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Research Article
Prevalence of Ochratoxin A in Poultry Feed and Meat from Jordan

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Abstract

Background and Objective: Ochratoxin A (OTA) is a fungal metabolite produced in feed and could be transmitted to food chain through animal and considered as one of the potent carcinogenic compound. This study aimed to evaluate the levels of Ochratoxin A in both poultry feed and meat in Jordan. **Materials and Methods:** About 129 samples representing feed, corn and poultry meat were collected from different location of Jordan market and evaluated for Ochratoxin A by ELISA. **Results:** About 38.5% of feed sample, 50.0% of corn sample, 100.0% of feed sample found under sun light were containing an Ochratoxin A (OTA) with an average concentration of 2.90±0.26, 2.35±0.32, 10.30±0.59 μg kg^{-1}, respectively. Also the results showed that between 66.0% (12 sample) to 100.0% (54 sample of the analyzed organs meat sample contains OTA with a concentration ranging from 1.89±0.07-7.68±0.12 μg kg^{-1}. **Conclusion:** The results indicated that none of the tested samples exceeded the maximum limit set by the EU limits of <50.0 ng km^{-1} in poultry feeds.

Key words: ELISA, poultry meat, ochratoxin A, toxicity, contamination

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In Jordan, poultry production considered as one of the major industrial sectors that is important in the national economy and a competitive products to meet the nutritional needs for consumer because poultry protein is cheap compared with other animal proteins[1].

Poultry feed is the corner stone of poultry industry, which account of more than 60% of the production process expenses, that used to be imported from countries includes India, European countries to full fill the requirements of poultry sector from feed to sustain and improve poultry industry in Jordan[1].

Worldwide including Jordan, the contaminated poultry feed gain attention as major source for toxins hazards associated with human health and increases the number of cancer cases registered in various countries including Jordan[2].

Poultry feed contamination with mycotoxins such as ochratoxin is of concerns in agricultural various produce[3], ochratoxin are groups of fungal metabolite consists of three types, A, B and C . Ochratoxin A (OTA) is the most prevalent and the most toxic[4-6] and considered as a secondary toxic metabolite produced mainly by some strains of fungi such as *Aspergillus ochraceus* It is the main producers to ochratoxin at moderate temperatures with high water activity and Preferably growing in oilseeds (peanuts and soybeans) more than in grain crops, such as wheat and corn[7-10] and *Penicillium verrucosum* species which grows at cool temperate regions (5-30°C) with law water activity Thus produce ochratoxin in wheat and corn[11,12]. Also, nutritional, humidity, water activity and integrity of the seeds are factors that influence on mold growth and produce ochratoxin[13].

The cereal and cereal products are the main contributors to human toxicity risk by OTA compared to other foodstuff of plant origin[14]. Generally, OTA formation occurs mainly as a result of a direct fungal contamination to crops and animal feed during growth in the field or post-harvest especially when favorable environmental conditions for growing fungi are available[15,16].

Food and contaminated poultry feed with Ochratoxin is potentially hazardous to the health of humans as well as poultry because of their carcinogenic effects, where in broilers, major clinical signs of feed consumption with OTA are poor growth, reduced feed efficiency and increased water consumption which is a consequence of damaged kidneys[17,18] also when, long-term exposure to Ochratoxin A could result in high mortality rate[4], while other animals such as cows and sheep's as a ruminant animals are generally capable to hydrolyze OTA to the non-toxic metabolites by the rumen micro flora in the stomachs before absorption and release it into the blood[19-21].

The International Agency for Research on Cancer has classified OTA as a possible carcinogen and placed in-group 2B carcinogen to humans[22].

The major risk to human health not only through the intake of contaminated foods of vegetable origin with ochratoxin A but also through eating meat of animals that fed on contaminated feed with OTA such as pork and poultry and dairy products[14,23]. Digesting food contaminated with ochratoxin by human for a long time established to disease known as ochratoxicosis, that mainly could damage the kidneys[4-6].

Level of safe intake of ochratoxin A set at100 ng kg^{-1} body weight per week and this level prevent the health and economic effects[24]. Also the European Commission has established a regulation list for the maximum tolerable limits of OTA in foodstuff commodities, such as cereals (5 μg kg^{-1}), cereal products (3 μg kg^{-1}), dried fruits (10 μg kg^{-1}) and foodstuff for baby and children less than three years of age of 0.5 μg kg^{-1} [25].

