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Assessment of Heavy Metals and Estrogen Content in Liver of Chicken from Industrial Farms in Ilam, Iran

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ABSTRACT

Introduction: This study aimed to measure the amount of estrogen and heavy metals (cadmium, mercury, lead, and arsenic) in the liver of industrial chickens from the selected areas of Ilam City, Iran.

Materials & Methods: This study is a cross-sectional study conducted on 30 liver tissue samples randomly selected from poultry farms in Ilam, Iran. The concentrations of the heavy metals (mg/kg) and 17-beta estradiol (ppb) were measured using atomic absorption spectrometer (AAS) and high-performance liquid chromatography (HPLC), respectively. The SPSS software version 21 was used for the data analysis.

Results: The mean level of 17-beta estradiol was 3.578 ± 0.756 ppb compared to the standard limit of 0.24 ppb ($p < 0.05$). The mean level of arsenic was 0.942 ± 0.842 mg/kg versus the standard limit of 0.1 mg/kg versus the standard limit of 0.24 ppb ($p < 0.05$). The mean concentrations of lead, mercury, and cadmium were 0.03±0.03, 0.11±0.13, and 0.015±0.02 mg/kg, respectively, and the differences were not significant compared to the standard values (all p values >0.05). The results showed no correlation between mean heavy metal levels and 17-beta estradiol. The target hazard quotient for arsenic was calculated to be 1.33.

Conclusion: The results show that the average levels of 17-beta estradiol and arsenic in the samples were higher than the standard limit. This may threaten the general health of residents in the area.

Keywords: Chicken, Estrogen, Heavy metal, Lead, Mercury, Cadmium, Arsenic

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Introduction

In recent decades, the consumption of poultry meat among animal protein sources has increased more than ever due to the low price of these products compared to red meat and seafood products (1). Driven by the growing demand for these products, the poultry industry is constantly developing worldwide to maximize production at minimal cost to producers (2-4). The production is highly dependent on factors such as feed formulation, veterinary drugs, and synthetic hormones that support animal growth, prevent disease, and improve final product yield (5, 6).

The history of the use of steroid hormones in agriculture dates back several decades. Estradiol 17-β, as a natural hormone, is used alone or in growth-promoting cocktails with some active compounds at low concentrations to accelerate muscle growth. The use of estrogen is beneficial for poultry farmers; however, the consumption of these products, which contain varying levels of hormonal residues, can cause serious health problems for consumers (7-9). Since 1988, they have not been authorized in the European Union (EU) and have been banned in some other countries after their negative effects on human health were proven (7). Excessive consumption causes symptoms such as nausea, vomiting, anorexia, and mental depression and can also lead to asthma, endometriosis, fibroids, heart and liver diseases, and even cancer (10-13). In fact, estrogen exposure is recognized as the most important risk factor for breast cancer in women and a contributing factor in prostate cancer in men (8, 9).

Accordingly, the National Toxicology Program, the U.S. government, and the National Institute of Environmental Health Sciences have added it to the list of known human carcinogens (14, 15). In addition, the development of the poultry industry requires sustainable protein sources to provide animal feed. For this purpose, some producers use seafood by-products. Despite having basic nutritional components, these products can contain heavy metals such as mercury, lead, cadmium, or arsenic, which can affect the chemical safety of poultry products (16, 17).

Heavy metals are toxic, widespread, and persistent elements in ecosystems, known to induce oxidative stress and carcinogenesis (18, 19). Mercury, lead, and cadmium, even at low concentrations, are highly toxic and pose a serious threat to human health. Their adverse effects on the kidneys, nerves, immune system, and reproduction make them important indicators of chemical contaminants in food (20). In addition, cadmium and lead can bind to many proteins, alter their activity, disrupt enzymatic processes, and cause disease (21). Metalloid arsenic is another important neurotoxicant found in drinking water and food sources with carcinogenic potential. Adverse effects of arsenic exposure include muscle weakness, decreased appetite, nausea, mucosal inflammation, and skin lesions (22).

According to current knowledge, a diet containing meat and other animal products provides the highest human exposure to heavy metals (19). Therefore, given the importance of poultry meat in the human diet and the possibility of chemical contamination of these products, monitoring their safety levels is essential. In this regard, the present study investigated the levels of heavy metals and estrogen in the liver of industrial chickens produced in Ilam, Iran.

Materials and methods

Study Design

This was an analytic cross-sectional study.

Setting and Participants

The study was conducted on 30 liver tissue samples of chickens collected from Ilam, a small city in Iran.

Sample Size

The minimum sample size was calculated as 30 samples with a power of (1-β) of 95% and a significance level of α = 0.05. For this purpose, three chickens from 10 poultry farms (out of 64 farms in the city) were randomly selected. In the sample farms, the chickens that were to be sent to the slaughterhouse were randomly selected.

