HEAVY METALS POLLUTION IN MUSCLE, CRAW, KIDNEY, LIVER AND PLANT FOOD GIVEN TO CHICKENS IN THE AREA AROUND PROBISHTIP

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ABSTRACT

Permanent intake of residues through meat, even if only of one of them and in small quantities, can over time cause the appearance of new chemical compounds that can cause chronic disease in humans due to cumulation and synergic action. Symptoms usually appear even when it is too late to intervene. The issue here is the fact that in this case there is no so-called alarm signal which is characteristic of infections caused by microorganisms and which would make a person feeling it seek help from a doctor. The increasingly more frequent fatigue attributed to hard work, a growing number of anxious people because of a deadly pace of life, headaches the occurrence of which is explained in different ways, heart pain etc., are probably consequences of the new nutritional era, i, e, of the effect of biological debris introduced into the human organism through meat and other products.

Toxic metals get into meat through contaminated cattle feed, and partly through contaminated water or air. Of toxic metals which can be found in meat selenium should be mentioned first because it is often given to animals as prevention from certain phenomena that negatively affect the quality of the meat. As samples for proving the presence of selenium in meat, fat (the amount should be less than 1mg/kg) and muscle tissue is taken, as well as liver and kidney. It should be noted that during the inspection of meat, the largest amounts of toxic trace elements can be found in the kidney, then liver and in other organs.

The paper will show the results for the content of lead, cadmium, arsenic and mercury in 11 different samples of muscle, craw, kidney, liver and plant food given to poultry (chickens) in the area around Probishtip.
Key words: heavy metals, pollution, lead, cadmium, arsenic, mercury, muscle, craw, kidney, liver, plant food, chickens, coefficients of correlation

Introduction

Lead is a toxic metal that can be found everywhere in the environment. Overexposure to lead continues to be an important worldwide problem. Food is an important source of lead and determination of lead in food can be used for the estimation of lead exposure. The level of lead in the Earth’s crust is about 20 μg/g. The Industrial Revolution gave rise to an increase in the amount of lead in the environment and an even bigger increase occurred around 1920 when leaded gasoline was introduced. Leaded gasoline is still not banned everywhere in the World, and it still used in the developed countries. In areas where leaded gasoline is banned, the major exposure pathways of nonsmoking adults are from food and water. Several studies were done to determine the concentration of lead in foods [10, 14] and to study its dangerous effects. Recently, the USA, all European countries and many developing countries have outlawed or strictly regulated the use of leaded petrol. In such countries, levels of lead in food and drinking water are closely monitored. Lead may reach and contaminate plants, vegetables, fruits and canned food through air, water and soil during cultivation and also during industrial processing and packaging. Fruits and vegetables grown in polluted soils may become contaminated as a result of plant uptake of lead from soils or direct deposition of leaded dust onto plant surfaces. Therefore, through these diverse mechanisms, lead deposited in soil becomes a persistent and long-term source of lead exposure for humans.

Lead exposure can occur through food, water, soil, air and the relative contributions from individual sources may depend on lifestyle and socioeconomic status. It was reported that the main sources of exposure for an adult are food (ranging from 0.4 μg/kg bw/week to 10.1 μg/kg bw/week) and water (ranging from 0.23 μg/kg bw/week to 0.35 μg/kg bw/week). The main potential sources of exposure to lead in children are food, air, water and dust or soil.

Watanabe *et al*. [26] reported that people, especially those who consume rice as a staple food for daily energy, are inevitably exposed to significant amount of lead via rice. Rice crops, even from non polluted areas, may be contaminated because of fertilizers that are used in forms containing lead. Amongst other foods fish are constantly exposed to lead from polluted water. Lead accumulates in their bodies tissues in different amounts depending on the size and age of fish [23, 24]. Canned fish, especially canned tuna fish, is abundantly consumed because of its convenient and affordable use. In the same respect, several studies [17-19] have reported that the lead content in different type of chocolates varied between 0.07 and 4.0 μg/g product.

