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Determination of Ochratoxin A Residues in Locally Broiler Liver in Baghdad Province

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Abstract | The main objectives of the present research were to determination of Ochratoxin A (OTA) residues in locally broiler liver by high-performance liquid chromatography (HPLC). A total of 50 samples were collected randomly from various markets Baghdad province from each sector Al-Kirkh and Al-Rusafa. All liver samples were positive for OTA and the result showed that there were significant differences (P ≤ 0.05) in the residual levels (ppm) of OTA between Al-Kirkh and Al-Rusafa. The highest values were recorded in Al-Rusafa (3.240 ± 0.0071), followed by Al-Kirkh (3.188 ± 0.0128). This research indicated that the presence of OTA in poultry liver increases the risk of poison for human beings and we need to protect the stored cereal from any conditions which help fungal growth and mycotoxin production.

Keywords | Ochratoxin A, Broiler liver, High-performance liquid chromatography, Mycotoxin, Baghdad.

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INTRODUCTION

The world would be a very different place without fungi, these organisms play vital roles as decomposers, breaking down all sorts of organic matter from roots and leaves to crop residues and wood, as well as the bodies of dead mammals, fish, and insects (WHO, 2010). The process of decomposition releases the nutrients stored within decaying organic matter, through this invaluable service, fungi help provide the foundation for the diversity of species living within an ecosystem and the capacity of one generation of life to sustain the next (Ramesh, 2012). The most known mycotoxins in poultry food chain are Aflatoxin (AF) and Ochratoxin A (OTA). Exposure to mycotoxins may result in acute, overt disease, or as is usually the case, chronic, insidious exposure that impairs poultry productivity. The severity of any of these effects in poultry production systems will depend on the level of mycotoxin present in the feed supply chain, the duration of exposure, the physiological status of the animal and other environmental and disease factors that impact on the uptake, biotransformation, deposition and excretion of theses toxins (Bryden, 2012). These contaminants of agricultural commodities have attracted worldwide attention because of the significant losses associated with their effects on human health and livestock (Schat and Skinner, 2014). Animal feed is the first link of food chain; therefore the risk of contaminant carryover from contaminated feeds to animal tissues and biological fluids, and eventually to products intended for human consumption (meat, eggs) is a matter of concern (Arroyo-Manzanares et al., 2015). Due to a high consumption of poultry liver in Iraq, therefore, this research was conducted to determine of OTA in locally broiler liver of Baghdad province by HPLC.

MATERIAL AND METHODS

Collection of Samples

A total of 50 liver samples; comprising locally broiler (30-45 days) were collected randomly from various markets located in different locations of Baghdad province during November 2017 to March 2018, fifty samples (10 samples for each month) 5 samples from each sector Al-Kirkh and Al- Rusafa. All of collected meat samples were locally slaughtered and transported by plastic bags in ice container and were stored at 4°C until analyze by HPLC.

Extraction of OTA and Deterimanation By Hplc

According to (Giacomo et al., 2016), Chickens were sacrificed by cervical dislocation, liver were collected, weighed and frozen for subsequent OTA analysis. Five gram of raw materials, homogenized with 5 mL of 1 M phosphoric acid, for 5 mints. A 2.5 gm of the homogenate was transferred into a centrifuge tube, extracted twice with 5 mL of ethyl acetate, and centrifuged for 5 mints. at 3000 rpm. The organic phase was removed, the residue re-extracted, as above, and the organic phases combined. The volume of the organic phase was reduced to approximately 3 ml and back-extracted with 3 ml of 0.5 M $NaHCO_3$ pH 8.4, and centrifuged for 10 mints. at 3000 rpm.The aqueous extract was acidified to pH 2.5 with H_3PO_4 85% and sonicated to strip the CO_2 formed.OTA was finally back-extracted into 5 ml ethylacetate, vortexed for 1 mint, and centrifuged for 10 min at 3000 rpm. The organic phase was evaporated to dryness under nitrogen stream, reconstituted in 1000ml of mobile phase, and a 100ml injected into HPLC.

Statistical Analysis

The data were analyzed using one way ANOVA. Differences were considered significant at ($P \leq 0.05$). SPSS (version 22) was used for statistical assessments.

