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NATURAL CONTAMINATION OF SOME BROILER'S FEED COMMODITIES WITH OCHRATOXIN

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ABSTRACT

Ninety eight samples of feed commodities (wheat, soybean and corn) were collected during the period 2000–2004 from various broiler farms showed clinical signs of ochratoxicosis. Feed samples were divided into two equal parts, one for mycological study, for detection of feed commodities contamination with *A. ochracous*, and the other part for ochratoxin analysis using Enzyme-linked immunosorbent assay. Mycological results showed that wheat samples show the higher percentage of contamination with *A. ochraceus* (73%) with log 10 CFU/gm of 2.77. Toxicological analysis shows that wheat samples had the highest rate of ochratoxin contamination (86%) followed by soybean (76%) and then corn samples (70%). Ochratoxin levels in all feed samples were ranged from < 100 ppb to 400 ppb. The importance of ochratoxin in poultry health was discussed.

التلوث الطبيعي لبعض مكونات أعلاف فروج اللحم بسموم الاوكرا

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الخلاصة

تمت دراسة تلوث ٩٨ نموذج لمكونات اعلاف فروج اللحم (ذرة، صويا و حنطة) خلال الفترة ٢٠٠٠-٢٠٠٤ من حقول أظهرت أعراض سريريه وصفات تشريحية مشابهـــة للتسمم بالاوكراتوكسين. قسمت عينات الاعلاف الى قسمين احدهما لدراسة تلوثها بفطر *Asperiglus ochraceous* والجزء الثاني لتحديد مستوى تلوث هذه الاعلاف بسمـــوم الاوكرا باستخدام تقنية Enzyme linked ELISA) Immunosorbent assay) اظهرت نتائج الفحص الفطري ان عينات الحنطة كانت الاكثر تلوثا (٧٣%)" وكانت اعداد العفن لوغارتم2.77 مستعمرة / غرام علف. اما نتائج تلوث العينات بسم الاوكرا فقد اتضح ان عينات الحنطة اظهرت اعلى نسب بالتلوث ٨٦% تلاها الصويا ٧٦% ثم الذرة ٧٠% عاكسة بذلك نسب تلوث هذه العينات بالعفن، وتراوحت مستويات التلوث بالسم بين اقل من ١٠٠ الى ٤٠٠ جزء بالبليون لجميع العينات. و نوقشت اهمية سم الاوكرا على صحة الدواجن.

INTRODUCTION

The mycotoxin ochratoxin A (OTA) is produced by the fungus *Aspergillus ochraceus* (now known as *A. alutaceus*) and *Penicillum verrocosum* has carcinogenic, nephrotoxic, teratogenic and immunosuppressive properties (1). These two fungi and other *Aspergilli* spp.(*A.carbonarius*) and other *penicillum* spp., are the principle producers of OTA (2). Ochratoxin A was first reported in1965 (3). The non-toxic dechloro analogue, ochratoxin B(OTB), and the ethyl ester, ochratoxin C (OTC), are also fungal products. *Penicillum verrucosum* is the principle source of OTA contamination of stored foods in temperate climates while *Aspergillus* spp. predominate in wormer countries. There is abundant information on the natural occurrence of OTA in foods and feeds including cereal grains, beans, coffee, animal feeds, human and animal blood plasma, animal products and mothers milk (4). Ochratoxin A causes ochratoxicosis in farm animals (5). It is considered as the most toxic mycotoxin for domestic fowl. In terms of lethality, which is the simplest measures of toxicity, OTA is more toxic than aflatoxin and comparable in toxicity to the trichothecene mycotoxin diacetoxyscirpenol (DAS). In broilers LD50 value for aflatoxin, DAS and OTA have been reported to be 6.8 (6), 2.0 (7), and 2.1 mg/kg (8), respectively. Hamilton et al. (9) reported several natural episodes of ochratoxicosis affecting broiler chickens, laying hens, and turkey in the United States. The level of OTA in suspected feed and ingredients ranged from <200 to 16000 ppb. In year 2000 till now many commercial broiler flocks in MOSUL governorate suffered form characteristic clinical syndrome, each time the case being clustered over a period of several months with being involved in specific imports of grains. Clinical signs were most often characteristic of typical ochratoxicosis. The aim of the present study was to draw a relationship between these signs and the natural contamination of broilers feed commodities with *A. ochraceous* and OTA.

