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DETECTION OF HEAVY METALS AND MYCOTOXINS POLLUTION IN BROILER CHICKEN GIBLETS IN RETAIL SHOPS OF EL-BEHEIRA GOVERNORATE

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ABSTRACT

The present study aimed to investigate the level of heavy metals bioaccumulation, mycotoxins contamination and macro-mineral composition of broiler chicken giblets (heart, liver and gizzard) from retail markets in El-Beheira Governorate. Ninty-six fresh samples of liver, gizzard and heart (thirty-two of each) were randomly collected from retail markets of four different cities (Damanhour, Kafr-El Dawar,Hosh Esa and Itay Elbarod) in EL-Beheira Governorate, Egypt. The concentrations of metals; Lead (Pb), cadmium (Cd), iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), Selenium (Se), arsenic (As) and macro-mineral composition; sodium (Na), potassium (K), calcium (Ca), magnesium (Mg) were determined by using atomic absorption spectrophotometer and phosphorous (P) by a colorimetric method. However, mycotoxins [Aflatoxins (AFs) and Ochratoxin A (OTA)] were determined by fluorometer. The significant elevation in the concentration level of Cd and Pb limit in livers samples from retail market of Damanhour and Kafr El-Dawar cities was above the tolerable. As and OTA were not detected. The mean concentration of Fe, Cu, Zn, Mn, Se and AFs were found below the tolerable limit in all tested chicken giblet samples. However, Fe, Cu, Zn levels significantly increased, while, Se levels significantly decreased in Hosh Esa and Itay-Elbarod giblet samples. Metals, macrominerals and AFs concentration were the highest in liver followed by gizzard and heart of chickens except Na which was the highest in heart followed by gizzard and liver. In addition, aflatoxins significantly increased while, P and Ca significantly decreased in Hosh Esa and Itay-Elbarod giblet samples. In conclusion, chicken giblet samples from Damanhour and Kafr El-Dawar retail markets were the worst contaminated samples with Pb and Cd. However, chicken giblet samples from Hosh Esa and Itay-Elbarod were the worst contaminated samples with AFs.

Keywords:

Broiler chicken giblets, Metals, Mycotoxin, Residues, Macro-minerals.

INTRODUCTION

Cadmium and lead are some of the most common heavy metals causing environmental and food contamination because they cannot be degraded and trigger many acute or chronic disorders in humans due to their accumulation in long-term periods in various organs such as liver, heart and muscles **(Akan *et al.,* 2010)**. Aflatoxin contamination in food and foodstuffs represents a major threat to the health of exposed people. AFB1 was detected in chicken liver and gizzard samples, thus confirming the poultry's exposure through feed or feed ingredients **(Augusto *et al.,* 2014)**. Generally, birds can be exposed to heavy metals both externally, by physical contact, and internally, by consumption of contaminated water, poultry feed, seweage water and industrial effluents **(Khan *et al.,* 2015)**. Exposure to heavy metals through poultry consumption may lead to health risks especially in areas with expanding industrial and agricultural activities **(Mahmoud and Abdel-Mohsein, 2015)**.Mineral contents of chicken giblets were, in most cases, higher than chicken meat **(Göncü Karakök *et al.,* 2010)**. The level of minerals in tissue may vary not only according to the mineral content of feeds but also according to the way animals are housed, their breed, sex and health, slaughter procedures, and type of tissue **(Połtowicz and Doktor 2013)**.

Variation of freezing temperatures may lead to spoilage of chicken meat and giblets and production of mycotoxins. Besides, feeding on mycotoxins contaminated feed, bad sanitary measures in abattoirs as contaminated water,air sources,contaminated tools,equipment, mishandling of carcasses on preparation after slaughtering beside improper evisceration increases mycotoxins levels **(Hussain *et al.,* 2016).** Mycotoxins are fungal toxic metabolites naturally contaminate food and feed. Aflatoxicosis and ochratoxicosis are the most common mycotoxicosis in commercial poultry **(El-Tras *et al.,* 2011).** Acute or chronic aflatoxicosis in poultry birds results in decreasedmeat/egg production,immunosuppressant,and hepatotoxicosis **(Khan *et al.,* 2010)**. Ochratoxin A (OTA) contaminates agricultural products and due to its accumulation in food,represents a serious threat to human and animal health worldwide. Youngpoultryaremoresensitivetoochratoxiningestionthanadults**(Pattison *et al.,* 2008)**.

OTA in poultry diets leads to reduction in growth rate, feed consumption and feed efficiency and increased mortality. OTA inhibits protein synthesis, and inhibits normal renal uric acid secretion. A decrease in the concentration of proteins, calcium, phosphorus and potassium is followed by an increase in the level of uric acid **(Elaroussi, 2008)**.

