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ORIGINAL PAPER

MINERAL COMPOSITION OF THE HAIR COAT OF POLISH KONIK HORSES RAISED IN NATURE RESERVES AND BARNS*

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Abstract

The aim of this study was to determine the effect of environmental and management conditions on the mineral composition of the hair coat of Polish Konik horses at the Research Station for Organic Farming and Conservation Breeding of the Polish Academy of Sciences in Popielno. The hair mineral content was compared in Koniks raised in a barn and a nature reserve. The foals bred in the reserve were divided into two groups based on the area occupied by the herds from which they originated. Hair samples were collected from 53 Koniks born over two consecutive years. Green forage and hair samples were analyzed to determine the concentrations of macroelements (P, K, Mg, Ca and Na) and microelements (Cu, Zn, Mn and Fe). Water quality was evaluated based on its pH and the concentrations of $N\text{-}NO_3$, $N\text{-}NH_4$ $P\text{-}PO_4$, K, Ca, Mg, Na and Cl. The hair coats of foals raised in the reserve had a significantly higher content of K and Na than the hair coats of stabled Koniks. Significant differences in the concentrations of K, Na, P and Ca in the hair coat were also noted between the two groups of horses in the reserve. The hair coats of foals born and raised in a stable had a higher content of Fe, Mn, and Cu. No significant differences in the hair macromineral content were found between the sexes. As regards microminerals, higher concentrations of Fe and Mn were determined in the hair coats of mares. Environmental and management conditions exerted a significant effect on the mineral composition of the hair coat of Polish Konik horses. Differences were observed not only between foals raised in a barn and a nature reserve, but also between Koniks kept in the reserve.

Keywords: horse, Polish Konik, hair, minerals, nature reserves, barn.

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INTRODUCTION

A horse's diet should supply adequate amounts of energy, protein and essential minerals, which perform important structural, catalytic and regulatory functions (Reis et al. 2010). Mineral elements are found in the structural components of bones and soft tissues, they are involved in enzyme activity, regulate the pH and osmotic pressure of body fluids and tissues. In horses, the most important macronutrients are calcium and phosphorus. They are essential for bone health, the heart muscle and other muscles, blood clotting and transmission of nerve impulses **(**Dobrowolski et al. 2009). The absorption of calcium and phosphorus is regulated by magnesium, which is important for the proper central nervous system activity. Sodium and chlorine are necessary for maintaining the acid-base balance and the osmotic pressure of interstitial fluid.

Micronutrients are also essential for healthy growth and development. Copper, an antioxidant nutrient, is required for normal bone and cartilage growth, and it plays an important role in the immune system's function. Iron forms complexes with molecular oxygen in hemoglobin and myoglobin, which affect the central nervous system's function and enhance immune responses. Zinc is essential for the immune and reproductive systems (Ott, Johnson 2001, Danek 2002, Ali et al. 2013). Manganese activates enzymes that catalyze many redox reactions, and regulates the functions of the central nervous and reproductive systems (Cieśla, Janiszewska 2000*a*).

The mineral status of horses can be determined using appropriate biological material such as hair. The high diagnostic value of the hair mineral content has been demonstrated by numerous authors, including Stachurska et al. (2004), asano et al. (2005), Dunnett (2005) and topczewska (2012). Asano et al. (2006) emphasized high stability of the mineral composition of a horse's hair coat. Hawkins, Ragnarsdottir (2009) pointed to high concentrations of minerals in a horse's hair coat as compared with other biological materials, and high accumulation of hair minerals in horses. Biogenic elements are incorporated into growing hair, and hair analysis provides information about changes in mineral concentrations over longer periods of time (Dunnett, Lees 2003, Budzyński et al. 2006). Non-invasive sample collection and storability of samples, if needed, are important considerations (Gabryszczuk et al. 2010). The results of hair mineral analysis can be used to evaluate the metabolic status of horses and to determine mineral deficiencies in their habitats, thus contributing to health prophylaxis (Dunnett, Lees 2003).

