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# INFLUENCE OF HORSE BREED AND HOUSING SYSTEM ON THE LEVEL OF SELECTED ELEMENTS IN HORSE'S HAIR

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## Abstract

The aim of the study was to compare the levels of selected toxic elements in hair of horses of two breeds, held in areas free of industrial pollution. The research was conducted on free range Hucul mares ( $n=40$ ) and Purebred Arabian mares ( $n=40$ ) kept in a mixed stable-pasture system. The content of 9 elements (arsenic, barium, cadmium, mercury, germanium, lithium, lead, tin and strontium) was determined in each trial. The hair of Purebred Arabian mares was characterized by significantly higher levels of arsenic, barium, mercury, lead, tin and strontium. The differences were statistically significant. Cadmium and germanium, on the other hand, were significantly higher in the hair of Hucul mares. Lithium was found to be on a similar level in both breeds i.e.,  $0.150 \text{ mg kg}^{-1}$  of dry mass in Hucul horses and  $0.142 \text{ mg kg}^{-1}$  of dry mass in Purebred Arabian horses. There was a significant positive correlation between the levels of mercury and arsenic observed in the hair of horses of both breeds. Arabian mares displayed a very high correlation between the levels of barium and lead, barium and tin, and between tin and lead.

Keywords: horses, breed, hair, toxic elements.

## POZIOM WYBRANYCH PIERWIĄTKÓW W SIERŚCI KONI Z UWZGLĘDNIENIEM RASY I SYSTEMU UTRZYMANIA

### Abstrakt

Celem badań było porównanie poziomu wybranych metali toksycznych w sierści koni dwóch ras utrzymywanych na terenach wolnych od zanieczyszczeń przemysłowych. Badaniom poddano klacze rasy huculskiej ( $n=40$ ) utrzymywane systemem bezstajennym i klacze czystej krwi arabskiej ( $n=40$ ) utrzymywane systemem stajenno-pastwiskowym. Określono poziom 9 pierwiastków (arsenu, baru, kadmu, germanu, rtęci, litu, ołowiu, cyny, strontu) w każdej z prób. W sierści klaczy czystej krwi arabskiej wykazano znacznie wyższą zawartość arsenu, baru, rtęci, ołowiu, cyny i strontu. Różnice te były statystycznie istotne. Natomiast zawartość kadmu i germanu była wyraźnie wyższa w sierści klaczy huculskich. Na zbliżonym poziomie u obu ras oznaczono lit ( $0,150 \text{ mg kg}^{-1}$  s.m. u koni huculskich i  $0,142 \text{ mg kg}^{-1}$  s.m. u czystej krwi arabskiej). W sierści koni obu ras stwierdzono istotną dodatnią zależność między poziomem arsenu i rtęci. U klaczy czystej krwi arabskiej wykazano bardzo wysokie korelacje między poziomem baru i ołowiu, baru i cyny, cyny i ołowiu.

Słowa kluczowe: konie, rasa, sierść, pierwiastki toksyczne.

## INTRODUCTION

Trace elements are crucial for the proper functioning of all living organisms. Some of them (especially heavy metals), however, can induce metabolic disorders. Both humans and animals derive trace elements from plants, whose mineral composition is associated with the natural abundance of elements in soils or with industrial pollution. The growing public awareness on the hazards of environmental contamination compels monitoring of elements and other toxic substances in air, soil, food and living organisms. Tissues of herbivores can be efficient bio-indicators of current levels of environmental pollution (MAIA et al. 2006, SMITH et al. 2010).

Several sources imply that hair and fur are as good biological material as blood, plasma, enamel and soft tissues (ASANO et al. 2005a, FARMER and FARMER 2000, VERMEULEN et al. 2009, GABRYSZUK et al. 2010, KOŚLA et al. 2011). They are characterized by non-invasive sampling as well as ease of transportation and storage. Moreover, the concentration of most trace elements in hair is much higher than in blood (CIZDZIEL, GERSTENBERGER 2004). In addition, the natural process of hair growth is a reflection of the ongoing metabolic changes which occur in the body over considerably long periods of time (COMBS et al. 1982, COMBS 1987).

