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CONCENTRATION OF CADMIUM AND LEAD IN HORSE BLOOD SERUM AND HAIR IN RELATION TO SEASON AND ENVIRONMENT

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ABSTRACT

Cadmium and lead levels were examined in blood serum and hair of 79 horses reared in 7 breeding centres. Blood and hair samples were collected twice a year: in summer and in winter. The concentration of the metals was determined with the ICP EAS emission spectrophotometer, model JY 24. Mean concentration of cadmium in the horses' blood serum in summer ranged from 0.027 to 0.07 micromole per litre, and in winter – from 0.003 to 0.049 micromole per litre. Mean concentration of this element in hair of horses was observed in the range 0.039–0.997 milligram per kilogram of fresh weight in summer, and 0.0–0.111 milligram per kilogram in winter. Average lead values found in blood serum were from 0.09 to 0.44 micromole per litre (summer) and from 0.06 to 0.17 micromole per litre (winter). Mean lead concentration recorded in the hair of horses ranged from 0.0 to 10.7 milligram per kilogram in summer and from 0.0 to 2.46 milligram per kilogram in winter. The study allows concluding that significantly high concentration of cadmium and lead in the horses' blood serum and hair in summer may indicate adverse effects of environmental pollution. A differentiating influence of season and environment has been found on the level of analysed elements in the serum and hair of horses. Correlation coefficients between the level of the elements in the horses' serum and hair found in this study showed that horse hair is a good indicator of cadmium and lead content in the body.

Key words: horses, cadmium, lead, blood serum, hair.

INTRODUCTION

Technological and scientific developments we have been witnessing over the recent years allow detailed understanding of the role and transformations of various elements in the body. It is commonly known that some of the elements, so–called heavy metals, may be particularly toxic to people and animals. Such elements include cadmium and lead, which are frequently released into the environment with various types of pollution. Horses are exposed to environmental effects due to the long time during which they are used, prolonged time spent grazing, and feeding on foods manufactured in households. Kozielec *et al.* [9] reported toxic metal content in human hair to be significantly correlated with its corresponding content in blood, therefore the analyses made on hair provide reliable indication of an individual's exposure to the metals in question. In the case of horses, their hair is easily available, and hair sampling is not stressful for the animal.

The objective of this study was to determine summer and winter levels of cadmium and lead in the blood serum and hair of horses kept at various breeding centres.

MATERIALS AND METHODS

The study covered 79 half–bred horses aged from 3 to 10 years. The horses were kept at 7 breeding centres. At each centre, a group of 10 to 13 horses was selected. Each group consisted of mares and geldings, healthy and in good condition, moderately saddle–exercised. Blood and hair sampling was preceded by a general veterinary check–up. In summer, apart from the time spent exercising, the horses stayed on pastures and in the paddock, while in the wintertime they spent 2–3 hours per day in the paddock. All the horses were fed on standard feeds consisting of hay, barley, and green forage, the feed rations being calculated based on Horse Feeding Standards (1991).

Blood and hair samples were collected twice during the year: in summer, end of September – beginning October, and in winter, end of March – beginning April. A total of 152 samples of blood serum and 152 samples of hair were analysed.

The samples were collected at the same time at each centre, between 8:00 and 10:00 hours. The blood was drawn from the external jugular vein. The serum obtained was frozen and the necessary amount was thawed prior to each analysis. The hair was sampled from the neck, from underneath the mane. Hair samples were weighed and combusted in a mixture of concentrated acids: 65% HNO_3 and 72% $HC1O_4$.

The analyses were performed in ICP EAS emission spectrophotometer (model JY 24). The concentrations in blood serum were expressed in micromole per litre, while those in the hair are given in milligram per kilogram of wet weight.

The results were treated statistically using the non–orthogonal design, one–way and two–way analysis of variance; statistically significant differences were further processed with Duncan's multiple range test. All the statistical analyses were run with Statgraphics 5.0 computer software.

RESULTS AND DISCUSSION

Cadmium

Cadmium in horse blood

As shown in Table 1, the lowest blood cadmium content in summer (0.027 micromole per litre) was recorded in the horses bred at Nowielice, while the highest concentrations were found in the horses kept at Bartoszewo and Ińsko (0.07 micromole per litre). In the wintertime, cadmium concentrations in the blood serum showed more variation, but were lower than those found in summer. The minimum and maximum values were 0.003 and 0.049 micromole per litre, respectively. The relevant Polish literature lacks data on cadmium concentrations in horse blood, except for our earlier studies [3], in which blood serum cadmium concentrations determined for Wielkopolski and Polish Konik horses kept in rural area did not exceed the detection limit. Maylin *et al.* [11] reported cadmium concentrations in horse blood to vary from 0.013 to 0.02 micromole per litre; the authors failed to show any increase in blood cadmium content after feeding the horses on cadmium–contaminated food for 6 weeks. In the opinion of Anke *et al.* [1], cadmium concentrations in horse blood in non–polluted areas is lower than 0.22 micromole per litre. Our results are much lower than that, both in the summer and in winter (Table l).

