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Accumulation of Zinc, Copper, Cadmium and Lead in Liver and kidney of the Iberian Hare (*Lepus granatensis*) from Spain

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ABSTRACT

Knowledge of trace and toxic metal concentrations in wild animals is important for assessing the effects of contaminants in wild animal's health and also contaminant intakes by humans. Concentrations of lead, cadmium, zinc and copper in liver and kidney of Iberian hares in relation with the origin region, sex and age were investigated. Over a period of two years 65 hares, 30 males and 35 females were analyzed. For the trace elements, mean zinc levels ranged from 18.52 to 21.82 mg/kg wet tissue in kidney and 23.32 to 25.02 mg/kg wet tissue in liver. The corresponding mean copper concentrations ranged from 2.42 to 2.72 mg/kg wet tissue and from 2.94 to 3.08 mg/kg wet tissue in kidney and liver, respectively. For the toxic elements, mean cadmium levels ranged from 0.35 to 0.76 mg/kg wet tissue in kidney and 0.05 to 0.12 mg/kg wet tissue in liver. The corresponding mean lead concentrations ranged from 0.06 to 0.10 mg/kg wet tissue and from 0.08 to 0.23 mg/kg wet tissue in kidney and liver, respectively.

In general, heavy metals contents in liver and kidneys of hares from area 1 (cereals) were lower than in the same hares from the other study areas (brushwood and perennial vegetation). Sex had almost no effects on the amount of toxic metal accumulated except that kidney cadmium concentrations were higher in females than in males. In relation to age, we observed a significantly higher concentration of cadmium in the liver as well in the kidney in adult animals in comparison with juvenile animals. Overall, the levels of zinc, copper, cadmium and lead in Iberian hares in Spain do no constitute a risk for hare health.

INTRODUCTION

Industrial and agricultural development has been largely responsible for pollution of the environment with toxic metals, although some contamination is derived from natural geological sources. The anthropogenic load of metals from industrial emissions to the atmosphere has significantly increased the aerial deposition of lead, cadmium, and zinc and to a lesser extent of copper and nickel during the twentieth century [1]. Some metals are essential for life, others have unknown biologic function. Those causing poisonings are the ones, which accumulate in the body through the food chain, water and air [2,3]. In terms of potential adverse effects on animal and human health, cadmium and lead are amongst the elements that caused most concern.

All components of the environment, including wild animals are continuously exposed to pollutants. Wild animals that have unrestricted movement in the environment have a greater chance to graze in places contaminated with toxic metals from industry via emissions, or from agriculture fertilizers [4]. Wild animals are important as indicators as they respond quickly to such pollution [5]. In comparison of wild and farm animals higher levels of toxic metals were detected in the organ of wild animals [4]. Metal levels in farm animals have been measured in Spain [6,7]. However, although hares are a good bio-indicator of pollution, metals levels in hares have been measured on a limited number of countries but not in Spain [1,8-12].

The aim of this study was to determine the concentrations of a variety of toxic and trace elements in the body tissues of hares from three different regions of Spain.

MATERIAL AND METHODS

Sample collection

Our study aimed to measure concentrations of cadmium, lead, copper and zinc in muscle, kidney and liver of Iberian hares (*Lepus granatensis*) from three areas of Spain. In area 1, hares find nourishment from cultivated lands, eating mainly grass and cereals. In area 2, they eat mainly sugar beet, alfalfa, brushwood and some grass. Perennial vegetation, pasture ground, vineyard and olive plantation are the most important nourishment from Iberian hares in the area 3. These are hunting areas with optimum soil and climate conditions for this game species. Tissues samples were taken from animals that had been shot by hunters at the hunting place, using appropriate tools and all standard precautions were followed to avoid contamination. The samples were packed separately and stored at -18ºC until analysis. The age determinations were performed by investigating the ossification stage of the radius and ulna [9].

Analytical procedure

Samples were dry-ashed and acid leached as described by Niemi et al. [13]. Metal concentrations in the digest were determined by atomic spectrophotometry (AAS) with Zeeman Effect (Hitachi Z-8100). Cadmium and lead were determined by graphite furnace AAS. The atomization temperatures and the wavelengths used were 2000 ºC at 228.8 nm for cadmium and 1500 C at 283.3 nm for lead. Copper and zinc were determined by flame AAS in an oxidizing air-acetylene flame at wavelengths of 324.8 and 213.9 nm respectively. All metals were quantified using calibration curves from analytical standards.