The United Nations World Health Organization (WHO) has also given recommendations for maximum lead content in food and water [3, 27]. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) [28] has established a provisional tolerable weekly intake (PTWI) of 25 μg/kg bw/ week for lead. PTWI is an estimate of the amount of a contaminant that can be ingested over a lifetime without appreciable risk. An intake above the PTWI does not automatically mean that health is at risk. Transient excursion above the PTWI would have no health consequences provided that the average intake over long period is not exceeded as the emphasis of PTWI is a lifetime exposure. Developing countries have been slow in putting in place regulatory restrictions on leaded products, and leaded petrol is still used in some cases. There is very little data on the magnitude of the contamination levels of lead in environment and in food in these countries. Lead can be very harmful even at low concentrations when ingested over a long time. After ingestion, the absorption rate of lead ranges from 3% to 80%, whereas the typical absorption rates of dietary lead in adults and infants are 10% and 50%, respectively. After absorption, lead is initially distributed to soft tissues throughout the body via blood, and then deposited in bone. Lead is excreted through the kidney and to a lesser extent in the bile while non-absorbed dietary lead is excreted in the feces. Organic lead may be metabolized to inorganic lead. The concentration of lead in blood is commonly used as a biomarker of exposure. Lead is a classical chronic toxic chemical. It may cause damages to kidneys, the cardiovascular, immune, hematopoietic, central nervous and reproductive systems. Short term exposure to high level of lead can cause gastrointestinal distress, anemia, encephalopathy and death. The effect of exposure to lead varies according to dose and the age of the exposed person. Manifestations of lead poisoning are nonspecific abdominal pain, constipation, irritability, malign, muscle aches, headache, anorexia, and decreased lipid concentrations. The Kingdom of Saudi Arabia (KSA) imports a lot of foodstuffs from several countries. These foods may be subjected to lead contamination as described above. The objective of this work was to estimate the levels of lead that may be present in some foods available in local markets in Riyadh city, to determine the food daily intake from the Food Consumption Survey (questionnaire) conducted in local population in Riyadh city 2010, and consequently the daily lead intake from food (questionnaire) and finally, to compare lead intake from the questionnaire with that from the food balance sheet of the KSA provided by the FAO.

Lead is ubiquitous in the environment, persists indefinitely, and can be found at low levels in almost all living organisms [5]. Sources of lead contamination of air, water, and soil include internal combustion engines, oil burners, smelters, lead pipes, glass and alloy processing plants, incinerators, industrial effluents, and smokestack fallout [11]. Lead is found in the soil, plants and grains grown on contaminated soil, and tissues of animals that eat contaminated plants and feed grains [1]. Because of widespread environmental exposure, low levels of lead can be demonstrated in tissues of clinically normal birds and animals [5] Lead toxicities occurs when an animal or a bird inhales or ingests a concentrated source of lead. Concentrated lead sources include lead-based paint, lead arsenate crop sprays, lead plates in automotive batteries, fishing sinkers, lead shotgun pellets, drapery weights, sewage sludge, and lead mine tailings [15].

Chickens are susceptible to lead intoxication. As little as 1.0 mg/kg lead in the diet can cause significant depression in the growth of broiler chickens and consistent reduction in blood d-aminolevulinic acid dehydratase, an erythrocyte enzyme sensitive to lead.1 Clinical signs of acute lead poisoning in chickens include muscle weakness, ataxia, and loss of appetite, followed by marked weight loss and eventual cessation of egg production. A severe anemia may develop. Young chickens are more susceptible than adult chickens [17, 20]. Long-term lead intoxication of chickens results in degeneration of motor nerves in the spinal cord and loss of axons in peripheral nerves without demyelination. In addition, muscles show atrophy and degeneration of fibers.6 Attempts to measure the effects of lead on the chicken's cell-mediated immune response, humoral immune response, and interferon production have yielded inconsistent results [24,28]. Lead ingested by chickens is deposited in bones, soft tissues, and eggs and produces elevated blood lead levels.1 Bone lead concentrations are by far the highest, followed by kidney and liver. The lowest concentration of lead is found in skeletal muscle [24]. Eggs accumulate lead in their shells, yolk, and albumen, with the highest concentration occurring in the shells.

