

Existence and control of some heavy metals in chicken breast meat and giblets

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ABSTRACT

Heavy metals are toxic residues that exert harmful effects because of their accumulation in the biological tissues. This study aimed to investigate the fitness of chicken breast meat, gizzard and liver for the human consumption in relation to their heavy metal residues. In addition, biodegradable effect of *Lactobacillus rhamnosus* on the heavy metals concentrations in chicken fillet samples was assessed. Lead, cadmium and arsenic concentrations were investigated in a total of ninety random samples of raw chilled chicken meat, gizzard and liver (30 of each), which were collected from poultry slaughter shops in Benha city, Qalubiya governorate, Egypt by the atomic absorption technique. Furthermore, *in vivo* degradable effect of *L. rhamnosus* on heavy metal residues in chicken fillet was investigated during refrigeration storage. Liver samples had significant higher levels of heavy metals than gizzard and meat, with the incidences of 56.7%, 46.7% and 16.7% for lead, cadmium and arsenic, respectively. Moreover, 28.9%, 24.4% and 28.9% of the examined samples were unfit for human consumption based on their heavy metal contents, and in accordance with the respective Egyptian standards. In addition, *L. rhamnosus* had a significant reduction effect on arsenic, cadmium and lead with the reduction (%) of 56, 71.5 and 82.6% at the end of the experiment, respectively. Accordingly, regular investigation of heavy metals levels in commercial meat products and their feed stuffs is recommended. Furthermore, *L. rhamnosus* showed promising diminishing effect on heavy metal accumulation in meat products, where advanced research on its effect on the environment is recommended.

Introduction

The commercial poultry business is well-developed around the world, and it is the greatest supply of animal protein as well as a good source of vital amino acids, vitamins, and minerals for human consumption in the form of meat and eggs. Its importance is magnified in under-developed nations because chicken is relatively cheap and can be reared in a small area, typically offering a good source of high quality and most appealing animal protein due to its high meat production, little cooking loss, and low cost. Although chicken meat has an economic advantage over beef meat, it may contain heavy metals and other elements that are naturally found in air, water, soil, and poultry diet, which is mainly reach it as a result of the human industrial and agricultural activities (Alagawany *et al.*, 2020).

Mercury, lead, cadmium, tin, and arsenic are metals of particular concern in terms of their negative effects on health and are often referred to as "heavy metals." These metals' toxicity is owing in part to the fact that they accumulate in living tissues, a process known as bioaccumulation. Metal bioaccumulation occurs in all living food animals and humans because of metal exposure in food and the environment. The harmful effects of these metals include damage to the central and peripheral nervous systems, the gastrointestinal and genital systems, hepatic and renal toxicity, immunodeficiency, and carcinogenesis (Kortei *et al.*, 2020; Jalali *et al.*, 2023).

Heavy metal pollution has become one of the most important global environmental issues. The human health risk posed by heavy metals encountered through the food chain and occupational and environmental exposure is increasing, resulting in a series of serious diseases. Ingested heavy metals might disturb the function of the gut barrier and cause toxicity to the extra-intestinal organs or tissues. Probiotics, including lactic

acid bacteria (LAB), can be used as an alternative strategy to detoxify heavy metals in the host body due to their safety and effectiveness (Wang *et al.*, 2023). Because of their ability to cling to or bind distinct metallic compounds, lactic acid bacteria (LAB) have been shown in numerous studies to play an important role in the body's immunity, detoxification, and removal of heavy metal residues (Petrova *et al.*, 2022). Previous research by Samir *et al.* (2021) demonstrated the potential of *Lactobacillus rhamnosus* to chelate several additional harmful chemicals from food, including aflatoxin B1 and mutagens.

In view of the presented facts, the current study was performed in order to determine the concentration of some of the most health hazardous heavy metals in chicken meat and edible giblets; followed by investigation of the biodegradability of *L. rhamnosus* on the selected heavy metals in chicken fillet model.

Materials and methods

Collection of samples

A total of 90 random samples of chicken breast meat, gizzard and liver (30 of each) were collected from poultry slaughter shops in Benha city, Qalubiya governorate, Egypt. The collected samples were transferred to the laboratory for determination of their residues of heavy metals (lead, cadmium and arsenic).

Determination of heavy metals

The basis of wet weight was used for determination of lead, cadmium, and arsenic (mg/Kg) in the examined samples.

Washing procedures of the used equipment were performed accord-

ing to AOAC (2006) using deionized water and hot diluted HNO_3 (10%) for 24 hours and then dried to ascertain that all the equipment were metal free.