A strict quality assurance and control procedure was carried out during the study. Standard reference materials were analyzed in duplicate with each sample series. Blank samples were also analyzed with each sample series to check the contamination.

One-wave analysis of variance was used to test differences in tissue metal concentration between organ hares from the three study areas, between sexes and ages (SPSS 11.5 Windows). In order to determine which established groups were significantly different from each other, a post-hoc comparison with the Scheffe's test was carried out. For all tests, p-value of <0.05 was used to determine significant differences.

RESULTS

Over a period of one year 65 hares, 30 males and 35 females were analyzed. Concentrations of zinc, copper, cadmium and zinc in Iberian hares in relation to the area of study are listed in **Tables 1-4**. The differences of metal content in relation to age and sex are given in **Tables 5-12.**

Table 1. Concentration of zinc in Iberian hare in relation to the study area (M: arithmetic mean; SD: standard deviation; Min: Minimum; Max: Maximum. *p<0.05).

Study area		Zinc (mg Kg^{-1} wet tissue)							
		Kidney				Liver			
	n	M	SD	Min	Max	M	SD	Min	Max
1	27	18.52	3.09	13.46	26.24	23.32	4.94	11.92	33.43
2	16	19.28	4.46	8.01	24.49	24.37	5.42	10.49	31.99
3	22	21.82*	2.85	19.03	26.89	25.02*	9.29	17.28	51.72

Table 2. Concentration of copper in Iberian hare in relation to the study area. (M: arithmetic mean; SD: standard deviation; Min: Minimum; Max: Maximum).

Study area		Copper (mg Kg^{-1} wet tissue)							
		Kidney				Liver			
	n	M	SD	Min	Max	M	SD	Min	Max
1	27	2.72	0.62	0.91	3.74	3.03	1.20	0.45	4.78
2	16	2.49	0.75	1.19	3.62	3.08	1.15	1.36	6.01
3	22	2.98	0.41	2.18	3.83	2.94	0.78	1.90	4.29

Table 3. Concentration of cadmium in Iberian hare in relation to the study area. (M: arithmetic mean; SD: standard deviation; Min: Minimum; Max: Maximum. *p<0.05).

Study area		Cadmium (mg Kg^{-1} wet tissue)							
		Kidney				Liver			
	n	M	SD	Min	Max	M	SD	Min	Max
1	27	0.35*	0.57	0.02	2.16	0.05	0.06	0.01	0.24

2	16	0.66	0.51	0.14	1.85	0.09	0.07	0.02	0.26
3	22	0.76	0.52	0.09	1.88	0.12	0.08	0.03	0.26

Table 4. Concentration of lead in Iberian hare in relation to the study area. (M: arithmetic mean; SD: standard deviation; Min: Minimum; Max: Maximum. *p<0.05).

Study area		Lead (mg Kg^{-1} wet tissue)							
		Kidney				Liver			
	n	M	SD	Min	Max	M	SD	Min	Max
1	27	0.06*	0.03	0.002	0.12	0.08	0.04	0.03	0.14
2	16	0.08	0.04	0.02	0.14	0.12	0.08	0.03	0.26
3	22	0.10	0.05	0.05	0.23	0.23*	0.04	0.04	0.17

Table 5. Concentration of zinc in Iberian hare in relation to the sex. (M: arithmetic mean; SD: standard deviation; Min: Minimum; Max: Maximum).

Sex		Zinc (mg Kg^{-1} wet tissue)							
		Kidney				Liver			
	n	M	SD	Min	Max	M	SD	Min	Max
Male	30	19.82	3.75	11.29	28.13	24.50	3.65	17.32	30.78
Female	35	19.34	3.63	8.01	26.65	24.03	8.77	10.49	59.18

Table 6. Concentration of copper in Iberian hare in relation to the sex. (M: arithmetic mean; SD: standard deviation; Min: Minimum; Max: Maximum)

Sex		Copper (mg Kg^{-1} wet tissue)							
		Kidney				Liver			
	n	M	SD	Min	Max	M	SD	Min	Max
Male	30	2.83	0.65	1.19	3.88	2.64	0.57	0.90	3.36
Female	35	3.07	0.86	0.86	4.65	2.94	1.20	0.45	6.01

Table 7. Concentration of cadmium in Iberian hare in relation to the sex. (M: arithmetic mean; SD: standard deviation; Min: Minimum; Max: Maximum).

