

HEAVY METAL STATUS OF POLISH KONIK HORSES FROM STABLE-PASTURE AND OUTDOOR MAINTENANCE SYSTEMS IN THE MASURIAN ENVIRONMENT

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Abstract

Among domestic animals, cattle and horses kept on pasture and fed with hay and straw are exposed most prominently to environmental pollution. Determination of the status of heavy metals in bodies of horses is important for two reasons. Firstly, it helps to assess the level of environmental contamination. Secondly, heavy metals can have considerable influence on animals living outdoors. The aim of the study has been to verify whether the level of Cr, Mn, Cu, Cd, Pb and Ni in the coat hair and cornified structures of hooves may differ between horses maintained alternately in a stable and on pasture and living outdoors all year long, with Polish Koniks from Mazury (the Masurian Lake District) taken as an example. Possible effects of the feeding season and age of horses were considered.

In total, 35 Polish Konik horses were examined, either kept in a stable-pasture system in Popielno or outdoors in Wojnowo. Sixty-two samples of summer and winter coat hair, 28 samples of hoof horn, as well as 10 samples of pasture plants and water were analyzed. The data were elaborated with the use multi-factor analysis of variance, T-Tukey-Kramer test and Pearson's correlation.

The results show that Polish Konik horses bred in Mazury have a low heavy metal concentration in the coat hair and hoof horn. The stable-pasture and outdoor maintenance systems hardly differentiate the element content, whereas the winter feeding results in

an elevated concentration of the elements in the hair. Yearlings show higher Mn and Cd content than mares and foals at foot. The dam's impact on the heavy metal concentration in the hair and hoof horn in their foals is usually non-significant.

Key words: horse, Polish Konik, heavy metal, coat hair, hoof horn.

ZAWARTOŚĆ METALI CIĘŻKICH U KONIKÓW POLSKICH W STAJENNO-PASTWISKOWYM I BEZSTAJENNYM SYSTEMIE UTRZYMANIA W ŚRODOWISKU MAZUR

Abstrakt

Spośród zwierząt gospodarskich bydło i konie utrzymywane na pastwisku oraz żywno sianem i słomą są najbardziej narażone na zanieczyszczenie środowiska. Zagadnienie koncentracji metali ciężkich u koni jest ważne pod dwoma względami: zanieczyszczenia środowiska i jego wpływu na zwierzęta utrzymywane w chowie bezstajennym. Celem pracy było ustalenie, czy poziom Cr, Mn, Cu, Cd, Pb i Ni w sierści i rogu kopytowym może różnić się u koni utrzymywanych w warunkach stajenno-pastwiskowych oraz żyjących przez cały rok w chowie bezstajennym, na przykładzie koników polskich na Mazurach. Uwzględniono możliwy wpływ sezonu żywieniowego i wieku konia.

Łącznie zbadano 35 koników polskich utrzymywanych w systemie stajenno-pastwiskowym w Popielnie i bezstajennym w Wojnowie. Przeanalizowano 62 próbki sierści letniej i zimowej, 28 próbek rogu kopytowego oraz 10 próbek roślinności pastwiskowej i wody. Dane opracowano wykorzystując wieloczynnikową analizę wariancji, test *T*-Tukeya-Kramera i korelacje Pearsona.

Wykazano, że koniki polskie hodowane na Mazurach mają niską zawartość metali ciężkich w sierści i rogu kopytowym. Stajenno-pastwiskowy i bezstajenny system utrzymania wpływa na niewielkie zróżnicowanie stężenia pierwiastków, natomiast żywienie zimowe ma wpływ na wzrost poziomu badanych pierwiastków w sierści. U roczniaków wykazano wyższą zawartość Mn i Cd niż u kłaczy dorosłych i sysaków. Wpływ kłaczy matek na koncentrację metali ciężkich w sierści i rogu kopytowym źrebiąt jest zazwyczaj nieistotny.

