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ESTIMATION OF SOME MYCOTOXINS AND HEAVY METALS IN THE LIVERS OF LOCAL AND IMPORTED CHICKENS AVAILABLE IN THE LOCAL MARKETS

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ABSTRACT

The current study aimed to estimate the concentrations of each of the metallic elements lead (Pb), copper (Cu), zinc (Zn) and cadmium (Cd) using the atomic absorption spectrometer (AAS) technique, and the residues of some mycotoxins using the High-performance liquid chromatography (HPLC) In samples of fresh, local and frozen chicken livers imported from different origins, which are sold in the local markets of Baghdad, the lead level ranged between (0.1470- 0.3768) µg/g, fresh weight of liver for local and Brazilian, respectively, and for the copper element (0.5032 - 1.8351) µg/g for the local and Brazilian, respectively, and the level of zinc ranged between (3.2890-4.1016) µg/g for the Brazilian and local, respectively, and the level of cadmium was (0.0031-0.1230) µg / g for the Brazilian and local liver, respectively, While the results showed the levels of mycotoxin residues (AFB1: Aflatoxin B1, AFB2: Aflatoxin B2, AFG1: Aflatoxin G1, AFG2: Aflatoxin G2) for liver samples under local study (1.35, 1.86, 1.02 and 0.91) µg/kg, respectively, for AFB1 toxins., AFB2, AFG1 and AFG2, and they reached (1.53, 1.35, 1.10 and 0.83) µg/kg in the Turkish liver, respectively, while in the Brazilian it showed (1.73, 1.62, 1.22 and 0.97) µg/kg.

Keywords: chicken livers, heavy metals, mycotoxin residues.

تقدير بعض السموم الفطرية والمعادن الثقيلة في اكباد الدجاج المحلي والمستورد المتوافرة في الاسواق المحلية

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الخلاصة

هدفت الدراسة الى تقدير تراكيز العناصر المعدنية الرصاص Pb، النحاس Cu، الزنك Zn والكادميوم Cd باستعمال تقنية الامتصاص الذري Atomic Absorption Spectrometer، ومتبقيات بعض السموم الفطرية باستعمال كروماتوكرافي السائل عالي الاداء High-performance liquid chromatography (HPLC) في عينات اكباد الدجاج الطازجة المحلية والمجمدة المستوردة من المناشئ المختلفة المتوافرة في الاسواق المحلية لمدينة بغداد، اظهرت النتائج ان مستوى الرصاص تراوح بين (0.1470 -0.3768) مايكروغرام/ غم، وزن رطب للكبد المحلي والبرازيلي على التوالي، ولعنصر النحاس (0.5032 –1.8351) مايكروغرام/غم للمحلي والبرازيلي على التوالي، وتراوح مستوى الزنك ما بين (3.2890 -4.1016) مايكروغرام/غم للبرازيلي والمحلي على التوالي، وكان مستوى الكادميوم (0.0031-0.1230) مايكروغرام/غم للكبد البرازيلي والمحلي على التوالي، في حين اظهرت نتائج مستويات بقايا السموم الفطرية (AFB1: Aflatoxin B1, AFB2: Aflatoxin B2, AFG1: Aflatoxin G1, AFG2: Aflatoxin G2) لعينات الاكباد قيد الدراسة للمحلي (1.35، 1.86، 1.02 و 0.91) مايكروغرام/ كغم على التوالي

لسموم AFB1 ، AFB2 ،AFG1 و AFG2 وبلغت في الكبد التركي (1.53، 1.35، 1.10 و 0.83) مايكروغرام/ كغم على التوالي، فيما اظهرت للبرازيلي (1.73، 1.62، 1.22 و 0.97) مايكروغرام/ كغم على التوالي.

الكلمات المفتاحية: اكباد الدجاج ، العناصر الثقيلة، بقايا السموم الفطرية.

