

HUMAN HEALTH RISK ASSESSMENT OF HEAVY METALS AND TRACE ELEMENTS RESIDUES IN POULTRY MEAT RETAILED IN SHARKIA GOVERNORATE, EGYPT

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Abstract: The objective of this study was to estimate the residual concentrations of copper (Cu), zinc (Zn), cadmium (Cd), lead (Pb) and mercury (Hg) in muscles and livers of chicken (broilers and layers), turkeys and quails marketed in Egypt. Metal-metal correlations were further analyzed. Moreover, metal's dietary intake and health risk assessment among Egyptian consumers were calculated. Livers had higher metal residues compared with the muscles in all species examined. The ranges ($\mu\text{g/g ww}$) of the elemental concentrations in the livers of the examined bird species were 0.03-0.46 (Cu), 1.77-10.33 (Zn), 0.05-1.09 (Pb), 0.02-0.15 (Cd) and 0.13-0.99 (Hg). Such ranges ($\mu\text{g/g ww}$) in the muscle samples were 0.007-0.60 (Cu), 0.69-4.64 (Zn), 0.01-0.55 (Pb), 0.02-0.13 (Cd) and 0.11-0.94 (Hg). Correlation analysis among metals revealed both tissue-dependent and inter-species differences for the accumulation patterns of metals. The potential risk assessment of all investigated metals in poultry meats revealed no significance risk on Egyptians. However, intake of repetitive small concentrations of metals may lead to severe toxicological implications.

Key words: poultry; heavy metals; risk assessment

Introduction

Poultry meat is an excellent source of high quality animal protein that is required for feeding convalescents, adults, young children and infants (1). Increasing the popularity of poultry meat is mainly due to its ideal chemical composition including essential polyunsaturated fatty acids, essential amino acids, as well as easy digestibility, availability, acceptance and low price compared with other meat (2).

Contamination of meat with heavy metals is a serious hazard because of their toxicity, bioaccumulation and biomagnification in the food (3). The main sources of poultry exposure

to heavy metals are feed, drinking water and litter contamination (4,5). These metals find their way to the human food mainly via the consumption of contaminated meat (6).

Toxic metals with severe health hazards include lead (Pb), cadmium (Cd) and mercury (Hg) (7). Human exposure to heavy metals may lead to several acute or chronic adverse effects (8). The main toxic effects of lead include hypertension, reproductive dysfunction, gastrointestinal track damage, nephropathy, and damage of central and peripheral nervous systems (9). The toxicity of cadmium is linked to its interactions with zinc and other essential elements leading to severe renal dysfunction and

respiratory symptoms (10). Mercury is a neurotoxin that causes neuro-behavioral alterations, renal damage and gastrointestinal toxicity (11). Zinc (Zn) and copper (Cu) are essential trace elements that play several physiological and biological roles in maintenance of animal performance and health, however, high intake of these elements can cause health problems (12).

As humans are on the top of the ecosystem, it is essential to evaluate their dietary exposure to such toxicants (13). Moreover, the human health risk assessment due to consumption of poultry meat contaminated with heavy metals is a major task of food hygiene. Therefore, this study aimed to estimate the concentrations of metals (Cu, Zn, Cd, Pb and Hg) in fresh poultry meat samples (muscle and liver) collected from Sharkia Governorate, Egypt and to evaluate the risks associated with consumption of such meat. Furthermore, metal-metal correlations were analyzed to investigate their inter-species differences in metal accumulation pattern.

Material and methods

Chemicals and reagents

The standards of the tested metals and other reagents including nitric and perchloric acids were of analytical grade and purchased from Merck, Darmstadt, Germany.

