Journal of Elementology ISSN 1644-2296

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. DOI: 10.5601/jelem.2015.20.1.914

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

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Abstract

The aim of the study was to determine the effect of an organic form of mineral supplement containing copper, zinc and manganese on the concentration of these elements in the coat (hair) of Polish Konik horses. Possible impact of the rearing conditions of 24 Polish Konik foals (which originated from stables and nature reserves), their sex and duration of the supplementation on the coat's mineral composition was considered. The research included 24 horses: 12 stabled (six fillies and six colts) and 12 captured in a nature reserve (seven fillies and five colts). Ten samples of fodder and 60 samples of coats were analysed. The influence of the rearing system on the concentration of elements in the coat was observed, for example stabled horses had a higher content of copper in the coat while the content of zinc and especially manganese was lower. All horses, both reared in a stable and from the nature reserve, showed higher copper concentrations in their coat ($P \leq 0.05$) after supplementation with copper, while an increased amount of zinc was observed only in animals reared in a stable. All horses (both stabled and wild ones) demonstrated an evident change in the manganese content of their coat after supplementation with a chelate. The content grew significantly ($P \leq 0.01$) with the supplementation time. An interaction between the sex and chelate supplementation time was observed only in the case of manganese administered to animals reared in a stable.

Keywords: Polish Konik horses, hair, copper, zinc, manganese.

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INTRODUCTION

The lack of mineral balance in a horse's body or deficiency of elements may result in energy imbalance, worse physical condition, inferior immunity and many other adverse changes. Like all farm animals, horses, especially young ones, must be supplied mineral compounds which are engaged in the body's metabolic processes, in amounts which maintain an animal's homeostasis. In addition to macroelements, trace amounts of microelements are needed for the body's proper development and healthy condition.

For an analysis of an animal's mineral metabolism to provide reliable data for an assessment of health or determination of changes in the body's metabolism, it must be made on adequate biological material, the selection of which often raises doubts. Analyses include determinations of mineral components in blood, urine or individual tissues.

Blood is the biological material most often used for this purpose because the level of macro- and microelements in serum reflects their content in the body (Janiszewska, Betlejewska-Kadela 1993, Cieśla, Janiszewska 2000, Forrer et al. 2001). However, results of blood analyses can be questionable due to active homeostatic mechanisms that compensate for the level of elements in blood at the expense of their reserve in tissues. Thus, blood does not always reflect well the amounts of absorbed elements.

The results of monitoring a body's mineral status indicate that the coat (hair) is better for this purpose (Farmer, Farmer 2000, Asano et al. 2005, Vermeulen et al. 2009, Gabryszuk et al. 2010, Kośla et al. 2011). Its mineral composition does not undergo sudden fluctuations resulting from the uptake of elements with fodder or from disease. Conversely, hair is characterized by considerable stability and, very importantly, the amount of bioelements in coat is much higher than in serum and other biological material (Puchyr et al. 1998, Chyla, Zyrnicki 2000, Cizdziel, Gerstenberger 2004).

According to Kaproń et al. (2010), analysis of mineral components in coat of horses may be useful for establishing the metabolic profile of horses and can serve to determine mineral deficiencies in their habitats as well as to optimize their nutrition. At the same time, amounts of specific elements in hair provide information on changes in the body that have occurred over a longer period of time (Combs 1987).

Use of coat as source material is also justified by the fact that collection of hair is usually stress-free for horses. Hair can be stored longer and is easily transported (Feng et al. 1997). These characteristics improve the reliability of the results (Asano et. al. 2005, Topczewska, Krupa 2013).

Moreover, coat is a fairly stable biological material in comparison to other animal tissues as its mineral components remain on relatively constant levels and are not subject to sudden changes resulting from a recent uptake of fodder. An analysis of elements in hair enables researchers to determine levels of elements that are difficult to detect in serum due to to significantly

lower amounts of bioelements accumulated in body fluids than in coat (Wichert et al. 2002).

Deficiency of mineral compounds relative to the needs of animals often present in fodders as well as low assimilability of compounds necessitates supplementation of feed rations. Most mineral supplements for horses used previously were composed of inorganic forms of elements, which were characterized by low assimilability. This has stimulated an interest in organic forms of these types of additives.

A beneficial effect of organic additives in equine nutrition on the mechanical properties of hair was observed by Kania et al. (2009), while a resulting improvement of the mineral composition of coat was reported by Marycz et al. (2009), Wagner et al. (2011) or Jancikowa et al. (2013).