Assays of the elemental composition of human hair for assessment of the level of minerals in a whole organism have been quite meticulously studied (MIEKELEY et al. 1998, CHOJNACKA et al. 2006, SZYNKOWSKA et al. 2009, DESHMUKH et al. 2010, GOW et al. 2010). It is also claimed that determinations of the mineral content of farm animals' hair could be helpful to assess

their nutritional status versus the requirements for certain substances or to determine their toxicity (HINTZ 2000, PALACIOS et al. 2002). Hair may also serve as a reliable source of data which facilitates monitoring of the body's mineral status owing to a relatively long period of deposition of bio-elements as well as its stability in comparison to blood.

Hair and fur are used to control the abuse of animals for therapeutic purposes as well as the application of steroids (DUNNETT, LEES 2003, ANIELSKI et al. 2005, GRATECOS-CUBRASI et al. 2006).

Providing that concentrations of certain elements in hair of horses may be taken as an indication of the need for their supplementation, the impact of such factors as breed, sex, hair colour, exposure to pollution and nutrition cannot be neglected (ASANO et al. 2002, MAIA et al. 2006).

The objective of the research was to compare the levels of selected trace elements in the hair of horses from two breeds kept in different housing systems, in a region free of industrial pollution.

## **MATERIAL AND METHODS**

### **Animals**

The research included 40 mares from each of two herds of horses. All the animals were kept in areas free of industrial pollution and grazed on pastures far from motorways. The Purebred Arabian horses between 3 and 25 years of age came from state-owned stables. All the horses were fed the same rations (3.5 kg oats, 4.0 kg hay, vitamin and mineral supplements), had an unlimited access to pasture and were kept in similar conditions: on pasture during the day and in stables overnight. The Hucul horses were kept on a private farm located in a mountainous area. The mares were aged 4-15 years. They had an unlimited access to grass on pasture and additionally received salt licks. They were kept in a free-range system all year round and were all healthy.

### **Sampling and mineral analysis**

The hair designated for analysis was collected from all horses in early autumn. Hair samples of 500 mg each were obtained from the nape area nearest to the skin. The content level of 9 elements i.e., arsenic, barium, cadmium, germanium, mercury, lithium, lead, tin, and strontium was determined in each sample. Elemental analysis was conducted using an atomic emission spectrometer with inductively coupled plasma (ICP-OES) Optima 5300 DV by Perkin Elmer. The method was based on the readings achieved by atomic emission spectrometry using the optical technique.

### Statistical analysis

The results were analyzed statistically, given the mean, standard deviation, and extreme values (minimum – min. and maximum – max.) Univariate analysis of variance ANOVA was performed. The significance of differences between means in herds was calculated using the student's t-distribution test. In order to estimate relationships between the levels of elements, their coefficients of correlation were calculated. The calculations were made using a Statistica 9.0 statistical package.

## RESULTS

The average concentrations of the analyzed trace elements in horses' hair are summarized in Table 1. The hair of Purebred Arabian mares was characterized by significantly higher more arsenic, barium, mercury, lead, tin and strontium, and the differences were statistically significant. In contrast, the levels of cadmium and germanium were distinctly higher in hair of Hucul mares. Lithium was found to be on a similar level in both breeds (0.150 mg kg<sup>-1</sup> of dry mass in Hucul horses and 0.142 mg kg<sup>-1</sup> of dry mass in Purebred Arabian horses).

Table 1

Content of selected elements (mg kg<sup>-1</sup> of dry mass) in horses' hair

Elements	Breed								Significance of difference	
	Hucul				Purebred Arabian				P value	
	$\bar{x}$	SD	min	max	$\bar{x}$	SD	min	max		
As	0.011	0.002	0.008	0.014	0.070	0.045	0.010	0.200	***	0.0000
Ba	0.144	0.085	0.057	0.431	0.204	0.135	0.060	0.800	*	0.0217
Cd	0.024	0.028	0.004	0.151	0.012	0.022	0.000	0.110	*	0.0354
Ge	0.023	0.007	0.011	0.037	0.015	0.002	0.011	0.020	***	0.0000
Hg	0.050	0.039	0.0004	0.121	0.096	0.009	0.070	0.120	***	0.0000
Li	0.150	0.080	0.054	0.493	0.142	0.159	0.013	0.760	ns	0.7798
Pb	0.081	0.064	0.000	0.229	0.124	0.074	0.048	0.479	**	0.0064
Sn	0.026	0.004	0.002	0.036	0.057	0.018	0.026	0.118	***	0.0000
Sr	2.393	0.958	0.890	5.530	2.775	0.712	1.670	4.690	*	0.0475