Słowa kluczowe: koń, konik polski, metale ciężkie, sierść, róg kopytowy.

INTRODUCTION

Out of domestic animals, cattle and horses maintained on pasture and fed with hay and straw are most prominently exposed to the environment pollution (ZNAMIROWSKA et al. 2004). Chemicals affect animals through the digestive and respiratory systems, as well as through the skin, hair and horn. The concentration of heavy metals is analyzed most often in blood, since the material can be collected *in vivo* (SUTTLE et al. 1996). However, the plasma content changes frequently, being highly influenced by the physiological status of a given organism (JAŚKOWSKI et al. 1993). The material showing more persistent concentration of elements in horses and easily sampled is the hair and hoof horn (WICHERT et al. 2002, ASANO et al. 2005). The content of some heavy metals in hair is influenced by the feeding season and –

in some cases – by the age of an animal (CIEŚLA, JANISZEWSKA 1997, 2000). The hoof horn grows continuously and special means would be necessary to determine which part appeared in a certain season of the year (STACHURSKA et al. 2009). However, the horn can probably absorb some elements from the ground at a rate that differs from season to season, depending on the presence of grass, mud, snow, etc.

The Polish Konik horses are a unique primitive breed, which originates from wild Tarpan living in the Central Europe and becoming extinct in the late 18th century. They are bred under stable-pasture conditions, outdoors on a pasture or paddock all year long or a free-ranging animals in Polish reserves, mainly in the regions called Mazury (N-E Poland) and Roztocze (S-E Poland). The environment around the largest Polish Konik breeding centers in Poland is dominated by forests and is assumed to be clean. The issue of the heavy metal status of horses is important for two reasons. First, it will attest whether the environmental contamination is low. Second, it will demonstrate how the given pollution affects animals living outside stables. According to KRUMRYCH et al. (1995), the Polish Koniks are highly responsive to environmental changes. However, the latest study by STACHURSKA et al. (2009) performed on the Polish Koniks in Roztocze National Park indicates that the heavy metal concentration in hair is only slightly affected by seasonal changes in the feeding. The generally low concentration the elements does not reveal possible influence of the differences between the reserve and stable-pasture maintenance systems or the animals' age.

The aim of the study has been to determine if the levels of Cr, Mn, Cu, Cd, Pb and Ni in the coat hair and hoof horn may differ between horses kept in a stable and on pasture versus the ones living outdoors, on pasture, all year. The Polish Koniks in Mazury served as an example.

MATERIAL AND METHODS

In total, 35 Polish Konik horses were examined: 21 in Popielno (9 dams, 12 foals) and 14 in Wojnowo (8 dams and 6 foals) in the Mazury region. The samples were taken twice at six-month interval, hence sucklings became yearlings in the second year. In total, there were 17 dams, 12 yearlings and 18 sucklings. The horses were clinically sound during the collection of the material.

Popielno lies 20 km away from Wojnowo. The horses examined in Popielno were maintained under stable-pasture conditions, whereas those in Wojnowo were kept outdoors. The maintenance conditions could differ in three aspects: the length of the time when horses stayed outdoors (connected with a possible impact of heavy metals in the air, rain, snow and ground), the content of elements in the feed and their content in drinking water.

In summer, the horses are on pasture all day and night in both centers. In Popielno, during winter, they are mainly in a stable, released to a paddock for a few hours a day. In Wojnowo, during winter, they stay on pasture all day and night. With respect to their nutrition, in neither of the centers the horses are fed additionally in summer. In winter, they are given oats, hay and some straw in Popielno. They also receive some carrots in November and December. In winter, in Wojnowo, they eat dry grass which remained after autumn on the pasture. Sometimes they have to dig it out from under the snow cover. Hay and some straw are given when the snow cover is thick. In Popielno, the horses drink water taken up from a water supply system and poured to a bath, as well as rainwater collected in ditches. In winter, they are given water from the supply system. In Wojnowo, the horses drink water from natural small ponds and ditches all year long. If they are frozen, the horses are given water taken from a supply system.

