Scientific Works. Series C. Veterinary Medicine. Vol. LXVI (2), 2020
ISSN 2065-1295; ISSN 2343-9394 (CD-ROM); ISSN 2067-3663 (Online); ISSN-L 2065-1295

RESEARCHES REGARDING THE CONCENTRATIONS OF HEAVY METALS IN GAME MEAT (DEER AND WILD BOAR)

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Abstract

The purpose of this article is to contribute to the subsequent establishment by the European Commission of maximum admitted limits for heavy metals in game meat. Heavy metals residues (Lead, Cadmium) are present in game meat because of the environmental excessive pollution and because of the hunting methods. Game meat samples (deer and wild boar meat) from Harghita county were analysed by: atomic absorption spectrophotometry. The results were compared with other categories of meat for which the maximum admitted limits are specified in the Regulation EC no. 1881/2006. The results were below the maximum admitted limits for other types of meat for which there is a specific European legislation, as following: cadmium concentration was highly below the highest admitted limit for meat categories. However for some samples, lead concentrations were comparable to the maximum admitted limit. Consequently, repeated analyses were performed to validate the results. The high quantities of lead residues could be explained by the hunting methods, but also by the samples collection close to the wounds produced by firearms. The hunting methods and the place for sampling too close to the wound caused by firearms (including the bullets) are responsible for finding high concentrations of residual lead in the analysed samples.

Key words: *lead, cadmium, game meat, atomic absorption, Regulation EC*

INTRODUCTION

Hunting is practiced by humans since the Palaeolithic era in order to obtain their food. At the same time, it is a conscious activity that exploits a renewable natural resource.
The game meat is an ever-expanding food alternative, due to its high nutritional properties, particular taste and interest in the livestock industry and the consumer's exploitation of hunting resources at the industrial level.
Therefore, the control and expertise of the quality of game meat is an important link in ensuring food security at national and international level, taking into account the density of the food supply, the traditional specificity and the effective functioning of the market (Stewart et al., 2011).
The game meat started to become very popular in Romania since the mid-2000s, when it appeared in hypermarkets or restaurants with specific hunting characteristics in some tourist areas. Seen at first perhaps with distrust, the hunting meat caught the attention of nutritionists very quickly, who appreciated its qualities and the important role it played in the diets of those who want to have a healthy lifestyle.
In addition, game meat is rich in vitamins A and D, zinc and iron, Omega 3, Omega 6, vitamins B1, B2, B3, B5, B7, B9 and B12 (which we must take from the diet, because our body does not process them naturally), calcium, potassium, magnesium etc. Very important is the fact that the game meat is high in proteins and low in fats. (Müller-Graf et al., 2017)

Table 1. Chemical composition of game meat (Popa G., Stănescu V., 1981)

Species	The constituent elements (%)			
	Water	*Proteins*	*Lipids*	*Mineral salts*
Wild boar	72.55	20.08	6.63	1.10
Deer	75.76	20.55	1.92	1.13

Some important things about game meat:
- The hunting meat comes from animals and birds raised close to their natural habitat (it could not happen otherwise, because hunting leads to completely different living conditions than farmed animals).

- The hunting meat is not treated with antibiotics or growth hormones.
- Wild game meat comes from strictly legally controlled hunting grounds - which is why animals feed on natural grass and fodder (Müller-Graf et al., 2017).

Concerning game meat, there is a continuous concern for the identification and quantitative and toxicological evaluation especially of heavy metals, the nutritional toxicity of these mineral elements (especially Pb, Cd, Hg) being directly influenced by their varying concentrations in water, air and habitat soil. In animals, the accumulation of heavy metals depends on the concentration in their food, the duration of exposure or the age of the animal.

Regarding the environmental excessive pollution in the last years, this is the main reason for the increasing the presence of chemical residues, including heavy metals, in game meat (Haldimann et al., 2002).

The hunting meat is recognized for its more intense coloration (Figures 1 and 2). Its variations depending on the type of the musculature, the diet, the age of the animal or the method of conservation.