Collection and preparation of samples

Eighty samples from breast muscles and liver of layers, broilers, turkeys and quails (10 samples, each) retailed for human consumption were randomly collected from Zagazig city, Sharkia Governorate, Egypt. After being sacrificed in poultry meat shops, 10 grams from each sample were identified and wrapped separately in polyethylene bags, then stored at -20°C until metal extraction and measurements. The analysis of heavy metals (Cu, Zn, Cd, Pb and Hg) was estimated by Atomic Absorption Spectrometry (AAS) (Perkin Elmer model (spectra-AA10, USA) in the Central Laboratory, Faculty of Veterinary Medicine, Zagazig University, Egypt.

Analytical procedures

One gram of each tissue sample was digested overnight in 5 mL volume of a mixture 3 volumes of 65% nitric acid and 2 volume of 70% perchloric acid (14, 15). The tubes were moist heated for 3 h at 70°C followed by cooling and filtration. The filtrates were kept at 4°C until measurement of the metals. Levels of Hg were measured using hydride generation/cold vapor atomic absorption spectroscopy, while in case of Cu, Zn, Pb and Cd, a graphite furnace was used (Perkin Elmer® PinAAcle™ 900T atomic absorption spectrophotometer (Shelton, CT, USA).

Quality assurance and quality control

The standard reference (DORM-3, fish protein) (National Research Council, Canada) was used for validation of the analytical method. The recovery rates (%) for Cu, Cd, Pb, Zn and Hg were 98%, 98%, 97%, 96% and 95, respectively. The instrumental limits of detection (LOD) were 0.02, 0.005, 0.01, 0.02 and $0.02\text{ }\mu\text{g/g}$ for Cu, Cd, Pb, Zn and Hg, respectively. The concentrations of Cu, Cd, Pb, Zn and Hg were expressed as $\mu\text{g/g}$ wet weight (ww). Residual metal concentrations in the examined samples were compared with the maximum permissible limits of metals set by FAO/WHO (16).

Metal's dietary intakes and health risk assessment

The potential health risks of Egyptian adults due to consumption of metal-contaminated poultry meat were assessed based on calculation of the estimated daily intake (EDI), hazard quotient (HQ), and hazard index (HI) of the metals examined. EDI was calculated using the equation described by the United States Environment Protection Agency (USEPA) (17) as follows

$$\text{EDI} = (\text{C} \times \text{FIR}) / \text{BW}$$

Where, BW is the body weight of the Egyptians adults, which was set as 70 kg. C is the concentration of the metal in the tested samples ($\mu\text{g/g}$ ww). FIR is the rate of the ingestion of poultry meat (muscle) in Egypt,

which was estimated to be 39.53 g/day (18) and the ingestion rate of liver tissue was set as 0.1 g/day (19). EDI values were expressed as ($\mu\text{g/kg BW/day}$) for the heavy metals. EDI values were then compared with metal's tolerable daily intakes (TDIs) (FAO/WHO) (20).

The assessment of heavy metal-related non-cancer risks followed the guide lines recommended by USEPA (21) using the following equation:

$$\text{THQ} = \frac{\text{ED} \times \text{C} \times \text{FIR} \times \text{EF}}{\text{RFD} \times \text{BW} \times \text{AT}} \times 10^{-3}$$

Where THQ is the target hazard quotient; C is the heavy metal concentration in poultry meat ($\mu\text{g/g}$); FIR is the food ingestion rate (g/day); ED is the exposure duration (70 years, average lifetime); EF is exposure frequency (365 days/year); RFD is the oral reference doses for Cu, Cd, Pb, Zn and Hg (0.04, 0.001, 0.004, 0.3 and 0.0016 mg/kg BW/day) (22). AT is the average exposure time (365 days/ year \times exposure years, assumed as 70 years); BW is the average adult body weight (70 kg).

Hazard index (HI) was generated to evaluate the risk of the combined metals using the following equation: $\text{HI} = \sum \text{HQ}_i$, Where i represent each metal. When the value of HQ and/or HI exceeds 1.0, there is a concern for potential health effect (23).

