AN ASSESSMENT OF HEAVY METALS IN POULTRY CHICKEN GIBLETS USING ICP-MS

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Name of Candidate: **Nwokoma Oliver Chijioke**

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ABSTRACT

Heavy metals concentrations in chicken giblets were assessed to determine their potential toxic level and impact to the inhabitants of Selangor and Kuala Lumpur via consumption of chicken giblets. The concentration of 14 heavy metals such as; Fe, Cu, Mg, Zn, Al, Hg, Sb, As, Pb, Se, Ni, Sr, Cr and Sb were determined in 280 samples of poultry chicken giblets; liver, gizzard and heart samples that were collected from different local markets of Selangor and Kuala Lumpur, Malaysia. The analysis was performed using the Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) and the obtained results were compared with the permissible limits set by World Health Organization (WHO**)** and The Australia Total Dietary Survey (ATDS). The result shows that all liver samples contain all toxic metals with EDI concentration in (µg/kg) as Al (0.22), Cr (0.00508), As (0.00202), Se (0.00475), and Zn (0.4605), Cd (0.00204), Hg (0.03297), Pb (0.00187), Cu (0.047), and Sb (0.5187) followed by heart with values of Al (0.19037), Cr (0.01725), Se (0.00414), Zn (0.620), Cd (0.01659), Hg (0.014658), Pb (0.0472), Cu (0.06476) and Sb (0.69076) with no As detected. The gizzard samples show no traces of Pb and Cd. However, the heart were found to have the highest average concentration of Sb as 0.371±0.150 µg/g and followed by the Zn 0.3340±0.06 µg/g in the same sample. The estimated daily intake (EDI) of antimony as 0.690 µg/kg in heart and 0.518 µg/kg in liver samples respectively represents a concern worthy of regular monitoring within the study region.

ABSTRAK

Kepekatan logam berat dalam giblets ayam telah dinilai untuk menentukan tahap toksik potensi mereka dan impak kepada penduduk Selangor dan Kuala Lumpur melalui penggunaan giblets ayam. Kepekatan empat belas (14) logam berat seperti; Fe, Cu, Mg, Zn, Al, Hg, Sb, As, Pb, Se Ni, Sr, Cr dan Sb telah ditentukan dalam dua ratus lapan puluh (280) giblets ayam; sampel hati, hempedal dan jantung yang dikumpulkan dari beberapa pasar tempatan Selangor dan Kuala Lumpur, Malaysia. Analisis telah dijalankan menggunakan Spektrometri Gandingan Terinduksi Plasma-Jisim (ICP-MS) dan keputusan yang diperolehi dibandingkan dengan had yang ditetapkan oleh Pertubuhan Kesihatan Sedunia (WHO) dan kajiselidik pemakanan menyeluruh Australia (ATDS). Hasil kajian mendapati bahawa kesemua sampel hati mengandungi semua logam toksik dengan kepekatan EDI dalam (µg/kg) sebagai Al (0.22), Cr (0.00508), As (0.00202), Se (0.00475), dan Zn (0.4605), Cd (0.00204), Hg (0.03297), Pb (0.00187), Cu (0.047) dan Sb (0.5187) diikuti dengan jantung dengan nilai-nilai Al (0.19037), Cr (0.01725), Se (0.00414), Zn (0.620), Cd (0.01659), Hg (0.014658), Pb (0.0472), Cu (0.06476) dan Sb (0.69076) dengan tiada As dikesan. Sampel hempedal menunjukkan tiada kesan Pb dan Cd. Walau bagaimanapun , jantung didapati mempunyai kepekatan tertinggi Sb sebagai 0.371±0.150 µg/g dan diikuti dengan Zn 0.3340±0.06 µg/g dalam sampel yang sama. Pengambilan harian yang dianggarkan (EDI) antimoni sebagai 0.690 µg/kg di dalam jantung dan 0.518 µg/kg dalam sampel hati masing-masing mewakili kebimbangan yang layak pemantauan berkala dalam kawasan kajian.

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NWOKOMA OLIVER CHIJIOKE.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS AND ABBREVIATIONS

ICPMS	:	Inductively Coupled Plasma Mass Spectrometry
DIM	:	Daily Intake of Metal
EDI	:	Estimated Daily Intake
TDI	:	Tolerance Daily Intake
ATDS	:	Australia Total Dietary Survey
WHO	:	World Health Organization
ND	:	Not detected
SD	:	Standard Deviation
±	:	Both Plus and Minus Operation
µg/kg	:	Microgram per Kilogram
µg/g	:	Microgram per Gram
Ppm	:	Parts per Million
Bw	:	Body Weight
Oz	:	Ounce

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.1 Introduction

Poultry meat is widely available, relatively inexpensive; a good sources of protein, essential and polyunsaturated fatty acids especially the omega (n)-3 fatty acids (Marangoni et al. 2015). These nutrients are known as trace minerals and vitamins, and can be of central importance for impoverished people to meet the shortfalls of essential nutrients (Uluozlu et al. 2009). The incidence of several common metabolic diseases associated with deficiencies of critical dietary minerals, vitamins and amino acids can be reduced by the use of poultry meat in dietary habits (Barroeta 2007).

Thus, poultry chicken is playing a key role as a major component of daily diet to human. It is a versatile and delicious choice - ready to be grilled, barbecued, roasted, stir-fried, microwaved, poached or sauted, which plays an important role in many international cuisines. It is also used in the production of several chicken seasoning grains globally (Farrell & K. T. 1999). Therefore, the high demand of poultry chicken has also influenced their production and has been enhanced extensively by several technological contributions and inputs (Eales et al. 1988).

Feed ingredients for poultry diets are selected for the nutrients they can provide, the absence of anti-nutritional or toxic factors, their palatability or effect on voluntary feed intake, and their cost. Feed ingredients are broadly classified into cereal grains, protein meals, fats and oils, minerals, feed additives, and miscellaneous raw materials such as roots and tubers (Kellems et al. 2002). The quality of cereal grains especially rice, oat, wheat, wild rice, corn etc. largely depend on seasonal and storage conditions (Cakmak & I. 2008).

Poor growing or storage conditions can lead to grains with a lower than expected energy content or contamination with mycotoxins or toxin-producing organisms such as fungi and ergots (Henson & Caswell 1999)

Poultry feeds are made from cereal and legume grain crops like corn, soya bean, rice, oat, wheat, barley, millet, bean, wild rice, teff, fonio, spelt, lentils, carob, peanuts, tamarind etc (Amerah et al. 2007). Soil is the major reservoir of heavy metals in the ecosystem, and the raw foodstuffs are harvested from the soil (Nicholson et al. 2003). Genetic and environmental factors also affect not only the contents of nutrients in grains but also the nutritive value, which takes into account the digestibility of nutrients contained in an ingredient in the target animal. The accumulation of toxic substances such as heavy metals in poultry feeds, on the other hand, are great cause for concern from the nuclear fall-out to the terrestrial, aquatic, ecosystem and heavy metal intake (Krivolutzkii & A.P. 1992).

Heavy metals from manmade pollution sources are continuously released into aquatic and terrestrial ecosystems and therefore, the concern about the effect of anthropogenic pollution on the ecosystems is growing (Babich et al. 1980). Heavy metals enter into plant, animal and human tissues via air inhalation, diet and manual handling. Motor vehicle emissions are also source of airborne contaminants including arsenic (As), cadmium (Cd), cobalt (Co), nickel (Ni), lead (Pb), antimony (Sb), vanadium (V), zinc (Zn), platinum (Pt), palladium (Pd) and rhodium (Rh). Again, heavy metals are naturally occurring elements that have a high atomic weight and a density at least 5 times greater than that of water (Tchounwou et al. 2012). Their multiple industrial, domestic, agricultural, medical and technological applications have led to their wide distribution in the environment; raising concerns over their potential effects on human health and the environment (Guan et al. 2014). Their toxicity depends on several factors including the daily intake, weekly intake and monthly intake, route of exposure, and chemical species. Heavy metals are found naturally in the earth, and become concentrated as a result of human caused activities (Kärenlampi et al. 2000). The toxic effects of arsenic, mercury and lead were known to the ancients but methodical studies of the toxicity of some heavy metals appear to date from only 1868.

Animals and humans require some amounts of metals like Iron (Fe), Magnesium (Mg), Cobalt (Co), and Manganese (Mn) between 8-18 mg/kg daily (Sat & I. G. 2008). All metals are toxic at higher concentrations, while excessive levels can be damaging to the body. Other metals such as Hg, Cd, Pb, Ni, As and Sb are toxic even in a low concentration of 0.05-1 mg/kg/bw daily, and have no known vital or beneficial effect on humans (Cohen M. 2007). Their accumulation over time in the bodies of animals and humans can cause serious bio-toxic effect. The bio-toxic effects of metals refer to the harmful effects to the body when consumed above the bio-recommended limits (Buchet et al. 1983). High exposures to these metals are also potent carcinogenic and mutagenic to humans. Metals are accumulating in food chain through uptake at primary producer level and then through consumption at consumer level. The toxicity of inessential metals is attributed to the fact that it interferes with the normal function of the enzymes, also toxic to the blood, nervous, urinary, gastric and genital systems (Nordberg & Gunnar 1976).

Heavy metals are serious threat because of their toxicity, bioaccumulation and bio magnifications. Metals can enter into the human body through food consumption (Gibney et al. 2009). Copper and zinc serves either as cofactors and enzymatic activator during biochemical process in the human body. A cofactor is a non-protein chemical compound that is required for the protein's biological activity. These proteins are commonly enzymes, and cofactors can be considered "helper molecules" that assist in performing certain necessary reactions that enzyme cannot perform alone.

High concentration intake of Cd can cause Itai Itai disease and also increased Systolic Blood Pressure (SBP) in the absence of significant renal disease. Mercury intake Lead to Minamita disease, damage to the Central Nervous System (CNS) neurotoxicity. Short-term exposure to high levels of Pb can cause brain disorder, while longer term of Pb exposure can cause damage to the kidneys, reproductive and immune systems in addition to effects on the nervous system (Maret 2013).

Low level of As in the body leads to headaches, confusion, severe diarrhea and drowsiness while high level of As for longer term exposure can cause vomiting, blood in the urine, cramping muscles, hair loss and stomach pain (WHO 2001). Excessive content of other toxic metals beyond maximum permissible level leads to a variety of cardiovascular, neurological and bone diseases as well as mutagenic effects in lungs, kidneys, liver and other vital organs in humans.

Toxic metals have the largest availability in soil and aquatic ecosystem and to relatively smaller proportion in atmosphere at particular vapors (Loska et al. 2004). Some metals that is normally toxic for some specific organisms or under certain conditions, may be beneficial for others. In recent years, levels of contaminants in chicken internal organs/giblets are of particular interest because of the potential risk to humans who consume them, while attention has focused on liver, kidney, intestine and muscle of the chicken eaten by humans.

Considering that the major source of ingestion of heavy metals in humans is via food chain, this study was carried out to assess heavy metals concentration in poultry chicken liver, gizzard and heart using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). This is because ICP-MS is a type of mass spectrometer which is capable of detecting metals and several non-metals at concentrations as low as one part in 10^{15} (part per quadrillion, ppq) on non-interfered low background isotopes (Wolf & R.E. 2005). Compared to Atomic Absorption Spectrometry (AAS) and other techniques, ICP-MS has greater speed, precision, and sensitivity (Jenner et al. 1990). So far, this particular study on heavy metals contents in chicken hearts, gizzards and livers is very rare within the Selangor and Kuala Lumpur region, in Malaysia.

1.2 Objectives

1. To measure the concentrations of heavy metals in poultry chicken liver, gizzard and heart.
2. To estimate the daily intake of toxic metals via the consumption of chicken liver, gizzard and heart by humans.
3. To determine the potential toxic impact to humans by the comparison of recommended values.

1.3 Scope of the Study

This study focuses on fresh poultry chicken livers, hearts and gizzards of minimum slaughter age of seven (7) weeks. Market survey has been made for Selangor and Kuala Lumpur areas where the samples were collected. It took few weeks to collect the samples from each of the four markets mention due to insufficient funding and means of transportation. Also these markets do not operate in same days; therefore adequate timing and dates were prepared for the collection of samples from the different markets.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Manmade activities such as industrial wastes, industrial spillage, bush burning and mining can create a potential source of heavy metal pollution in the aquatic and terrestrial environment (Zhuang et al. 2009). It was reported that heavy metals are ubiquitous and easily get into human and animal feedstuff, and are consequently passed out during excretion and defecation. Heavy metal contamination of feedstuffs used in making animal feed may occur as a result of the use of metal-based herbicides and pesticides, metal contaminated fertilizers, atmospheric deposition, irrigation with metal-laden water etc (Curtis & Smith 2002).

A general survey of literature shows a number of studies that has been emphasizing the assessment of heavy metals in chicken liver, kidney, muscle, tissue and meat. Livers and kidneys of chickens and other poultry animals were found to have the highest significant levels of metals (Akan et al. 2010). A low concentration of Pb below CODEX/WHO permissible level was determined in the samples of liver, kidney, bone and muscle tissue of chicken (Zraly et al. 2008), while the levels of Pb in some analyzed chicken products were also below the permissible levels.

2.2 Previous Studies on Chicken Hearts, Livers and Gizzards

Few years back, a detailed study conducted in Kasuwan Shanu , Maiduguri Metropolis, Nigeria showed that the concentration of both Mn; 4.11±0.44 µg/g and Fe; 4.65±0.30 µg/g were below the tolerable limits in the liver of chicken collected from Kasuwan Shanu market. The result showed that concentration of other metals like; Pb, Ni, Co, As, Cu, & Zn were below their tolerable limits set by WHO except for Cr; 0.65±0.04 µg/g which calls for regular monitoring (Akan et al. 2010).

