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Monitoring of Heavy Metal Residues in the Meat of Some Game Birds with Insight into Their Health Risk Assessment

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Abstract

Due to their great nutritional value, distinct scent and taste, and lower price in contrast to other usual sources of protein, game birds' meat products are in higher demand. Toxic metals including lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As) are still present in these items, but there is a glaring lack of information on their presence. Therefore, this analysis was done to determine the residual level of lead, mercury, cadmium and arsenic present in three different types of game bird's meat, including pigeons, quails and sparrows. According to the study's findings, Pb, Cd, Hg, and As were detected at variable concentrations in the tested samples. Residual concentrations of the tested metals in several samples are over the suggested maximum permissible limits (MPL). However, Cd level was low in all samples and did not exceed MPL. Furthermore, the Hazard Quotient (HQ) ranged from 0.04 to 0.22 for cadmium, 1.16 to 1.34 for arsenic, 0.06 to 0.07 for lead and 0.05 to 0.35 for mercury. Although HQs of studied heavy metals except for arsenic did not exceed 1, it supposedly demonstrates that eating the meat of game birds does not pose a significant health risk to people for ingesting the particular metals.

KEYWORDS
Game meat, Heavy metals, Pigeons, Quails, Risk assessment.

INTRODUCTION

Whether native or non-native, game birds available in the market today are those raised for their meat, eggs, or to be released by state wildlife agencies or in hunting areas as 'flight-ready" birds. In the past, game birds referred to wild game or attractive fowl. Some types of game birds include guinea fowl, peacocks, partridges, pheasants, pigeons and doves, quail or squab (a young pigeon), swans, wild turkeys, and some ducks, such as mallards or wood ducks. Only a tiny fraction of pheasants, partridges, and quail types are brought up as flight-ready birds; an astounding range of species and variants are raised for the "decorative pet" exhibit or hobby market (Park *et al.*, 2008).

Game bird meat is the perfect solution to meet the need for healthy foods. Game birds have higher protein and lower fat content than other meats; quail, for example, has a high iron content. While game bird industry can expand its specialized market by diversifying, the meat will still be considered an exceptional item. Different nations produce and consume game bird meat in different ways. Compared to the UK, where the game bird business is significant, Finland's consumption of game bird meat is still quite low (Horigan *et al.*, 2014). Game bird meat typically promotes local production and is connected to healthy, ethical meat production. It is still an uncommon delicacy, though, and is typically given to consumers and restaurants straight from hunters. The slaughter of game birds is often carried out by hunters and is less regulated than that of farm animals (Sauvala *et al.*, 2021).

Ingestion of chemical substances, containing zinc, or less frequently, lead is the most common cause of heavy metal toxicity in birds. Due to their curiosity and natural urge to forage, companion parrots that eat or chew on metal objects can develop acute heavy metal toxicity. The most common victims of chronic lead poisoning are free-ranging animals including ducks, geese, swans, and loons. The late autumn and early spring migratory periods are when lead toxicity in wild birds is most frequently observed. Toxicity can be seen at any time of the year in highly contaminated regions. Upland game birds including pheasants, quail, wild turkeys, and mourning doves (Zenaida macroura) can infrequently get lead toxicity as well. Raptors and small animals like raccoons (Procyon lotor) have also been recorded to suffer from lead poisoning, most likely by consuming lead-contaminated prey (Stevenson, 2002).

Heavy metals such as lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (AS) are distinguished by their bioaccumulation and biomagnification properties.

