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BIOAVAILABILITY OF HEAVY METALS (Pb AND Cd) IN WILD ROE DEER MEAT

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Abstract

Intense concern in recent years for quantitative and toxicological identification and evaluation, especially for heavy metals from animal origin products represent a side that needs to be studied continuously, especially for game meat, nutritional toxicity of those mineral elements (mainly Pb and Cd) being directly influenced by their variations in habitat water, air and soil. The goal of the study is motivated by the inexistence of information regarding appreciation of contamination degree with heavy metals of game meat, having in view the limited checkout of feeding of game animals exploited in their natural environment, as well as the polluting environmental factors. The analytical results regarding the quantitative evaluation of the Cd concentration of the muscle samples indicate that the average values found in male carcasses are higher than those in female carcasses, except the Trapezius cervicalis muscle. The average concentration of Cd residues ranged from 0.020 ± 0.0028 to 0.040 ± 0.017 mg/kg DM, both limits being attributed to the Triceps brachii muscles. The range of averages corresponding to the other muscle groups are described by an amplitude of the variation of 0.018 mg/kg DM.

***Key words**: Roe deer, toxicology, xenobiotic.*

INTRODUCTION

Heavy metals are components naturally present in the environment, having a dual role in this regard. Although low levels of some of them have been considered essential for animal health and growth, having multiple functions in the body (Theron et al., 2012; Govind & Madhuri, 2014; Neila et al., 2017), intensification of human activities and complexity modern industries have led to a continuous increase in their environmental concentrations, with important ecotoxicological effects on wildlife and implicitly on human health (Perez-Lopez et al., 2016).

Even as essential elements for maintaining physiological processes, some heavy metals can be dangerous to the matrices of the ecosystem by their presence in excessive concentrations, so that numerous studies have highlighted their particular toxicity for the entire food chain.

According to Baker et al. (2003), given their structural complexity and their properties, the transfer of metallic elements can be genuinely done from the environment to the biota, being favoured by different parameters. Therefore, considering their persistence and the peculiar capacity for bioaccumulation in trophic elements, concerns about them have been arisen due to their expression within the food chain structure.

Ali et al. (2019) carried out reviews in this subject on various peculiarities of heavy metals, namely their persistence in the environment, toxicity to living organisms and bio-cumulative potential for all elements of the food chain, in this way, highlighting the characteristics of each compound and the subsequent physiological mechanisms developed by the body for their removal.

The animal organism is not exposed to the action of a single chemical substance, but a whole series of chemical compounds present in food, water and air. Each chemical element in the group of heavy metals has its specificity as a way of presentation, manifestation, level of contamination and toxicity on the health of consumers.

According to Aprile & De Bellis (2020), metallic contaminants bind to cellular structures in the human body, mainly affecting the performance of essential biological functions. The toxic effects caused by heavy metals are enhanced by the level of exposure to the source (Wang & Fowler, 2008), being materialized mainly by changes in physiological processes, combined with several other important disorders in cardiovascular or bone systems (Bouchard et al., 2009). In the same context, Ismail et al. (2017) described the toxic impact on human health and the prevalence level of different metals (Cd, Pb, As, Hg, Ni, Co and Cu) assessed by researchers in food matrices. Since the animal organism functions as an absorbent of all these compounds (Aftab et al., 2011; Ismail et al., 2014), through this study, high proportions of them were found in animal products, especially meat and milk.

To assess the degree of contamination with heavy metals, as well as to quantify their impact, a key element could be their transfer to animals and subsequently, through their production, to humans. Mammals such as wild boar (*Sus scrofa*), deer (*Capreolus capreolus*), foxes (*Vulpes vulpes*) or red deer (*Cervus elaphus*) can be excellent bioindicators of heavy metal contamination, as well as for the concentrations in which they are found in the environment (Srebocan et al., 2011; Bakowska et al., 2016; Friends et al., 2012).

From the environment, the transfer of heavy metals to animal tissues takes place mainly through the digestive tract, mainly due to the intake of contaminated feedstuffs (Latif et al., 2013); in this point of view, for wild animals, food has a particular role (Bakowska et al., 2016). Exposure of plant matter, part of wildlife feed to the many substances with potential environmental pollutants, makes it difficult to manage the generated risks, especially to quantify the diversity of ecosystem contaminants (Voda et al., 2019).

