BIOACCUMULATIN OF TRACE ELEMENTS IN TISSUES OF CHICKEN AND QUAIL AND ESTIMATE HEALTH RISKS FROM THE CONSUMPTION OF BIRDS VISCERA

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Keywords: trace element, chicken, tissues, health risk.

ABSTRACT

Food safety issues and potential health risks in avian tissues are one of the most serious environmental concerns, this paper carries out probabilistic risk analysis methods to quantify trace elements bioaccumulation in chicken liver, gizzard, and lung content to assess the range of exposures for the people who consume the contaminated chicken. The concentrations of Al (aluminum), Mn (manganese), Cu (copper), Co (cobalt), and Zn (zinc) were measured using inductively coupled plasma mass spectrometry (ICP-MS), using the stock standard solution of heavy metals and blank sample. Results show the contents of elements in bird tissues samples were in the range of (18.68-62.24, 1.6-18.6, 0.12-0.61, 2.12-24.95, 35.10-93.85 μ g/g for Al, Mn, Co, Cu, and Zn respectively. A risk assessment on human health beings due to consumption of chicken was performed using toxic reference benchmark, namely the reference dose (RfD). The hazard index (HI), sum of the hazard quotients calculated for all pollutants have shown that the risks of fowls consumption were generally low and are within safe limits.

INTRODUCTION

The tissues of chicken and other domestic birds are valuable food source of humans because they are rich in essential nutrients including proteins (essential amino acids), minerals (e.g., iron, zinc, selenium), vitamins (e.g., vitamin E), and fats (essential fatty acids, such as Omega 3 fatty acids) (1). However, poultry meat accounts for only 30% of global meat consumption. Furthermore, food and agriculture organization (FAO) (2) reported that the consumption of chicken meat in 2005 increased to 31.8 kg/person compared with the 20.1 kg/person that were consumed in 1990. The purpose of risk assessment in the present study is to estimate the severity and the possibility of harm of these exposures to human health. The ingestion of these contaminants by animals results to the deposition of heavy metal in meats (3, 4). Target hazard quotients (THQ) were developed by the environmental protection agency (EPA) in the US for the estimation of potential health risks associated with long term exposure to chemical pollutants. The THQ is a ratio between the measured concentration and the oral reference dose, weighted by the length and frequency of exposure, amount ingested and body weight. The THQ value is a dimensionless index of risk associated with long term exposure to chemicals based upon reference upper safe limits. The present study was carried out to determine the levels of some heavy metals (Al, Mn, Co, Cu and Zn) in chicken and quail gizzard, liver and lung to assess the risk to estimate the severity and the possibility of harm of these exposures to human health from consumption of chicken.

MATERIAL AND METHODS

2.1 Reagents and Apparatus

The reagents with suprapur quality, analytical grade Nitric acid (70%) and hydrogen peroxide (30%) were acquired from Merck (Darmstadt, Germany) along with the stock standard solutions of Al, Mn, Co, Cu and Zn, in concentrations of 1,000 mg/l. All the plastic and glassware were cleaned by soaking in dilute HNO3 (10%) and were rinsed with de-ionized water prior to use. A Perkin Elmer model Elan 9000 inductively coupled plasma-mass spectrometry (ICP-MS, USA) was used in current study. After calibrating the apparatus with standard solutions derived from commercial materials, it was optimized according to the manufacturing standards the cones and tubes were carefully cleaned to get rid of any possible residues.

2.2 Sample collection and preparation

A total of 120 adult chickens, 120 adult quails, were collected from different farms and local markets in Selangor, Malaysia. The chickens collected were immediately slaughtered. The chickens were dissected to remove their organs and tissues, including the liver, gizzard and lung. The tissues were washed with deionized water to remove blood. The organs were cut to pieces and stored in ice until further use. Tissue samples were oven dried at 70 °C for 24 h or until a constant dry mass was achieved. Then, the dried samples were pulverized using a mortar. All samples weighed about 0.5 g per tissue. The individual tissue samples were digested with 70% nitric acid and 30% hydrogen peroxide (2:1) according to a standard analytical method (5) and left at room temperature overnight. The samples were completely digested in a block thermostat (150 °C; SASTEC, ST Dbmak 200) for 4 h until the solutions became clear. After cooling, the

solution was diluted to 50 ml with deionized water. Following digestion, the solutions were filtered through a 0.45 µm acid-resistant filter paper. The samples were stored at 4 °C for subsequent metal analyses in labeled, acid-washed, metal-free bottles. All materials and tools were soaked in 10% nitric acid solution for 48 h and rinsed in deionized water before use. The concentrations of Al, Mn, Co, Cu, and Zn, were determined by ICP-MS (PerkinElmer, model Elan 9000 A). Each analysis was carried out in duplicate; standard and blank samples were analyzed every 20 samples.

