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Assessment of Gender Effects and Reference Values of Mane Hair Trace Element Content in English Thoroughbred Horses (North Caucasus, Russia) Using ICP-DRC-MS

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Abstract

The objective of the present study was assessment of gender differences in hair trace element content in English Thoroughbred horses (North Caucasus, Russia) using ICP-DRC-MS and calculation of the reference values. Trace element content in mane hair of 190 stallions and 94 mares (3–7 years old) bred in North Caucasus (Russia) was assessed using inductively coupled plasma mass spectrometry. Mane hair Co, Cr, Mn, Li, Si, and Sr levels in mares exceeded those in stallions by 77%, 63%, 64%, 42%, 39%, and 64%, respectively. Hair Fe and Si content was nearly twofold higher in female horses as compared to the males. Only hair Zn content was 5% higher in stallions as compared to mares. In addition, mares were characterized by 63%, 65%, 29%, and 40% higher levels of As, Pb, Sn, and Ni levels in hair as compared to the respective values in stallions. In turn, hair Al and Hg were more than twofold higher in mares than in stallions. The reference intervals of mane hair content (μg/g) for Co (0.006–0.143), Cr (0.028–0.551), Cu (4.17–6.84), Fe (10.11–442.2), I (0.026–3.69), Mn (0.551–12.55), Se (0.108–0.714), Zn (97.43–167), Li (0.011–0.709), Ni (0.060–0.589), Si (0.665–29.12), V (0.006–0.584), Al (1.98–168.5), As (0.006–0.127), Cd (0.002–0.033), B (0.494–16.13), Pb (0.018–0.436), Sn (0.002–0.144), Sr (1.0–9.46), and Hg (0.0018–0.017) in the total cohort of horses were estimated using the American Society for Veterinary Clinical Pathology Quality Assurance and Laboratory Standard Guidelines. The reference intervals were also estimated for stallions and mares bred in North Caucasus (Russia) and may be used for interpretation of the results of hair trace element analysis in horses.

Keywords Horses · Mares · Stallions · Trace elements · Reference range

Introduction

Trace elements and minerals play a significant role in equine health and performance [1]. Our earlier data demonstrated that trace element levels in hair are significantly associated with speed in trotter horses [2]. Rhabdomyolysis is also associated with altered trace element status in Arabian horses [3].

Therefore, monitoring of trace element status of horses is considered as an essential tool for analysis of equine nutrition and exposure. The earlier studies demonstrated that supplementation with Fe, K, and Zn chelated with glycine resulted in a significant increase in hair metal levels in horse hair [4]. Hair Cu content responded differentially not only to the overall dose of copper but also to its particular form [5]. Similarly, assessment of hair Se content may be used for evaluation of Se toxicosis for up to 3 years after exposure [6].

Similarly, environmental pollution was also associated with increased hair toxic element content. In addition, hair Cd and Pb levels were characterized by a significant correlation with serum concentrations [7].

Hair is considered as the potential indicator of trace element nutrition due to its stability [8], although certain contradictions associated with a high variability of hair trace element content exist [9]. Particularly, a variety of factors have a

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significant effect on hair trace element content in horses [10]. The existing data demonstrate that equine age [11], as well as breed, housing system [12], and season [13], has a significant impact on hair trace element content. At the same time, gender effects on hair trace element content in horses are insufficiently studied [14].

In order to increase the adequacy of hair trace element analysis for nutritional and toxicological biomonitoring, the appropriate reference values for a particularly homogenous group should be determined. At the same time, the reference values for hair trace element content in horses are limited [15]. Moreover, the reference values estimated using American Society for Veterinary Clinical Pathology Quality Assurance and Laboratory Standard Guidelines [16] are absent.

Therefore, the objective of the present study was assessment of gender differences in hair trace element content in English Thoroughbred horses (North Caucasus, Russia) using ICP-DRC-MS and calculation of the gender-specific reference values.

Materials and Methods

The protocol of the present investigation was approved by the Institutional Ethics Committee (Orenburg State University, Orenburg, Russia). All procedures involving animals were performed in agreement with the ethical standards by the Declaration of Helsinki (1964) and its later amendments (2013).

