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Human health risk from dietary exposure to heavy metals through poultry eggs: Evidence from commercial farms in Wakiso District, Uganda

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ABSTRACT

Uganda's projected annual egg consumption of 1.9 kg per capita by 2025 raises concerns over heavy metal contamination in poultry products. Wakiso District, a peri-urban hub supplying Kampala, faces potential exposure risks due to inconsistent feed quality. This study sampled 53 poultry farms in Wakiso District. Egg and feed samples were analyzed for chromium (Cr), lead (Pb), mercury (Hg), arsenic (As), and cadmium (Cd) using ICP-MS. Weekly intake estimates were derived from FAO consumption data and compared against WHO/JECFA and Codex thresholds. Spatial variation was assessed using the Kruskal–Wallis H test. Cr levels peaked at 6.72 ± 4.91 mg/kg in Kajansi, with intake of 237.6 µg/week, below the JECFA limit of 2100 µg. As reached 1.36 ± 0.48 mg/kg, with intake up to 42.3 µg/week, under the WHO threshold of 1050 µg. Cd exposure peaked at 0.15 ± 0.10 mg/kg, translating to 2.69 µg/week, below the FAO limit of 2695 µg. Significant spatial differences were observed for Cr (H = 41.77), As (H = 49.82), and Cd (H = 31.94), all $p < 0.001$. While overall intake remains within safety limits, localized contamination in Kajansi and Katabi suggests cumulative risk, hence need for regulations and feed and residue monitoring.

1. Introduction

The poultry industry is undergoing significant global expansion, driven by accelerating human population growth, enhanced consumer purchasing power, and the rising worldwide demand for protein-dense food sources, (Wu, Cui, Zhou & Ying, 2022; Attia et al., 2022). This expansion is especially pronounced in Uganda, where the population has risen markedly from 24.2 million in 2002 to an estimated 45.6 million in 2023. Such demographic growth has generated a parallel increase in the demand for eggs and other poultry products, which in turn has driven a notable rise in the national poultry stock, from 47.4 million birds in 2021 to 48.1 million in 2023, (Tainika & Duman, 2019; UBOS, 2024).

Notwithstanding the considerable nutritional and economic advantages associated with the poultry industry, the contamination of poultry feeds by heavy metals constitutes a serious public health and environmental challenge, (Aljohani, 2023; Kabeer et al., 2021). Heavy metals, including mercury (Hg), lead (Pb), cadmium (Cd), and arsenic (As), are recognised as highly toxic substances that pose serious health risks. Exposure to these elements has been associated with neurological impairment, organ dysfunction, and an elevated incidence of non-communicable diseases (Balali-Mood et al., 2021). The principal origin of this contamination arises from the mechanical degradation of cast iron and steel milling equipment employed in feed processing, whereby heavy metals are directly introduced into the feed (Mugume, Byamugisha, Omara & Ntambi, 2023; Oniya et al., 2018). These elements exhibit resistance to thermal treatment and other processing techniques, thereby facilitating their persistence within poultry feeds and subsequent transfer into eggs, ultimately reaching consumers and presenting significant food safety concerns (Korish & Attia, 2020).

The presence of these chemical residues has had a significant economic impact on Uganda. International markets have imposed quality standards that act as non-tariff trade barriers, leading to restrictions on the country's poultry products when heavy metal levels exceed permissible limits (Humphrey, 2017). This has resulted in considerable revenue losses and economic setbacks for farmers, traders, and the government (Diana, 2023).

Sample preparation and analytical technique selection are critical for accurate heavy metal determination in food matrices, (El Hosry et al., 2023). Acid digestion using nitric acid–hydrogen peroxide under controlled heating was employed to ensure complete breakdown of

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organic matter and efficient release of trace metals from eggs and feed, (Gonzalez et al., 2022). Among available instruments include Atomic Absorption Spectroscopy (AAS); Microwave Plasma Atomic Emission Spectroscopy (MPAES), and Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The ICP-MS was chosen for its superior sensitivity, multi-element capability, and low detection limits, (Wilschefski & Baxter, 2019). This combination provides reliable quantification of Cr, As, Cd, Hg, and Pb, supporting robust health risk assessment.

While extensive research exists on heavy metal contamination in other animal-source foods like milk and meat (Tahir & Alkheraije, 2023), similar data for poultry feeds and eggs in Uganda is limited. This significant research gap hinders the development of evidence-based regulatory frameworks necessary to ensure food safety and protect public health. Therefore, this study aims to fill this critical gap by assessing the levels of heavy metals in poultry feeds and chicken eggs from selected commercial farms in Wakiso District, Uganda.

2. Materials and methods

2.1. Study area

This study investigated heavy metal contamination (Cr, As, Cd, Hg, and Pb) in poultry feeds and eggs within Wakiso District, a *peri*-urban region of Central Uganda. The district was purposively selected as the study site due to its exceptionally high population density (3,519,300 inhabitants as of 2023), its extensive poultry farming activities, and the strong consumer demand for poultry products, which together render it a representative context for assessing food safety risks in rapidly urbanising settings. Wakisospans an area of 2,807.75 km^2 and is geographically situated at 00°24′N, 32°29′E, positioning it adjacent to Kampala, the national capital. This location provides both a critical supply base for urban markets and a nexus of intensive production systems, thereby justifying its inclusion as a focal area for examining pathways of heavy metal exposure through poultry products

2.2. Study design and population

A concurrent sequential study design was adopted to investigate heavy metal contamination in poultry products. The qualitative component comprised key informant interviews (KIIs) and focus group discussions (FGDs) with local leaders, animal health practitioners, and poultry farmers, thereby providing contextual insights into farming practices and perceived risks. In parallel, quantitative data were obtained through structured questionnaires administered to poultry farmers, enabling systematic characterisation of production systems and feed utilisation. To substantiate these findings, laboratory analyses of poultry feeds and eggs collected from the same farms were conducted using Inductively Coupled Plasma–Mass Spectrometry (ICP-MS), ensuring precise quantification of chromium (Cr), arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb). The study population encompassed 2,545 commercial poultry farmers in Wakiso District, purposively selected due to its intensive poultry production and high consumer demand. Furthermore, estimates of daily and weekly heavy metal intake among consumers were derived by integrating measured concentrations in eggs with per capita egg consumption data reported by the Food and Agriculture Organization (FAO). These intake estimates were subsequently benchmarked against tolerable limits established by the World Health Organization (WHO) and the European Union (EU), thereby facilitating an assessment of the public health implications of heavy metal exposure within the study area.

2.3. Sampling technique

Wakiso District was purposively selected as the study site owing to its geographical proximity to Kampala City, its high concentration of commercial poultry enterprises, and the diversity of poultry feed sources and processors operating within the area. A sampling frame was constructed in collaboration with district veterinary officers, private animal health practitioners, and local leaders, from which participating poultry farms were selected using a simple random sampling technique to ensure representativeness. All interviews and quantitative data collection activities were conducted at these farms. In addition, purposive sampling was employed to identify participants for key informant interviews (KIIs) and focus group discussions (FGDs), specifically targeting farmer representatives, community leaders, and animal health workers with detailed knowledge of feed utilisation practices. Feed and egg samples were subsequently collected directly from the same randomly selected farms for laboratory analysis, thereby ensuring consistency between survey data and analytical measurements.

