Original Research

Journal of Advanced Veterinary Research
(2024) Volume 14, Issue 3, 486-490

Monitoring heavy metal residues in domesticated and game birds' meat from Damietta Governorate, Egypt

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ARTICLE INFO

Recieved: 03 November 2023

Accepted: 06 February 2024

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Keywords:

Heavy metals
ICP metal analysis
Estimated daily intake
Target hazard quotient

ABSTRACT

Concerns about potential health risks have made heavy metal contamination in food a prominent issue. The primary route of human exposure to metals is through food consumption. Hence, the objective of this study was to assess metal concentrations in game bird meat and calculate daily intake and associated health risks. An ICP-OES was employed to measure the levels of mercury (Hg), lead (Pb), arsenic (As), cadmium (Cd), chromium (Cr), magnesium (Mn), zinc (Zn), copper (Cu), cobalt (Co), and iron (Fe). The study's results indicated that As, Zn, and Fe were present at relatively high levels in the studied groups, while Co, Hg, Cr, and Cd had comparatively lower mean concentrations. Among the detected heavy metals, there were no statistically significant differences between the three studied groups, except for Cr, Co, and Zn, which exhibited statistically significant variations. Most quails and pigeons stayed within the designated permissible limits (PL) for Hg, while sparrows exceeded these limits. Both quails and sparrows also exceeded the PL for Pb, whereas pigeons remained within it. In the case of Zn, Cu, and Co, the majority of the three species adhered to the PL, while they exceeded the PL for As. However, all three species remained within the PL for Cd and Cr. Despite the Hazard Quotients (HQs) for the tested heavy metals, with the exception of As, Fe, and Zn, not exceeding 1, this suggests that consuming game bird meat is unlikely to significantly elevate the risk of human exposure to these specific metals, indicating a lower potential health hazard.

Introduction

Game birds, including pigeons and doves, quails, squab (a young pigeon), and upland game birds, are vital not only for maintaining ecological balance but also for human consumption. Therefore, it is crucial to ensure that these birds are not contaminated with dangerous levels of heavy metals, which can pose significant risks to both wildlife and human health (Park *et al*., 2008). These species are vulnerable to toxic metals such as mercury, cadmium, and arsenic within their habitats, where these metals frequently accumulate in high concentrations. Furthermore, numerous studies have documented lead ingestion by both domesticated and game birds, highlighting the presence of heavy metals and their potential impact on a wide range of bird species (Morshdy *et al*., 2022; Mohamed *et al*., 2023). Thus, monitoring and controlling heavy metals in game meat is imperative to shield consumers from potential toxicity (Branciari and Ranucci, 2022).

The use of game birds in monitoring heavy metal pollution has increased due to their wide range in the food chain, distribution, and their sensitivity to environmental changes (Kanstrup *et al*., 2019). Methods for monitoring heavy metals in game birds vary, but generally involve the collection and analysis of tissue samples from targeted bird populations. One approach to monitoring heavy metals in game birds is to focus on lead levels in organs and tissues, as lead is a component of hunting ammunition. Another heavy metal of concern in game birds is mercury. Although it receives less attention, mercury can also accumulate in the tissues of game birds and pose significant health risks (Tajchman *et al*., 2023).

The presence of potential pollutants such as mercury, lead, arsenic, chromium, magnesium, cobalt, and potential trace elements in meat and meat products has raised significant concerns in recent times regarding public health (Morshdy *et al*., 2018; Hussein *et al*., 2023a). To address the issue of heavy metal contamination in game birds, studies have focused primarily on lead, which is commonly present in hunting ammunition, representing the most significant risk of harmful metals entering game meat. Additionally, heavy metals have been recognized as a hazard to wildlife, particularly in the case of lead shot poisoning in waterfowl. However, little attention has been given to the potential exposure issues of heavy metals in upland game birds and mourning doves (Żarski *et al*., 2017).

