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Original article

Morphology and elemental analysis of free range and stabled Polish Konik horses hair using Energy-dispersive X-ray spectroscopy (EDS)

N. Siwińska[1], A. Żak[1], M. Słowikowska[1], K. Kubiak[1], Z. Jaworski[2], A. Niedzwiedź[1]

[1] Department of Internal Medicine and Clinic of Diseases of Horses, Dogs and Cats, Faculty of Veterinary Medicine, University of Environmental and Life Sciences, Pl. Grunwaldzki 47, 50-366 Wroclaw, Poland
[2] Department of Horse Breeding and Riding, University of Warmia and Mazury, Prawocheńskiego 2, 10-720 Olsztyn, Poland

Abstract

Hair is a more biologically stable material than other tissues and contains a relatively constant level of minerals, drugs or toxins. The content of essential elements in hair depends on dietary and non-dietary factors. The aim of this study was to assess and compare the effect of diet on the elemental composition and morphological properties of the hair of healthy Polish Konik horses. Mane hair was collected from 19 horses living in The National Park in Popielno in Poland. Six of these horses were free-ranged all year with permanent access to pasture, while 13 horses were kept in the stable and fed oats, hay and straw. The samples were analyzed using a Scanning Electron Microscope combined with Energy-dispersive X-ray spectroscopy (SEM-EDS). Each hair was analyzed for its microscopic appearance and elemental Mg, Si, S, Ca, Mn, Co, Zn and Se content. No significant changes in the morphological properties and elemental composition were observed. There was inter-individual variability in element content between horses in both groups, but this did not affect their health. The comparison of the present results with other studies shows that the level of elements in the hair of Polish Konik horses is significantly lower than in healthy horses of other breeds. The traditional feeding plan containing pasture, oats and hay does not contain a sufficient amount of main mineral elements. Further investigation is needed to explain the effect of diet and maintenance on the mineral balance of horses.

Key words: diet, elemental composition, hair, horses, Polish Konik, shortages

Correspondence to: N. Siwińska, e-mail: natalia.siwinska@upwr.edu.pl

Introduction

Nutrition is one of the most important factors affecting health and welfare. Particular attention should be paid to essential elements and trace minerals, which are compounds that maintain proper functioning of tissues and metabolic processes (Asano et al. 2002, Marycz et al. 2013, Topczewska and Krupa 2013). A mineral imbalance in the body can cause a variety of metabolic disorders and diseases (Asano et al. 2002, Topczewska and Krupa 2013). Herbivores obtain trace elements from plants, whose mineral composition depends on the natural abundance of the elements in soils (Janiszewska and Ciesla 2002, Stachurska et al. 2009, 2011, Topczewska and Krupa 2013). The elemental composition of the body can be estimated based on an examination of body fluids and tissues, including hair and/or hooves. Hair analyses have been routinely performed over the past decades to assess the nutritional status of the subjects (Dunnett 2001). The analysis of trace elements in hair provides additional information about the physiological state and homeostasis of the organism. In addition, hair can be collected noninvasively, easily transported and stored (Marycz et al. 2013, Topczewska and Krupa 2013). Furthermore, they contains more trace elements than internal organs or body fluids (Dunnett and Lees 2004a). In addition, the post-mortem-mineral concentration in hair does not change (Roug et al. 2015). A hair examination not only determines the nutritional status of an organism, but it also allows for a retrospective assessment. Moreover, a hair examination enables the detection and quantification of substances months or years after their administration. This retrospective aspect of this analysis highlights the unique features of hair in comparison with other matrices, such as blood, urine or faeces (Dunnett and Lees 2004a). Hair analyses permit “long-term” observations of the nutritional status of the organism, which minimizes the influence of a brief dietary change on the examination result. Elemental analysis of hair determines the concentration of those minerals which are unidentifiable in the blood due to their low content in body fluids compared to their accumulation in the hair (Wichert et al. 2002).

Hair follicles are metabolically active tissues that require nutrients to support both structural and functional activities (Dunnett 2001, Galbraith 2008). Nutrition affects the rate of hair growth as well as their quality and quantity. Poor nutrition may result in a dull, dry, brittle, thin or depigmented hair (Manson and Zlotkin 1985, Dunnett 2001).