2.4. Sample collection

Ten eggs were selected via stratified random sampling across pens and laying times to capture within-farm variability and 200 g of feed were collected per study farm, sealed in polythene bags (food-grade, inert to trace metals), and stored in ice cool boxes at 2 °C during transportation to the laboratory for analysis.

2.5. Reagents, standards, and solvents

All reagents used in this study were of analytical or ultra-pure grade to ensure accuracy and reproducibility of heavy metal quantification. Aqua regia ($HCl:HNO_3$, 3:1) and hydrogen peroxide (H_2O_2, 30 %) were employed for sample digestion. The hydrochloric acid and nitric acid were of trace-metal grade ($\geq$99.999 % purity), supplied by Sigma-Aldrich (USA). Hydrogen peroxide was procured from Fisher Scientific (UK). For calibration, multi-element ICP-MS standard solutions (0–50 ppb) were prepared from certified reference materials traceable to NIST (National Institute of Standards and Technology, USA). Ultra-pure nitric acid ($\geq$69 %, sub-boiling distilled, trace-metal grade) was used for dilution and matrix stabilization, typically sourced from Merck (Germany). High-quality deionized water (18.2 MΩ·cm resistivity) was produced using a Millipore Milli-Q system (USA), ensuring minimal background contamination.

2.6. Sample preparation

For egg samples, the yolk and albumen were thoroughly homogenised to ensure uniformity. Feed samples collected from each farm were similarly homogenised prior to analysis. A subsample of one gram (1 g) was accurately weighed from both egg and feed homogenates. Digestion was performed using aqua regia ($HCl:HNO_3$, 3:1) supplemented with 4 ml of hydrogen peroxide, followed by heating at 140 °C for four hours; for mercury (Hg) and arsenic (As), digestion was conducted at a reduced temperature of 60–80 °C to minimise volatilisation losses. The digested solutions were subsequently filtered, diluted to appropriate volumes, and subjected to elemental analysis using Inductively Coupled Plasma–Mass Spectrometry (ICP-MS; Thermo Scientific iCAP RQ, model 03358), (English, 2021).

2.7. ICP-MS analysis

Heavy metal quantification (Cr, Pb, Hg, As, and Cd) was performed using Inductively Coupled Plasma–Mass Spectrometry (ICP-MS) on a Thermo Scientific iCAP RQ 03,358 instrument. The method was optimized to achieve high sensitivity and reproducibility in food/feed matrices, (Belkouteb et al., 2024).

2.7.1. Instrumental conditions

Typical operating parameters employed were: Plasma power – 1550 W; Nebulizer gas flow rate – 1.05 L/min (argon); Auxiliary gas flow rate – 0.9 L/min; Plasma gas flow rate – 14 L/min; Sample uptake rate – 1.0

mL/min using a PFA concentric nebulizer; Dwell time per isotope – 20–50 ms; Collision/reaction cell mode – He gas (4.5 mL/min) to minimize polyatomic interferences; and Monitored isotopes – Cr-52, Pb-208, Hg-202, As-75, Cd-114.

2.7.2. Preparation of standards

Calibration standards (0, 5, 10, 20, 30, and 50 ppb) were prepared from certified multi-element ICP-MS stock solutions (Inorganic Ventures, USA; NIST traceable). Standards were diluted in 1 % ultra-pure HNO3 prepared with sub-boiling distilled nitric acid (Merck, Germany) and 18.2 MΩ·cm deionized water (Milli-Q, USA). Calibration curves were constructed for each analyte with correlation coefficients (R^2) consistently >0.999.

2.7.3. Detailed sample preparation and quality control

Samples were digested in aqua regia (HCl:HNO_3, 3:1) with 4 mL of H_2O_2 at 140 °C for 4 h (Hg and As at 60–80 °C) followed by filtration and dilution to 25 mL with 1 % HNO_3. Procedural blanks were included in each batch to monitor contamination. At least 10 % of samples were run in duplicate to assess reproducibility. Matrix spikes were prepared by fortifying samples with known concentrations (20 ppb) of each analyte to evaluate recovery. Poultry feed Certified Reference Material (CRM): (NIST SRM 1548a) was analyzed alongside samples to verify accuracy.

2.7.4. Method validation

Validation was performed according to ISO 17,025 and AOAC guidelines. Limits of detection (LOD) and quantification (LOQ) were calculated as 3σ and 10σ of the blank signal, respectively. Recovery and precision were assessed using spiked samples and CRMs, as shown in Table 1.

2.8. Daily/Weekly human heavy metal intake

Data on per capita egg consumption were obtained from Food and Agriculture Organization (FAO) reports (FAO Statistics (FAOSTAT) 2025). These figures were utilised to estimate the daily and weekly intake of heavy metals through egg consumption in Uganda. The calculated intake values were subsequently correlated with laboratory-derived concentrations of heavy metals in eggs, thereby providing a basis for evaluating the public health implications associated with contaminated egg consumption among Ugandan populations.

According to the FAO data, human egg consumption per capita in Uganda increased from 0.550 kg in 2005, to 1.11 kg in 2011, to 0.79 in 2020, and 0.770 kg in 2021[3]. Through projection, this value is expected to increase to 1.9 kg in the year 2025 (FAO Statistics (FAOSTAT) 2025). Hence, the daily/weekly eggs intake was calculated as follows: 1.9 kg/365days = 0.0052 kg/day or 5.2 *g*/day or 36.4 *g*/week. However, across different income groups, the poor consume only 0.7 pieces/capita/week compared to 1.1, 1.2, 1.5 and 2.2 for the moderately poor, middle quintile, moderately rich and the richest quintile respectively (FAO, 2018; Jet al., 2012). Considering that an average egg is approximately 52 *g*, this translates to 36.4 g, 57.2 g, 62.4 g, 78.0 g, and 114.4 g for the poor, moderately poor, middle quintile, moderately rich and the richest quintile respectively. Based on these values, the estimated daily/weekly heavy metal intake values were calculated by the following equation (Samad Abedi et al., 2023; Hoseini et al., 2023a):

Table 1
Method validation results.

Parameter	Cr	Pb	Hg	As	Cd
LOD (μg/kg)	0.15	0.1	0.08	0.12	0.05
LOQ (μg/kg)	0.5	0.35	0.25	0.4	0.15
Recovery (%)	95.2	97.5	93.8	96.1	98.4
Precision (RSD%, *n* = 6)	3.2	2.8	4.1	3.5	2.6
Calibration R^2	0.9992	0.9995	0.999	0.9993	0.9996

$$\mathrm{D/WIHM} = C{*}\mathrm{A},$$

where D/WIHM stands for the daily/weekly intake of heavy metals,

C represents the concentration of metal in the egg (μg/kg), and

A is the average per capita consumption of eggs (g/day or g/week).

The WHO Provisional Tolerable Weekly Intake (PTWI) for the heavy metals of interest are: Chromium (Cr) at 30 μg/kg body weight per week; Lead (Pb) at 25 μg/kg body weight per week; Cadmium (Cd) at 7 μg/kg body weight per week; Mercury (Hg) at 4 μg/kg body weight per week and Arsenic (As) at 15 μg/kg body weight per week (Balali-Mood et al., 2021; Marini, Angouria-Tsorochidou, Caro & Thomsen, 2021; Hoseini et al., 2023b).