Other metals like cadmium, zinc, and copper are widely recognized for their bioaccumulation capabilities. Exposure to these metals from both natural and human-induced sources contributes to the release of these heavy metals into the environment, potentially leading to various toxicological effects on humans (Hussein *et al*., 2023b). Cadmium (Cd) is a poisonous toxin that can have hazardous effects when ingested through contaminated food. It is classified as a class B1 carcinogen by the United States Environmental Protection Agency (Samet *et al*., 2020).

The biochemistry and physiology of living organisms are significantly influenced by copper as an essential element. The respiration process in cells also requires copper. However, continual exposure to Cu may harm the cell's organelles through oxidation (Darwish *et al*., 2014). Zinc (Zn) is used by the body as a crucial trace element, serving as a catalyst for over a hundred enzymes. Additionally, zinc is necessary for controlling gene expression in various cell components and maintaining the cell wall. Zinc deficiency is a severe concern in many developing nations, leading to conditions such as hypogonadism, dwarfism, reduced immunity, and anemia. Meat products used for human consumption are known to be a significant source of zinc (Pogorzelska-Nowicka *et al*., 2018).

Addressing heavy metal contamination in game birds is essential to safeguard both human health and wildlife. Therefore, monitoring heavy metals in game birds is crucial in order to assess the potential risks associated with consuming game meat and evaluate the risk related to the consumption of game meat, especially in developing countries where the consumption of game birds is common.

Materials and methods

Samples collection

In the Damietta Governorate, 45 randomly chosen samples of pigeon, quail, and sparrow muscle (each weighing 1 gram) were collected. Each sample was carefully placed in a polypropylene bag, with its type indicated on an identification card. This study was designed to be cross-sectional on game birds at Department of Food Hygiene, Safety, and Technology, Faculty of Veterinary Medicine, Zagazig University.

Washing procedure

Given that the analysis and determination of the examined metals require delicate techniques to prevent any lingering contamination, washing plays a crucial role in reducing the risk of contamination in tubes, plasticware, and glassware. The first two steps involve washing with water and soap. According to El-Seady (2001), plastic and glassware were soaked for several hours in this mixture before being thoroughly rinsed with sterile water. After cleaning the experiment's containers, a solution consisting of eighty milliliters of hydrogen peroxide, two hundred milliliters of concentrated HCL, and approximately five hundred milliliters of deionized water was used to rinse them. The containers were then meticulously rinsed with de-ionized water and allowed to air dry in an incubator free from any potential sources of contamination or dust (El-Mowafi, 1995).

Digestion procedure

Individual samples, each weighing about 1 gram, were digested using the Speed Wave MWS-2 microwave digestion system after being dried in an oven at 40°C. These dried samples were temporarily placed in cleaned digestion jars. For acid digestion, 5 mL of (65%) nitric acid (HNO3) and 1 mL of (30%) hydrogen peroxide (H2O2) were used. The digestion jars were sealed and arranged on a 10-position turntable for a temperature program, involving a ramp to 160°C for five minutes and then to 190°C for fifteen minutes. After cooling, the samples were transferred into polypropylene tubes and diluted with Milli-Q water to a final volume of 10 mL. The same procedure was followed to create a reagent blank.

Procedures

A set of calibration solutions was prepared, with each solution containing exactly 1 gram of muscle samples. These solutions were used to measure the concentrations of selected metals. The range of concentrations for each element in the set was chosen to include the expected concentration of that element in the sample solutions, if known. The next step in ICP metal analysis involved introducing the calibration solutions and sample solutions into the plasma and measuring the intensities of light at the appropriate analytical wavelengths. For example, the wavelength for iron (Fe) might be 238.204 nm, for lead (Pb) 220.253 nm, and for copper (Cu) 324.754 nm. Calibration curves were prepared for each element based on the emission intensities of the calibration solutions. The concentrations of the elements in each sample solution were determined from these calibration curves. Finally, the concentrations in the original samples were calculated using the measured concentrations of the elements in the sample solution and the known dilution factor. All substances, including standard stock solutions, were of analytical reagent quality from Wako Pure Chemicals, Osaka, Japan. The detection limits (in µg/L) for chromium, manganese, iron, cobalt, copper, zinc, arsenic, cadmium, and lead were 0.003, 0.025, 0.154, 0.0004, 0.007, 0.226, 0.002, 0.001 and 0.001, respectively. Metal concentrations were expressed in mg/kg wet weight (mg/kg ww).