Sixty-two samples of coat hair (34 from the stable-pasture and 28 from the outdoor system; 28 in summer and 34 in winter; 18 from sucklings, 12 from yearlings and 32 from dams) and 28 samples of hoof horn were collected at the beginning of October and in the April next year. The hoof horn was collected from dams and sucklings in October. Simultaneously, three samples of plants from different pastures and seven samples of water from various sources were taken. The coat hair was cut from the horses' neck under the mane. The autumn coat shedding in Polish Koniks living in reserves begins in late August and lasts till mid-October (DETKENS 1967, STACHURSKA et al. 2006). The winter coat is shed from January until June. Hence, two main seasons of feeding were distinguished: summer season and winter season. The October samples contained the hair grown during the summer feeding and the April samples during the winter feeding. The horn was trimmed from the toe of a front hoof during routine trimming. The pasture plants, the major feed in summer and used for making hay for the other seasons, as well as all the water sources available for the horses were examined to randomly assess the heavy metal pollution of the environment. The plants were cut from 1 m² squares from sites most often grazed by the horses. The water samples were collected in plastic vessels.

The coat hair and hoof horn samples (the latter after fragmenting) were washed in 1% Triton-X100 solution and acetone. Then, they were rinsed three times in redistilled water, air-dried, crushed and homogenized. 0.5 g samples weighted at ± 0.0001 accuracy were digested in a Multiwave 3000, Anton Paar microwave stove. The level of Cr, Mn, Cu, Cd, Pb and Ni was determined in an atomic absorption spectrometer SpectrAA 220Z (electrothermal atomization, Zeeman background correction), Varian. Samples of plants were air-dried, crushed and homogenized. They underwent a similar mineralization procedure before determination of the heavy metal content as hair and horn samples. The water samples were filtered through a 0.45 μm Milipore filter. The AAS method was used directly after collecting the samples to define the heavy metal levels.

The SAS procedure, 9.1 version, was used to perform a multi-factor analysis of variance. The following effects were investigated: the maintenance system (stable-pasture, outdoor), the feeding season (summer, winter) and the horse's age (dam, yearling, suckling). The data were reported as means \pm SD (Standard Deviation). Multiple *T*-Tukey-Kramer test was performed to identify homogenous groups. The heavy metal levels in the hair and hoof between dams and their sucklings, between dams and yearlings, as well as between the hair and the horn, were analysed with Pearson's correlation coefficients.

RESULTS

Mean concentrations of heavy metals in the coat hair and hoof horn in Polish Konik horses, as well as in pasture plants and water available for the horses, are shown in Table 1. The Ni content in the samples was below the detection level. Since the concentrations were determined in completely different materials, they were not compared statistically. The Mn level was higher in the hoof compared to the hair, whereas that of Cu was lower. The content of the other elements in the hair and hoof was in a similar range. In pasture plants, Mn and Cd concentrations were relatively higher than in the hair and hooves. The Mn level in pasture plants was particularly high.

Table 1

Mean concentration of heavy metals in Polish Konik horses, pasture plants and water

Sample	N	Cr		Mn		Cu		Cd		Pb	
		mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
Coat hair (mg kg ⁻¹ d.m.)	62	1.03	0.66	9.39	5.90	2.92	0.43	0.01	0.00	0.22	0.41
Hoof horn (mg kg ⁻¹ d.m.)	28	1.31	0.45	21.13	9.62	1.10	0.36	0.00	0.00	0.39	0.13
Pasture plants (mg kg ⁻¹ d.m.)	3	0.84	0.30	245.45	77.23	2.05	0.48	0.22	0.13	0.35	0.06
Water sources (μ g dm ⁻³)	7	0.44	0.55	54.07	27.29	4.73	2.71	0.63	0.10	1.53	1.02

