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COMPARISON OF ZINC HAIR MINERAL LEVELS IN CATS WITH LIVER AND KIDNEYS DISORDERS

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Abstract

This research's main objective was the assessment of zinc levels in hair from cats with different liver and kidneys disorders, compared to control group. Zinc (Zn) hair content analysis of the cats with liver disorders (n = 6), cats with kidneys disorders (n = 9), and clinically healthy cats as control (n = 6), was performed by inductively coupled plasma-optic emission spectrometry (ICP-OES). Zn concentrations in hair were significantly different (p = 0.015) between males with liver disorders (22.10 mg kg^{-1}) and males with kidneys disorders (6.181 mg kg^{-1}), and no significant differences have been observed compared to zinc mean concentrations in clinically healthy males (10.60 mg kg^{-1}). Significant differences were observed when comparing Zn mean levels in males with different kidneys disorders to Zn levels in clinically healthy males. No significant differences depending on health status or age were found in either studied category. The highest zinc mean value was found in hair samples from male cats with liver disorders, and the lowest zinc mean value was found in male cats with kidneys disorders. This research on zinc assessment from hair, an easy and non-stressfully collected sample, shows that this kind of sample could be appropriate for the evaluation of the mineral status of cats with different organ dysfunctions in urban areas. Hair zinc levels found in this study may contribute to the database of reference concentrations of minerals in cats from Romania.

Key words*: hair, cats, zinc, liver disorders, kidneys disorders.*

INTRODUCTION

Zn is an essential metal necessary for the proper functioning of organisms. Zinc is relatively abundant in nature and is widely used in industry and agriculture (Sloup *et al*., 2017).

Zn is a cofactor of over 3000 proteins or enzymes (Beyersmann & Haase, 2001; Andreini *et al.*, 2006; Mohommad *et al.*, 2012) being the only metal that is part of all six classes of enzymes: oxidoreductase, transferase, hydrolase, lyase, isomerase, ligase (Webb, 1992; Mohommad *et al*., 2012; Cornish-Bowden, 2014). Zn is also essential for cell proliferation and differentiation processes, especially for DNA synthesis and mitosis (Beyersmann & Haase, 2001; Mohommad et al., 2012).

Unlike other transition metals, such as Fe or Cu, Zn has no redox activity (Berg & Shi, 1996).

In patients with chronic kidney disease, Zn deficiency/altered metabolism is well documented, especially in those with nephrotic disease and uremia (Mahajan, 1989), and is also observed in patients with many types of liver disease (Mohommad *et al*., 2012).

The change in Zn metabolism can be determined by the decreased dietary and intestinal absorption levels, increased endogenous secretion and urinary excretion of Zn (Mahajan, 1989; Mohommad et al., 2012), activation of certain zinc transporters, and induction of hepatic metallothionein (Mohommad *et al.*, 2012). Zn is excreted mainly through urine, but also through perspiration, saliva, and is incorporated into the hair (Goran & Crivineanu, 2016c; Sloup *et al.*, 2017).

The liver and kidneys' roles in xenobiotics metabolism and excretion expose them to high levels of toxic substances and their metabolites (Osweiler, 1996a; 1996b; Goran & Crivineanu, 2016a; 2016b).

The mineral composition of the hair reflects the mineral content of the body's tissues. If there is a mineral deficiency or excess in the hair, it usually indicates a mineral deficiency or excess inside the body, although sometimes it can mean the opposite.

Thus, the concentration of minerals and heavy metals in animal hair reflects the presence of these elements in the surrounding forage and soil and varies from area to area, providing information on pollution in the area (Rashed & Soltan, 2005).

Because of hair's easy and non-stressful sampling way, it is used for evaluation of the mineral content of the animal organisms. Hair mineral analysis has become a routine analysis since the early 1970s and it is increasingly used, both for the assessment of pollution in the area, as well as for the evaluation of the involvement of minerals/metals in various pathologies in animals and humans (Combs *et al.*, 1982; Foo *et al.*, 1993; Poon *et al.*, 2004; Adams *et al.*, 2006; Goran & Crivineanu, 2007; Długaszek *et al.*, 2008; Skibniewska *et al.*, 2011; Kosla *et al.*, 2011; Kolachi *et al.*, 2012; Skibniewski *et al.*, 2013; Panhwar *et al.*, 2013; Wołowiec *et al.*, 2013; Roug *et al.*, 2015; Badea *et al.*, 2016; Badea *et al.*, 2017; Goran *et al.*, 2017a; Goran *et al.*, 2017b).