Statistical analysis

Heavy metals concentrations were recorded as means \pm standard errors (SE). Results were analyzed using one-way analysis of variance (ANOVA) (SPSS for Windows 21.0). Duncan's test was used for statistical analysis. Comparing metals in muscles and liver of the same bird was done by T-test. The value of $P < 0.05$ was used to indicate statistical significant differences. Correlation analyses among the measured metals were calculated based on Spearman's coefficient using JMP program (SAS Institute, Cary, NC, USA).

Results and discussion

Levels of the tested heavy metals

Residual concentrations of copper ($\mu\text{g/g ww}$) were recorded in Table (1). Samples collected from turkeys had the highest Cu residues (0.13 ± 0.12) compared with other bird species, particularly in the muscle. The recorded concentrations in the present study were far below the values recorded in muscles of broilers and quails in Egypt (6, 24). However, copper residues were below the detection limit on livers of broilers in Philippines (25). All examined samples in the present study had copper residues within the permissible limits set by FAO/WHO and Egyptian authorities (16,26)

Table 1: Heavy metal residual concentrations in the examined meat samples from different poultry species (n = 10 each)

Item		Cu		Zn		Pb		Cd		Hg	
		Mean \pm SE (Range)	%	Mean \pm SE (Range)	%	Mean \pm SE (Range)	%	Mean \pm SE (Range)	%	Mean \pm SE (Range)	%
Layers	Muscle	0.08 \pm 0.02 ^{ab} (0.06-0.14)	0	2.59 \pm 0.10 ^b (2.26-2.86)	0	0.10 \pm 0.05 ^a (0.01-0.24)	40	0.06 \pm 0.01 ^{ab} (0.02-0.10)	80	0.45 \pm 0.14 ^a (0.11-0.83)	80
	Liver	0.18 \pm 0.03 ^{a*} (0.11-0.25)	0	5.53 \pm 0.45 ^{a*} (4.81-7.32)	0	0.21 \pm 0.05 ^a (0.05-0.34)	0	0.06 \pm 0.01 ^a (0.02-0.09)	0	0.41 \pm 0.07 ^b (0.20-0.60)	20
Broilers	Muscle	0.04 \pm 0.02 ^b (0.007-0.11)	0	2.46 \pm 0.30 ^{bc} (1.26-2.88)	0	0.31 \pm 0.04 ^a (0.17-0.4)	100	0.09 \pm 0.01 ^a (0.05-0.13)	80	0.62 \pm 0.11 ^a (0.23-0.94)	100
	Liver	0.21 \pm 0.03 ^{a*} (0.10-0.29)	0	4.76 \pm 0.44 ^{a*} (3.69-5.86)	0	0.34 \pm 0.06 ^a (0.19-0.48)	0	0.10 \pm 0.01 ^a (0.04-0.15)	0	0.65 \pm 0.12 ^a (0.23-0.99)	80
Turkeys	Muscle	0.13 \pm 0.12 ^a (0.04-0.60)	0	3.70 \pm 0.33 ^a (2.94-4.64)	0	0.18 \pm 0.10 ^a (0.01-0.55)	80	0.07 \pm 0.01 ^{ab} (0.03-0.1)	60	0.44 \pm 0.11 ^a (0.17-0.79)	80
	Liver	0.26 \pm 0.08 ^a (0.19-0.46)	0	5.56 \pm 1.21 ^a (3.51-10.33)	0	0.45 \pm 0.19 ^{a*} (0.16-1.09)	20	0.10 \pm 0.01 ^a (0.06-0.14)	0	0.47 \pm 0.13 ^{ab} (0.13-0.88)	40
Quails	Muscle	0.10 \pm 0.05 ^{ab} (0.02-0.28)	0	1.13 \pm 0.35 ^c (0.69-2.54)	0	0.18 \pm 0.08 ^a (0.01-0.46)	40	0.04 \pm 0.01 ^b (0.03-0.08)	60	0.52 \pm 0.05 ^a (0.43-0.71)	80
	Liver	0.15 \pm 0.05 ^a (0.03-0.27)	0	2.40 \pm 0.38 ^b (1.77-3.82)	0	0.34 \pm 0.05 ^a (0.19-0.45)	0	0.08 \pm 0.02 ^a (0.04-0.12)	0	0.70 \pm 0.15 ^a (0.14-0.97)	40