International guidelines for heavy metal intake vary significantly across organizations. The WHO/FAO recommends daily limits of 50 μg/kg for mercury (Hg), 250 for lead (Pb), 120 for chromium (Cr), 55 for cadmium (Cd), and 150 for arsenic (As). The EU/WHO sets a lower mercury threshold at 16 μg/kg/day, maintains the same lead and arsenic limits, but raises cadmium to 70 μg/kg/day, with no specified value for chromium. EU/Europe adopts the most stringent standards, allowing only 2 μg/kg/day for mercury, 10 for lead, 4.1 for cadmium, and 10 for arsenic, with chromium again unspecified. Meanwhile, WHO/JECFA provides weekly intake limits: 4 μg/kg for mercury, 25 for lead, 30 for chromium, 7 for cadmium, and 15 for arsenic.

To rule out the possibility of heavy metal intake by the poultry from other sources other than the feeds, farmers were interviewed and they all mentioned that they do not feed their poultry with any other supplements except feeds bought from wholesale stores and other retail outlets.

2.9. Monte Carlo simulation

A Monte Carlo simulation was done for a more nuanced understanding of health risks by incorporating uncertainty and variability in metal concentrations, consumption patterns, body weight, and cooking effects.

Exposure Dose Calculations

i. Estimated Weekly Intake (EWI):

$$\mathrm{EWI_m} = \mathrm{C_m} \times \mathrm{I_{wk}}$$

Estimated Daily Intake (EDI):

$$\mathrm{EDI_m} = \mathrm{EWI_m}/(\mathrm{BW} \times 7)$$

Where:

C_m: Metal concentration in eggs (mg/kg, converted to μg/g)
I_{wk}: Weekly egg intake (g/week)
BW: Body weight (kg)

ii. Non-Cancer Risk

Hazard Quotient (HQ):

$$\mathrm{HQ_m} = \mathrm{EWI_m}/(\mathrm{PTWI_m} \times \mathrm{BW})$$

Hazard Index (HI):

$$\mathrm{HI} = \sum \mathrm{HQ_m}$$

iii. Lead Margin of Exposure (MOE)

$$\mathrm{MOE_{Pb}} = \mathrm{BMDL}/\mathrm{EDI_{Pb}}$$

iv. Arsenic Lifetime Cancer Risk (LCR)

$$LCR_{As} = EDI_{As} \times SF_{iAs}$$

Input Parameters and Distributions

Metal Concentrations: Lognormal distribution based on mean ± SD per administrative unit. Non-detects imputed as $LOD/\sqrt{2}$; Egg Intake: Wealth-stratified (36.4–114.4 g/week), modeled as normal with 20 % CV; Body Weight: Normal distribution (mean 60 kg, SD 10 kg); Cooking Retention Factors: Triangular distributions per metal: Pb: 0.7–1.0 (mode 0.9); Cd: 0.8–1.0 (mode 0.95); Hg: 0.6–0.9 (mode 0.8); As: 0.7–0.95 (mode 0.85); and Cr: 0.8–1.0 (mode 0.9); Speciation Scenarios: As: 30 % inorganic (base), 10 % (optimistic), 70 % (worst-case); and Cr: 100 % Cr (III) vs. 10 % Cr(VI) (worst-case)

Simulation Design: 100,000 iterations per administrative unit and wealth quintile; Outputs:

- ○ Medians and 95th percentiles for HQ, HI, MOE, and LCR
- ○ Exceedance probabilities vs. WHO/JECFA PTWIs
- ○ Sensitivity analysis via Partial Rank Correlation Coefficients (PRCCs)

2.10. *Analysis of results*

Normality tests were performed to determine whether the results followed a normal distribution to decide whether to use parametric tests or non-parametric tests for comparative analysis of data from different administrative units. The Q-Q plots and Histograms were used to assess linearity, while the Shapiro–Wilk Test and the Anderson–Darling Test were used for skewness and kurtosis tests of results. The Kruskal–Wallis H-test was used to compare heavy metal concentrations across administrative units. The Means, Median and Interquartile Range (IQR) were used for comparative analysis.

2.11. *Quality control*

Quality control was ensured through standardized ICP-MS protocols, validated calibration curves, and duplicate sample analysis. Assurance measures included ethical approvals, randomized sampling, and statistical rigor via non-parametric tests. All procedures adhered to international safety thresholds, ensuring reliability, reproducibility, and integrity of both laboratory and field data across Wakiso District.

3. Results and discussion

3.1. *Feed ingredients used in the Wakiso peri-urban areas*

The survey revealed that the most frequently utilised feed ingredients in the study area were maize bran (16.33 % of respondents), lime (12.76 %), broken maize (10.71 %), sunflower (8.67 %), and maize kernel (7.65 %) (Fig. 1). These components constitute the staple inputs in poultry and livestock feed formulations, serving primarily as sources of energy, protein, and mineral supplementation. In contrast, less commonly employed ingredients included concentrates (2.04 %), cottonseed cake (1.53 %), fish (1.53 %), eggs (1.02 %), meat (0.51 %), blood meal, poultry core (0.51 %), toxin binders (0.51 %), calcium (0.51 %), and various synthetic feed additives, (Afolabi et al., 2021). Comparable findings have been reported in West Africa, where feed formulations typically comprise cereals (predominantly maize), soybean meal, fishmeal, meat and bone meal, shell grit, calcium and dicalcium phosphate, defluorinated rock phosphate, bone meal, trace mineral premixes, salt, sodium bicarbonate, vitamin premixes, methionine, lysine, threonine, enzymes, and antibiotics (Ravindran, 2013). The observed similarities underscore the reliance on cereal-based energy sources and protein supplements across different regions, while also highlighting the incorporation of mineral and synthetic additives to enhance nutritional balance. It is important to note, however, that certain feed ingredients may present a heightened risk of heavy metal contamination, depending on their mode of production, storage conditions, and processing practices. This raises critical concerns regarding feed safety and the potential transfer of contaminants into poultry products, thereby necessitating rigorous monitoring and quality assurance measures within feed supply chains.

The following are the key observations pointing towards the utilization of the feed ingredients in the study area. High-Risk Feed Types: Maize Bran and Maize Kernel - These cereals are prone to contamination with cadmium (Cd) and lead (Pb), especially from pesticide residues, contaminated soils, and improper storage (Aslam et al., 2021). Heavy metal accumulation in cereals may pose chronic toxicity risks when consumed by animals, leading to bioaccumulation in poultry products