MATERIALS AND METHODS

Lead concentration was extracted from the samples (muscle, liver, kidney, spleen and heart) according to the method of.[14]. Samples were homogenized separately and 5-10 g of the fresh homogenate were weighed into quartz dishes and evaporated to dryness in an oven at 100° C (~16 h). Dried samples were ashed in a muffle furnace at 450-500°C for 8-12 h. Ashed samples were cooled to room temperature and 1.0 ml of concentrated nitric acid was added and the volume was adjusted to 25 ml with deionized water. The metal was measured by atomic absorption spectrophotometer (Perkin Elmer 5000). Lead was measured at wavelength 217.0 nm with Hollow Cathode Lamp of lead. The limit of detection was 0.06 mg/kg for lead. The recovery of lead was studied by adding known amounts of standard solution to different samples under investigation. The added amounts of lead were selected so that they would be close to the amounts normally found in the different samples. Recoveries in muscle, liver, kidney, spleen and heart ranged from 94-98%. All the results obtained were corrected according to the percentage of recovery.

The material for analysis (fresh tissue from muscle, liver, kidney, spleen and heart of three domestic animals – swine, sheep and goat) were taken from three localities in the vicinity of the town Probishtip.

1. The first and second measuring point was the industrial zone in the town of Probishtip – flotation of the lead-zinc ore.
2. The third measuring point was the locality in the vicinity of the waste landfill near the village of Strmos.
3. The fourth measuring point was the control measuring point, located at 10 km from the town of Probishtip where there are no sources of pollution with heavy metals.

RESULTS AND DISCUSSION

Results presented in Tables (1-4) show the mean concentrations of lead, cadmium, arsenic and Hg analyzed samples collected from heavy traffic, urban and industrial areas from the different investigated chicken. The highest concentrations of lead were detected in gaster, followed by liver samples.

Table 1: Concentrations of lead (Pb) in organs of chicken (mg·kg^{-1} fresh mass)

M.Point	Muscle1	Muscle2	Muscle3	Gaster	Cor	Liver	Plant 1	Plant 2
Probishtip 1	0,225	0,209	0,375	13,93	0,389	15,149	42,69	38,79
Probishtip 2	1,17	0,544	1,98	58,50	0,466	17,25	54,62	62,15
Strmos	2,58	1,59	3,26	60,25	0,89	20,540	88,216	124,31
Control	0,154	0,128	0,147	5,24	0,131	4,123	0,941	0,647

In respect of the measuring stations the highest values of lead content were measured in Strmosh in whose immediate vicinity is the old tailings pond of the lead and zinc mine “Zletovo”. The lead dust is carried by wind to greater distances and contaminates the environment with heavy metals. The highest values are measured in the stomach of chickens, 60.25 mg/mk fresh mass at the measuring place Strmosh which is located near the landfill, while the value at the control point of measurement that is considered relatively clean environment is 5.24 mg/kg. The plants that chickens feed on are heavily contaminated with lead in Strmosh and the value is from 88.216-124.31 mg./kg dry mass. At other measuring points relatively low values of lead content are measured (42.16-62.15 mg/kg dry mass in Probishtip), while at the control measuring point the value is only 0.647 mg/kg.