Means within the same sample (muscle or liver) carrying different subscripted letter are significantly different ($p < 0.05$) based on Duncan's multiple comparisons. *indicates significant difference among muscle and liver of the same bird species. % indicates percentage of samples exceeding maximum permissible limits (MPL) for the tested heavy metals set by Egypt organization for Standardization (EOS 2010)(26) and FAO/WHO (2000) (16). MPL for Cu, Cd, Pb, Zn and Hg ($\mu\text{g/kg ww}$) in muscles were set to be 15.00; 0.05, 0.1, 50, 0.2, respectively; in liver, they were set to be 15.00, 1.00, 0.5, 50, 0.5, respectively.

In the present study, residual concentrations of zinc were the highest among the examined elements as shown in Table (1). This may be attributed to the addition of zinc to the poultry feed formula for improvement of the physiological body functions (27). Similar to copper, turkeys had the highest residual concentrations of zinc in the muscles among the examined avian species ($3.70 \pm 0.33 \mu\text{g/g ww}$). The detected levels of zinc in the current investigation were comparable to 1.29 and 1.28 ($\mu\text{g/g ww}$) in the muscles of quails reared in either battery or deep litter systems, respectively in Egypt (4) and lower than those recorded in broilers examined in; Egypt (24), India (28, 29) and Zambia (30) and in free-range chicken from Ghana (31) and Nigeria (32). Zinc levels were within the accepted permissible limits of zinc in meat (16).

Residual concentrations of cadmium were recorded in Table (1). The ranges ($\mu\text{g/g ww}$) of Cd residual concentrations in the livers and muscles of the examined bird species were 0.02-0.15 and 0.02-0.13, respectively. Levels of

Cd in livers and muscles of the examined species were comparable to that recorded in broilers (33) and quails (6) examined in Egypt and in broilers from India (29). However, higher Cd concentrations were recorded in the livers of free-range chicken collected from Poland (34), Zambia (30), Ghana (31), Egypt (35) and Nigeria (32) and in turkeys collected from Spain (36). Levels of cadmium recorded in muscles were 80%, 80%, 60% and 60% in broilers, layers, turkeys and quails, respectively (Table 1) that exceeded maximum permissible limits of cadmium (16,26) This is may be attributed to the high levels of cadmium in poultry ration, drinking water and/or environment (6,37).

Lead is an environmental toxin that is linked to several health adverse effects (15). The obtained concentrations of lead in the present study were shown in Table (1). The results indicated that turkeys had the highest lead residues among the examined avian species. The average Pb residual concentrations ($\mu\text{g/g ww}$) in the muscles and livers of turkeys were

0.18±0.10 and 0.45±0.19, respectively. The recorded concentrations in the present study were in agreement with the recorded concentrations in poultry meat samples collected from Poland (34), India (29), Egypt (38). However, lower concentrations of Pb were reported in turkeys from Spain (36), quails from Egypt (6) and broilers from Philippines and Zambia (25,30). Lead had exceeded the established MPL (16,26) in all examined bird species with different percentages as indicated in Table (1). This might be due to the contamination of birds' feed and water with lead that is commonly used in the manufacturing of water pipes (39) and might be contained in bone and fish meals (40).

Mercury occurs naturally in the environment at low levels and characterized by its high bioaccumulation and biomagnification nature (41), it can find its way to food chain via industrial wastes (42), water, feed, dust and soil (31,43). The mean concentrations of Hg in the current study were declared in Table (1). The recorded mercury concentrations in the livers of broilers (0.65±0.12 µg/g ww) were lower than that recorded in broilers in India (28) and in free-range chicken from Ghana (31) and higher than those recorded in poultry meat in Philippines, Zambia and Nigeria (25, 30, 32). In the present study, 80%, 100%, 80% and 80% of the examined samples of layers, broilers, turkeys and quails, respectively were higher than the recommended maximum permissible limits of Hg (16,26).