Concerning game animals, assessments on their tissues can provide a relevant perspective on the degree of environmental pollution. Given the specificity of game animals and their living in a free environment, the presence of high levels of toxic elements in their tissues may be a genuine indication for quantifying pollution threats to human health (Amici et al., 2012). The assessment of heavy metals in different tissues can be performed through various techniques, adapted to a specific analyte or desired level of detection. Thus, the concentrations identified in the evaluated matrices could serve to evaluate more accurately the humans' potential exposure to heavy metals and several prevention and reduction actions could be implemented (Demirel et al., 2008; Kazi et al., 2009).

The study of game populations in certain environments that could potentially be altered or damaged by anthropogenic activities provides relevant information on the viability and balance of these ecosystems. Knowledge on the degree of diffusion of heavy metals in soil, water and air, as well as into animal tissues and organs is an important aspect to be taken into account when the safety of game meat products is assessed (Danieli et al., 2012).

Studies related to the proportions of heavy metals identified in tissues of game mammals sampled from heavily polluted areas, specified levels of 1.6-2.1 mg/100 g Pb and 1.6 to 3.0 and 10 mg/100 g Cd, particularly in certain internal organs, such as liver and kidney. In slightly polluted areas, the concentrations ranged between 0.1-0.5 mg/100 g Cd or within the 0.4-1 mg/100 g limits for Pb (Rajaganapathy et al., 2011). For other game species, Florijancic et al. (2015) evaluated the heavy metals content in wild boars muscles and found concentrations of 0.0107-0.0209 mg Pb/100 g, of 0.1425-0.1799 mg Cd/100 g, as well as lower concentrations of Hg (0.0041-0.0097 mg/100 g) or As (0.0130-0.0158 mg/100 g live weight). Similarly, Durkalec et al. (2015) studied the level of bioaccumulation of Pb, Cd and Hg in the muscle tissues of deer and wild boars in areas with different levels of toxic metal pollution and found average concentrations of 0.07-0.43 mg Cd /100 g and of 0.12-0.57 mg Pb /100 g. Mitrănescu et al. (2011) investigated the occurrence of Cd and Pb residues in game meat and found average values of 0.254 mg Cd/100 g and 2.079 mg Pb/100 g, within the maximal limits specified by the Regulation (EC) 1881/2006 establishing maximum levels for certain contaminants in foodstuffs.

The intense concern in recent years for the identification, quantification and toxicological evaluation of heavy metals in animal products is an aspect that needs to be studied continuously, especially for game meat, because their nutritional toxicity is directly influenced by variations of their proportions in water, air and soil habitat. In animals, the accumulation of heavy metals depends on their concentration in feed, on the duration of exposure or on the age of the animal (Pokorny et al., 2000; Taggart et al., 2011; Lehel et al., 2015; Giżejewska et al., 2017; Pilarczyk et al., 2020).

Given that game species are known as bioindicators for heavy metal toxicity (Millan et al., 2008; Perez-Lopez et al., 2016), both in terms of their distribution areas and in terms of feeding specificity (Schley & Roper, 2003; Baubet et al., 2004), they can be used in various biomonitoring studies (Froslie et al., 2001).

MATERIALS AND METHODS

The purpose of the research is motivated by the lack of information on the assessment of the degree of heavy metal contamination of game meat, taking into account the limited control of the diet of game exploited in its natural environment, as well as other environmental pollutants.

To achieve the proposed goal, investigations were performed on a total of 11 adult individuals of deer (*Capreolus capreolus* L.), out of which 6 males and 5 females. Four muscle areas were taken in the analysis (*Longissimus dorsi*, *Semitendinosus*, *Triceps brachii*, *Trapezius cervicalis*). The individuals were harvested in hunting parties organized in the N ÷ E area of Romania (Suceava Forestry Department, 24 Frasin Hunting Fund) in the hunting seasons 2018-2019 and 2019-2020, according to the provisions of Law no. 407/2006 amended and supplemented by G.E.O no. 102/2010. Analysis of the metal content of the lyophilized muscle tissue samples was performed by the atomic absorption spectrometric method (AAS) on a GBC-AVANTA type atomic absorption spectrometer, provided with burners for flame analysis and a gas source. The principle of the method consists in calcining the samples dissolving the ash in HCl and evaporating to dryness the obtained solution. The final residue is redissolved in 0.1 mol/L nitric acid and the metal content is determined by the atomic absorption method, according to RS EN 14082/2003. The working method included the separation of metal cations from muscle samples (the stage of transition of metals into the ionic state) and their actual determination (instrumental analysis by spectrometer), working simultaneously in duplicate each muscle sample analyzed. The separation of the metals consists of the calcination operation of the biological samples of lyophilized muscle tissue, a process that determined the passage of the metals in ionic or ionizable form. This process allowed the global gravimetric evaluation of the mineral content, because the calcination residue contains all the mineral substances in the form of salts.