The mean values of arsenic in breast, thigh, liver and gizzard samples collected from Elkharga chicken butchers, New Valley governorate, Egypt were 0.36±0.02 µg/g, 0.49±0.01 µg/g, 0.77±0.06 µg/g and 0.85±0.05 µg/g, respectively. While the respective mean values of cadmium were 0.03±0.01 µg/g, 0.04±0.02 µg/g, 0.05±0.03 µg/g and 0.02±0.01 µg/g (Elsharawy 2012). All examined samples were laid within the permissible limits set by the Egyptian Organization for food standards.

Cadmium concentration distribution in kidney of chicken (layer breeds) in Maiduguri, Nigeria was revealed to have the highest amount, while in liver tissues were in the range of 0.003±0.001 mg/g to 0.004±0.000 mg/g (Mohammed et al. 2013). Concentration of Cu, Zn, Mn and Fe were found in domestic and broilers chickens collected from District Bannu, Khyber Pakhtunkhwa, Pakistan. Ag, Pb, and Cd were also present in small concentration in domestic chickens. Similarly Zn, Pb, and Cu were found in higher concentration in broiler. Liver and Gizzard samples of domestic chickens showed higher accumulation of Zn, Mn, Cu and Fe than broiler and Ag was the only heavy metal that showed higher concentration in broiler than domestic chickens (Hameed & A.R. 2013). The concentrations of Cd, Pb, Ni, Zn, Cr and Mn were determined in 120 meat samples comprising of broiler, cockrel layers and local chicken muscles, gizzard and liver using flame atomic absorption spectrophotometry. The mean concentration of metal contents in the different parts of chickens in (mg kg^{-1}) for Cd was (0.0236±0.0016), (0.0457±0.0092) and (0.0162±0.0008); for Pb (0.2867±0.0176), (0.3012±0.0172) and (0.2151±0.0167); for Mn (0.1265±0.0096), (0.4150±0.0283) and (0.2657±0.1068); for Zn (1.9399±0.0376), (2.3245±0.0676) and (1.5701±0.0558), for Ni (0.0615±0.0038), (0.1079±0.0220) and (0.0620±0.0074) in chicken gizzard, liver and muscle respectively. The results of the study indicate that chickens raised in the oil rich Rivers State Nigeria have concentrations of Cd, Ni, Zn and Mn below the permissible FAO/WHO levels except Pb which is slightly higher than the 0.2 mg kg^{-1} stipulated by FAO/WHO (Oforka et al. 2012).

Using flame atomic absorption spectrometer (FAAS), the concentrations of Pb and Cd were estimated in 60 samples of chicken giblets comprising of broiler livers, gizzards and hearts collected randomly from retail markets in Ismailia city, Egypt. The greatest Pb concentrations were found in liver samples 0.8762±0.2089 ppm, whereas gizzard samples contain 0.3186±0.1462 ppm and lowest levels of Pb were detected in heart samples as 0.1733±0.06777 ppm. Cd deposited in liver samples reached to 0.040714±0.0290 ppm. The result interpreted that Pb residual concentration, particularly in chicken liver sold in Ismailia city of Egypt, is more than the permissible limit (0.5 ppm) in the Codex Alimentarius international food standards (Ismail & Abolghait 2013).

2.3 The Importance of Chicken Liver as Nutritional Diet

Figure 2.1: Collected liver samples for this study

Fresh chicken liver samples are shown in fig 2.1. Scientists have proved that one chicken liver contains 73 kilo calories (Sara & D.M. 2005). While chicken is one of the most commonly eaten meats, the liver is often overlooked as an undesirable part of the bird. Chicken liver does contain a large amount of cholesterol, but it also supplies healthy doses of many essential vitamins and minerals (Nitsan et al. 1991).

2.4 Calories

There are many scientific terms that define a calorie, but the precise definition is better replaced by a simpler standard definition. A calorie is a unit of measure for energy that our body uses for all of our vital process. It has been proved and reported that we are constantly burning calories, even during sleep, although the rate at which calories are burned falls dramatically while at rest. The rate at which calories are used is constantly changing to meet the energy requirements of our body (Rena et al. 2006). This varies from person to person and during the different stages of life.

2.5 Calories the Measurement of Energy

When we speak of calories in relation to a chicken liver particularly, we actually mean the amount of energy that it provides for our body to fuel its metabolic processes (Nordqvist 2016). So weight control actually comes down to a process of energy. If we take in more energy than we use, the rest gets stored as fat. If we burn more energy than we consume, our body relies on our energy stores to make up the deficit (Count 2004). Also it was reported that chicken livers are high in protein and a rich store of folate, which is important for fertility and helps prevent certain birth defects (Jenkins et al. 2010). Food Standards Authority advises pregnant women not to eat liver because too much vitamin A can harm the baby (Suharno et al. 1993). Livers are also loaded with iron to give you energy and a treasure trove of certain B vitamins, most notably B12 . This nutritional profile makes them a good choice for anyone prone to anaemia. Chicken livers are also one of the top sources of vitamin A, which helps eye health (Lessard et al. 1997).

2.6 The Importance of Chicken Gizzard as Nutritional Diet

Figure 2.2: Collected gizzard samples for this study

Fresh chicken gizzard sample for this study are shown in fig 2.2. A gizzard is an organ found in the digestive tract of a chicken. Similar to a stomach, the gizzard is used to grind up the foods the bird eats. Gizzards are considered a delicacy in certain cultures, and provide a healthy dose of certain vitamins and minerals. Although there are drawbacks from eating chicken gizzards, because most people prefer eating chicken meats rather than its giblets and this has led to decrease in nutritional value healthily. It was reported that A 100-gram serving of chicken gizzards, which is equal to about 3.5 ounce (oz), contains 2.68 grams of total fat, less than 1 gram of which is saturated (Sara & D.M. 2005). It means that, it is good for those looking for lower fat ways to add protein to their diet; chicken gizzards are a healthier option than high-fat cuts of beef or pork . The same serving of chicken gizzards also has 370 milligrams of cholesterol, which is significantly more than the 300 milligrams or less you should limit yourself to each day (Pereira et al. 2002). A diet low in saturated fat and cholesterol can reduce your risk of heart disease, stroke and certain types of cancer.

2.7 Nutrients

Scientists have also proved that a 3.5 oz serving of chicken gizzards supplies you with 3.19 milligrams of iron and 4.42 milligrams of zinc. An ounce (oz) is a unit of weight equal to one sixteenth of a pound; 1 ounce is equal to 437.5 grains or 28.349 grams. Women need 18 milligrams of iron and 8 milligrams of zinc each day (Thompson & L.D. 2010). Men need 8 milligrams of iron and 11 milligrams of zinc on a daily basis. These nutrients support a healthy immune system, promote wound healing and aid in cell division. Each day our body needs about 2.4 micrograms of Vitamin B12 for treating and preventing deficiency, a condition in which vitamin B12 levels in the blood are too low (Vicki & R.L. 1999). Vitamin B12 is essential for a healthy immune system and for proper neurological function.

2.8 Low Calorie Protein

Also it is stated that for those looking to save calories the gizzards make a better choice. A 3.5-ounce portion of simmered chicken hearts contains 185 calories, while the same portion of simmered chicken gizzards contains 153 calories (Thompson & L.D. 2010). Even though there is a 32 calorie in the gizzard, the chicken gizzards are low energy dense foods, which means their calorie content is low when compared to weight. They are full of protein and B vitamins, not too fatty, either. They are not more popular because they take some care in preparation, take a while to cook, and there are easier proteins available, though few are cheaper. Chicken gizzards also contain high amount of iron. Iron is needed to produce blood cells and hemoglobin and myoglobin, two proteins that carry oxygen throughout your body. The United States Department of Health and Human Services recommends between 8 and 18 milligrams of iron per day for adult men and women (N.I.H. 2016). A 4-ounce serving of chicken gizzards contains almost 3 milligrams of iron.

2.9 The Importance of Chicken Heart as Nutritional Diet

Figure 2.3: Collected heart samples for this study

Fresh chicken heart samples for this study are shown in fig 2.3. Chicken heart is a very good source of protein, the B vitamins, riboflavin, zinc, selenium and iron and malic enzyme (Ma X. et al. 1990). Chicken hearts also provide folate, magnesium, manganese, phosphorus, potassium, sodium and copper. Consuming chicken hearts is a great way to rack up amino acids that can improve metabolism and compounds that aid the production of collagen and elastin, which fight wrinkles and aging in the body system (Wang et al. 2005). This mixture of unique nutrients helps build muscle, store energy and boost stamina and endurance.

2.10 High-Quality Protein

The chicken hearts are also high in protein similar to the gizzards. A 3.5 oz portion of simmered chicken hearts contains 26 grams of protein, and the same portion of simmered chicken gizzards contains 27 grams. As an animal protein, both the hearts and gizzards provide all the essential amino acids, making it a complete source of protein (I.C.A. 2009). Protein is found in every cell in human body, and the protein in your diet is used to help replace and maintain protein levels.

CHAPTER 3: MATERIALS AND METHOD

3.1 Sample Collection Map (Kuala Lumpur and Selangor in West Malaysia)

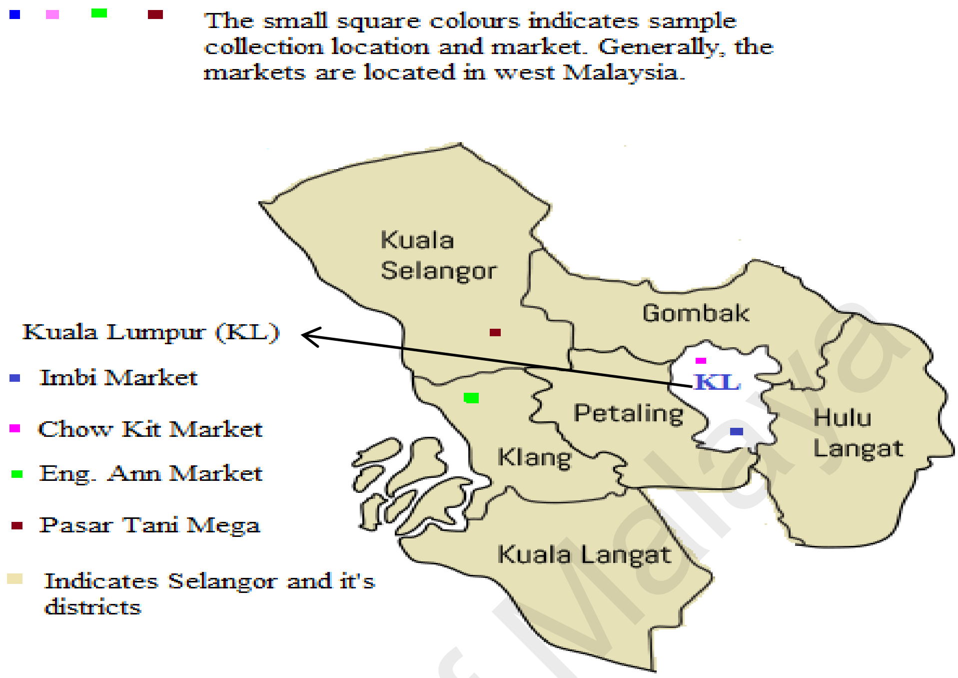

Figure 3.1: Location of chicken samples

Gombak, Hulu Langat, Kuala Langat, Petaling, Klang, and Kuala Selangor are districts in Selangor State in fig.3.1.

Figure 3.2: Poultry Chicken

In fig. 3.2 shows poultry chicken in their age of minimum 7 weeks ready to be transported to the various markets for slaughtering.

3.2 Collection of Samples

A total of 280 unit fresh samples consisting of poultry chicken liver, gizzard and heart were collected from four different local markets in Selangor and Kuala Lumpur. Tables 3.1-3.4 illustrate type of samples, quantities (unit), wet weight (kg), and dry weight (g) after proper drying, dehydration in the microwave oven and proper grinding into a powered form.

Figure 3.3: Google map showing Chow Kit Market 2016

Table 3.1: Chow Kit Market (Code: M01)

No	Type of Sample	Quantity Collected (unit)	Wet Weight (kg)	Dry Weight (g)
1	Liver	25	1.5	130.25
2	Gizzard	25	1.75	107.5
3	Heart	20	1	58.5
Total		70	4.25	296.25

Normally, fresh samples have more weight than when it is properly dried, dehydrated in the microwave oven and grinded into a powered form due to the existence of some water molecules in it. This means that the dried samples weighted in grams (g), while the wet/fresh samples in kilograms (kg).

The demand for gizzards and livers consumption by the people of Selangor and Kuala Lumpur are higher than that of heart. Therefore only 20 samples of hearts from each market mentioned were selected for this study.