Thus, the objective of the present study was to investigate the extent of heavy metals bioaccumulation, mycotoxins contamination and macro-mineral composition of chicken giblets (heart, liver and gizzard) from retail markets in El-Beheira Governorate.

MATERIAL AND METHODS

Samples collection:

In this survey, ninty six fresh samples of liver, gizzard and heart (thirty-two of each) were randomly collected between June and Juli 2018 from retail markets of four different cities (Damanhour,Kafr-El Dawar, Hosh Esa, Itay Elbarod) in EL-Beheira Governorate,Egypt. Organs were separated, packed in into sterile plastic bags (Ziploc type), frozen and stored at -20 °C until further analysis. After thawing, organs were cut into small pieces and each sample was homogenized

Determination of heavy metal and macro-minerals:

Two grams of each sample was dried and added to 10 ml of the digestion mixture (3:2 nitric acid (HNO3) 65% v/v and perchloric acid (HCIO4) 70% v/v. and was digested for 3 h in a water bath adjusted to 70 C°. After cooling, the deionized water was added to 20 mL. Lead (Pb), cadmium (Cd), iron (Fe), copper (Cu),zinc (Zn),manganese (Mn),Selenium (Se), arsenic (As), and mineral composition (sodium (Na), potassium (K),calcium (Ca), magnesium (Mg) concentration were determined by atomic absorption spectrometer (Sens AA), Australia **(Capar and Szefer,2011)** in Toxicology unit, Biochemistry Department, Animal Health Research Institute. The total phosphorous (P) content of the giblets tissues was determined by a colorimetric method after dry ashing mineralization of samples, according to **ISO 13730 method (1996).**

Quantification of mycotoxins using fluorometry method:

Each sample was ground and each 10 g was extracted with 10 ml of a methanol/water mixture (80:20 v/v). After shaking, the mixture was filtered through a filter paper (Whatman no.1), the filtrate was diluted (1:4) with phosphate buffer saline and 2 ml was used to determine total aflatoxins using Aflatest-P immunoaffinity column (VICAM,Watertown, MA, USA).

Ochratoxin A was determined using Ochraprep Immunoaffinity Column Procedures (VICAM, Water-town, MA, USA). The quantities of total aflatoxins and ochratoxin A were determined using recalibrated VICAM Series-4 fluorometer set at 360 nm (excitation) and 450 nm (emission).The samples were analyzed for total aflatoxins and ochratoxin A using immunoaffinity method based on method of **Scott and Trucksess (1997)** in Feed Deficiency Unit, Biochemistry Department, Animal Health Research Institute.

Statistical analysis:

Data were expressed as mean± standard error (SE) and analyzed using one-way analysis of variance (ANOVA) followed by Duncan's test as a post-hoc test using IBM SPSS Statistics 22.0 software package.

RESULTS

Tabes (1-3) indicated that Pb was not detected in all tested broiler chicken giblets except livers samples from retail market of Damanhour and Kafr El-Dawar cities which contain Pb levels above the maximum permissible level (0.5 ppm) according to **FAO (2004),EFSA (2006) and EOSQC (2010)**.However, Cd was not detected in all broiler chicken giblets samples except broiler chicken giblets samples from retail market of Damanhur and Kafr El-Dawar cities in which it was within the permissible limits except liver samples with Cd levels exceeds the permissible limits (0.5 ppm) according to **EFSA (2006) and EOSQC (2010)**. Fe, Cu, Zn, Mn and Se were below the permissible limits (30 mg/kg) stipulated by **(EOSQC, 2010)** for Iron, 15 mg/kg by **(EOSQC, 2010)** for Cu**,** 20 mg/kg, by (**EFSA, 2006)** for Zn, 10 mg /kg by **(EOSQC, 2010)** for Mn and 2 µg/ g by **(ANZFA, 2001)** for Se. In addition, Fe, Cu, Zn levels significantly higher. Se levels were significantly lower in Hosh Esa and Itay-Elbarod giblet samples in comparison to other samples (P <0.05). Furthermore, Arsenic was not detected in all tested broiler chicken giblet samples.

Table (1): Heavy metals concentrations (mg/kg) in broiler chicken liver samples collected from retail markets of 4 cities in El-Beheira Governorate.