In general, liver had higher accumulation pattern for metals compared with muscle in the examined avian species, this is may be attributed to the fact that liver is the organ of metabolism and detoxification of xenobiotics in humans and animals (6,15)

Correlation analysis among metals in poultry meat

In order to investigate the inter-metal's tendency of accumulation, Spearman's coefficient factors were calculated in both liver and muscle tissues of the different birds as indicated in Tables (2 and 3). Interestingly, the achieved results revealed both interspecies and tissue-dependent variations in the tendency of accumulation of the tested metals. For instances, significant negative correlations were observed between the essential trace elements as copper and zinc from one side and the toxic ones from the other side as clear in case of Cd-Cu ($r = -0.21$, $p = 0.74$ in layers and $r = -0.67$, $p = 0.22$ in layers); Cd-Zn ($r = -0.87$, $p = 0.05$) and Hg-Zn ($r = -0.60$, $p = 0.28$). This may be attributed to the physiological trials of the body for detoxification of such toxicants. Essential elements such as copper and zinc were reported to induce metal binding proteins and antioxidant enzymes (6). These findings agreed with the inter-metal correlations detected in the livers of cattle and sheep (15), chicken (31) and quails (6).

Table 2: Correlation analysis among the examined metals in the muscle tissues of different poultry species

		Layers		Broilers		Turkey		Quails	
		<i>R</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
Cd	Cu	-0.15	0.81	0.81	0.01	-0.80	0.11	0.11	0.87
Pb	Cu	0.9	0.04	0.45	0.45	0.45	0.45	-0.11	0.87
Pb	Cd	0.15	0.81	0.05	0.93	-0.05	0.93	-1.00	<.0001
Zn	Cu	-0.51	0.39	-0.45	0.45	0.11	0.86	0.71	0.01
Zn	Cd	-0.56	0.32	-0.62	0.27	0.11	0.87	-0.11	0.87
Zn	Pb	-0.77	0.05	-0.11	0.87	-0.31	0.62	0.11	0.87
Hg	Cu	0.31	0.62	-0.89	0.04	-0.67	0.02	-0.20	0.75
Hg	Cd	0.41	0.49	-0.62	0.27	0.15	0.81	-0.40	0.51
Hg	Pb	0.44	0.51	-0.7	0.05	-0.71	0.05	0.41	0.51
Hg	Zn	-0.90	0.04	0.61	0.04	0	1.00	0.50	0.39

r: spearman's correlation factor; *p*: probability and Values in bold show significant correlation between each two metals.

Table 3: Correlation analysis among the examined metals in the livers of different poultry species

		Layers		Broilers		Turkeys		Quails	
		<i>r</i>	<i>P</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
Cd	Cu	-0.21	0.74	-0.67	0.22	0.58	0.31	-0.21	0.74
Pb	Cu	-0.20	0.75	-0.56	0.32	0.65	0.02	0.31	0.62
Pb	Cd	-0.41	0.49	0.68	0.02	-0.14	0.82	-0.15	0.81
Zn	Cu	-0.31	0.62	-0.60	0.28	-0.86	0.05	0.31	0.62
Zn	Cd	-0.87	0.05	0.87	0.05	-0.29	0.63	0.67	0.02
Zn	Pb	0.51	0.39	0.56	0.32	-0.55	0.34	0.31	0.62
Hg	Cu	-0.11	0.87	0.15	0.80	-0.59	0.29	0.1	0.87
Hg	Cd	0.67	0.22	-0.13	0.83	-0.34	0.57	0	1.0
Hg	Pb	-0.40	0.50	-0.29	0.64	-0.08	0.89	0.31	0.61
Hg	Zn	-0.60	0.28	0.36	0.55	0.87	0.05	-0.36	0.55

r: spearman`s correlation factor; *p*: probability and Values in bold show significant correlation between each two metals.