According the above information about toxicity and health risk of Ochratoxin A on consumer health and because these compounds have great heat-stable to heat treatment such as process of cooking and sterilization, which makes the consumption of mycotoxin contaminated animal-derived food a potential health hazard on consumer health[8]. Although the presence of OTA is increasingly regulated in most the countries of Europe and America and there are a lot of studies which related directly or indirectly to each other but up until now, in most Middle East countries, including Jordan no regulation limits are in force about level of ochratoxin in poultry feed and meat which prevent the health and economic effects also the laboratory tests for detection of OTA not available in most countries regional and studies are very limited. Thus this study designed: to evaluate the extent of contamination of poultry feed and meat circulated in Jordan market with Ochratoxin A by using Enzyme Linked Immunosorbent Assay and compare them with maximum level recommended by the European Commission and some countries in the region. This study will contribute to raising awareness about the importance of legislation and laws that show the level of ochratoxins allowed in poultry feed and meat at national and regional level.

MATERIALS AND METHODS

Sample collection: A total of 57 poultry feed samples of 1 kg each were collected from different poultry chicken grower

(broiler and layers) representing different regions in Jordan governorate of which 6 samples collected from bags placed under sunlight and 72 poultry meat samples collected from different commercial sources were stored in ice box during transportation and stored in plastic bags at 4°C, until detection of Ochratoxin A.

The sample collected during spring and summer seasons from April, 2016 until August, 2016 and analyzed at Jordan University.

Sample preparation for ELISA analysis: About100-200 g from each samples were ground and mixed prior to the extraction procedure then grinded feed passed through a sieve of 1 mm opening using Poltron miller (Kinematica, Lucerne, Switzerland).

Five gram of ground sample was extracted with 12.5 mL of 70% methanol by shaking vigorously for 3 min, the mixture was filtered using a filter paper (Whatman No. 1, Germany). One milliliter of the filtrate was mixed with 1 mL of deionized water.

The prepared samples were tested using microplate enzyme-linked immunosorbent assay (ELISA) quantitative test kits (Ridascreen , r-biofarm, Germany) and ELISA reader (Expert plus, Switzerland). The quantitative analyses for Ochratoxin A (Sigma Chemical. St. Louis, MO, USA)[26], procedure was followed for extraction of OTA from flesh samples.

The samples were analyzed using the Ochratoxin A test procedure (Art. No. R5402) as described by r-biofarm test procedure kit (RIDASCREEN® FAST, Ochratoxin A 10-01-27)[27].

Ochratoxin A working standard solutions: Five working standard dilutions of 0 (zero standard), 5, 10 , 20 , 40 μg kg^{-1}, Ochratoxin A in methanol /water were provided with ELISA kit. Ochratoxin A was used and immunoassayed in triplicate (RIDASCREEN® FAST, Ochratoxin A 10-01-)[27].

Test procedure: Pipit 50 μL of standard dilutions or prepared sample into separate wells, then add 50 μL of enzyme conjugate and add 50 μL of anti-Ochratoxin A antibody solution to each well. Mix gently by shaking the plate manually and incubate for 10 min at room temperature (20-25°C). Add 100 μL of substrate/chromogen to each well. Mix gently by shaking the plate manually and incubate for 5 min at room temperature (20-25°C), 100 μL of stop solution was added to each well, mix gently by shaking the plate manually and read the absorbance at 450 nm (RIDASCREEN® FAST, Ochratoxin A 10-01-27)[27].

Statistical analysis: The results for OTA obtained by ELISA reader were statistically analyzed and given as the arithmetic means and standard deviations by using statistical analysis program the SPSS software version 9.5 (SPSS, Cary, NC, USA)

RESULTS AND DISCUSSION

Ochratoxin A in poultry feed: The results obtained for the concentration of Ochratoxin A in poultry feed are presented in Table 1. It showed that 38.5% of feed samples, 50.0% of corn and 100.0% of feed samples collected from lots found under sun light were contaminated with OTA. The mean concentration of the contaminated samples tested range between 2.35-10.31 (μg kg^{-1}) compared to minimum and maximum concentration of OTA of 1.54-27.11 μg kg^{-1} with the maximum level of OTA was found in feed samples exposed to sun light. Although that Farmers are often tempted to incorporate moldy grain of low price into animal diets to reduce feed costs. However, this practice causes a risk for mycotoxin contamination and alters nutrient content of the grain[28]. The results found are in agreement with Published report by Domijan *et al.*[29], it results showed that the contaminated feed with OTA ranges between 0.42-6.19 μg kg^{-1}. While in Italy Schiavone *et al.*[30], who found that OTA in poultry feed samples ranges between 0.04-6.5 μg kg^{-1} which is in agreement of the values found in the current study. Also Jaimez *et al.*[31], who found the level of OTA in feed samples in Spain was 1.53 μg kg^{-1}.