Research findings have confirmed that the determination of a mineral status can support the diagnosis of diseases and disorders in horses, which are usually accompanied by changes in mineral concentrations. Suzuki et al. (2007) demonstrated that the content of Ca, Cu, Mg and Zn in mane hair is correlated with the severity of second-degree atrioventricular block and could help predict a horse's susceptibility to this disease long before the develop-

ment of any symptoms. According to Marycz et al. (2013), analysis of horse hair can support the diagnosis of the Equine Metabolic Syndrome (EMS). Asano et al. (2006) reported an increase in Ca and Zn concentrations in mane hair in horses diagnosed with atrial fibrillation. Microminerals are analyzed in hair coats more frequently than macrominerals (Humann-Zehank et al. 2008), and copper and zinc are considered to be reliable indicators of an adequate micronutrient supply in horses (Cieśla, Janiszewska 2000b).

The present research was undertaken to test the hypothesis whether the diet of Polish Konik horses, related to management and habitat conditions, affects the mineral composition of their hair. The objective of this study was to determine the effect of environmental and management conditions (barn vs. nature reserve) on the concentrations of microminerals and macrominerals in the hair coats of Polish Koniks.

MATERIAL AND METHODS

A total of 53 Polish Konik horses (Table 1), born over two consecutive years in a barn and nature reserve under different environmental conditions, were examined in the study. Nature reserve A comprised grasslands which could be used for grazing horses and extensive areas occupied by peatland and mid-forest meadows, including marshy grounds surrounding Lakes Śniardwy and Warnołty. Nature reserve B comprised areas which were more elevated and drier, with a lower share of meadows, where horses could graze grass in the forest and in the littoral belt around Lake Bełdany. In both years of the study, observations were carried out in the summer months, when horses born in a nature reserve remained inside the reserve, and horses born in a barn grazed on pasture. The sex of horses was also taken into account in the analysis. The experimental materials consisted of 10 replicate samples of green forage harvested in reserves A and B and in pastures grazed by stabled horses, and 500 mg hair samples cut out in the withers region. Hair samples were treated with 1% Triton X-100 and acetone, rinsed with redistilled water, dried and ground.

Fodder and hair samples were analyzed to determine the concentrations of macroelements (P, K, Mg, Ca and Na) and microelements (Cu, Zn, Mn and

Table 1

Experimental design

Experimental factor	Barn		Nature reserve A		Nature reserve B	
Sex (S)	♀	♂	♀	♂	♀	♂
Location (L)	+	+	+	+	+	+
Number of horses in used in experiment	16	11	5	9	5	7

Fe). The content of K, Mg Na, Ca, Zn, Cu, Fe and Mn in the samples was determined by atomic absorption spectrometry with the use of a VARIAN AA240FS atomic absorption spectrophotometer, by measuring the absorbance of a given element at a specific wavelength after sample mineralization in strong acids HCl+HNO_3 (3:1 ratio) in a MARS microwave mineralizer. Microelements were determined at the following wavelengths: Zn – 2013.8 nm, Cu – 327.4 nm and Mn – 279.5 nm. The phosphorus content was measured colorimetrically with the use of an Epoll 20 BIO spectrophotometer.

All animals had free access to water. Ten replicate water samples were collected from lakes surrounding the peninsula and from drinkers in the barn, to determine the standard quality parameters of water, including the concentrations of K, Ca, Mg, Na and Cl, pH, N-NO_3, N-NH_4 and P-PO_4. The content of K, Ca, Mg and Na was analyzed using a VARIAN AA240FS flame atomic absorption spectrometer, while the Cl content was determined by the potentiometric method with an Orion 4 Star ion meter. Water pH was determined using a Hanna Instruments HI 9321 microprocessor pH meter. The concentrations of N-NO_3 and N-NH_4 were analyzed by flow spectrometry using a Skalar San plus analyzer. The P-PO_4 content was determined by spectrophotometry with a Specol 11 spectrocolorimeter.

The data were analyzed by one-way ANOVA and two-way MANOVA for non-orthogonal designs. Arithmetic means were calculated. The significance of differences between means was determined by the Duncan's test. All calculations were performed using Statistica ver. 10.0 PL software.