Table 1. Mean concentration of cadmium in horse blood serum depending on the stud-farm and season (micromole per litre)

Stud farm		Summer	Winter	Total
Nowielice	n	11	7	18
	$\bar{x}$	**0.027**	**0.003**	**0.017**
	S	0.032	0.0068	0.028
	V	122.00	264.58	161.98
Bielin	n	13	13	26
	$\bar{x}$	**0.051 A**	**0.015 A**	**0.033**
	S	0.021	0.0107	0.024
	V	42.07	73.99	75.86
Witkowo	n	12	11	23
	$\bar{x}$	**0.035 A**	**0.013 A**	**0.024**
	S	0.0100	0.0137	0.0162
	V	28.83	104.27	67.41
Bartoszewo	n	10	9	19
	$\bar{x}$	**0.07**	**0.049**	**0.06**
	S	0.027	0.0203	0.0256
	V	38.68	41.33	42.75
Osowo	n	13	13	26
	$\bar{x}$	**0.048**	**0.04**	**0.044**
	S	0.0634	0.0179	0.0459
	V	131.34	45.11	104.36
Ińsko	n	10	10	20
	$\bar{x}$	**0.070 A**	**0.043 A**	**0.056**
	S	0.0182	0.0194	0.023
	V	26.0	45.39	40.93
Gliwice	n	10	10	20
	$\bar{x}$	**0.042 A**	**0.011 A**	**0.026**
	S	0.0283	0.0102	0.0262
	V	67.47	95.03	99.34
Total	n	79	73	152
	$\bar{x}$	**0.048 A**	**0.025 A**	**0.037**
	S	0.0357	0.0219	0.0319
	V	73.88	87.34	86.11

Explanations:
n – number of horses,
x – mean,
S – standard deviation,
V – coefficient of variation,
←- - - → Means are significant at P below 0.05 (between stud farms),
a – Means in rows are significant at P below 0.05 (between seasons),
←——→ Means are significant at P below 0.01 (between stud farms),
A – Means in rows are significant at P below 0.01 (between seasons),
* – values are below detection level.

Cadmium in horse hair

Anke *et al.* [1], who studied cadmium concentrations in various horse tissues, concluded that hair was a very good indicator of exposure. In their opinion, cadmium concentrations in the hair of the horses kept in non–polluted areas should not exceed 0.1 milligram per kilogram.

Mean cadmium concentrations in the horse's hair found in this study are shown in Table 2. In summer, the lowest mean content (0.039 milligram per kilogram) was recorded in the horses kept at Ińsko, while the highest mean concentrations (0.997 milligram per kilogram) was found in the horses kept at Bielin. The lower winter cadmium concentrations in the blood serum were concurrent with the lower concentrations found in the hair. It was only at Osowo that the concentrations in both seasons were similar. In winter, the mean content at Ińsko did

not exceed the detection limit, while the maximum concentrations were recorded in the hair of the horses kept at Gliwice (0.111 milligram per kilogram). The overall mean cadmium concentrations in winter were significantly lower than those recorded in summer. Recently, lower winter cadmium concentrations (averaging 0.0056 milligram per kilogram) have been recorded in the hair of stallions of various breeds [4].

Table 2. Mean concentration of cadmium in horse hair depending on the stud-farm and season (milligram per kilogram wet weight)

Stud farm		Summer	Winter	Total
Nowielice	n	11	7	18
	$\bar{x}$	**0.270 A**	**0.015 A**	**0.163**
	S	0.212	0.042	0.206
	V	78.34	282.84	126.54
Bielin	n	13	13	26
	$\bar{x}$	**0.997 A**	**0.083 A**	**0.54**
	S	0.402	0.104	0.548
	V	40.28	125.27	101.43
Witkowo	n	12	11	23
	$\bar{x}$	**0.305 A**	**0.005 A**	**0.162**
	S	0.159	0.009	0.19
	V	52.04	171.34	117.43
Bartoszewo	n	10	9	19
	$\bar{x}$	**0.270**	**0.054**	**0.168**
	S	0.396	0.100	0.309
	V	146.64	186.72	184.1
Osowo	n	13	13	26
	$\bar{x}$	**0.084**	**0.086**	**0.085**
	S	0.081	0.116	0.098
	V	95.82	134.81	115.02
Iñsko	n	10	10	20
	$\bar{x}$	**0.039**	**0.0 ***	**0.019**
	S	0.068	0.0	0.051
	V	174.07	100	260.65
Gliwice	n	10	10	20
	$\bar{x}$	**0.231**	**0.111**	**0.171**
	S	0.223	0.192	0.213
	V	96.67	173.28	124.47
Total	n	79	73	152
	$\bar{x}$	**0.328 A**	**0.055 A**	**0.196**
	S	0.393	0.11	0.322
	V	119.90	201.06	164.57