SE – standard error
d.m. – dry matter

The mean Cr content in the coat hair in Polish Konik horses was higher in the stable-pasture system than in the outdoor one, but similar in the hoof horn from both groups (Figure 1). More Cr was found in the hair grown in winter than in summer. The levels of the elements in the hair did not differ between the age groups. The Cr concentration in hooves was higher

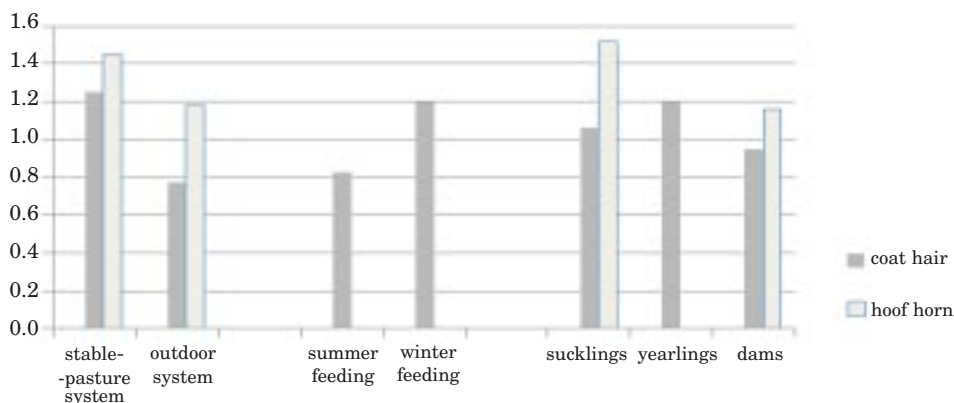


Fig. 1. Cr content (mg kg^{-1} d.m.) in coat hair and hoof horn in Polish Koniks. Significant differences in the hair between maintenance systems, feeding seasons and in the horn between age groups ($P=0.05$)

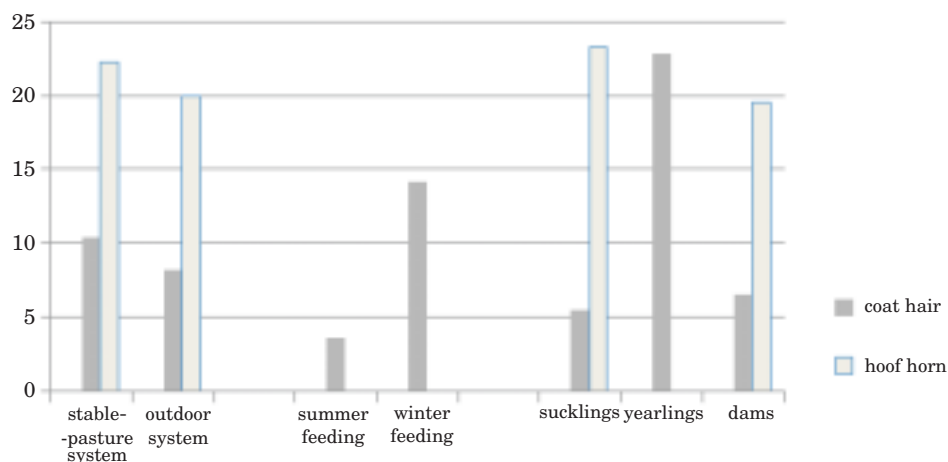


Fig. 2. Mn content (mg kg^{-1} d.m.) in coat hair and hoof horn in Polish Koniks. Significant differences in the hair between feeding seasons and age groups ($P=0.05$)

in sucklings than in dams. Differences in the Mn level occurred only in the hair between the summer and winter feeding and between the age groups (Figure 2). There was more Mn in winter hair and in yearlings compared to the other groups. The average Cu content was balanced except for the feeding seasons in the hair: it was higher in winter than in summer (Figure 3). In the coat hair, relatively more Cd occurred in the outdoor system, in winter and in yearlings (Figure 4). In the hoof horn, Cd was below the detection level. Considering the mean Pb content, differences were found with regard to the seasons, as well as to the maintenance systems

