

## The evaluation of Cu, Zn, Mn, and Se concentrations in the hair of South American camelids

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### Abstract

The aim of this study was to monitor the content of trace elements in the hair of South American camelids and to evaluate the effect of coat colour, species, age, and sex on their concentration in the hair. The samples were collected from 77 animals – 23 llamas (*Llama guanicoe f. gllama*) and 54 alpacas (*Llama guanicoe f. pacos*) during a spring health check. The concentrations of copper (Cu), zinc (Zn), and manganese (Mn) were determined by Flame Atomic Absorption Spectrometry and selenium (Se) by Hydride Generation Atomic Absorption Spectrometry. We found the following concentrations (mean  $\pm$  standard deviation; mg/kg dry matter) in the llama hair: Cu  $9.70 \pm 4.69$ ; Zn  $145.20 \pm 21.07$ ; Mn  $12.49 \pm 10.14$ ; Se  $0.25 \pm 0.14$ . In alpaca hair we found the following concentrations: Cu  $10.22 \pm 2.90$ ; Zn  $129.81 \pm 19.01$ ; Mn  $12.67 \pm 13.85$ ; Se  $0.48 \pm 0.24$ . We found a significant difference between llamas and alpacas in Zn and Se concentrations in the hair. From all the evaluated factors we found that coat colour had the highest effect on Mn and Se concentration. Dark haired animals had significantly higher concentrations of these trace elements than other coloured groups. The evaluation of the concentration of trace elements in the hair of llamas has a potential to be used for the evaluation of long-term status of trace elements in the body; however, it is necessary to continue with experimental work in this area. Our findings can serve as a pilot study for further works in this field.

*Llama, alpaca, trace elements, Atomic Absorption Spectrometry, coat colour*

The content of trace elements in the body is a very important factor for the health state evaluation of South American camelids. Usually, blood is used for the determination of the trace element content in the organism; however, this method has some limitations. One of them is the fact that blood sampling requires fixation, which might be quite stressful for the animal. Therefore, other means of trace element monitoring may be helpful. Some authors recommend the monitoring of trace element concentration in the hair as an indicator of the long-term status of trace elements in the body (Pavlata et al. 2011; Ghorbani et al. 2015).

It is not easy to determine the level classifying deficiency of microelements. When an animal's diet fails to meet its needs for a certain mineral, four overlapping phases of deprivation can often be distinguished: a) depletion, when stores in the tissues or body fluids are diminishing; b) deficiency, when mineral concentrations normally kept within close limits in transport pools fall; c) dysfunction, when mineral-dependent functions in the tissues or body fluids become rate-limited; d) disorder, when animals appear abnormal or perform poorly. Transition between the phases is often gradual and consequently, disorders develop gradually, with only the most vulnerable individuals being visibly affected (Suttle 2010). This makes the assessment of a mineral deficit difficult and more information in this field is always helpful.

The concentration of trace elements in blood shows the actual homeostasis of trace elements, but it is also influenced by other factors. For example, copper bound to

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caeruloplasmin accounts for about 88% of plasma copper (Telfer et al. 1996). Caeruloplasmin is an acute phase protein and its concentration in blood is affected during inflammation. Therefore, copper concentration in blood is not a good indicator of the body's supply of copper. Determination of copper from the liver is suggested as an optimal choice. However, this method has limitations in the monitoring of live animals as the liver biopsy is required. Similarly, liver tissue is recommended for the evaluation of manganese content in the body as manganese concentration in blood is a poor indicator of the body's status (Pitropovska et al. 2014). The concentration of zinc in plasma is widely used for monitoring the Zn content in the body; however, Clauss et al. (2004) recommend the use of hair samples as well. Blood is a good indicator of selenium status and Husakova et al. (2014) recommend measuring Se together with glutathione peroxidase for the evaluation of Se intake in alpaca. However, Haenlein and Anke (2011) showed that Se deficiency can also be reliably detected by analysing hair samples and according to Nasli-Esfahani et al. (2011), hair is the best biological sample for trace element analysis especially for Se and Mn due to high accumulation of these elements in hair. Based on the above facts we decided to use hair as an alternative biological material for assessing the content of microelements in the body. This biological material remains isolated from metabolic activities and therefore indicates the element concentration over a long-term period.