Health risk assessment

The evaluation of health hazards associated with local consumption of game birds was based on the Hazard Quotient (HQ). The HQ serves as a comparison between the determined pollutant dosage and a reference dose level. If the HQ is less than 1, the exposed population is unlikely to experience overtly harmful effects. The United States Environmental Protection Agency (USEPA) report established a risk evaluation method using high-quality data. This method is based on the equation:

THQ= (EFr *ED *FIR *MC)/ (RfD* BW*AT) *10-3 .

Here, THQ represents the target hazard quotient, EFr is exposure frequency (365 days/year), ED is exposure duration (70 years), FIR is food ingestion rate (g/person/d), MC is the average concentration of metal in food (µg/g, on a fresh weight basis), RfD is the oral reference dose (mg/kg/d), BW is the average body weight in adults (60 kg), and AT is the average exposure time (365 days/year * number of exposure years, assuming 70 years in this study). Oral reference doses were based on 0.0003, 0.001, 0.0005, 0.004, and 0.14 mg/kg/day for As, Cd, Hg, Pb, and Mn, respectively (USEPA, 2010).

Statistical analysis

The data underwent normalization using the Shapiro-Wilk test. Continuous variables are presented as means with standard deviations for normally distributed data and as medians with an interquartile range (25th to 75th percentile) for data that do not follow a normal distribution. Group means and standard deviations were compared using the ANOVA test. Correlation studies were conducted using the Spearman's rank correlation test. Categorical variables are expressed as numbers and percentages (%). Statistical significance was defined as a p-value less than 0.05. All results were analyzed using SPSS, Version 25.

Results

The mean concentration of arsenic, zinc, and iron was high in the study groups, whereas the mean concentrations of cadmium, cobalt, mercury, and chromium was low. Table 1 compares the mean and standard deviation of the concentrations of heavy metals among the studied groups. No significant statistical distinction exists between the three species regarding the measured heavy metals, except for chromium, cobalt and zinc, where statistically significant differences were observed among the three groups.

Results indicated the presence of non-normally distributed data. In the subsequent Table 2, we presented statistics using additional values in addition to mean ± SD. Each group comprised 15 samples. Pigeons exhibited a median mercury reading of 0.00 with an IQR of 0.17, illustrating variations among the samples. Additionally, lead concentrations had an IQR of 0.57 and a median of 0.12, while cadmium concentrations in the same setting showed an IQR of 0.07 and a median of 0.04. The median concentration of arsenic, at 3.62 with an interquartile range of 4.63, was slightly higher compared to other heavy metals. Consistent with previous findings, this group demonstrated a low incidence of cobalt and chromium intoxication, indicated by median values of 0.04 for both elements. However, arsenic, zinc, and iron showed a higher incidence across the three groups.

Table 3 indicates that pigeons and quails in PL tend to be free from mercury. Conversely, most sparrows exhibit higher mercury levels than PL. Both quails and sparrows show lead levels surpassing PL. However, pigeons meet the lead target set by PL. Concerning zinc, copper, and cobalt, the majority of all studied groups fall within PL; however, all three species surpass PL levels for arsenic. For cadmium and chromium, each sample from the three species falls within PL. The Spearman correlation test revealed no statistically significant discrepancies among the studied groups, as depicted in the following Table 4.

The EDI and HQ values for the examined heavy metals after ingesting game birds from the three species under consideration are shown in Table 5. The observation that the majority of heavy metal HQ values did not surpass 1 suggests that ingesting the metals from game bird flesh does not pose any serious health hazards to people. Calculations show that the combined HQ of all studied heavy metals for the three species did not exceed one, except for arsenic, zinc, and iron.

Discussion

There has been debate surrounding the potential poisoning of domesticated and game birds by heavy metals, as documented in previous studies. This study was designed to address this dispute within a specific sample representing domesticated and game birds in Egypt.