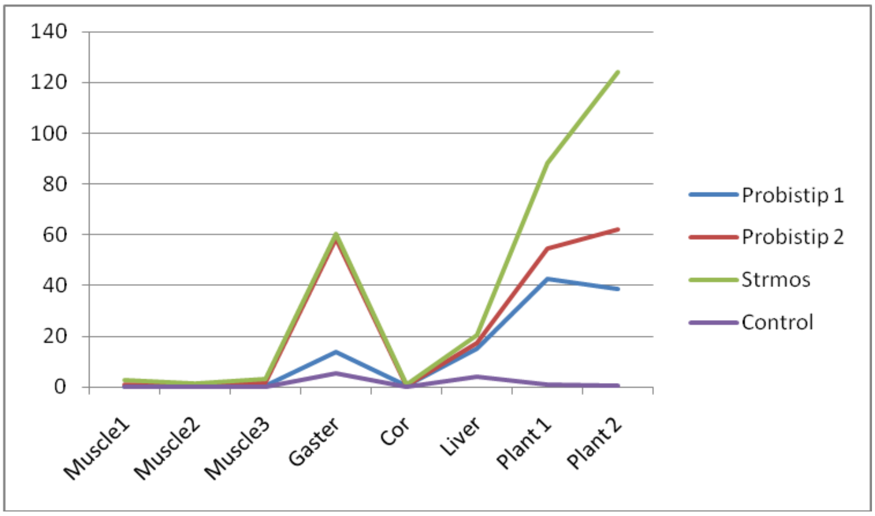

Figure 1. Concentrations of lead (Pb) in organs of chicken (mg·kg^{-1} fresh mass)

The levels of lead varied according to the species of tissues and the locality (Fig. 1).

Table 2: Concentrations of cadmium (Cd) in organs of chicken (mg·kg^{-1} fresh mass)

M,Point	**Muscle1**	**Muscle2**	**Muscle3**	**Gaster**	**Cor**	**Liver**	**Plant 1**	**Plant 2**
Probishtip 1	2,56	2,89	3,12	1,24	0,26	4,89	7,56	5,88
Probishtip 2	3,25	2,98	2,84	1,78	0,23	5,66	8,47	6,95
Strmos	5,48	6,12	4,58	2,02	0,515	7,92	8,54	7,56
Control	0,05	0,12	0,09	0,496	0,026	0,54	1,24	0,98

Table 2 gives the results for the content of cadmium in muscle tissue, stomach, heart, liver and two types of plants that chickens were fed on. As for the measuring points, the highest values are measured in Strmosh and in muscle tissue they vary from 4.58 to 6.12 mg/kg fresh mass. In internal organs the highest values are measured in liver and they vary from 4.89 mg/kg (Probishtip 1) to 7.92 mg/kg (Strmosh). At the control measurement point that is located outside any source of pollution with heavy metals the lead content in liver is 0.54 mg/kg. The lowest values are measured in heart, from 0.026 mg/kg at the control measuring point to 0.515 mg/kg at the most polluted measuring point (Strmosh).

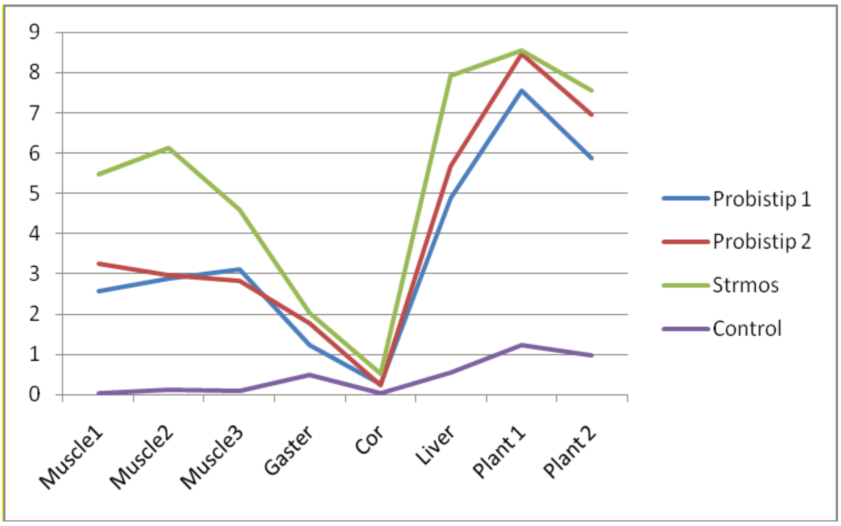

Figure 2. Concentrations of cadmium (Cd) in organs of chicken (mg·kg^{-1} fresh mass)

Table 3: Concentrations of arsen (As) in organs of chicken (mg·kg^{-1} fresh mass)

