Assessment and ICP-MS Determination of Toxic Metal Content (Cd, Cr, Ni, and Pb) in Turkish Chicken Meat for Use as Bioindicator for Human Health

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INTRODUCTION

Food is usually the main source of human exposure to elements. Among foodstuffs, chicken meat is often the most significant source of protein. It is less expensive than red meat but can result in adverse health effects and cause peripheral artery disease, atherosclerotic heart disease, vascular disease, gut disease and hypertension. Poultry production is one of the main sources of global agribusiness. According to official data, world poultry production increased considerably and reached 98 million tons in 2010 (1). Turkey is among the 10 largest chicken producers in the world with approximately one billion head and about 1.7 million tons, with exports up to 330.000 tons in 2012 (2-3). The essential elements of Zn, Cu, and Fe act as enzyme cofactors, Zn and Cu are the organic molecule stabilizers for proteins, Co for vitamin B-12, and Mn, Fe, Cu, Se as structural component and participants in redox reactions (4-7). Compared to adults, children are more susceptible to heavy metal toxicity which interferes with the incompletely developed blood-brain barrier (BBB) and the central nervous system (CNS), and increased absorption of Pb from the intestine. Lead has a non-biological function (as well as As, Cd, and Hg) and is one of the most toxic metals. Lead is well absorbed via ingestion, inhalation, and through the skin, and can be transported to the CNS via similar pathways as the essential metals. Pb²⁺

ABSTRACT

The aim of this study was to determine the toxic metal concentrations in chicken meat consumed by the Turkish population and to establish health risk assessment. The concentrations of cadmium (Cd), chromium (Cr), nickel (Ni), and lead (Pb) in chicken meat produced by seven different suppliers were determined using inductively coupled plasma mass spectrometry (ICP-MS). The Cd concentrations in all samples were found to be lower than the maximum allowable concentration (MAC) of 50 $\mu\text{g kg}^{-1}$. The Cr and Ni concentrations in all samples were lower than 700 and 450 $\mu\text{g kg}^{-1}$, respectively, with the exception of Cr in the samples from one producer and of Ni from two other producers. The Pb concentrations (max. 413 $\mu\text{g kg}^{-1}$) in the samples from three producers were found to be higher than the MAC of 100 $\mu\text{g kg}^{-1}$. Although the concentrations of lead in some samples exceed the limits set by the authorities, risk of developing cancer over a human lifetime was found to be not significant (between 3 to 16 per 1,000,000 persons).

can inhibit the synthesis of hemoglobin, interferes with the normal development of children's brains, their behavior, including lack of attentiveness and possibly low IQ. All trace and essential elements can become toxic when ingested in excess, or may lead to deficiencies when taken in insufficient amounts (4-5, 8-10). Recent findings report significant interaction with metals in relation to pediatric health (11).

The main source of metals in chicken meat arises from the contamination of feed, drinking water, and processing. The pollutants such as Cd, Cr, Ni, and Pb bioaccumulate in chickens and subsequently transfer to the human being. Thus, analysis of chicken samples for their elemental content is very important in order to ensure adequate intake of essential elements and prevent exposure to harmful ones. Toxicity and/or adverse effects of many elements including Cd, Pb, Ni, Cr, Cu, Fe, Mn, Se, and Co (12-16) in foods and biological matrices (17-21) have been well documented. However, comprehensive studies on the metal concentrations in chicken meat from Turkish sources are relatively few (22-26).

Flame atomic absorption spectrometry (FAAS), electrothermal atomic absorption spectrometry (ETAAS), and inductively coupled plasma atomic emission spectrometry (ICP-AES) are commonly used to determine the metal concentrations in various foods, biological and environmental matrices (26-31). However, inductively coupled plasma mass spectrometry (ICP-MS) is a superior technique for determining metal concentrations at the $\mu\text{g L}^{-1}$ levels due to its high sensitivity, reliability, and wide linear dynamic range (32-35).

The present study has two primary objectives: (a) to obtain preliminary information on the concentration of four toxic trace metals (Cd, Cr, Ni, and Pb) in chicken meat obtained from seven different producers in Turkey and (b) to conduct a screening-level evaluation of the potential hazards

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associated with Pb in this food, particularly for children. The chicken meat was obtained at different stores in different cities in Turkey. Further, seasonal variations in toxic metal concentrations of chicken muscle samples were also studied. A microwave oven was used for digestion of the tissues and ICP-MS was used for the determination of Cd, Cr, Ni, and Pb in clear solutions.