Trace element concentration is higher in hair compared to blood or urine, and so it better reflects the content in the body than the other biological materials. Hair analysis provides information about intracellular accumulations of trace elements and has been used to evaluate the trace element status in the body (Senofonte et al. 2000; Ozmen et al. 2013). Kempson and Lombi (2011) summarise that hair is a good source of tissue samples, it is easy to obtain, the sample is robust and its storage and preparation for analysis is simplified because no special preservation techniques are required and it has the potential of offering a convenient and unique medium for assessment of both individuals and populations.

Generally, the beginning of hair analysis as a potential for new useful method dates from the early 1960s, but the most significant development appeared from 1990. In this time the knowledge of human hair structure and physiology improved as well as the method to detect trace elements in biological samples (Bencze 1990; Chyla and Zyrnicki 2000). According to the above mentioned studies, hair can be used for monitoring the content of bioelements in human and animal organisms (Senofonte et al. 2000; Ikemoto et al. 2004; Kosla et al. 2011; Ozmen et al. 2013; Ghorbani et al. 2015). The hair is even recommended as a non-invasive biomarker by various institutions – including US Environmental Protection Agency (EPA), World Health Organization, and Global Environmental Monitoring Systems of United Nations Environment Programme (Batzevich 1995; Ghorbani et al. 2015).

To the authors' knowledge, no study monitoring trace elements in alpaca and llama hair was done — in South American camelids. The objective of our work was to offer an alternative method for determination of trace element concentrations in the body, causing minimum stress to these animal species. The aim of present study was to monitor the concentration of copper, zinc, manganese, and selenium in the hair of llamas, to establish "normal" values and to determine the influence of species, coat colour, age, and sex on the concentration of chosen trace elements.

#### **Materials and Methods**

The study was performed at the University of Veterinary and Pharmaceutical Sciences Brno. The samples were analysed in the AAS laboratory at the Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno.

### Animals and sampling

A total of 77 llamas from 20 farms in the Czech Republic were included in this study. The samples were collected from 23 llamas (*Llama guanicoe f. glama*) and 54 alpacas (*Llama guanicoe f. pacos*) during regular preventative spring health check. All the examined animals were clinically healthy and showed no symptoms of trace element deficiency. The animal feed during the year consisted of hay in the winter season, of hay in combination with pasture forage in the spring season, and throughout the whole year the animals were fed also a supplemental mixture with trace elements. Samples of llama hair were taken from the shoulder area with stainless steel scissors. For each animal the age, sex, and coat colour was recorded. The animals were divided into groups according to age as crias ( $\leq 1$  year) and adult animals ( $> 1$  year) as recommended by Husakova et al. (2014). Each group was further divided according to coat colour into four subgroups (white, beige, brown, black). The numbers of tested animals in individual groups are presented in Table 1.

Table 1. Numbers of examined animals.

	Crias		Adults		Colours			
	Males	Females	Males	Females	White	Beige	Brown	Black
Llama	0	1	7	15	3	5	15	0
Alpaca	3	4	15	32	16	6	19	13
$\Sigma$	3	5	22	47	19	11	34	13

### Analytical procedure

All chemicals used in this work were of analytical grade. Washing solutions were Triton X-100 (Lab Mark a.s., Czech Republic) and acetone (Analytika s.r.o., Czech Republic). Other chemical reagents were nitric acid (65% v/v) (Analytika s.r.o., Czech Republic), hydrogen peroxide (30% v/v) (Analytika s.r.o., Czech Republic) and hydrochloric acid (36% v/v) Analpure (Analytika s.r.o., Czech Republic). Deionized water from a GenPure Ultrapure Water Purification System (Thermo Scientific) with a resistivity of 18 M $\Omega$  was used for all necessary diluting and cleaning of the samples. As reference materials, SRM 1577c Bovine Liver and SRM 1566b Oyster Tissue were used.