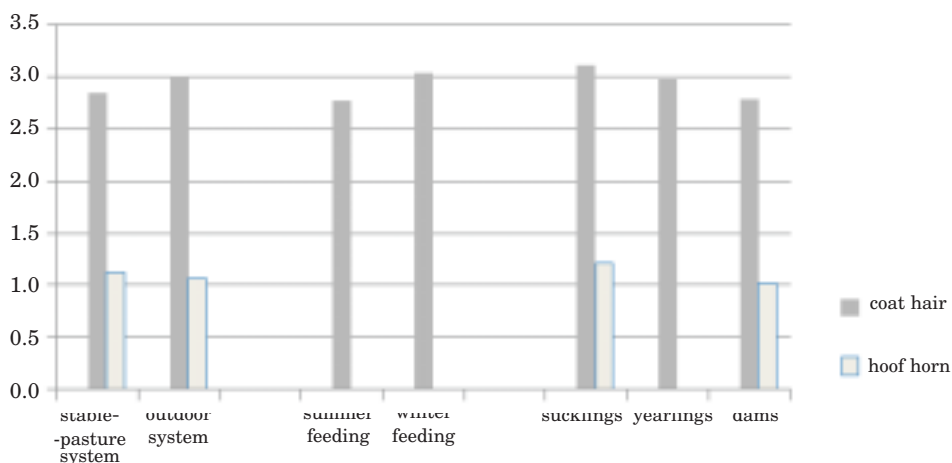


Fig. 3. Cu content (mg kg^{-1} d.m.) in coat hair and hoof horn in Polish Koniks. Significant differences in the hair between feeding seasons ($P=0.05$)

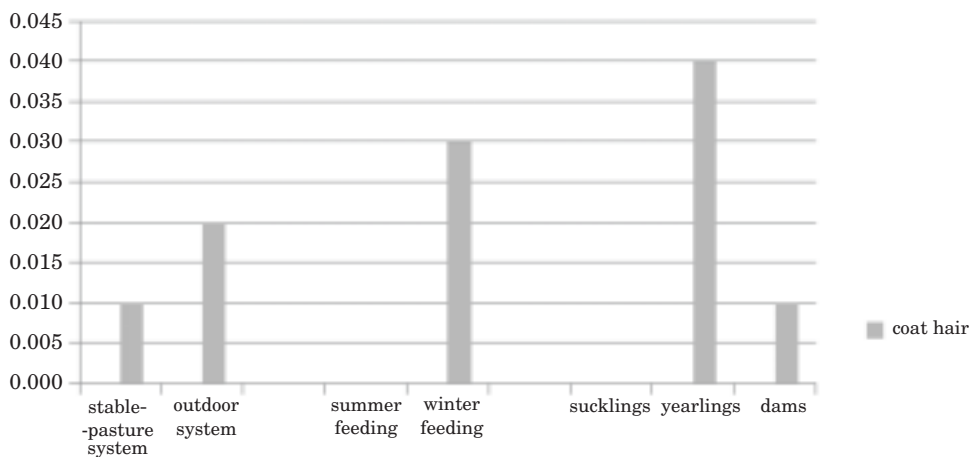


Fig. 4. Cd content (mg kg^{-1} d.m.) in coat hair in Polish Koniks. Significant differences in the hair between maintenance systems, feeding seasons and age groups ($P=0.05$)

(Figure 5). There was more Pb in winter hair and in the horn of horses kept in the outdoor system.

In the hair, significant correlations were found between the concentration of Cr versus Mn, Cd and Pb, of Mn versus Cd and Pb, as well as of Cd versus Pb (Table 2). In the hoof horn, it was only the correlation between Cr and Mn content that proved significant. The correlation between the hair and the hoof in their levels of particular elements was significant only in the case of Cr.