2.3 Certified reference materials for quality control

Certified reference materials were used to check the accuracy of the method for heavy metal determination. The analytical procedures were verified using the Certified Reference Materials for lobster hepatopancreas (TORT-2, National Research Council Canada). The recoveries of all metals were within a satisfactory range table 1.

Table (1)Analytical results for the Certified Reference Materials (CRM) and itscertifiedValues for each metal (µg/g dry weight)

Metals	Measured value	Certificate value	Recovery %
Mn	12.23 ± 0.32	13.6 ± 1.2	89.92 ± 2.65
Со	0.42 ± 0.020	0.51 ± 0.09	83.92 ± 4.81
Cu	83.14 ± 1.758	106 ± 10	78.44 ±2.11
Zn	140.43 ± 0.74	180 ± 6	78.01 ± 0.67

Note: The CRM for Al is not available

2.4 Assessment of health risk from avian viscera

To estimate the human health risk from consuming metal-contaminated birds viscera, the metal concentration used for risk assessment was on a dry weight basis weight. The methodology for estimating the target hazard offers an indication of the risk level because pollutant exposure data are available in US EPA Region 111 Risk-based Concentration Table US EPA 2000. The estimation can be calculated using the following equation: Target hazard quotient (THQ) = $(EF \times ED \times FIR \times C)/(RFD \times BW \times TA)$

where EF is the exposure frequency (from 365 days/year for people who eat chicken seven times a week to 108 days/year for people who eat chicken two times a week; ED is the exposure duration (70 years), equivalent to the average lifetime; FIR is the food ingestion rate in this study (chicken tissues ingestion rate = 32.7 g/person/day, *C* is the metal concentration in tissues of bird (µg/g dry weight); RFD is the oral reference dose (6,7); BW is the average body weight (64 kg, the reference weight for the age categories derived from several local studies in Malaysia) (8); and TA is the average exposure time for noncarcinogens (365 day/year × ED). To estimate the human health risk from consuming metal-contaminated chicken, the estimated exposure doses were calculated for 5 metals. The THQ is defined as the ratio of daily intake to the RFD value. If THQ is less than one, toxic effects are not expected to occur. THQ > 1.0 indicates a potential risk associated with that metal (9).

2.5 Daily intake of metals (DIM)

The DIM was calculated by the following equation:

 $DIM = (C \text{ metal} \times D \text{ food intake})/B \text{ average weight}.$

Where *C* is the metal concentration in chicken tissue, *D* is the food intake, and *B* is the average body weight. The heavy metal concentration in chicken tissues (μ g/g), daily intake of chicken (kg/person), as well as average body weight (kg/person) were calculated (10).

2.6 Statistical analysis

All calculations were performed using SPSS for Windows (vers. 18.0, SPSS Ltd., Woking, Surrey, UK). The descriptive statistics (mean values) for values of chicken liver, gizzard and lung analyzed by one-way analysis of variance followed by the Tukey honestly significant difference test. Differences were considered significant at the p<0.05 level.

RESULT

3.1 Trace metals concentration in viscera of domestic birds

Generally, result showed that tissues of two species accumulated relatively dissimilar levels of metals. The results of the statistical analysis showed significant differences (P <0.05) the content of zinc has been found in high levels in all tissues of two species, the Co burden occurred in fewer levels. Table (2) shows that the lung of chicken had significantly (P<0.05) higher Al concentration ($30.55\mu g/g$) than liver and gizzard. Quail gizzard had significantly (p<0.05) elevated Al levels ($62.24 \mu g/g$) than chicken tissues, no significant(p>0.05) differences for Al level between quail tissues. Chicken liver had 18.6 µg/g Al whereas gizzard had 21.8 µg/g Al. The maximum Mn level was absorbed in quail liver (18.6 μ g/g dry wight) followed by chicken liver. The minimum Mn level was found in chicken lung (1.6 μ g/g). Gizzard of chicken and quail collected 2.93 and 12.91µg/g of Mn respectively. The highest Co level existed in quail liver (0.61 μ g/g), whereas the lowest concentration of 0.12 μ g/g was recorded in chicken lung. Chicken and quail gizzard was gathered 0.14 and 0.53µg/g respectively. Quail tissues accumulated significantly (p<0.05) highest levels of Cu than chicken tissues. The highest concentration of Zn was occurred in gizzard of quail and chicken $(93.8-81.6\mu g/g)$ respectively), while the lowest concentration of Zn was found in lung of two birds .in general, Quail tissues were accumulated significantly (p<0.05) higher concentrations of most metals than chicken tissues.