Digestion technique was performed according to Tsoumboris and Papodoulou (1994) using a digestion mixture of 60 ml of 65% Nitric acid + 40 ml of 70% perchloric acid in a screw capped tube with vigorous mixing, followed by filtration of the digested sample with Whatman filter paper No. 42. The filtrates were collected in Pyrex glass test tubes until analyzed for their lead, cadmium and arsenic concentrations.

Blank and standard solutions were prepared according to Shibamoto and Bjeldanes (1993). Blank solution consisted of 10 parts of nitric acid and 1 part of H_2O_2 then was diluted with 25 parts of deionized water and filtered. Furthermore, the standard solutions using pure certified metal standards at different strengths were prepared by 10 parts of nitric acid and 1 part of H_2O_2 then was diluted with 25 parts of deionized water and filtrated.

Analysis of heavy metal concentrations in the examined samples was conducted using Flame Atomic Absorption Spectrophotometer (VARIAN, Australia, model AA240 FS) and analyzed for lead, cadmium and arsenic concentrations.

Quantitative determination of heavy metal residues

The absorbance of lead, cadmium and arsenic was recorded directly from the digital scale of AAS and their concentrations were calculated according to the following equation: $C = R \times (D/W)$

Where, C=concentration of lead (mg/kg) wet weight, R=reading of digital scale of AAS, D= Dilution of prepared sample, W= Weight of the sample.

N. B. The concentration of each heavy metal in the blank solution was also calculated and subtracted from each analyzed sample.

Experimental part (effect of *Lactobacillus rhamnosus* on the concentrations of lead, cadmium and arsenic which were experimentally inoculated into chicken fillets samples).

Preparation of bacterial suspension to harvest a 10^7 CFU/ml bacterial suspension was performed according to Halttunen et al. (2007).

Binding assay

The bacterial pellet (10^7 CFU/ml), 50 mg/Kg ionic lead, 20 mg/Kg ionic cadmium, and 10 mg/Kg of arsenic solutions were mixed with chicken fillet samples according to Halttunen et al. (2008).

Experimental design

Chicken fillet samples were experimentally inoculated with metals was served as a control positive group (G1), the test groups represented chicken fillets inoculated with *L. rhamnosus* (10^7 CFU/g) and lead (50 mg/kg) (G2), chicken fillets inoculated with *L. rhamnosus* (10^7 CFU/g) and cadmium (20 mg/kg) (G3), and chicken fillets inoculated with *L. rhamnosus* (10^7 CFU/g) and arsenic (10 mg/kg) (G4) were served as treated groups. The samples were examined at specific time points for each metal by atomic absorption spectrophotometer.

Statistical analysis

Using the SPSS program for Windows, version 16.0, for expressing heavy metal concentrations as the mean \pm SE. The results were analyzed statistically using one-way analysis of variance (ANOVA) with Duncan comparison tests.

$$\text{Reduction rate (\%)} = (B-A)/A \times 100$$

B = mean value of the next heavy metal level.

A = mean value of the previous heavy metal level.

Results

Referring to the recorded results in Tables 1, 3 and 5, liver samples had a significant higher levels of heavy metals accumulation with the incidences of 56.7%, 46.7% and 16.7%, with the mean values (mg/kg) of 0.54, 0.39 and 0.15 for the examined lead, cadmium and arsenic, respectively; followed by gizzard and meat samples, respectively. Moreover, out of the total of the examined samples, 28.9%, 24.4% and 28.9% were unfit for human consumption in relation to the lead, cadmium and arsenic levels, and in accordance with the respective Egyptian standards following the recorded data in Tables 2, 4 and 6.

Table 1. Incidence and levels of lead (mg/Kg) in the examined samples of chicken carcasses (n=30).

Chicken samples	+ve samples		Min	Max	Mean \pm S.E
	No	%			
Meat	13	43.3	0.01	0.33	0.16 \pm 0.01 ^c
Gizzard	14	46.7	0.02	0.57	0.31 \pm 0.01 ^b
Liver	17	56.7	0.04	0.95	0.54 \pm 0.01 ^a

*Means with different superscript letters in the same column are significantly different (P<0.05).

Table 2. Fitness of the examined chicken samples based on their levels of lead (n=30).

Chicken samples	MRL (mg/Kg)*	Fit samples		Unfit samples	
		No.	%	No.	%
Meat	0.1	24	80	6	20
Gizzard	0.1	21	70	9	30
Liver	0.1	19	63.3	11	36.7
Total (90)		64	71.1	26	28.9

* Maximum Residual Limit (EOS, 2010).