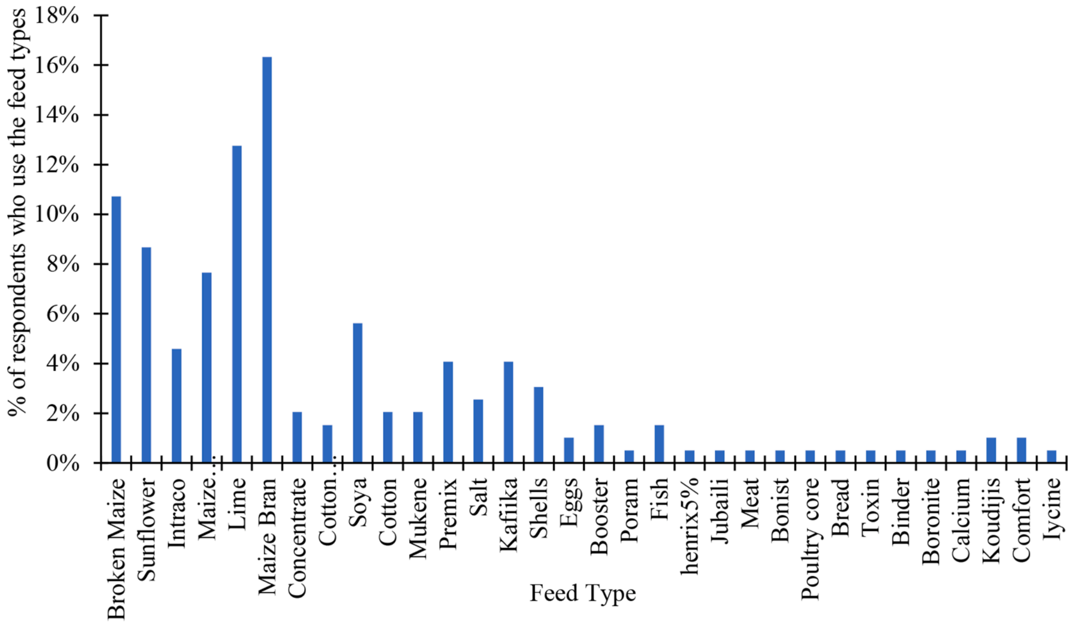

Fig. 1. The feed types and the percentage of respondents who mentioned using them at their farms.

such as eggs, (Akenga et al., 2017; Adam, Sackey & Ofori, 2022).

Lime, Shells, and Calcium Supplements: These sources are often derived from limestone or marine sources, which can contain lead (Pb), mercury (Hg), and arsenic (As) due to natural mineral composition or industrial contamination (Rehman, Adnan, Khalid & Shaheen, 2011). Sunflower and Cottonseed Cake - Oilseed crops are prone to accumulating cadmium (Cd) and arsenic (As) from soil, fertilizers, or industrial pollution (Mubeen, Ni, He & Yang, 2023). Mukene (Silver Cyprinid Fish) and Fishmeal - Fishmeal can accumulate mercury (Hg), arsenic (As), and lead (Pb), depending on the aquatic source (Plessl et al., 2019).

Premix and Concentrates: Premixes (containing vitamins, minerals, and additives) may be contaminated with lead (Pb), chromium (Cr), and arsenic (As) due to synthetic processing (Dai et al., 2016). Other sources of heavy metal contamination are milling machines which transfer debris from their surfaces to the feeds (Oniya et al., 2018).

Lower-Risk Feed Types: Soybean, Poram, Henrix 5 % (Protein Sources) – Lower risk of contamination if sourced from certified suppliers. However, genetically modified soy may introduce pesticide residues (Świątkiewicz et al., 2021). Eggs, Meat, Bread (Unconventional Feeds) – these have minimal heavy metal risk unless from contaminated sources.

The current study shows that maize bran (16.33 %), maize kernel (10.71 %), lime (12.76 %), and sunflower (8.67 %) were the most used feed types, but they pose high risks of heavy metal contamination. Feeds derived from cereals, marine sources, oilseeds, and premixes are prone to contamination with cadmium, lead, arsenic, and mercury (Adamse, Van der Fels-Klerx & de Jong, 2017) . Mitigation strategies, including regular testing, proper storage, and careful sourcing, are necessary to prevent toxicity and ensure safer poultry production.

3.2. Heavy metals in poultry feeds identified in Wakiso peri-urban areas

The Kruskal–Wallis H-test, a non-parametric method suitable for comparing more than two groups without assuming normal distribution, was applied to determine if heavy metal concentrations in poultry feeds varied significantly across administrative units in Wakiso District. The test results indicated statistically significant differences in concentrations for Chromium (Cr), Arsenic (As), and Cadmium (Cd), each with p-values below the $\alpha = 0.05$ threshold (0.033, 0.015, and 0.030 respectively). This suggests spatial variability in contamination levels, potentially linked to differences in feed sourcing, handling practices, or environmental exposure in specific areas.

In contrast, the p-value for Lead (Pb) and Mercury (Hg) were 0.095, and 0.098 exceeding the 0.05 cutoff, indicating no statistically significant variation in their concentrations across locations. This could be attributed to a more uniform exposure pattern and low concentrations detected across farms, Table 3.

The findings emphasize the localized nature of contamination for Cr, As, and Cd, likely stemming from inconsistent feed ingredient sources such as maize bran, lime, and sunflower cake, or differences in milling equipment hygiene and input monitoring. These disparities highlight the need for targeted regulation and surveillance at the sub-county level to address site-specific risks and enhance feed safety across the poultry production value chain.

All observed concentrations of Pb (range: 0.03 mg/kg in Kakiri to 0.08 mg/kg in Katabi), Hg (range: Not detected in Gombe – 0.02 mg/kg in Kajjansi), As (range: 0.53 mg/kg in Kakiri to 1.22 mg/kg in Katabi) , and Cd (0.01 mg/kg in Katabi to 0.23 mg/kg in Kakiri) in poultry feeds across Wakiso District's administrative units are within permissible limits outlined by FAO/WHO Codex, EU, and US FDA standards, as shown in Table 2. While Cr is less commonly regulated, the values in this study (range: 1.70 mg/kg in Gombe to 7.59 mg/kg in Mende) also remain below the 10 mg/kg threshold used by China and adopted in Codex-aligned literature (Codex Alimentarius, 2020).

Chromium (Cr): The highest mean recorded was in Mende (7.59 mg/kg) and Kajansi (6.71 mg/kg), suggesting localized inputs like mineral premixes or contamination from processing equipment. Still below the 10 mg/kg ceiling, but these elevated warrant targeted source tracking, especially in mixed feed operations. A previous study (Korish & Attia, 2020) recorded 2.63 mg/kg in layer starter feeds as Cr concentration. In another study, the concentrations of Cr and Pb in broiler and laying hen diets ranged from 1.51 to 5.20 mg/kg and 2.09 mg/kg to 5.27 mg/kg, which were below the upper tolerable levels of 10–300 mg/kg and 10–1000 mg/kg for Cr and Pb respectively (Korish & Attia, 2020). A similar study also identified 2.31 ± 1.23 μg/g dry weight Pb (Chinyelu Igwemmar, Kakulu & Dauda, 2022) in livestock feeds.

Lead (Pb): Lead (Pb) was detected consistently at low levels (0.03–0.08 mg/kg). There were no observed exceedances of the 10 mg/kg limit. These trace levels could be attributed to environmental dust, water supplies, or storage materials (Kumar et al., 2022). Although not immediately alarming, sustained accumulation could still pose chronic exposure risks to birds. In a study conducted in Nigeria, 44.4 % of the poultry feed samples had Pb, 29.1 % had Cd and 21 % of samples had Cr levels above permissible limit (Ifie et al., 2022). Another study (Korish & Attia, 2020) recorded 3.02 mg/kg Pb in layer starter.