The findings of this study highlight high average concentrations of mercury in pigeons, quails, and sparrows. However, it's crucial to consider the limitations and uncertainties associated with using birds as bioindicators. Although birds tend to accumulate heavy metals in their tissues, the implications of such contamination on their overall health and well-being are not always clear. Additionally, the presence of high mercury concen

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

Metals	Pigeons (N = 15)			Quails (N = 15)			Sparrows (N = 15)			P value
	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	
Hg	0.09	0.15	0.07	0.11	0.13	0.09	0.19	0.16	0.11	0.2
Pb	0.39	0.51	0.23	0.5	0.52	0.34	0.4	0.45	0.31	0.8
As	3.55	1.33	0.45	3.44	1.84	0.23	3.8	2.25	0.33	0.9
Cd	0.01	0.02	0.01	0.02	0.02	0.01	0.02	0.02	0.01	0.06
Mn	1.58	1.12	0.30	1.54	1.07	0.28	1.79	1.21	0.41	0.8
Cr	0.07	0..09	0.01	0.14	0.29	0.09	0.39	0.47	0.14	0.03
Zn	106	69.00	44.00	148.00	91.00	53.00	194	101.00	76.00	0.03
Cu	2.63	1.06	0.57	2.04	1.06	0.41	1.8	1.1	0.36	0.11
Co	0.06	0.07	0.01	0.28	0.44	0.09	0.47	0.57	0.12	0.03
Fe	164	116	77	197	110	81	163	115	72	0.6

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

Investigated metals	Pigeons*	Quails*	Sparrows*
	(N = 15)	(N = 15)	(N = 15)
Hg	0.00 (0.00, 0.17)	0.06 (0.00, 0.20)	0.18 (0.02, 0.34)
Pb	0.12 (0.00, 0.56)	0.41 (0.00, 0.73)	0.17 (0.00, 0.78)
As	3.62 (2.47, 4.63)	3.12 (2.23, 4.83)	3.87 (2.16, 5.36)
Cd	0.04 (0.00, 0.07)	0.00 (0.00, 0.03)	0.02(0.00, 0.04)
Mn	1.22 (0.69, 2.57)	1.19 (0.69, 2.25)	1.44 (0.80, 2.49)
Cr	0.04 (0.00, 0.14)	0.00 (0.00, 0.16)	0.13 (0.04, 0.63)
Zn	88.00 (61, 146)	114.00 (84, 198)	179.00 (106, 276)
Cu	2.85 (1.81, 3.59)	2.13 (1.06, 2.86)	1.87 (0.80, 2.67)
Co	0.04 (0.00, 0.09)	0.00 (0.00, 0.46)	0.08 (0.00, 1.05)
Fe	146.00 (102, 219)	175.00 (131, 236)	156.00 (82, 220)

*Median (IQR)

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

Investigated metals	Pigeon		Quails		Sparrows		PL*
	Within PL	Exceed PL	Within PL	Exceed PL	Within PL	Exceed PL	
Hg	66.60%	33.30%	53.30%	46.60%	26.60%	73.30%	0.05
Pb	53.30%	46.60%	33.30%	66.60%	46.60%	53.30%	0.1
As	0.00	100%	0.00	100%	0.00	100%	1.0
Cd	100.00%	0.00	100%	0.00	100%	0.00	0.1
Mn	13.30%	86.60%	6.60%	93.30%	0.00	100%	0.3
Cr	100%	0.00%	100%	0.00	100%	0.00	200
Zn	80.00%	20%	73.30%	26.60%	53.30%	46.60%	200
Cu	66.60%	33.30%	80.00%	20.00%	86.60%	13.30%	3.0
Co	80.00%	20.00%	66.60%	33.30%	66.60%	33.30%	0.6
Fe	40.00%	60.00%	26.60%	73.30%	46.60%	53.30%	140

* Egyptian Standard (ES No.7136, 2010)