Sex		Cadmium (mg Kg^{-1} wet tissue)							
		Kidney				Liver			
	n	M	SD	Min	Max	M	SD	Min	Max
Male	30	0.50	0.51	0.02	1.85	0.08	0.07	0.01	0.26
Female	35	0.59	0.60	0.02	2.16	0.08	0.08	0.01	0.27

Table 8. Concentration of lead in Iberian hare in relation to the sex. (M: arithmetic mean; SD: standard deviation; Min: Minimum; Max: Maximum).

Sex		Lead (mg kg^{-1} wet tissue)							
		Kidney				Liver			
	n	M	SD	Min	Max	M	SD	Min	Max
Male	30	0.08	0.06	0.003	0.23	0.09	0.05	0.03	0.20
Female	35	0.08	0.04	0.01	0.18	0.10	0.06	0.01	0.26

Table 9. Concentration of zinc in Iberian hare in relation to the age. (M: arithmetic mean; SD: standard deviation; Min: Minimum; Max: Maximum).

Age		Zinc (mg Kg^{-1} wet tissue)							
		Kidney				Liver			
	n	M	SD	Min	Max	M	SD	Min	Max
Juvenile	36	19.11	4.09	8.01	28.13	27.99	8.28	10.49	59.18
Adult	29	20.69	2.96	15.38	26.65	23.33	4.48	13.07	33.44

Table 10. Concentration of copper in Iberian hare in relation to the age. (M: arithmetic mean; SD: standard deviation; Min: Minimum; Max: Maximum).

Age		Copper (mg Kg^{-1} wet tissue)							
		Kidney				Liver			
	n	M	SD	Min	Max	M	SD	Min	Max
Juvenile	36	2.60	0.69	0.90	3.74	2.96	1.23	0.45	6.01
Adult	29	2.89	0.45	1.77	4.12	3.04	0.80	1.62	4.77

Table 11. Concentration of cadmium in Iberian hare in relation to the age. (M: arithmetic mean; SD: standard deviation; Min: Minimum; Max: Maximum. *p<0.05).

Age		Cadmium (mg Kg^{-1} wet tissue)							
		Kidney				Liver			
	n	M	SD	Min	Max	M	SD	Min	Max
Juvenile	36	0.34	0.49	0.02	1.80	0.06	0.05	0.01	0.27

Adult	29	0.69*	0.62	0.04	2.16	0.09*	0.08	0.01	0.26

Table 12. Concentration of lead in Iberian hare in relation to the age. (M: arithmetic mean; SD: standard deviation; Min: Minimum; Max: Maximum).

Age		Lead (mg Kg^{-1} wet tissue)							
		Kidney				Liver			
	n	M	SD	Min	Max	M	SD	Min	Max
Juvenile	36	0.09	0.06	0.002	0.23	0.10	0.06	0.03	0.26
Adult	29	0.07	0.03	0.01	0.17	0.08	0.04	0.03	0.23

Table 13. Correlation between the concentration of zinc, copper, cadmium and lead in Iberian hare. (K: Kidney; L: Liver. *p <0.01).

	ZnK	ZnL	CuK	CuL	CdK	CdL	PbK	PbL
ZnK	1	0.255	0.512*	0.1	0.287	0.440*	0.290	-0.57
ZnL		1	0.66	0.244	-0.136	-0.19	-1.21	0.85
CuK			1	-0.08	0.78	0.254	0.112	-0.150
CuL				1	-0.08	-0.93	0.127	0.316
CdK					1	0.756*	-0.51	-0.198
CdL						1	0.65	-0.177
PbK							1	0.445*
PbL								1