The samples of hair were purified by optimized cleaning procedure shown in Table 2. The washing cycles were done at room temperature in the beakers with the use of shaker. As a main washing solution 0.5% Triton X-100 was used, which was suggested as a suitable agent for animal hair pre-treatment in multianalysis by Chyla and Zyrnicki (2000).

Table 2. Sample processing – cleaning procedure and drying.

Washing solution	Procedure	Repetitions
0.5% Triton X-100	Washing 60 min	3
0.5% Triton X-100	Washing 15–20 h	1
0.5% Triton X-100	Washing 60 min	2
Distilled water	Washing 60 min	1
Acetone	Washing 60 min	1
Distilled water	Washing 60 min	2
	Drying (75 °C) 60–90 min	

Hair sample mineralization was performed by microwave digestion in Microwave Labstation Ethos SEL (Milestone, Italy). For the mineralization of hair samples, a 0.25 g sample of treated hair was used for Cu, Zn, and Mn determination, and a 0.5 g sample for determination of Se, always with 6 ml HNO<sub>3</sub> and 2 ml H<sub>2</sub>O<sub>2</sub>.

The digested samples for Cu, Zn, and Mn determination were diluted with deionised water into 25 ml volumetric flasks and the concentration of Cu, Zn and Mn was measured by Flame AAS technique with acetylene air as the carrier gas.

The digested samples for Se determination were evaporated to eliminate residues of nitric acid and then the samples were diluted with deionised water. The reduction of Se<sup>6+</sup> to Se<sup>4+</sup> was performed by the addition of 12.5 ml 6M HCl for 20–24 h at room temperature (Pechova et al. 2005). Selenium concentration in reduced

samples was measured by Hydride Generation AAS technique with hydride generation unit HS 600 with argon as the carrier gas. Trace elements were measured using an Atomic Absorption Spectrometer ContrAA 700 (Analytik Jena, Germany).

#### Statistical evaluation

The data set was divided by the age (crias, adults), sex (male, female), coat colour (white, beige, brown, black) and species (llama, alpaca). The basic statistical parameters for all trace elements within all categories (mean, standard deviation) were calculated. The data are shown as mean values with standard deviation in mg/kg of dry matter (DM). Significance of differences between categories was tested by multifactorial ANOVA and Student-Newman-Keuls *post hoc* test. Analysis was performed in the programme QC Expert 3. 2 and Unistat 6.0.

## Results

The concentrations of microelements in whole set of data are presented in Table 3. Generally, we found the following concentrations (mean  $\pm$  standard deviation): Cu  $10.07 \pm 3.48$  mg/kg DM, Zn  $134.41 \pm 20.62$  mg/kg DM, Se  $0.41 \pm 0.24$  mg/kg DM and Mn  $12.62 \pm 12.71$  mg/kg DM. Our results show that from the evaluated trace elements, zinc was at the highest concentration followed by manganese and copper, whereas selenium was at the lowest concentration in the hair. Although all tested animals were clinically healthy, we found a relatively high variability among all the monitored elements. In order to identify factors that could affect the concentration of microelements we made a statistical evaluation of the impact of species, sex, age, and hair colour. We found a significant influence of the species on the concentration of zinc ( $P \leq 0.05$ ) and selenium ( $P \leq 0.001$ ), therefore, we divided the data also according to the species. The results for each species are shown in Tables 4 and 5. The biggest difference between llamas and alpacas was found in selenium, the concentration of which was almost twice as high in alpacas ( $0.48 \pm 0.24$  mg/kg DM) compared to llamas ( $0.25 \pm 0.14$  mg/kg DM). The difference in zinc concentration was relatively small.

The sex and age of animals influenced the concentration of monitored elements only to a small extent, significant differences were found only in manganese for the entire

Table 3. The concentrations of Cu, Zn, Se and Mn in hair (mg/kg dry matter) of South American camelids (n = 77) and the influence of age, sex, coat colour, and species.