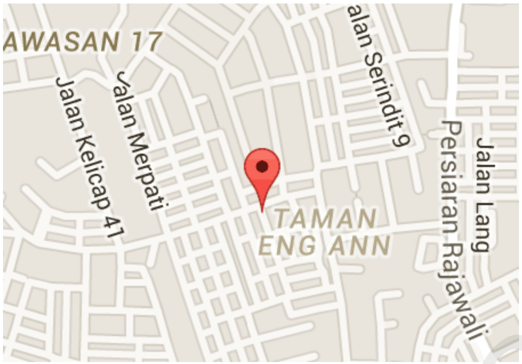

Figure 3.4: Google map showing Eng Ann Market 2016

Table 3.2: Eng Ann Market (Code: M02)

No	Type of Sample	Quantity Collected (unit)	Wet Weight (kg)	Dry Weight (g)
1	Liver	25	1.2	130
2	Gizzard	25	1.70	106.5
3	Heart	20	1	58
Total		70	3.9	294.5

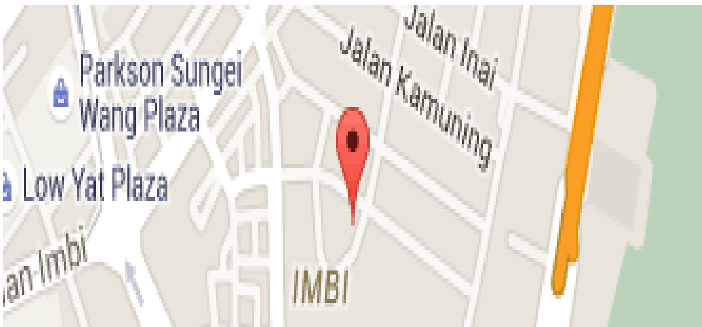

Figure 3.5: Google map showing Imbi Market 2016

Table 3.3: Imbi Market (Code: M03)

No	Type of Sample	Quantity Collected (unit)	Wet Weight (kg)	Dry Weight (g)
1	Liver	25	1.85	131.5
2	Gizzard	25	1.72	107
3	Heart	20	1	58.4
Total		70	4.57	296.9

Figure 3.6: Google map showing Pasar Tani Mega Market 2016

Table 3.4: Pasar Tani Mega Market (Code: M04)

No	Type of Sample	Quantity Collected (unit)	Wet Weight (kg)	Dry Weight (g)
1	Liver	25	1.45	129.25
2	Gizzard	25	1.83	109
3	Heart	20	1	59.1
Total		70	4.28	297.35

Table 3.5: The table shows the quantity of all sample types (unit), wet weight (kg), and dry weight (g) collected from the whole markets (Code: M01, M02, M03, and M04)

No	Sample type	Quantity of all samples (unit)	Wet weight for all samples (kg)	Dry weight for all samples (g)
1	Liver	100	6	521
2	Gizzard	100	7	430
3	Heart	80	4	234
Total		280	17	974

There were 100 liver, 100 gizzard and 80 heart samples collected from all market making a total of 280 giblets. The samples were collected and each type were put in different polyethylene bags and transported to the laboratory for subsequent preparation and analyses by ICP-MS.

3.3 Preparation and Treatment of Samples

The collected samples were washed with distilled water to remove any contaminated particles, and then were cut to small pieces using a clean stainless steel knife. Samples were dried in a programmable microwave oven after cutting at 75^{o}C for 2 days. Then after the samples were properly dried and dehydrated in the microwave oven, the samples were grained into a fine powder form using a Blender Grinder. Then after, the total weight for all samples collected from the whole markets was taken after proper drying and grinding: livers 521g, gizzards 430g and hearts 234g and afterward, these were stored in polyethylene containers until used for acid digestion. Seven (7) steps were taken to achieve the result of this study.

Step 1: Samples were dried properly in a microwave oven at 75^{o}C for 2days.

Figure 3.7: Microwave Oven

Step 2: Samples were grind into a fine powder form using blender grinder.

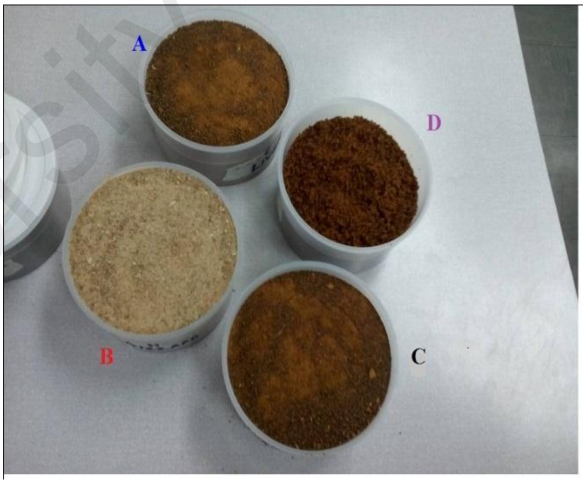

Figure 3.8: Polyethylene Containers

Picture of some polyethylene containers, containing liver, heart and gizzard samples were taken. Container (A) contains liver, (B) gizzard, (C) liver and (D) heart samples in fig 3.8.

Step 3: An electronic balance were used to collect the weight of liver, gizzard and heart samples portion between 0.4-0.5g from each market: Chow Kit Market: M01, Eng Ann Market: M02, Imbi Market: M03 and Pasar Tani Mega Market: M04 after proper drying and grinding prior to acid digestion (Fig 3.9). An electronic balance is a device used to find accurate measurements of weight. It is used very commonly in the Labourites for weighing chemicals to ensure a precise measurement of those chemicals, and it is also used to weigh food, and samples.

The grinding process was carried out after samples were dried and dehydrated in the microwave oven. Then few portion of each type of samples were carefully transferred into blender grinder and was grinded repeatedly until a powered form was obtained. Same method was applied for all samples type until all samples were properly grinded into powered form.

Figure 3.9: Digital electronic balance (ADAM-AQT 200)

Step 4: The 0.4-0.5g (dry weight) of collected samples were transferred into digestion flask and then 9 ml of 65% HNO_3 and 4 ml of 30% H_2O_2 were added.

Figure 3.10: Digestion Flask

The digestion flasks containing the samples were placed on a Hot Plate after acid mixture at 120 °C (Fig 3.10).

Figure 3.11: Yellow-line MAG HS 7; IKA Products+ Instruments

Step 5: fig 3.11 shows the digestion flask placed on a hot plate at 120 ^{o}C. The digestion flask was heated at 120 °C for 8 hours until a clear solution was obtained.

Step 6: Following a cooling to room temperature, the content of the flask were filtered into an empty clean 50ml volumetric flask, and then the digested samples were diluted with 12ml distilled water to a total volume of 25 ml (Fig 3.12).

Figure 3.12: Filtered Sample in Volumetric Flask Beaker

The solution may contain some undigested biological substances which cannot be seen with the natural eyes. Therefore, filtration process was adopted to achieve better result by removing any undigested biological substance or contaminants (Fig 3.12).

Step 7: Analyses with the ICP-MS.

Figure 3.13: Inductively Coupled Plasma Mass Spectrometer (ICP-MS)

Inductively coupled plasma mass spectrometry is an analytical technique used for elemental determinations. The technique was commercially introduced in 1983 and has gained general acceptance in many types of laboratories. Geochemical analysis labs were early adopters of ICP-MS technology because of its superior detection capabilities, particularly for the rare-earth elements (REEs). ICP-MS has many advantages over other elemental analysis techniques such as atomic absorption and optical emission spectrometry, including (ICP) Atomic Emission Spectroscopy (ICP-AES). An ICP-MS combines a high temperature inductively coupled plasma (ICP) source with a mass spectrometer (Batsala et al. 2012). The ICP source converts the atoms of the elements in the sample to ions. These ions are then separated and detected by the mass spectrometer.

3.4 Principle of ICP-MS

Using ICP-MS, all kinds of materials can be measured. Solutions are vaporized using a nebulizer, while solids can be sampled using laser ablation. Gasses can be sampled directly. The sampled material is introduced into high energy argon plasma that consists of electrons and positively charged argon ions. In the plasma, the material is splitted into individual atoms. These atoms will lose electrons and become (singly) charged positive ions. Most elements ionize efficiently (> 85%) in the hot plasma (Ammann & Adrian 2007). To allow their identification, the elemental ions produced in the plasma (ICP) must be transferred from 7000 K to room temperature and from atmospheric pressure to high vacuum. To do so, the ions are extracted through a number of apertures. Besides ions also photons are produced in the plasma. Photons also pass through the apertures. They are not removed by vacuum and produce high background signal when they reach the detector. To minimize this background, a so-called photon-stop is present. This is a small metal plate placed in the centre of the ion beam, which reflects the photons away from the detector (Batsala et al. 2012). The positive ions are not stopped by the photon-stop because a positively charged cylinder lens guides them around it.

Subsequently, the ion beam enters the quadrupole mass analyser. In the quadrupole, the ions are separated on the basis of their mass-to-charge ratio. Each element has its own characteristic isotopes and masses, and will therefore produce its own mass spectrum. After passing the quadrupole, the ions hit a special detector. It contains two stages to allow simultaneous measurements of high and low signals (GieBmann & Greb 1994). This allows simultaneous detection of main components and ultra-trace elements in a single run, which makes the ICP-MS a perfect tool for survey analysis of totally unknown samples.

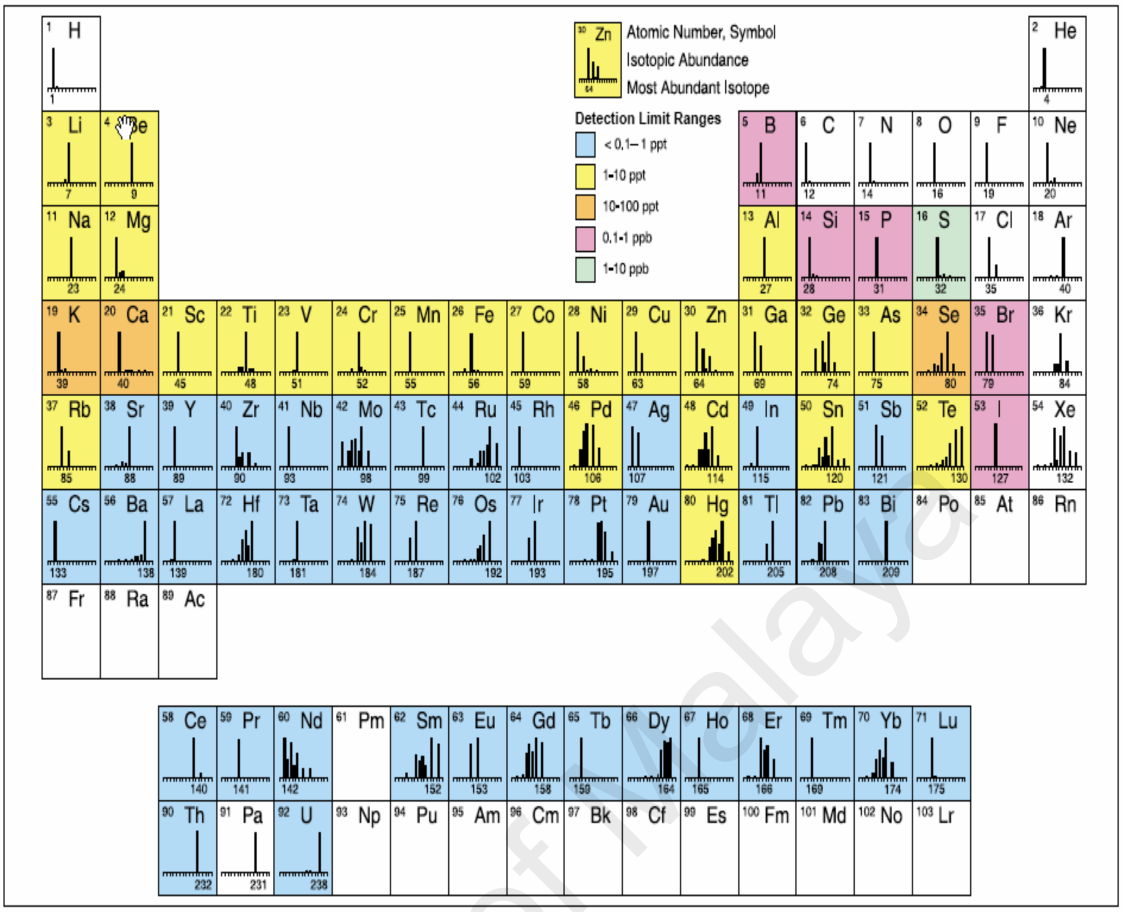

Figure 3.14: Representation of ICP-MS Technical Specifications in a Periodic Table

Fig 3.14 shows approximate detection capabilities of the energy, style, and enthusiasm (ELAN) 6000/6100 quadrupole ICP-MS of each element represented in a periodic table. (Courtesy of PerkinElmer, Inc.).

3.4.1 Technical Specification

An ICP-MS consists of the following components:

1. Sample introduction system – consist of the peristaltic pump, nebulizer, and spray chamber that introduces sample to the instrument
2. ICP torch – generates the plasma which serves as the ion source of the ICP-MS, converting the atoms to be analyzed to ions,

3. Interface – the sample ions are extracted from the central plasma channel and separated from the bulk ions by cooled conical aperture plates with aperture openings of 1/0.8 mm in the vacuum interface (vacuum <2mbar),
4. Vacuum system – provides high vacuum for ion optics, quadrupole and detector,
5. Quadrupole – the high frequency quadrupole acts as a mass filter to sort ions by their mass-to-charge ratio (m/e). The mass resolution with constant peak widths from 0.5 to 1 amu at 10% peak height can be set in three steps,
6. Detector- after passing mass filter the ions are either detected through direct current measurements on the ion collector or the ions generate secondary electrons that are propagated in the multiplier. Together, both methods can cover an intensity range from a few ions/s to 10^{12} ions/s.
7. Data handling and system controller.

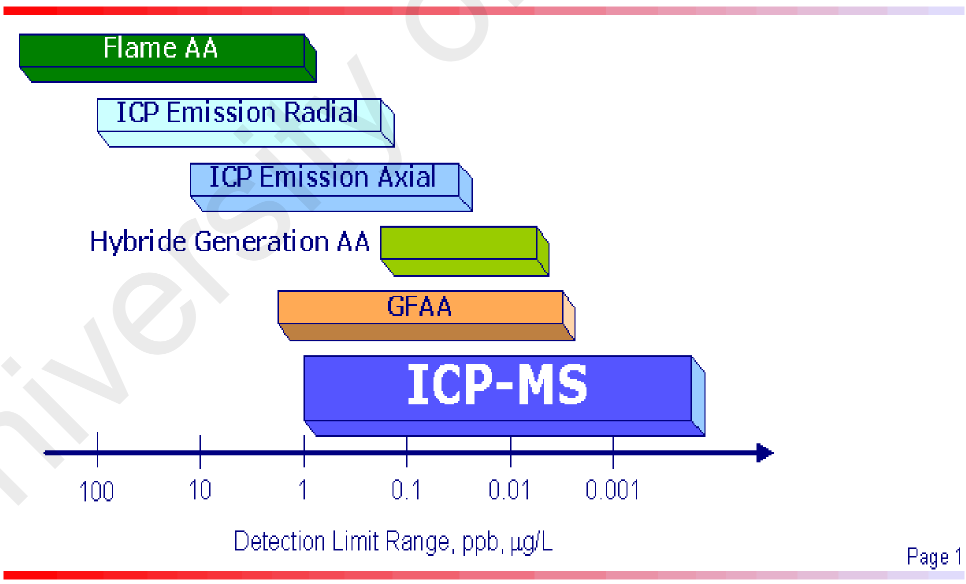

Figure 3.15: Typical Detection Limit Ranges for the Major Atomic Spectroscopy Techniques

3.5 Summarized ICP-MS Operations

1. Starting the Instrument
2. Auto-turning for sensitivity detection
3. Creating a method
4. Setting up a sequence
5. Running a sample analysis
6. Viewing spectra
7. Viewing time chart
8. Creating report/database.