The content of trace elements in the human and animal organisms mostly depends on the type of housing and the quality of the diet. The evaluation of the mineral content of the hair of chosen farm animals may not only determine their nutritional status, but also indicate mineral deficiencies or their toxicity (Hintz 2000). Trace elements in the mane hair of horses have also been evaluated in certain diseases and certain metabolic disorders (equine metabolic syndrome) (Asano et al. 2002, Marycz et al. 2009, 2013). The aim of the present work was to compare the hair morphology and the concentration of selected elements in horse hair, depending on the type of diet and housing system used.

Materials and Methods

Animals

In total, 19 Polish Konik horses (*Equus cabalus gmelini Ant.*) from The National Park in Popielno in North-East Poland were included in this study. The first group contained six horses which were kept outdoors day and night in a state-owned park in a free-range system all year round. They had unlimited access to grass on pasture. Hay and straw were given in winter when the snow cover was thick. Horses in this group drank water from natural ponds, except in the winter, when they were given water from a supply system. The second group consisted of 13 horses kept in the same stable without access to pasture. They were fed oats, hay and straw (*ad lib.*). Additionally, they received some carrots. Horses from this group drank tap water from an automatic waterer. Horses were chosen randomly for the study. They were clinically healthy – without any evident symptoms of disease and in good condition. The animals in both groups were adults and were aged 4-13 years. The horses did not receive vitamin or mineral supplements, except for permanent access to salt licks. The area of The National Park in Popielno is located far from motorways and industrial pollution. All animal experimental procedures in this study were performed with the approval of the 2nd Local Ethics Committee on Animal Experimentation in Wroclaw (permission No. 1/2012).

Hair analysis

The hair designated for analysis was collected from all the horses in the summer, after their shedding of the winter coat. For standardization of the sampling method the hair was collected from the same autonomous region in the middle of the mane. Nineteen hairs were removed from each horse (six from the first group and 13 from the second group)

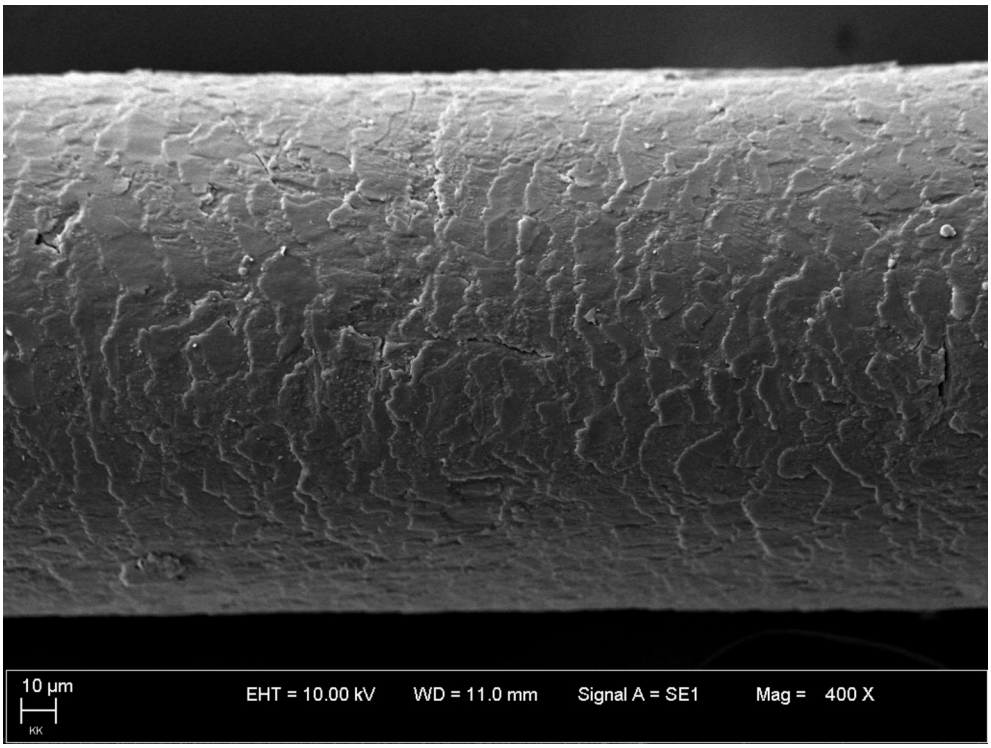

Fig. 1. Surface of mane hair with irregular distribution of keratin cells and size reduction.