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

Investigated metals	Correlation	Quails/Pigeon	Sparrow/ Pigeon	Sparrow/Quails
Hg	Spearman	-0.67	-0.35	-0.05
	P- value	0.01	0.19	0.84
Pb	Spearman	-0.07	0.24	-0.18
	P- value	0.8	0.37	0.51
As	Spearman	0.47	0.32	0.39
	P- value	0.07	0.23	0.14
Cd	Spearman	0.28	0.39	-0.13
	P- value	0.30	0.14	0.64
Mn	Spearman	-0.25	0.47	-0.37
	P- value	0.35	0.07	0.17
Cr	Spearman	0.28	0.01	0.31
	P- value	0.31	0.98	0.26
Zn	Spearman	0.16	-0.10	0.18
	P- value	0.56	0.71	0.49
Cu	Spearman	0.23	0.02	-0.22
	P- value	0.4	0.94	0.41
Co	Spearman	0.07	0.04	0.22
	P- value	0.79	0.87	0.42
Fe	Spearman	0.09	-0.18	0.27
	P- value	0.73	0.51	0.32

Table 5. Estimated daily intake (μg/kg BW/day) and hazard analysis of investigated heavy metals due to meat consumption of examined species.

Investigated metals	Risk	Pigeons	Quails	Sparrows
Hg	EDI	0.08	0.09	0.12
	HQ	0.14	0.12	0.36
Pb	EDI	0.43	0.62	0.38
	HQ	0.12	0.13	0.17
As	EDI	3.90	4.20	3.70
	HQ	12.20	13.40	12.30
Cd	EDI	0.01	0.02	0.01
	HQ	0.01	0.02	0.01
Mn	EDI	1.61	1.55	1.77
	HQ	0.01	0.16	0.33
Cr	EDI	2.80	2.50	3.10
	HQ	0.01	0.03	0.06
Zn	EDI	34.30	36.20	32.80
	HQ	16.20	17.50	15.70
Cu	EDI	2.90	3.20	2.70
	HQ	0.15	0.22	0.13
Co	EDI	0.05	0.08	0.12
	HQ	0.01	0.01	0.03
Fe	EDI	55.70	61.20	65.20
	HQ	23.20	27.40	27.90
Hazard index (HI)*		0.44	0.79	1.09

EDI: Estimated daily intake; HQ: Hazard quotient; HI: Hazard index
* Hazard index (HI) = Σ HQ (Hg + Pb + Cd + Mn + Cr + Cu + Co)

trations in these birds doesn't necessarily indicate a direct risk to other wildlife species or humans. It's important to note that these findings may not apply to all bird species. Different species may have varying capacities to accumulate heavy metals, and their diets and habitats play significant roles in their exposure to contaminants. These findings concur with Kitowski *et al.* (2015) discovery of mercury accumulation in various game bird tissues. Although Kanstrup *et al.* (2019) found low mercury levels in Danish birds, their conclusions that birds were not expected to be susceptible to mercury toxicity can be reconciled with their data.

Regarding lead, the mean concentration was notably high among three species, with quails displaying the highest concentration, evidenced by about two-thirds of quails exceeding the PL limit. These findings are in line with Mohamed *et al.* (2023), which also reported high mean concentrations across the three species, with 50% of pigeons and sparrows surpassing the PL limit. According to analyses of field and laboratory data, there are two possible outcomes when lead is ingested: The bird may regurgitate the Pb-containing grit/pellets consumed, or the ingested grit/pellets containing Pb might be continuously absorbed over an extended period. This is due to the fact that for Pb to be harmful, it needs to be in a form that will be maintained in the gizzard, causing long-term continuous absorption (Kendall *et al.*, 1996).

The residual concentrations of arsenic in the measured samples were significantly high. The statistics clearly demonstrate that the examined specimens displayed elevated arsenic concentrations. The arsenic levels in the samples investigated in this study were consistent with previously reported data, showing higher arsenic contents in both chicken and game bird samples. In each case, every sample exceeded the permissible limit (PL), which might be attributed to the high arsenic concentration in game bird meats, as indicated in studies by Hu *et al.* (2018) and Mohamed *et al.* (2023).