EXPERIMENTAL

Instrumentation

All measurements were performed using a PerkinElmer® ELAN® 9000 ICP-MS (PerkinElmer, Inc., Shelton, CT, USA), equipped with a Rytan® double-pass spray chamber and cross-flow nebulizer. The ICP-MS operating conditions were taken from the manual. A microwave digestion system (CEM MARSPress, Matthews, NC, USA) was used to digest the chicken samples. Ultrapure water was obtained with a Millipore® 1 Direct-Q® water purification system (Millipore Corporation, Bedford, MA, USA).

Reagents and Standard Solutions

Unless otherwise stated, all chemicals used throughout the study were of high-purity reagent grade. All glassware was kept permanently full of 1.0 mol L⁻¹ nitric acid when not in use. Concentrated nitric acid (65%, E. Merck, Darmstadt, Germany) was used in the digestion of the chicken meat samples. The working solutions of the elements were prepared from their stock (1000 mg L⁻¹) solutions (Merck, Darmstadt, Germany). A solution of 10 mg L⁻¹ Ir (Merck, Darmstadt, Germany) was employed.

Sampling and Digestion of Samples

Chicken meat samples were collected during each month in the autumn of 2008, the winter of 2008, and the autumn of 2009. The

muscle is preferred for consumption because it is the main edible part of the chicken. The chicken meat samples from each supplier were randomly purchased at large supermarkets and traditional open markets in Elazig city, Turkey (population of 500,000). It should be mentioned that the production centers of the producers are in different cities of Turkey, and the produced meat is distributed to all cities in Turkey. After collection of the samples, the edible parts were separated and the tissues such as fat, skins, tendons, and bones removed. The samples were transported immediately to the laboratory and stored in a refrigerator until analysis. Three to five different samples of 0.50 g from one chicken muscle were taken to represent a few hundred grams of chicken muscle. Thus, the results are given as the mean values of three to five different digestions for one sample.

The samples were digested using a microwave oven in order to prevent possible loss of the elements of interest in the dry digestion procedure. For this purpose, 0.50 g of fresh chicken sample was transferred into a digestion vessel and 5.0 mL of concentrated nitric acid added. After removal of the nitric oxide gases over a period of a few minutes, the vessels were closed and placed into the microwave oven. The samples were irradiated to digestion at 800 W and 180 °C for 15 minutes. The digested samples were left to cool to room temperature. Then, the digests were evaporated to 1.0 mL, followed by dilution to 20 mL with ultrapure water. The vessels were cleaned after each run using the same reagents as well as the program applied for sample decomposition, followed by rinsing with ultrapure water. Subsequently, the samples were analyzed for Cd, Cr, Ni, and Pb by ICP-MS using Ir as the internal standard. Blank analysis was

also performed to determine the background contribution of the reagents and treated the same way as the samples.

Analytical Performance

There are three methods to check the accuracy of the results obtained. These are (a) the use of standard reference material (SRM), (b) comparison of the results with those obtained by an independent method for the same samples, and (c) the spike recovery test. In this study, the SRM NCS ZC73016 Chicken Trace Elements (P.R. China) was analyzed to verify the accuracy of the metal determinations by ICP-MS. Further, the spiked recovery values were determined. The blank digests were prepared and analyzed in the same way. Each sample obtained in each month for one season was analyzed in triplicate. Thus, an average of the results of nine samples was used as the mean value on the basis of the original fresh weight for each sample from each season.

Health Risk Analysis

Analysis of health risks associated with consumption of chicken containing lead was performed in accordance with the procedure provided by the United States Environmental Protection Agency (US EPA) Region III Risk-based Concentration Table (36). Lead was included in the risk analysis because of the toxicological data available in the Integrated Risk Information System (IRIS) (36). In general, the analysis for determining non-carcinogenic health risks was assessed by the HQ=Hazard quotient. This means the ratio of a single substance exposure level over a specified time period (sub-chronic) to a reference dose (RfD) for that substance derived from a similar exposure period (HQ=EED (estimated exposure dose)/RfD). The system is based on whether there may be a concern for poten-

tial non-carcinogenic effects if the exposure level exceeds this threshold value. The RfD is the oral reference dose: 1.0×10^{-3} mg/kg/day for Cd and 4.0×10^{-3} mg/kg/day for Pb (36). The RfD represents an estimate of daily oral exposure that is likely to occur without an appreciable risk of adverse health effects over a lifetime. The EED was calculated using the following equation (36):

$$\text{EED} = \text{Conc.} \times \text{IR} \times \text{EF} \times \text{ED} / (\text{BW} \times \text{AT})$$

where:

EED=Estimated lead exposure dose (mg/kg/day)

Conc.=Lead concentration in chicken (mg/kg, wet weight)

IR=Ingestion rate (adult, 0.227 kg chicken per day; child, 0.1135 kg chicken per day)

EF=Exposure frequency (156 days/year for people who eat chicken three times per week)

ED=Exposure duration (adult, 70 years; child, his/her age (see Table I)

BW=Body weight (adult, 70 kg; child, his/her age (see Table I)

AT=Averaging time or the period over which cumulative exposures are averaged ($\text{AT} = \text{ED} \times 365$ days/year for non-cancer risk and $\text{AT} = 70$ years $\times 365$ days/year for lifetime cancer risk)

For carcinogens, risks are estimated as the incremental probability of an individual to develop cancer over a lifetime as a result of exposure to potential carcinogens. The acceptable risk levels for carcinogens range from 10^{-4} (risk of

developing cancer over a human lifetime is 1 in 10,000) to 10^{-6} (risk of developing cancer over a human lifetime is 1 in 1,000,000). The lifetime cancer risk (CR) was obtained by multiplying the estimated exposure dose of the chemical slope factor (SF) according to the equation of:

$$\text{CR} = \text{EED} \times \text{SF}$$

where the SF is the oral carcinogenic slope factor from the IRIS database (8.5×10^{-3} (mg/kg/day for Pb) (36).

RESULTS AND DISCUSSION

The accuracy of the Cr, Ni, and Pb concentrations in NCS ZC73016-Chicken Trace elements reference standard material was $541 \mu\text{g kg}^{-1}$, $138 \mu\text{g kg}^{-1}$, and $100 \mu\text{g kg}^{-1}$, while the certified values are $590 \mu\text{g kg}^{-1}$, $150 \mu\text{g kg}^{-1}$, and $110 \mu\text{g kg}^{-1}$, respectively. Because the recoveries were more than 90%, the Cr, Ni, and Pb determinations in this study were considered to be accurate. In the recovery test, the spiked metal concentrations to the chicken muscle sample were found to be more than 90% for all studied metals. The limits of detection (LODs) calculated as three times the standard deviation of the blank were found to be (as $\mu\text{g L}^{-1}$) 0.02 for Cd, 0.17 for Cr, 0.06 for Ni, and 0.03 for Pb. The limits of quantitation (LOQs) were found to be (as $\mu\text{g L}^{-1}$) 0.05 for Cd, 0.5 for Cr, 0.2 for Ni, and 0.1 for Pb. The repeatability values were found between 8–17% depending on the metal species. Due to the high sensitivity of the ICP-MS method, the microwave oven could be applied for digestion of the chicken tissue samples without problems using samples of less than 0.50 g. The advantages of microwave digestion instead of the classical dissolution methods include use of a shorter period of time, less consumption of acid, and being able to keep volatile compounds in the solution (37).

TABLE I
Estimated Exposure Dose (EED), the Hazard Quotient (HQ),
and Lifetime Cancer Risk (CR) for Children
Based on an Average Intake of 0.4 mg kg^{-1} Pb in Chicken Muscle

Child Age (Year)	BW (kg)	EED	HQ=EED/RfD (0.004 for Pb)	R=EEDxSF (8.5×10^{-3})
1	10	1.94×10^{-3}	0.49	1.65×10^{-5}
2	13	1.49×10^{-3}	0.37	1.27×10^{-5}
3	15	1.29×10^{-3}	0.32	1.10×10^{-5}
4	17	1.14×10^{-3}	0.29	9.69×10^{-6}
5	19	1.02×10^{-3}	0.26	8.67×10^{-6}
6	20	9.70×10^{-4}	0.24	8.25×10^{-6}
7	23	8.44×10^{-4}	0.21	7.17×10^{-6}
8	26	7.46×10^{-4}	0.19	6.34×10^{-6}
9	30	6.47×10^{-4}	0.16	5.50×10^{-6}
10	35	5.54×10^{-4}	0.14	4.71×10^{-6}
11	40	4.85×10^{-4}	0.12	4.12×10^{-6}
12	44	4.41×10^{-4}	0.11	3.75×10^{-6}
13	49	3.96×10^{-4}	0.10	3.37×10^{-6}
14	53	3.66×10^{-4}	0.09	3.11×10^{-6}
Adult	70	5.54×10^{-4}	0.14	4.71×10^{-6}

BW = Average body weight (kg).