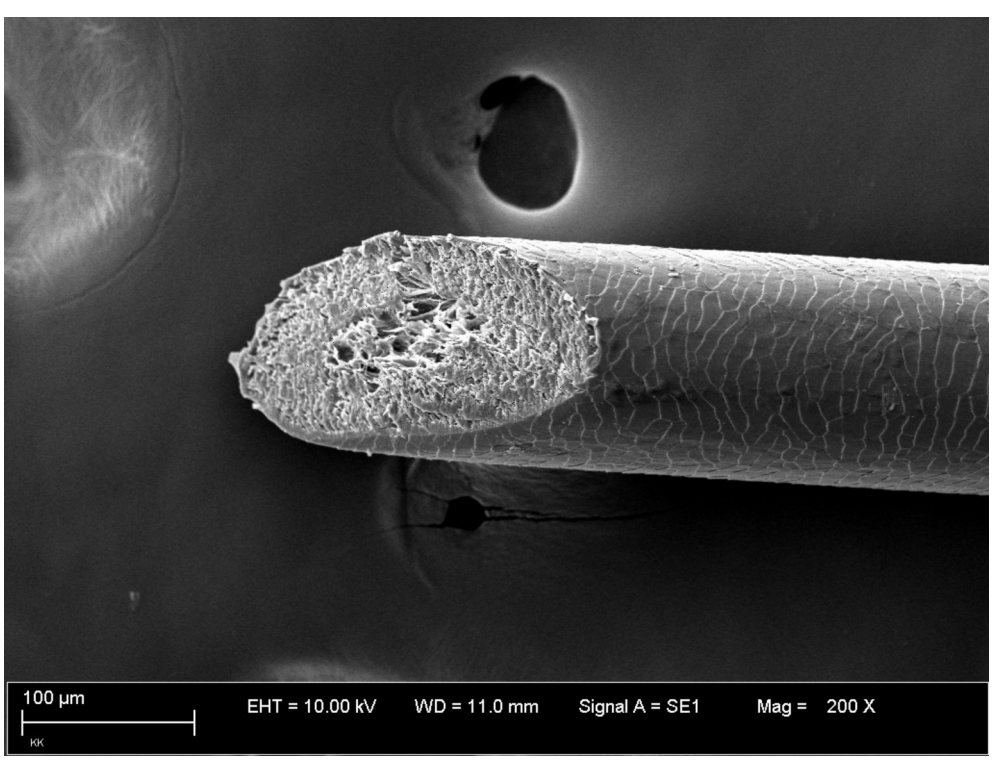

Fig. 2. Cross section of mane hair with irregular medulla structure and low medulla/hair shaft diameter ratio.

using sterile gloves. Samples were not in contact with the ground or other materials that could cause contamination. The hair was washed in distilled water and degreased in demineralized water with a detergent, rinsed three times and dried. The elemental constitution was then analyzed in the samples using a Scanning Electron Microscope (Evo LS 15) combined with a Bruker 129 eV microroentgenographic detector (SEM-EDS). The following features of hair morphology were analyzed: the appearance of the

hair cuticle including the shape and size of the keratin scales, the structure of the medullar cells, the width of the medulla as well as the diameter and appearance of the hair shaft. Subsequently, the peribulbar part of each hair was analyzed for its Mg, Si, S, Ca, Mn, Co, Zn and Se elemental content. The measurement was done in five different areas of each hair under 20 kV.

Statistical analysis

The results of the hair morphology were examined using descriptive analysis. The results of the elemental composition were processed in order to obtain the mean value, standard deviation, and extreme values (minimum – min. and maximum – max.). The significance of the differences between the means in the two groups of horses was calculated using Student's t-distribution test (MS Excel). The results were considered to be statistically significant when $p<0.05$. In order to estimate the relationship between the levels of elements, their coefficients of correlation were calculated and considered to be significant at $p\leq0.05$.