RESULTS AND DISCUSSION

Green forage samples differed ($P \leq 0.01$) in their content of macrominerals**.** Fodder fed to stabled horses had higher concentrations of P, K and Ca (Table 2). Smaller differences were found between green forage samples collected in the reserve. Forage harvested in drier areas, with a lower share of

Table 2

Macromineral content of green forage (X ± SD)

Forage origin	Macrominerals (g kg^{-1} d.m.)				
	P	K	Mg	Ca	Na
Pasture (stabled Koniks)	3.62^A ± 0.42	37.66^A ± 1.44	1.88 ± 0.40	7.63^A ± 1.41	0.19^B ± 0.06
Nature reserve A	2.28^B ± 0.41	20.52^B ± 1.84	1.88 ± 0.30	5.49^B ± 0.59	0.18^B ± 0.07
Nature reserve B	2.76^B ± 0.47	21.35^B ± 1.62	1.90 ± 0.41	4.76^B ± 0.42	0.28^A ± 0.07
SEM	0.13	1.69	0.06	0.27	0.02
Significance of differences	**	**	n.s.	**	**

X – arithmetic means, SD – standard deviation, SEM – standard error of mean, $^{**\,(A,\,B)}$ $P \geq 0.01$, n.s. – non-significant differences

meadows, where horses could graze grass in the forest and in the littoral belt around Lake Bełdany (nature reserve B), had a higher content of P, K and Na. Greater differences ($P \leq 0.01$) were observed in the content of microminerals in fodder (Table 3). In comparison with the forage harvested in

Table 3

Micromineral content of green forage (X ± SD)

Forage origin	Microminerals (mg kg^{-1}d.m.)			
	Cu	Zn	Mn	Fe
Pasture (stabled Koniks)	$7.51^{A} \pm 0.48$	$32.32^{B} \pm 1.87$	$143.77^{B} \pm 17.38$	$182.3^{Aa} \pm 17.92$
Nature reserve A	$6.08^{B} \pm 0.48$	$46.79^{A} \pm 2.99$	$444.51^{A} \pm 38.87$	$138.20^{B} \pm 27.74$
Nature reserve B	$6.44^{B} \pm 0.35$	$44.68^{A} \pm 2.21$	$397.58^{A} \pm 28.31$	$166.35^{b} \pm 28.85$
SEM	0.19	1.44	25.84	5.56
Significance of differences	**	**	**	**

X – arithmetic means, SD – standard deviation, SEM – standard error of mean, $^{**\ (A, B)}$ $P \geq 0.01$

reserves A and B, the forage harvested in pastures grazed by stabled horses had a higher Cu content (7.51 mg kg^{-1} d.m. vs. 6.08 and 6.44 mg kg^{-1} d.m., respectively) and a higher Fe content. The Mn content of fodder samples collected in reserves A and B was nearly three-fold higher than that determined in fodder consumed by stabled horses (444.51 and 397.58 mg kg^{-1} d.m. respectively, vs. 143.77 mg kg^{-1} d.m.).

Despite the differences in the concentrations of biogenic elements in green forage, the noted values were consistent with the relevant standards (Nutrient Requirements of Horses (2007), Brzóska et al. 2015). The parameters of water consumed by horses, presented in Table 4, indicate that its quality conformed to the standards for class 1 surface water bodies. The pH of water samples (7.75-7.82) remained within the range typical of class 1 surface water bodies (6.0-8.5) and was consistent with the Regulation of the Minister of Environment (Journal of Laws 2014, item 1482). The concentrations of nitrate nitrogen, ammonium nitrogen and phosphates were also within the limit values established for class one surface water bodies. Water from the stable contained statistically more K, Ca, Mg than water from reserves A and B. There were no significant changes in the concentration of Na and Cl depending on the origin of water.

The concentrations of biogenic elements in hair samples collected from Koniks varied widely with regard to both microminerals and macrominerals. In the group of macrominerals, no significant differences were found in the Mg content of hair samples between groups (0.73-0.80 g kg^{-1}), and the noted values were consistent with those obtained by Cieśla, Janiszewska (2000*b*) but slightly higher than those reported by Kupczyński et al. (2014). Highly significant differences were noted in the content of K and Na, which was higher in the hair coats of horses raised in the reserve.