3.6 Elemental Analysis

The analysis was performed by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS). Calibration of the ICP-MS was performed using multi-element calibration standard (Agilent Technologies, 7500 Series USA, Part No. 8500-6940). After calibrating the instrument with standard solutions derived from commercial materials, it was optimized according to the manufacturing standards (Van & H.J. 2003).

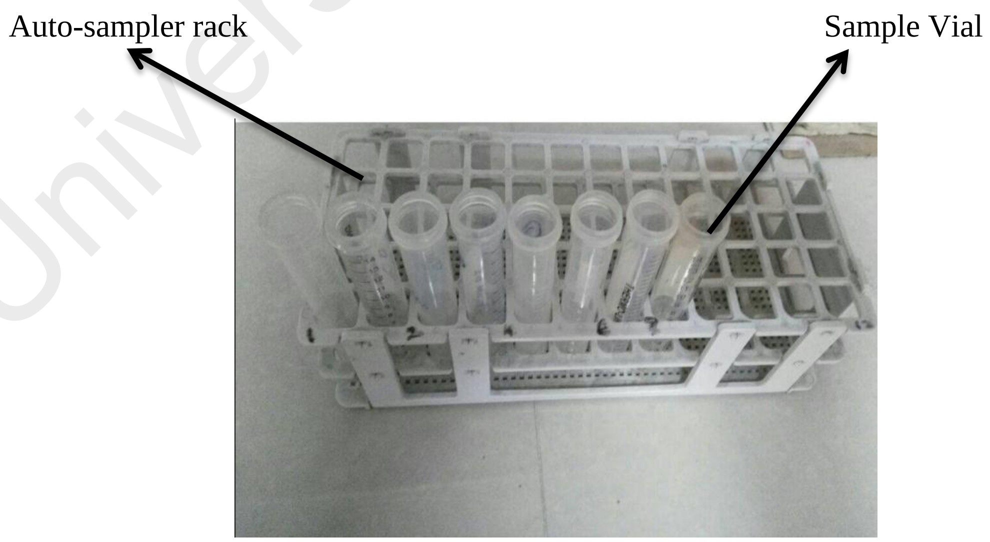

Figure 3.16: Auto-sampler Rack and Sample Vial

Auto Sampler Rack: It was used to support the sample vials during ICPMS analysis. The auto-sampler rack was placed in the auto-sampler chamber of ICPMS after the sample vials containing the filtered samples have been placed in the rack properly.

Sample Vials: Filtered samples were transferred into the sample vials accordingly before it was placed in the auto-sampler rack carefully and gently. See fig 3.16.

Figure 3.17: Auto-Sampler Chamber of ICPMS

In fig 3.17, shows auto-sampler chamber of the ICPMS and how samples are placed for analysis.

The auto-sampler vials and tubes were carefully cleaned to purge of any possible residues before starting the instrument for analysis. Appropriate quality assurance procedure and precautions were carried out to ensure reliability of the results. In all metal determination, analytical blanks were prepared in a similar manner. All glassware was carefully cleaned with a solution of 10% nitric acid for 48 h followed by rinsing with deionized water. In addition, a recovery study of the total analytical procedure was carried out for metals in selected samples by spiking analyzed samples with aliquots of metal standards and then reanalyzed the samples. A recovery of greater than 93.4% was achieved. After then, the assessment of concentrations of the following 14 metals: Al, Mg, Cr, Ni, As, Se, Sr, Cd, Hg, Pb, Fe, Zn, Cu, and Sb were performed.

CHAPTER 4: RESULTS AND DISCUSSION

Results of metal concentrations of chicken liver, gizzard and heart, collected from different markets in Selangor, Malaysia, are presented in Table 4.1, 4.2, and 4.3. Magnesium (Mg), Nickel (Ni), Strontium (Sr), and Iron (Fe) are essential metals that our body need for metabolic processes, and are therefore, not discussed in details in this study.

4.1 Concentration of Metals in Liver Samples

Table 4.1: Mean Concentration in the Livers (μg/g dry weight)

Metal	M01	M02	M03	M04	Mean±SD
Mg	4.456	4.543	4.189	4.256	4.361±0.17
Al	0.089	0.05206	0.1653	0.173	0.12±0.058
Cr	0.00433	0.00105	ND	ND	0.00269±0.002
Ni	0.00247	0.00285	ND	ND	0.0026±0.0002
As	ND	0.00109	ND	ND	0.00109
Se	0.00415	0.00229	0.0023	0.0013	0.0025±0.001
Sr	0.0024	0.00626	ND	0.0019	0.0035±0.002
Cd	ND	0.0011	ND	ND	0.0011
Hg	0.04423	0.01789	0.0072	0.0015	0.017±0.018
Pb	ND	0.00101	ND	ND	0.00101
Fe	1.161	0.5982	0.4239	0.425	0.652±0.349
Zn	0.231	0.239	0.301	0.221	0.248±0.036
Cu	0.03376	0.01232	0.0109	0.0442	0.0253±0.016
Sb	0.2039	0.3491	0.3212	0.243	0.279±0.0674

4.2 Concentration of Metals in Gizzard Samples

Table 4.2: Mean Concentration in the Gizzards (µg/g dry wt)

Metal	M01	M02	M03	M04	Mean±SD
Mg	6.061	6.228	5.789	5.695	5.943±0.245
Al	0.04378	0.0806	0.2507	0.2267	0.1504±0.103
Cr	ND	0.00575	ND	0.00123	0.00349±0.0031
Ni	ND	0.00218	ND	ND	0.00218
As	ND	0.00106	ND	ND	0.00106
Se	0.00762	0.00942	0.00295	0.0029	0.00572±0.0033
Sr	0.00145	0.00198	ND	ND	0.00171±0.0003
Cd	ND	ND	ND	ND	ND
Hg	0.02293	0.03498	0.00891	ND	0.0222±0.013
Pb	ND	ND	ND	ND	ND
Fe	1.153	0.76	1.129	0.62098	0.9157±0.27
Zn	0.198	0.2529	0.22	0.2959	0.2417±0.0425
Cu	0.0439	0.04728	0.05098	0.04647	0.0471±0.0029
Sb	0.176	0.2206	0.1648	0.1983	0.189±0.0247

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4.3 Concentration of Metals in Heart Samples

Table 4.3: Mean Concentration in the Hearts (μg/g dry wt)

Metal	**M01**	**M02**	**M03**	**M04**	**Mean±SD**
Mg	5.402	5.155	5.211	4.982	5.1875±0.173
Al	0.1115	0.07912	0.1764	0.04301	0.1025±0.057
Cr	0.00118	0.02555	0.00114	ND	0.00929±0.014
Ni	0.00289	0.02709	0.00209	ND	0.0106±0.0142
As	ND	ND	ND	ND	ND
Se	0.00316	0.00131	ND	0.00222	0.0022±0.00092
Sr	0.0014	0.02936	0.00288	0.0113	0.0112±0.0128
Cd	0.00233	0.01554	ND	ND	0.0089±0.0093
Hg	0.01832	ND	0.00235	0.00301	0.00789±0.009
Pb	ND	0.02543	ND	ND	0.02543
Fe	1.294	0.5705	0.753	0.411	0.757±0.3842
Zn	0.3039	0.2755	0.3557	0.401	0.3340±0.06
Cu	0.02393	0.01821	0.04265	0.0547	0.0348±0.0168
Sb	0.5613	0.2095	0.307	0.41	0.371±0.150

Heavy metals concentrations of in chicken liver, gizzard, and heart samples collected from different markets of Selangor in Malaysia**.** ND denotes not detected (< detection limit, ppm), and the codes: M01, M02, M03 and M04 represents each local market mentioned.

Mean concentration of aluminum (Al) in chicken liver was 0.120±0.058 µg/g, while its mean values in gizzard, and heart were 0.150±0.103, and 0.1025±0.057 µg/g, respectively in (Table 4.1, 4.2 & 4.3). The results obtained in this study for Al concentration in chicken liver, gizzard and heart, is comparable to that of similar study reported (Uluozlu et al. 2009) revealing that various parts of chicken samples and chicken products were to be in the range of 0.10 - 1.90 µg/g for aluminum.

Chromium (Cr) in chicken liver, and heart samples recorded mean concentration value of 0.0026±0.002 and 0.0092±0.014 µg/g respectively, while mean concentration of Cr in the heart samples was slightly higher, with a value of 0.0092±0.014 µg/g in (Table 4.3). It was reported that Cr concentration in chicken giblets in a previous study was 0.38±0.08 µg/g (Mousa et al 2010), while 0.03±0.002 µg/g were revealed for Cr concentration in giblets by (Uluozlu et al. 2009) in Tokat, Turkey. Fortunately, the values for Cr content in present study shows a very low level in giblets. Content of arsenic (As) in heart was virtaully not detected, while a very low concentration values of 0.001 µg/g in liver, and gizzard samples was detected for As (Table 4.1 & 4.2). Cadmium (Cd) was not detected in the gizzard, while a low concentration was revealed for heart and liver 0.0089±0.0093 and 0.0011 µg/g. The content of Cd in the heart samples in this study is comparable to a similar study in Iraq with values 0.004 and 0.015 µg/g (Reem Th., et al (2012).

Furthermore, mean concentration of lead (Pb) in liver and heart samples were 0.001, and 0.025 µg/g respectively. In a previous study, estimation of Pb residual level in chicken giblets at retail markets in Ismailia, Egypt was slightly higher with values 0.0789 and 0.5770 µg/g (Ismail et al. 2013). Mercury (Hg) recorded mean concentration value of 0.017 µg/g in the liver samples (Table 4.1). Average concentration values of 0.046, 0.025, and 0.035 µg/g, were recorded respectively, for copper (Cu), in chicken gizzard, liver, and heart samples. Antimony (Sb) on the other hand, has significant concentrations in all the studied chicken giblets. Mean concentration values for Sb were 0.279±0.0674, 0.189±0.0247 and 0.371±0.150 µg/g, respectively in liver, gizzard and heart samples. The result for heart samples obtained in this study reveals that Sb content level is higher than that of a previous study in Jakarta, Indonesia with value of 0.10±0.06 µg/g by (Surtipanti et al. 1995).

4.4 Estimated Daily Intake (EDI)

The daily intake of metals (Mg, Al, Cr, Ni, As, Se, Sr, Cd, Hg, Pb, Fe, Zn, Cu, and Sb) depends both on the metal concentration level and the amount of consumption.

The EDI for adults was estimated using the following formula:

$$\text{EDI} = \frac{C \times W}{m} \quad (1)$$

Where *C* is the average concentration of heavy metals in each sample, *W* represents the daily average consumption of giblets (130 g) and *m* is the adult's body weight 70 kg (Khandaker et al. 2015). Estimated daily intake (EDI) of heavy metals through consumption of poultry chicken liver, gizzard and heart are presented in Tables 4.4-4.6, respectively. Also presented in Tables 4.4-4.6, are the tolerable daily intake (TDI) for the studied metals. The TDI is regulatory value of a substance in food or drinking water, expressed on a body-mass basis (usually mg kg^{-1} body weight), which can be ingested daily over a lifetime by humans without appreciable health risk.

Table 4.4: EDI and TDI (μg/kg) of heavy metals in liver samples

Metal	Average Concentration (μg/g)	EDI body weight daily (μg/kg)	TDI (μg/kg/daily)
Al	0.120	0.22	500[e]
Cr	0.00269	0.00508	500[a]
As	0.00109	0.00202	10[b]
Se	0.00251	0.00475	20[b]
Zn	0.248	0.460	260[b]
Cd	0.0011	0.00204	5[b]
Hg	0.0177	0.0329	10[d]
Pb	0.00101	0.00187	10[c]
Cu	0.0253	0.047	500[e]
Sb	0.2793	0.518	10[b]

As it was earlier said, finding the EDI of a particular metal is to multiply the average concentration of that metal by the average daily consumption of the sample (130g), divide by adult body weight (70kg).

Table 4.5: EDI and TDI (μg/kg) of heavy metals in gizzard samples

Metal	Average Concentration (μg/g)	EDI body weight daily (μg/kg)	TDI (μg/kg/daily)
Al	0.1504	0.279	500[e]
Cr	0.00349	0.00648	500[a]
As	0.00106	0.00196	10[b]
Se	0.00572	0.01062	20[b]
Zn	0.2417	0.449	260[b]
Cd	ND	ND	5[b]
Hg	0.022	0.0413	10[d]
Pb	ND	ND	10[c]
Cu	0.0471	0.0875	500[e]
Sb	0.189	0.3527	10[b]

Table 4.6: EDI and TDI (µg/kg) of heavy metals in heart samples

Metal	Average Concentration (µg/g)	EDI body weight daily (µg/kg)	TDI (µg/kg/daily)
Al	0.1025	0.1903	500[e]
Cr	0.00929	0.01725	500[a]
As	ND	ND	10[b]
Se	0.00223	0.00414	20[b]
Zn	0.3340	0.620	260[b]
Cd	0.00893	0.0165	5[b]
Hg	0.00789	0.01465	10[d]
Pb	0.02543	0.0472	10[c]
Cu	0.0348	0.0647	500[e]
Sb	0.3719	0.690	10[b]

Sources: (Choi Y.Y. 2011)[a], (A.N.F.A. 2001)[b], (Abbott et al. 2003)[c], (WHO 2007)[d], and (Mendez et al. 2005)[e]. ND represent no detected.