Explanations as in Table 1.

Mean cadmium concentrations found during this study, particularly those recorded in winter, are comparable with the data we obtained previously from the hair of the Wielkopolski horses, ponies, and the Polish Koniks [6]. The concentrations in human hair were also found within the range of 0.01–1.66 milligram per kilogram [8, 15], i.e., the range comparable to that found in horses, both in previous studies and in this work.

Lead

Lead in horse blood

Mean summer and winter lead concentrations in the horse blood serum at different breeding centres are shown in Table 3.

Mean summer lead concentrations in the horse blood serum were found in the range from the minimum 0.09 micromole per litre, at Gliwice, to the maximum 0.44 micromole per litre, at Bielin. In winter, a decrease in the mean lead content in the blood serum was recorded at most of the centres. Mean winter lead concentrations were

found to vary from 0.06 to 0.17 micromole per litre. At two centres only a non–significant increase in the blood serum lead concentration in winter was found. Overall, the mean winter content of lead in the horse blood was significantly lower than that in summer.

Table 3. Mean concentration of lead in horse blood serum depending on the stud-farm and season (micromole per litre)

Stud farm		Summer	Winter	Total
Nowielice	n	11	7	18
	$\bar{x}$	**0.42**	**0.07**	**0.28**
	S	0.444	0.078	0.384
	V	106.73	105.79	135.74
Bielin	n	13	13	26
	$\bar{x}$	**0.44**	**0.07**	**0.25**
	S	0.780	0.109	0.579
	V	176.41	168.64	228.29
Witkowo	n	12	11	23
	$\bar{x}$	**0.12**	**0.17**	**0.14**
	S	0.089	0.19	0.148
	V	76.79	110.66	102.91
Bartoszewo	n	10	9	19
	$\bar{x}$	**0.30 A**	**0.06 A**	**0.19**
	S	0.232	0.091	0.216
	V	76.67	163.67	116.39
Osowo	n	13	13	26
	$\bar{x}$	**0.31**	**0.11**	**0.21**
	S	0.378	0.103	0.289
	V	123.67	96.15	140.49
Ińsko	n	10	10	20
	$\bar{x}$	**0.21 a**	**0.06 a**	**0.14**
	S	0.173	0.064	0.148
	V	81.38	99.02	106.91
Gliwice	n	10	10	20
	$\bar{x}$	**0.09**	**0.16**	**0.13**
	S	0.079	0.145	0.119
	V	84.27	88.19	92.34
Total	n	79	73	152
	$\bar{x}$	**0.28 A**	**0.10 A**	**0.19**
	S	0.411	0.125	0.319
	V	149.02	122.29	166.11

Explanations as in Table 1.

For most of the centres, the mean lead blood serum concentrations found in summer exceeded the physiological level, determined by Bohosiewicz [2] as 0.19 micromole per litre, while the winter concentrations were below that value at all the centres. The results obtained in our previous studies [3, 6] are comparable with the mean winter concentrations. Higher concentrations (0.217 micromole per litre), close to the summer means of this study, were reported by Tomaszewska–Guszkiewicz *et al.* [14] in the blood serum of the Polish Konik and the Norwegian Fjord horses.

Lead in horse hair

Mean lead concentrations in the hair of horses kept at different centres for the two seasons are shown in Table 4. Neither in summer, nor in winter did the lead concentrations of the horses kept at Ińsko exceed the detection limit, while the highest concentrations were recorded in the horses kept at Bielin (10.7 milligram per kilogram in summer and 2.46 milligram per kilogram in winter). At most of the centres, the mean lead content in the horses hair was significantly lower in winter than in summer.

