Title: The Measurement of Cadmium, Zinc and Silver in Chicken Meat in Isfahan Province, Iran

Running Title: Cadmium, zinc and silver in chicken meat

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ABSTRACT

Background: One of the concerns of consumers of animal protein sources is the residues of heavy metals in meat. The accumulation of these elements in meat and the consumption by humans can cause acute and chronic disorders in the function of vital organs.

Methods: For the estimation of cadmium, zinc and silver in the poultry meat products, 100 samples of chicken breasts, thighs, hearts and livers were collected from meat markets in Isfahan province. The tissues were digested and passed through filter paper, and the cadmium, zinc and silver contents were measured by an atomic absorption spectrophotometer.

Results: The mean contents of cadmium in the chicken thigh, breast, liver and heart samples were 0.055 ± 0.21, 0.048 ± 0.023, 0.074 ± 0.031, and 0.012 ± 0.004 mg/kg, respectively. The mean contents of zinc in the chicken thigh, breast, liver and heart samples were 17.19 ± 8.10, 15.70 ± 5.75, 22.10 ± 10.87, and 19.70 ± 10.38 mg/kg, respectively. The concentrations of cadmium and zinc were below the international permissible limits in all samples. The mean concentrations of silver in some chicken thigh, breast, liver and heart samples were 0.0080 ± 0.0044, 0.0048 ± 0.0013, 0.012 ± 0.009 and 0.0036 ± 0.006, respectively. Of note, silver was not detected in most chicken samples.

Conclusions: This study did not find cadmium, zinc and silver in the chicken meat samples above the international permissible limits. However, frequent monitoring for heavy metals in poultry meat sold in public markets is warranted to prevent their transmission to the human food chain.

Keywords: Chicken meat, Cadmium, Zinc, Silver, Toxic contaminations
INTRODUCTION

Nowadays, due to increases in environmental contaminants and the possibility of transmitting them to the human food chains, there is a growing demand for the marketing and consumption of healthy food products. The side effects of heavy metals on human health have been proven. Due to the toxicity of heavy metals, they can cause numerous diseases in humans and animals even at trace concentrations [1]. Humans and animals can be contaminated with heavy metals through inhalation, drinking, skin contact, or consuming contaminated foods [1]. Sources of heavy metals are usually contaminated water, soil, and air; however, foods can be the most likely source of heavy metals transmission to humans due to environmental pollutions and exposure to industrial waste products [2].

Heavy metals accumulate in water, soil and foods, such as vegetables, grains, fruits, fish, shellfish and most importantly, meats, causing major health problems, including cancers and even death. Studies have shown that some heavy metals, such as mercury and lead, play a major role in the development of autoimmune diseases, such as rheumatoid arthritis and disorders of the liver, kidneys, blood and nervous systems [3]. Cadmium, zinc and silver are among the heavy metals that can be transmitted to the human body via contaminated foods. Further, these metals can accumulate in such vital organs as liver, heart, kidneys and muscles as a result of long-term consumption of contaminated foods, leading to acute or chronic disease processes [4].

The public consumption of poultry meat is usually higher than red meat due to its protein, low fat and cholesterol contents, and low market prices [5]. Poultry diets usually contain agricultural products and other animal proteins, which can be contaminated with a variety of heavy metals and organophosphate compounds. Also, fish and oysters in contaminated waters can be the sources of heavy metals, which may be transmitted to poultry foods by adding fish powder to them as sources of protein and calcium [2]. Therefore, monitoring human food sources for a variety of toxic heavy metals is of vital importance to safeguard the public health. For this reason, the current study was conducted to evaluate and measure the cadmium, zinc and silver contents in the chicken meat and major organs.

MATERIALS & METHODS

Sampling: One hundred fresh chicken meat samples (50 breasts & thighs) and 100 samples of chicken heart and liver (50 each) were collected from various public markets in Isfahan province.

Measurement of Heavy Metals: The concentrations of cadmium, zinc and silver were determined based on the wet weight of the samples [6]. Specimens were collected separately in polyethylene bags and transferred to the laboratory. To digest the tissue samples, 200 mg of each sample was mixed with an equal volume of nitric acid (65%) and hydrogen peroxide (35%) and incubated at 160°C for 4 hours to complete dissolution. After cooling the mixture and centrifugation, the top solution was filtered through Whatman paper No. 1. The filtered solution was then diluted in 10 ml of double sterile distilled water. The cadmium, zinc and silver contents were measured for each sample with an atomic absorption spectrophotometer (Perkin Elmer 900, Akron, OH, USA). The standard solutions of cadmium, zinc and silver (Sigma, USA) were diluted with distilled water at appropriate concentrations (1000mg/L).
Statistical Analyses: The mean concentrations of the heavy metals for different organs were statistically analyzed, using SPSS, version 16.0, statistical software (Chicago, IL) and one-way analysis of variance (ANOVA) method. The statistical differences were analyzed by Tukey’s test at a confidence level of $P<0.05$.