IR = Ingestion rate (IR) of 0.1135 kg chicken per day for children and 0.227 kg for adult.

EF = Exposure frequency (EF) of 156 for the people who eat chicken three times per week.

ED = Exposure duration (ED) based on age of child.

The Provisional Tolerable Weekly Intake (PTWI) is the maximum amount of a contaminant to which a person can be exposed per week over a lifetime without any unacceptable risk of health effects. The PTWI is set by the Food and Agriculture Organization/World Health Organization (FAO/WHO) Joint Expert Committee on Food Additives (JECFA) (38). The WHO established PTWI of Cd and Pb at 5 and 25 $\mu\text{g kg}^{-1}$ body weight, respectively, for all humans which is equivalent to 350 and 1750 $\mu\text{g/week}$, respectively, for an adult weighing 70 kg (38). The JECFA has established a PTWI of 35 $\mu\text{g/kg/week}$ for Ni, which is equivalent

to 2450 $\mu\text{g/week}$ for an adult weighing 70 kg (4, 38). The official established Recommended Dietary Allowance (RDA) of Cr is 50–200 $\mu\text{g/day}$ for an adult weighing 70 kg (4, 38). According to the U.S. dietary guidelines of 2001 (39), the adequate daily intake of Cr was lowered from 50–200 μg to 30–35 μg for an adult male and to 20–25 μg for an adult female.

The maximum allowable concentrations (MAC) for each food is based on regional food consumption and the total amount of the metal of interest in those foods should not exceed the weekly amounts of 350 μg Cd, 1750 μg Pb, and 2450 μg Ni, and daily amounts

of 50–200 μg Cr, for an adult weighing 70 kg. In view of the fact that food consumption is dependent on income, household consumption, age group, and socio-economic and religious dietary factors, the MAC values established by authorities in the developing countries do not reflect the actual intakes in undeveloped countries.

Because the Cd concentrations in this study were in the range of 6–40 $\mu\text{g kg}^{-1}$ (lower than the MAC of 50 $\mu\text{g kg}^{-1}$), the Cd levels are not given as a separate figure. It can be seen from Figures 1 and 2 that the Cr and Ni concentrations were in the range of 246–1893 $\mu\text{g kg}^{-1}$ and 170–822 $\mu\text{g kg}^{-1}$, respectively. The Turkish Food Codex and other similar authorities have not yet established the MAC for both Cr and Ni in chicken meat (40). The variations of Pb in chicken muscle for seven different producers were found to be in the range of 90–413 $\mu\text{g kg}^{-1}$ (see Figure 3). According to the Turkish Food Codex and the European Commission Regulation, the MAC of Cd and Pb in chicken tissue is 50 and 100 $\mu\text{g kg}^{-1}$ (28, 40). The results of this study show that the MAC limit for Cd in all studied samples did not exceed the MAC, while the Pb level was in excess in the samples from three producers. From Figures 2–3, it can be seen

Fig. 1. Variations in Cr concentrations of chicken muscle depending on firms and seasons between 2008-2009.

Fig. 2. Variations in Ni concentrations of chicken muscle depending on firms and seasons between 2008-2009.

Fig. 3. Variations in Pb concentrations of chicken muscle depending on firms and seasons between 2008-2009.

that there is a decrease in Ni and Pb concentration for the samples collected from producers 1 and 6 in the autumn of 2009 in comparison to the autumn of 2008. For all other producers, there were no considerable changes found in Ni and Pb concentration. In addition, as seen in Figure 1, the Cr concentrations for samples from all of the studied producers did not show a considerable difference among the sampling seasons.

Among the more than 100 chicken samples analyzed (taking into consideration that some of the data points in Figures 1–3 are the mean value of 10 sub-samples), the highest metal concentrations based on fresh weight were found to be $40 \pm \mu\text{g kg}^{-1}$ Cd and $1893 \mu\text{g kg}^{-1}$ Cr for producer 3, $822 \mu\text{g kg}^{-1}$ Ni for producer 5, and $413 \mu\text{g kg}^{-1}$ Pb for producer 1. Assuming an intake of three portions of 100 g chicken per week, the weekly doses of Pb are calculated to be approximately $124 \mu\text{g Pb}$, which is 7% of PTWI ($1750 \mu\text{g Pb}$).