Human exposure to such metals might have a number of deleterious consequences (Morshdy *et al.*, 2018). Paint, plaster, galvanized wire, fishing weights, bullets, ammunition, costume jewelry, stained glass, bells or other toys, twist ties, and linoleum are just a few of the potential sources to heavy metals exposure. A comprehensive history is the first step in making the diagnosis of heavy metal toxicity. Depending on the type of bird and the amount of metal consumed, clinical symptoms can differ substantially. If the owner notes any chewing on baseboards or walls, any new toys or enclosures, or any construction that could release lead paint into the air, toxicity may become a more plausible concern. While chronic consumption of lower amounts may manifest as progressive neurologic symptoms, sudden ingestion of a high amount of metal may induce immediate symptoms or death (Kanstrup, 2012). In light of the aforementioned information, this study attempted to evaluate the residual mercury, lead, arsenic and cadmium content in three birds meat products encompassing quails, sparrows, and pigeons marketed in Egypt. Additionally, the health risks related to consuming these products were analyzed.

MATERIALS AND METHODS

Samples collection

Randomly selected samples of muscle (~30 grams each) were taken from pigeons, quails, and sparrows (10 of each group) from Damietta Governorate, Egypt. Each sample was preserved in a plastic bag and labelled on the identification card with the type of sample. After that, samples were delivered to the lab, where wet digestion was carried out. This cross-sectional study was performed on game birds at Department of Food Hygiene, Safety, and Technology, Faculty of Veterinary Medicine, Zagazig University, Egypt.

Washing procedures

Washing is a crucial step in preventing contamination of tubes, plastic items, and glasses since analysis and determination of heavy metals are extremely sensitive processes to any residual contamination. Water and soap are the first two washing steps. They were used to soak glassware and plastic containers for two hours before rinsing them repeatedly using sterile water (El-Seady, 2001). The containers used in the experiment were cleaned and then rinsed with a solution comprised of 520 ml of deionized water, 200 ml of concentrated HCL, and 80 ml of H_2O_2. After carefully cleaning the containers with de-ionized water, they were allowed to air dry in an incubator avoid any contaminating or dusty sources.

Digestion procedures

Following the acid mixture digestion of the samples, the remaining metal matter dissolves, and the resulting solution is aspirated into an atomic absorption spectrometer (AAS). Wet digestion has been chosen in this study as the preferred approach for volatile metals like Mercury and metals that create volatile salts when heated with acids like lead. There are many different methods of digestion, including dry and wet digestion. A computerized atomic absorption spectrometer (AAS) from Perkin Elmer model (spectra-AA10, USA) was used to identify heavy metals.

In a screw-capped tube, one gram of each sample was macerated with a sharp scalpel. The tissue sample received 5 ml of an acid digestion combination (3 ml HNO_3: $2HClO_4$) (Zantopoulos *et al*., 1996). The used tubes were firmly closed, and the contents were shaking erratically before being let to stand at room temperature for an entire night. To ensure that the materials were completely digested, these tubes were then heated for three hours in a water bath set at 70°C. Throughout the heating period, the digesting tubes were shaken ferociously every 30 minutes. After then, room temperature was reached for the tubes, with 20 ml of deionized water dilution, after which filter, paper was used to clean each tube. To analyze the filtrate for the presence of heavy metals, glass tubes were used to collect the filtrate, covered with polyethylene films, and maintained at room temperature (Tsoumbaris, 1990).

Determination

Instrumental protocols for various analyses were based on those recommended in the atomic absorption spectrophotometer's operator's manual. However, blanks and standards were made using the same procedures and chemicals as for wet digestion.

Preparation of blank solution

Nitric acid and perchloric acid were combined to make the blank solution, which was then diluted with 20 parts of deionized water using a method akin to wet digestion. The potential for heavy metals to be present in the chemicals used in aqueous digestion was evaluated using the blank. For each metal, standard solutions made with pure certified metals were produced.

Atomic absorption spectrophotometer (AAS)

The ideal option is to employ an atomic absorption approach due to its accessibility and simplicity. Heavy metals' quantitative determination was done by "Buck scientific 210VGP Atomic Absorption Spectrophotometer" at the Faculty of Veterinary, Zagazig University. By using an atomic absorption spectrophotometer (AAS), the heavy metal contents of the digest, blank, and standard solutions were aspirated were examined. At the Central Laboratory of the Faculty of Veterinary Medicine at Zagazig University, analyses of, mercury, lead, arsenic and cadmium were performed. Air/acetylene flow (5.5/1.11/m) flame (AAS) was used to conduct it.