Animals and Sampling

A total of 284 English Thoroughbred horses (190 stallions and 94 mares) bred in Northern Caucasus (Russia) were examined. The age of the animals varied from 3 to 7 years old. No significant group difference in age between male and female horses was observed. Only animals with black (bay) mane hair were studied as the earlier data demonstrate that mane hair color may have a significant impact on trace element and mineral levels [14]. The hair samples were collected in the period of 2017–2018 during the summer season in order to avoid the potential effect of different seasons [13] on hair element levels. The studied animals were maintained on similar diets for all periods adjusted to the final levels of trace elements and other nutrients with mineral premix (Table 1). Trace element content of the dietary items was generally adequate to the requirements but did not exceed maximal tolerable limits as estimated by recommendations of USSR State Agriculture Committee [17] and U.S. National Research Council [18].

Hair samples (not less than 0.4 g) were collected from mane at the level of the first cervical vertebra (C1). Hair were collected using stainless steel scissors precleaned with ethanol before each use. Only proximal parts of hair strands (15 mm) were collected in order to avoid the excessive impact of external binding of trace elements on the results of analysis.

Table 1 Characteristics of the horses' diet in relation to gender (per 100 kg of body weight/day)

Parameter	Stallions	Mares
Dry matter (kg)	2.2	2.2
Energy (MJ)	16.566	16.104
Crude protein (g)	206.8	220
Digestible protein (g)	145.2	154
Crude cellulose (g)	396	440
Salt (NaCl) (g)	4.62	5.28
Calcium (Ca) (g)	8.8	11
Phosphorus (P) (g)	6.6	7.7
Magnesium (Mg) (g)	2.2	2.86
Iron (Fe) (mg)	176	176
Copper (Cu) (mg)	18.7	18.7
Zinc (Zn) (mg)	84.7	66
Cobalt (Co) (mg)	0.44	0.88
Manganese (Mn) (mg)	66	88
Iodine (I) (mg)	0.44	0.66
Selenium (Se) (mg)	0.198	0.22
Carotene (mg)	27.06	42.9

Hair Pretreatment and Analysis

The obtained hair samples were washed in acetone (Sigma-Aldrich, Co., USA) and then rinsed thrice in deionized water (18 MΩ cm) in order to remove external contamination. After washing, the samples were dried at 60 °C to a stable weight. Fifty milligrams of hair samples was introduced into Teflon tubes containing 5 ml of concentrated nitric acid (Sigma-Aldrich, Co., USA) and subsequently digested in the microwave system Berghof SW-4 DAP-40 microwave system (Berghof Products + Instruments GmbH, Eningen, Germany). The obtained solutions were transferred into 15-ml polypropylene test tubes and adjusted to the final volume of 15 ml with deionized water and thoroughly mixed up by shaking in the closed test tubes.

Assessment of essential and conditionally essential (Co, Cr, Cu, Fe, I, Li, Mn, Se, Si, Sr, V, Zn) as well as toxic and potentially toxic (As, B, Cd, Hg, Ni, Pb, Sn) trace elements in the samples was performed using NexION 300D spectrometer (Perkin Elmer, USA) equipped with ESI SC-2 DX4 autosampler (Elemental Scientific Inc., Omaha, NE, USA). Calibration of the system was performed using standard solutions with different concentrations of the trace elements prepared from Universal Data Acquisition Standards Kits (PerkinElmer Inc., Shelton, CT 06484, USA). Internal online standardization was performed using 10 μg/l Yttrium (Y) and

Rhodium (Rh) Pure Single-Element Standard (PerkinElmer Inc., Shelton, CT, USA). Laboratory quality control was performed via permanent analysis of the certified reference material of hair GBW09101 (Shanghai Institute of Nuclear Research, Shanghai, China). Analysis was performed both before and after each set of analyses. The recovery rate for all studied trace elements was within the interval of 88–107%. The laboratory of the Center for Biotic Medicine (Moscow, Russia) is the IUPAC company associate, also being involved in the system of External Quality Assessment Schemes in the field of Occupational and Environmental Medicine (EQAS OELM).