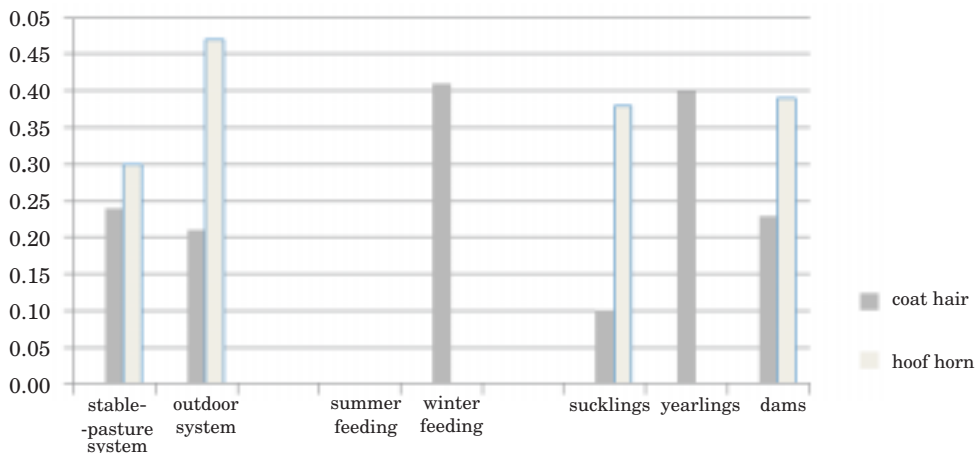


Fig. 5. Pb content (mg kg^{-1} d.m.) in coat hair and hoof horn in Polish Koniks. Significant differences in the hair between feeding seasons and in the horn between maintenance systems ($P=0.05$)

Table 2

Significant correlations ($P<0.01$) in heavy metal content within the coat hair (above the diagonal), within the hoof horn (below the diagonal) and between the coat hair and the hoof horn samples (along the diagonal)

Element	Hair-hoof	Cr	Mn	Cu	Cd	Pb
		coat hair				
Cr	hoof horn	0.52	0.43		0.31	0.61
Mn		0.64			0.66	0.38
Cu						
Cd						0.44
Pb						

The correlation of 0.68 ($P<0.01$) was found in the Cr level in the coat hair between dams and sucklings in summer. Correlations between the concentrations of the other elements in dams versus their sucklings and yearlings were not significant.

DISCUSSION

Generally, the content of heavy metals in coat hair and hoof horn of Polish Konik horses living in Mazury is low. The chromium levels found in the study are similar to the ones reported previously (ASANO et al. 2005,

STACHURSKA et al. 2009). The Mn content in the hair assessed in this study resembles the one determined in Roztocze (STACHURSKA et al. 2009), but is lower than 17.24 mg kg^{-1} reported by CIEŚLA and JANISZEWSKA (1997) or 14.19 mg kg^{-1} found by CIEŚLA (2002). However, it exceeds the concentration in the horse mane hair (1.24 mg kg^{-1}) observed by ASANO et al. (2005). According to KOWNACKI (1962), the Mn level in the hoof horn was 0.8 mg kg^{-1} in reserve horses and 1.0 mg kg^{-1} in stabled horses, hence it was several-fold lower than in the present study. SASIMOWSKI et al. (1987) also observed low Mn levels ($2\text{-}5 \text{ mg kg}^{-1}$) in hooves in cold-blood and warm-blood horses. It should be pointed out that Mn plays an important role in bone mineralization and reproduction, hence its deficiency may cause disorders in the organism (CIEŚLA 2002). The Cu concentration in the hair is slightly lower and in the hoof horn similar as in Roztocze (STACHURSKA et al. 2009). Meanwhile, the content of copper in the hair is approximately two-fold lower and in the hoof horn several-fold lower than observed by other researchers (SASIMOWSKI et al. 1987, CIEŚLA, JANISZEWSKA 1997, 2000, WICHERT et al. 2002). It can be suggested that there is some Cu deficiency in Polish Koniks maintained in the two analysed systems. REIWALD and RIOND (2002) show that a traditional ration of oats and hay as well as many commercial feeds do not contain sufficient amounts of copper for adult horses. Cu and Mn are antagonistic elements, although in the present study it does not seem that the high Mn level caused a low Cu content in the environment. Copper deficiency in horses may result in *physitis*, *osteocondrosis*, *osteoporosis*, as well as *fracturae spont* (GEHRKE 1997). The Cd level in the hair was slightly lower or not detectable compared to the horses living in Roztocze (STACHURSKA et al. 2009) or in Dobrzyniewo in Wielkopolska (CIEŚLA, JANISZEWSKA 1997). Concentrations of Pb are of a similar order as determined in Roztocze and lower than found by ASANO et al. (2005).