Total	Mean	SD	Min	Max	Age	Sex	C	S	Age x Sex x C x S
Cu	10.07	3.48	4.48	24.07	ns	ns	ns	ns	ns
Zn	134.41	20.62	86.14	211.30	ns	ns	ns	*	ns
Se	0.41	0.24	0.05	1.23	ns	ns	***	***	***
Mn	12.62	12.71	0.54	66.52	*	*	***	ns	ns

SD - standard deviation, C - colour, S - species; \* Significance  $P \leq 0.05$ ; \*\*\* Significance  $P \leq 0.001$ ; ns - not significant

Table 4. The concentrations of Cu, Zn, Se and Mn in hair (mg/kg dry matter) of llamas (n = 23) and the influence of sex and coat colour.

Llamas	Mean	SD	Min	Max	Sex	Colour	Sex x Colour
Cu	9.70	4.69	4.48	24.07	ns	ns	ns
Zn	145.20	21.07	115.30	211.30	ns	ns	ns
Se	0.25	0.14	0.05	0.44	ns	ns	ns
Mn	12.49	10.14	0.64	43.68	ns	ns	ns

SD - standard deviation, \* Significance  $P \leq 0.05$ , \*\*\* Significance  $P \leq 0.001$ , ns - not significant

Table 5. The concentrations of Cu, Zn, Se and Mn in hair (mg/kg dry matter) of alpacas (n = 54) and the influence of age, sex, and coat colour.

Alpacas	Mean	SD	Min	Max	Age	Sex	C	Age x Sex x C
Cu	10.22	2.90	6.17	18.93	ns	ns	ns	ns
Zn	129.81	19.01	86.14	169.70	ns	ns	ns	ns
Se	0.48	0.24	0.07	1.23	ns	ns	***	*
Mn	12.67	13.85	0.54	66.52	ns	ns	*	ns

SD - standard deviation, C - colour; \* Significance  $P \leq 0.05$ , \*\*\* Significance  $P \leq 0.001$ , ns - not significant

data set ( $P \leq 0.05$ ). After dividing the animals by the species, no significant influence of the age or sex on manganese hair concentration was found. The mean concentrations of monitored elements in alpacas divided according to the age are presented in Table 6, and according to sex in llamas and alpacas in Table 7. The animals' age was not evaluated in the llamas because of the small number of crias. As mentioned, our results show that sex and age of the animals can affect the concentration of manganese in hair ( $P \leq 0.05$ ), but for a more accurate assessment, a larger number of animals would be required.

Table 6. The concentrations of Cu, Zn, Se and Mn in alpaca hair (mg/kg dry matter) by age.

	Alpacas ( <i>Llama guanicoe f. pacos</i> )	
	$\leq 1$ year (n = 7)	$> 1$ year (n = 47)
Cu	12.32 $\pm$ 3.99	9.91 $\pm$ 2.62
Zn	138.33 $\pm$ 15.28	128.54 $\pm$ 19.32
Se	0.44 $\pm$ 0.13	0.49 $\pm$ 0.26
Mn	22.08 $\pm$ 17.71	11.27 $\pm$ 12.84

The greatest influence of the studied factors on the concentration of the monitored elements was found for the coat colour (Table 8), which significantly ( $P \leq 0.001$ ) influenced the concentration of selenium and manganese in the evaluation of the entire set of animals (Table 3) and also in the evaluation of alpacas (Table 5). A significant effect of coat colour was not found in the separate evaluation of llamas, which was probably due to the smaller number of animals

Table 7. The concentrations of Cu, Zn, Se and Mn in hair of llamas and alpacas (mg/kg dry matter) by sex.