Table 4. Mean concentration of lead in horse hair depending on the stud-farm and season (milligram per kilogram wet weight)

Stud farm		Summer	Winter	Total
Nowielice	n	11	7	18
	$\bar{x}$	**0.98**	**0.09**	**0.61**
	S	1.43	0.257	1.17
	V	145.06	282.84	192.00
Bielin	n	13	13	26
	$\bar{x}$	**10.7** A	**2.46** A	**6.58**
	S	4.88	2.1	5.59
	V	45.65	85.23	84.92
Witkowo	n	12	11	23
	$\bar{x}$	**9.1** A	**1.01** A	**5.23**
	S	7.03	1.06	6.5
	V	77.24	104.39	124.29
Bartoszewo	n	10	9	19
	$\bar{x}$	**4.64**	**0.39**	**2.63**
	S	6.37	0.484	5.02
	V	137.24	124.3	190.87
Osowo	n	13	13	26
	$\bar{x}$	**5.80**	**1.36**	**3.58**
	S	8.16	2.33	6.3
	V	140.61	170.83	175.8
Ińsko	n	10	10	20
	$\bar{x}$	**0.0** *	**0.0** *	0.0
	S	0.0	0.0	0.0
	V	100	100	100
Gliwice	n	10	10	20
	$\bar{x}$	**3.8** A	**0.67** A	**2.23**
	S	2.53	1.21	2.51
	V	66.56	180.92	112.55
Total	n	79	73	152
	$\bar{x}$	**5.27** A	**0.96** A	**3.18**
	S	6.28	1.62	5.11
	V	119.28	168.99	160.73

Explanations as in Table 1.

The data on lead concentrations in animal hair are generally scarce, which refers to horses as well. In our previous studies [3], detectable lead concentrations were found in the hair of few individuals, while the mean content was 1.99 milligram per kilogram. Concentrations recorded in human hair were found to range from 0.11 to 22.5 milligram per kilogram [8, 15].

The decrease in the mean horse blood serum lead content in winter, found at most of the centres, corresponded with observations reported by Monkiewicz and Żyła [12]. During 3 consecutive years of their 4–year study, they found significantly higher concentrations of lead in the blood serum of cows during the grazing season. They contended that the result was due to atmospheric lead being inhaled by the respiratory system of the cows and to lead–containing substances deposited on the hair and then licked off. During the grazing season, such behaviour is a substantial source not only of lead, but of other heavy metals as well.

It is very likely that the significantly lower winter concentrations of cadmium and lead in the bodies of the studied horses resulted from the limited exposure of the animals to atmospheric pollution by the minimal length of time they spent out of doors. In addition, effects of antagonistic elements, such as zinc, magnesium, selenium, iron, and calcium [7, 10] could be involved as well.

The correlation and regression coefficients calculated for the concentrations of the two heavy metals in horse blood serum and hair are presented in Table 5. The low positive correlation between cadmium and lead was reflected in the concurrent decrease in concentrations of the two metals in the blood and hair in winter.

Table 5. Correlation coefficients for contents of cadmium and lead in the horse blood serum and hair (n=152)

Cadmium	Lead
+0.102	+0.185

RECAPITULATION

The number of factors responsible for differences in cadmium and lead concentrations in horses was restricted – by using exclusively mares and geldings of the same breed – to those related to the season and the environment. Feeding is commonly known to be one of the environmental factors controlling concentrations of various elements in the animal body.

Pollutants emitted to the atmosphere by various industrial plants and cars are both an environmental factor and a substantial source of heavy metals. The present study confirmed this by demonstrating significantly higher concentrations of cadmium and lead in the horse blood and hair in summer when the animals, staying on pastures and in paddocks for most of the day, were exposed to atmospheric pollution.

Doubtlessly, an interpretation of the results obtained in this study is difficult due to the absence of generally accepted standards of physiological levels of cadmium and lead in the horse blood serum and in the hair, as well as of other heavy metals in the latter. The scant literature on cadmium and lead concentrations in the tissues in question makes unequivocal concluding difficult and justifies further studies along these lines.

The present study allows concluding that:

1. Mean concentrations of cadmium and lead in the horse blood serum in winter do not exceed critical limits.
2. Significantly higher blood and hair concentrations of cadmium and lead in summer demonstrate an adverse effect of exposure of the horses to environmental pollution.
3. The breeding centre whose horses showed the lowest cadmium and lead levels was the one situated in the Iński Landscape Park, an area considered environmentally clean.
4. To reduce the toxic effects of cadmium and lead in summer, magnesium, zinc or selenium supplementation of diets could be introduced, as these elements are antagonistic against cadmium and lead.
5. This study showed the horse's hair to be a good indicator of cadmium and lead concentrations in the body.
6. It is important that similar studies be continued to establish standards physiological levels of various elements in the horse hair, an easily available test material.

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