After complete calcination of the muscle tissue sample, 5 mL HCl (6 mol/L) was added. The resulting mixture was evaporated in a sea bath and subsequently dissolved in a set volume of HNO3 (0.1 mol/L). The melting pot with the resulting solution was left to stand up for 2 hours, after which, the solution was transferred to a plastic container. The solutions thus prepared were introduced into the apparatus to be read from the constructed calibration curve.

The construction of the calibration curve initially involved the achievement of the optimal operating parameters, provided in the device manual. This step was followed by the successive measurement of the absorbance of each standard solution. The data were statistically processed to achieve the main descriptors (mean, standard mean error, variation coefficient) and were subsequently introduced to analysis of variance, for males vs. females comparisons, using the ANOVA single factor algorithm, under the GraphPad Prism 8.0 statistical analysis software for Windows.

RESULTS AND DISCUSSIONS

Cd is a microelement that is part of the heavy metal group, specifically the seventh most toxic heavy metal (Jaishankar et al., 2014). Rarely present in nature, it is considered one of the most noxious elements, along with Pb, Hg and As. On the other hand, Pb is also considered a metal compound with high toxicity, the sources of contamination, in this case, being more frequent.

The main criteria for which heavy metals are considered dangerous are bioaccumulation, toxicity and persistence, which are considered "risk elements", with toxic effects on both animals and humans. Contamination of animal tissues with Cd occurs as its transmission from the soil, air or water to heavily industrialized areas. The statistical estimators of the values attributed to the Cd and Pb concentration defined an extended dispersion of the data compared to the average, with limits of the coefficient of variation of 45.97 ÷ 114.79% for Cd (Table 1), respectively 82.97 ÷ 162.45% for Pb (Table 2).

The analytical results of the Cd concentration in muscles indicate higher concentration of this element in males samples, in comparison with the females ones, the single exception occurring in *Trapezius cervicalis* samples. For the analyzed muscles, the average concentration of Cd residues showed values in the range 0.020 ± 0.0028 to 0.040 ± 0.017 mg/kg DM, both limits reached in the *Triceps brachii* muscles, the range of averages corresponding to the other muscle groups being described by an amplitude of the variation of 0.018 mg/kg DM.

Compared to the maximum allowed limit of 0.05 mg/kg, regulated for the farm animals meat (EC Reg. No. 1881/2006), the average Cd concentration in game meat was lower.

Table 1. Cd content (mg/kg DM) in deer meat (males and females)

	Muscles	Gender	$\bar{x} \pm s_{\bar{x}}$	V%	Min.-Max.	The significance of the differences (Males vs. Females)
Cd	LD	M	**0.031**±0.017	71.685	0.008 – 0.062	$\hat{F} = 0.475$; $F_{0.05}(1;19) = 4.38$; $\hat{F} < F_{0.05} \Rightarrow$ **ns**
		F	**0.022**±0.012	91.957	0.001 – 0.048	
	ST	M	**0.034**±0.008	65.825	0.009 – 0.056	$\hat{F} = 0.578$; $F_{0.05}(1;19) = 4.38$; $\hat{F} < F_{0.05} \Rightarrow$ **ns**
		F	**0.023**±0.015	114.795	0.001 – 0.067	
	TB	M	**0.040**±0.017	45.976	0.02 – 0.060	$\hat{F} = 2.697$; $F_{0.05}(1;19) = 4.38$; $\hat{F} < F_{0.05} \Rightarrow$ **ns**
		F	**0.020**±0.028	75.458	0.009 – 0.041	
	TC	M	**0.027**±0.024	79.309	0.005 – 0.062	$\hat{F} = 0.215$; $F_{0.05}(1;19) = 4.38$; $\hat{F} < F_{0.05} \Rightarrow$ **ns**
		F	**0.036**±0.013	62.421	0.018 – 0.070	
MAL* = 0.05 mg/kg (ppm)						

[1]**LD** = m. *Longissimus dorsi,* [2]**ST** = m. *Semitendinosus,* [3]**TB** = m. *Triceps brachii,* [4]**TC** = m. *Trapezius cervicalis;*
[5]**M** – males, [6]**F** – females, [7]**MAL*** = maximum allowed limit Reg. EC no. 1881/2006.