INTRODUCTION

Food quality and safety is a public health issue of increasing importance, and this issue has become very important in the food sector as a result of the globalization of the food supply and the increase in the complexity of the food chain, as the consumer seeks to buy safe products that do not involve any kind of risk to health (**Alkhafaji, 2020**). Meat of various kinds, including poultry meat and its products, is one of the most important main food sources for humans, which is consumed on a large scale, due to its nutritional value, which is attributed to its content of proteins, fats, carbohydrates, vitamins and minerals. Industrialized countries consume increasing amounts of meat, nearly double the amount consumed in developing countries, apart from poultry meat and its products, which constitute an important part of the human diet as well as an important source of a wide range of nutrients, it may also carry some toxic substances, although the level of these toxic substances in the muscles is generally low, some products such as eggs and viscera such as liver and kidneys showed a higher concentration of toxins compared to other poultry products, and therefore the intake of these toxic substances and their entry into the diet is one of the most important aspects of environmental pollution for humans, which sits on top The food pyramid, and because its food sources are multiple and varied, which increases the variety of pollutants to which it is exposed and increases their accumulation in its various organs **(Mohammed & Abu-Almaaly, 2016; Abbas, 2017).** Environmental pollution with heavy metals is very dangerous because these pollutants are not viable to decompose or break, thus the arrival and accumulation of its residues to the organs of the chicken body through feeding on crops that grow in soil irrigated from the water of artesian wells or drinking water contaminated with heavy metals, as well as from agricultural fertilizers and pesticides, most of the pollutants are characterized by accumulating in the food chains, the farther the location of the food source in the food chain, the greater the concentration of the pollutants in it **(Ali Karm & Alsaffar, 2018)**, although the mineral elements are necessary for animal health, survival and production due to their participation in the biological, structural, motivational and regulatory functions of the organism, the heavy metals in the group of trace elements (iron Fe, copper Cu, zinc Zn, and manganese Mn) are among the needs of the body of the organism, and they play an important role in the physiological functions of the body, but the harmful effect on the organs of the body may also result from exposure to high levels of these elements, heavy metals often have direct physiological toxic effects and are stored or incorporated into living tissues. According to the classification of the European Union, drinking water contaminated with heavy metals, especially lead (Pb) and cadmium (Cd), is dangerous and toxic even at low concentrations (**Al-Soufi *et al*., 2015), (Adekoya *et al*., 2018; Duman, 2019)**, consumption of food and feed contaminated with mycotoxins affects human and animal health due to their potential for carcinogenicity, toxicity, and other adverse effects. Consumption of food and feed contaminated with mycotoxins affects human and animal health due to their potential for carcinogenicity, toxicity, and other harmful effects, and it has been estimated that annually many of the mentioned crops and about 25% of the food supply in the world are contaminated with mycotoxins **(Alaboudi *et al*., 2022).** Mycotoxins are toxic metabolites produced by certain types of fungi as byproducts during their growth on agricultural crops in the field or when storing these crops, the growth and production of these toxins depends on environmental

factors (temperature, humidity, precipitation) and farm management practices (crops, harvest, and storage conditions), and fungal proliferation and subsequent secretion of mycotoxins can occur at any stage of the crop production and marketing chain **(Al-Musawi, 2017; Jallow *et al.*, 2021)**, among the fungi that produce aflatoxins that cause pollution and poisoning mainly are *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius*, and are among the most important and dangerous environmental toxic substances that cause health risks to both humans and animals **(Sineque *et al.*, 2017),** chicken livers are available in the local markets at cheap prices, and they are either imported from different countries through many commercial companies, or they are obtained from slaughtered local chicken, given the openness of the markets to many products and from different origins, this study targeted chicken livers for being the most important in metabolic processes and one of the evidences of any disorder in the animal's body, as well as one of the indicators of the level of environmental pollution through the accumulation of these pollutants in the living tissues of organisms, based on the foregoing, this study was conducted to estimate the concentrations of some mineral elements and residuals of some mycotoxins in fresh local and frozen chicken livers imported from some different origins and their compliance with the permissible limits as stipulated in local and international laws and specifications.

MATERIALS AND METHODS

Sample collection

Thirty samples were collected from fresh local and frozen chicken livers imported from the origins of Turkey and Brazil available in the local markets of Baghdad city, with a weight of 250 each, with three replicates for each sample. They were transported in a refrigerated box to the laboratory and placed in sterile dishes, left at room temperature in order to thaw them, each sample was dried with filter paper, weighed, and the required analyzes were performed on it.

Determination of heavy metal elements

The samples were prepared using the method of wet digestion by heating them in the presence of strong acids and oxidizing agents. The weight of 2 g of liver samples in digestion flasks of 100 mL capacity was digested using 65% nitric acid according to the method mentioned by (**Ali *et al.*, 2020**), the mixture was left for 24 h at room temperature, then the samples were heated on a hot plate with the addition of distilled water gradually and the heating continued to get rid of the nitric acid completely, the mixture was evaporated to a size of 20-25 mm. The digested samples were kept separately in order to estimate their content of metallic elements Pb, Cu, Zn and Cd with the AAS7000 flame atomic absorption device equipped by the Japanese Shimadzu company, according to the measurement conditions approved in the device using acetylene gas, air and length of λ max waveforms for elements: Pb 217.0 nm, Cu 324.8 nm ,Zn 213.9 nm and Cd 228.8 nm.