Mercury (Hg): Uniformly detected at trace levels (<0.02 mg/kg) across all units. The low values suggest minimal industrial or contaminated fishmeal input, likely due to limited marine product use in these feed formulations, (Chisanga et al., May 2025). In a study conducted in Bangladesh, (Al Mamun et al., Dec., 2024) the Pb, Cd, Cr, As, and Hg contents in mg/kg of fresh poultry feed weight were 0.481–1.067, 0.025–0.118, 0.069–0.319, 0.002–0.019, respectively, comparable to

Table 3

Mean and standard deviations of heavy metal concentrations in feeds (mg/kg).

Administrative Unit	Cr (Mean ± STD)	Pb (Mean ± STD)	Hg (Mean ± STD)	As (Mean ± STD)	Cd (Mean ± STD)
Gombe (mg/kg)	1.70 ± 1.25	0.05 ± 0.02	–	0.46 ± –	0.05 ± 0.02
Kajansi (mg/kg)	6.71 ± 4.27	0.05 ± 0.03	0.02 ± 0.01	0.76 ± 0.54	0.04 ± 0.03
Kakiri (mg/kg)	3.45 ± 4.08	0.03 ± 0.03	0.01 ± 0.01	0.53 ± 0.80	0.23 ± 0.17
Katabi (mg/kg)	1.75 ± 1.60	0.08 ± 0.04	0.01 ± 0.01	1.22 ± 0.61	0.01 ± 0.01
Mende (mg/kg)	7.59 ± 6.04	0.05 ± 0.06	0.01 ± 0.01	0.79 ± 0.59	0.12 ± 0.08
Wakiso(mg/kg)	3.67 ± 2.38	0.06 ± 0.04	0.01 ± 0.00	0.59 ± 0.35	0.17 ± 0.10

Table 2

International standards for heavy metals in animal feeds (mg/kg).

Organization / Source[**]	Cr (mg/kg)	Pb (mg/kg)	Hg (mg/kg)	As (mg/kg)	Cd (mg/kg)	Ref
WHO (1996)	1.30 (plants)	2	–	–	0.02 (plants)	(WHO, 1996)
EU (Directive 2002/32/EC)	–	10	0.1	2	1	(Ioannis et al., 2012)
FAO/WHO Codex	–	10	0.1	2	1	(Codex Alimentarius, 2020)
China (GB 13,078–2017)	10	10	0.1	2	1	(Codex Alimentarius, 2020)
US FDA (Guidance)	–	10	0.1	2	1	(Choi, 2012)

[**] *Chromium is not commonly regulated in feed by WHO or Codex; values for Cr are often derived from soil or plant standards. The EU and Codex align closely on Pb, Hg, As, and Cd limits in feedstuffs.*

what was observed in this study.

Arsenic (As): Katabi stood out with a higher mean (1.22 ± 0.61 mg/kg) and individual values up to 2.22 mg/kg were observed. While the mean was still below the 2 mg/kg threshold, these levels imply possible historical use of arsenic-based growth promoters or contaminated water. Katabi may benefit from follow-up residue studies. In another study (Al Mamun et al., Dec., 2024) As levels in the range 0.007–0.071 mg/kg were identified in poultry feeds. Other studies (Korish & Attia, 2020) recorded 2.03 mg/kg in layer starter feeds, (Saipan, Tengjaroenkul & Prahkarnkaeo, Aug., 2014).

Cadmium (Cd): Detected in nearly all units, with higher means in Kakiri (0.23 ± 0.17 mg/kg) and Wakiso(0.17 ± 0.10 mg/kg). While below thresholds of 1.0 mg/kg, cadmium's cumulative toxicity and potential food chain persistence merit ongoing surveillance, (Kubier, Wilkin & Pichler, Sep., 2019). Another study (Chinyelu Igwemmar, Kakulu & Dauda, 2022) identified Cd with a mean of 0.43 ± 0.20 µg/g dry weight. A similar study, (Korish & Attia, 2020) recorded 0.055 mg/kg in layer starter feeds. Cd has a long biological half-life and accumulates in kidneys and liver, creating a risk for consumers of animal organs (Genchi et al., 2020).

Urban runoff and industrial waste from peri-urban Wakisoareas like Kajansi and Katabi is a likely contributor to the heavy metals observed in this study, especially given high Cr and As near Lake Victoria (Angiro, Abila & Omara, 2020).

3.3. *Heavy metal contamination levels in chicken eggs*

The dataset included concentrations of heavy metals (Cr, Pb, Hg, As, Cd) in both poultry eggs from various administrative units. While the heavy metal concentrations in eggs vary between administrative units, most units exhibit very low or non-detectable levels, indicating either minimal contamination or differences in agricultural practices, Table 4. However, certain areas like Kajansi and Katabi display higher concentrations, particularly of Cr, and As in eggs.

Chromium concentrations in poultry eggs were mostly non-detectable in Gombe and Mende. The higher concentrations in of Cr in eggs in Kajansi (6.72 ± 4.91 mg/kg), is correlated with high Cr concentrations in feeds. The Cr contamination, particularly in feeds, could result in a gradual accumulation in poultry tissues, including eggs, (Kirov, Karadjov, Hristov & Alexandrova, May 2023). Chronic exposure to high levels of Cr can lead to health issues in both poultry and humans, including potential liver and kidney damage, carcinogenic risks, and immune system disruption. In one study (Hossain et al., 2017), chromium concentrations in eggs was 0.2174 to 1.08 mg/kg and the highest concentration of chromium 2.4196±0.0019 mg/kg was found in egg yolk.

Pb concentrations were consistently low in eggs, with concentrations mostly at or near zero. However, certain villages/divisions like Wakisoand Kajansi exhibited detectable levels of Pb in feeds (e.g., Kajansi with Pb at 0.02 ± 0.02 mg/kg in eggs and Wakisowith Pb as 0.03 ± 0.01 mg/kg in feeds), (Hoseini et al., 2023a). Lead exposure, even at low levels, poses significant risks to both poultry and human health. In humans, lead is a neurotoxin and can cause developmental issues in children, renal problems, and cognitive impairments. In poultry, it may impact growth, egg quality, and reproduction (Wani, Ara & Usmani, 2015).

Table 4
Mean concentration of heavy metals in chicken eggs.

Administrative Unit	Cr mg/kg	Pb mg/kg	Hg mg/kg	As mg/kg	Cd mg/kg
Gombe	ND**	ND	ND	0.16 ± 0.12	ND
Kajansi	6.72 ± 4.91	0.02 ± 0.02	0.02 ± 0.01	1.36 ± 0.48	0.04 ± 0.01
Kakiri	2.81 ± 2.29	0.01 ± 0.01	0.01 ± 0.01	0.72 ± 0.65	0.06 ± 0.02
Katabi	4.40 ± 3.22	0.01 ± 0.01	0.02 ± 0.01	1.09 ± 0.64	0.02 ± 0.03
Mende	ND	0.02 ± 0.00	ND	0.11 ± 0.07	ND
Wakiso	3.56 ± 3.86	0.03 ± 0.01	0.01 ± 0.01	1.01 ± 0.52	0.15 ± 0.10

** *ND means "Not Detected".*

Mercury concentrations in eggs were generally very low, while in most administrative units, no Hg was detected. The mean concentrations observed in Kajansi was 0.02 ± 0.01 mg/kg while those in Wakiso-samples were 0.01 ± 0.01 mg/kg. Mercury is highly toxic even at low levels and is bio-accumulative. It can affect the nervous system, particularly in growing organisms (Fernandes Azevedo et al., 2012). Poultry exposed to high mercury levels can suffer from neurological impairments, and humans consuming contaminated eggs might experience similar health issues, including developmental delays and cognitive dysfunction (Oliveira et al., 2018).