Table 2. Pb content (mg/kg DM) in deer meat (males and females)

	Muscles	Gender	$\bar{x} \pm s_{\bar{x}}$	V%	Min.-Max.	The significance of the differences (Males vs. Females)
Pb	LD	M	**0.045**±0.017	82.971	0.003 – 0.084	$\hat{F} = 0.275$; $F_{0.05}(1;19) = 4.38$; $\hat{F} < F_{0.05} \Rightarrow$ **ns**
		F	**0.070**±0.0275	85.781	0.003 – 0.13	
	ST	M	**0.051**±0.028	126.784	0.005 – 0.16	$\hat{F} = 0.549$; $F_{0.05}(1;19) = 4.38$; $\hat{F} < F_{0.05} \Rightarrow$ **ns**
		F	**0.025**±0.017	162.453	0.004 – 0.101	
	TB	M	**0.037**±0.023	128.596	0.001 – 0.112	$\hat{F} = 0.054$; $F_{0.05}(1;19) = 4.38$; $\hat{F} < F_{0.05} \Rightarrow$ **ns**
		F	**0.044**±0.024	114.585	0.009 – 0.137	
	TC	M	**0.041**±0.025	144.57	0.001 – 0.133	$\hat{F} = 0.075$; $F_{0.05}(1;19) = 4.38$; $\hat{F} < F_{0.05} \Rightarrow$ **ns**
		F	**0.053**±0.031	132.142	0.006 – 0.165	
MAL* = 0.1 mg/kg (ppm)						

[1]**LD** = m. *Longissimus dorsi,* [2]**ST** = m. *Semitendinosus,* [3]**TB** = m. *Triceps brachii,* [4]**TC** = m. *Trapezius cervicalis;*
[5]**M** – males, [6]**F** – females, [7]**MAL*** = maximum allowed limit Reg. EC no. 1881/2006.

Lead is another chemical element with a metallic character. In small quantities, it does not have an essential function for plants or animals even though it is naturally and permanently found in the body tissues. As it is not a constituent of living matter, Pb is considered a non-essential element in food. Lead is a toxicant with a certain aggressiveness towards living organisms, being found on the list of carcinogenic elements. Quantitatively, the *Longissimus dorsi*, *Triceps brachii* and *Trapezius cervicalis* muscles in carcasses expressed higher average Pb concentration than in males. In the shoulder muscles, the values were quite reversed, and the maximum difference between genders was attributed to the shoulder muscles (Table 2).

The statistically processed data indicated the average value obtained within the interval 0.025 ± 0.017 (m. *Semitendinosus*) to 0.07 ± 0.027 mg/kg DM (m. *Longissimus dorsi*), the amplitude of variation reaching 0.034 mg/kg DM. The statistical significance of the differences between males and females for the values of Cd and residual Pb concentrations was tested through the ANOVA dispersed unifactorial analysis. Thus, for both pollutant heavy metals, the samples did not differ significantly between genders. For both elements, cadmium and lead, the differences between males and females for each type of muscle were analyzed, respectively for m. *Triceps brachii*, m. *Trapezius cervicalis*, m. *Longissimus dorsi* and m. *Semitendinosus*, were 100% statistically insignificant.

CONCLUSIONS

From a toxicological point of view, the analyzes confirmed the presence of heavy xenobiotic metals in deer meat (Cd and Pb), while the average concentrations were LMA compliant, ranging between 0.020 ÷ 0.040 mg/kg DM for Cd and 0.025 ÷ 0.07 mg/kg DM for Pb. Despite the identified proportions, the sources of exposure to heavy metal contamination of wild animals could not be easily quantified, thus highlighting the outstanding persistence and storage capacity of these xenobiotics in the environment. Between the sexes, the differences for the cadmium and lead concentration values were insignificant, for the four analyzed muscle areas.

Although the data obtained are a direct consequence of their bioaccumulation, their low level does not endanger the health of animals, and consequently of the consumer.

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*** RS EN 14082/2003 Food products. Determination of microelements. Determination of lead, cadmium, zinc, copper, iron and chromium by atomic absorption spectrometry (SSA) after calcination.