Statistical Analysis

Statistical treatment of the data was performed using Statistica 10.0 (StatSoft Inc., USA) software package. Data distribution was assessed using Shapiro-Wilk test, being indicative of non-Gaussian data distribution. Therefore, median and the respective 25th and 75th percentile boundaries (interquartile range) were used as descriptive statistics. Gender group comparison was performed using non-parametric Mann-Whitney U test at the level of significance of $p < 0.05$. False discovery rate (FRD) adjustment for p value was applied due to multiple comparisons.

The reference intervals were estimated using the American Society for Veterinary Clinical Pathology Quality Assurance and Laboratory Standard Guidelines [16]. Briefly, after percentile two-sided exclusion of outliers, the robust method was applied for assessment of reference intervals and 90% confidence intervals (90%CI) for the lower and upper limits. The assessment of the reference intervals was performed using Reference Value Advisor [19] for MS Excel (Microsoft Inc., USA).

Results and Discussion

The obtained data demonstrate that hair essential trace element content was characterized by a significant gender difference (Table 2). Particularly, hair mane Co, Cr, Mn, Li, Si, and Sr levels in mares exceeded those in stallions by 77%, 63%, 64%, 42%, 39%, and 64%, respectively. At the same time, hair Fe and Si content was nearly twofold higher in female horses as compared to the males. Only hair Zn content was 5% higher in stallions as compared to mares. No significant group difference was observed in hair Cu, I, and Se.

The gender patterns of hair toxic trace element content were generally similar to those observed for essential elements (Table 3). Mares were characterized by 63%, 65%, 29%, and 40% higher levels of As, Pb, Sn, and Ni levels in hair as compared to the respective values in stallions. In turn, hair Al and Hg were more than twofold higher in mares than in stallions. No significant gender difference for hair Cd and B was observed.

Table 2 Mane hair essential trace element content in stallions and mares

Element	Stallions ($n = 190$)	Mares ($n = 94$)	p
Co	0.013 (0.01–0.0219)	0.0231 (0.012–0.0895)	0.000001*
Cr	0.089 (0.0597–0.136)	0.145 (0.0923–0.304)	0.000001*
Cu	5.62 (5.09–6.04)	5.57 (5.04–6.02)	0.531811
Fe	21.77 (14.94–37.61)	42 (19.91–208)	0.000003*
I	0.221 (0.0935–0.721)	0.395 (0.224–0.859)	0.002866
Li	0.05 (0.0257–0.103)	0.071 (0.0416–0.174)	0.000412*
Mn	1.33 (0.927–2.08)	2.18 (1.33–7.08)	< 0.000001*
Se	0.446 (0.359–0.531)	0.4460 (0.331–0.539)	0.676430
Si	8.55 (4.95–16.47)	11.91 (5.17–20.63)	0.044921*
Sr	2.6 (1.89–3.89)	4.26 (2.56–5.79)	< 0.000001*
V	0.024 (0.0132–0.0575)	0.057 (0.0177–0.284)	0.000016*
Zn	130 (121–141)	124.5 (113–137)	0.004285*

*Significant group difference at $p < 0.05$

The observed gender difference in mane hair trace element content corresponds to different trace element requirements in male and female horses [17] and nutrient intake (Table 1). It has been demonstrated that dietary intake has a significant effect on hair trace element content in horses [20]. Gender may have a significant impact on trace element metabolism. Particularly, an earlier study demonstrated significantly higher serum haptoglobin concentrations in mares [21]. Serum copper levels were also found to be higher in female horses [22]. Gender variations in trace element and especially heavy metal levels may be related to metallothionein metabolism [23] that is known to be regulated by sex hormones [24]. Correspondingly, Anke et al. [25] demonstrated a significant gender difference in hair Cd content in horses. The effect of gender on hair trace element content was demonstrated in multiple studies in other species including humans [26] and ruminants [27]. Gender difference in sex hormones production seems to be the most likely cause of the observed gender-specific hair trace element levels [28]. It has been demonstrated that serum essential trace element levels in mares differ between the reproductive phases (estrual, fertile, pregnancy, infertility) [29]. Although equine studies on the potential role of sex hormones in gender difference of trace element metabolism are insufficient, the existing human data corroborate this suggestion. Particularly, it has been demonstrated that the levels of trace elements are interrelated with sex hormones during maturation [30] and normal estrous cycle [31]. Correspondingly, impaired hormonal profile in polycystic ovary syndrome was associated with altered serum trace element levels in women [32]. Estrogens are capable of induction of metal transporter protein expression including Ctr1 and DMT1 [33]. Testosterone also has a significant impact on