Cities / Metals	Damanhour	Kafr-El Dawar	Hosh Esa	Itay-Elbarod	F-Sig.	MRL
Pb	**0.68± 0.03 a**	**0.73± 0.05a**	**ND b**	**NDb**	**0.000***	**(0.5mg/kg) [1]**
Cd	**0.53± 0.01 a**	**0.54± 0.02a**	**ND b**	**ND b**	**0.000***	**(0.5mg/kg) [1]**
Fe	**20.73± 0.24 a**	**22.46± 0.21b**	**25.23± 0.99 c**	**25.09± 0.10 c**	**0.000***	**(30 mg/kg) [1]**
Cu	**4.52± 0.02 a**	**4.49± 0.02a**	**5.44± 0.13 b**	**5.31± 0.13 b**	**0.000***	**(15 mg/kg)[1]**
Zn	**12.74± 0.04 a**	**12.83± 0.09 a**	**16.94± 0.02 b**	**17.00 ± 0.09 b**	**0.000***	**(20 mg/kg) [2]**
Mn	**3.54± 0.11 a**	**3.34± 0.16 a**	**3.63± 0.01 a**	**3.77± 0.01 a**	**0.168**	**(10 mg/kg)[1]**
Se	**1.95± 0.03 a**	**1.99± 0.01 a**	**1.72± 0.02 b**	**1.64± 0.02 c**	**0.000***	**(2 µg/ g) [3]**
As	**ND**	**ND**	**ND**	**ND**	**ND**	**-**

Values are epresented as means of samples ± SE *significantly difference using ANOVA test at P <0.05. Mean in the same row with different letters are significantly different (Duncan multiple range test (P < 0.05). EOSQC (2010)[1] , EFSA (2006)[2], (ANZFA, 2001) [3] ND=not detected, MRL=maximum residue limit. F-Sig =significance of variance between groups.

Table (2): Heavy metals concentrations (mg/kg) in broiler chicken gizzard samples collected from retail markets of 4 cities in El-Beheira Governorate.

Cities / Metals	Damanhour	Kafr-El Dawar	Hosh Esa	Itay-Elbarod	F-Sig.	MRL
Pb	ND	ND	ND	ND	-	(0.5mg/kg) [1]
Cd	0.17± 0.01 a	0.23± 0.08 a	ND b	ND b	0.000*	(0.5mg/kg) [1]
Fe	19.70± 0.23 a	22.05± 0.09 b	24.40± 0.23 c	24.63± 0.22 c	0.000*	(30 mg/kg) [1]
Cu	4.41± 0.07 a	4.40± 0.04 b	5.08± 0.09 c	5.24± 0.14 d	0.000*	(15 mg/kg) [1]
Zn	10.29± 0.17 a	11.42± 0.23 a	12.31± 0.26 c	13.88± 0.04 d	0.000*	(20 mg/kg) [2]
Mn	3.29± 0.05 a	3.18± 0.04 a	3.25± 0.01 a	3.29± 0.01 a	0.132	(10 mg/kg) [1]
Se	1.91± 0.05 a	1.92± 0.02 a	1.64± 0.02 b	1.56± 0.02 b	0.000*	(2 µg/ g) [3]
As	ND a	ND a	ND a	ND a	ND a	-

Values are epresented as means of samples ± SE *Significantly difference using ANOVA test at P <0.05.

Mean in the same row with different letters are significantly different (Duncan multiple range test (P < 0.05). EOSQC (2010)[1], EFSA (2006)[2], (ANZFA, 2001)[3]. ND=not detected. MRL=maximum residue limit. F-Sig =significance of variance between groups

Table (3): Heavy metals concentrations (mg/kg) in broiler chicken heart samples collected from retail markets of 4 cities in El-Beheira Governorate.

Cities / Metals	Damanhour	Kafr-El - Dawar	Hosh Esa	Itay-Elbarod	F-Sig.	MRL
Pb	**ND**	**ND**	**ND**	**ND**	**-**	**(0.5mg/kg)** [1]
Cd	**0.11± 0.006 a**	**0.12± 0.003 b**	**ND**	**ND**	**0.000***	**(0.5mg/kg)** [1]
Fe	**18.65± 0.22 a**	**20.35± 0.22 b**	**22.28± 0.16 c**	**21.13± 0.13 d**	**0.000***	**(30 mg/kg)** [1]
Cu	**4.05± 0.04 a**	**4.13± 0.05 a**	**5.04± 0.09 b**	**4.91± 0.15 b**	**0.000***	**(15 mg/kg)**[1]
Zn	**9.38± 0.21 a**	**8.70± 0.24 a**	**10.42± 0.46 b**	**10.77± 0.20 b**	**0.000***	**(20 mg/kg)** [2]
Mn	**3.12± 0.06 a**	**3.05± 0.03 a**	**3.09± 0.03 a**	**3.15± 0.09 a**	**0.000***	**(10 mg/kg)**[1]
Se	**1.78± 0.03 a**	**1.85± 0.02 a**	**1.55± 0.08 b**	**1.22± 0.04 c**	**0.000***	**(2 µg/ g)** [3]
As	**ND**	**ND**	**ND**	**ND**	**ND**	**-**

Values are epresented as means of samples ± SE *significantly difference using ANOVA test at P <0.05.
Mean in the same row with different letters are significantly different (Duncan multiple range test (P<0.05).EOSQC (2010)[1], EFSA (2006)[2],(ANZFA,2001)[3]. ND=not detected, MRL=maximum residue limit. F-Sig =significance of variance between groups.