The aim of this study has been to determine the effect of an addition of copper, zinc and manganese chelates to a feed ration on the content of these elements in the hair coats of Polish Konik foals reared in a stable or in a nature reserve.

MATERIAL AND METHODS

The study was conducted at the Research Station of the Polish Academy of Sciences in Popielno, from 20 December 2013 to 7 April 2014.

The research animals were 24 foals born in the spring of 2013 and including 12 animals born in a stable (six fillies and six colts) and 12 animals born in a nature reserve (seven fillies and five colts), which were 8-9 months old at the onset of the experiment. The design of the experiment is presented in Table 1.

Table 2 presents the basic composition of the fodder. The animals were fed traditionally with meadow hay (3-6 kg) and oats (0.4-1.4 kg), with a periodic addition of small amounts of carrot. Microelement concentrations per kg DM of the diet were as follows: 3.16-3.19 mg of copper, 23.86-24.21 mg of zinc, 70.78-73.47 mg of manganese; the diets were copper- and zinc-deficient (Horse Nutrient Requirements, 1991)

Table 1

The design of the experiment

Experimental factor										
Place of rearing	stable						nature reserve			
Collection of hair after days of supplementation	1st - 0 days		2nd – 38 days		3rd – 108 days		1st – 0 days		2nd – 70 days	
Sex	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Number of animals	6	6	6	6	6	6	7	5	7	5
Applied supplement	Chelandmix 3 g animal daily^{-1}									

Table 2

Fodder supplied to Polish Konik horses

Details	Meadow hay	Oats	Carrot
Basic chemical composition (g kg^{-1})			
Dry matter	870.9	872.1	105.3
Crude protein	102.4	95.6	11.0
Crude fibre	315.7	108.2	9.8
Crude fat	14.2	36.8	1.1
Crude ash	47.2	63.2	12.3
Microelements (mg kg^{-1} DM)			
Cu	3.1	3.6	0.5
Zn	23.3	28.1	3.0
Mn	77.9	40.3	2.0

At the beginning of the study (December 2013), 500 mg control samples of coat (hair) were collected from an area within the withers of 12 stable foals. Hair samples were collected from the coat on the neck, in the withers area, under the mane. Then a feed ration for those foals was supplemented with an addition of Chelandmix formulation, comprising zinc (20 mg kg^{-1}), manganese (20 mg kg^{-1}) and copper (3 mg kg^{-1}). Diets supplemented with Chelandmix were fed to horses on a daily basis.

After 38 days of the supplementation, another series of samples from the stud stable animals and control samples from the reserve foals caught in this period (January 2014) was collected. Since then, the supplementation was also administered to the foals from the nature reserve. The last coat sample collection was performed after 70 days. The total time of mineral supplementation of the feed rations was 108 days for foals reared in a stable, and 70 days for foals reared in the reserve.

Concentrations of the minerals were determined in purified material. The determination of Zn, Cu and Mn in the coat was performed with a flame atomic spectroscopy technique using a VARIAN AA240FS atomic absorption spectrometer. In this method, the absorption intensity of a particular element at its specific wavelength was determined in solutions of samples after mineralization with strong acids HCl+HNO_3 (at a volume ratio of 3:1) in a MARS microwave digestion system. Microelements were determined at the following wavelengths: 2013.8 nm for zinc, 327.4 nm for copper and 279.5 nm for manganese.

The analytical data were submitted to bivariate analysis of variance (MANOVA) in a non-orthogonal system. Arithmetic means were used in the statistical analysis. The significance of differences was determined using the Duncan's range test. All calculations were performed with Statistica ver. 10.0 PL.

RESULTS AND DISCUSSION

Copper

The average content of copper in the coats of the stabled Polish Konik foals equalled 7.26 mg kg^{-1} (Table 3). A significant increase in the copper

Table 3

The content of mineral components in the coats of stabled Polish Konik horses (mg kg^{-1} DM)