Results

In the morphological analysis there were no significant differences in the hair coat appearance and hair ultrastructure between the free-living and stabled horses. The hair shaft, cuticle and medulla were slightly damaged in the hair of the horses in both groups, but no serious damage or rupture was found. An irregular distribution of the keratin scales was noted in 67% of the horses in the first group and in 69% in the second group (Fig. 1). Although most horses had normal scales, a similar percentage of animals in both groups showed changes in their shape. An analysis of the cross sections of the hair revealed a reduction in the medulla/hair shaft diameter ratio, which occurred in 67% of the free-ranged horse and in 62% of the horses kept in the stable (Fig. 2). 50% of the horses from the first group and 56% of the horses from the second group had irregular medullar cells.

The average concentrations of the analyzed trace elements in the horse hair are summarized in Table 1. There were no statistical differences in the content of the examined elements in the hair between the two groups of horses. Attention should be paid to the difference between the extreme values (minimum and maximum) of the content of the examined minerals and the differences in the content of the elements in individual horses. A high variability of the evaluated components was observed mainly in the second group. In the first group, the concentration of sulfur and selenium exceeded the mean in 67% of animals, while the calcium level was below the mean value in 67% of animals. Similarly, in the second group, the calcium (67%) as well as the manganese and zinc (62%) values were below the mean in most animals. Silicon and selenium were the most abundant elements present in the horse hair.

The correlation coefficients between the concentrations of the elements analyzed in the hair of the free range Polish Konik horses are provided in Table 2. There were significant positive correlations between the level of magnesium and silicon as well as between cobalt and selenium. In this group there was a negative correlation between the concentration of calcium and sulfur. The coefficients of correlation between the concentrations of the trace elements analyzed in the hair of the Polish Konik horses living in the stable are summarized in Table 3. A significant positive correlation, similar to that in the first group, was confirmed between the level of magnesium and silicon.

Discussion

Evaluation of the concentrations of trace minerals can be an important tool when assessing individual or population health in free-ranging or stabled animals (Roug et al. 2015). Hair has been evaluated as an indicator of trace mineral and heavy metal status in livestock, and the reliability of hair as a measure of the mineral levels varies with animal species, geographic location, hair color, age and sex (Manson and Zlotkin 1985, Combs 1987, Janiszewska and Betlejewska-Kadela 1993, Asano et al. 2002, Wichert et al. 2002, Janiszewska and Ciesla 2002, Dobrzanski et al. 2005, Stachurska et al. 2011). To our best knowledge, research on the effects of diet and the maintenance system on morphology and elemental content of the hair of horses, excluding the impact of geographical location and breed, has not been assessed. To date, the SEM-EDS has been used to evaluate horse hair in two other reports (Marycz et al. 2013, 2014).

The average mineral content in both groups was equal, which indicates that there was no influence of the environment or diet on the morphology and the level of concentration of trace elements in horse hair. To date, only two studies have been published reporting the impact of environmental conditions on the amount of trace elements in horse hair (Janiszewska and Betlejewska-Kadela 1993, Dobrzanski et al. 2005). Few studies have focused on the relationship between the living environment and the content of heavy metals in the hair of horses (Stachurska et al.

Table 1. Content of selected elements (wt./%) in Polish Konik horses' hair.

Elements	Group of Polish Konik horses								Significance of difference	
	free-ranged				stabled					
	$\bar{x}$	SD	min.	max.	$\bar{x}$	SD	min.	max.	*P* value	
Mg	0.24	0.11	0.07	0.37	0.27	0.1	0.04	0.39	ns	0.541
Si	0.19	0.07	0.09	0.28	0.21	0.09	0.05	0.34	ns	0.594
S	6.96	0.52	6.13	7.37	6.64	1.24	4.09	9.42	ns	0.428
Ca	0.17	0.07	0.1	0.29	0.13	0.07	0.08	0.33	ns	0.344
Mn	0.08	0.02	0.07	0.11	0.09	0.02	0.06	0.15	ns	0.698
Co	0.12	0.02	0.09	0.15	0.13	0.02	0.09	0.18	ns	0.503
Zn	0.15	0.03	0.1	0.18	0.18	0.05	0.11	0.25	ns	0.129
Se	0.88	0.46	0.29	1.39	0.72	0.43	0.28	1.42	ns	0.471

Table 2. Coefficients of correlations between levels of selected elements in free-living Polish Konik horses.