MATERIALS AND METHODS

Feed sampling: Ninety eight samples of ground feed commodities (corn, Soya beans and wheat) in approximately 1kg were sent from broiler farms during the period 2000-2004 (24 samples in 2000; 25 samples in 2001, 2002 and 24 in 2004) to the college of veterinary medicine, department of public health. These farms show clinical signs of restlessness, huddling, growth retardation, birds with different weights, decreased feed consumption, diarrhea, dehydration, wet litter and increased water consumption with some neural abnormalities. Post-mortem findings of necropsed birds from these farms were characterized by emaciation, dehydration, dry firm gizzard with erosions, proventricular hemorrhages. Hydro pericardium and ascents. The kidneys were pale, swollen and enlarged and changed in color from normal mahogany to tan. Livers were enlarged, pale and friable or hemorrhagic, while the gall bladders were distended with bile. Some birds show accumulation of urates on the serosal surface of several organs .Catarrhal enteritis with fragile intestine (Fig. 1). Feed commodity samples drawn from these broiler farms were taken from their grain stores, and equally divided into two parts, one for mycological examination (for detection and enumeration of *A.ochaceus*), and the other for toxilogical examination (for detection of natural OTA contamination of these feed commodities).

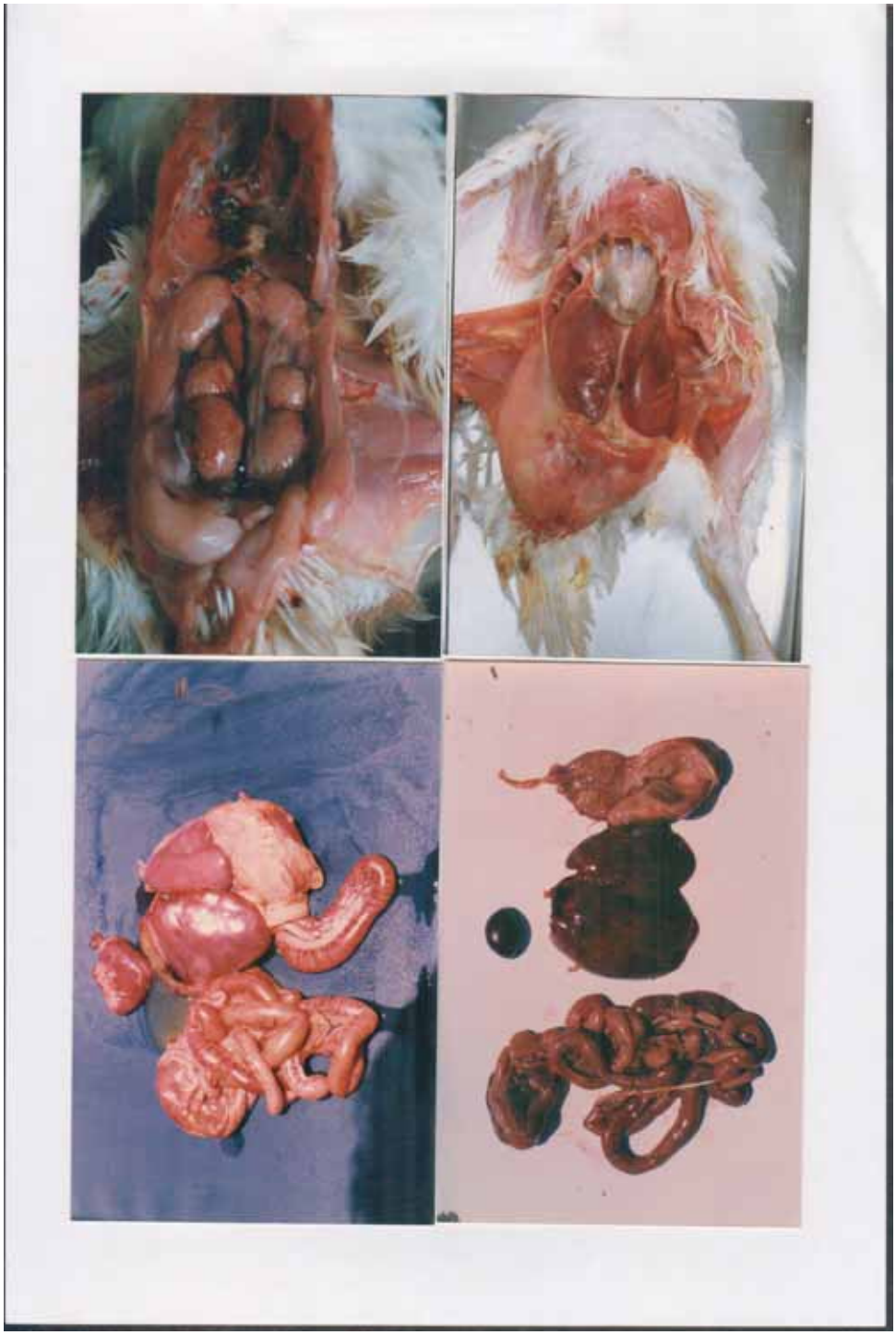

Figure 1**:** Gross lesions associated with natural field ochratoxicosis of broiler chicks**.** (A) kidneys are pale, swollen and enlarged and changed in color from normal mahogany to tan; (B) hydropericardium, hemorrhaged liver; (C) Enlarged, pale and fibrosed liver and ascetic fluid; (D) Dry firm gizzard with erosions, proventricular hemorrhages, catarrhal enteritis with fragile intestine.

Mycological examination: Ten grams of each ground feed commodity samples (corn, Soya beans and wheat) were soaked for 60 min. with 0.1 peptone (10), then blended for 60 sec. Serial delusions were carried out at a rate of 1:10 (=1+9). Spread plating method on Rose Bengal yeast extract succarose agar **(**PRYS**)** (11) was used for enumeration of *A. ochraceus* by plating 0.1 ml inoculums. Plates gave 30 to 40 colonies were chosen for enumeration. The identification key of *A. ochraceus* was based primarily on the standardized procedure described by Pitt (1979) (12). Cultures were grown for 7 days on three standered media, at 5C°, 25C° and 37C°; Czapk Yeast Extract Agar (CYA) (Pitt, 1973) (13); Malt Extract Agar (MEA) (14) and 25% Glycerol Nitrate Agar (G25N) (13). Results were expressed as Log^{10} colony forming unit/gm (CFU/gm) of feed sample.

Ochratoxin 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, otherwise stored at -20C° until analysis. Samples were ground so that at least 75% of them passes through a 20 mesh sieve. After grinding, samples were blended with 100 ml of 50% methanol /water solution (50/50) for 2 minutes in a high speed blender. Extract was filtered by pouring at least 5 ml through whattman no. 1 filter paper then filtrate was collected. The level of ochratoxin 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 ochratoxin in the samples and controls was allowed to compete with enzyme- labeled ochratoxin (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 ochratoxin. The test was read in a micro well reader (ELx800) to yield optical densities. The optical densities of the controls from the standard curve, and the sample optical densities are plotted against the curve to calculate the exact concentration of ochratoxin.

RESULTS

Mycological results: The percentages of feed commodities contamination with *A. ochraceus* are presented in Fig. 2. Wheat samples show the highest percentages of contamination, being 73% of the tested samples. In the second position, were Soya beans samples with 69% contamination, while corn samples were in the third order with the lowest percentage of contamination (52%). The number of colonies per gram of ground feed commodities (CFU/gm) were presented as log 10 CFU/gm in Fig. 3. The highest enumeration rate was noticed with wheat samples, followed by Soya beans and then corn. Only wheat samples reveal log 10 CFU/gm of 2.7. Enumeration of Soya beans samples reached up to log 10 CFU/gm of 2.6, while corn samples did not exceed Log 10 CFU/gm of 2.3.