Quantitative determination

Lead, cadmium, mercury, arsenic concentrations were calculated using the following equation and recorded immediately from the AAS digital scale: R*D/W = element (ppm). Where, R = Digital AAS scale reading of element concentration in ppm. D is the prepared sample's dilution. W is the sample's weight. Each sample that was subjected to analysis had its concentration or absorption values for heavy metals calculated and removed. Lead, cadmium, mercury, and arsenic registered values were given as μg/g wet weight (ppm).

Health risk assessment

The non-cancer risks associated with the consumption of game bird meat products contaminated with metal among the Egyptian population were identified using the US EPA (2010) recommendations. The hazard ratio (HR) was calculated by comparing the estimated daily intake to the recommended reference doses (RfD) (0.001 for Cd, 0.004 for Pb, 0.0003 for As, and 0.0005 mg/kg/day for Hg). HR= EDI/RfD*10-3. A hazard index (HI) can be created by adding the hazard ratios to estimate the health hazards posed by combined pollutants. HI = ΣHRi, where i stands for each meal. A result of one or less shows no risk, whereas a value of more than one suggests possibly endangering people's health.

Statistical analysis

The Shapiro-Wilk test was utilized to normalize the data. For regularly distributed data, continuous variables are expressed as a mean with standard deviation, and for non-normally distributed data, they are expressed as a median with an interquartile range (25th-75th percentile). The means and standard deviations of the groups will be compared using the ANOVA test. The Spearman's rank correlation test was used to conduct the correlation analysis. Numbers and percentages (%) are used to express categorical variables. P-values lower than 0.05 were regarded as statistically significant. SPSS, Version 25, was used to analyze each and every outcome.

RESULTS

Table 1 shows mean and standard deviation of heavy metals concentrations between three groups, in which mean concentration of arsenic in the three groups was high on the other hand the mean concentration of cadmium was low in the three groups. there is no statistically significant difference between three types of game birds regarding each element of measured heavy metals.

Table 2 shows an overview of heavy metal residues statistics using other values than mean and standard deviations due to presence of non-normally distributed data in pigeons, quails and sparrows. Each group includes ten samples. Among pigeons, there were differences between samples, as evidenced by their median mercury concentrations 0.00 with IQR 0.13. Add to that lead concentrations median was 0.2 with IQR 0.87. in the same context cadmium concentrations median was 0.00 with IQR 0.04. on the other hand, arsenic median concentration was slightly higher than other measured heavy metal which was 4.24 with IQR 2.27. Among quails. In harmony with the previous results of pigeons, there were low incidence of mercury, lead and cadmium intoxication among this group evidenced by median 0.00, 0.42 and 0.005 respectively. But arsenic shows higher incidence evidenced by median concentration 4.06 with IQR 3.16. Among sparrows. The median concentration of mercury was 0.16 with IQR 0.33. However, the median AS was relatively high 4.15 with IQR 4.6., the median concentration of lead and cadmium were 0.00 and 0.02 respectively.

Table 3 shows that the majority of both pigeons and quails within PL regarding mercury. On the other hand, majority of sparrows exceed PL regarding mercury. Equal percentage in both pigeons and sparrows exceed PL regarding lead. But majority of quails exceed PL regarding lead. All samples of the three species exceed PL regarding arsenic. In contrast, all samples of three species within PL regarding cadmium.

Table 4 shows matrix spearman correlation between three groups regarding heavy metals intoxication (Hg, Pb, Cd, As), in which there is no statistically significant difference between groups. In Table 5, the EDI and HQ for lead, cadmium, Arsenic, and Mercury that result following the ingestion of game birds

Table 1. Statistical analytical results of some heavy metal residues in the examined game birds' samples (µg/g wet weight).