Table 3. Incidence and levels of cadmium (mg/Kg) in the examined samples of chicken carcasses (n=30).

Chicken samples	+ve samples		Min	Max	Mean \pm S.E
	No	%			
Meat	9	30	0.01	0.24	0.10 \pm 0.01 ^c
Gizzard	12	40	0.01	0.38	0.22 \pm 0.01 ^b
Liver	14	46.7	0.02	0.72	0.39 \pm 0.01 ^a

*Means with different superscript letters in the same column are significantly different (P<0.05).

Table 4. Fitness of the examined chicken samples based on their levels of cadmium (n=30).

Chicken samples	MRL (mg/Kg)*	Fit samples		Unfit samples	
		No.	%	No.	%
Meat	0.05	26	86.7	4	13.3
Gizzard	0.05	22	73.3	8	26.7
Liver	0.05	20	66.7	10	33.3
Total (90)		68	75.6	22	24.4

* Maximum Residual Limit (EOS, 2010).

An experimental study was conducted for investigation the biodegradable effect of *L. rhamnosus* on the examined heavy metals, where it revealed significant reduction values of the injected lead, cadmium and arsenic with a reduction percent (%) of 82.6, 71.5 and 56.0% at the end of the experimental period, respectively as was recorded in Figures 1, 2 and 3.

Table 5. Incidence and levels of arsenic (mg/Kg) in the examined samples of chicken carcasses (n=30).

Chicken samples	+ve samples		Min	Max	Mean ± S.E
	No	%			
Meat	2	6.7	0.01	0.07	0.04 ± 0.01 ^C
Gizzard	4	13.3	0.01	0.16	0.09 ± 0.01 ^B
Liver	5	16.7	0.03	0.25	0.15 ± 0.01 ^A

*Means with different superscript letters in the same column are significantly different (P<0.05).

Table 6. Fitness of the examined chicken samples based on their levels of arsenic (n=30).

Chicken samples	MRL (mg/Kg)*	Fit samples		Unfit samples	
		No.	%	No.	%
Meat	0.05	29	96.7	1	3.3
Gizzard	0.05	27	90	3	10
Liver	0.05	26	86.7	4	13.3
Total (90)		82	71.1	8	28.9

* Global Agricultural Information Network "GAIN" (2015)

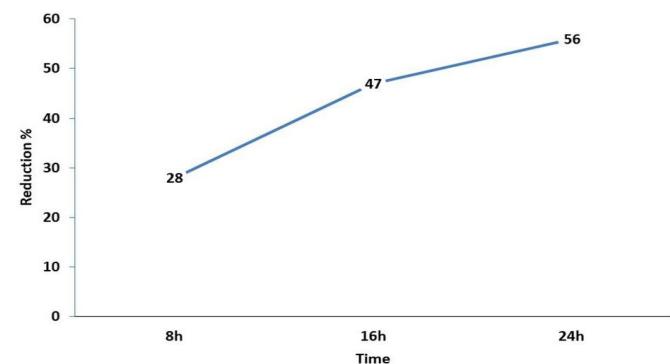


Fig. 1. Reduction % of arsenic concentration in the examined fillet samples.

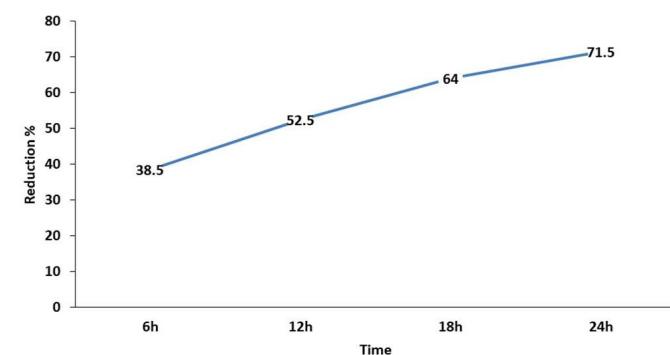


Fig. 2. Reduction % of cadmium concentration in the examined fillet samples.