In the literature, few reports particularly with regard to toxic metal concentrations in chicken muscle samples have been published. Batista et al. (2012) evaluated the concentration of nonessential and essential elements in chicken, pork, and beef produced in Brazil (32). They reported that the metal concentrations in chicken meat were considerably dependent on whether the poultry was free-range or confined chicken. Free-range chickens had a higher Cu, Co, As, Cd, and Pb concentration than confined chickens. The free-range chickens were in the metal ranges of (as $\mu\text{g kg}^{-1}$) 1.2–7.4 (mean 4.5) for Cd and 5.7–32 (mean 15.3) for Pb (32). Yabe et al. (2013) reported free-range chicken muscles (as $\mu\text{g kg}^{-1}$) containing 1–12 (mean 5) for Cd, 23–913 (mean 228) for Pb, 13–42 (mean 23) for Ni, and 57–106 (mean 75) for Cr (29). Yilmaz and Gecgel (2011) reported

trace metals in chicken meat as $13 \mu\text{g kg}^{-1}$ Cd and $27 \mu\text{g kg}^{-1}$ Pb (26). It is clear that the results of the present study were higher than the results found by Yabe et al. (2013) and Yilmaz and Gecgel (2011) (26, 29). Uluzozlu et al. (2009) (10) reported mean metal concentrations in chicken meat (as $\mu\text{g kg}^{-1}$) of 6.09 for Cd, 400 for Pb, 2080 for Ni, and 70 for Cr (10). The present study obtained much lower values for Ni than by Uluzozlu et al. (2009) (10).

In the present study the potential health risk from metals in chicken meat was calculated only for lead, because it surpassed or is close to the international and national limits used for human health protection (Figure 3). The EED, HQ, and CR from the RfD and SF values as set by the USEPA-IRIS (2007) for Pb (36) were calculated and are listed in Table I. The estimated exposure dose (EED), the hazard quotient (HQ), and the lifetime cancer risk (CR) for children were calculated according to an average of 0.4 mg kg^{-1} Pb in chicken meat, the ingestion rate (IR) of $0.1135 \text{ kg chicken per day}$ for children and 0.227 kg for adult, the exposure frequency (EF) of 156 for people who eat chicken three times a week, the exposure duration (ED) in years of a child, and the RfD (reference dose) of 4×10^{-3} for Pb and SF (Slope Factor) of 8.5×10^{-3} . A cancer risk value higher than the 10^{-6} is an acceptable risk for cancer occurring. The HQ values were found to be lower than 1.0 which indicates that there is no need for concern with regard to causing cancer or other systemic effects including neurotoxicity. The risks of developing cancer over a human lifetime as given in Table I are in the range of 3–16 in 1,000,000. Thus, this study verifies that Pb contamination levels in chicken should be carefully and regularly monitored to protect safe human health.

CONCLUSION

The results obtained in this study are of great importance for the protection of human health from overdose of Cd, Cr, Ni, and Pb from chicken meat produced and consumed in Turkey. It was also verified whether the metal levels exceeded the maximum allowable levels (MAC) ($50 \mu\text{g kg}^{-1}$ Cd, $100 \mu\text{g kg}^{-1}$ Pb) as set by national and international authorities. A comparison was made with provisional tolerable weekly intake (PTWI) levels (5, 25, and $35 \mu\text{g/kg}$ body weight/week for Cd, Pb and Ni, respectively). In other words, weekly amounts of $350 \mu\text{g Cd}$, $1750 \mu\text{g Pb}$, and $2450 \mu\text{g Ni}$, and daily amounts of $50\text{--}200 \mu\text{g Cr}$ for an adult weighing 70 kg are recommended by the JECFA. It was found that the Pb concentration in chickens from three producers was higher than the MAC values ($100 \mu\text{g kg}^{-1}$). Although the concentrations of lead in some samples exceeded the limits set by the authorities, risk of developing cancer over a human lifetime was found not significant (between 3 to 16 in 1,000,000).

Compliance With Ethics Requirements

This article does not contain any studies with humans or animals. The chicken muscles were purchased from markets as cut and dead.

The authors declare that they have no conflict of interest.

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