Arsenic concentrations in poultry eggs were generally low, with the highest recorded in Kajansi as 1.36 ± 0.48 mg/kg. Arsenic is a carcinogen and poses long-term health risks, including skin, lung, and bladder cancer. Chronic exposure can also lead to organ damage, particularly affecting the liver and kidneys (Hong, Song & Chung, 2014; Speer et al., 2023).

Cadmium concentrations in poultry eggs were largely undetectable, similar to Mercury though small amounts were present in some regions like Mende and Wakiso. Cadmium is a heavy metal with toxic effects on the kidneys and bones. Even low levels of exposure over time can result in kidney dysfunction, bone demineralization, and potential cancer risks (Nordberg et al., 2018). Other studies that detected heavy metals in eggs are shown in Table 5.

3.4. *Heavy metal intake in the different administrative units through egg consumption*

3.4.1. *Statistical significance*

The non-parametric comparisons of heavy metal intake across administrative units was done using the Kruskal–Wallis H test. The results reveal statistically significant differences ($p < 0.001$) in Cr, Hg, As, and Cd intake, confirming that geographic location influences exposure levels. Chromium and arsenic exhibit the highest H-statistics, suggesting strong spatial variability, possibly due to localized feed compositions or environmental inputs. Cadmium variation is notably driven by outlier regions with elevated concentrations. In contrast, lead shows only mild differences ($p = 0.015$), indicating more uniform but low-level exposure. These findings underscore the need for targeted surveillance and

Table 5
Heavy metals in eggs identified in other studies.

Heavy metal	Concentration	Matrix	Reference	Year
Pb (µg/kg)	0.54 ± 0.19	Hen egg yolks	(Kılıç Altun, Paksoy & Aydemir, 2024)	2024
Pb (µg/kg)	7.16 ± 0.248	Hen eggs	(Hoseini et al., 2023a)	2023
Cd (µg/kg)	2.83 ± 0.151	Hen eggs	(Hoseini et al., 2023a)	2023
Cd (mg/kg)	< 0.001	Hen eggs	(Samad et al., 2023)	2023
As (mg/kg)	0.033±0.004	Hen eggs	(Hashish, Abdel-Samee & Abdel-Wahhab, 2012)	2012
As (mg/kg)	0.04–0.5	Hen eggs	(Samad et al., 2023)	2023
Cr (µg/kg)	0.48–8.45	Hen eggs	(Aliu, Dizman, Sinani & Hodolli, 2021)	2021
Cr (mg/kg)	0.01–0.89	Hen eggs	(Samad et al., 2023)	2023
Hg (µg/kg)	0.27–6.54	Hen eggs	(Aliu, Dizman, Sinani & Hodolli, 2021)	2021

region-specific feed safety interventions.

3.4.2. Human heavy metal intake variation

Chromium (Cr): Highest weekly intake was in Kajansi (mean 237.6 μg), followed by Katabi and Wakiso. There was no measurable Cr intake in Gombe or Mende. The data suggests localized sourcing of Cr through chicken feed composition, likely from mineral premixes.

Lead (Pb): Overall low intakes, all well below international safety thresholds. Wakisoexhibited the highest intake (1.06 μg/week), which remained modest. Kajansi, Kakiri, and Katabi also showed measurable Pb exposure, likely via environmental residue in feed ingredients. Despite low absolute values, any detection of Pb is a concern, especially in vulnerable populations such as children and pregnant women, (WHO).

Mercury (Hg): The Hg was observed in all administrative units except Gombe and Mende. The highest average were in Kakiri and Katabi (0.61–0.63 μg/week), though still at trace levels. The consistency of detection suggests low-level contamination from additives like fishmeal, (Eboigbe et al., 2025).

Arsenic (As): The As intake was universal across units, ranging from 4.5 μg/week (Mende) to 42 μg/week in Kajansi. Kajansi's As intake was the highest, with moderate intake variability. These values may reflect differences in feed formulations and ingredient sourcing between peri-urban and rural zones, (Cubadda et al., 2017).

Cadmium (Cd): Detected mainly in Kakiri (1.67 μg/week) and Wakiso(2.69 μg/week), the latter with high variability, (Satarug, Vesey & Gobe, 2017). Trace amounts in Kajansi and Katabi. No cadmium was observed in Gombe or Mende.

3.4.3. Comparative analysis of heavy metal intakes against international limits

To enable a robust comparison, the tolerable intake limits were harmonized to weekly equivalents based on a 70 kg adult as standard: Weekly intake limits for heavy metals vary widely: WHO/FAO allows up to 12,250 μg of lead and 7350 μg of arsenic, while EU/WHO sets stricter mercury and cadmium thresholds. Europe's strictest standards cap lead at 490 μg. WHO/JECFA recommends even lower weekly limits, especially for mercury (280 μg) and cadmium (490 μg).

All intake values were well below international regulatory thresholds, including the WHO/FAO's generous PTWI thresholds (based on general population); the EFSA's more conservative European limits, often used in precautionary risk assessments and the WHO/JECFA guidance for a standard 70 kg adult. This suggests that poultry consumption alone, under current feed contamination levels, is unlikely to pose acute toxicological risk for the general Ugandan consumer population.

Chromium (Cr): Kajansi's mean intake (237.61 μg/week), was less than 5 % of even JECFA's strict threshold (2100 μg/week), though it was higher than 0.017 – 0.308 range obtained in a similar study (Aliu, Dizman, Sinani & Hodolli, 2021). However, Cr speciation matters, the hexavalent chromium (Cr^{6+}) is far more toxic than trivalent (Cr^{3+}) (Sharma, Singh, Parakh & Tong, 2022). The data from this study lacks speciation granularity, raising a flag for further chemical characterization. Though there is no threshold breach, enhanced surveillance is prudent, especially near industrial or peri-urban zones.

Lead (Pb): Wakiso's 1.06 μg/week is far below the EFSA limit of 490 μg/week, signaling no immediate concern. The results from this study were however, higher than 0.26 ± 0.01 μg/week identified in another study, (Hoseini et al., 2023a). Despite low levels, lead is bioaccumulative and known to affect cognitive and developmental health, especially in children. Continued biomonitoring in feed chains and cross-sectional surveillance in eggs/meat is warranted.

Mercury (Hg): Katabi's average (0.63 μg/week) is less than 0.1 % of the WHO threshold. Another study (Aliu, Dizman, Sinani & Hodolli, 2021) found 0.010 – 0.238 μg/week. Likely source: marine-based feed components (e.g., fishmeal). Hg poses a risk via biomagnification, especially in aquatic food chains, but risk here remains minimal based on observed values.