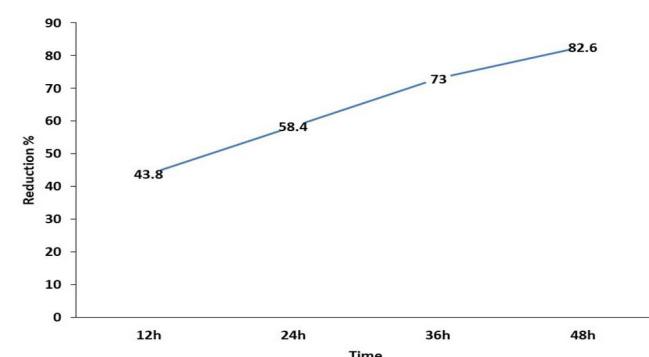


Fig. 3. Reduction % of lead concentration in the examined fillet samples.

Discussion

The mean values of heavy metal residual levels in the examined samples revealed significantly ($P \leq 0.05$) higher concentrations in liver samples than gizzard and meat, respectively; where, lead was the mostly detected metal with the highest concentrations and existence in the examined samples.

Lead has been shown to bio-accumulate in human tissues and organs, particularly the liver and bones, resulting in a variety of illnesses. In the human body, absorbed lead has a biologic half-life of roughly 27 years in bone. Lead encephalopathy in children is characterized by irritability, ataxia, convulsion, and altered state of consciousness, but lead toxicity in adults results in neuropathy, which causes wrist and foot drop. Other disorders such as hemolytic anemia, atherosclerosis, liver apoptosis, renal toxicity, and ovarian shrinkage may occur (Gasmi et al., 2022; WHO, 2022).

The obtained results of lead concentrations (mg/kg) came lower than those previously recorded by Korish and Attia (2020) (16.51 in meat samples), Morshdy et al. (2022) (0.73 in chicken meat samples, and Kamaly and Sharkawy (2023) (2.57 and 0.146 in chicken meat and liver samples, respectively). While the obtained results of cadmium in the examined samples came in the same line with those recorded results of Hassanin et al. (2014) (0.11 in chicken meat samples) and Abbas (2017) (0.07 and 0.05 in liver and meat, respectively); while, were higher than those obtained by Kamaly and Sharkawy (2023) (0.05 and 0.1 mg/kg in meat and liver samples, respectively).

Cadmium appears to be a non-essential element that is lacking at birth, but accumulates in diverse tissues with aging, a process known as tissue specific bioaccumulation. Cadmium causes severe respiratory symptoms, renal failure (nephrotoxicity, glycosuria, aminoaciduria, and decreased glomerular filtration rate), hypertension, liver injury, and lung damage. Furthermore, a teratogenic dose of Cadmium chloride caused considerable changes in detoxifying enzymes in the liver and kidneys. Also, cadmium causes osteoporosis and osteomalacia, which is referred to as Itai-Itai illness (CDC, 2012).

Arsenic is a naturally occurring element in the earth's crust and is widely spread throughout the environment, including the air, water, and land. Long-term arsenic exposure from polluted drinking water and foods is typically seen in the skin as pigmentation changes, skin lesions, and hyperkeratosis. Cancers of the bladder and lungs are also possible. Developmental impacts, diabetes, lung disease, and cardiovascular disease are among the other negative health outcomes (WHO, 2022).

Referring to the current obtained results of arsenic residual levels in the examined samples, similar results were recorded by Liu et al. (2016). Other investigators obtained some lower arsenic concentrations such as Mariam et al. (2004), Akan et al. (2010).

An experimental study was conducted to investigate the arsenic, cadmium and lead levels degradation under the effect of *L. rhamnosus* in chicken fillet samples. Results were recorded in Figures (1 to 3) and showed a significant diminishing effect on arsenic, cadmium and lead levels with reduction rates of 56%, 71.5%, and 82.6%, respectively.

The degradation potential of *L. rhamnosus* was previously recorded by Samir et al. (2021) who recorded a significant reduction in lead and cadmium levels after interaction with *L. rhamnosus* for 24h in refrigeration storage, with reduction % of 84.3 and 72%, respectively. Moreover, potential significant reduction in the prevalence of genotoxicity and hepatotoxicity rat model by cadmium after inoculation of *L. rhamnosus* was recorded by Jama et al. (2012).

The diminishing interaction effect of LAB with heavy metals can be attributed to its ability to bind with the metals intra- or extracellularly by bio-sorption the passive no metabolically mediated binding process of metal ions to the LAB cell wall, so preventing any harmful interaction in the host cell (Mrvicic et al., 2012).

Conclusion

L. rhamnosus is of a great value in reducing the heavy metal load, particularly for arsenic, cadmium, and lead.

Conflict of interest

The authors declare that they have no conflict of interest.

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