Experimental factor	Statistical measures	Ingredient		
		Cu	Zn	Mn
Time of supplementation (*C*)				
0	x^*	7.26^b	137.58^a	22.75^C
38 days	x	7.92^a	143.33^b	35.42^B
108 days	x	8.03^a	135.58^a	62.58^A
Sex (*P*)				
fillies	x	7.53	139.11	37.39
colts	x	7.95	138.56	43.11
SEM		0.134	1.258	3.266
Variance analysis				
Time of supplementation (*C*)		0.029	0.034	0.000
Sex (*P*)		0.093	0.818	0.071
Interaction (*C* x *P*)		0.420	0.590	0.029^{**}

a, *b*, *c* – $P \leq 0.05$, *A*, *B*, *C* – $P \leq 0.01$, x^* – arithmetic mean, ** – $P \leq 0.05$

concentration was observed during the first 38 days of supplementation (7.92 mg kg^{-1} DM). After 108 days of feed supplementation with the copper-containing chelate, the amount of copper in coat only slightly increased (8.03 mg kg^{-1} DM). The coats of fillies contained less copper than those of colts (7.53 vs. 7.95 mg kg^{-1} DM).

The level of copper in the coat control samples from the foals reared in the reserve was slightly higher than in the coat of the stabled horses (6.94 vs. 7.26 mg kg^{-1} DM) – Table 4. The 70-day application of the mineral supplement resulted in a significant increase in the concentration of copper in coat, from 6.94 mg kg^{-1} DM to 7.40 mg kg^{-1} DM.

The initial average content of copper in the coats of the investigated horses (6.94-7.26 mg kg DM^{-1}) can be considered high and normal. Considerably lower concentrations of copper were observed by Stachurska et al. (2011) in coats of the Polish Konik (2.98 mg kg^{-1} DM), and by Budzyńska et al. (2006) in the coats of Arabian horses (approx. 2 mg kg^{-1} DM). In comparison to the results reported by Janiszewska and Betlejewska-Kadela (1993), Danek et al. (1996) and Wichert et al. 2002, we noted greater differences in the copper content of equine coats, ranging from 1.0 to 3.5 mg kg^{-1} DM).

Table 4

The content of mineral components in the coats of Polish Konik horses in a nature reserve (mg kg^{-1} DM)

Experimental factor	Statistical measures	Ingredient		
		Cu	Zn	Mn
Time of supplementation (*C*)				
0	x^*	6.94^b	143.08	34.41^B
70 days	x	7.40^a	141.67	56.58^A
Sex (*P*)				
fillies	x	7.21	144.21	46.21
colts	x	7.11	139.80	44.50
SEM				
Variance analysis				
Time of supplementation (*C*)		0.041	0.585	0.000
Sex (*P*)		0.634	0.288	0.655
Interaction (*C* x *P*)		0.749	0.235	0.256

a, b – $P \leq 0.05$, A, B – $P \leq 0.01$, x^* – arithmetic mean

According to Truchliński et al. (2004), the normal level of copper in a horse's coat is 13.6 mg kg^{-1}

An increase in the copper content observed after the addition of a mineral supplement to a feed ration was found by Jančíková et al. (2012), who also demonstrated an increase in the copper concentration in coats of horses after an organic supplement was given. Moreover, a more beneficial effect of the organic form and better absorption of copper in comparison to the inorganic supplement were noted. After the addition of a an inorganic copper supplement, a higher level of copper in serum and a lower level excreted in faeces were observed.

A higher level of copper detected in the coats of the stabled Polish Konik horses is not consistent with the observations of Stachurska et al. (2009), who found that coats of Polish Konik horses living in a nature reserve had a higher content of copper than coats of stabled horses.

The relevance of an analysis of the amount of copper in coat as a suitable indicator of the supply this element to the body was proven experimentally by Cieśla and Janiszewska (2000), who demonstrated a positive correlation between the content of copper in serum and coat. Supplementation of this element in a feed ration was recommended in cases when levels of copper were below the physiological norm.

Zinc

The amount of zinc in the coats of the stabled Polish Konik horses significantly increased in the first period of supplementation, and after 38 days

it rose significantly from 137.58 mg kg^{-1} DM to 143.58 mg kg^{-1} DM. However, after 108 days the zinc content was actually slightly lower and similar to the result in the control sample (135.58 mg kg^{-1}DM). There was no increase in zinc in hair of the nature reserve horses after 70 days of using the supplement. In fact, the level of this mineral even slightly decreased, from 143.08 mg kg^{-1} DM to 141.67 mg kg^{-1} DM.