	Mg	Si	S	Ca	Mn	Co	Zn	Se
Mg	x	0.97	ns	Ns	ns	ns	ns	ns
Si		x	ns	Ns	ns	ns	ns	0.7
S			x	-0.97	ns	ns	ns	ns
Ca				x	ns	ns	ns	ns
Mn					x	ns	ns	ns
Co						x	ns	0.92
Zn							x	ns
Se								x

Table 3. Coefficients of correlations between levels of selected elements in stabled Polish Konik horses.

	Mg	Si	S	Ca	Mn	Co	Zn	Se
Mg	x	0.89	ns	ss	ns	ns	ns	ns
Si		x	ns	ns	ns	ns	ns	ns
S			x	ns	ns	ns	ns	ns
Ca				x	ns	ns	ns	ns
Mn					x	ns	ns	0.74
Co						x	ns	ns
Zn							x	ns
Se								x

2009, 2011, Topczewska and Krupa 2013). Studies that assessed the elemental content in the hair of Wielkopolska horses depending on the seasons found that there were statistically significant differences in the levels of Cu, which we did not measure (Janiszewska and Betlejewska-Kadela 1993). Dobrzanski et al. (2005) did not observe any significant differences in the concentration of essential minerals in hair derived from two different breeds of horses, living in different environments and receiving different mineral feed additives. The concentration of heavy metals in the coat of horses kept in a stable-pasture and outdoor system are in most cases similar (Stachurska et al. 2011). This finding corresponds to that determined for stabled and free-living Polish Konik horses in Roztocze (Stachurska et al. 2009). Only one study shows significant differences in the heavy metal concentration in the hair of free-living and stabled horses (Topczewska

and Krupa 2013). However, this could stem from the fact that the study was carried out on two different breeds of horses. Many factors affect hair content including its color, diameter, rate of growth, season of year, geographic location, and age and gender of the individual (Manson and Zlotkin 1985). In order to eliminate the influence of non-dietary factors in our study, we chose animals of the same breed living in a defined geographic area (Niedzwiedz et al. 2013). Some authors reported that Polish Konik horses are highly responsive to environmental changes, as indicated in our study of the seasonal variability of the element levels in equine blood (Dunnett and Lees 2004b). However, our results revealed that different housing systems did not affect the element content in the studied horse hair. Unlike blood, hair is stable biological material that is not affected by acute and transient factors (Dunnett 2001). However, the heavy metal content of horse hair has been shown to be affected by seasonal variation in a number of horse breeds (Janiszewska and Betlejewska-Kadela 1993, Ciesla and Janiszewska 1997, Dobrzanski et al. 2005). This could be explained by the time the horses spend on pastures, where they are exposed to atmospheric pollution. Considering that in our study the diet and environmental factors did not affect the elemental content in the hair of Polish Konik horses, it may be assumed that this breed requires a basic feed and is adaptable to various environmental conditions (Pilarczyk et al. 2014).

Despite no statistically significant differences in the elemental content in the hair of the horses kept on pasture and in a stable, we found significant differences in the elemental content between horses in the same group. The large differences in the Mg, Se and S content did not affect the animals clinically. Significant differences between the physiological levels of certain trace elements between individuals were also noted in studies conducted in 2000 by Ciesla et al. (2000). In a study performed in 2005 by Asano et al. (2005) which analyzed the concentrations of several elements in horse hair, a significant influence of the hair color was observed. For example, hair from horses with a grey coat contain lower Se and Ca levels than hair from horses with a pigmented coat (Combs et al. 1982). The impact of the coat color on the elemental content can be overlooked in our study, since all Konik Polish horses have a distinct mouse-gray coat. It is also suspected that gender, age or genetic factors may impact the content of trace elements. Studies of the content of micro- and macro elements on adult Purebred Arabian mares, have also shown that the presence of individual variability indicated that sex, breed and age should not be considered as factors that would influence concentrations of trace elements in mane hair (Asano et al. 2002, Asano et al. 2005a,b, Krupa et al. 2006). Hence, it seems unlikely that sex and individual differences should influence the elemental content in the studied Polish Konik horses.