Table 4

Water quality parameters (X ± SD)

Water origin	pH	Content (mg dm^{-3})							
		$N\text{-}NO_3$	$N\text{-}NH_4$	$P\text{-}PO_4$	K	Ca	Mg	Na	Cl
Barn	7.82 ± 0.10	0.18 ± 0.08	0.08 ± 0.05	0.21 ± 0.05	3.47^{A} ± 0.24	89.26^{A} ± 17.73	10.06^{a} ± 0.98	4.89 ± 1.12	21.67 ± 0.97
Nature reserve A	7.82 ± 0.25	0.17 ± 0.07	0.15 ± 0.02	0.17 ± 0.03	2.98^{ABa} ± 0.47	70.39^{Ba} ± 9.13	9.41^{ab} ± 0.36	5.51 ± 1.11	13.77 ± 1.87
Nature reserve B	7.75 ± 0.19	0.21 ± 0.10	0.12 ± 0.08	0.19 ± 0.11	2.17^{Bb} ± 0.20	64.92^{Bb} ± 11.56	8.62^{b} ± 0.84	4.34 ± 1.57	8.90 ± 1.82
SEM	0.06	0.02	0.01	0.01	0.17	3.03	0.39	3.20	0.69
Significance of differences	n.s.	n.s.	n.s.	n.s.	**	**	*	n.s.	n.s.

X – arithmetic means, SD – standard deviation, SEM – standard error of mean, $^{**\,(A,\,B)}$ $P \geq 0.01$, $^{*\,(a,\,b)}$ $P \geq 0.05$, n.s. – non-significant differences

Table 5

Hair mineral content in Polish Konik horses (X ± SD)

Minerals	Location (L)			Sex (S)		SEM	Variance analysis P≥		
	barn	nature reserve A	nature reserve B	♀	♂		L	S	L x S
Macrominerals (g kg^{-1})									
K	$1.35^{A} \pm 0.32$	$4.42^{B} \pm 1.26$	$6.83^{C} \pm 2.14$	3.36 ± 1.32	3.53 ± 1.32	0.380	0.00	0.07	0.25
Mg	0.79 ± 0.12	0.80 ± 0.19	0.73 ± 0.20	0.79 ± 0.15	0.77 ± 0.18	0.022	0.52	0.77	0.26
Na	$0.48^{A} \pm 0.15$	$1.00^{B} \pm 0.18$	$1.31^{B} \pm 0.16$	0.82 ± 0.12	0.81 ± 0.12	0.085	0.00	0.38	0.65
P	$0.47^{B} \pm 0.04$	$0.46^{B} \pm 0.10$	$0.32^{A} \pm 0.06$	0.42 ± 0.09	0.44 ± 0.07	0.018	0.00	0.45	0.01
Ca	$0.36^{b} \pm 0.06$	$0.35^{b} \pm 0.06$	$0.21^{a} \pm 0.07$	0.31 ± 0.05	0.33 ± 0.08	0.025	0.05	0.44	0.64
Microminerals (mg kg^{-1})									
Fe	$331.37^{Bb} \pm 11.53$	$265.70^{Ba} \pm 12.08$	$148.02^{A} \pm 17.53$	282.6 ± 18.06	261.0 ± 14.60	15.92	0.00	0.32	0.02
Zn	117.0 ± 10.14	117.7 ± 9.77	112.6 ± 9.23	116.9 ± 10.78	115.5 ± 9.18	1.36	0.45	0.63	0.89
Mn	$33.39^{B} \pm 5.98$	27.87 ± 3.88	$18.60^{A} \pm 4.82$	30.44 ± 6.61	26.68 ± 5.91	1.96	0.02	0.82	0.39
Cu	$6.55^{B} \pm 0.77$	$5.76^{A} \pm 0.84$	$5.31^{A} \pm 0.72$	5.96 ± 0.94	6.14 ± 0.93	0.12	0.00	0.09	0.88