No difference between the sexes was observed in the zinc content in coat of the Polish Konik horses. The level of zinc (137.58-143.08 mg kg^{-1} DM) was similar to the values determined by Wichert et al. (2002) and De Souza et al. (2014), and only slightly higher than the values reported by Asano (2002). The level of zinc in the study by Dobrzański et al. (2005) was slightly lower and equalled approx. 120 mg. Moreover, the authors observed a beneficial effect of the mineral supplement on an increase in the zinc concentration in coat. According to Truchliński et al. (2004), a normal zinc content in hair coat is 160 mg kg^{-1} DM.

An increase of zinc in the coat observed during the initial period of chelate supplementation and a further decrease in its concentration seems to indicate appropriate absorption and adequate use of this element by the body.

According to Danek and Wiśniewski (1992) or Cieśla and Janiszewska (2000), zinc is a good indicator of supply because of the confirmed positive correlation between the content of this element in blood and coat. The enrichment of a diet with an organic formulation containing zinc and copper in the study by Marycz et al. (2009) indicated a significant correlation between the concentrations of these elements in a diet and in hair.

Manganese

The most remarkable response of the animals to the supplementation of the mineral component occurred with respect to manganese. After 38 days, the manganese content in coat increased from 22.75 mg kg^{-1} DM to 35.42 mg kg^{-1} DM, and after 108 days it rose to even 62.58 mg kg^{-1} DM. The differences observed were statistically highly significant. The coats of the colts had a significantly higher concentration of manganese.

The manganese level in the coats of the nature reserve horses was higher by over 10 mg kg^{-1} DM than in the coats of the stabled horses, and after 70 days of supplement intake, a highly significant increase in the manganese concentration was observed (from 34.41 mg kg^{-1} DM to 56.58 kg kg^{-1} DM).

The results concerning the concentration of manganese in the investigated horses differed from those reported in literature. Smaller amounts were observed by Cieśla and Janiszewska (2000), Janiszewska and Betlejewska-Kadela (1993) or Budzyński and Truchliński (2004), while an average Mn level in horses' coats was below 20 mg kg^{-1} DM. Similar results were obtained by Stachurska et al. (2009).

The increasing tendencies for manganese concentrations obserevd in the coats in specific periods seem to indicate a sufficient Mg supply in equine nutrition and limited absorption of the element to the tissues.

The results concerning the concentration of mineral components in relation to the sex of Polish Konik horses only partially confirm the results of ASANO et al. (2002), who found no effect of this factor on the concentration of Cu, Zn or Mn in the coat. In the current research, different levels of these components in coats of colts and fillies were observed. However, the results were unequivocal as to the method of rearing Polish Konik horses because coats of the stabled colts contained more copper and manganese in comparison to coats of the fillies. Conversely, for horses born in the reserve, coats of the fillies contained more copper, zinc and manganese.

The results concerning the beneficial effect of an organic form of copper, zinc and manganese chelates are confirmed by ARMELIN et al. (2003), who used organic chelated minerals (Cu, Fe, K, Mg, Mn) and reported results that indicated an improved absorption of microelements in the body of animals which received that kind of supplementation in their feed rations.

While the levels of copper in coats of the stabled and nature reserve horses were similar, higher amounts of zinc and manganese were found in the nature reserve horses. The response to supplementation with the mineral additive was similar, including an increase in the copper concentration during the supplement application and absence of a clear effect of the supplement on zinc levels. However, a significant effect was observed in the case of manganese, whose amount in coat increased most remarkably.

The stabled colts had coats with a higher content of copper and manganese, compared to the coats of the stabled fillies, while the coats of the fillies born in the nature reserve contained more copper, zinc and manganese than the coats of wild colts.

Our statistical analysis indicated an interaction between the sex and duration of the supplementation with respect to manganese in coats of the Polish Konik horses reared in a stable.

CONCLUSION

The results concerning relationships between the concentrations of copper, zinc and manganese in the horses' coats and supplements in the feed rations suggest that results of hair analysis enable researchers to assess the mineral status of the body and can reveal possible deficiencies of these elements. This will facilitate desiging an optimum equine nutrition and observe changes in the metabolism of the body, which are suggestive of physiological abnormalities. Observation of changes in the mineral composition of hair, which undergoes modifications as the hair grows, provides information on

the changes occurring during a longer period of time, in comparison with other biological materials used for analyses of mineral composition, such as blood.

Analysis of the content of bioelements in coat can help to achieve precise and non-invasive determination of a horse's mineral status, which will enable one to monitor and optimize the nutrition of Polish Konik horses.

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