(Tables 4 - 6) indicated that P and Ca content were significantly lower in chicken giblets from Hosh Esa and Itay-Elbarod (P <0.05) in comparison to other samples. Heavy metals and macrominerals levels were the highest in liver followed by gizzard and heart of chicken except Na which was the highest in heart followed by gizzard and liver.The order of macro- minerals and heavy metals in all analysed tissues was K> P> Na> Mg> Ca> Fe> Zn> Cu> Mn> Se> Pb> Cd (Tables 1 - 6).

Table (4): Minerals levels (mg/kg) in broiler chicken liver samples collected from retail markets of 4 cities in El-Beheira Governorate.

Cities / Macro-minerals	Damanhour	Kafr-El Dawar	Hosh Esa	Itay-Elbarod	F-Sig
Na	1042.01±22.24 a	1054.75±2.14 a	1035.50±2.10 a	1033.00±2.12 a	0.546
K	2870.00±20.38 a	2836.50±21.11 a	2842.50±24.62 a	2825.25±9.36a	0.456
Ca	130.00±1.29 a	130.25±0.85 a	115.36±0.93 b	116.75±0.95 b	0.000*
P	2738.50±14.80 a	2733.25±12.47 a	2234.50±1.85 b	2235.25±1.31 b	0.000*
Mg	255.00±2.087a	256.14±2.27 a	256.71±0.09 a	254.75±1.89 a	0.942

Values are epresented as means samples ± SE *significantly difference using ANOVA test at P <0.05.

Mean in the same row with different letters are significantly different (Duncan multiple range test (P < 0.05). F-Sig =significance of variance between groups.

Table (5): Macro-minerals levels (mg/kg) in broiler chicken gizzard samples collected from retail markets of 4 cities in El-Beheira Governorate.

Cities / Macro-minerals	Damanhour	Kafr-El-Dawar	Hosh Esa	Itay-Elbarod	F-Sig
Na	1028.75±1.70 a	1029.75±085 a	1031.50±1.19 a	1031.25±0.63 a	0.442
K	2107.00±1.29 a	2107.50±1.29 a	2106.50±2.84 a	2108.50±1.32 a	0.888
P	1632.75±12.68 a	1636.09±10.38 a	1284.30±3.24 b	1282.25±4.70 b	0.000*
Ca	109.25±2.25 a	111.50±1.32 a	88.31±0.89 b	87.25±2.29 b	0.000*
Mg	228.25±6.17 a	229.75±4.33 a	231.75±5.69a	230.75±4.34 a	0.969

Values are represented as means of samples ± SE *significantly difference using ANOVA test at P <0.05.

Mean in the same row with different letters are significantly different (Duncan multiple range test (P < 0.05). F-Sig =significance of variance between groups.

Table (6): Macro-minerals levels (mg/kg) in broiler chicken heart samples collected from retail markets of four cities in El-Beheira Governorate.

Cities / Macro-minerals	Damanhour	Kafr-El Dawar	Hosh Esa	Itay-Elbarod	F-Sig
Na	**1120.50± 0.65 a**	**1163.00± 30.97 a**	**1123.75± 1.49 a**	**1122.00± 0.82 a**	**0.221**
K	**2450.00± 12.91 a**	**2443.00± 12.87 a**	**2456.25± 5.54 a**	**2458.50± 8.84 a**	**0.729**
P	**1529.25± 10.01 a**	**1536.50± 10.28 a**	**1189.50± 4.33 b**	**1181.75± 4.50 b**	**0.000***
Ca	**97.50± 1.19 a**	**98.75± 2.02 a**	**87.32± 0.80 b**	**86.25± 1.85 b**	**0.000***
Mg	**216.00± 2.16 a**	**223.50± 4.72 a**	**222.25± 4.87 a**	**222.75± 3.04 a**	**0.519**

Values are represented as means of samples ± SE *significantly difference using ANOVA test at P <0.05.

Mean in the same row with different letters are significantly different (Duncan multiple range test (P < 0.05). F-Sig =significance of variance between groups.