Estimation of mycotoxin residues

A mashing process was carried out on the homogenized samples, and then they were frozen at -18 ºC and kept in sterile dark containers until the extraction process was carried out according to the method mentioned by (**Sineque *et al.*, 2017)**, as all the glassware used in the experiment was washed with detergents and distilled water, and then sterilized with the autoclave, mix 2 g of previously mashed samples separately with 8 mL of methanol 87.5% in a 15 mL centrifuge tube with vigorous shaking for 3 min with a vortex device, the supernatant

was centrifuged at a speed of 4000 rpm for 10 min at room temperature, then the supernatant was mixed with a mixture of concentrated methanol and shaken for a min using a vortex, mycotoxin residues were estimated using the German-made Sykam HPLC system by estimating the retention time in comparison with the standard toxins for each of the B1, B2, G1 and G2, using a separation column of the type (C18-ODS) with dimensions (250×4.6) mm to separate the phenols, where the fluorescence detector (Ex=365nm,Em= 445nm) was used, with a mobile phase consisting of acetonitrile: distilled water (70: 30) (v:v) respectively, at a flow rate of 0.7 mL/min, after mixing it with a mixer before use to get rid of bubbles invasive.

Statistical analysis

To detect the differences between studied chicken liver samples in the concentrations of heavy metals and mycotoxin residues, the Statistical Analysis System- **SAS (2018)** program was used. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare between means in this study.

RESULTS AND DISCUSSION

The results showed in (Table,1) the concentrations of heavy metal elements in fresh local and frozen chicken liver samples imported from the origins of Turkey and Brazil estimated (µg/g , wet weight), and the metal concentrations recorded significant differences at ($P \leq 0.05$) between samples from different origins, the lead level ranged between 0.1470 and 0.3768 µg/g for the liver of the local and Brazilian, respectively, while the copper element was 0.5032 and 1.8351 µg/g for the local and Brazilian, respectively,

Table (1): Concentration of heavy metals (µg/g, wet weight) in local and imported chicken's livers samples.

Origins of chicken liver samples	Heavy Metals (µg/g)			
	Pb	Cu	Zn	Cd
Local	0.1470 ±0.04 b	0.5032 ±0.10 b	4.1016 ±0.31 a	0.1230 ±0.03 a
Turkey	0.3681 ±0.07 a	1.4610 ±0.17 a	4.1175 ±0.27 a	0.0200 ±0.003 b
Brazil	0.3768 ±0.07 a	1.8351 ±0.23 a	3.2890 ±0.26 b	0.0031 ±0.001 b
LSD value	0.163 *	0.502 *	0.492 *	0.0239 *
Means having with the different letters in same column differed significantly. * ($P \leq 0.05$).				

Zinc level ranged between (3.2890-4.1016) µg/g for the Brazilian and local, respectively. Cadmium levels were recorded as 0.1230 and 0.0031 µg/g for Brazilian and local livers, Pollution with heavy metal elements is one of the most important challenges that threaten human health in Iraq, and the main source of entry for most metal elements into the body is through the diet, and one of the sources of these metals in the environment is the combustion of fossil fuels, mining industries, waste disposal, and sewage, agriculture and forestry also contribute to the mineral content in the environment due to the fertilizers and pesticides used **(Jawad *et al.*, 2021),** it was observed through the results that the concentration of lead is the lowest in the local liver compared to other origins that showed high levels of it (Table, 1), noting that the levels of lead in the samples of Turkish and Brazilian livers were higher than the permissible limits approved by the World Health Organization (WHO), which It should not exceed 0.1 µg/g. perhaps the reason for the presence of these levels of lead in the liver is due to the proximity of pollution sources to poultry farming fields, such as industrial and chemical laboratories, which in turn leads to contamination of soil, water, and diets, and it may also reach the air that poultry inhales, in addition to pollution during the manufacturing

stages in the countries from which it was imported, or to the physiological difficulty of poultry in getting rid of it, which causes the accumulation of this element in the liver, and these percentages are not allowed to be consumed according to what was indicated by the World Health Organization (WHO) and the Food Organization Food and Agriculture Organization (FAO) Food Standards Australia New Zealand **(Faten *et al.*, 2014; Elsharawy, 2015).**

The results of the current study showed that the concentration of copper ranged from (0.5032 - 1.8351) µg/g in the liver samples of local and imported chickens, which is within the permissible limits of 10 µg/g. Copper is an essential element important for the health of all living organisms (humans, plants, animals and microorganisms). it is one of the essential elements required for the activity of many metalloenzymes, but it is toxic in the case of high concentrations because it produces oxidative damage to biological systems, including the oxidation of fats or other large molecules, and copper is often added to poultry feed to promote growth and stimulate immunity **(Agbetiameh *et al.*, 2018; Naqeebullah Khan & Samiullah Khan, 2020).**

Zinc concentration in all studied samples showed that it was within the permissible limits in food and did not exceed (10-50) µg/g in wet weight, as it ranged between 3.2890 - 4.1175 µg/g, (**Supriya *et al.*, 2017)** found that the concentration of zinc in samples of chicken livers of one of the states in India ranged from 0.009 to 0.091 µg / g, which is within acceptable limits and indicated that it is one of the necessary elements for the function of mammalian enzymes.