Table 3 Gender difference in toxic and potentially toxic trace element content in mane hair of horses

Element	Stallions ($n = 190$)	Mares ($n = 94$)	p
Al	8.13 (4.49–18.89)	21.36 (5.63–119)	0.000102*
As	0.0168 (0.0111–0.025)	0.0274 (0.0167–0.0697)	< 0.000001*
B	2.12 (1.44–3.16)	2.215 (1.46–3.59)	0.682590
Cd	0.0052 (0.0031–0.01)	0.0062 (0.0035–0.0113)	0.180272
Hg	0.0018 (0.0018–0.0036)	0.0052 (0.0018–0.0091)	0.000004*
Ni	0.125 (0.0929–0.175)	0.175 (0.116–0.382)	< 0.000001*
Pb	0.05 (0.0352–0.0813)	0.0824 (0.0552–0.192)	< 0.000001*
Sn	0.0141 (0.0068–0.0284)	0.0182 (0.0104–0.0319)	0.014540*

*Significant group difference at $p < 0.05$

metabolism of metals and especially iron [34]. In addition, reduced testosterone levels are associated with higher hair Cu and Cu-to-Zn ratio [35]. Circulating testosterone concentrations were also found to correlate directly with urinary Cd and Cr levels [36].

At the same time, Asano did not observe any significant difference in hair levels of both toxic and essential elements between male and female horses [11] possibly due to a low number of observations.

The reference intervals for hair trace element content for mares and stallions were also different due to gender-specific differences in hair element content (Table 4).

The reference intervals for all studied animals ($n = 284$) were compared to the earlier data on hair trace element content (Table 5). None of the earlier studies have used ICP-MS for analysis. The studies by Asano et al. [11, 14, 15] provide particular values for the majority of trace elements studied. As the authors have not used the American Society for

Table 4 Reference intervals for mane hair trace element content (μg/g) in English Thoroughbred stallions and mares calculated in agreement with the American Society for Veterinary Clinical Pathology Quality Assurance and Laboratory Standard Guidelines

	Stallions ($n = 190$)			Mares ($n = 94$)		
Element	Reference interval	Lower limit 90% CI	Upper limit 90% CI	Reference interval	Lower limit 90% CI	Upper limit 90% CI
Essential elements						
Co	0.006–0.112	0.005–0.008	0.102–0.154	0.0062–0.173	0.0057–0.008	0.139–0.175
Cr	0.026–0.40	0.02–0.035	0.319–0.52	0.0263–0.665	0.024–0.036	0.56–0.706
Cu	4.29–6.78	4.0–4.52	6.65–7.23	4.06–7.88	3.31–4.17	6.69–8.83
Fe	9.99–268.2	9.09–10.32	242–299	9.46–591.7	8.76–11.4	451–656
I	0.02–3.87	0.015–0.029	2.52–4.82	0.079–3.47	0.054–0.088	2.09–4.49
Mn	0.48–9.35	0.407–0.585	5.66–12.67	0.613–14.6	0.562–0.966	12.01–17.68
Se	0.127–0.732	0.09–0.251	0.664–0.771	0.077–0.71	0.075–0.119	0.618–0.768
Zn	100.7–170.8	89.9–105	160–181	93.95–166.7	82.06–101.0	153–171
Li	0.01–0.31	0.008–0.013	0.283–0.374	0.012–1.02	0.01–0.015	0.713–1.22
Ni	0.054–0.464	0.05–0.064	0.398–0.639	0.075–0.653	0.074–0.09	0.538–0.71
Si	0.532–29.18	0.048–0.821	25.35–29.77	1.59–30.1	1.17–2.29	24.49–32.02
V	0.007–0.328	0.005–0.008	0.268–0.404	0.0052–0.823	0.005–0.008	0.685–0.929
Toxic elements						
Al	2.23–136.0	1.54–2.44	105–151	1.72–192.7	1.67–2.23	174–203
As	0.006–0.099	0.005–0.007	0.07–0.118	0.0066–0.181	0.005–0.012	0.13–0.183
Cd	0.002–0.03	0.0015–0.002	0.025–0.045	0.002–0.054	0.0018–0.0024	0.025–0.059
B	0.464–18.9	0.349–0.53	12.39–21.52	0.512–5.96	0.496–0.704	5.3–6.37
Pb	0.016–0.5	0.015–0.02	0.294–0.796	0.023–0.436	0.0228–0.029	0.405–0.443
Sn	0.0016–0.2	0.0006–0.0025	0.125–0.446	0.0017–0.133	0.00095–0.0055	0.091–0.146
Sr	0.926–7.02	0.797–1.05	6.34–8.57	1.33–13.49	1.28–1.62	9.5–14.18
Hg	0.0018–0.016	0–0.0018	0.012–0.019	0.0018–0.02	0–0.0018	0.017–0.023