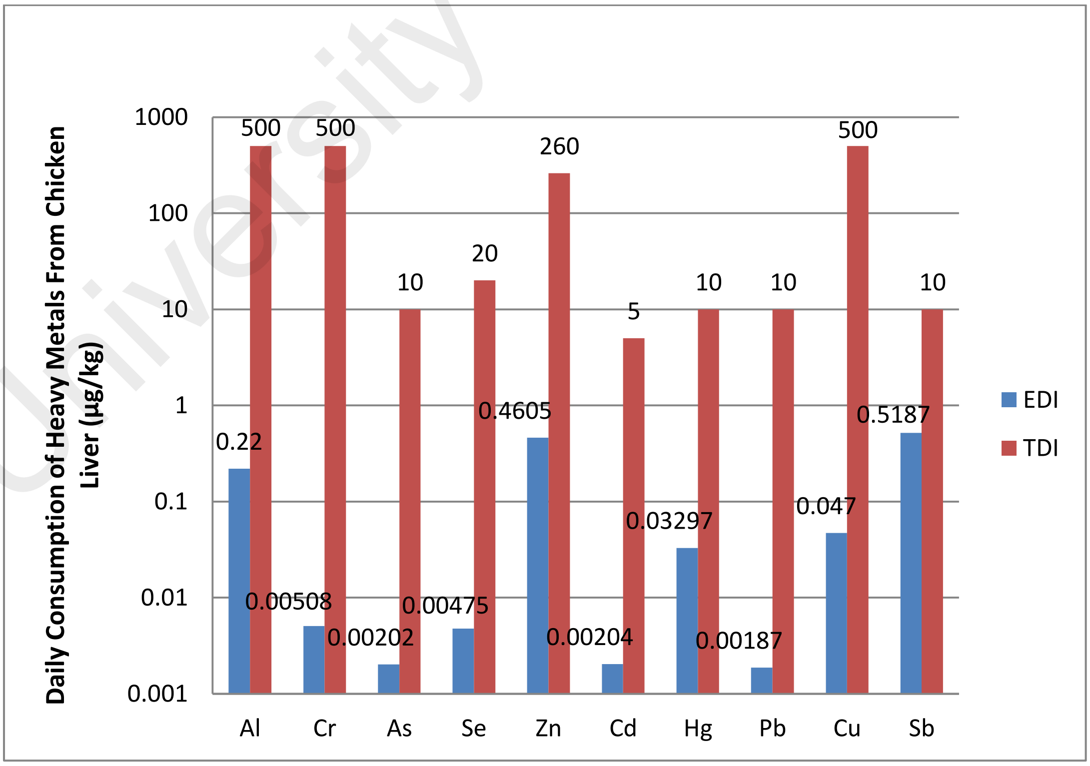

Figure 4.1: Comparison of Estimated Daily Intake (EDI) with the Tolerable Daily Intake (TDI) of Heavy Metals in liver

All targeted heavy metals for this study were revealed and detected in the liver samples. Sb has the highest EDI level 0.516 µg/kg in the liver, followed by Zn: 0.46 and Al: 0.22 µg/kg (Fig 4.1).

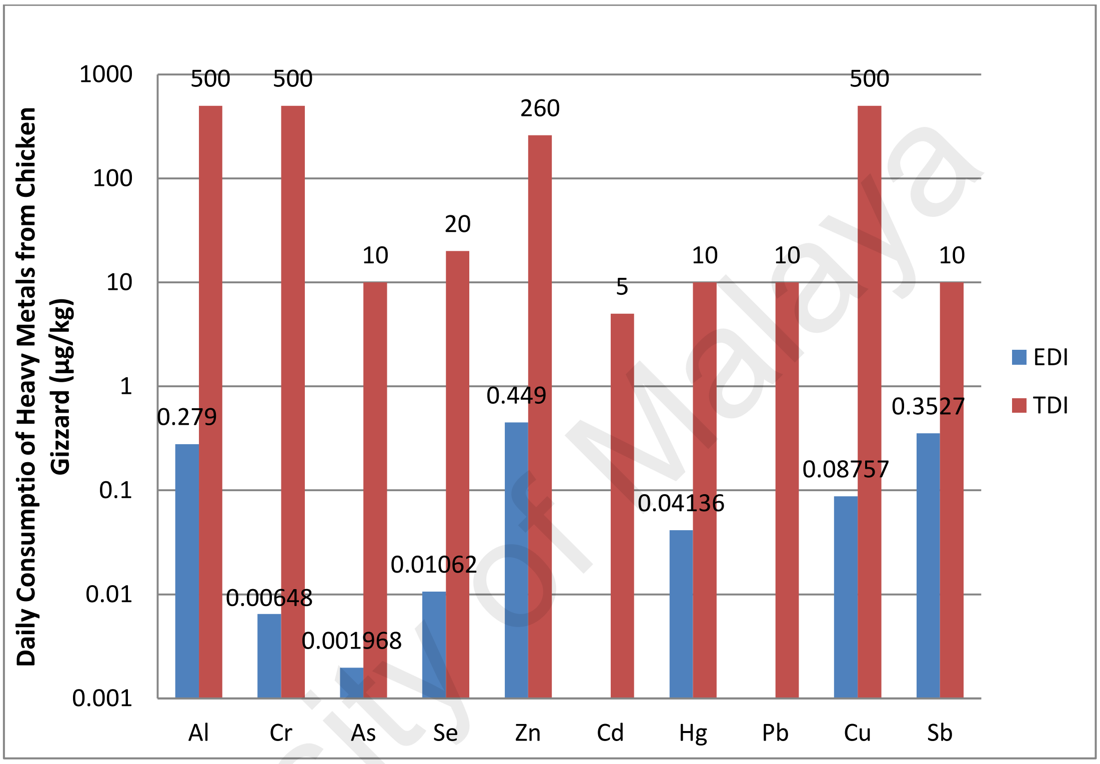

Figure 4.2: Comparison of Estimated Daily Intake (EDI) with the Tolerable Daily Intake (TDI) of Heavy Metals in gizzard

In fig 4.2, Zn has the highest EDI concentration 0.449 µg/kg in the gizzard samples, followed by Sb and Al with values of 0.35 and 0.27 µg/kg. Furthermore, Cd and Pb were virtually not detected in the gizzard samples. In addition, the result for this study shows that all targeted heavy metals concentrations are below the TDI limits, except for Sb which calls for regular monitoring procedures for all sample types.

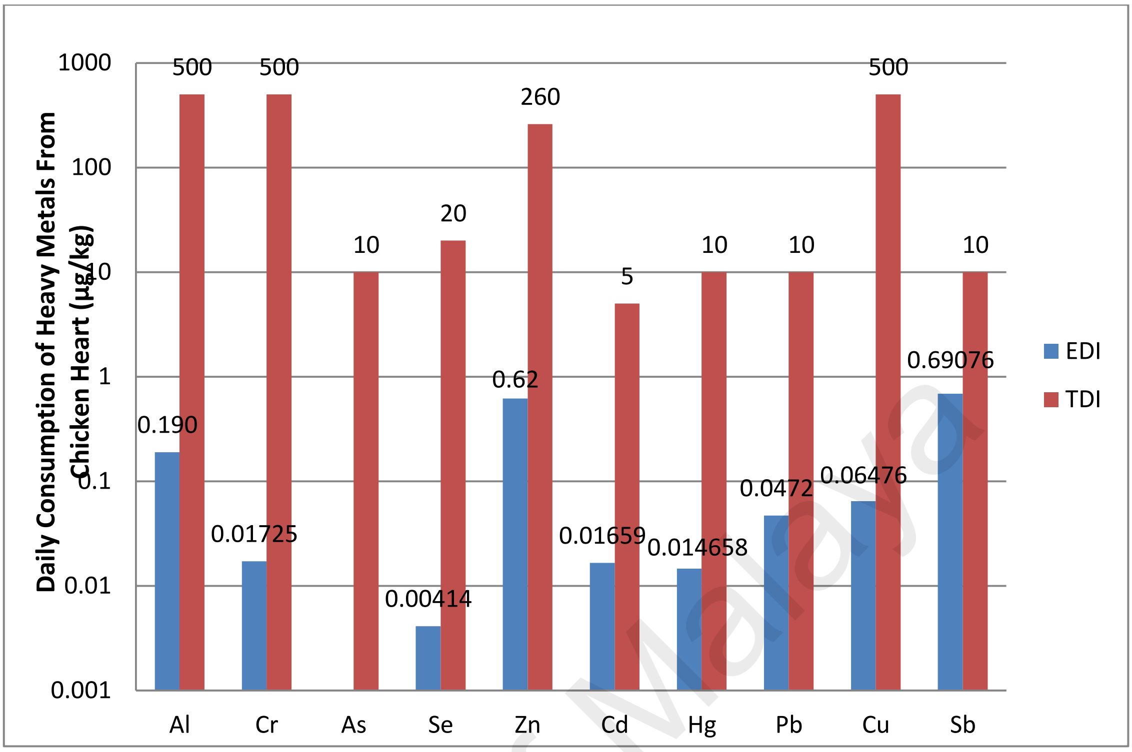

Figure 4.3: Comparison of Estimated Daily Intake (EDI) with the Tolerable Daily Intake (TDI) of Heavy Metals in heart

The heart samples were found to contain more Sb concentration in this study. The EDI level for element Sb in this case, has the highest EDI concentration of 0.69 µg/kg in the heart. The result also indicates that the heart contains the highest content of Sb than in liver and gizzard samples. Furthermore, all targeted heavy metals for this study was detected except for As which is not detected (Fig 4.3). It is advisable to carry out some regular monitoring procedures for the element Sb

4.4.1 Aluminum

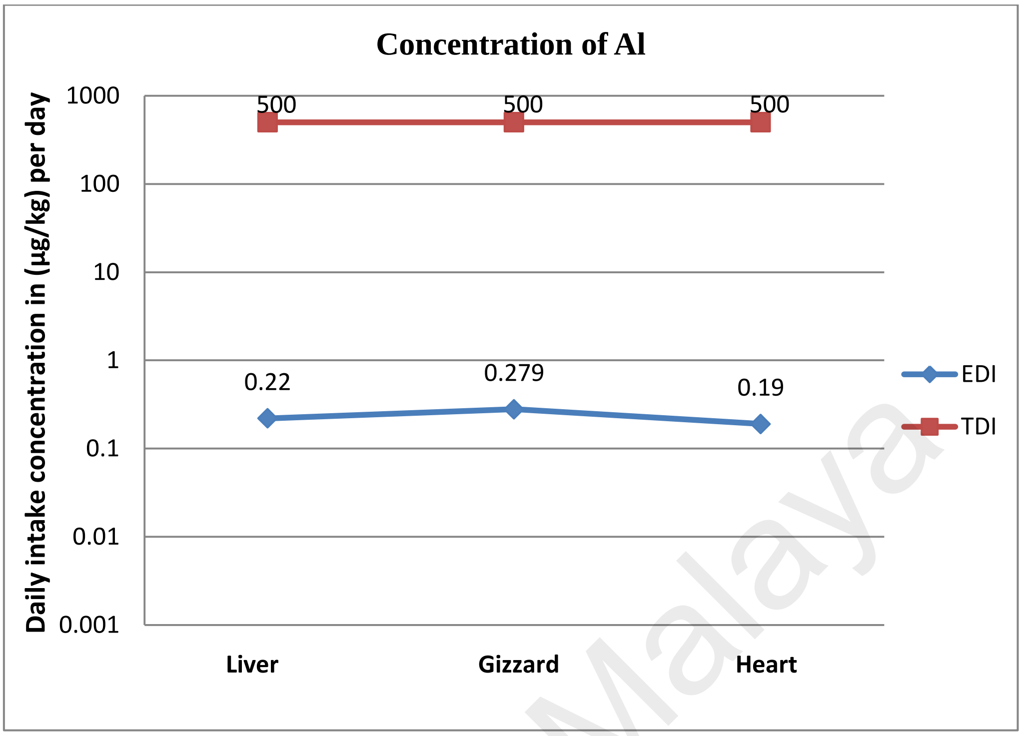

Figure 4.4: Line Chart of TDI and EDI for Al in all chicken giblets

It has been found to adversely affect the reproductive and nervous systems in human body. Some studies have also suggested a potential association between Al and Alzheimer's disease (Krewski et al. 2007). High intake of Al can cause dialysis encephalopathy, a degenerative neurological syndrome, neurotoxic effects that may contribute to declines in cognitive, and lung problems, such as coughing or changes that show up in chest X-ray (Ganrot 1986).

In a similar study, concentration of Al was 0.10±0.01, 0.23±0.02 & 0.14±0.01 µg/g respectively in heart, gizzard and liver of chicken products from Turkey, while comparison of recommended limits shows that it is below the tolerance limit (Uluozlu et al. 2009).

This study shows that EDI content of Al in gizzard sample were higher 0.279 µg/kg than the liver and heart 0.22 and 0.1903 µg/kg dry weight. However, comparing the revised WHO value with the (EDI) and (TDI) values from consumption of chicken giblets pose no significant health challenge to the consumers.