(Table 7-9) revealed that OTA was not detected in all tested broiler chicken giblet samples. On the other hand, AFs were not detected in all broiler chicken giblets samples from retail market of Damanhour and Kafr El-Dawar cities. AFs were 100%positive in all samples from retail market of Hosh Esa and Itay-Elbarod except in heart samples from Itay-Elbarod which were 75% positive. AFs were significantly higher in all giblet samples from retail market of Hosh Esa and Itay-Elbarod (P <0.05) in comparison to other samples and were below the maximum residue level 4 ppb according to **(FAO,2004)**. In this survey, liver of boiler chickens concentrated AFs, more than did gizzard and heart.

Table (7): Mycotoxins levels (ppb) in broiler chicken liver samples collected from retail markets of four cities in El-Beheira Governorate.

Cities / Mycotoxins	Damanhour	Kafr-El Dawar	Hosh Esa	Itay-Elbarod	F-Sig	MRL
Alfatoxins	ND a	ND a	2.50± 0.29 b	1.25± 0.25 c	0.000*	(4-10ppb)[1]
Ochratoxin A	ND a	ND a	ND a	ND a	-	-

Values are epresented as means of samples ± SE *significantly difference using ANOVA test at P <0.05.

Mean in the same row with different letters are significantly different (Duncan multiple range test (P < 0.05).

[FAO, 2004, EOSQC (2010), EFSA (2006)][1]. ND=not detected, MRL=maximum residue limit.

F-Sig =significance of variance between groups.

Table (8): Mycotoxins levels (ppb) in broiler chicken gizzard samples collected from retail markets of four cities in El-Beheira Governorate.

Cities / Mycotoxins	Damanhour	Kafr-El Dawar	Hosh Esa	Itay-Elbarod	F-Sig	MRL
Alfatoxins	ND a	ND a	1.25± 0.25 b	1.50± 0.29 b	0.000*	(4-10ppb)[1]
Ochratoxin A	ND a	ND a	ND a	ND a	-	-

Values are epresented as means of samples ± SE *significantly difference using ANOVA test at P <0.05. Mean in the same row with different letters are significantly different (Duncan multiple range test P < 0.05). [FAO, 2004, EOSQC (2010) , EFSA (2006)][1]. ND=not detected, MRL=maximum residue limit. F-Sig =significance of variance between groups.

Table (9): Mycotoxins levels (ppb) in broiler chicken heart samples collected from retail markets of four cities in El-Beheira Governorate.

Cities / Mycotoxins	Daman hour	Kafr-El Dawar	Hosh Esa	Itay-Elbarod	F-Sig	MRL
Alfatoxins	**ND a**	**ND a**	**0.75± 0.25 b**	**1.25± 0.25 b**	**0.001***	**(4-10ppb)[1]**
Ochratoxin A	**ND a**	**ND a**	**ND a**	**ND a**	**-**	**-**

Values are epresented as means of 15 samples ± SE*significantly difference using ANOVA test at P <0.05.

Mean in the same row with different letters are significantly different (Duncan multiple range test (P < 0.05).

[FAO, 2004, EOSQC (2010), EFSA (2006)][1]. ND=not detected, MRL=maximum residue limit. F-Sig =significance of variance between groups.

DISCUSSION

Kafr El-Dawar is one of the major industrial cities on the Nile Delta in northern Egypt. This area is unfortunately suffering from factories that let out their waste water into the irrigation canal and agricultural drains. Water resources were mainly contaminated by Cd. According to Cd values, moderate risk was expected even when the water resources was used in short- run. On the other hand, the low values of Zn, Fe, Ni, Pb and Cu of all water resources indicating long use are safe **(Abdelhady El-Hady *et al.,* 2017)**. Al-Mahmoudia canal is located at the northern edge of Beheira Governorate. The canal receives pollutants from different sources. These pollutants lead to significant deterioration of the quality of the water in the canal. The point source of pollutants is Edko drain in El- Beheira Governorate **(Abukila, 2012)**. Significant associations were found between essential metals in the different tissues and non-essential metals. The interactions between them perhaps indicate that mineral balance in the body is regulated by important homeostatic mechanisms in which toxic elements compete with the essential metals, even at low levels of metal exposure **(Abduljaleel *et al.,* 2012).**

At low levels of dietary lead, increased levels of dietary iron and copper was able to reduce lead absorption. However, only when both copper and irons are at optimal levels in the diet, the effect of Pb on Fe metabolism completely inhibited. Pb exposure has been associated with decreased biosynthesis of the hormonal metabolite of vitamin D that is responsible for the metabolism of Ca **(Silva *et al.,* 2005)**.