Table 5 Comparison of the reference values for hair trace element content obtained for horses within the present study and earlier data (µg/g)

	Present study	Asano et al. (2005a) [15]	Asano et al. (2005b) [14]	Asano et al. (2002) [11]	Stachurska et al. (2011) [37]	Wichert et al. (2002) [38]	Jančíková et al. (2013) [5]
Method	ICP-MS	PIXE	PIXE	ICP-AES	AAS	AAS	AAS
Country	Russia	Japan	Japan	Japan	Poland	Germany	Czech Republic
Color	Bay	Bay	n.a.	n.a.	n.a.	n.a.	n.a.
Breed	Thoroughbred	n.a.	Thoroughbred	Thoroughbred	Polish Konik	Various	Bohemian
n	284	27	26	24	35	106	20
Co	0.006–0.143	0.193 ± 0.304	0.46 ± 0.36	n.a.	n.a.	n.a.	n.a.
Cr	0.028–0.551	0.375 ± 0.306	0.44 ± 0.32	0.22 ± 0.16	1.03 ± 0.66	n.a.	n.a.
Cu	4.17–6.84	6.330 ± 1.388	6.00 ± 0.14	4.8 ± 1.3	2.92 ± 0.43	7.3 ± 1.7	13.46 ± 1.974
Fe	10.11–442.2	30.363 ± 16.426	39 ± 45	69.0 ± 83.0	n.a.	n.a.	202.86
I	0.026–3.69	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Mn	0.551–12.55	1.204 ± 1.109	1.37 ± 1.38	5.20 ± 3.35	9.39 ± 5.90	n.a.	n.a.
Se	0.108–0.714	0.658 ± 0.299	0.64 ± 0.26	1.29 ± 0.56	n.a.	0.284 ± 0.136*	n.a.
Zn	97.43–167	189.662 ± 38.108	164 ± 26	86.0 ± 24.0	n.a.	126 ± 38	168.25 ± 26.139
Li	0.011–0.709	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Ni	0.060–0.589	0.330 ± 0.523	0.18 ± 0.15	0.26 ± 0.09	n.a.	n.a.	n.a.
Si	0.665–29.12	90.403 ± 64.977	97 ± 127	20.2 ± 19.2	n.a.	n.a.	n.a.
V	0.006–0.584	0.295 ± 0.362	0.54 ± 0.56	n.a.	n.a.	n.a.	n.a.
Al	1.98–168.5	65.529 ± 43.479	82 ± 74	64.5 ± 77.0	n.a.	n.a.	n.a.
As	0.006–0.127	n.a.	n.a.	1.16 ± 0.44	n.a.	n.a.	n.a.
Cd	0.002–0.033	n.a.	n.a.	0.12 ± 0.12	0.01 ± 0.00	n.a.	n.a.
B	0.494–16.13	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Pb	0.018–0.436	0.833 ± 0.943	1.63 ± 1.13	0.93 ± 0.78	0.22 ± 0.41	n.a.	n.a.
Sn	0.002–0.144	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Sr	1.0–9.46	3.577 ± 1.108	3.48 ± 1.15	n.a.	n.a.	n.a.	n.a.
Hg	0.0018–0.017	0.412 ± 0.529	1.02 ± 0.58	0.31 ± 0.02	n.a.	n.a.	n.a.