Measurements & Validity and Reliability

Chicken liver tissue was measured for heavy metal concentrations including mercury, arsenic, lead, cadmium, and 17β-estradiol levels.

All chickens were 40 days old. Feed composition was usually a combination of corn, soybean meal, salt, fish powder, and soybean oil.

The liver tissue was taken and placed in a clean polythene bag. Then, the samples were transported to the pharmacology research center of Ilam Medical School.

Chemical reagents (of analytical reagent grade) used in this study, including standard solutions of Cd, Pb, As, and 17β-estradiol, 1000 ± 5 mg L^{-1} solution, SRM from NIST, $Cd(NO_3)_2$ in HNO_3 0.5 mol L^{-1}, $Pb(NO_3)_2$ in HNO_3 0.5 mol L^{-1}, $As(NO_3)_2$ in HNO_3 0.5 mol L^{-1}, and methanol, were purchased from Merck Company, Germany. Deionized water (Milli-Q Millipore 18.2 MΩ cm^{-1} resistivity) was used in the chemical modifier solution preparation (1% mg L^{-1}).

The concentration of heavy metals, including mercury, arsenic, lead, and cadmium, was determined based on the wet weight of the samples. In detail, a quantity of 100 mg of the test sample was mixed with an equal volume (1:1) of nitric acid (65%) and hydrogen peroxide (35%) and incubated at 160°C for 4 hours for digestion. After cooling, the solution was vigorously shaken and diluted with 10 ml of sterile distilled water. Finally, Cd, Pb, Hg, and As concentrations in prepared solutions were measured by an atomic absorption spectrophotometer (Perkin-Elmer Analyst 800 atomic absorption spectrometer, including an AS-800 Autosampler equipped with Zeeman-effect background correction) at 228.8 (23), 283.3 (24), 253.7 (24), and 193.7 nm (25), respectively. The limit of detection and the limit of quantitation of the atomic absorption device were 0.08 ppm and 0.15 ppm, respectively.

To quantify the estrogen 17β-estradiol levels, 10 ml of methanol and water in the ratio of 8:2 were added to 0.5 g of chicken liver sample. After that, the samples were mixed with a shaker for 10 minutes and were then centrifuged at 10000 g for 10 min at 4°C.

After centrifugation, the mixture was separated into two phases. The upper phase was completely dried by passing the N_2 gas through, and the residue was re-dissolved in 0.5 ml of methanol, and 4 ml of water was added. For each sample, approximately 100 µL of the solution was injected into the HPLC device (Waters 2487, Autosampler Waters 717, a controller with gradient pumps, and a diode array (PDA) detector) to calculate the 17β-estradiol concentration.

The maximum standard limit of 17β-estradiol was considered 0.24 ppb as in accordance with reference number (1), and the standard levels of mercury, lead, arsenic, and cadmium were set as References (26, 27) and the COMMISSION REGULATION (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/200 (28).

Statistical and Data Analysis

Data were analyzed using SPSS-21 software (Chicago, Illinois, USA). All hypothesis tests were two-tailed, and $P < 0.05$ indicated a statistical significance level. Data analysis was performed using a one-sample t-test, and the mean levels of heavy metals and estrogen were compared with standard values. Also, the values of each farm were compared separately with the standard value. Pearson correlation analysis was used to find the relationship between the levels of the measured items in chicken liver.

Results

TTable 1 summarizes the mean concentrations (mg/kg) and the samples' minimum and maximum levels of the studied heavy metals compared with the international standard values. The results in Table 2 present the values obtained for 17β-estradiol in the collected samples.

Table 1. Amounts of heavy metals measured in the liver of chickens and their standard limits.

Heavy metal	Min /Max (mg/kg)	mean ± SD (mg/kg)	Standard limit (mg/kg)	95% Confidence Interval	P value
lead	0.005 /0.174	0.031 ± 0.028	0.10	-0.079 - -0.058	<0.001
mercury	0.004 /0.748	0.106 ± 0.129	0.10	-0.041 – 0.053	0.793
cadmium	0.009 /0.119	0.015 ±0.020	0.30	-0.291- -0.277	<0.001
arsenic	0.642 /1.041	0.942 ±0.842	0.10	0.639- 1.05	<0.001

Heavy metal measurement unit: mg/kg, Min: minimum, Max: Maximum, SD: standard deviation

Table 2. The average concentrations and the minimum and maximum amounts of 17β-estradiol in the samples.

Variable	Min /Max (ppb)	mean ± SD (ppb)	Standard value (ppb)	95% Confidence Interval	P value
17β-estradiol	1.650 /5.000)	3.578 ±0.756	0.24	3.05- 3.62	<0.0001

Estrogen measurement unit: ppb, Min: minimum, Max: Maximum, SD: standard deviation

The results showed that the average levels of arsenic and 17β-estradiol were significantly higher than the standard limit. The average levels of cadmium, lead, and mercury metals were within the standard permissible limits. As shown in Figure 1, the average concentration of estrogen in chicken liver was more than 15 times the standard level, and in the case of arsenic, 9.5 times the standard level.