X – arithmetic means, SD – standard deviation, SEM – standard error of mean, ^{A,B} $P \geq 0.01$ ^{a,b} $P \geq 0.05$

The habitat conditions in the reserve affected the K concentration in hair samples. The K content of hair of stabled foals (1.35 g kg^{-1}) and foals raised in reserves A and B (4.42 and 6.83 g kg^{-1}) can be considered as high. According to Krupa et al. (2006), the average K content of a horse's hair coat is 0.09 g kg^{-1}, and according to Budzyński, Truchliński (2004), it is 0.24 g kg^{-1}. In the reported study, the average Na content of horse's hair was determined at 0.22 g kg^{-1}, whereas higher Na concentrations were noted in our study: 0.48 g kg^{-1} in stabled horses, and 1.00 and 1.31 g kg^{-1} in horses raised in reserves A and B, respectively.

The content of Ca and P was higher in the hair coats of Koniks in reserve A, and the noted values were similar to those reported by Dobrowolski et al. (2009) in Thoroughbred yearlings. Significant differences in the concentrations of K, Na, P and Ca in the hair coat were also found between the two groups of Polish Konik horses in the reserve. It seems that the observed differences, at similar levels of minerals determined in green forage and water samples, could result from the fact that the horses grazed on large areas and could choose various forages.

Habitat and management conditions exerted varied effects on the hair micromineral content in Polish Konik horses. Considerable differences were found in Fe concentrations between the groups. In comparison with the value reported by Krupa et al. (2006) and Budzyńska et al. (2006), the hair coats of Koniks raised in a barn and reserve A had very high Fe content (331.37 and 265.70 mg kg^{-1}, respectively). A similar Fe concentration (346.88 mg kg^{-1}) was determined by Jancikowa et al. (2012) in mane hair, and in an earlier study by Kownacki (1962), who reported the hair Fe content of 150 - 400 mg kg^{-1}. Substantially lower hair Fe content was noted in horses raised in reserve B (148.02 mg kg^{-1}). There were no significant differences in the Zn levels in the hair coats of Koniks (barn - 117.0, reserve A – 117.7, reserve B – 112.6 mg kg^{-1}), which is consistent with the findings of Stanek et al. (2016) and Asano et al. (2006). (2005). According to Truchliński et al. (2004), the hair Zn content lower than 160 mg kg^{-1} may point to an insufficient Zn intake. The hair Mn content was higher in stabled horses, and similar to that reported by Stanek et al. (2016). Cieśla, Janiszewska (1997), Kupczyński et al. (2014) noted a substantially lower content of Mn in a horse's hair coat. In a study by Stachurska et al. (2009), the hair Mn content was below 20 mg kg^{-1}. The hair Cu content in Polish Konik horses ranged from 5.31 to 6.55 mg kg^{-1}, and was comparable with that reported by Cieśla et al. (2000b). The values noted by Budzyński, Truchliński (2004) differ considerably from our findings, whereas in a study by Budzyńska et al. (2006) the Cu concentration in the coats of Arabian horses reached approximately 2 mg kg^{-1}. Biricik (2005) demonstrated that environmental factors had no significant influence on the hair micromineral content in horses. The results of the current study do not support the above findings. No significant differences in hair macromineral content were found between the sexes. However, higher concentrations of Fe and Mn were determined in the hair coats of mares.

According to Hintz (2000), analyses of the effects of a diet and environmental conditions on the mineral composition of the hair coat of horses should account for the impact of other factors that could also affect concentrations of biogenic elements in hair.

It should be noted, however, that the possibility to determine the influence of forage consumed by horses in the nature reserve on the mineral composition of their hair coats is limited because the areas where animals could graze were extensive and characterized by high species diversity. Grazing Koniks could select forage whose mineral composition had not been comprehensively analyzed.

CONCLUSIONS

The results of this study indicate that environmental and management conditions exert a significant effect on the mineral composition of the hair coat of Polish Konik horses.