Similar to the present study, the chicken liver samples in the local markets of Basrah city, Iraq, Pb contents were in the range of 0.171-3.269 mg/kg. Also, maximum permissible limit of Cd was determined 0.5 mg/kg for internal organs **(Hussain *et al.,* 2012)**. In retail markets in Ismailia city, Egypt, the maximum estimated concentrations of Pb were found in livers followed by broiler chicken gizzards and hearts samples **(Loutfy *et al.,* 2017)**. Lower levels of Pb have been reported in the range of 0.01-0.40 ug/g in chicken samples in Turkey **(Uluozlu *et al.,* 2009).** Higher Pb values 1.234 mg/kg were recorded in liver by **(Khan *et al.,* 2015)**. At Libya country, Pb levels in chicken samples were lower than the Saudi Arabia standards 1.0 mg/kg and the highest concentrations of Cd were found in liver and heart. The levels of Cd in liver were exceeding the permitted limits according to some European regulations. It was also indicated that the high concentrations of Cd in some tissues were due to the effect of high levels of these metals in the feed of birds **(Abdolgader *et al* 2013)**.

Liver may accumulate significant amounts of heavy metals followed by gizzard, then heart **(Sadeghi *et al.,* 2015)**. In chicken fed rice contaminated with Pb, Cd, Zn and Cu, the highest levels of Cd and Zn were found in liver which is known specific target organs of metal bioaccumulation. This indicated that dietary exposure pathway poses a potential health risk to local residents. Tissue Pb and Cd concentrations of chickens fed with metal-enriched diets can reflect environmental contamination of poultry production sites **(Zhuang *et al.,* 2014).**

Iron has been reported to play important role as an essential element in all the living system **(Kanakaraju *et al*, 2008)**. Most of broiler chicken by-products had considerably higher amounts of Fe content. The iron in internal organs (e.g., liver and heart etc.) is heme iron whose absorption into the intestinal lumen is several times greater than non-heme iron present in other foods **(Simpson and McKie, 2009).**

Lower than our result, Iron level in liver was 4.65 mg/kg as recorded by **Akan *et al.* (2010)** and Iron ranging from 2.1099 to 7.2834 ppm in the heart of chicken **(Al-Zuhairi *et al.,* 2015)**, this might be due to change in nutrition. Regarding to copper, food is the main source of copper as an essential micronutrient element for man and animal, required for normal biological activity for several enzymes. Excess amount of Cu in food may lead to copper toxicity. The concentration ranges of the elements were: 23.59–97.72 mg/kg for Fe; 0.01-5.15 mg/kg for Cu; 4.95 - 48.23 mg/kg for Zn; 0.01-1.37 mg/kg for Mn; 0.01-5.68 mg/kg for Cd; 0.01 - 4.60 mg/kg for Pb and Cu levels were (1.22 to 4.71 mg/kg) in poultry liver samples from Romania and Belgium as recorded by **Ghimpeteanu *et al.,* (2012)**. But our results were lower than those concentrations (4.90, 9.67 mg/kg) in the gizzard and liver of broiler chicken samples from Malaysia which recorded by **Abduljaleel *et al.* (2012)** and higher than average concentrations of copper in liver of broiler chicken samples were found to be 1.35, 1.861 and 2.254ppm from three selected areas from Pakistan **(Khan *et al.,* 2015)**.The high concentration of Cu was observed in liver samples and that may be due that, the liver is the first organ to encounter the ingested nutrients, drugs, and environmental toxicants that enter the hepatic portal vein from the digestive system **(El - Okle and Lebda, 2014)**.

Concerning zinc, it is constituent of all cells and is an essential trace element, it acts as a co-factor for number of enzymes and it is involved in well over one hundred different reactions in the body **(Khan *et al.*, 2015)**. The higher bioavailability of organic Zn associated with its higher concentration in the hepatic tissues **(Ivanišinová *et al.,* 2016)**.

In the gastrointestinal tract, the inorganic Zn combined with phytic acid found in most of the broilers grains based diets thus impair zinc and calcium absorption **(McDowell, 2003).** In accordance to our result, **Uluozlu *et al.* (2009)** recorded Zn content (7.5–24.3 ug/g) in various parts of chicken samples and chicken products from Turkey and **Akan *et al.* (2010)** who recorded that Zn concentration for chicken samples liver was 9.865mg/kg.

Manganese is involved in skeletal integrity. The degree of mineral absorption based on the synergism (Zn and Mn) or antagonism (Zn and Cu) of different minerals **(Gajula *et al.,* 2011)**. Manganese is a very common compound that can be found everywhere on earth.