	Llamas ( <i>Llama guanicoe f. gllama</i> )		Alpacas ( <i>Llama guanicoe f. pacos</i> )	
	Males (n = 7)	Females (n = 16)	Males (n = 18)	Females (n = 36)
Cu	9.17 $\pm$ 2.34	9.92 $\pm$ 5.46	10.20 $\pm$ 3.26	10.23 $\pm$ 2.76
Zn	159.30 $\pm$ 29.02	139.03 $\pm$ 13.45	130.82 $\pm$ 22.18	129.31 $\pm$ 17.54
Se	0.23 $\pm$ 0.12	0.25 $\pm$ 0.15	0.47 $\pm$ 0.21	0.48 $\pm$ 0.26
Mn	18.56 $\pm$ 13.25	9.83 $\pm$ 7.49	17.07 $\pm$ 17.44	10.48 $\pm$ 11.31

in this group. However, the mean values showed similar tendencies as in alpacas. Generally, the Se and Mn concentrations were significantly higher for dark hair compared to fair hair. The concentrations of evaluated elements in llama and alpaca hair for various coat colours are shown in Table 8.

## Discussion

Hair analysis has been studied in various animal species: livestock (Combs 1987; Christodouloupoulos et al. 2003; Cygan-Szczegieliński et al. 2014), sheep (Hawkins and Ragnarsdóttir 2009), goats (Pavlata et al. 2011; Pitropovska et al. 2014), bison

Table 8. The concentrations of Cu, Zn, Se and Mn in hair of llamas and alpaca (mg/kg dry matter) by coat colour.

	Llamas ( <i>Lama guanicoe</i> f. <i>glama</i> )			Alpacas ( <i>Lama guanicoe</i> f. <i>pacos</i> )				
	White (n = 3)	Beige (n = 5)	Brown (n = 5)	White (n = 16)	Beige (n = 6)	Brown (n = 19)	Black (n = 13)	
Cu	6.11 ± 1.22	10.68 ± 4.63	10.09 ± 4.99	11.39 ± 2.93	10.02 ± 2.15	9.55 ± 2.24	9.86 ± 3.78	
Zn	141.13 ± 16.56	129.18 ± 8.36	151.35 ± 22.43	132.77 ± 14.90	118.63 ± 21.25	130.78 ± 17.31	129.91 ± 24.61	
Se	0.16 ± 0.14	0.23 ± 0.13	0.27 ± 0.15	0.32 ± 0.02 <sup>abb</sup>	0.34 ± 0.15 <sup>dd</sup>	0.50 ± 0.20 <sup>ace</sup>	0.72 ± 0.25 <sup>bb,dd,ee</sup>	
Mn	2.49 ± 0.80	9.00 ± 7.87	15.65 ± 10.37	3.26 ± 2.82 <sup>abb</sup>	15.79 ± 25.07	14.19 ± 12.17 <sup>a</sup>	20.61 ± 12.60 <sup>bb</sup>	

<sup>a</sup>  $P \leq 0.05$ , <sup>aa</sup>  $P \leq 0.01$  The same superscripts in one row show significant differences between groups

(Kosla et al. 2011), camels (Faye and Seboussi 2009), mule deer (Roug et al. 2015), horse (Dunnnett and Lees 2003; Asano et al. 2005) and seals (Ikemoto et al. 2004). Some of these studies show the diagnostic potential of hair analysis.

It has been found that hair colour influences the concentration of microelements (Chyla and Zyrnicki 2000; Christodouloupoulos et al. 2003; Asano et al. 2005; Hawkins and Ragnarsdottir 2009; Skibniewska et al. 2011; Farag et al. 2015). Some studies evaluated also the relationship between microelement concentrations in hair and different biological tissues/fluids. Copper concentration in hair was found to correlate with liver Cu in rats (Jacobs et al. 1978). Selenium concentration in hair was found to correlate with blood selenium in cows (Christodouloupoulos et al. 2003) or with blood and liver selenium in deer (Roug et al. 2015). Zinc concentration in hair correlated with Zn contents in the liver and kidneys in Caspian seals (Ikemoto et al. 2004) and with Zn concentration in bones and testes of rats (Deeming and Weber 1977). Roug et al. (2015) found the correlations between liver and hair manganese of the deer. Because of a lack of information about the concentrations of trace elements in South American camelid hair, we compared our data with studies on other animal species.