Concentrations of heavy metals in the coat hair and hoof horn of horses maintained in stable-pasture and outdoor systems are in most cases similar. It seems that the element levels, except for Mn in the hoof horn, are too low to show significant differences between the two breeding centers. The two systems, i.e. stable and pasture or entirely outdoors, do not differ in the effect on the horse's status regarding heavy metals. This finding is similar to that determined for stabled and reserve Polish Konik horses in Roztocze (STACHURSKA et al. 2009).

In turn, the impact of the feeding season on levels of elements in hair shows that in winter the content of heavy metals is higher than in summer. This regularity appeared both centers (the data not presented in tables due to the paper's limited length). Probably, the differences result from the environmental seasonal changes. In Roztocze, it was only Mn that occurred in higher concentration in winter hair (STACHURSKA et al. 2009). CIEŚLA and JANISZEWSKA (2000) found a higher Cu level in the coat hair in halfbred horses in summer than in winter.

Heavy metals, particularly Mn and Cd, tend to accumulate in higher amounts in hair of yearlings compared to dams or sucklings. In the hoof, the element levels in dams and sucklings are similar in nearly all the cases. In Rostocze (STACHURSKA et al. 2009), the Mn concentration was higher in yearlings' hair as compared to dams. In a growing organism, the demand for nutritional components, including minerals, is the highest (GEHRKE 1997). That may explain the elevated Mn content in the yearlings' coat. However, ASANO et al. (2005) did not find the age effect on the content of heavy metals in the hair.

In general, the differences between levels of heavy metal in the feed versus the hair and hoof horn were small, except an exceptionally high Mn content in plants. It is likely that the Mn and Cd concentrations in the pasture flora are not high enough to accumulate in considerable amounts in the horses' hair and hoof. It has been documented that exposure to elevated levels of heavy metals in the environment is reflected by high concentrations of these elements in horses, although the path from ingestion or absorption to deposition in animals' tissues is not direct (WICHERT et al. 2002, STACHURSKA et al. 2009). Therefore, differences in the degree of heavy metal contamination between the feed and the animal can appear.

In both centers, the water drunk by the horses can be classified as class one (the highest) in the Polish water purity classification system with regard to Cr, Cu and Pb, between class one and two considering the Mn content and class two in respect of Cd (*Regulation of the Minister for the Environment, 11 February 2004*). Hence, the water does not pose a threat to the horses in terms of toxicology.

The correlations between levels of different elements are mainly found in the hair, therefore suggesting that in the hooves they can be more strongly dependent on external factors, e.g. concentrations of metals in the ground. Since the concentrations of the analysed elements in the hair and in the hoof were most often not correlated between dams and their foals, it seems that the effect produced by dams is weak.

CONCLUSION

In conclusion, the Polish Konik horses bred in Mazury have low heavy metal concentrations in the coat hair and hoof horn. The stable and pasture or the outdoor maintenance system hardly differentiated the content of elements, whereas the winter feeding resulted in elevated concentrations of the elements in the hair. Yearlings showed a higher Mn and Cd content than mares and foals at foot. The dams' impact on the heavy metal concentration in the hair and hoof horn in their foals is usually negligible.

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