Data analysis also conducted a baseline human health risk assessment for arsenic. This can be effectively achieved by calculating the estimated daily intake (EDI) and the target hazard quotient (THQ). For arsenic at an average concentration of 0.94 mg/kg, the EDI for a 70 kg adult consuming 0.03 kg of liver per day would be:

EDI = (0.94 mg/kg × 0.03 kg) / 70 kg = 0.0004 mg/kg body weight per day. Comparing this value with the reference dose (RfD) for inorganic arsenic (0.0003 mg/kg body weight per day, US EPA) results in THQ = 0.0004 / 0.0003 ≈ 1.33 (moderate risk).

To find the correlation between heavy metals and estrogen in chicken liver, we performed a correlation analysis, the results of which showed no correlation (all $p>0.05$).

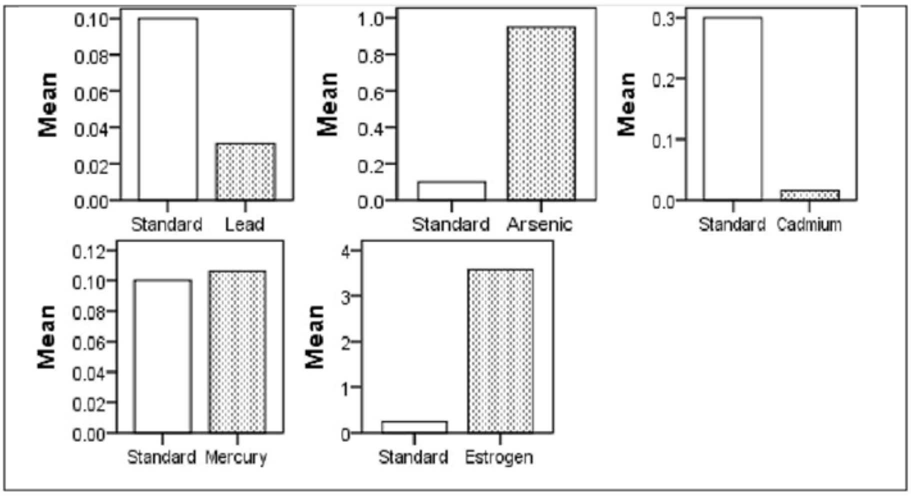

Figure 1. Mean levels of heavy metals and estrogen in chicken liver compared to standard level.

Discussion

The results of our study showed that the levels of arsenic and 17β-estradiol in chicken liver samples were higher than the standard values. The levels of cadmium, lead, and mercury were lower than the standard values. Although the standard value of estrogen is set at 0.24 ppb, the average level of estrogen detected in our samples was 3.578 ± 0.756 ppb, which was significantly higher compared to the standard level. Chicken meat consumption has increased significantly in most parts of the world and is expected to increase in low-income developing countries in the coming decades. Poultry meat and eggs provide high-quality protein in addition to important vitamins and minerals (29). Poultry meat has also become an important and growing component of meat demand in Iran. This has led to the growth of the poultry industry in the region. 17β-estradiol is known to be the main estrogen involved in the reproductive function of avian species. It also supports improved growth rates in industrial poultry. In previous studies, the use of oral contraceptives has been shown to improve growth rates in farm animals and lead to the formation of high estrogen residues in muscle and liver (31). In a comparative study on the estimation of estrogen and progesterone residue levels, it was found that estrogen levels in the liver were significantly higher than in muscle compared with controls (32). However, little change was observed in the progesterone residues of liver and muscle tissues, indicating that estrogen is the dominant sex steroid that acts directly on the tissues. The use of steroid hormones is currently illegal worldwide, including in the United States, Europe, and Iran. Contrary to popular belief, no country officially uses hormones in broiler chickens to accelerate growth. In our study, the diet given to the chickens on the farm consisted of a mixture of corn, soybean meal, salt, fish meal, and oils such as soybean oil. Soy contains phytoestrogens, which are plant compounds that mimic the action of estrogen in the body. The main isoflavones found in soy are genistein and daidzein, which can have weak estrogen-like effects. Although soy does not contain true estrogen, its phytoestrogens may affect estrogen levels. Several clinical studies have shown that consuming up to 50 grams of soy daily does not cause adverse effects in humans (33). However, on the farm, very large amounts of soybean meal are used in the daily diet of the chickens. It is possible that the high serum estradiol levels in the study samples were related to the excessive intake of soy in the chickens' diets.