Figure 1. Wild boar meat - macroscopic aspect

Figure 2. Deer meat - macroscopic aspect

Heavy metal toxicity presents serious consequences in the human body. The most affected systems and organs are: the central nervous system, leading to mental disorders, the blood components, which may damage the liver, lungs, kidneys and other vital organs, leading to serious systemic diseases (ATSDR, 1999).

Lead poisoning can be chronic or acute disorder. Acute exposure of lead can cause headache, loss of appetite, abdominal pain, fatigue, sleeplessness, hallucinations, vertigo, renal dysfunction, hypertension and arthritis while chronic exposure can result in birth defects, mental retardation, autism, psychosis, allergies, paralysis, weight loss, dyslexia, hyperactivity, muscular weakness, kidney damage, brain damage, coma and may even cause death (ATSDR, 2007; Lanphear et al., 2005).

In evaluation of its toxicity in humans, it was found that bone to blood mobilization increases during pregnancy, lactation, physiological stress, chronic disease, along with advanced age (Gulson et al., 2003).

In wild animals, **cadmium** concentrates in liver and kidneys. The literature indicates that in highly polluted areas, there is a Pb and Cd content of the mammalian organs (liver and kidney) of 20 and 16, respectively 30 and 100 mg/kg. In some low polluted areas, the values are between 1-5 mg/kg for Cd, respectively 4-10 mg/kg for lead (EFSA, 2010).

MATERIALS AND METHODS

For this article, there were analysed lead and cadmium concentrations from the following types of game meat: deer and wild boar (muscular tissue), coming from Harghita county, Romania.

In collected samples, lead and cadmium values were established by using the Graphite Furnace Atomic Absorption Spectrophotometry technique (Figures 3 and 4).

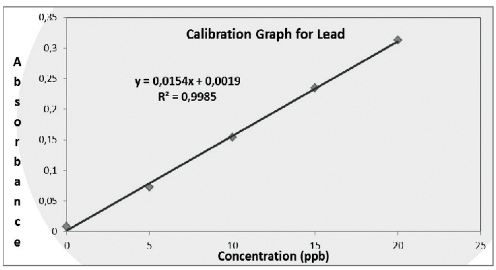

Figure 3. Calibration curve of lead performed by graphite furnace atomic absorption spectrometry

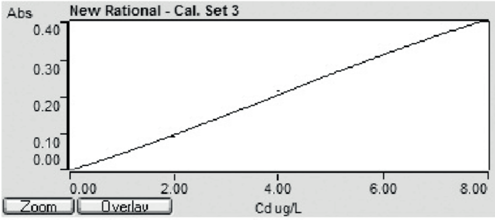

Figure 4. Calibration curve of cadmium by GFAAS

Processing the analyses

The stock solution of Cd of 1 µg/ml (1 ppm) is prepared from the concentrated solution (1000 mg/l), using nitric acid (0.1 mol/l).

The stock solution of Lead of 10 µg/ml (10 ppm) is prepared from concentrated solution (1000 mg/l), with nitric acid (0.1 mol/l).

From the stock solution of Cd (1 µg/ml), the calibration solution of 0.005 µg/ml is prepared using 0.5 ml of stock solution and HNO_3 0.1 mol/l.

From the stock solution (10 µg/ml) of Pb, the calibration solution with a concentration of 0.05 µg/ml is prepared using 0.5 ml of stock solution and 0.1 mol/l HNO_3.

Fresh calibration solutions will be prepared each day when making determinations on the Graphite Furnace Atomic Absorption Spectrophotometer.

Dry mineralization

The drying and calcination of the samples take place at an initial temperature of no more than 100 degrees Celsius.

Then, the temperature increases with a maximum speed of 50 degrees Celsius per hour up to 450 degrees Celsius and is allowed to the sample to stand overnight at this temperature.