In current study, low levels of cadmium were found in the analyzed samples. The cadmium residue levels in the examined samples for all three species were 100% within the permissible limits (PL). These results are consistent with Bortey-Sam *et al.* (2015), where the average level of Cd in chicken kidney (0.73-0.81 mg/kg ww) fell within the acceptable range of concentrations for offal intended for human consumption, as per the standards set by the European Commission (2006) (which ranges from 0.5 to 1.0 mg/kg ww). Cadmium traces were found in fourteen percent of all muscle and ninety percent of chicken gizzard. When compared to goat and sheep, the levels of cadmium in chicken liver and kidney were significantly higher (Wang *et al.*, 2013). Furthermore, there have been theories suggesting that cadmium could accumulate in the renal systems of animals exposed to cadmium due to the presence of free protein-thiol groups, promoting the effective binding of heavy metals (Bortey-Sam *et al.*, 2015).

The role of Mn in neuropsychiatric disorders is also documented (Klos *et al.*, 2006). Mn was detectable in almost all samples, which aligns with the findings of Bortey-Sam *et al.* (2015), showing that all samples of offal and muscles contained manganese. According to this study, the mean Manganese contents in chicken and goat ranged from 0.15 to 3.51 and 0.62–2.43 mg/kg ww, respectively. Chromium levels were within permissible limits (PL) in all samples, aligning with the findings of Uluozlu *et al.* (2009), where all measured samples fell within the accepted range. Chromium is considered an essential trace element crucial for insulin function and lipid metabolism. The recommended daily intake of chromium is 50–200 µg (Uluozlu *et al.*, 2009).

In comparison with related field studies conducted in various nations, similarities between the results of this study and those from research by Husain *et al.* (1996) in Kuwaiti goat and sheep were observed, with some variations (except for copper, zinc, and cadmium), and surpassing findings from studies by Uluozlu *et al.* (2009) and Villar *et al.* (2005) in poultry. Additionally, when contrasted with research by Okoye and Ugwu (2001); Abou-Arab (2001), and Caggiano *et al.* (2005) in Egypt, this study found reduced levels of cadmium (Cd) and lead (Pb) in goats and sheep.

The health risks associated with consuming the examined game birds were assessed based on the hazard quotient (HQ). An exposed population is unlikely to experience obvious adverse effects if the HQ value is below 1 (USEPA, 2000). Although the calculated HQ values were below 1.0, the values for arsenic, iron, and zinc exceeded 1. These three metals were measured in total, with ninety percent in inorganic form, which is less hazardous than the organic form representing ten percent. Therefore, the intake of these species might not pose a significant risk to human health (FAO/WHO, 2013). Additionally, this study has some limitations, including a small sample size and a limited variety of game bird species. Consequently, additional research with a larger sample size and broader inclusion of game bird species is necessary to reinforce the conclusions drawn from our research.

Conclusion

The study results revealed that arsenic, zinc, and iron exhibited relatively high levels in the studied groups, while the mean concentrations of cobalt, mercury, chromium, and cadmium was comparatively low among these groups. In terms of each detected heavy metal, there were no statistically significant differences among the three studied groups, except for chromium, cobalt, and zinc, which showed statistically significant variations between the groups. The majority of quails and pigeons adhered to the targeted permissible limits (PL) for mercury, whereas sparrows exceeded the PL for mercury. Additionally, both quails and sparrows surpassed the PL for lead, while pigeons remained within the PL for lead. As for zinc, copper, and cobalt, the majority of the three species adhered to the PL, whereas they exceeded the PL for arsenic. On the other hand, all three species remained within the PL for cadmium and chromium. Despite the Hazard Quotients (HQs) for the tested heavy metals, except for arsenic, iron, and zinc, remaining below 1, this suggests that the consumption of game bird meat is unlikely to significantly elevate the risk of human exposure to these specific metals, indicating a lower potential health hazard.

Acknowledgments

The authors are thankful for the assistance provided by the Department of Food Hygiene, Safety, and Technology at the Faculty of Veterinary Medicine, Zagazig University.

Conflict of interest

The authors declare that they have no conflict of interest.

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