The hair coats of foals raised in the reserve had a higher content of K and Na than the hair coats of stabled Koniks. Significant differences in the concentrations of K, Na, P and Ca in the hair coat were also noted between two groups of horses in the reserve. The hair of horses born and raised in a stable had a higher content of Fe, Mn, and Cu. No significant differences in the hair macromineral content were found between the sexes, but higher concentrations of microminerals Fe and Mn were determined in the hair coats of mares. Apart from differences resulting from the environmental and management conditions, differences were also observed in the mineral composition of the hair coat between Koniks kept in the reserve, which were related to their diet and habitat conditions.

Changes in the concentrations of mineral elements in the hair coats of Polish Konik horses, depending on environmental factors and feeding choices, suggest that a hair mineral profile has a high diagnostic value and could contribute to effective disease prevention and treatment in horses.

REFERENCES

Ali F., Lodhi L.A., Qureshi Z.I., Ahmad I., Hussain R. 2013. *Serum mineral profile in various reproductive phases of mares.* Pak. Vet. J., 33: 296-299.

Asano K., Suzuki K., Chiba M., Sera K., Matsumoto T., Asano R., Sakai T. 2005. *Correlation between 25 element contents in mane hair in riding horses and atrioventricular block.* Biol. Trace Elem. Res., 108(1): 127-135.

Asano K., Suzuki K., Chiba M., Sera K., Asano R., Sakai T. 2006. *Relationship between trace elements status in mane hair and atrial fibrillation in horse.* J. Vet. Med. Sci., 68(7): 769-771.

Biricik H., Ocal N., Gucus A I., Ediz B., Uzman M. 2005. *Seasonal changes of some mineral status in mares.* J. Equine Vet. Sci., 25(8): 346-348.

Brzóska F., Strzetelski J.A., Borowiec F., Jamroz D. 2015. *Feeding recommendations for horses and feed tables.* Kraków (in Polish).

Budzyńska M., Krupa W., Sołtys L., Sapuła M., Kamieniak J., Budzyński M. 2006. *Level of bioelements in purebred Arabian horses' hair*. Ann. UMCS Lublin, sect. EE, 24(28): 199-207.

Budzyński M., Sołtys L., Budzyńska M., Mazurek E., Sapuła M., Kamieniak J. 2006. *Relationship between nervous excitability and the level of minerals in hair Arabian horses*. Ann. UMCS Lublin, sect. EE, 30: 217-225. (in Polish)

Budzyński M., Truchliński J. 2004. *Assessment of the composition of bioelements contained in the horse body on the basis of their analysis in the hair coat*. Ann. UMCS Lublin, sect. EE, 27: 253-261.

Cieśla A. , Janiszewska J. 1997. *Comparing the level of selected elements in the blood serum and hair of horses of the Polish Wielkopolska and Polish Konik breeds*. Zesz. Nauk. AR Szczecin Zoot., 35: 259-265. (in Polish)

Cieśla A., Janiszewska J. 2000*a*. *Relationship between magnesium-lead and zinc-cadmium in hair of halfbred horses*. Fol. Univ. Agric. Stetin. Zoot., 38: 21-26. (in Polish)

Cieśla A., Janiszewska J. 2000b. *Concentrations of some elements in stallion's coat of different*. Fol. Univ. Agric. Stetin., Zoot., 40: 273-276.

Danek J. 2002. *The importance of zinc in a breeding stallion*. Med. Wet., 58: 840-844. (in Polish)

Dobrowolski M., Jodkowska E., Marycz K., Lisowska K. 2009. *Effect of nutrition on calcium and phosphorus contents in coat and hair of thoroughbred yearling*. Zesz. Nauk. UP Wrocław, 59(575): 77-86.

Dunnett M. 2005. *Hair analysis for screening horses for exposure to dietary toxic residues*. Pferdeheilkunde, 21: 457-467.

Dunnett M., Lees P. 2003. *Trace element, toxin and drug elimination in hair with particular reference to the horse*. Res. Vet. Sci., 75: 89-101.

Gabryszczuk M, Sloniewski K, Metera E, Sakowski T. 2010. *Content of mineral elements in milk and hair of cows from organic farms*. J Elem., 15(2): 259-267.