RESULTS

The HPLC analyses revealed that all 50 liver samples were positive for OTA (Table 1). The highest range values (ppm) of OTA were recorded in Al-Rusafa (3.22 - 3.26), followed by Al-Kirkh (3.15 - 3.22) at March/ 2018, while the lowest range values (ppm) of OTA were recorded in Al-Rusafa (2.48 - 2.57), followed by Al-Kirkh (0.49 - 0.52) at November/2017.

Table 1: Levels of Ochratoxin A residues (ppm) in broiler liver samples

Sector	No. of samples	Months	Range
Al-Kirkh	5	November/2017	2.43 - 2.59
	5	December/2017	2.55 - 2.59
	5	January/2018	2.65 - 2.69
	5	February/2018	2.89 - 2.99
	5	March/2018	3.15 - 3.22
Al- Rusafa	5	November/2017	2.48 - 2.57
	5	December/2017	2.57 - 2.61
	5	January/2018	2.67 - 2.71
	5	February/2018	2.96 - 2.99
	5	March/2018	3.22 - 3.26
Total	50		

The results revealed that there were a significant differences ($P \leq 0.05$) between all study months. The highest levels were recorded at March, followed by February, January, December, and November in Al-Rusafa sector (3.240 ± 0.0071), (2.972 ± 0.0074), (2.690 ± 0.0071), (2.590 ± 0.0071), and (2.526 ± 0.0163) respectively. Moreover, the results showed that, the highest mean levels of Ochratoxin A Al-Kirkh sector were recorded in March, followed by February, January, December, and November (3.188 ± 0.0128), (2.938 ± 0.0185), (2.668 ± 0.0066), (2.574 ± 0.007), and (2.510 ± 0.028) respectively (Table 2).

Table 2: Comparisons of Ochratoxin A residues (ppm) in liver samples between the months of the study period

	Mean ± SE	
Months	Al-Rusafa	Al-Kirkh
November	2.510 ± 0.0163 **a**	2.526 ± 0.028 **a**
December	2.590 ± 0.0071 **a**	2.574 ± 0.007**a**
January	2.690 ± 0.0071 **a**	2.668 ± 0.0066 **a**
February	2.972 ± 0.0074 **a**	2.938 ±0.0185 **a**
March	3.240 ± 0.0071 **a**	3.188 ± 0.0128 **b**
Total mean ± SE	2.935 ± 0.0145	2.643 ± 0.009

*Vertically the mean difference is significant at the 0.05 level

DISCUSSION

The present study showed that all liver samples were positive for OTA when analyses by the HPLC. The highest levels of OTA in liver were recorded at Al-Rusafa, followed by Al-Kirkh. The differences between Baghdad sectors could be attributed to several reasons such as difference in age of animals, the site that animals come from, source of water, type of rearing, feed component, farmers having low formal education and differences in withdrawal times. Previous studies confirmed the present results by reported that the Ochratoxin are fungal secondary metabolites that contaminate grains, legumes, coffee, dried fruits, beer, wine, and meat. Ochratoxin A (OTA) is a widely-spread mycotoxin all over the world causing major health risks (Bozzo et al., 2008). The same pattern of OTA tissue distribution was also established by (Rosi et al., 2006), who had administered 200 µg OTA/kg of feed during the period within which the animals' mean live weight increased from 146.5 kg to 165 kg. The highest OTA concentration of 9.6 ± 2.7 µg/kg was determined in the kidney, followed by 6.3 ± 1.7 µg/kg in the liver and 1.9 ± 0.6 µg/kg in the muscle tissue, while the lowest concentration of 1.1 ± 0.6 µg/kg was found in the adipose tissue.

The reason for increased the levels of OTA in poultry liver in March followed February, January, December, and November due to in this month's rain fell many with poor storage of feed in fields, this result in growth of mould in feed that lead to produce mycotoxin and transfer to poul-

try tissues. Moulds are ubiquitous in nature and universally found where environmental conditions are conducive to mould growth. Because moulds are present in soil and plant debris, and its spores are spread by wind currents, insects, and rain, they are frequently found in/on foods together with their associated mycotoxins (Habib et al., 2015), so the environmental variations can affect the concentration of OTA residues.

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CONFLICT OF INTEREST

None of the authors have any conflict of interest to declare.

AUTHORS CONTRIBUTION

Anfal Jamal Ibrahem: Plan of work, execution, HPLC analysis, and manuscript preparation. Dalia Abdul-Kareem Abdul-Shaheed: Plan of work, technical assistance and manuscript preparation.

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