M.Point	**Muscle1**	**Muscle2**	**Muscle3**	**Gaster**	**Cor**	**Liver**	**Plant 1**	**Plant 2**
Probishtip 1	0,118	0,092	0,089	2,57	0,010	0,056	21,58	29,54
Probishtip 2	0,124	0,115	0,098	3,152	0,014	0,076	24,58	31,59
Strmos	0,154	0,126	0,113	5,842	0,017	0,098	36,59	45,51
Control	0,026	0,012	0,016	0,458	0,008	0,005	2,54	3,24

Arsenic can get into the flesh by means of food that is contaminated with pesticides, by treating animals with drugs that have arsenic and fattening them using feed that contains arsenic. The meat should not have more arsenic than 1mg/kg, and in organs 0,5 mg/kg.

The survey results show that relatively high values are measured in the stomach (from 2.57 mg/kg in Probishtip 1 to 5.842 mg/kg in Strmosh) versus the values obtained at the control point of measurement of 0.458 mg/kg. In plants that chickens were fed on relatively high levels of arsenic were measured, from 29.54-45.51 mg/kg dry mass of plants.

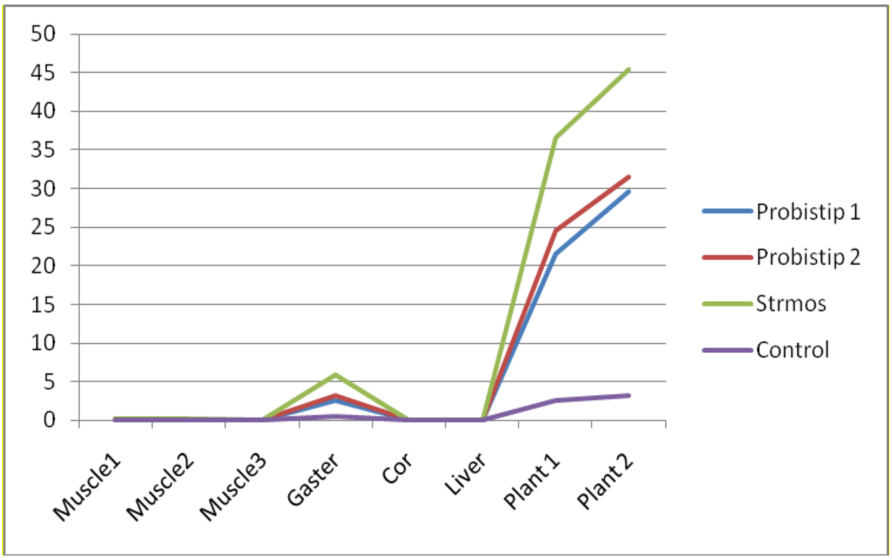

Figure 3. Concentrations of arsen (As) in organs of chicken (mg·kg^{-1} fresh mass)

Chart 3 clearly shows that in terms of the examined tissues of chickens highest values are measured in liver at all measuring points, somewhat lower values in the stomach, while muscle tissue contains the lowest values.

Table 4: Concentrations of mercury (Hg) in organs of chicken (mg·kg^{-1} fresh mass)

M,Point	**Muscle1**	**Muscle2**	**Muscle3**	**Gaster**	**Cor**	**Liver**	**Plant 1**	**Plant 2**
Probishtip 1	0,0025	0,0028	0,0014	0,009	0,0025	0,0145	0,0358	0,0587
Probishtip 2	0,0031	0,0036	0,0023	0,010	0,0031	0,0159	0,0475	0,0662
Strmos	0,0098	0,0087	0,0085	0,068	0,0078	0,0568	0,0845	0,0921
Control	0,001	0,001	0,001	0,002	0,001	0,003	0,0012	0,0023

Mercury contamination of food chain can representa risk factor both for animal and human health. For farm animals, European Commission Directive 2005/8/EC permitted the maximum content of Hg 0.1 mg/kg of complete feedstuffs. Until now, there have been no claims that Hg is an essential constituent of the diet for any species (Underwood and Suttle, 1999).