Hawkins D.P., Ragnarsdottir K.V. 2009. *The Cu, Mn and Zn concentration of sheep wool: Influence of washing procedures, age and colour of matrix*. Sci Total Environ. 407: 4140- 4148.

Hintz H.F. 2000. *Hair analysis as an indicator of nutritional status*. J. Equine Vet. Sci., 2: 199.

Humann-Zehank E., Genter M., Hennig-Pauka I., Binder A. 2008. *Trace mineral status and liver and blood parameters in sheep without mineral supply compared to local roe deer (Capreolus capreolus) populations*. Small Rumin. Res., 75: 185-191.

Jančíková P., Horký P., Zeman L. 2012. *The effect of various copper sources on the trace elements profile in the hair, plasma and faeces and copper activity in the organism of horses*. Acta Univ. Agric. Silvic. Mendelianae Brun, 6: 145-151.

Kownacki M. 1962. *An estimation of the supply of some minerals in horses, based on the level of these elements in the horn tissue of the hoof and in the coat*. Rocz. Nauk Rol., 80(B-4): 519-538. (in Polish)

Krupa W., Sołtys L., Budzyńska M., Sapuła M., Kamieniak J., Budzyński M. 2006. *Rating mineral composition of hair of Arabian mares including genealogical lines*. Ann. UMCS Lublin, sect. EE, 24(29): 209-216. (in Polish)

Kupczyński R., Śpitalniak K., Śmieszek A., Popiel J. 2014. *Chemical-biological evaluation of foragers mineral supplements*. Przem. Chem., 93(7): 1219-1222. (in Polish)

Marycz K., Toker N.Y., Czogaa J., Michalak I., Nicpon J., Grzesiak J. 2013. *An investigation of the elemental composition of horse hair affected by Equine Metabolic Syndrome (EMS) using SEM EDX and ICP-OES*. J. Anim. Vet. Adv., 12(2): 146-152.

Nutrient Requirements of Horses. 2007. Sixth Revised Edition. National Research Council

Ott E.A., Johnson E.L.2001. *Effect of trace mineral proteinates on growth and skeletal and hoof development in yearling horses*. J. Equine Vet. Sci., 21: 287-291.

Regulation of the Minister of Environment of 22 October 2014 on the classification of surface

water bodies and environmental quality standards for priority substances. Journal of Laws 2014, item 1482.

Reis L.S.L.S., Pardo P.E., Camargos A.S., Oba E. 2010. *Mineral element and heavy metal poisoning in animals.* J. Med. Med. Sci., 1: 560-579.

Stachurska A., Pięta M., Jaworski Z., Ussing A.P., Bruśniak A., Florek M. 2004. *Colour variation in blue dun Polish Konik and Biłgoraj horses.* Livest. Prod. Sci., 90: 201-209.

Stachurska A., Wałkuska G., Chałabis-Mazurek A., Jaworski Z., Cebera M. 2009. *Heavy metal concentration in coat hair and hoof horn in stabled and reserve Polish Konik horses.* Pol. J. Vet. Sci., 12(3): 369-377.

Stanek M., Jaworski Z., Sobotka W., Lipiński K., Olenkowicz R. 2016. *Influence of an organic supplement of copper, zinc and manganese in feed rations on concentrations of these elements in the coat of Polish Konik horses.* J. Elem., 21(2): 549-558.

Suzuki K., Yamaya Y., Asano K., Chiba M., Sera K., Matsumoto T., Sakai T., Asano R. 2007. *Relationship between hair elements and severity of atrioventricular block in horses.* Biol. Trace Elem. Res., 115(3): 255-264.

Topczewska J. 2012. *Effects of seasons on the concentration of selected trace elements in horse hair.* J. Cent. Eur. Agric., 13(4): 671-680.

Truchliński J., Budzyński M., Rzucidło M. 2004. *Dietary supplementation of minerals for horses based on the analysis of the composition of elements contained in the hair coat.* Ann. UMCS Lublin, sect. EE, 22: 263-270.