Health risk assessment

Daily intake of heavy metals

Estimation of dietary intake of metals is considered as a good tool for identification of the human exposure frequencies to heavy metals. The estimated daily intakes of Cu, Zn, Cd, Pb and Hg were assessed based on the average concentrations of the accumulated metals as indicated in Table (4). The estimated metal intake from consumption of muscles and

livers of poultry were lower than the tolerable daily intake of Cu (500), Cd (1.00), Pb (3.57), Zn (300-1000), and Hg (0.71) (20). This may indicate that such metals had minimum potential health risks from consumption of poultry meat in the study area. Similarly, Darwish *et al.* (6) reported that the EDIs of Pb and Cd from consumption of the edible tissues of the quails were below TDI.

Table 4: Estimated daily intake (EDI) ($\mu\text{g}/\text{kg BW}$) of different tested metals tested

Examined samples		Cu	Zn	Pb	Cd	Hg
Layers	Muscle	0.045	1.463	0.057	0.034	0.254
	Liver	0.0003	0.008	0.0003	0.0001	0.001
Broilers	muscle	0.023	1.389	0.175	0.051	0.350
	liver	0.0003	0.007	0.001	0.0001	0.001
Turkeys	muscle	0.073	2.089	0.102	0.040	0.249
	liver	0.0004	0.008	0.001	0.0001	0.001
Quails	muscle	0.057	0.638	0.102	0.023	0.294
	liver	0.0002	0.003	0.001	0.0001	0.001
TDIs		500	300 - 1000	3.57	1	0.71

EDI: estimated daily intake and TDI: tolerable daily intake.

Target hazard quotient and hazard index

Heavy metals were highly accumulated in the livers compared with the muscle. However, the potential risks from consumption of the livers were minor when compared with the muscle owing to their extremely low ingestion

rates (5). The non-carcinogenic risks from consumption of poultry meat were assessed based on the THQs and HI as indicated in Table (5). Amongst all the examined metals, Hg in muscle of layers posed the highest potential health risk (0.219), while Cd was the lowest (Table 5). In general, THQ and HI values were

far below one, which indicate minimum potential hazards for Egyptian consumers from poultry meat. However, this finding should be handled carefully due to the extensive consumption of poultry meat to compensate the shortage in the red meat in Egypt, which may

lead to higher accumulation for metals. Therefore, continuous screening for metal accumulation in the poultry meat should be conducted. The recorded THQ and HI values corresponded with that reported in Ghana, Nigeria and Egypt (6,31,32).

Table 5: Hazard analysis due to consumption of poultry meat in Egypt

Examined samples		THQ Cu	THQ Zn	THQ Pb	THQ Cd	THQ Hg	HI
Layers	muscle	0.001	0.005	0.014	0.034	0.159	0.213
	liver	>0.000	>0.000	0.001	>0.000	>0.000	0.001
Broilers	muscle	0.001	0.005	0.043	0.051	0.219	0.319
	liver	>0.000	>0.000	0.0001	>0.000	0.001	0.001
Turkeys	muscle	0.002	0.007	0.02	0.040	0.155	0.229
	liver	>0.000	>0.000	0.0002	0.000	>0.000	0.001
Quails	muscle	0.001	0.002	0.025	0.023	0.184	0.235
	liver	>0.000	>0.000	>0.000	>0.000	>0.001	0.001
RFD		0.04	0.3	0.004	0.001	0.002	

THQ: target hazard quotient; RFD: oral reference dose for each metal; HI: hazard index.

Conclusion

The present study revealed accumulation of Cu, Zn, Cd, Pb and Hg in the livers and muscles of layers, broilers, turkeys and quails marketed in Egypt. Metals varied in their accumulation tendency based on tissue and species. The recorded concentrations had exceeded maximum permissible limits for Cd, Pb and Hg. Non-carcinogenic risk assessment of the examined metals revealed minimum risk among Egyptian populations due to consumption of poultry meat.

Conflict of interest

The authors declare no conflict of interest.

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