The definition of a mineral element deficiency and the minimum content of mineral elements in the hair of clinically healthy horses are still under discussion. The difficulties in defining these concepts result from the lack of uniform referential ranges, especially for examinations carried out using the SEM-EDS. The comparison of our results to other research carried out by Marycz et al. (2013) using the SEM-EDS in horses shows a significant difference. The level of Ca, Zn, Mg in both groups of Polish Konik horses is approximately 50% lower than in healthy horses of other breeds. These results are more similar to those obtained in a group of horses diagnosed with EMS (Marycz et al. 2014). None of the horses included in the study had symptoms or a history of metabolic disease. Our results may confirm the study by Niedzwiedz et al. (2013) that Polish Konik horses are more resistant to unfavorable environmental conditions than other breeds of horses. Due to a lack of specific reference values given in wt/% of the element content in Polish Konik hair, it is difficult to definitely determine whether some horses had trace element deficiencies. It is likely that the reference values differ from those in horses of other breeds. A study by Niedzwiedz et al. (2013) assessing chosen blood parameters in Polish Konik horses found that this breed had different reference values of certain parameters compared to other horse breeds. On the other hand, according to the literature, the mineral content of mane hair may be an early indicator of deficiency or metabolic diseases (Asano et al. 2002). Hence, the low levels of chosen microelements in the Polish Konik hair may indicate the need to carry out further observations in these animals in order to rule out subclinical diseases and to prevent the development of diseases. The low elemental content may also be a result of low levels of trace minerals in the plants ingested by the Polish Konik horses from the Nature Park in Popielno. The mineral concentration of plants, including hay, is strongly influenced by the soils and geology in a given location (Sutton et al. 2002, Madejón et al. 2009). Reinwald and Riond (2002) showed that the traditional portions of oats and hay did not contain sufficient amounts of main mineral elements. This finding is similar to that determined for horses eating only oats and hay and horses with additional supplementation (Marycz et al. 2009). Similarly, pasture plants have a varied mineral content. It has been shown that grass contains fewer minerals than other plants (Madejón et al. 2009). We did

not evaluate the mineral composition of the feed and plants consumed by the studied horses. The microscopic morphological analysis of the horse hair revealed changes including irregular distribution of the keratin scales, decreased medulla/hair shaft ratio and irregularity of medullar cells in most horses. These changes may be caused by a mineral deficiency. It is well known that diet has a large impact on the quality and morphology of the skin and hair (Marycz et al. 2009). A diet enriched with specific elements, such as zinc or copper, positively influenced to the morphology, mechanical properties and composition of the hair (Janiszewska and Betlejewska-Kadela 1993, Wichert et al. 2002, Marycz et al. 2009, Topczewska and Krupa 2013). Subclinical mineral deficiencies can cause a rough hair coat, and poor growth (Dunnett 2001, Marycz et al. 2009). Changes in nutritional regimens have an impact on the concentration of elements in the equine hair (Marycz et al. 2009).

Unfortunately, it was also found that micro- and macro element supplementation does not always increase the level of these elements in the animal tissues (Wysocki and Klett 1971, Pearce et al. 1998). The mineral tissue content is affected not only by the quality and quantity of the elements in the feed, but also by their chemical form, their availability and by factors that affect their absorption in the gastrointestinal tract (Dobrzanski et al. 2008, Marycz et al. 2009). In general, hair examination is a good ancillary diagnostic tool in human and veterinary medicine (Seidel et al. 2001). Some authors claim that an elemental analysis of hair can serve as a good indicator of the nutritional status of humans and animals (Hintz 2000, Asano et al. 2002). An evaluation of the mineral content in biological material enables early detection of mineral deficiencies and metabolic diseases (Asano et al. 2002). There are numerous advantages in the use of hair for carrying out an elemental analysis (Dunnett and Lees 2004). The main advantage of this method is its non-invasiveness. Collection of other types of biological material, e.g. blood from free living horses, who have limited contact with humans, could be difficult and unsafe for the examiner.