Arsenic (As): While Kajansi (42.29 μg/week) has the highest As mean, it's still <5 % of even the strictest WHO/JECFA limit. A similar study (Samad et al., 2023) identified 1.46 – 18.20 μg/week range As intake. Past use of arsenic-based growth promoters (like roxarsone) in poultry feeds has been phased out globally, but their residues may persist. Persistent low-level As intake is linked to cardiovascular and cancer risks, so routine residue testing in feeds and poultry tissues is advisable.

Cadmium (Cd): Wakiso's Cd intake (2.69 μg/week) is 1000 times lower than the WHO/FAO PTWI (2695 μg/week), and is also far lower than the JECFA's tighter limit (490 μg/week) when using conservative benchmarks. In another study (Hoseini et al., 2023a), 0.10 ± 0.01 μg/week was observed. Cd tends to accumulate in kidneys and livers of exposed poultry, posing risk to human consumers of organ meats. It is bioaccumulative nature and renal toxicity call for continued surveillance (Genchi et al., 2020; Rahimzadeh, Rahimzadeh, Kazemi & Moghadamnia, 2017; Charkiewicz et al., 2023; Rasin et al., 2025), particularly in Kakiri and Katabi, where moderate levels (1–3 μg/week) were consistently recorded. Other studies that computed weekly heavy intake are shown in Table 6.

3.5. Monte Carlo simulation

3.5.1. Health risk characterization

The probabilistic health risk assessment revealed that non-cancer risks associated with egg consumption across Wakiso District were generally low. Hazard Index (HI) medians remained below 1 for all administrative units and wealth quintiles, with 95th percentile values also staying under the threshold of concern. Notably, arsenic (As) and chromium (Cr) emerged as dominant contributors to cumulative risk in Kajansi and Katabi, consistent with localized concentration elevations, (Samad Abedi et al., 2023; Formisano et al., 2025).

For lead (Pb), the calculated Margin of Exposure (MOE) values were significantly greater than 1 across all strata, indicating negligible risk for developmental neurotoxicity. This aligns with EFSA's benchmark dose modeling, which suggests MOE values above 1000 are generally considered of low concern, (Kwon et al., 2024).

The lifetime cancer risk (LCR) for arsenic, under the base assumption

Table 6
Heavy metal intake from other studies.

Metal	Heavy Metal Concentration in eggs	Computed Weekly Intake (μg/week)**	Matrix	Ref.	Year
Lead (Pb)	7.16 ± 0.25 μg/kg	0.26 ± 0.01	Hen eggs	(Hoseini et al., 2023a)	2023
Cadmium (Cd)	2.83 ± 0.15 μg/kg	0.10 ± 0.01	Hen eggs	(Hoseini et al., 2023a)	2023
Arsenic (As)	40–500 μg/kg	1.46 – 18.20	Hen eggs	(Samad et al., 2023)	2023
Chromium (Cr)	0.48–8.45 μg/kg	0.017 – 0.308	Hen eggs	(Aliu, Dizman, Sinani & Hodolli, 2021)	2021
Mercury (Hg)	0.27–6.54 μg/kg	0.010 – 0.238	Hen eggs	(Aliu, Dizman, Sinani & Hodolli, 2021)	2021

** *Computed based on the FAO data that an average Ugandan consumes 36.4 g/week of eggs and converted to μg/week.*

of 30 % inorganic As, fell within the 10^{-7} to 10^{-6} range, well below the commonly accepted de minimis risk threshold of 10^{-4} for foodborne exposures. However, under a worst-case scenario (70 % inorganic As), LCR values approached 10^{-5} in Kajansi, warranting further investigation into speciation and potential feed or water sources contributing to elevated As levels, (Speer et al., 2023).

Regarding chromium, the conservative assumption of 10 % Cr(VI)—the more toxic form—resulted in increased HQs but did not exceed 1 at the 95th percentile. This suggests that even under precautionary modeling, Cr-related risks remain within acceptable bounds, though speciation confirmation is recommended given Cr(VI)'s carcinogenic potential, (Sun, Brocato & Costa, 2015).

3.5.2. *Equity and consumption patterns*

Stratification by wealth quintile revealed that egg intake increased with socioeconomic status, yet overall risk remained concentration-driven rather than intake-driven. This finding underscores the importance of source control (e.g., feed quality, environmental contamination) over consumption restrictions, especially given eggs' nutritional value for lower-income households (FAO 2018).

4. Conclusions

This study investigated the presence and dietary intake of toxic heavy metals: chromium (Cr), lead (Pb), mercury (Hg), arsenic (As), and cadmium (Cd), in poultry feeds and eggs from commercial farms in Wakiso District, Uganda. The results revealed significant variation in heavy metal concentrations across different administrative units, with localized hotspots exhibiting elevated levels of Cr, As, and Cd. Despite these findings, all calculated intakes for these metals remained below WHO/JECFA's Provisional Tolerable Weekly Intake (PTWI) thresholds, suggesting minimal acute health risks under current consumption patterns.

However, the detection of Pb in 18 % of egg samples raises concern, as there is no established safe threshold for lead exposure due to its cumulative neurotoxic effects. Cr intake was notably elevated in WakisoS/C30 (78.88 μg/day), while Cd intake peaked at 9.10 μg/week in the same area. Katabi T/C18 recorded the highest As intake at 81.90 μg/week. These values, although within international safety limits, highlight potential chronic exposure risks, especially in populations consuming eggs regularly from these regions.

Feed analysis confirmed elevated concentrations of heavy metals in commonly used ingredients like maize bran, lime, and fishmeal, supporting the hypothesis of dietary transmission. Normality testing indicated that all heavy metal intake data were significantly non-normal, justifying the use of non-parametric tests which confirmed significant geographical differences in contamination levels.

This study was subject to certain limitations that warrant consideration. First, non-responsiveness among a proportion of farmers, attributable to factors beyond the scope of this investigation, constrained the breadth of primary data collection. Future research should therefore prioritise strategies to enhance response rates, thereby enriching the representativeness and robustness of findings. Second, limited resources restricted the geographical coverage of the study and precluded the application of advanced laboratory techniques such as speciation analyses (e.g., Cr(III)/Cr(VI), inorganic versus organic As). Such methods would enable more refined risk estimates and provide greater insight into the toxicological relevance of heavy metal exposure. Finally, while the sample size employed was sufficient for this study, larger datasets are essential to improve statistical precision, facilitate the detection of outliers, and strengthen the reliability of conclusions. Subsequent studies should thus incorporate expanded sampling frameworks, with particular attention to extreme values and regulatory thresholds, and increase the number of egg samples analysed to better inform public health decision-making.

Overall, while current health risks from egg consumption are low, ongoing monitoring, improved feed regulation, and public health interventions are critical to prevent long-term toxicological effects in both poultry and consumers.

Ethics approval and consent to participate

i. The study protocol obtained clearance from the Institutional Animal Care and Use Committee (IACUC) from the School of Veterinary Medicine and Animal Resources before commencing data collection.
ii. The researchers contacted the district veterinary office and the district local government and obtained administrative clearance. The district veterinary office then provided a guide to introduce the research team to the farmers and community. Upon arrival on the farmers the guide from the district veterinary office introduced the researchers to the selected farmer respondent. The research team explained the purpose of the visit and clarified that confidentiality would be maintained throughout the research process and while handling the data collected.
iii. Researchers requested for farmers' participation in the study and requested for their consent.
iv. After obtaining consent, the researchers used a combination of participatory observation of the selected study farm environment and infrastructure before obtaining eggs and feed samples for laboratory analysis from farm owners. Chickens on the study farms were not touched or manipulated.
v. The researchers also respected the socio-cultural context of the study area based on the exposure during the reconnaissance visits and through consultation and guidance by the district veterinary office guides and local area leaders.