RESULTS

The highest contents of cadmium, zinc and silver, as detected in the liver samples, were 0.270, 42.0 and 0.60 mg/kg, respectively. Although silver and cadmium were not found in most tissue samples, zinc was detected in all of them.

Cadmium Content: The mean cadmium contents in the samples of thigh, breast, liver, and heart were 0.055±0.21, 0.048±0.023, 0.074±0.031 and 0.012±0.004 mg/kg, respectively. The highest and lowest cadmium concentrations were found in the liver and heart, respectively, were statistically different ($P<0.05$) between the two tissues and the samples from other tissues (Table 1).

Table 1: The cadmium, zinc and silver contents of chicken breast, thigh, heart and liver samples.

<table>
<thead>
<tr>
<th>Heavy Metal (mg/kg)</th>
<th>Liver</th>
<th>Thigh</th>
<th>Breast</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium</td>
<td>0.074±0.031</td>
<td>0.055±0.021</td>
<td>0.048±0.023</td>
<td>0.012±0.004</td>
</tr>
<tr>
<td>Zinc</td>
<td>22.10±10.87</td>
<td>17.90±8.10</td>
<td>15.7±5.75</td>
<td>19.70±10.38</td>
</tr>
<tr>
<td>Silver</td>
<td>0.036±0.006</td>
<td>0.0080±0.0044</td>
<td>0.0048±0.0013</td>
<td>0.012±0.009</td>
</tr>
</tbody>
</table>

Zinc Content: The mean zinc contents in the samples of thigh, breast, liver, and heart were 17.9±8.10, 15.7±5.75, 22.10±10.87 and 19.7±10.38 mg/kg, respectively. The highest zinc content was found in the liver; the difference was statistically significant ($P<0.05$) compared to those found in other organ samples (Table 1).

Silver Content: The mean silver contents in the thigh, breast, liver, and heart samples were 0.0080±0.0044, 0.0048±0.0013, 0.036±0.006 and 0.012±0.009 mg/kg, respectively. The highest and lowest concentrations of silver were found in the liver and breast, respectively, which were statistically different ($P<0.05$) between the two tissues and other organ samples (Table 1).

DISCUSSION

In principle, heavy metals, such as cadmium, are not present in the body at birth, since these elements are not necessary but harmful to the body. It is important to check the amounts of toxic heavy metals in meat and other human food chains and draw strategies to prevent them from entering the human body via contaminated foods. These elements cannot be decomposed during the cooking process and accumulate in the body after consumption of the contaminated products, causing pathologic conditions, including kidney dysfunction, skeletal damages and reproductive defects [7].
In this study, the cadmium contents in the analyzed samples ranged from zero to 0.27 mg/kg. The amount of cadmium in the heart tissue was trace, with the lowest being 0.122 mg/kg. The highest amount of cadmium found in the liver was 0.074 mg/kg. The mean amount of cadmium in the chicken breasts and thighs were 0.048 and 0.055 mg/kg, respectively, which were less than the international limits of 0.5 mg/kg permissible by FAO and WHO [13]. To date, there is little information known about the levels of heavy metals in poultry meats consumed in Iran.

In 2015, Sadeghi et al. [8] reported cadmium levels in chickens supplied in Mashhad at approximately 2 to 3 mg/kg. Sinkakarimi, et al. [13] showed cadmium levels in the breast at 0.0099 mg/kg and in the liver at 0.004 mg/kg. Ismail and Abolghai [9] examined the lead and cadmium levels in the internal organs of poultry in Ismailia, Egypt. They reported a mean cadmium concentration of 0.40 mg/kg in the liver samples. The mean cadmium and lead contents in the chicken samples collected from different locations in Spain have been reported to be 3.16 and 4.15 mg/kg, respectively [10]. The differences in the concentrations of cadmium reported by earlier studies might be due to varying amounts of cadmium present in chicken feeds used in poultry farming worldwide [11]. Also, the amount of cadmium in the poultry meat increases with the increasing the chickens age [11]. In a recent study, the highest levels of cadmium and other heavy metals found in chicken liver samples were consistent, mostly reported by older studies [9, 12]. Sinkakarimi, et al. [13] examined the presence of heavy metals in chickens in Sanandaj province, Iran, and reported the highest amounts of zinc, lead and cadmium in the liver samples. However, in another experimental study, the highest amount of cadmium was found in the kidneys [14], which was not evaluated in the current study. It is likely that the high blood circulation through the liver and the high metabolic activity can play a major role in the accumulation of heavy metals in the liver compared to that in other organs [9].