If the sample is not completely burned, the ash is moistened with 1 ml to 3 ml deionized water or hydrogen peroxide, put the crucible back in the oven at no more than 200 degrees Celsius and gradually increase the temperature to 450 ± 10 degrees Celsius for 1-2 hours or more.

Repeat this operation until the sample is completely burned, which means that the entire ash is white/gray.

If there are black spots in the crucible that do not disappear after repeated burns, there is the possibility that they may actually be pieces of lead bullets, resulting at the time of the impact during the hunt. In this case, it is highly recommended to repeat the analysis (Trinogga et al., 2013).

Mineral processing

Add 5 ml HCl, conc. 6 mol/l so that all the ash comes in contact with the acid. The acid is evaporated on the sand bath. The residue is dissolved in 10 ml of 0.1 mol/l nitric acid. All ash must come in contact with the acid.

Allow the sample to stand from 1 hour to 2 hours. After this time, the solution is ready to be analysed.

In parallel, calibration solutions (cadmium of 0.005 µg/ml and lead 0.05 µg/ml), the matrix modifier (if applicable) are manually prepared.

The preparation of the calibration solutions for making the curve is automatically performed, the equipment is performing alone the dilutions prescribed in the calibration solution preparation program, indicating the concentration of the calibration solution used. To perform the calibration curve, the readings will be repeated until a correlation coefficient of minimum 0.990 is obtained.

For the analyses performed in this study, it was used the following matrix modifier: mixture of ammonium acid phosphate 1% and magnesium nitrate 0.1%.

All analyses were done in the Veterinary Sanitary and Food Safety Laboratory, Ploiesti, Prahova county. The interpretation of the results was done according to the current European legislation, more exactly to the Regulation EC no. 1881/2006.

This Regulation gives reference information regarding the maximum admitted limit of different contaminants in food products for bird meat, mutton, pork, lamb and beef.

RESULTS AND DISCUSSIONS

The obtained results have been related to the number of figures provided in the present regulations: Regulation (EC) 1881/2006 with subsequent amendments and completions.

For each reported result, the percentage of recovery obtained on the set of samples processed under the same conditions will be specified.

In the following table and charts there are presented details regarding average values of lead and cadmium concentrations found in muscular tissue samples of the game species.

Table 2. Distribution of deer and wild boar muscle samples (Lead, Cd) according to their concentrations

Sample	Sex		Age	Collection place	No. of samples	Assessed parameters	
						Lead (mg/kg)	Cd (mg/kg)
Deer	11 F	13 M	3.5-9 years	Harghita county	24	0.02 (min. conc., 10 samples) - 0.08 (max. conc., 6 samples)	0.001 (min. conc., 7 samples) - 0.03 (max. conc., 3 samples)
Wild boar	10 F	13 M	4-11 years	Harghita county	23	0.01 (min. conc., 5 samples) - 0.09 (max. conc. - 5 samples)	0.009 (min. conc., 8 samples) - 0.02 (max. conc., 3 samples)
Admitted limit according to EC Regulation no.1881/2006						0.100	0.050

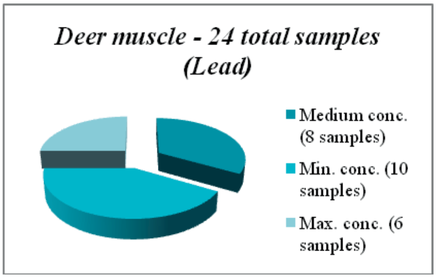

Chart 1. Distribution of deer muscle samples (Lead) according to their concentrations

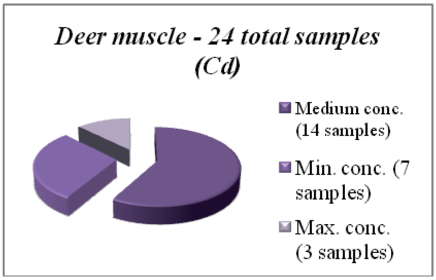

Chart 2. Distribution of deer muscle samples (Cd) according to their concentrations

As it can be seen in this table, all the analysed samples had lead and cadmium within the maximum allowed limits.
The values are within the ranges: wild boar 0.01-0.09 (lead), 0.009-0.02 (cadmium), deer 0.02-0.08(lead), 0.001-0.03 (cadmium).
The high values from these intervals are probably due to the bioaccumulation of heavy metals in the body. If the collection of the muscle tissue samples had taken place in the area near a wound obtained by shooting with lead bullets, the values of the lead would have far exceeded the maximum allowed limit.