Consent for publication

The manuscript does not contain any personal information in form of photos or videos of respondents.

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CRediT authorship contribution statement

Shadad Mugabi: Writing – review & editing, Validation, Investigation, Conceptualization. **Sylvia Angubua Baluka:** Writing – review & editing, Supervision. **Andrew Tamale:** Writing – review & editing, Supervision. **Antony Nyombi:** Writing – review & editing, Writing – original draft, Software, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Annex

Tests for normality for heavy metals in feeds

A. *Visual Inspections of plots*

The Fig. 2 below shows a histogram that was used for symmetry and outlier testing of the obtained results and a Q-Q Plot superimposed on the histogram to assess linearity of quantiles.

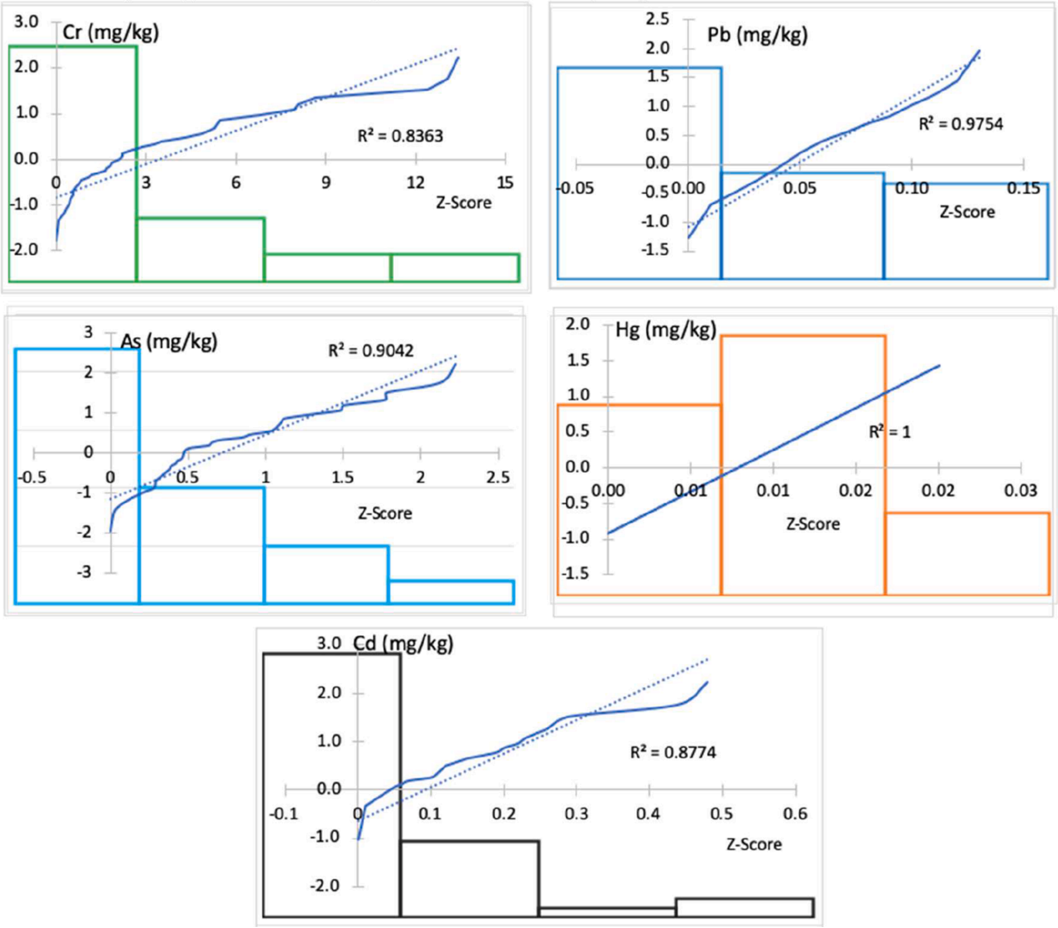

Fig. 2. Normality testing of obtained feed heavy metal concentration values from the study ($n = 40$).

In the Fig. 2 the Cr, Pb, As and Cd concentration values have a slight positive skewness. However, the Hg concentrations were well aligned to the reference line giving a regression of approximately one ($R = 1$), hence, normally distributed. The slight positive skewness and mild kurtosis is further shown in the Table 7, especially for Cr, As, Cd concentration values. Mercury (Hg) values again showed a mild negative skew approximating to a symmetric distribution. The descriptive statistics and the Shapiro-Wilk and the Anderson-Darling tests are shown in Tables 8 and 9.

B. Descriptive statistics

Table 7
Descriptive statistics.

Heavy Metal	N	Mean (mg/kg)	Median	Skewness	Kurtosis
Cr	50	4.26	2.18	1.95	4.59
Pb	48	0.058	0.05	0.67	2.9
Hg	46	0.009	0.01	–0.56	2.7
As	50	0.72	0.51	1.02	3.4
Cd	48	0.12	0.11	1.39	4.1

Additionally, the Shapiro–Wilk Test (sensitive for $n < 50$) and the Anderson–Darling Test (good for tail sensitivity).

C. The Shapiro–Wilk Test and the Anderson–Darling Test

Table 8
Summary of p-values from the Shapiro–Wilk Test and the Anderson–Darling Test.

Heavy Metal	Shapiro–Wilk p-value	Anderson–Darling (A^2)	Interpretation
Cr	< 0.001	2.94	The low p-value and large A^2 statistic show that chromium intake is significantly non-normal, likely right-skewed with heavy tails.
Pb	0.041	1.31	The Shapiro–Wilk p-value is just below 0.05, suggesting the distribution of lead values was mildly non-normal, potentially with slight skewness
Hg	0.228	0.51	No significant deviation from normality. The p-value was above 0.05 and low A^2 suggest that mercury values followed a reasonably normal distribution.
As	0.006	1.83	Arsenic values were heavily skewed consistent with environmental exposure data where some locations showed spikes.
Cd	0.008	1.74	Cadmium concentration values showed clear non-normality, likely due to its concentration in a few geographic hotspots, resulting in skewness and kurtosis.

Normality Tests (Shapiro-Wilk Test) for heavy metal intake in this study.

Table 9
The normality result of Shapiro-Wilk tests for each heavy metal intake.

Metal	W-statistic	p-value	Interpretation
Cr (μg/day)	0.645	<0.001	Not normally distributed
Pb (μg/day)	0.622	<0.001	Not normally distributed
Hg (μg/week)	0.665	<0.001	Not normally distributed
As (μg/week)	0.894	<0.001	Not normally distributed
Cd (μg/week)	0.531	<0.001	Not normally distributed

All distributions deviate from normality ($p < 0.05$), due to high number of zeros (where no heavy metal intake was observed) and skewness in high-exposure sites.
Given the non-normal distributions, non-parametric methods were used.

Data availability

Data and materials are available and can be shared upon request or a reasonable notice.

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