Different housing systems and different diets did not affect the elemental content of hair in the Polish Konik horses. Absence of statistically significant differences between the groups in our study may be due to the small sample size. It should be noted that that only a few elements were examined in our study, and it must be assumed that the content of the remaining unexplored minerals may be different in the two groups of horses. The inter-individual differences in the elemental content and the decreased values of the elements in all the studied Polish Konik horses compared to healthy horses of different breeds may indicate that Polish Konik horses are resistant to diseases and highly adaptable to changing environmental conditions. In order to confirm these findings, further studies on a larger group are required. Furthermore, reference values for elements in Polish Konik horses should be determined. Establishing these values would facilitate carrying out routine tests to rule out mineral deficiencies in herds of horses, which could be treated by changing the diet. However, there is still too little information available to explain the effect of diet and maintenance on horses mineral status.

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References

Asano K, Suzuki K, Chiba M, Sera K, Asano R, Sakai T (**2005b**) Twenty-eight element concentrations in mane hair samples of adult riding horses determined by particle-induced X-ray emission. Biol Trace Elem Res 107: 135-140.

Asano K, Suzuki K, Chiba M, Sera K, Matsumoto T, Asano R, Sakai T (**2005a**) Influence of the coat color on the trace elemental status measured by particle-induced X-ray emission in horse hair. Biol Trace Elem Res 103: 169-176.

Asano R, Suzuki K, Otsuka T, Otsuka M, Sakurai H (**2002**) Concentrations of toxic metals and essential minerals in the mane hair of healthy racing horses and their relation to age. J Vet Med Sci 64: 607-610.

Ciesla A, Janiszewska J (**1997**) Comparing the level of selected elements in the blood serum and hair of Wielkopolski and Polish Konik horses. Zesz. Nauk. AR Szczecin. Zootechnika 177: 259-265.

Ciesla A, Janiszewska J (**2000**) The level of copper in horses blood sera and hair. Med Wet 56: 589-592.

Combs DK (**1987**) Hair analysis as an indicator of mineral status of livestock. J Anim Sci 65: 1753-1758.

Combs DK, Goodrich RD, Meiske JC (**1982**) Mineral concentrations in hair as indicators of mineral status: a review. J Anim Sci 54: 391-398.

Dobrzanski Z, Jankowska D, Dobicki W, Kupczynski R (**2005**) The influence of different factors on the concentration of elements in hair of horses. Proccedings of the ISAH 2005 Warsaw, pp 450-453.

Dobrzanski Z, Korczynski M, Chojnacka K, Gorecki H, Opalinski S (**2008**) Influence of organic forms of copper, manganese and iron on bioaccumulation of these metals and zinc in laying hens. J Elemental 13: 309-319.

Dunnett M (**2001**) The diagnostic potential of equine hair: a comparative review of hair analysis for assessing nutritional status, environmental poisoning, and drug use and abuse. In: Pagan J, Georg RJ (eds) Advances in

equine nutrition – III. Equine Research Inc., Kentucky pp 85-106.

Dunnett M, Lees P (**2004a**) Equine hair analysis: current status and future prospects. Equine Vet J 36: 102-103.

Dunnett M, Lees P (**2004b**) Hair analysis as a novel investigative tool for the detection of historical drug use/misuse in the horse: a pilot study. Equine Vet J 36: 113-117.

Galbraith H (**1998**) Nutritional and hormonal regulation of hair follicle growth and development. Proc Nutr Soc 57: 195-205.

Hintz HF (**2000**) Hair analysis as an indicator of nutritional status. Nutr Sci 21: 191.

Janiszewska J, Betlejewska-Kadela K (**1993**) Effects of season feeding on the content of Mg, Zn, Cu, Mn and Co in horse hair. Med Wet 49: 522-523.

Janiszewska J, Ciesla A (**2002**) Concentration of cadmium and lead in horse blood serum and hair in relation to season and environment. Electronic Journal of Polish Agricultural Universities, Animal Husbandry 5.