The highest concentration of cadmium in the local liver under study reached 0.1230 µg/g, while the lowest concentration was in the Brazilian liver 0.0031 µg/g. It accumulates with age, and it has been found that cadmium can affect the metabolism of calcium and phosphorus in the bones, especially in people exposed to it in an environment contaminated with this element, noting that the permissible limit should not exceed 0.5 µg/g **(Faten *et al.*, 2014), (Hossain *et al.*, 2022**) found the concentration of Cd, Pb and Zn in different genres of chicken in Dhaka, Bangladesh reached to 0.351, 1.372 and 64.423 µg/g respectively, it was higher than the recommended value of WHO/FAO and that heavy metals continue to transport from chicken to the human body through the food chain.

Mycotoxins

The results of the total mycotoxins in local and imported livers chicken samples in (Table, 2) showed significant differences at (P≤0.05) among them, the levels of mycotoxins AFB1 increased in the livers of Brazilian chicken, as it reached 1.73 µg/g, the Turkish 1.53 µg/g and the local 1.35 µg/g.

Table (2): Total mycotoxins residues (µg/g) in the local and imported chicken's livers samples.

Origins of chicken liver samples	Mycotoxin residues (µg/g)			
	AFB1	**AFB2**	**AFG1**	**AFG2**
local	1.35 ±0.06 b	1.86 ±0.09 a	1.02 ±0.04 a	0.91 ±0.13 a
Turkey	1.53 ±0.04 ab	1.35 ±0.07 b	1.10 ±0.02 a	0.83 ±0.10 a
Brazil	1.73 ±0.09 a	1.62 ±0.07 ab	1.22 ±0.04 a	0.97 ±0.15 a
LSD value	0.287 *	0.319 *	0.277 NS	0.207 NS
Means having with the different letters in same column differed significantly. * (P≤0.05).				

(El-Tawaab *et al.*, 2019) found that the percentage of aflatoxins AFB1, AFB2, AFG1, AFG2 in the livers of Egyptian chickens amounted to (27, 40.43, 59.42 and 92.15)%, respectively, and its concentration exceeded 10 µg/kg in all samples, which is the maximum allowable level in animal foods, (**Hasanen *et al.*, 2016)** when studying the accumulation of mycotoxins in the tissues and organs of Egyptian chickens, indicated that the liver is a home for the remains of these toxins, especially AFB1, despite its presence in the muscles, stomach, kidneys, and fatty tissues. (6.1, 13, and 2.2) µg/g respectively, for AFB1, AFB2, AFG1, AFG2, **Darwish *et al.*, (2016)** also confirmed in his study in Egypt that the total concentration of aflatoxin was in the liver 3.4 µg/g, which is higher in the kidneys compared to gizzards, while the lowest concentrations were in breast and thigh meat at a concentration of 0.5 µg/g, (**El-Tawaab *et al.*, 2019**) stated that the liver and kidneys of poultry containing mycotoxins intended for human consumption should not be allowed to be consumed, they also indicated that feeding poultry on feed contaminated with mycotoxins is the reason for the high concentrations of toxins in these organs, poor refrigeration is another cause of mold contamination, whereby food spoilage occurs, which promotes the production and increase of mycotoxins such as aflatoxin. The accumulation of aflatoxin residues in human tissues and organs is fatal in acute cases due to poisoning, and in chronic cases it leads to hepatocellular carcinoma, the World Health Organization considered AFB1 to be one of the most toxic and carcinogenic mycotoxins, in addition to its toxic effects, the International Agency for Research on Cancer (IARC) provided sufficient evidence that aflatoxins cause cancer in humans, and the consumption of feed contaminated with AFB1 poultry may lead to significant economic losses due to a decrease in growth performance and meat quality, poor use of feed and an increase in the incidence of diseases in poultry **(Sineque *et al.*, 2017)**.

CONCLUSION

This study found that the concentration of cadmium and lead in different types of local and imported chicken livers was higher than the value recommended by the World Health Organization and the Food and Agriculture Organization (0.1 µg/g for lead and 0.01 µg/g for cadmium), as for copper and zinc, their levels were within acceptable limits (2 µg/g for copper and 50 µg/g for zinc), and the percentage of mycotoxins in all local and imported liver samples was within the limits allowed by the World Health Organization (WHO) (10 µg/kg).

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