The concentrations of zinc in liver ranged from 23.32 to 25.02 mg/kg wet tissue and in kidneys from 18.52 to 21.82 mg/kg wet tissue **(Table 1).** The highest mean concentration of zinc was found in hares from area 3 (perennial vegetation), but the differences were only statistically significant with hares from area 1 (cereals). Copper concentrations in the tissues of Iberian hares varied from 2.49 to 3.08 mg/kg wet tissue **(Table 2).** There were no major differences in copper contents between areas and also no differences between juvenile and adult hares. The concentrations of cadmium in livers ranged from 0.05 to 0.12 mg/kg wet tissue and in kidneys from 0.35 to 0.76 mg/kg wet tissue **(Table 3).** The cadmium content of kidneys was clearly lower in hares from area 1 (cereals) than hares from areas 2 (brushwood) and 3 (perennial vegetation). Sex did not have a significant effect on renal cadmium although accumulation of cadmium in kidney was slightly higher in females than in males **(Table 7).** The cadmium content of liver and kidney was significantly higher in adult than in young hares **(Table 11).** Lead concentrations in the body tissues of Iberian hares varied from 0.04 to 0.1 mg/kg wet tissue **(Table 4).** Accumulation of lead in liver was significantly higher in hares from area 3 (perennial vegetation) than area 1 (cereals). The lead content of kidneys was clearly lower in hares from area 1 (cereals) than hares from areas 2 (brushwood) and 3 (perennial vegetation). There were no significant differences between males and females in any of the organs analyzed **(Table 8).** Correlation analysis **(Table 13)** showed significant correlations ($p < 0.01$) between the concentration of lead in kidney and liver, as well as the level of cadmium in kidney and liver. Zinc in kidney is significantly correlated with cadmium in liver. All metals are in significant correlation in kidney.

DISCUSSION

Heavy metals are natural components of the environment, but in recent years industrial, agricultural and zootechnic development has been responsible for the diffusion of these substances in the environment, causing pollution of water, soil and atmosphere. These elements are accumulated in soils and edible plants, and when animals eat these plants, they accumulate high levels of toxic metals in their organism.

Copper and zinc are essential elements whose deficiency results in impairment of biological functions. Nevertheless, when present in excess, essential metals may become toxic [14]. Cadmium and lead have no known physiological role and at high doses they are acutely and lethally toxic.

The liver and kidneys were examined because these organs often accumulate the highest concentrations of heavy metals [15,16]. The environment and diet of Iberian hares differ from each study area, and therefore the investigation provides information about the concentrations of heavy metals in forest and cultivated lands.

In general, heavy metals contents in liver and kidneys of hares from area 1 (cereals) were lower than in the same hares from the other study areas. The difference probably results from the different diets because cereals are sowed yearly, whereas for example brushwood and olive tree are exposed to the influence of air pollution for longer periods, during which the accumulate heavy metals. In area 1 (cereals), hares feed nearly exclusively one year plants. The young plant is less contaminated than an older one.

Zinc and copper concentrations in Iberian hares were in the lower range of those reported for hares from Finland [1] and Serbian [11]. The higher copper and zinc concentrations recorded in area 3 (perennial vegetation) are evidently the result of long distance transport, as there are no local sources of emissions. There were no major differences in copper and zinc contents between males and females and also no differences between young and adult hares. However, although it was not statistically significant copper accumulation in liver and kidney were higher in females than in males. These sex-related effects may be due to the influence of female estrogens on copper metabolism [17]

Lead poisoning has been a part of history since 4000 years before Christ. With increasing awareness of the toxicity associated with lead, it is one of the most common toxicants in large and small animals. Our values were similar to those reported by Venäläinen et al. [1] and Massányi et al. [10] and clearly lower than those described by Bukovjan [18] and Baikov et al. [8]. This can be at least partly a consequence of the fact that the consumption of unleaded petrol has increased during the lasts years.

No significant differences between males and females were observed in accumulation of lead in any organs analyzed. Similar results were also described in cattle [6,7,19], in goats [20] and in sheep [15]. However, Massányi et al. [10] observed a significant higher lead level in liver as well as in kidney in males brown hares.

Cadmium levels were depending on age, food quality and the conditions of the environment. Many authors have described higher accumulation of cadmium in hares in other European countries [4,9-12]. Our values were similar to those described in non-industrialized regions of Europe [1] perhaps because cadmium reaches the environment mainly by industrial release from concentration and refining processes of metals. In agreement with other works [4] kidney and liver levels of cadmium were higher than those described in the same organs in farm animals from Spain [6,7]. We recorded the highest cadmium levels in kidney and the same view was in other kind of animals [4,6,7,10]. The most typical feature of chronic cadmium intoxication is kidney damage. In general, kidney damage is assumed to occur at cadmium concentrations of 80-200 mg/kg wet tissue [21]. In cadmium we reported significant age dependent accumulation similarly as it is described by other authors [6,10,22].

According to Lutz and Slameka [23] and Massányi et al. [10] we observe higher concentrations of cadmium in kidneys of female hare. This is perhaps due to a longer biological half-life of cadmium in females [14]. Increased retention of cadmium by females could be due to more efficient metalothionein synthesis [14].

Overall, the levels of zinc, copper, cadmium and lead in Iberian hares in Spain do no constitute a risk for hare health.

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