It is not only necessary for humans to survive, but it is also toxic when too high concentrations are present in a human body. The concentration of Mn ranged from 3.59 to 32.77 mg/kg. It was higher in the liver of broiler chicken followed by gizzard **(Abduljaleel *et al.,* 2012)**. Similar to our results, Mn content (0.05–3.91ug/g) was recorded in various parts of broiler chicken samples and broiler chicken products from Turkey. Manganese and copper being part of the essential trace minerals are also detected in the entire feed samples **(Uluozlu *et al.,* 2009).**

The high levels of zinc, copper, manganese and iron reflecting their addition to meet animal nutrient requirements**(Alkhalaf*etal.,*2010)**.Selenium isrecognizedasan essential micronutrient in animal and humans, playing important biological roles as antioxidant.

In Malaysia, Se concentration in the broiler chicken tissues were 1.18 and 2.01 mg/kg in the gizzard and liver respectively and Se was below the permissible limit in broiler chicken liver and gizzard and was higher in liver **(Abduljaleel *et al.,* 2012)**. In accordance with this study, the concentrations of Fe, Mn, Cu, and Zn were below the permissible limits while those of Cd, and Pb in some samples were at levels above the permissible limits **(Iwegbue *et al.,* 2008)**. The mineral composition liver was the highest for K, P, Ca, Mg, Fe, Zn, Cu and Mn, while heart was the highest for Na content. K was quantitatively the most abundant mineral in broiler chicken giblets. The order of the minerals in all analysed tissues was K > P > Na > Mg > Ca >Fe > Zn > Cu > Mn. Iron was considered to be the highest mineral in liver and lowest in gizzard. The highest Zn, Cu, Mn content was in liver and lowest in heart.

The lowest copper content was in gizzard, while both heart and gizzard were at the same level regarding manganese content (0.65 mg/kg) **(Jokanović *et al.,* 2014)**.

Arsenic is very important and usually found in the environment in organic and inorganic forms with different bioavailability. Phenyl arsenic acids (organic) are used as poultry feed additive as it controls coccidiosis, improves production performance **(Ghosh *et al*., 2012).** In the present study arsenic was not detected because organic arsenic in the form of arsenobetaine does not undergo biotransformation to other forms in animal body, and it is readily excreted through urine **(Arai *et al.,* 2003).**

Aflatoxins (AFs), a kind of mycotoxins, are the main toxic secondary metabolites of some *Aspergillus* moulds such as *Aspergillus flavus*, *Aspergillus parasiticus* and the rare *Aspergillus nomius.* These fungi are ubiquitous in the environment and produce aflatoxin in warm and high-humidity conditions. Generally, presence of aflatoxins in animal or human bodies cause a disease named Aflatoxicosis **(El-Tras *et al.,* 2011).** The increases of AFB1 in the diet resulting in higher residue levels in animal tissues. Besides the toxic effects, consumption of AFB1 contaminated feeds by poultry may lead to significant economic losses due todecreases in growth performance and meat quality, poor feed utilization and an increase in the incidence of disease in poultry **(Herzallah *et al.,* 2014)**.

In Egypt, 45% of broiler chicken liver, 32% gizzard and 25% heart samples collected by **El-Desouky *et al.* (2014)** from different retail markets were contaminated with AFB1. Aflatoxin residues, especially AFB1 and its metabolites, may be present in the tissues of animals fed with AFB1-contaminated diets to become a potential human health hazard.

In Pakistan, the maximum level of AFB1 and total aflatoxins found in the livers 2.98±0.76 and 3.23±0.82 µg/kg, respectively. This emphasizes the importance of monitoring aflatoxins and their metabolites in poultry products. Overall, the relative high frequency of contamination and AFB1 levels in livers samples from local sector producers-proportionally twice as many as in broiler chicken livers from industrial sector producers-may be explained by the fact that feeding practices and poultry feeds can be major source of aflatoxins **(Iqbal *et al.*,2014)**.The limits of AFB1 and total AF in foods are 5 and 10 µg/kg, respectively, in more than 75 countries around the world whilst they are 2 and 4 µg/kg in the European Union (EU) **(Herzallah,2009)**. Liver is the reservoir place of total aflatoxins residues. The means average of total aflatoxins residues of broiler chicken liver samples were much higher than our result (22.8±4.1µg/kg) **(Hasanena *et al.,* 2016)**. Liver is also the main site where AFB1 is accumulated, metabolized and/or conjugated to nucleic acids and proteins. Residues of AFB1 and some of its metabolites have been detected in liver and gizzard, as a consequence of the direct intake of feed contaminated with aflatoxins **(Yunus *et al.* 2011)**. The residual concentrations of AFB1 and total aflatoxins were higher in liver than in gizzard. In addition to bad chilling, mould contamination of frozen broiler chicken giblets has particular public health significance in the field of food safety due to its related food spoilage and production of mycotoxins as aflatoxins **(Darwish *et al.,* 2016)**.