4.4.2 Chromium

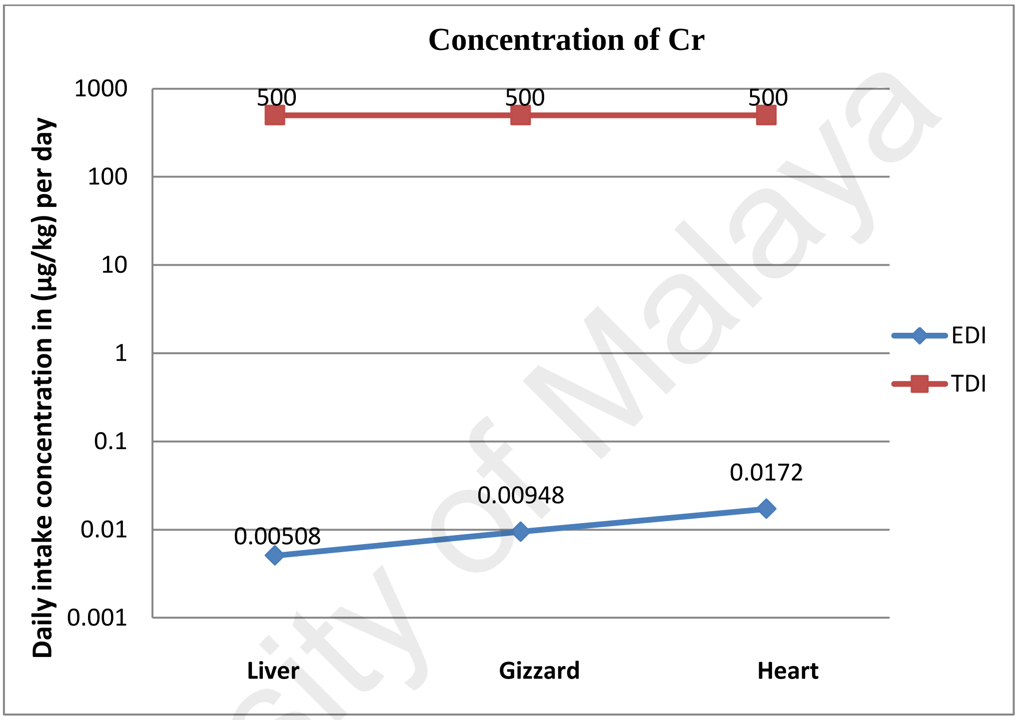

Figure 4.5: Line Chart of TDI and EDI for Cr in all chicken giblets

A low amout of Cr 0.05 µg/kg dry weight in the body can enhace the action of insulin, a hormone critical to the metabolism and storage of carbohydrate, fat, and protein in the body (Dwyer et al. 2005). Also, Cr is directly involved in carbohydrate, protein and fat metabolism. The toxicity of chromium is mainly attributable to the Cr(VI) form. It can be absorbed by the lung and gastrointestinal tract in human body, and even to a certain extent by intact skin. Again, high intake of Cr can cause several bio-toxic effects like: renal, hepatic, gastrointestinal, cardiovascular, hematological, reproductive and developmental effects (US Department 1999). Also in a similar study, levels of Cr in chicken meat and gizzard consumed in southern Nigeria was above the permissible limits with the following values given: 0.01–3.43 mg/kg (Iwegbue et al. 2008).

Presently this study is rare in Selangor Malaysia, while the present result shows that chromium were available in all samples of chicken, and fig 4.5; indicating the highest value for chromium in heart 0.0172 µg/kg dry weight. Thus comparing the results of this study with the international standards for heavy metals in foods value represents a current safe level of consumption.

4.4.3 Arsenic

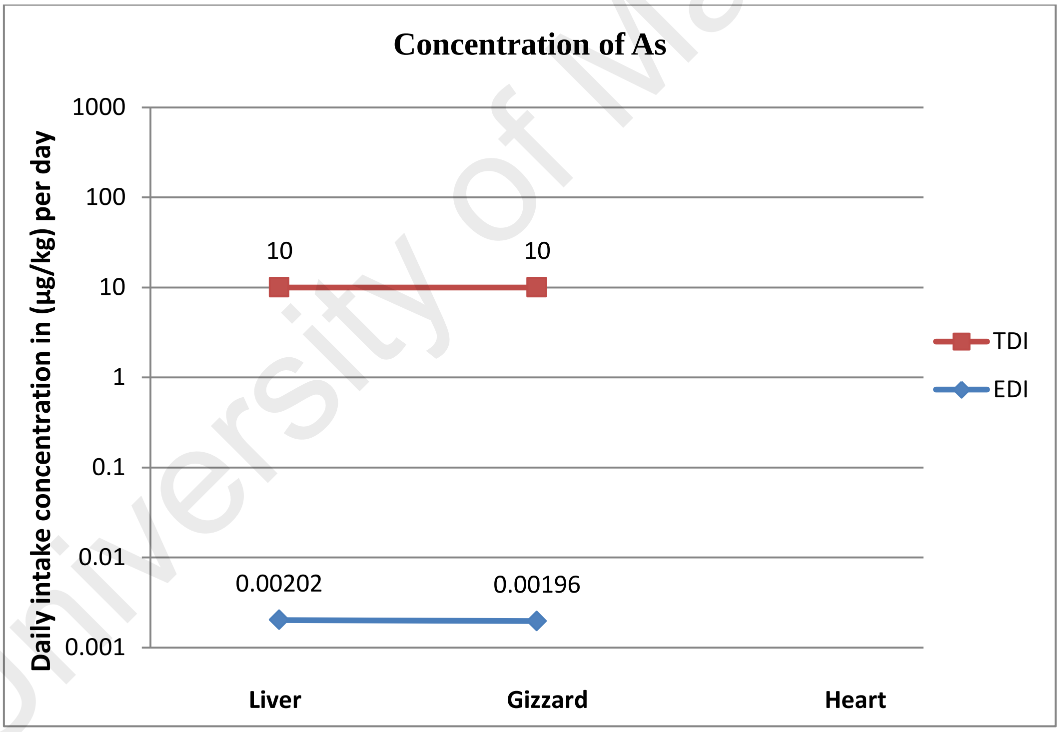

Figure 4.6: Line Chart of TDI and EDI for As in all chicken giblets

A non-essential element because of its known metabolic function in the human body (Hughes 2002). Also it was reported that As is a cancer-causing toxic metal that is fatal in high intake, can also cause reproductive effects and teratogenicity carcinogenicity even at a low concentration 0.5 µg/kg dry weight daily (George et al. 2014). Only in one case in Malaysia shows As concentration in gallus chicken gizzard and liver (0.238 and 0.515 µg/kg dry weight), which is half below the recomended limits set by the Australia New Zealand Food Authority (A.N.F.A. 2001).

In this study, EDI concetrations of As were determined only in liver and gizzard samples of poutry chicken in above metioned location in Malaysia, the values were: 0.0020 and 0.00196 µg/kg, respectively (Fig 4.6). This may be as a reuslt of depletion of As in the chicken feeds. Thus, comparing the revised WHO and The 19th ATDS value with the estimated values from liver and gizzard consumption, the current As level per kg body weight can be considered to remain at a safe level.

4.4.4 Selenium

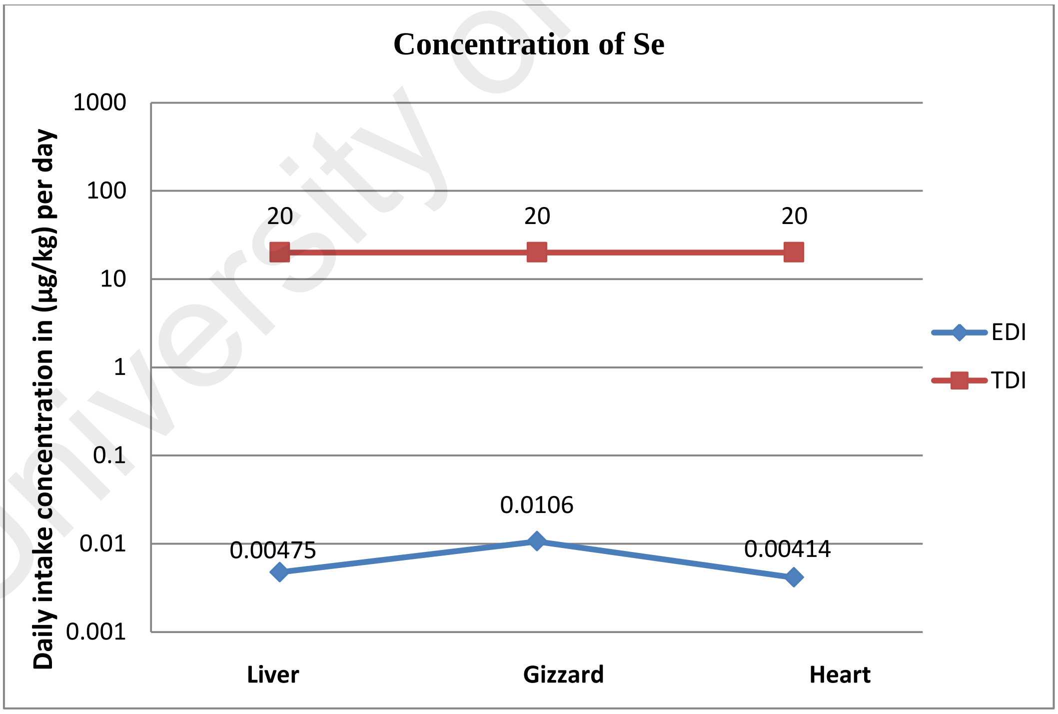

Figure 4.7: Line Chart of TDI and EDI for Se in all chicken giblets

An extremely vital mineral for the human body as it increases immunity, takes part in antioxidant activity that defends against free radical damage and inflammation, and plays a key role in maintaining a healthy metabolism (Feller et al. 1987). According to studies, consuming plenty of naturally occurring Se has positive antiviral effects, is essential for successful male and female fertility and reproduction, and also reduces the risk of cancer, autoimmune and thyroid diseases. Se is able to play such a protective role in the body because it increases antioxidant capabilities and the quality of blood flow, therefore enhancing the body's resistance against diseases and stress. Se is often praised for its role in antioxidant activity which lowers free radical damage and inflammation (Rayman 2000).

Exceeding the Tolerable Intake Level of 20 µg/kg per day can lead to selenosis. Symptoms of selenosis include a garlic odor on the breath, gastrointestinal disorders, hair loss, sloughing of nails, fatigue, irritability, and neurological damage (Sakurai & Tsuchya 1974). Extreme cases of selenosis can result in cirrhosis of the liver, pulmonary edema, and death. Acute Se intoxication is followed by adverse effects on the nervous system with special clinical relevance, while the neurotoxicity of long-term overexposure is less characterized and recognized (Vinceti et al. 2014).

In recent study, reports about acute Se intoxication include suicide attempts, consumption of Se-containing dietary supplements, intake of food sources with very high Se content like Brazil nuts, occupational exposures and rarer etiologies. Selenium concentration was very low below the permissible limits in chicken meat, intestine and liver from local market of Jakarta, Indonesia; with values 0.05 ± 0.03 and 0.06 ± 0.03 µg/g (Surtipanti et al. 1995).

Herein, a relatively low concentration of Se has been detected in all of the samples heart, gizzard and liver found to be of insignificant risk via TDI and EDI. The values of

Se in the samples were 0.00414 µg/kg for heart, liver 0.00475 and 0.01062 µg/kg dry weight daily for gizzard. The observed EDI and TDI values suggest Se levels through consumption of chicken giblets is yet below the harmful level.

4.4.5 Zinc

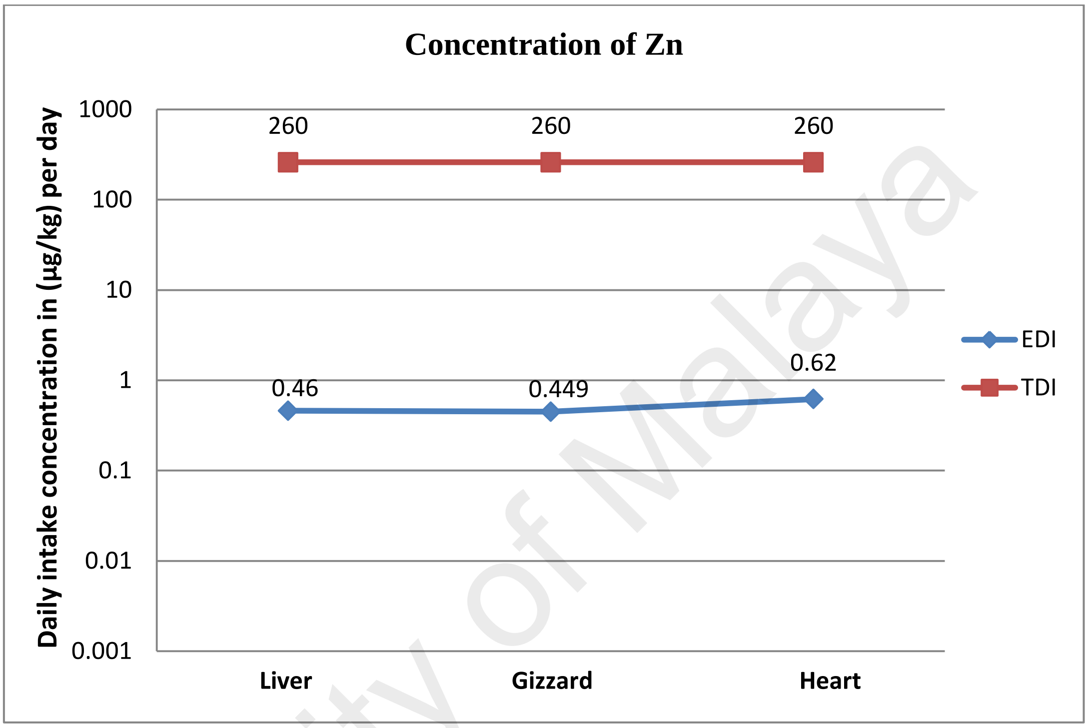

Figure 4.8: Line Chart of TDI and EDI for Zn in all chicken giblets

Compared to several other metals with similar chemical properties, Zn is relatively harmless. Zn is extraordinarily useful in biological systems. It is involved in many biochemical processes that support life and required for a host of physiological functions including normal immune function, and sexual function (FAO 2001). Zn is an essential component which supplies more proteins and metalloenzymes including alkaline phosphatase, lactate dehyrogenase, carbonic anhydrase, carboxypeptides, and DNA and RNA polymerases found in most body tissues. Only a high intake of Zn has toxic effects, making acute zinc intoxication a rare event (Plum et al. 2010).