Cereals, oilseeds contaminated with OTA or products of animal origin such as poultry when animals are fed on contaminated feed with OTA is the major cause of that exposure of these OTA to animals and therefore ultimately to humans[21,32].

The concentration of OTA found in animal feeds varies from country to country but the levels recommended by the EU legislation (2006/576/EC, <50 μg kg^{-1}) [12]. However, none of the samples that collected contained more than the maximum level recommended by the European Commission.

Also the results found are means that the feed storage under the sunlight will increases the risk of mold growth and ochratoxin A production. Which do not degrade under sunlight, The results found are in agreement with previews report by Schiavone *et al.*[30], While Herzallah *et al.*[33] showed that mycotoxin such as aflatoxin after 30 h of exposure to sunlight more than 60% is degraded.

Ochratoxin A in poultry meat: The levels of OTA in poultry meat obtained in this study are presented in Table 2. The results showed that the 100% of thigh and legs,

Table 1: Ochratoxin A concentration in different poultry feed*

Feed	Total number of sample tested	Number of sample positive (%)	Number of sample negative (%)	Mean value concentration (μg kg^{-1})	Minimum (μg kg^{-1})	Maximum (μg kg^{-1})
Feed	39	15 (38.5)	24 (61.5)	2.90±0.26	1.72± 0.10	3.70±0.33
Corn feed	12	6(50.0)	6 (50.0)	2.35±0.32	1.54±0.14	3.18±0.51
Feed exposed to sun light	6	6 (100.0)	0 (0.0)	10.3±0.59	2.10±0.19	27.11±1.05
Total	57	27 (47.4)	30(52.6)			

*Values are Means±SD of a triplicate

Table 2: Incidence of OTA in tissue of slaughtered chicken*

Feed	Number of samples	Number of positive (%)	Number of sample negative (%)	Mean value concentration (μg kg^{-1})	Minimum (μg kg^{-1})	Maximum (μg kg^{-1})
Thigh and legs	18	18 (100.0)	0	2.610±0.270	1.90±0.14	2.98±0.50
Liver	18	18 (100.0)	0	5.860±0.390	4.06±0.66	7.68±0.12
Gizzard	18	18 (100.0)	0	2.070±0.133	1.89±0.07	2.26±0.19
Breast	18	12 (66.0)	6 (44.00)	3.062±0.300	2.81±0.52	3.31±0.18
Total	72	64 (88.886)	6 (8.40)			

*Values are Means±SD of a triplicate

Liver, gizzard samples and 66.6% of breast samples were contaminated with OTA with an average concentration of 2.61±0.27, 5.86±0.39 , 2.073±0.13 and 3.062±0.30 μg kg^{-1} respectively, with minimum and maximum concentration were between 1.891±0.07 and 7.681±0.12 μg kg^{-1} for gizzard and liver, respectively. Also the results showed that OTA levels in liver were higher than those found in breast, thigh, legs and gizzard. These results were in agreement with the results obtained by Iqbal *et al.*[34].

About 41% chicken meat samples were contaminated with OTA. and OTA level in liver of chicken was highest mean level (2.41±0.72 μg kg^{-1}) Markov *et al.*[35], who found that OTA level in commercial sausage samples was 7.83 μg kg^{-1}. On the contrary the Persi *et al.*[36] found that OTA level in liver samples was 13.77 μg kg^{-1}. It is higher than results these study Joo *et al.*[37] found that the level of OTA in animal tissues increased as the OTA level in feed increase.

All samples found to contain OTA less than (10 ng kg^{-1}) recommended by the European Commission (FAO/WHO)[38].

Human exposure to Ochratoxin A occurs mainly through consumption of contaminated crops or food derived from animals exposed to contaminated feedstuffs such as meat and meat produce[39]. Also (Iacumin *et al.*[40] found that molds such as *Aspergillus ochraceus* produce OTA in meat speck and Vipotnik *et al.*[41], who found that *Aspergillus westerdijkiae* produce OTA in dry-cured ham flesh.

CONCLUSION

The daily intake of OTA through chicken meat depends on the OTA concentration in the food, the amount OTA consumed and the frequency of consumption OTA. In addition, the concentration of OTA in chicken tissues is generally low and the total of Ochratoxin A intake from chicken products is very small compared with other sources. The level of OTA in all samples used in the study were contained less than the maximum level recommended by the European Commission but higher the safe intake of 100 ng kg^{-1} body weight per week recommended.

SIGNIFICANCE STATEMENT

This study found that feed and poultry flesh samples found contaminated with OTA of the possibilities of exceeding the permitted levels set by EU. The data found in this study will provide valuable information for the government agencies responsible for feed food quality to consider the presence of OTA in the feed source and in order to evaluate the risk of OTA contamination on consumer health.

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