*Originally reported in µg/kg; *n.a.*, data not available; *ICP-MS*, inductively coupled plasma mass spectrometry; *PIXE*, particle-induced X-ray emission; *ICP-AES*, inductively coupled plasma atomic emission spectrometry; *AAS*, atomic absorption spectrometry

Veterinary Clinical Pathology Quality Assurance and Laboratory Standard Guidelines [16], comparison is sometimes complicated. However, the obtained values for the majority of essential elements correspond to the earlier data by Asano and colleagues [11, 14, 15]. The obtained data on hair Cu content generally correspond to the values obtained for Polish Konik horses [37], whereas the German [38] and Czech [5] studies revealed higher values. The values of hair Mn [37] and Se [38] content in hair obtained by AAS were nearly similar to the obtained reference ranges. A majority of contradictions were observed in the case of toxic trace element levels in hair of horses. Particularly, the obtained data and reference ranges for hair As, Cd, Pb, and Hg content in hair of horses from Russia (North Caucasus) were significantly lower than those observed by Asano et al. [11, 14, 15]. At the same time, Stachurska et al. [37] reported nearly similar hair Pb levels. The observed difference in hair toxic metal content may be associated with background metal pollution in the studied regions as demonstrated in humans [39] and animals [40]. Particularly, earlier data on hair trace element content in humans demonstrated the lowest levels of toxic metals including mercury in inhabitants of Northern Caucasus as compared to the other regions of Russia, being in agreement with low rate of toxic metal emission from industrial and other anthropogenic sources [41].

Generally, the methodology of trace element and mineral analysis may also have a significant impact on the data. Particularly, it has been demonstrated that ICP-MS is a more sensitive and reproducible technology as compared to ICP-OES and AAS, although being much more expensive. Moreover, potential atomic interferences in ICP-MS are removed by the use of collision-cell or dynamic reaction-cell technology (ICP-DRC-MS) [42] as used in the present study. Despite a lower reproducibility of PIXE method, it is also considered as the sensitive method for trace element content assessment [43]. It is also notable that comparative analysis of the use of XRF, PIXE, and ICP-OES demonstrated that the usefulness of the methods may be element specific [44].

The obtained data demonstrate significant gender difference in hair trace element content in mane hair in horses. Therefore, only gender-specific reference ranges of hair trace element content should be used for interpretation of the results of hair trace element analysis in horses. At the same time, further studies aimed at assessment of the mechanisms underlying the observed gender differences are highly required. Finally, the obtained results demonstrate that English Thoroughbred horses from Northern Caucasus are characterized by very low levels of toxic metals, being indicative of the favorable conditions for horse breeding.

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Compliance with Ethical Standards

The protocol of the present investigation was approved by the Institutional Ethics Committee (Orenburg State University, Orenburg, Russia). All procedures involving animals were performed in agreement with the ethical standards by the Declaration of Helsinki (1964) and its later amendments (2013).

Conflict of Interest The authors declare that they have no conflict of interest.