Investigated heavy metals	Species of game birds									P value
	Pigeon			Quail			Sparrow			
	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	
Mercury	0.06	0.12	0.04	0.08	0.14	0.04	0.16	0.16	0.05	0.31
Lead	0.39	0.46	0.14	0.57	0.51	0.16	0.38	0.51	0.16	0.68
Arsenic	3.96	1.20	0.38	3.95	1.85	0.58	3.62	2.71	0.84	0.93
Cadmium	0.01	0.02	0.01	0.01	0.02	0.01	0.02	0.02	0.01	0.91

Table 2. Overview of heavy metal residues (µg/g wet weight) statistics among different species of game birds.

Species	Metal	Minimum	Maximum	Median	IQR
Pigeon	Hg	0	0.38	0	0.13
	Pb	0	1.16	0.20	0.87
	As	2.23	5.75	4.24	2.27
	Cd	0	0.06	0	0.04
Quail	Hg	0	0.36	0	0.23
	Pb	0	1.27	0.42	1.13
	As	1.21	6.27	4.06	3.16
	Cd	0	0.06	0.01	0.04
Sparrow	Hg	0	0.38	0.16	0.33
	Pb	0	1.38	0	0.70
	As	0.42	7.21	4.15	4.60
	Cd	0	0.06	0.02	0.04

Table 3. Percentage of samples within or exceeding the maximum permissible limits of heavy metals in the examined samples.

Species	Mercury		Lead		Arsenic		Cadmium	
	Within PL	Exceed PL	Within PL	Exceed PL	Within PL	Exceed PL	Within PL	Exceed PL
Pigeon	70%	30%	50%	50%	0	100%	100%	0
Quails	70%	30%	20%	80%	0	100%	100%	0
Sparrows	30%	70%	50%	50%	0	100%	100%	0
PL*	0.05		0.1		1		0.2	

* Permissible limit for copper according to Egyptian standards (ES No 7136/2010).

Table 4. Correlation analysis of different heavy metals among the examined three groups

Samples		Hg		Pb		As		Cd	
		Spearman	P value	Spearman	P value	Spearman	P value	Spearman	P value
Quails	Pigeon	-0.41	0.23	-0.18	0.61	0.51	0.13	-0.20	0.57
Sparrow	Pigeon	0.02	0.95	-0.28	0.42	0.51	0.13	-0.17	0.63
Sparrow	Quails	-0.49	0.15	0.35	0.31	0.09	0.81	-0.05	0.88

Table 5. Estimated daily intake (μg/kg BW/day) and hazard analysis of Cd, Pb, As, and Hg due to meat consumption of examined species.

Samples	Hg		Pb		As		Cd		HI*
	EDI	HR	EDI	HR	EDI	HR	EDI	HR	
Pigeon	0.06	0.12	0.40	0.10	4.10	13.22	0.01	0.01	0.23
Quails	0.08	0.16	0.55	0.15	4.05	13.02	0.02	0.02	0.33
Sparrow	0.16	0.33	0.35	0.10	3.70	12.30	0.01	0.01	0.44

Hg: Mercury, Pb: lead, As: Arsenic, Cd: cadmium, EDI: Estimated daily intake, HR: Hazard ratio, HI: Hazard index

* Hazard index (HI) = Σ HR (Pb + Cd + Hg)

from the three species under consideration. The HQ for cd varied between 0.04 to 0.22, for arsenic between 1.16 and 1.34, regarding lead between 0.06 and 0.07, and for Hg from 0.05 to 0.35. Though HQs of the investigated heavy metals did not surpass 1, this presumably proves that humans don't face significant risks to their health from the metals' intake through ingesting game birds' flesh. According to estimates, the three species' combined HQ of As from eating game birds was over one.