Inorganic forms of Hg are poorly absorbed; the range was variously quoted 5–15% of intake (Clarkson, 1987) and 1–3% (Kostial et al., 1978), but in very young animals 30–40% of intake (Kostial et al., 1978). Methylmercury is a more available form of Hg (Underwood and Suttle, 1999). Houserova et al. (2005) found the highest mercury concentration (39.2 mg/kg dry matter) in liver of an adult population of cormorant (*Phalacrocorax carbo*) while the content of mercury in younger individuals it was approximately six-times lower (5.8 mg/kg DM). Srebocan et al. (2007) mentioned that with the increasing age the concentration of Hg in the organism of bluefin tuna (*Thunnus thynnus*) increased as well. Goutner et al. (2001) did not find significant correlation of mercury levels in feathers of squacco heron (*Ardeola ralloides*) with age. We did not find any literature data on Hg accumulation in chickens.

The objective of this research was to determine the differences in toxic dynamics of mercury in the body of chickens with different measuring point.

The results obtained indicate relatively low levels of mercury content in the examined tissues at all measuring points compared to the control point of measurement.

Influence of age on the retention of total Hg was investigated in laying- and meat-type chickens. To evaluate the effect of age as exactly as possible, it is necessary to carry out a great number of estimations within very short time intervals during a longer period of life.

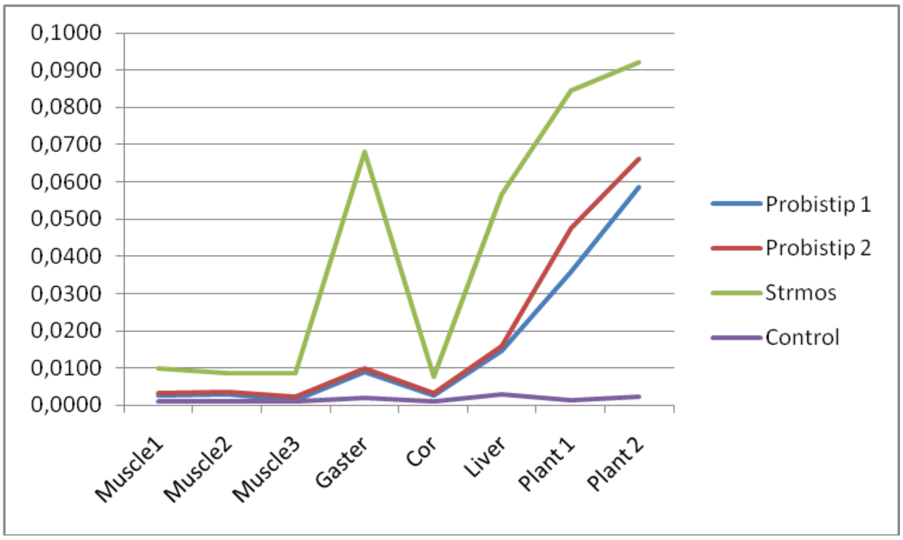

Figure 4. Concentrations of mercury (Hg) in organs of chicken ($mg \cdot kg^{-1}$ fresh mass)

CONCLUSION

1. The results of research on the content of lead, cadmium, arsenic and mercury in muscle 1, muscle 2, muscle 3, liver, and cor, gaster, show a variation of values in a relatively wide range between respective measurement sites. In terms of research, the highest values were obtained in the stomach and liver, and lowest values were measured in heart tissue.
2. Relatively high values of all tested elements - lead, cadmium, mercury and arsenic were measured in muscle tissue of chickens in Probistip and Strmosh areas. Muscle tissue is most consumed (chicken meat) by man and therefore should not be used in the diet. All this is very harmful to human health and can have serious consequences upon future generations.
3. These significantly high concentrations of lead in the stomach and liver in the industrial zone of Probishtip and the vicinity of the village Strmosh suggest that an appeal to the population should be made to avoid the consumption of these organs.
4. At the control point of measuring the lead content in all examined tissues from the organs of chickens is significantly low compared to the industrial zone of Probishtip and the vicinity of the village Strmosh.
5. All the above mentioned facts require greater commitment to remediate the harmful effects of heavy metals on the environment and man.

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