The goal of this study was to evaluate the levels of zinc in hair samples from cats with different liver and kidneys disorders, compared to control samples, using inductively coupled plasma-optic emission spectrometry (ICP-OES).

MATERIALS AND METHODS

Sampling and samples preparation

Analysis of hair Zn content of cats with liver disorders (n = 6), cats with kidneys disorders (n = 9), and clinically healthy cats as control (n = 6) was performed by ICP-OES.

The number of animals in this study broken down into categories depending on age and sex in each studied group are presented in Table 1.

The cats with liver and kidneys disorders showed symptoms that led to the suspicion of organ injuries, and the diseases were confirmed by biochemical blood tests and ultrasound examination.

For all studied animals, the hair samples were collected as close to the skin as possible from the flank region, placed in labelled paper envelopes, and transported to the laboratory, where the hair samples were stored in a dark, dry place, with a constant temperature.

The samples were prepared initially by degreasing, washing, rinsing, and drying, and then they were weighed and mineralized. All hair samples were digested using a Speedwave MWS-2 Berghof microwave oven as following: Step 1: 120ºC, power 50%; Step 2: 180ºC, power 75%; Step 3: 100ºC, power 40%. The samples were then analyzed to assay the Zn levels by ICP-OES.

Table 1. Studied cats depending on health status, age and sex

HA	6	**< 8**	4	**M**	1
				F	3
		> 8	2	**M**	1
				F	1
LD	9	**< 8**	2	**M**	1
				F	1
		> 8	7	**M**	4
				F	3
KD	6	**< 8**	1	**M**	0
				F	1
		> 8	5	**M**	1
				F	4

*HA - clinically healthy animals; LD - animals with liver disorders; KD - animals with kidneys disorders; < 8 - animals below the age of 8; > 8 - animals above the age of 8; M - male cats; F - female cats.

For all studied animals, the hair samples were collected as close to the skin as possible from the flank region, placed in labelled paper envelopes, and transported to the laboratory, where the hair samples were stored in a dark, dry place, with a constant temperature.

The samples were prepared initially by degreasing, washing, rinsing, and drying, and then they were weighed and mineralized. All hair samples were digested using a Speedwave MWS-2 Berghof microwave oven as following: Step 1: 120ºC, power 50%; Step 2: 180ºC, power 75%; Step 3: 100ºC, power 40%. The samples were then analyzed to assay the Zn levels by ICP-OES.

Spectrometric analysis

For mineralization all hair samples were treated with 5 mL HNO_3, 0.8 mL HCl and 1 mL H_2O_2, then diluted to 10 mL with ultrapure water and analyzed using a Thermo iCAP ICP-OES spectrometer (RF1100 W; reading time 30 s, washing time 30 s, nebulizer gas flow 0.5 L/min; auxiliary gas flow 0.5 L/min; sample injection pump flow 50 rpm). Calibration curves were developed using standard solutions of 0.001 mg kg^{-1}, 0.01 mg kg^{-1}, 0.1 mg kg^{-1}, 1 mg kg^{-1}, 5 mg kg^{-1}, 10 mg kg^{-1}, 50 mg kg^{-1} obtained by dilution

from a multi-element ICP MERCK standard containing 1000 mL/L of Zn.

Statistical analysis

VassarStats software: Website for Statistical Computation (http://vassarstats.net/) was used for performing One-Way ANOVA for all samples' mineral concentrations. In order to verify the ANOVA assumptions, Student's t-test (Microsoft Excel) with unequal sample sizes, unequal variances (Welch's *t*-test), was used.

RESULTS AND DISCUSSIONS

The mean Zn contents of hair samples from clinically healthy cats and those with different liver and kidneys disorders are presented dependent of age and sex in Table 2 and expressed as mg kg^{-1}.

Mean Zn levels found were higher in HA and LD cats compared to the cats with KD but with no significant differences ($p = 0.168$).

The highest mean Zn concentration was found in the group of LD cats (11.916 mg kg^{-1}).