However, manifestations of over toxicity symptoms (nausea, vomiting, epigastric pain, lethargy, and fatigue) will occur with extremely high Zn intakes of greater than 260 µg/kg daily of Zn (Tulchinsky & T.H. 2010). Chronic and Subchronic toxicity ingestion of zinc and zinc-containing compounds can result in a variety of chronic effects in the gastrointestinal, hematological and respiratory systems along with alterations in the cardiovascular and neurological systems of humans (Chandra 1984).

Zn is mostly found in all poultry foods and meats. In fig 4.8, heart contains the highest EDI of Zn 0.62 µg/kg, followed by the liver 0.460 µg/kg and gizzard 0.449 µg/kg dry body weight. Comparing the EDI values to the 19th ATDS and the WHO tolerable limits, it can be considered to remain at a safe level.

4.4.6 Cadmium

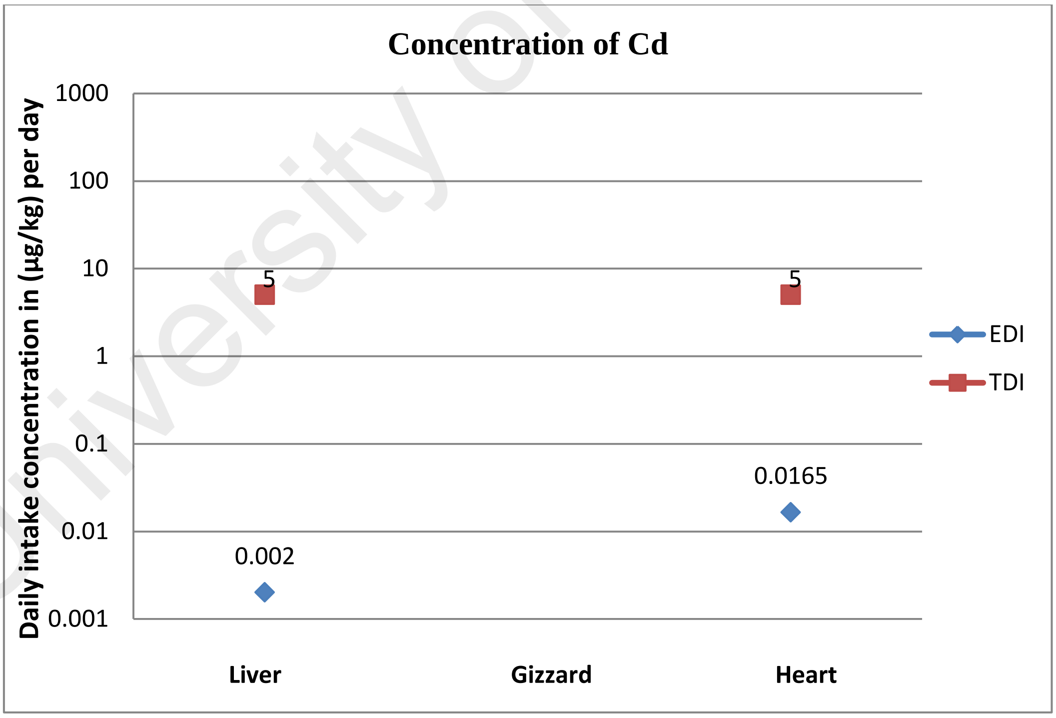

Figure 4.9: Line Chart of TDI and EDI for Cd in all chicken giblets

The principal toxic effect of Cd is its toxicity to the kidney (Godt et al. 2006). It was reported that Cd has also been associated with lung damage, including induction of lung tumours, causing renal damage of the kidney and skeletal changes in high intake (Bernard 2008). It is almost absent in the human body at birth, however accumulates from age 24. Cd accumulates in the human kidney and liver over long time (US Department 1999).

Similarly to this study, Cd deposited in liver samples collected from retail markets in Ismailia city, Egypt reached 0.0407 ppm; however gizzard and heart samples contain negligible Cd concentrations 0.0041 and 0.0036 ppm, respectively (Ismail and Abolghait 2013). In this study, Cd was found only in heart and liver samples 0.01659 µg/kg and 0.00204 µg/kg dry weight respectively. No data has been recorded so far for Cd in Chicken liver and heart in Selangor. Comparison of EDI and TDI of the present study, remains at a safe level recommened by Joint FAO/WHO and The 19th ATDS.

4.4.7 Mercury

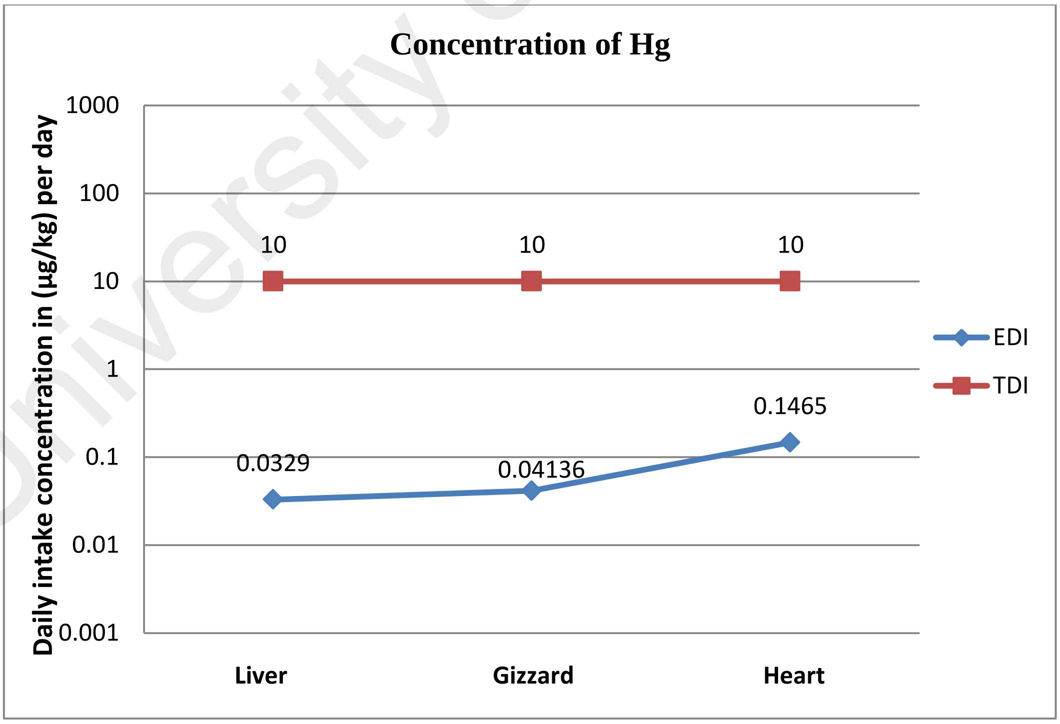

Figure 4.10: Line Chart of TDI and EDI for Hg in all chicken giblets

The toxicity effect of Hg when excessively present in the body leads to a wide spectrum of adverse health effects that include damage to the central nervous system (neurotoxicity) and the kidney (Ismail & Abolghait 2013). High rate of ingestion of Hg can be fatal to humans, but even relatively low rate of mercury containing compounds can have serious adverse impacts on the developing nervous system, and have recently been linked with possible harmful effects on the cardiovascular, immune and reproductive systems (Holmes et al. 2009).

Hg and its compounds affect the central nervous system, kidneys, and liver and can disturb immune processes; cause tremors, impaired vision and hearing, paralysis, insomnia and emotional instability (Ratcliffe et al. 1996). During pregnancy, Hg compounds cross the placental barrier and can interfere with the development of the foetus, and cause attention deficit and developmental delays during childhood (Menon 2016).

There is no known specific reported safe level, while the 19^{th} ATDS has set a limit of reporting for Hg of 0.01 mg/kg dry wt/day. There is no recorded data of Hg concentration in chicken giblets within Selangor Malaysia due to low rate of industrial activities within the study area. Fig 4.10: reveals that EDI concentration of Hg in liver, gizzard and heart samples were 0.03297, 0.04136, and 0.01465 µg/kg dry wt/day, respectively shows it is safe for consumption and the concentrations lie below the recommended limits by WHO and The 19^{th} ATDS.

4.4.8 Lead

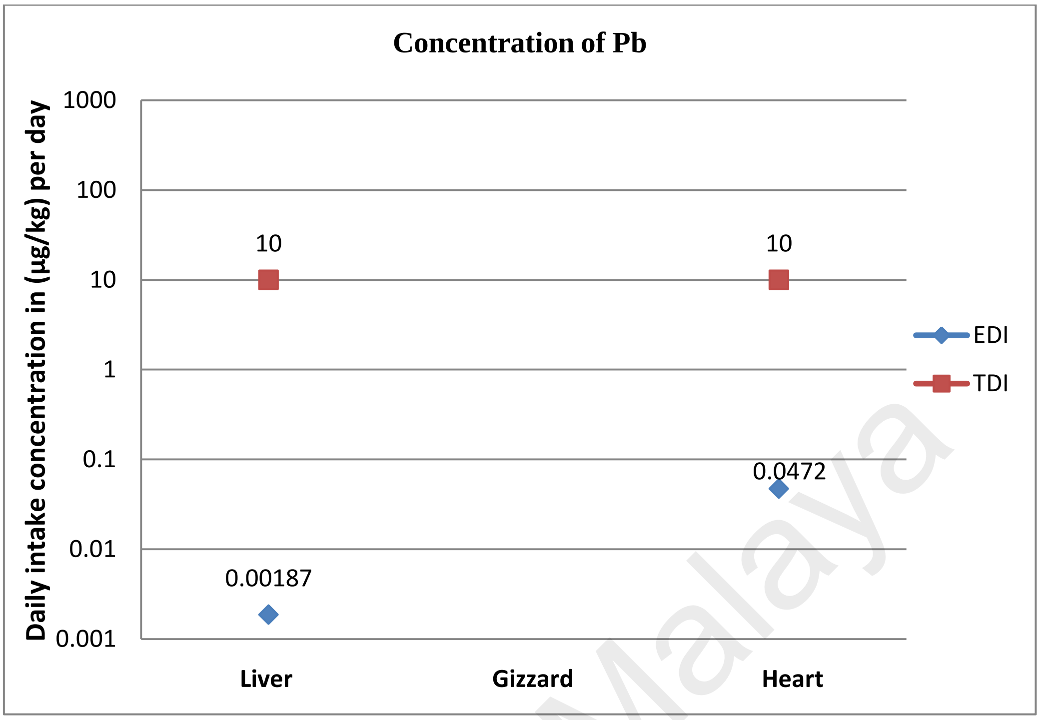

Figure 4.11: Line Chart of TDI and EDI for Pb in all chicken giblets

It fulfills no essential function in the human body, but rather it can do harm after uptake from food. High exposure to Pb can cause several unwanted effects, such as: Disruption of the biosynthesis of haemoglobin and anemia, a rise in blood pressure, kidney damage, miscarriages and subtle abortions, disruption of nervous systems, brain damage, declined fertility of men through sperm damage, diminished learning abilities of children, behavioral disruptions of children, such as aggression, impulsive behavior and hyperactivity etc (US Department 1999).

Pb is devastating to the human body, inhibiting oxygen and calcium transport and altering nerve transmission in the brain. It was also reported that lead builds up in soft tissue, kidneys, bone marrow, liver, and brain as well as bones and teeth. Pb absorption rates vary; the gastrointestinal tracts of adults typically absorb 10-15 percent of ingested

Pb, while those of pregnant women and children can absorb up to 50 percent (Tong et al. 2000).

Even low concentrations of Pb can cause permanent damage including reduced IQ, learning disabilities, and shortened attention span. Some scientists believe that low level chronic Pb exposure in childhood can alter secretion of the human growth hormone, stunting growth and promoting obesity. In rare cases, children with high, untreated blood-lead concentrations (150 micrograms per deciliter) can die from encephalopathy or massive brain damage (Pirkle et al. 1994).

It was reported in a similar study titled “quantitative determination of Cd, and Pb concentration in the tissues of thigh, breast, liver, heart, gizzard, neck and skin of chicken”. Samples were collected in the summer of 2004 from three different commercial farms of chickens production located in different areas of EL-Jabal Alakhder region at Libya. The results revealed that the highest concentrations of Cd were found in neck, liver and heart while the neck and skin tissues contained the highest level of Pb. However, the tissues of thigh and breast flesh had the lowest level of metals. The levels of Pb in the different tissues ranged from: 0.093 to 2.391 ppm for Pb. The results revealed that the levels of Pb in the neck and skin from all farms were exceeding the permitted limits according to some European regulations (Abdolgader et al. 2013).

Also, the mean concentration of Pb in the liver, heart and muscle of chicken samples collected from four popular brands distributed in different markets in Mashhad Iran were: 3.79 ± 3.64, 2.65 ± 1.88 and 1.65 ± 1.09 mg/kg respectively, in a similar study. Result indicating higher heavy metals levels in liver and heart samples compared to those of muscle ones. The contents of some heavy metals including Pb in chicken samples are warning that highlights its public health risk in this region of Iran (Sadeghi et al. 2015).

Also recently, the concentration of Pb in chicken gallus gizzard and liver within Selangor were 0.300 and 0.51 µg/kg, respectively (Abduljaleel et al. 2012), which is well below the recommended limits set by FAO/WHO. The fig 4.11 indicates that Pb was not available in the gizzard samples, while the concentration of Pb in liver and heart were (0.00187 µg/kg and 0.047 µg/kg dry wt/day). This shows that the highest EDI concentration of lead was found in heart. However, this is presently at a level of limited concern.