Correlation coefficients between concentrations of the elements analyzed in hair of Hucul horses are given in Table 2. Significant positive correlations were found between the levels of arsenic and mercury, arsenic and lithium as well as between arsenic and tin. Similar tendencies were noted

Table 2

Coefficients of correlations between levels of selected elements in Hucul horses

Elements	As	Ba	Cd	Ge	Hg	Li	Pb	Sn	Sr
As	<i>x</i>								
Ba	ns	<i>x</i>							
Cd	ns	ns	<i>x</i>						
Ge	ns	ns	ns	<i>x</i>					
Hg	0.3996* <i>p</i> =0.011	-0.3368* <i>p</i> =0.034	ns	0.3741* <i>p</i> =0.017	<i>x</i>				
Li	0.3709* <i>p</i> =0.018	ns	ns	0.4395* ** <i>p</i> =0.005	0.3855* <i>p</i> =0.014	<i>x</i>			
Pb	ns	ns	ns	ns	-0.4275* ** <i>p</i> =0.006	ns	<i>x</i>		
Sn	0.5922* ** <i>p</i> =0.000	ns	ns	0.3716* <i>p</i> =0.018	0.4900* ** <i>p</i> =0.001	ns	ns	<i>x</i>	
Sr	ns	ns	ns	ns	ns	0.4324* ** <i>p</i> =0.005	ns	ns	<i>x</i>

\*significant at  $P \leq 0.05$ ; \*\* significant at  $P \leq 0.01$ 

Table 3

Coefficients of correlations between levels of selected elements Purebred Arabian horses

Elements	As	Ba	Cd	Ge	Hg	Li	Pb	Sn	Sr
As	<i>x</i>								
Ba	ns	<i>x</i>							
Cd	ns	ns	<i>x</i>						
Ge	ns	ns	ns	<i>x</i>					
Hg	0.4827* ** <i>p</i> =0.002	ns	ns	ns	<i>x</i>				
Li	ns	ns	ns	-0.3783* <i>p</i> =0.016	ns	<i>x</i>			
Pb	ns	0.7852* ** <i>p</i> =0.000	ns	ns	ns	ns	<i>x</i>		
Sn	ns	0.7809* ** <i>p</i> =0.000	ns	ns	ns	ns	0.7626* ** <i>p</i> =0.000	<i>x</i>	
Sr	ns	ns	ns	ns	0.3320* <i>p</i> =0.036	ns	ns	ns	<i>x</i>

\*significant at  $P \leq 0.05$ ; \*\* significant at  $P \leq 0.01$

in respect of correlations between concentrations of germanium and mercury, germanium and lithium as well as between germanium and tin. Statistically significant positive correlation was also observed between concentrations of lithium and strontium. Negative correlation was, however, found between concentrations of mercury and barium. A similar tendency was observed between levels of mercury and lead.

The coefficients of correlation between concentrations of trace elements analyzed in the hair of Purebred Arabian horses are summarized in Table 3. Significant positive correlation, similarly to Hucul mares, was confirmed between the levels of arsenic and mercury. Significant positive correlation was also traced between the concentrations of barium and lead, barium and tin, tin and lead as well as between tin and lead, and mercury and strontium. Negative correlation was determined between the concentrations of lithium and germanium in the hair of Purebred Arabian mares.

## DISCUSSION

In general, hair of herbivores is considered a better indicator of possible environmental pollution than human hair because these animals feed directly on plants (NAGEEB RASHED, SOLTAN 2005). Animal hair is also considered good biological material for analysis of both essential and toxic elements (COMBS 1987, LIU 2003, ASANO et al. 2005a, DOBRZAŃSKI et al. 200, KAPROŃ et al. 2010). Some authors also claim that elemental analysis of hair can serve as a good indicator of the nutritional status of animals (HINTZ 2000), especially herbivorous ones, for which green forage is the basic source of nutrients.