### Copper

The concentration of copper in the hair of South American camelids was found within the range 4.5–24.1 mg/kg DM with the mean value of 10.1 mg/kg DM. Liu et al. (1994) found a lower copper concentration of  $3.5 \pm 1.0$  mg/kg DM in camel hair. Similar values as in our work were also found in ruminants. Patkowska-Sokola et al. (2009) reported a Cu concentration of 5.3–10.3 mg/kg DM in sheep wool and Cygan-Szczegieliński et al. (2014) found a Cu concentration of 10.2–32.0 mg/kg DM in cow hair. In the study of Roug et al. (2015), the Cu content in mule deer hair was in the range of 0.01–13.0 mg/kg DM and Asano et al. (2005) found  $6.6 \pm 2.2$  mg/kg DM in horse mane hair. The mean copper content in rat hair was found to be  $9.4 \pm 1.8$  mg/kg (Jacob et al. 1978) and a higher mean value (17.1 mg/kg) was found in the study of Chyla and Zyrnicki (2000) monitoring the Cu content in the dog hair.

The concentration of copper was not influenced by any of our monitored factors. Jacob et al. (1978) found correlations between Cu in the liver and in the hair of rats, and also Ramirez-Perez et al. (2000) reported that the Cu concentration in hair is dependent on the Cu content in the animal diet. We suggest that the Cu content in llama hair is influenced by the copper intake and that it can be the reason for the wide range of values in our study. Even if the animals in our study did not show clinical signs of any health problems, we were not able to exclude subclinical deficiency. Our values can serve as a pilot study of “normal” values, however, more detailed research in this area is required.

## Zinc

The concentration of zinc in the hair of South American camelids was found within the range of 86.1–211.3 mg/kg DM with the mean value of 134.4 mg/kg DM. Values reported in ruminants vary according to the species. Patkowska-Sokola et al. (2009) reported a lower range of 75.0 to 88.8 mg/kg DM in sheep wool, whereas the range of Zn concentrations in cow hair was broader, namely 125.7–427.4 mg/kg DM (Cygan-Szczegielniak et al. 2014). The Zn concentration in mule deer hair in California was found with the range of 57–180 mg/kg DM by Roug et al. (2015). Pavlata et al. (2011) found the mean value of  $97.9 \pm 10.1$  mg/kg DM in goat hair, and Ikemoto et al. (2004) found  $98.1 \pm 26.4$  mg/kg DM in hair of Caspian seals. On the other hand, the mean Zn concentration in the hair of pet and feral cats in the study of Skibniewska et al. (2011) was much higher than the concentration in the hair of llamas (238.9 mg/kg DM). Very similar result (234 mg/kg DM) was described in the study of Chyla and Zyrnicki (2000) in dog hair, whereas in horse mane hair the mean Zn concentration was slightly lower ( $200.9 \pm 47.8$  mg/kg DM) according to Asano et al. (2005). Based on our results, the concentration of zinc was affected only by the animal species. On the basis of studied literature (Scott 1991; Ikemoto et al. 2004; Klevay et al. 2004) we suggest that zinc concentration in hair is influenced by the concentration of this element in the body.