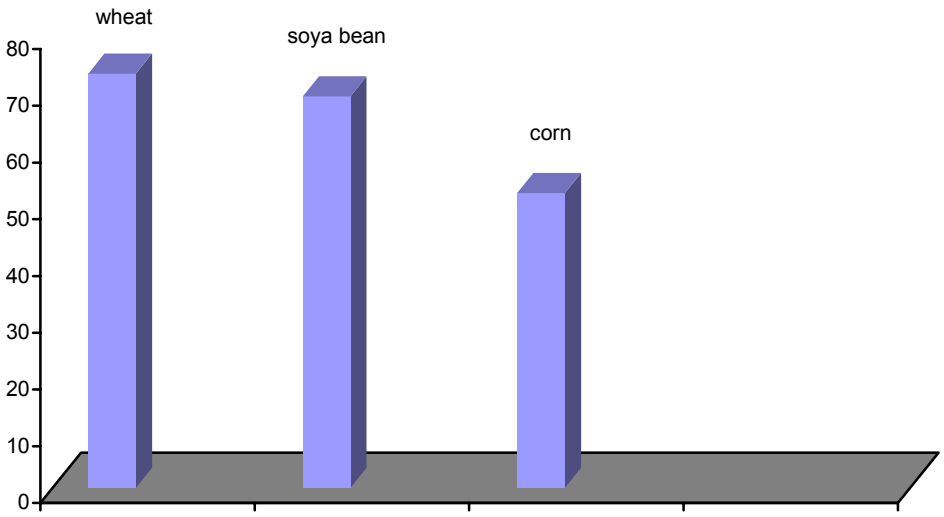

Figure 2: Percentages of feed commodities contaminated with *A. ochracious.*

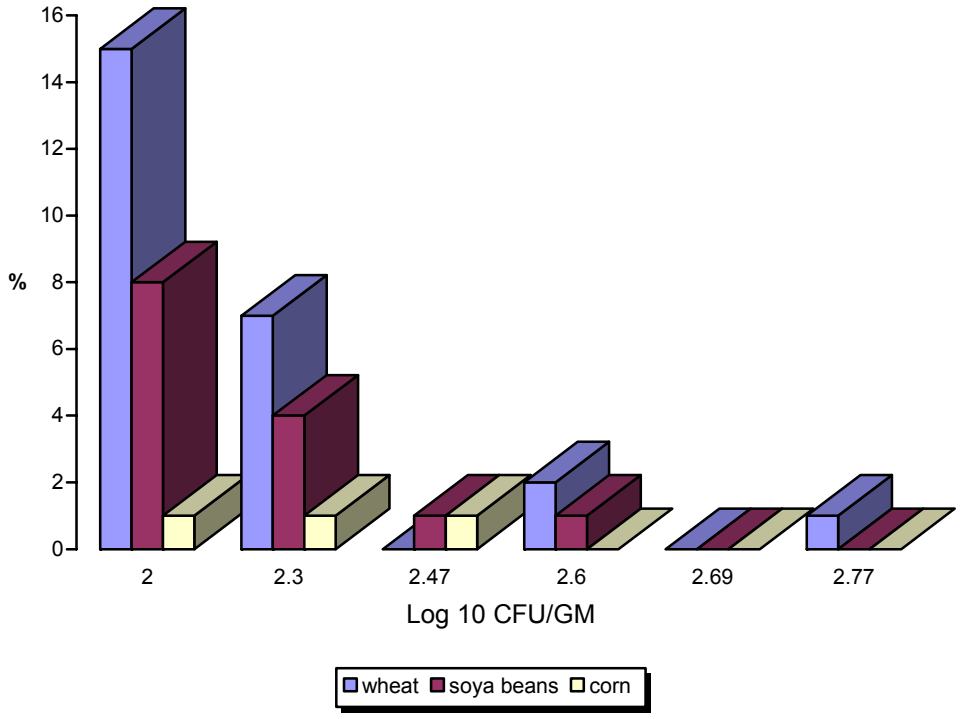

Figure 3: Logarithmic distribution of *A. ochraceus* number in feed commodities.

Toxicological results: Percentages of positive feed commodities for natural ochratoxin contamination is illustrated in Fig. 4 & 5. It is evident that wheat samples show the highest percentage of natural OTA contamination (86%), followed by soya beans (76%) and then corn (70%). The distribution of OTA contamination into three specific concentration of low (<100ppb); medium (100-400ppb), and high (>400ppb) are shown in Fig. 4. All feed commodities were contaminated with OTA at two specific concentrations, low and medium ranges, and no samples of tested commodities had OTA concentration more than 400 ppb. Wheat samples show the lowest percentage (43%) of contamination with low OTA level (<100ppb), but had the highest percentage (56%) of contamination with medium OTA level (100-400ppb). Corn samples show the opposite position to those of wheat samples, having the highest percentage of contamination (50%) with the low OTA level of (<100ppb) and the lowest percentages (44%) with the medium OTA levels. Soya beans samples occur in the medium order between percentages of wheat and Soya beans samples in contamination with OTA at low and medium OTA levels.