Krupa W, Soltys L, Budzynska M, Sapula M, Kamieniak J, Budzynski M (**2006**) Assessment of mineral content in purebred Arabian mare's hair considering genealogical lines. Annales Universitatis Mariae Curie-Skłodowska Lublin-Polonia 26: 209-216.

Madejón P, Dominguez T, Murillo M (**2009**) Evaluation of pastures for horses grazing on soils polluted by trace elements. Ecotoxicology 18: 417-428.

Manson P, Zlotkin S (**1985**) Hair analysis – a critical review. Can Med Ass J 133: 186-188.

Marycz K, Moll E, Zawadzki W, Nicpon J (**2009**) The correlation of elemental composition and morphological properties of the horses hair after 110 days of feeding with high quality commercial food enriched with Zn and Cu organic forms. Electronic Journal of Polish Agricultural Universities 12.

Marycz K., Smieszek A., Nicpon J. (**2014**) Scanning X-Ray Electron Microscopy (SEM-EDX) as a therapeutic tool in the diagnosis of equine metabolic syndrome (EMS). In: Polychroniadis E, Oral A, Ozer M (eds) International Multidisciplinary Microscopy Congress. Springer Proceedings in Physics, Springer, Cham 154, pp 215-219.

Marycz K, Toker NY, Czogaa J, Michalak I, Nicpon J, Grzesiak J (**2013**) An investigation of elemental composition of horse hair affected by equine metabolic syndrome (EMS) using SEM-EDX and ICP-OES. J Anim Vet Adv 12: 146-152.

Niedzwiedz A, Jaworski Z, Filipowski H, Zawadzki M, Wrzosek M, Sluzewska-Niedzwiedz M, Nicpon J (**2013**) Serum biochemical reference intervals for the Polish Konik horse (Equus cabalus gmelini Ant.). Vet Clin Pathol 42: 66-69.

Pearce SG, Grace ND, Wichtel JJ, Firth EC, Fennessy PF (**1998**) Effect of copper supplementation on the copper status of pregnant mares and foals. Equine Vet J 30: 200-203.

Pilarczyk B, Tomza-Marciniak A, Stankiewicz T, Blaszczyk B, Gaczarzewicz D, Smugala M, Udala J, Tylkowska A, Kuba J, Ciesla A (**2014**) Serum selenium concentration and glutathione peroxidase activity and selenium content in testes of Polish Konik horses from selenium-deficient area in North-Western Poland. Pol J Vet Sci 17: 165-167.

Reinwald D, Riond JL (**2002**) Copper and zinc in animal feed for adult horses in Switzerland. Schweiz Arch Tierheilkd 144: 545-548.

Roug A, Swift PK, Gerstenberg G, Woods LW, Kreuder-Johnson C, Torres SG, Puschner B (**2015**) Comparison of trace mineral concentrations in tail hair, body hair, blood, and liver of mule deer (*Odocoileus hemionus*) in California. J Vet Diagn Invest 27: 295-305.

Seidel S, Kreutzer R, Smith D, McNeel S, Gilliss D (**2001**) Assessment of commercial laboratories performing hair mineral analysis. JAMA 285: 67-72.

Stachurska A, Walkuska G, Cebera M, Jaworski Z, Chalabis-Mazurek A (**2011**) Heavy metal status of Polish Konik horses from stable-pasture and outdoor maintenance systems in the masurian environment. J Elem 4: 623-633.

Stachurska A, Walkuska G, Chalabis-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: 369-377.

Sutton P, Maskall J, Thornton I (**2002**) Concentration of major and trace elements in soil and grass at Shimba Hills National Reserve, Kenya. Appl Geochem 17: 1003-1006.

Topczewska J, Krupa W (**2013**) Influence of horse breed and housing system on the level of selected elements in horse's hair. J Elem 2: 287-295.

Wichert B, Frank T, Kienzle E (**2002**) Zinc, copper and selenium intake and status of horses in Bavaria. J Nutr 132: 1776-1777.

Wysocki AA, Klett RH (**1971**) Hair as an indicator of the calcium and phosphorus status of ponies. J Anim Sci 32: 74-78.