The Zn hair concentrations reported by other authors were significantly different in some studies such as Skibniewska *et al.* (2011) who evaluated the Zn levels in pet and feral cats' hair. They reported much greater values compared to those found in our study. They determined that in feral cats were determined greater values (250.52 mg kg^{-1}) than in pet cats (227.28 mg kg^{-1}). Other studies on cats' hair mineral content from Bucharest (Romania) also reported mean hair Zn values comparable to those found in all these study groups (Badea *et al.*, 2016; Goran *et al.*, 2017a).

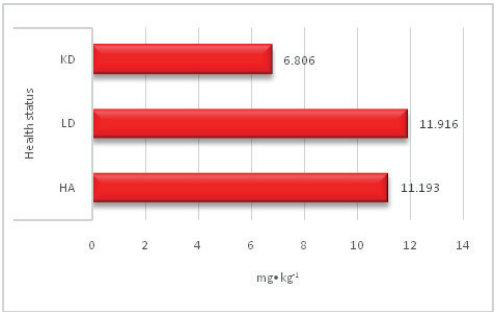
Table 2. Mean Zn levels in cat hair samples depending on health status, age and sex (mg kg^{-1})

Health status						
Element	**Health status**	**Mean**	**SD**	**Std err mean**	***p*-value**	**Welch t-test**
Zn	HA	11.193 [a]	2.839	1.159		HA/LD=0.289
	LD	11.916 [a]	2.517	0.839	*0.168*	HA/KD=0.009
	KD	6.806 [b]	8.472	3.459		LD/HD=0.05
Age						
Element	**< 8 years**	**Mean**	**SD**	**Std err mean**	***p*-value**	**Welch t-test**
Zn	HA	11.290 [a]	3.414	1.707		HA/LD=0.312
	LD	6.779 [a]	1.158	0.819	*0.334*	HA/KD=0.060
	KD	13.000 [a]	0	0		LD/HD=0.059
	> 8 years	**Mean**	**SD**	**Std err mean**	***p*-value**	**Welch t-test**
	HA	11.000 [a]	0.9	0.636		HA/LD=0.018
	LD	11.700 [b]	9.266	4.144	*0.442*	HA/KD=0.009
	KD	6.814 [b]	2.786	1.053		LD/HD=0.089
Sex						
Element	**Males**	**Mean**	**SD**	**Std err mean**	***p*-value**	**Welch t-test**
Zn	HA	**10.600** [a]	1.3	0.65		HA/LD=0.382
	LD	**22.100** [a]	0	0	***0.015***	HA/KD=0.039
	KD	**6.181** [ab]	3.075	1.375		LD/HD=0.265
	Females	**Mean**	**SD**	**Std err mean**	***p*-value**	**Welch t-test**
	HA	11.490 [a]	3.313	0.919		HA/LD=0.373
	LD	9.880 [a]	7.826	3.501	*0.662*	HA/KD=0.049
	KD	7.589 [ab]	1.154	1.537		LD/HD=0.111

*Levels not connected by the same letter are significantly different.
**LD - liver disorders; KD - kidneys disorders; HA - healthy animals.

Although a higher mean Zn hair content was observed in the group of younger KD cats (13.0 mg kg^{-1}), the age of cats did not influence the Zn hair level in a statistically significant way. As it is presented in Figure 2, the lowest mean Zn content was found in the group of LD cats, independent of age (6.779 mg kg^{-1} in cats below 8 years of age, and 6.814 mg kg^{-1} in cats above 8 years of age).

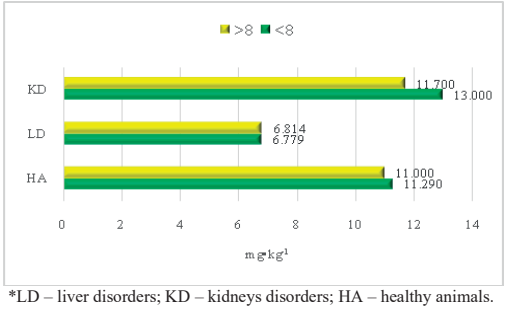
Another study on hair mineral content also has reported that in both studied groups below and above 5 years of age, mean Zn levels were higher than in the cats with kidneys failure (Badea *et al.*, 2016).