3.2 Risk estimation and Target hazard quotient (THQ)

Data on chicken consumption patterns were obtained from (11), which were based on the average chicken consumption reported by the Ministry of Health of Malaysia (2006). The daily intake of chicken tissues in the study area (Selangor) reached 32.77 g/day/person. The suggested that the daily consumption of other species' tissue was of the same rate. To estimate the human health risk from consuming Al-contaminated tissues of chicken, the reference dose of Al (7 mg/kg/day) was used to determine the hazard quotients (THQs). Different levels of exposure to these metals were considered based on the frequency of chicken consumption. The THQ calculated from Al contaminated tissue in a frequency quail of seven days a week was ranged from 0.0015 in chicken gizzard to 0.0045 in quail gizzard. In case the person consumed chicken two days a week, the THQ ranged from 0.0004 in chicken gizzard to 0.0013 in quail gizzard (Table 3). According to the results shown in Table 3, the THQ calculated from Mn-contaminated gizzard ranged within 0.010–0.047, and that from Mn-contaminated liver was 0.037– 0.067 for adults that consumed chicken seven days a week. The THQs were 0.01-0.02 if the person ate chicken two days a week. Table 4 shows the estimates of risks to human health caused by exposure to Co and Cu from consumption of chicken and quail gizzard, lung and liver. The THQ for Co-contaminated tissues consumed seven days a week ranged within 0.0052-0.0155. The THQ for tissues consumed two days a week were within 0.001–0.0046 (Table 4).THQ calculated for Cu in chicken tissues was 0.027-0.318

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for tissues consumed seven days a week and 0.0046-0.094 for tissues consumed two days a week. THQ calculated for Zn concentration in bird tissues was explained in Table 5. THQ for Zn in birds tissues ranged from 0.159-0.070 if the person consumed chicken seven day a week. While from 0.047 to 0.020 for chicken consumed two days a week.

These individual characterizations can be excellent indicators of potential problems related to contaminants, but not sufficient to express the combined risk of all elements in tissues. Therefore, from these individual elemental THQs, an aggregate hazard index (HI) was obtained. The HI shows the combined effect of contaminants by summing the THQs for individual elements. If the HI is less than one, negative effects of chronic ingestion of chicken are unlikely to occur (12). The results indicated that the HI values were as follows: chicken gizzard, 0.293; chicken liver, 0.331; chicken lung; 0.097 quail gizzard, 0.542; quail liver, 0.465; quail lung, 0.154.

3.3 Daily intake rate of metals (DIM)

Table 6 shows the estimated DIMs for heavy metals caused by the consumption of chicken gizzard, lung and liver. To assess the toxicological significance of various metals, the estimated intakes of diet in this study were compared with those in the recommendations of FAO, FAO/WHO (2, 13), which established a reference value for the tolerable intake of metals. For the heavy metals (Al, Mn, Co, Cu, and Zn) the daily intakes of Al were higher in lung than in gizzard and liver. While Co intake was low in chicken lung (0.061 μ g/ kg/day). Daily intake of Mn and Cu were higher in liver of chicken. Zn had high daily intake in gizzard than other tissues. All estimated daily intakes for Al, Mn, Co, Cu and Zn from gizzard, lung and liver, of chicken and quail were below the tolerable daily intake (Table 6).

Metal						
	Chicken			Quail		
	gizzard liver lung			gizzard	liver	lung
Al	21.81 b	18.68 ^b	30.55 ^a	62.24*	55.17*	55.9*
Mn	2.93 ^b	10.17 ^a	1.6 ^b	12.91 ^a	18.6 ^a	3.51 ^b
Со	0.14	0.206	0.12	0.53^{*}	0.61*	0.41*
Cu	8.61 ^a	11.88 ^a	2.12 ^b	24.95 ^a	21.25 ^{a*}	4.41b [*]
Zn	81.67 a	80.32 ^a	35.10 ^b	93.85 ^a	63.17 ^b	41.58 ^b

Table (2) concentrations of trace elements $(\mu g/g)$ dry weight in chicken and quail

tissues

Values with different letters in the same row are significantly (p<0.05) different (between tissues of each species)