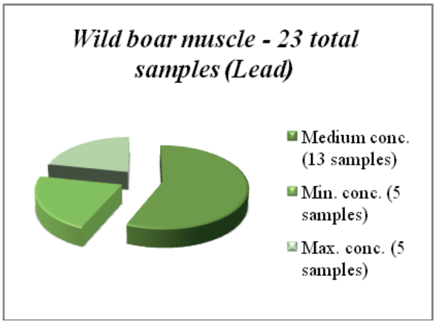

Chart 3. Distribution of wild boar muscle samples (Lead) according to their concentrations

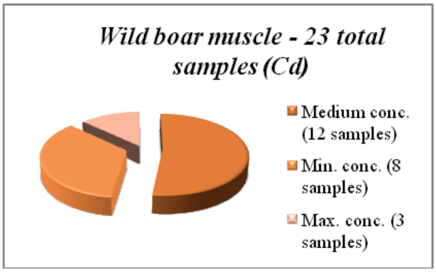

Chart 4. Distribution of wild boar muscle samples (Cd) according to their concentrations

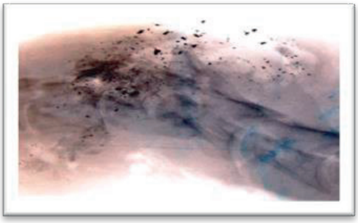

Figure 5. Lead bullet fragments - the dark spots

Figure 6. Lead bullet fragments removed from tissue

From this point of view, the sample collection was in conformity, the results expressing the value of the analyte given by the residual accumulation of the heavy metals.

According to the obtained results, the samples of wild game muscular tissue from wild boar and deer collected from the Harghita county fall within the maximum limits allowed by the European legislation on the field of muscular tissue.

CONCLUSIONS

• Lead and cadmium may occur in muscle tissue samples as a result of the residual bioaccumulation of these heavy metals. Regarding the lead, meat can also be contaminated with pieces of lead bullets, as a result of their dissemination at the time of the hunting impact.

• Lead and cadmium residues are present in normal range in the muscular tissue of the deer and wild bear in samples from Harghita county. There were not recorded overvalues for these analytes.

• The lack of a European regulation putting up the maximum permissible limits of lead and cadmium for the muscular tissue of these animal species is due to the deficient monitoring of the heavy metal residues in the meat and the food of these animals (Taggart et al., 2011).

• The capturing procedures are the main reason for getting lead at a value that exceeds very much the maximum permissible limit of this analyte regarding the muscle tissue.

• None of the analysed samples had results above the maximum permissible limit of lead or cadmium for the muscle tissue category.

• In case of exceeded results, it is necessary to repeat the analyses including a close examination of the muscular tissue at macroscopic level, before starting working protocol. It is necessary to make the difference between contamination with pieces of lead bullets and residual accumulation of heavy metals.

• For the analysis of each sample, the entire amount of sample was chopped and homogenized so that the harvesting for processing is done in the most objective way possible.

• Do not wash the sample before analysing it because the tissue fluid will be lost and it contains an important amount of analytes. Otherwise, the result is a wrong one (the dilution process occurs).

• The hunting meat is a complex matrix from the analytical process' point of view. Therefore, choosing the matrix modifier is very important to achieve an objective result. The final choice is a rigorous selection after several attempts.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Veterinary Sanitary and Food Safety Laboratory, Ploiesti, Prahova county.

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Figure 1 - www.roexpo.ro;

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Figure: 5,6 - www.huntingwithnonlead.org.

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