4.4.9 Copper

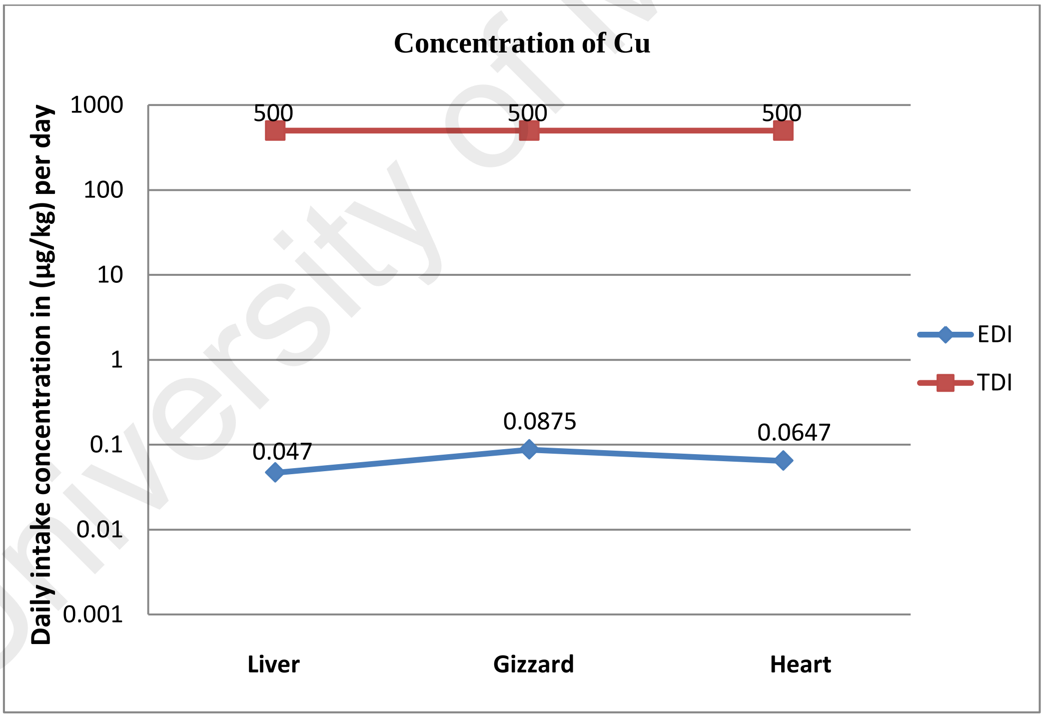

Figure 4.12: Line Chart of TDI and EDI for Cu in all chicken giblets

An essential metal in all organisms in trace amount, and particularly serves as a constituent of respiratory enzyme complex in the human body (Uauy et al. 1998). Due to its role in facilitating iron uptake, deficiency of Cu can lead to impaired growth, anemia-like symptoms, bone abnormalities, and vulnerability to infections. The health benefit of copper are crucial for an overall health existence, as this mineral enables normal metabolic process in association with amino acids and vitamins (Osredkar 2011). Cu is essential for proper growth of the body, efficient utilization of iron, proper enzymatic reaction, as well as improved health of connective tissues, hair and eyes.

Cu is also integral for preventing premature aging and increasing energy production. The toxicity of Cu is associated with high intake, which can cause anemia, low body temperature, brittle bones, osteoporosis, dilated veins, low white blood cell count, uneven heartbeat, elevated cholesterol levels, low resistance to infections, birth defects, low skin pigmentation, thyroid disorders (Nolan & K.R. 1983). The results of this study shows that Cu has been found in all sample for gizzard, liver and heart with concentrations values of 0.087, 0.047 and 0.0647 μg/kg dry weight daily in fig 4.12. Thus, the results are considered to remain at a safe level.

4.4.10 Antimony

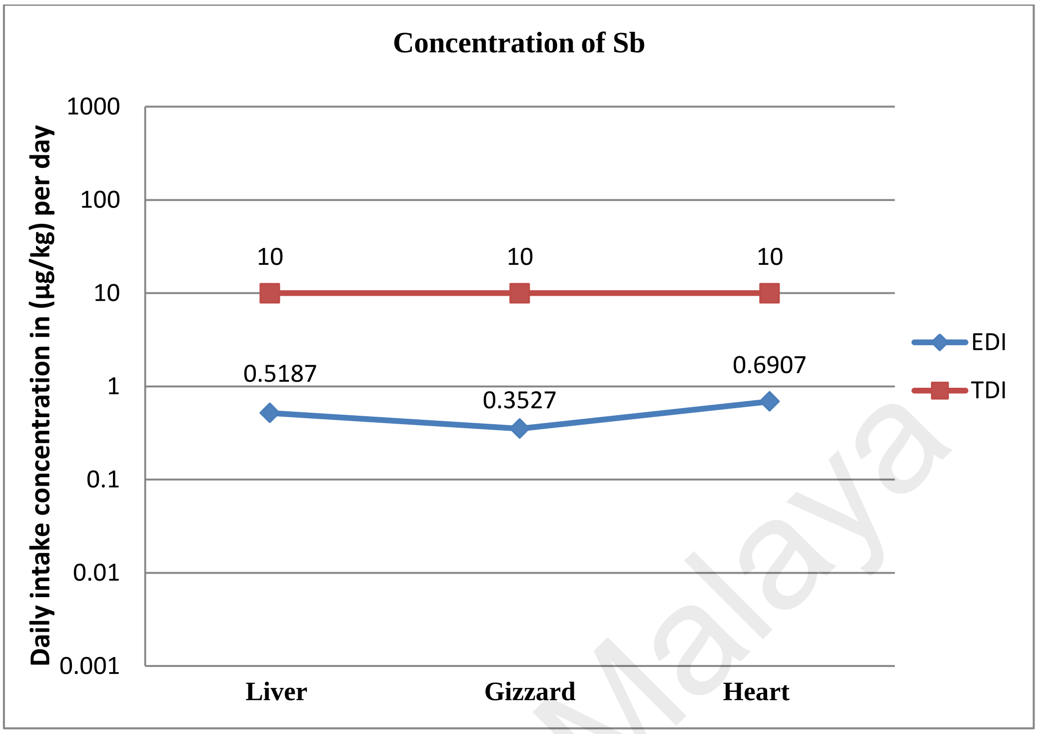

Figure 4.13: Line Chart of TDI and EDI for Sb in all chicken giblets

High exposure of Sb beyond the tolerable limits may cause respiratory irritation, pneumoconiosis, Sb spots on the skin and gastrointestinal symptoms (Sundar & C. 2010). In addition, Sb trioxide is possibly carcinogenic to humans (Cooper & H. 2009). No recorded data for Sb concentration in chicken giblets samples within the geographical area of Selangor Kuala Lumpur, while in this study Fig 4.13 shows that only the heart samples has the highest concentration of Sb. The estimated daily intake (EDI) of Sb in liver, gizzard and heart of poultry chickens in Selangor with the following values: 0.51, 0.35, and 0.690 µg/kg dry wt/day are below the TDI limits of 10 µg/kg set by WHO. However, the EDI of Sb as 0.690 µg/kg in heart and 0.51 µg/kg in liver samples represents a concern worthy of regular monitoring.

Table 4.7: Comparison of targeted heavy metals concentration with other literatures values (µg/g) in chicken livers

Metal	Mean Value (µg/g)	Literature Value (µg/g)	Study Area (Location)	Technic
Al	0.12±0.058	0.14±0.01[k]	Tokat, Turkey	**AAS**
Cr	0.0026±0.002	0.38±0.08[g]	El-Sheikh	AAS
As	0.00109	0.06 ± 0.004[k]	Tokat, Turkey	AAS
Se	0.0025±0.001	0.01742[q]	Kurdistan, Iraq	ICP-OES
Zn	0.248±0.036	5.27±0.59[g]	El-Sheikh	AAS
Cd	0.0011	0.004, 0.015-0.033[h]	Al Basrah, Iraq	AAS
Hg	0.017±0.018	0.0005±0.0002[f]	Jakarta	NA-AAS
Pb	0.00101	0.171, & 2.060[h]	Al Basrah, Iraq	AAS
Cu	0.0253±0.016	0.1583[h]	Kurdistan, Iraq	ICP-OES
Sb	0.279±0.0674	0.10±0.06[k]	Jakarta	NA-AAS

Sources: (Uluozlu et al. 2009)[k], (Mousa et al. 2010)[g], (Aljaff et al. 2014)[q], (Anas et al. 2015)[h], (Surtipanti et al. 1995)[f]

Table 4.7 illustrates the following: measured mean values for this study (µg/g), literature values in µg/g – ppm, location/study area, and technic used.

The content of Cr, Hg and Cu shows in table 4.7 shows not significant in the liver samples with the value of: 0.0026±0.002, 0.017±0.018 and 0.0253±0.016 µg/g.

Table 4.8: Comparison of targeted heavy metals concentration with other literatures values (µg/g) in chicken gizzards

Metal	Mean Value (µg/g)	Literature Value (µg/g)	Study Area (Location)	Technic
Al	0.1504±0.10	0.23 ± 0.02[k]	Tokat, Turkey	AAS
Cr	0.0034±0.003	0.05 ± 0.004[k]	Tokat, Turkey	AAS
As	0.001	0.118 ± 0.018, 0.196 ± 0.058[j]	South Nigeria	AAS
Se	0.0057±0.003	0.17 ± 0.01[k]	Tokat, Turkey	FAAS
Zn	0.334±0.06	3.05±0.33 & 3.14±0.38[m]	Manisa, Turkey	AAS
Cd	--	0.0211[i]	Ismailia, Egypt	FAAS
Hg	0.0078±0.009	0.039, 0.048-0.051[m]	Manisa, Turkey	AAS
Pb	--	0.1139-0.5176[i]	Ismailia, Egypt	FAAS
Cu	0.047±0.0029	0.445 ± 0.027[j]	South Nigeria	ASS
Sb	0.189±0.0247	--	--	--

Sources: (Okoye et al. 2015)[j], (Demirbas 1999)[m], (Ismail et al. 2013)[i],

The result reveals that content of Cr 0.0034±0.003, Hg 0.0078±0.009, and Al 0.1504±0.10 µg/g, are not significant in the gizzard samples. Furthermore, it is certain that the result indicates some accretion or depletion of Cr, Hg and Al in the analyzed sample (Table 4.8).

Table 4.9: Comparison of targeted heavy metals concentration with other literatures values (µg/g) in chicken hearts

Metal	Mean Value (µg/g)	Literature Value (µg/g)	Study Area (Location)	Technic
Al	0.102±0.05	0.10 ± 0.01[k]	Tokat Turkey	FAAS
Cr	0.009±0.01	0.03 ± 0.002[k]	Tokat, Turkey	AAS
As	--	0.06 ± 0.005 [k]	Tokat, Turkey	AAS
Se	0.002±0.0009	0.39 ± 0.02 [k]	Tokat, Turkey	AAS
Zn	0.33±0.06	2.23±0.26[g]	El-Sheikh, Egypt	AAS
Cd	0.008±0.009	0.0006-0.0071[i]	Ismailia, Egypt	FAAS
Hg	0.0078±0.009	--	--	--
Pb	0.025	0.0789-0.5770 [i]	Ismailia, Egypt	FAAS
Cu	0.034±0.016	1.77±0.26 [g]	El-Sheikh, Egypt	AAS
Sb	0.37±0.15	--	--	--

Table 4.8 shows literature values in µg/g – ppm, location/study area, measured mean value of the studied metals (µg/g), and technic used. The sign (--) denotes virtually not detected.

The mean and standard deviation for Hg 0.0078±0.009, Cd 0.008±0.009, and Cr 0.009±0.01 µg/g for this study indicates not significant in concentration in heart samples. Thus, this means that the content of these metals varies slightly in the heart samples due to some environmental sources (water, air or soil pollution) which can contaminate poultry feeds.

CHAPTER 5: CONCLUSION

Consumption of food is the major pathway for human exposure to heavy metals, and therefore threatens the toxic effect on human health. The level of heavy metals in poultry chickens liver, heart and gizzard in Selangor and Kuala Lumpur were determined using ICP-MS and compared with the permissible limits given by international regulatory agencies for human protection. Present study shows that most of the toxic metals (Al, Cr, As, Se, Zn, Cd, Hg, Pb, Cu and Sb) are available in the liver samples followed by the (Al, Cr, Se, Zn, Cd, Hg, Pb, Cu and Sb) in heart samples, and (Al, Cr, As, Se, Zn, Hg, Cu and Sb) in gizzard samples.

The results shows high average concentration for Sb in liver and heart samples with values of: 0.279±0.0674 and 0.37±0.15 µg/g, followed by Zn in the liver, gizzard and heart: 0.248±0.036, 0.334±0.06 and 0.33±0.06 µg/g. Furthermore, content for Cr in the liver, gizzard and heart samples was 0.0026±0.002, 0.0034±0.003 and 0.009±0.01 µg/g, followed by Hg in liver: 0.017±0.018, gizzard: 0.0078±0.009 and heart: 0.0078±0.009 µg/g. Average concentration for Cd in heart was 0.008±0.009 µg/g, while Al in the gizzard samples was 0.1504±0.10 µg/g.

Thus the values for Hg and Cr indicate not significant in concentration for all sample types. Only for Cd in heart and Al in the gizzard samples shows not significant. This can be as a result of some accretion or depletion of chemical contaminants in the studied samples via cantaminated feeds used during poultry feeding. In addition, the source of poultry feed contamination of heavy metals may come from environmental sources as mentioned (water, air or soil pollution) or manmade activities like (bush burning, oil spillage, mining operation or industrail hazard) etc.

Fortunately, the overall EDI data obtained for Al, Cr, As, Se, Zn, Cd, Hg, Pb, Cu, in chicken giblets in this investigation, were below prescribed the safety limits, showing a very low level in the concentration of heavy metals in all the studied samples except for Sb which requires regular monitoring procedures. Although there may not be internationally agreed safe levels for all of these metals. However, consumption of the chicken giblets does not therefore; pose any significant metal pollution or health challenge to the inhabitants of Selangor and Kuala Lumpur, Malaysia.

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