Zinc is one of the essential nutrients in the body for optimal immune function and is involved in the activity of more than 300 enzymes [15]. Also, zinc plays a pivotal role in the strength of protein structures by activating enzymes involved in protein metabolism and carbon dioxide transfer [1]. Zinc deficiency causes anorexia, anemia, slow wound healing, growth retardation, diarrhea, hair loss and skin rashes [15, 16]. Further, food sources with the right amount of zinc meet the dietary standard for this essential element. In our study, the concentration of zinc in the meet samples ranged between zero and 46 mg/kg. The lowest zinc concentration was found in the breast samples (6 mg/kg) and the highest in the liver samples (42 mg/kg). The mean level of zinc in the breast, thigh, liver, and heart samples ranged between 15.70±5.75 mg/kg (breast) and 22.10±10.87 mg/kg (liver), reflecting significant differences among these organs. A previous study conducted by Barik and Piscac [17] reported a normal zinc concentration in chicken meat samples at 35 and 45ppm. In our study, the zinc concentrations in the breast and thigh samples were between 6 and 24mg/kg, which were lower than those reported previously. In addition, Salisbury and Chan [18] reported that the zinc concentrations in the chicken meat and internal organs, such as liver and kidneys, were between 23 and 147 ppm. In our study, although the concentration of zinc in the chicken liver was higher than that in the chicken meat, no more than 42 mg/kg of this metal was observed. However, our results were very similar to the findings reported by another study [19]. In that study, the researchers reported the zinc levels in their chicken meat to be between 6.12 to 33.21 mg/kg. In our samples, we found the zinc contents ranged from 6.83 to 24.91 (breasts) and from 12.91 to 31.57 mg/kg (thighs).
In recent years, nano-silver compounds have been used for a variety of purposes in the poultry industry. Some of the most widely used compounds in this category are nano-silver-based disinfectants [20, 21]. These compounds are commonly used even in poultry farms that claim to produce healthy products devoid of antibiotics to prevent infectious diseases in chickens. Some poultry producers utilize these compounds to disinfect drinking water, grains, and spaces where chickens are kept. Since the application of these compounds in the poultry industry, there have been concerns about their indiscriminate use, which may lead to the accumulation of silver ions in the chicken meat and other organs, and the transmission to human food chains. Therefore, the assessment and monitoring of the silver content in the poultry products are warranted. Although there has been no published study on silver residues in poultry meat and organs, some studies have investigated the accumulation of silver ions in various animal organ tissues. Sharma, et al. [22] showed that feeding nano-silver compounds to chickens can accumulate in their tissues. In that study, silver was found in the liver, kidneys, spleen, and blood of chickens that were fed silver-contained supplements. In another study by Kim, et al. [23] in mice it was demonstrated that silver ions could be stored in the testicles, kidneys, liver, brain, lungs, stomach and blood, with the highest amount being found in the liver. Also, Sung, et al. [24] have reported that liver and lung tissues are the target of silver accumulation in experimental animals.

Fondevila, et al. [25] showed that in addition to the accumulation of silver ions in the liver, it is also stored in the muscles. They documented these results after 5 weeks of feeding nano-silver compounds to broiler chickens, observing that the amount of nano-silver deposited in the tissues depended on its dosage. Also, silver has been detected even in the control chickens, likely due to the overuse of these compounds in poultry farms. In one study, nano-silver ions were detected in liver, lung, and kidneys following the intravenous administration of the compound. However, the silver ions were cleared in all tissues except for the lungs after 3 days [26]. Ahmadi, et al. [27] have shown that consumption of 900 mg/kg of silver nano-particles in broiler chickens can lead to necrotic spots in the liver of some birds. Another study has reported necrosis of hepatocytes following the use of silver nano-particles [28]. Free radicals from silver nano-particles appear to affect hepatocytes and the urea cycle [29].

Previous studies have shown that nano-silver particles can be absorbed and metabolized through soft tissues, causing pathological defects in them. However, in numerous poultry farms nano-silver compounds are only sprayed to disinfect the spaces and reduce the microbial growth. These compounds are now used less in water and chicken feeds. However, according to two recent studies, the application of these compounds even on the mucosa can be absorbed and affect the chickens [30, 31]. Finally, the current accumulation of silver ions in meat and other edible tissues of chickens supplied in Isfahan, is very minimal. However, it is important to perform repeated monitoring of poultry products using sensitive and accurate techniques, such as inductively coupled plasma mass spectrometry, to ensure the consumers’ safety.

**Recommendations for Future Research:** Given our findings, further research is warranted on the safe concentration and use of nano-silver-based disinfectants in poultry industry versus the accumulation of silver in chicken meat.
CONCLUSIONS

The results of this study show that cadmium, silver and zinc ions can accumulate in meat and other edible tissues of broiler chickens, even though the amounts were less than the permissible limits in most of the samples. Since, the toxic and non-toxic heavy metals can accumulate in human body and cause pathologic changes, observing the standard principles for growing and processing of poultry feeds, and regular monitoring of heavy metals in poultry products are essential to the health of human consumers.

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Ethical statement:
This study was performed on ready-to-cook chicken meat in Isfahan province and no part of the study was performed on live chickens. The study protocol was approved by the Ethics Committee, Islamic Azad University, Shahrekord, Iran (Registered #: IR.iau.shk.1399.110).

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Conflict of Interests:
The authors declared no conflict of interest with any internal or external entity in conducting this study.

Authors’ Contributions:
M. Gholami designed the study and laboratory procedures. A. Ahmadi assisted with the laboratory experiments. S. Azizi wrote and revised the initial and final drafts of the manuscript. All authors reviewed and approved the final manuscript.
REFERENCES