*significant(p<0.05) difference between two species

Table (3) Health risk estimate for Al and Mn ingestion from chicken and quail viscera

Bird species	Level of exposure day/week	Mean Al conc. µg/g ±SE	THQ	Mean Mn conc. μg/g ±SE	ТНQ
Gallus gallus domesticus(gizzard	7	21.81±1.94	0.0015	2.93±0.34	0.010
	2		0.00047		0.0031
Gallus gallus domesticus(liver)	7	18.68±1.62	0.0013	10.17±0.68	0.037
	2		0.0004		0.0102
Gallus gallus domesticus(lung)	7	30.55±3.79	0.0022	1.6±0.16	0.0058
	2		0.00065		0.0017
Coturnix coturnix japonica(gizzard)	7	62.24±19.63	0.0045	12.91±1.55	0.0476
	2		0.0013		0.0139
<i>Coturnix coturnix japonica</i> (liver)	7	55.17±4.94	0.0040	18.6±1.25	0.0678
	2		0.0011		0.0200
Coturnix coturnix japonica(lung)	7	55.9±5.95	0.0040	3.51±0.266	0.0128
	2		0.0012		0.0037

RFD (mg/kg/day) for Al (7), RFD (mg/kg/day) for Mn (0.14)

Bird species	Level of exposure day/week	Mean Co conc. µg/g ±SE	ТНQ	Mean Cu conc. µg/g ±SE	THQ
Gallus gallus domesticus(gizzard	7	0.14±0.026	0.0035	8.61±1.83	0.109
	2		0.0010		0.065
Gallus gallus domesticus(liver)	7	0.206±0.016	0.00526	11.88±0.49	0.152
	2		0.00155		0.044
Gallus gallus domesticus(lung)	7	0.12±0.018	0.0030	2.126±0.13	0.027
	2		0.0009		0.008
Coturnix coturnix japonica(gizzard)	7	0.53±0.062	0.0135	24.95±3.87	0.318
	2		0.0040		0.094
Coturnix coturnix japonica(liver)	7	0.61±0.058	0.0155	21.25±1.24	0.271
	2		0.0046		0.080
Coturnix coturnix japonica(lung)	7	0.41±0.05	0.0104	4.41±0.30	0.0563
	2		0.0030		0.0666

Table (4) Health risk estimate for Co and Cu ingestion from chicken and quail viscera

RFD (mg/kg/day) for Co (0.02), RFD (mg/kg/day) for Cu(0.04)

Bird species	Level of exposure day/week	Mean Zn conc. µg/g ±SE	ТНQ
Gallus gallus domesticus(gizzard	7	81.67±2.47	0.138
	2		0.041
Gallus gallus domesticus(liver)	7	80.32±3.69	0.136
	2		0.040
Gallus gallus domesticus(lung)	7	35.10±2.20	0.0597
	2		0.0176
Coturnix coturnix japonica(gizzard)	7	93.85±3.80	0.159
	2		0.047
Coturnix coturnix japonica(liver)	7	63.17±3.88	0.107
	2		0.0317
Coturnix coturnix japonica(lung)	7	41.58±3.22	0.0706
	2		0.0209

Table (5) Health risk estimate for Zn	ingestion from chicken	n and quail viscera
	8	1

RFD (mg/kg/day) for Zn (0.3)

Table (6) Daily intake rate (µg/ kg/day) of heavy metals through consumption of contaminated chicken gizzard and liver and lung with recommended dietary allowances

Metals		PMTDI [*]		
	Daily int			
	Gizzard	Liver	Lung	
Al	11.14	9.544	15.60	0.2-1.5 mg/kg
Mn	1.497	5.196	0.817	5 mg/day
Со	0.071	0.105	0.061	500 µg/kg/day
Cu	4.39	6.069	1.086	0.5 mg/kg
Zn	41.69	41.028	17.93	0.3-1 mg/kg

PMTDI: Provisional Maximum Tolerable Daily Intake a: tolerable intake suggested by FAO/WHO, [#]Lenntech (2011), ^{}JOINT FAO/WHO Food standards programme codex committee on contaminants in food, 2011