WŁOSTOWSKI et al. (2006) suggest that a high concentration of cadmium in animals may be due to the low pH of soil, which accelerates migration of cadmium ions into plant tissues and therefore affects the quantity of cadmium absorbed by animals. This seems to confirm the results obtained in the tests reported herein. The soil under the pasture grazed by the Hucul horses were kept was characterized by a rather low pH. MADEJON et al. (2009) demonstrated higher concentrations of certain elements in green biomass during autumn, which is attributable to a slower growth rate of plants than in spring. Thus, it seems that grazing horses during summer and autumn periods can result in higher levels of certain bio-elements in their hair.

JANISZEWSKA and CIEŚLA (2002) reported higher concentrations of cadmium and lead, i.e., 0.196 mg kg<sup>-1</sup> of dry mass, in the hair of half-blood horses during summer compared to 0.024 mg kg<sup>-1</sup> d.m. for Hucul breed and 0.012 mg kg<sup>-1</sup> d.m. for Purebred Arabian horses. The reason could be the fact that horses are more exposed to various sources of pollution during these periods. The above authors recommend supplementation of horses' diets

with magnesium, zinc or selenium in order to limit the accumulation of potentially toxic cadmium and lead in animals.

STACHURSKA et al. (2011) obtained small differences in concentrations of heavy metals in hair and hoof horns of the Polish Konik depending on the housing system. The author also revealed that increased levels of minerals in hair, especially cadmium and lead, occurred during the winter feeding periods. In contrast, KAPROŃ et al. (2010) obtained lead concentrations at the level of  $0.22 \text{ mg kg}^{-1} \text{ d.m.}$  for Polish Konik horses kept in the wild during summer. Less lead was found in the hair of horses analyzed during the present study (Table 1).

GABRYSZUK et al. (2010), who investigated the levels of minerals in milk and hair of cows kept on conventional and organic farms, showed their relationship with the system of production. ANKE et al. (1989) confirmed differences in the levels of cadmium in hair of mares and geldings held in Central Europe. They also noted that the intensity of cadmium accumulation was influenced by the race type, a finding also fact confirmed in our studies.

ASANO et al. (2005b), when analyzing concentrations of several elements in hair of horses (using the PIXE method), observed a significant influence of the hair colour. Grey hair contained the lowest concentrations of Br, Ca, Se and Sr. With respect to the animals' gender, more mercury was found in hair of mares, lead was more abundant in hair of geldings while strontium was on a higher level in hair of stallions (ASANO et al. 2005a).

FARMER and FARMER (2000) analyzed the content of some elements in tissues of animals held in regions exposed to industrial pollution and reported that the level of lead depended on its concentration in plants. In addition, the concentration of lead in hair was markedly higher than in soft tissues. However, no such dependency was observed for cadmium, which may suggest that its accumulation in the body depended on other factors as well. This seems to be corroborated by the study of DOBRZAŃSKI et al. (2005) on the content of selected elements in hair of horses living in areas exposed to different levels of industrial pollution. The level of cadmium in the hair of horses from industrial areas was slightly higher, but the difference was not statistically significant. The authors cited above also observed positive correlation between levels of cadmium and lead, whilst in our study, correlation coefficients between these two elements were not significant. Because of the toxicity of lead, cadmium and mercury, concentrations of these metals in tissues of animals are often treated as realistic indicators of environmental pollution. The levels of arsenic, barium, germanium, lithium, tin and strontium in the hair of horses covered by the study show that both the housing system and nutrition may be modifying factors. This conclusion is supported the research conducted by KABATA-PENDIAS and PENDIAS (1993). However, the results obtained so far are difficult to interpret because not much research has been conducted on this question.

## CONCLUSION

The content of minerals in the hair of domestic animals may vary depending on the breed and dietary factors. Too little information is available to elucidate the effect of these factors on horses. Further research will also be needed to evaluate the effect of a diet, regional differences and the reproductive status of animals.

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