In accordance to the present study, the levels of calcium and phosphorus were decreased by AFs in broiler chicks that received AFs **(Guerre, 2016)**. The dose of AFs (1 ppm) caused a decrease in blood Ca and P levels and non-significantly change the blood potassium and sodium levels **(Yang *et al.,* 2012)**. Aflatoxin production is also induced by high zinc concentration in feed **(Pattison *et al.,* 2008)**. On the other hand, selenium as feed additive have been reported to reduce the toxicity of AFB1 in chickens **(Sumit *et al.,* 2010)**.

In broilers and layers mycotoxicosis (aflatoxicosis and ochratoxicosis) lowered serum calcium and phosphorus levels but non-significant changes occurred in the blood potassium level **(Qubih, 2012)**. Ochratoxicosis occurs less frequently in poultry than aflatoxicosis but is more lethal because of its acute toxicity **(Pattison *et al.,* 2008)**.

It is characteristic of poultry to have a more efficient and faster excretion of ochratoxins than other animals, approximately 48 hours. The non-detected ochratoxin in all tested samples may be due to that ninety percent of the ingested OTA is excreted **(Elaroussi, 2008)**.

CONCLUSION AND RECOMMENDATION

In conclusion, broiler chicken giblet samples from Damanhour and Kafr El-Dawar retail markets were the worst contaminated samples with Pb and Cd. However, giblet samples from Hosh Esa and Itay-Elbarod were the worst contaminated samples with aflatoxins. Pb and Cd were above the tolerable limit in liver samples from Damanhour and Kafr El-Dawar retail markets, while Fe, Cu and Zn content were in lower levels and within the permissible. However, the higher levels of AFs were accompanied by lower Se, Ca and P contents in liver samples from Hosh Esa and Itay-Elbarod. Heavy metals, macrominerals and AFs concentration were the highest in liver followed by gizzard and heart of broiler chicken except Na which was the highest in heart followed by gizzard and liver.

Regular monitoring of poultry meat by-product and the control of feed and water of poultry safety, assure that a consumer is protected against undesirable higher values of heavy metals and mycotoxins. Thus, strict hygienic precautions during evisceration should be applied to reduce mould contamination and mycotoxin production as aflatoxins.

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الكشف عن تلوث أحشاء الدجاج ببقايا المعادن الثقيلة و السموم الفطرية في محلات التجزئة بمحافظة البحيرة

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الملخص العربى

تم تجميع عدد تسع وستون عينة من أحشاء الدجاج (إثنان وثلاثون من كل من أكباد وقوانص و قلوب الدجاج) من محلات التجزئة فى أربع مدن من محافظة البحيرة (دمنهور,كفر الدوار, حوش عيسى, إيتاى البارود) وقد تم قياس بعض المعادن الثقيلة مثل الرصاص والكادميوم والحديد والنحاس والزنك والمنجنيز و السيلينيوم والزرنيخ وبعض الأملاح كالصوديوم والبوتاسيوم والكالسيوم والماغنيسيوم بجهاز الإمتصاص الذرى والفوسفوربجهاز السبكتروفوتوميتروالسموم الفطرية بجهاز الفلوروميتر فى جميع العينات.

وقد لاحظنا زيادة فى مستوى الرصاص والكادميوم فى أكباد الدجاج من محلات التجزئه فى مدن دمنهور و كفر الدوار وقد تعدت الحدود المسموح بها بينما لم يتم إكتشاف وجود الزرنيخ والأكراتوكسين أ فى كل العينات وكانت مستويات الحديد والنحاس والزنك و المجنيز والسيلينيوم والأفلاتوكسين الكلى فى حدود المسموح بها فى كل أعضاء وأنسجة الدجاج من المحلات المختلفة..

وقد حدثت زيادة ملحوظة فى مستويات الحديد و النحاس والزنك مصحوبا بنقص فى مستوى السيلينيوم فى أحشاء الدجاج من محلات مدن حوش عيسى وإتاى البارود. وقد كان الكبد أكثر الأعضاء إحتواءا على المعادن الثقيلة والأملاح والسموم الفطرية يليه القناصة ثم القلب فيما عدا الصوديوم وقد كان اعلى تركيز له فى القلب ثم القناصة ثم الكبد, بالإضافة إلى زيادة ملحوظة فى مستوى الأفلاتوكسين الكلى مصحوب بنقص ملحوظ فى محتوى الفوسفور والكالسيوم فى أحشاء الدجاج من مدن حوش عيسى وإتاى الباورود.