs. (15,16).The obtained level of T-2 toxin contamination here, could lead to variable adverse health conditions, like necrosis in the oral cavity (400 ppb and more), reduction in feed consumption and weight gain (1000-4000 ppb) (17). Under field conditions, there is the possibility of feed being contaminated with more than one mycotoxin. Fungal strains are often capable of synthesizing several mycotoxins and some Fusarium *sop.* can produce more than 8 mycotoxins (18). On the other hand, the use of multiple grain sources in poultry diets can lead to mixtures of mycotoxins in the feed. Chemical interactions between such toxins may then occur through several mechanisms. (19). Toxic synergism between T-2 toxin and other mycotoxins like aflatoxin was reported in broiler chickens (20), between T-2 toxin and ochratoxin or Cyclopiazonic acid (21; 22); and between T-2 toxin and deoxy nivalenol (DON) (23).

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