*LD - liver disorders; KD - kidneys disorders; HA - healthy animals

Figure 1. Mean Zn levels in hair samples from cats with LD or KD compared to HA

In a study on female cats' hair mineral content was reported a significantly lower mean Zn hair level in clinically healthy animals (10.1 mg kg^{-1}), compared to its value found in cats with chronic hepatitis (18.4 mg kg^{-1}) (Goran *et al.*, 2017a). Another study on cats' Zn hair content reported higher values in older cats (242.14 mg kg^{-1}) (Skibniewska *et al.*, 2011).



*LD – liver disorders; KD – kidneys disorders; HA – healthy animals.

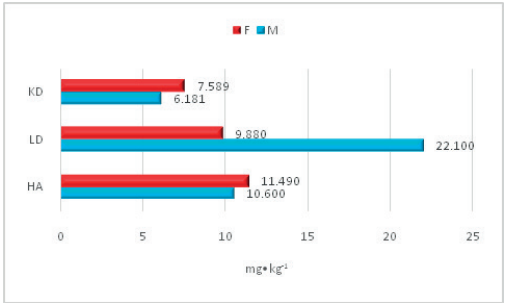
Figure 2. Mean Zn levels in hair samples from cats with LD or KD compared to HA, depending on age

Zn hair content provides useful information about the Zn body's levels, but the interpretation can be complex, as both high and low hair Zn levels may indicate low Zn body levels (Cutler, 2004). These Zn levels could be influenced by the disturbed capacities of liver metabolization in LD animals, and increased excretion in KD animals, correlated to the organism low capacity of Zn compounds use, which are also reflected in hair mineral content. Zn hair levels recorded significant differences between its levels ($p = 0.015$), the highest concentrations being found in male cats from LD group hair samples (22.1 mg kg^{-1}), followed by those in female HA cats, which were almost 2 times lower (Figure 3). The significant differences correlations between Zn hair levels could be made between hair samples from HA and LD cats vs. those from KD cats ($p < .05$). The Zn hair levels' differences between HA cats and LD cats were not statistically significant.

Skibniewska *et al.* (2011) have reported Zn hair levels much higher than those found in the present study, independent of health status, with higher levels in female feral cats (268.09 mg kg^{-1}), and lower in pet female cats (214.49 mg kg^{-1}).

Even the Zn hair levels found in females, independent of health status, had no significant differences ($p > 0.05$), it can be observed that Zn hair values were lower in LD and KD cats compared to HA animals.

Other researches on hair mineral content showed that Zn hair level in male cats (6.18 mg kg^{-1}) and female cats (7.59 mg kg^{-1}) with renal failure, were lower than clinically healthy animals (Badea *et al.*, 2016), which was not the case in hair samples of cats with chronic hepatitis (Goran *et al.*, 2017a).



*LD - liver disorders; KD - kidneys disorders; HA - healthy animals.

Figure 3. Mean Zn levels in hair samples from cats with LD or KD compared to HA, depending on sex

Some toxic metals and essential metals have common chemical characteristics, which could lead to interactions (Goyer, 1997). There was reported that interactions between essential and toxic metals indicate that toxic elements compete with the essential metals, but at the same time the mineral status assay needs to be realized on a specific tissue or organ (López Alonso *et al.*, 2004). Also, choosing potential target organs for the metals' concentrations assessment needs to be carefully analyzed for interfering elemental interactions (Elsenhans *et al.*, 1987).

CONCLUSIONS

This study presents investigations of the hair use as a mineral biomarker for the cats' hepatic and renal pathology in a Romanian urban area.
Generally, the highest Zn mean level was found in cats with liver disorders, and the lowest in cats with kidneys disorders.
The highest Zn mean value recorded significantly difference in hair samples from male cats with liver disorders compared to the lowest Zn mean value, found in male cats with kidneys disorders hair samples.
This research on Zn assessment from hair, an easy and non-stressfully collected sample, shows that this kind of sample could be appropriate for the evaluation of the mineral status of cats with different organ dysfunctions in urban areas.
Zn recorded insignificant differences between its levels in hair samples from female cats independent of the health status, and all animals independent of age and health status.
Mean Zn hair concentrations were below the determined levels in cats reported in studies in other countries.
Zn hair levels reported in the present research may *contribute* to a database of reference levels of minerals in cats in Romania.

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