## Selenium

The concentration of selenium in the hair of llamas was in the range of 0.05–1.23 mg/kg DM with the mean value of 0.41 mg/kg DM. Liu et al. (1994) reported a smaller range of Se in the wool of Bactrian camel (0.14–0.19 mg/kg DM). The difference in comparison with our values can be caused by hair colour, because we found similar values in white and beige llamas. Christodoulopoulos et al. (2003) also found an effect of colour in Holstein dairy cows, which had a lower Se content in white hair ( $0.23 \pm 0.16$  mg/kg DM) in comparison with black hair ( $0.37 \pm 0.17$  mg/kg DM). Roug et al. (2015) found the Se concentration in mule deer hair within the range 0.1–1.90 mg/kg DM. Asano et al. (2005) found the highest mean Se values ( $0.58 \pm 0.35$  mg/kg DM) in horse hair. From the factors monitored in our study we found only the coat colour to have a significant effect on the selenium concentration in llama hair. The Se concentration was directly proportional to the degree of hair colouration. In fair hair the mean Se concentration was the lowest and on the other hand, in dark hair we found the highest Se concentration. The effect of coat colour on Se concentration is probably due to the differences in the concentration of sulphur-containing amino acids, such as methionine and cysteine which are present in melanin molecules. These amino acids can be replaced by selenomethionine and selenocysteine, which represented some deposits of selenium (Christodoulopoulos et al. 2003). The amount of selenoaminoacids in the body is influenced by the intake of selenium and also by the form of Se in the ration (Sevcikova et al. 2011). Organic forms of Se (mainly Se-yeast) contain high amounts of Se-Met. Thus, Se hair concentration is influenced by the intake of Se and can serve as an indicator of long term supplementation. However, it is necessary to differentiate the colour for the evaluation.

## Manganese

The concentration of manganese in the hair of South American camelids was within the range 0.54–66.52 mg/kg DM with the mean value of 12.62 mg/kg DM. This element had the highest variability. Patkowska-Sokola et al. (2009) reported the range of 3.4 to 22.9 mg/kg DM in sheep wool; a similar range of 3.8–20.0 mg/kg DM was found by Cygan-Szczegielniak et al. (2014) for Mn concentrations in cow hair, which corresponds with our mean result. Roug et al. (2015) report a Mn concentration of 0.02–120.0 mg/kg DM in mule deer hair. Pitropovska et al. (2014) found  $3.14 \pm 0.19$  and  $4.25 \pm 0.36$  mg/kg DM

in kids of white short haired goats with different supplementations of manganese. These lower values are corresponding with our values for white haired llamas, supporting our finding of a significant effect of colour on the concentration of Mn in hair. The mean Mn concentration values were the lowest in white haired animals and the highest Mn values were found in dark haired ones. Chyla and Zyrnicki (2000) monitored the content of minor and trace elements in dog hair in relation to the hair colour and they also found that generally, black hair had higher element concentrations than fair hair. We have confirmed the same fact in the hair of alpacas and llamas only for Se and Mn concentrations. The mean values in studies monitoring the content of Mn in other animal hair were lower than in our study, e. g. the mean Mn concentrations in hair of Caspian seals were  $1.75 \pm 1.55$  mg/kg (Ikemoto et al. 2004); the values of Mn concentration in dog hair were 1.72 mg/kg (Chyla and Zyrnicki 2000); the Mn contents in hair of Weddell seal were  $1.15 \pm 0.41$  mg/kg (Gray et al. 2008); and the Mn concentrations in horse mane hair were  $1.24 \pm 1.12$  mg/kg (Asano et al. 2005). According to the literature, Mn concentrations in hair are similar for ruminants, and lower values are reported for other mammals in general. The effect of age and sex on Mn concentration in hair was found also in different animal species. According to several studies the effect of the age or sex on the hair concentrations of Cu, Zn, Se and Mn was not confirmed (Asano et al. 2002; Kosła et al. 2011; Skibniewska et al. 2011; Ghorbani et al. 2015).

This study reports the concentrations of Cu, Zn, Se and Mn in the hair of llamas and alpacas in the Czech Republic and evaluates the effect of species, coat colour, age, and sex on their concentrations. A significant difference was found in the Zn and Se concentrations between llamas and alpacas as species. From all the evaluated factors, coat colour had the highest effect, significantly influencing Mn and Se concentrations. The concentrations of both elements were higher in animals with darker hair. The evaluation of trace element concentrations in llama hair has a potential to be used for the evaluation of the long-term status of trace elements in the body, however, it is necessary to continue with experimental work in this area. Our findings can serve as a pilot study for further works in this field.

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