In addition, nowadays, ensuring food safety has become challenging due to heavy metals in food, especially chicken meat and its products. Cadmium, lead, mercury, and arsenic are among the most important heavy metals in food, which pose great risks to consumers (1). Considering the importance of their risks, in the present study, chicken livers distributed in Ilam City, Iran, were examined in terms of heavy metal levels and compared with international standard levels.

The results showed that the mean arsenic level was 0.94 mg/kg, which was significantly higher than the standard level of 0.1. The lowest and highest arsenic levels were 0.64 and 1.04 mg/kg, respectively. This indicates that the animals were exposed to arsenic in their natural environment. Previous studies have shown that variable arsenic concentrations in animal tissues depend on the amount of arsenic consumed in the diet, the rate of absorption, and the homeostatic mechanisms controlling arsenic in the body (35). Arsenic poisoning in drinking water and feed is considered the main source of increased arsenic in animal tissues (34). As the animal ages and arsenic accumulates, increased arsenic in tissues is observed (36-38). In a 2020 study, Nejati et al. reported arsenic concentrations in the liver of chickens obtained from different farms in Shiraz as 0.017 mg/kg (41), which was lower than the international standard. This discrepancy is likely due to the different arsenic contamination in local environments in Iran. Similar to our results, in a recent study conducted in Bangladesh, the mean arsenic content in the liver of three different breeds of broiler, layer, and rooster chickens was 0.943, 0.241, and 0.379 mg/kg, respectively, indicating values higher than the arsenic standard limit in that region (39). In the present study, arsenic values were higher than the standard value in all 10 sampled farms. On the other hand, the THQ value of arsenic was 1.33. A THQ value greater than 1 provides direct and quantitative evidence of potential non-carcinogenic health risks. This is a major concern. As a result, the water from these farms should be tested for arsenic levels.

The results showed that the cadmium content of the liver samples studied ranged from 0.119 to 0.009 mg/kg with an overall mean of 0.015, which did not exceed the standard value of 0.3 mg/kg set by the Food and Drug Administration and the World Health Organization (FAO/WHO). This value was significantly lower than the standard value in all sampled farms (except farm 7, which did not differ significantly). Our results are consistent with previous studies conducted in Sanandaj, Isfahan, and Shiraz (3, 40-41). Furthermore, in the analysis of trace metal content of poultry products in Turkey, cadmium content was found to be 0.25 to 6.09 µg/kg (42). In our study, the mean lead concentration in the samples was 0.031 mg/kg, which was significantly lower than the standard limit of 0.1 mg/kg and similar to the results of Nejati et al. in Shiraz (41). This value was significantly lower than the standard value in all sampled farms (except farm 2, which was not significantly different).

Mercury is another known toxic element that accumulates in the tissues of living organisms and has the potential to affect the food chain. Mercury can be easily absorbed by the human body from the gastrointestinal tract and widely distributed to the brain, causing damage to the central nervous system. Furthermore, mercury concentrations exceeding the permissible limit (30 µg/kg) in food and animal feed may lead to serious health problems such as vision loss, hearing loss, and mental retardation (43). In the present study, the mean mercury level was 0.11 mg/kg, which was not statistically different from the standard value (0.10 mg/kg). This value was also not significantly different from the standard value in any of the 10 sampled farms.

Limitations and Strengths

This study was the first in Ilam to investigate the concentrations of heavy metals and estrogen in the liver of poultry farms in Ilam. In addition, we calculated the target risk ratio (a basic assessment of human health risk), which provides the quantitative evidence needed to substantiate this important conclusion. Our study had limitations. Due to budget constraints, the sample size was small. It would also have been better to analyze the feed and water used by the poultry.

Conclusion

The results showed the average concentration of estrogen in chicken liver was more than 15 times the standard level, and in the case of arsenic, 9.5 times the standard level. Since there is no evidence of estrogen consumption in chickens, it is recommended to conduct frequent quality control assessments on the composition of feed and drinking water provided to chickens. Also, educational classes should be held for poultry farmers, and the serious risks and complications that water and food given to chickens can cause to human health should be explained. It seems that regulatory agencies should be more vigilant and follow the poultry industry more closely.

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CRediT authorship contribution statement

Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Resources, Software, Data Curation, Writing–Original Draft Preparation, Writing–Review & Editing, Visualization, Supervision, Project Administration: KM, NA, ZVS, SS.

Declaration of Generative AI and AI-assisted technologies in the writing process

No AI writing assistance was utilized in the production of this manuscript.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper. The authors confirm that the manuscript is original research that has not been published and is not under consideration elsewhere.

Ethical considerations

This study was approved by the Ethical Committee of Ilam University of Medical Sciences, Ilam, Iran with the code of IR.MEDILAM.REC.1399.323.

Data availability statement

Data will not be made available in a public repository as we have not obtained ethical clearance to share data publicly. However, on request from corresponding author data could be provided while maintaining anonymity.

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