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References

1. Coenen M (2013) Macro and trace elements in equine nutrition. In: Geog JR, Harris PA, Coenen M (eds) Equine applied and clinical nutrition. Saunders Elsevier, Philadelphia, pp 190–228
2. Kalashnikov V, Zajcev A, Atroshchenko M, Miroshnikov S, Frolov A, Zav'yalov O, Kilinkova L, Kalashnikova T (2018) The content of essential and toxic elements in the hair of the mane of the trotter horses depending on their speed. Environ Sci Pollut Res 25:21961–21967. https://doi.org/10.1007/s11356-018-2334-2
3. El-deeb WM, El-Bahr SM (2014) Selected biochemical indicators of equine rhabdomyolysis in Arabian horses: acute phase proteins and trace elements. J Equine Vet Sci 34:484–488. https://doi.org/10.1016/j.jevs.2013.09.012
4. Armelin MJA, Ávila RL, Piasentin RM, Saiki M (2003) Effect of chelated mineral supplementation on the absorption of Cu, Fe, K, Mn and Zn in horse hair. J Radioanal Nucl Chem 258:449–451. https://doi.org/10.1023/A:1026278914222
5. Jančíková P, Horký P, Zeman L (2013) 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 Mendel Brun 60:145–152. https://doi.org/10.11118/actaun201260060145
6. Davis TZ, Stegelmeier BL, Hall JO (2014) Analysis in horse hair as a means of evaluating selenium toxicoses and long-term exposures. J Agric Food Chem 62:7393–7397. https://doi.org/10.1021/jf500861p
7. Janiszewska J, Cieśla A (2002) Concentration of cadmium and lead in horse blood serum and hair in relation to season and environment. EJPAU 5:1–8
8. 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. https://doi.org/10.1016/S0034-5288(03)00074-2
9. Hintz HF (2001) Hair analysis as an indicator of nutritional status. J Equine Vet Sci 21:A1. https://doi.org/10.1016/S0737-0806(01)70122-0
10. Dobrzanski Z, Jankowska D, Dobicki W, Kupczynski R (2005) The influence of different factors on the concentration of elements in hair of horses. Anim Environ 2:153
11. 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. https://doi.org/10.1292/jvms.64.607
12. Topczewska J, Krupa W (2013) Influence of horse breed and housing system on the level of selected elements in horse's hair. J Elem 18:287–295
13. Topczewska J (2012) Effects of seasons on the concentration of selected trace elements in horse hair. JCEA 13:671–680. https://doi.org/10.5513/JCEA01/13.4.1110
14. Asano K, Suzuki K, Chiba M, Sera K, Asano R, Sakai T (2005) 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. https://doi.org/10.1385/BTER:107:2:135
15. Asano K, Suzuki K, Chiba M, Sera K, Matsumoto T, Asano R, Sakai T (2005) 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. https://doi.org/10.1385/BTER:103:2:169
16. Friedrichs KR, Harr KE, Freeman KP, Szladovits B, Walton RM, Barnhart KF, Blanco-Chavez J (2012) ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. Vet Clin Pathol 41:441–453. https://doi.org/10.1111/vcp.12006
17. National Research Council (2007) Nutrient requirements of horses: sixth revised edition. the National Academies Press, Washington, DC. https://doi.org/10.17226/11653
18. USSR State Agriculture Committee (1987) Temporary maximum allowable levels of certain chemical elements and gossypol in feeds for farm animals and feed additives. Gosagroprom USSR, Moscow
19. Geffré A, Concordet D, Braun JP, Trumel C (2011) Reference value advisor: a new freeware set of macroinstructions to calculate reference intervals with Microsoft Excel. Vet Clin Path 40:107–112. https://doi.org/10.1111/j.1939-165X.2011.00287.x
20. Ghorbani A, Mohit A, Kuhi HD (2015) Effects of dietary mineral intake on hair and serum mineral contents of horses. J Equine Vet Sci 35:295–300. https://doi.org/10.1016/j.jevs.2015.01.018
21. Cywinska A, Szarska E, Kowalska A, Ostaszewski P, Schollenberger A (2011) Gender differences in exercise–induced intravascular haemolysis during race training in thoroughbred horses. Res Vet Sci 90:133–137. https://doi.org/10.1016/j.rvsc.2010.05.004
22. Naseema U, Vairamuthu S, Balachandran C, Ravikumar G (2018) Effect of age and gender on serum mineral profile in thoroughbred horses. Indian Vet J 95:21–23
23. Jeffery EH, Noseworthy R, Cherian MG (1989) Age dependent changes in metallothionein and accumulation of cadmium in horses. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 93: 327–332. https://doi.org/10.1016/0742-8413(89)90242-9
24. Shimada H, Hashiguchi T, Yasutake A, Waalkes MP, Imamura Y (2012) Sexual dimorphism of cadmium-induced toxicity in rats: involvement of sex hormones. Arch Toxicol 86:1475–1480. https://doi.org/10.1007/s00204-012-0844-0
25. Anke M, Kosla T, Groppel B (1989) The cadmium status of horses from Central Europe depending on breed, sex age and living area. Arch Tierernahr 39:657–683. https://doi.org/10.1080/17450398909428337