DISCUSSION

Health risk estimates determined in this study were based on the metal levels found in the gizzard, liver and lung of two domestic avian species, as well as the levels found in daily chicken consumption. The mean Zn concentrations in tissues of chicken and quail were 21.8 and 62.2 μ g/g, respectively, which were lower than the permissible limits of 100 µg/g (FAO/WHO (14). whereas the Mn concentrations in chicken and quail liver were 10.1 ,18.6 μ g/g exceeded the standard value of 6.5 μ g/g by WHO (14). This study conducted a probabilistic risk analysis method to quantify the bioaccumulation of essential and non-essential metals viscera of domestic chicken and quail. Another purpose was to assess the range of exposures for people who eat contaminated chicken. A probabilistic bioaccumulation model was established to account for metal accumulation in chicken. A human health exposure and risk model that accounts for the THQ and lifetime risk for humans consuming contaminated chicken was also established. Food is an important route of exposure to several metals, particularly in populations eating contaminated foods. The risk assessment goal is to estimate the harshness and probability of harm to human health from exposure to a substance or activity under reasonable circumstances. Risk characterization involved quantitative estimates of exposure doses against a benchmark of toxicity. More metals are gradually entering the environment due to increasing industrialization. These metals remain permanently because they cannot degraded in the environment, therefore, threatening human and animal health and causing natural imbalance. Al has deleterious effects on the central nervous, skeletal, and

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hematopoietic systems of humans (15). Adult humans need aluminum between 2.5 and 13 mg/day via foods or other sources(16). Increased oral aluminum absorption has been suggested in Alzheimer's disease. The continued exposure to high levels of aluminum can also cause bone abnormalities (17) Aluminum and its compounds comprise about 8% of the earth's surface. Manganese is a naturally occurring element and an essential nutrient. However, exposure to high manganese levels is toxic. Eating too little manganese can interfere with normal growth, bone formation, and reproduction. Moreover, manganese substances can cause lung, liver, and vascular disturbances, declines in blood pressure, failure in development of animal fetuses, and brain damage (18).

Cobalt (Co) is favorable for humans because it is part of vitamin B12; thus essential for human health. Cobalt is used to treat anemia for pregnant women because it stimulates the production of red blood cells. The total daily intake of cobalt varies and may be as much as 1 mg (18). Hathaway *et al.* (19) reported that inhalation of cobalt metal fume and dust may cause interstitial fibrosis, interstitial pneumonitis, myocardial and thyroid disorders and sensitization of the respiratory tract and skin. A deficiency in zinc may cause no obvious symptoms; however when exposures exceed physiological needs, zinc can become toxic. The estimated average daily dietary intake of zinc in adult is 14.4 mg/day. Long-term intake of large amounts of zinc in pharmacological doses (150 mg/day to 2000 mg/day) results in sideroblastic anemia, leukopenia, and hypochromic microcytic anemia. Copper (Cu) is an essential trace element that is particularly toxic to organisms and organs at high doses. High concentration of copper can also cause public health hazards (20). Long-term exposure to copper can cause

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irritation of the nose, mouth, and eyes, as well as headaches, stomachaches, dizziness, vomiting, and diarrhea. Intentionally high uptakes of copper may cause liver and kidney damage and even death. Daily recommended intake of copper is 2 mg, and as little as 10 mg of copper can have a toxic effect.

No THQ value higher than one was found for all metals through the consumption of chicken viscera, in the present study, indicating no risk to humans. The same conclusion was reported in Nigeria by Oforka *et al* (21) who revealed that the exposure to excessive metals (Cd, Pb, Mn, Zn and Ni) via chicken meat, liver, and gizzard consumption do not pose any imminent health risk. In general, present results were consistent with our previous studies in Malaysia on domestic bird eggs(22) Abduljaleel and Shuhaimi-Othman concluded through the risk quotient that the intake of heavy metals by eating the eggs of domestic birds does not pose any apparent threat to local people.

CONCLUSION

We conclude that chicken and quail have a different capacity to cumulative concentrations of various trace metals in their tissues. However, liver of quail gathered higher level of Mn and Co. While gizzard of quail accumulated high concentration of Al, Zn and Cu. The THQ and HI that calculated in this study and the analysis of the dietary exposure of the population studied showed a low exposure of these metals from chicken and other domestic birds meat and liver. It seems that it is not any less imminent risk to health from exposure to excess consumption of chicken meat.

Acknowledgments

I take this opportunity to thank the Department of School of Environmental Science and Natural Resources, UKM, Malaysia for kindly providing all of the samples collected.

تراكم العناصر النزرة في أنسجة الدجاج والسمان وتقدير المخاطر الصحية من استهلاك أحشاء الطيور سلوى عبد الزهرة عبد الجليل قسم علوم الحياة ، كلية العلوم، جامعة البصرة ، البصرة ، العراق.

الخلاصة

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