DISCUSSION

Previous studies have shown that there was a conflict regarding heavy metal intoxication among game birds. Therefore, this study was drawn aiming to illustrate this conflict among specific sample size represent game birds in Egypt. The result of this study shows that high mean of mercury concentration among pigeons, quails and sparrows although it is within PL in majority of both pigeons and quails (70%) but minority of sparrows (30%) these results in agreement with Kitowski *et al.* (2015) which showed mercury accumulation among different tissues of game bird. In contrast, these results not in tandem with Kanstrup *et al.* (2019) which showed that birds were not likely to be at risk of mercury toxicity due to the low mercury levels measured, this can be explained by using different sample of Danish birds.

In terms of lead poisoning, there is a high mean concentration across the three species, with 50% of pigeons and sparrows are over the PL limit, while 81% of quails are. These findings concur with those found in grill edible tissues in India (Kumar *et al.* 2007). The various samples of broilers and free-range chicken collected from three different countries studies had greater Pb concentrations than those found in the current investigation (Mariam *et al.*, 2004; Zhuang *et al.*, 2009; Yabe *et al.*, 2013). Villar *et al.* (2005) found low Pb concentration in broilers that were obtained from the Philippines. Additionally, reduced Pb concentrations were found by Bortey-Sam *et al.* (2015) in chicken giblets from Tarkwa in Ghana. The mean Pb levels found in samples of the water and food were low in contrast to those found in West Bengal, India's industrial districts, but they were in line with other findings (Ahmed *et al.*, 2017; Kar *et al.*, 2018). Due to the widespread use of Pb in the production of water pipes, lead can enter quails through drinking water and air pollution (Villar *et al.*, 2005). Additionally, it might be present in fishmeal and bone-based animal feed (Ishii *et al.*, 2017).

The recorded data clearly show that the samples had much greater residual As concentrations. The amounts of As in the samples studied in this investigation were consistent with those previously reported by Bortey-Sam *et al.* (2015) and Hu *et al.* (2018) nevertheless, revealed higher As contents in samples of chicken edible tissues and animal feed taken from the Chinese province of Guangdong. Additionally, grill muscles in Pakistan have greater As concentrations than those elsewhere (Mariam *et al.*, 2004). Lower levels of As were reportedly found in the muscles of chicken and ducks that were imported from Argentina and Bangladesh (Islam *et al.*, 2015; Sigrist *et al.*, 2016). In the current investigation, 100% of the tissue samples had arsenic concentrations above the specified MPL. The widespread consumption of nutrients based on arsenic in quail meal could possibly be the cause of the elevated As concentration in samples (Hu *et al.*, 2018).

In the present research, Cd was found in a small number of the tested samples. The analyzed samples had amounts of cadmium residue that were 100 percent within PL in the three species. The recorded levels of Cd for all examined products were within the established PL for Cd according to WHO (2010). Likewise, Elhelaly *et al.* (2022) reported that Cd concentrations in Egyptian animal products wouldn't surpass PL and wouldn't endanger Egyptian customers which is similar with the results of the current investigation. In contrast these results not in agreement with Morshdy *et al.* (2022) who demonstrated that all samples evaluated contained Cd. Among the tested chicken meat products, pane had the highest Cd residual concentration. This is likely due to the fact that various samples had varying exposure levels.

According to estimates, game birds' eating of the three species resulted in HQ of As levels above 1, which may provide a health risk to consumers. This study may not be entirely accurate because the majority of the arsenic found in food is in its organic form, which is also the most hazardous. Nevertheless, the total arsenic is routinely measured using analytical techniques. 90% of the total amount of inorganic arsenic found in both fish and birds is found in the less hazardous form of organic arsenic (FAO/WHO, 2013).

CONCLUSION

The results of the study showed that Pb, Cd, Hg, and As were found in the analysed materials at various quantities. Multiple samples had residual metal concentrations that are higher than the recommended MPL. Cd was low in all samples, and did not surpass MPL. Although HQs of studied heavy metals except for arsenic did not exceed 1, which theoretically demonstrating that human does not acquire a major health hazard from the individual metals' ingestion throughout consumption of game birds' meat. However, this study has limitations, such as a small sample size and few types representing game birds. So further studies with larger sample size and applying on further types of game birds are required to validate the results of our study.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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