26. Skalny AV, Skalnaya MG, Tinkov AA, Serebryansky EP, Demidov VA, Lobanova YN, Grabeklis AR, Berezkina ES, Gryazeva IV, SkalnyAA SOA, Zhivaev NG, Nikonorov AA (2015) Hair concentration of essential trace elements in adult non-exposed Russian population. Environ Monit Assess 187:677. https://doi.org/10.1007/s10661-015-4903-x
27. O'Hara TM, Carroll G, Barboza P, Mueller K, Blake J, Woshner V, Willetto C (2001) Mineral and heavy metal status as related to a mortality event and poor recruitment in a moose population in Alaska. J Wildl Dis 37:509–522. https://doi.org/10.7589/0090-3558-37.3.509
28. Malter R, Rendon C, Aalund R (2005) A developmental study of sex differences in hair tissue mineral analysis patterns at ages six, twelve and eighteen. J Orthomol Med 20:245–254
29. Ali F, Lodhi LA, Qureshi ZI, Ahmad I, Hussain R (2013) Serum mineral profile in various reproductive phases of mares. Pak Vet J 33:296–299
30. Vivoli G, Fantuzzi G, Bergomi M, Tonelli E, Gatto MR, Zanetti F, Del Dot M (1990) Relationship between zinc in serum and hair and some hormones during sexual maturation in humans. Sci Total Environ 95:29–40
31. Michos C, Kalfakakou V, Karkabounas S, Kiortsis D, Evangelou A (2010) Changes in copper and zinc plasma concentrations during the normal menstrual cycle in women. Gynecol Endocrinol 26: 250–255
32. Zheng G, Wang L, Guo Z, Sun L, Wang L, Wang C, Zuo Z, Qiu H (2015) Association of serum heavy metals and trace element concentrations with reproductive hormone levels and polycystic ovary syndrome in a Chinese population. Biol Trace Elem Res 167:1–10
33. Arredondo M, Núñez H, López G, Pizarro F, Ayala M, Araya M (2010) Influence of estrogens on copper indicators: in vivo and in vitro studies. Biol Trace Elem Res 134:252–264
34. Gabrielsen JS (2017) Iron and testosterone: interplay and clinical implications. Curr Sex Health Rep 9:5–11
35. Chang CS, Choi JB, Kim HJ, Park SB (2011) Correlation between serum testosterone level and concentrations of copper and zinc in hair tissue. Biol Trace Elem Res 144:264–271
36. Zeng Q, Zhou B, Feng W, Wang YX, Liu AL, Yue J, Li YF, Lu WQ (2013) Associations of urinary metal concentrations and circulating testosterone in Chinese men. Reprod Toxicol 41:109–114
37. 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 16:623–633. https://doi.org/10.5601/jelem.2011.16.4.11
38. Wichert B, Frank T, Kienzle E (2002) Zinc, copper and selenium intake and status of horses in Bavaria. J Nutr 132:1776S–1777S. https://doi.org/10.1093/jn/132.6.1776S
39. Tamburo E, Varrica D, Dongarrà G (2015) Coverage intervals for trace elements in human scalp hair are site specific. Environ Toxicol Pharmacol 39:70–76. https://doi.org/10.1016/j.etap.2014.11.005
40. Patra RC, Swarup D, Naresh R, Kumar P, Nandi D, Shekhar P, Roy S, Ali SL (2007) Tail hair as an indicator of environmental exposure of cows to lead and cadmium in different industrial areas. Ecotoxicol Environ Saf 66:127–131. https://doi.org/10.1016/j.ecoenv.2006.01.005
41. Skalny AV, Kiselev VF (2012) Element status of population of Russia. Part III. Element status of population of North-Western, Southern, and North-Caucasian Federal districts. ELBI-SPb, Saint Petersburg, p 447
42. Brown RJ, Milton MJ (2005) Analytical techniques for trace element analysis: an overview. TrAC Trends Anal Chem 24:266–274
43. Saitoh K, Sera K, Gotoh T, Nakamura M (2002) Comparison of elemental quantity by PIXE and ICP-MS and/or ICP-AES for NIST standards. Nucl Instrum Methods Phys Res B 189:86–93
44. Elzain AH, Ebrahim AM, Eltoum MS (2016) Comparison between XRF, PIXE and ICP-OES techniques applied for analysis of some medicinal plants. J Appl Chem 9:6–12