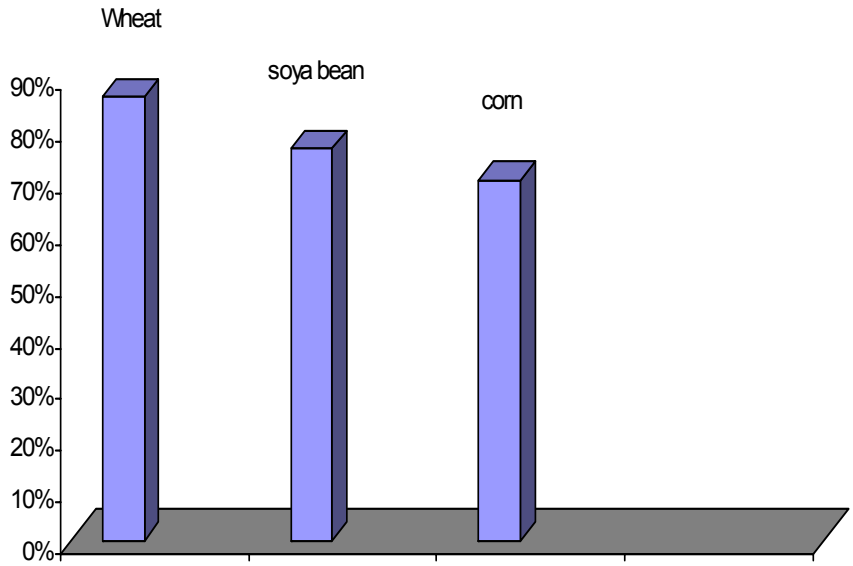

Figure 4: Percentages of OTA positive feed commodities.

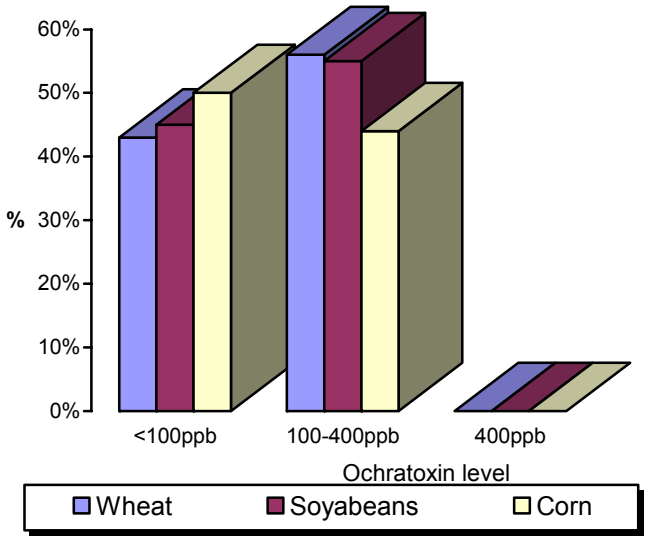

Figure 5: Percentage distribution of OTA levels of positive feed commodities.

DISCUSSION

In addition to *A. ochraceus*; *P. verrucosum*, *A. carbonarius* (and closely related *A. niger*) are OTA producing fungi. In our study we stressed only on *A. ochraceus*, due to the restriction of *A. ochraceus* for OTA production in tropical and subtropical regions (15), while *P. verrucosum* is primerly confined to temperate climates, and both *A.carbonarius* and *A. niger* are commonly found in graps and similar fruit at high temperature (13). In the studied poultry farms, bad managements in grain stores were practiced among these are; poor traditional methods of grain drying; poor ventilation insect and rodents damage; feed grains were stored in unsuitable sacks to perform fumigation, these factors are indeed promote the growth of different fungi in the stored grains (16). *Aspergillus ochareceus* was also reported by others to contaminate Soyabean (17); corn (18); and wheat (19). The high OTA level in our study in wheat samples could be regarded as a reflection to their higher *A. ochraceus* contamination rate, or due to the support of wheat commodity to OTA production, than on other substrates like corn and Soya beans (20). Ochratoxin A levels revealed here could be responsible for the signs of ochatoxicosis noticed in examined broiler flocks, since, these levels occur within the levels (<200ppb-16000ppb) referred by Hamilton et al. (9), which were responsible for field ochratoxicosis episodes in broiler chicks. Because of the stability of OTA and its long half – life in blood and tissues (kidney, liver and muscle) after long term administration of low OTA levels (50 ppb) to broilers (21). Ochratoxin A residue in poultry is of great public health concern. The toxicological status of OTA has been reviewed as a potentially nephrotoxic, carcinogenic, teratogenic, immunotoxic, affecting both humoral and cell-mediated immunity and protein synthesis inhibitor (22,23). So the protection of animal and human health is best assured by preventive measures to minimize the contamination with OTA- producing fungi and the conditions that give rise to production of the toxin.

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