Reference intervals of essential and toxic elements concentrations in mane hair and blood serum of Arabian purebred horses

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Abstract. The article presents the results of studies on the establishment of reference intervals of concentrations of the main essential and toxic elements in mane hair and blood serum of Arabian purebred horse stallions, calculated by Atomic emission spectroscopy (AES) and Inductively coupled plasma mass spectrometry (ICP-MS) methods. For mane hair, these values are Ca 81.28–187.5; K 57.2–213.4; Mg 93.92–115.4; Na 16.76–54.2; P 47.1–827.3; Co 0.008–0.201; Cr 0.022–1.3; Cu 0.936–9.73; Fe 18.55–363; I 0.006–0.367; Mn 0.407–8.34; Se 0.039–0.625; Zn 14.49–201; B 0.311–25.66; Li 0.027–0.82; Ni 0.05–0.712; Si 0.809–34; V 0.018–0.594; Al 5.98–208; As 0.009–0.116; Cd 0.001–0.021; Hg 0.002–0.008; Pb 0.02–0.571; Sn 0.001–0.097; Sr 0.311–5.94. For blood serum, these values are Ca 106.2–161.3; K 89.19–187.1; Mg 18.16–27.05; P 78.32–153.1; Co 0.0003–0.0101; Cr 0.0003–0.0101; Cu 0.7–1.46; Fe 1.35–3.51; Mn 0.0005–0.0043; I 0.007–0.019; Se 0.118–0.208; Zn 0.502–0.952; Al 0.01–0.028; As 0.001–0.004; Cd 0–0.0001; Hg 0.0002–0.0009; Pb 0.0001–0.0026; Sn 0–0.0001; Sr 0.14–0.367. It was found that the chemical elements concentration in hair is significantly higher (1.4–2707 times) compared to blood serum; it makes this biosubstrate more informative for the elemental status evaluation. The results of the correlation analysis indicate the absence of a reliable correlation between the content of the studied elements in hair and blood serum.

1. Introduction

With the development of science, it becomes obvious that a further stage in the theory of chemical elements development should be a technology development to increase the productivity of horses due to the study of the multi-element composition of biosubstrates with subsequent evaluation and correction of metabolism.

The greatest groundwork for the problem of data evaluating and interpreting the content of chemical elements in biosubstrates has been formed in medicine. One of the first such developments was a method for controlling the elemental status of populations based on data on the mineral composition of a human body prepared by a working group under the patronage of the Harvard University (USA) and the International Atomic Energy Agency (IAEA) [1]. The further development of the technology for
the detection and prevention of elementosis was obtained with the advent of high-precision analytical methods for studying the elemental composition of biosubstrates [2,3]. A significant volume of information on the biosubstrates’ elemental composition of the population has been accumulated in Russia. Only in 2009-2013, a comprehensive analytical study of the elemental status of 65 thousand people was carried out in the Russian Federation [4].

The successes achieved in animal husbandry are much more modest. So far, at the disposal of practical zootechnics and veterinary medicine, there is no information on the physiological norms of the content of chemical elements in biosubstrates of valuable horse breeds. Meanwhile, imbalances of trace elements pose a serious threat to the health and productive qualities of any farm animals [5], not only in terms of vital elements but also in toxic ones [6].

In this regard, the main goal of our research was to establish reference intervals for the concentrations of the main essential and toxic elements in the hair and blood serum of horses.

2. Materials and methods
The studies were carried out on purebred Arabian stallions. The live weight of stallions during the sampling period was 410.5 ± 37.8 kg, age 5-8 years.

The experimental part of the work was carried out in the conditions of the Terskoy breeding stud farm No. 169 of the Stavropol Territory. Clinically healthy Arabian purebred stallions (n is 120) bred in the same biogeochemical province (North Caucasus, Russia) were selected for research. Mane hair and blood serum taken from the jugular vein were applied as biosubstrates for research.

The conditions of feeding and keeping for all examined animals were identical. The consumed daily ration of feeding of experimental stallions in the two-month period preceding sampling contained Ca - 73.3-78.5 g, P - 50.3-55.8 g, Mg - 12.9-15.3 g, Fe - 1112 -1391mg, Cu - 115.1-128.9 mg, Co - 6.8-8.2 mg, Mn - 554-685 mg, I - 6.8-8.3 mg.

The elemental composition of mane hair and blood serum was determined by Atomic emission spectroscopy (AES) and Inductively coupled plasma mass spectrometry (ICP-MS) methods in the testing laboratory of ANO “Center for Biotic Medicine”, Moscow. The elemental composition of biosubstrates was investigated according to 25 parameters (Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, I, K, Li, Mg, Mn, Na, Ni, P, Pb, Se, Si, Sn, Hg, Sr, V, Zn).

Reference intervals of chemical elements concentrations in mane hair and blood serum were calculated in accordance with the IUPAC recommendations [7], and recommendations by M.G. Skalnaya (2003) [8].

Spearman’s correlation coefficients were calculated. The data were processed using the Statistica 10.0 software package (StatSoft Inc., USA).

3. Results
The concentration and reference intervals of chemical elements in the wool and blood serum of Arabian purebred stallions calculated in accordance with the IUPAC recommendations are presented in table 1.

Table 1. Reference intervals of essential and toxic elements concentrations in hair of Arabian purebred stallions, μg/g [7].

| Element | 2.5 (90 % CI) | 97.5 (90 % CI) |
|---------|---------------|---------------|
|         | Macronutrients |               |
| Ca      | 81.28 (105.6-56.90) | 1875 (1312-2437) |
| K       | 57.20 (40.04-74.36)  | 2134 (1494-2774)  |
| Mg      | 93.92 (65.74-122.1) | 1154 (807.8-1500) |
| Na      | 16.76 (11.73-21.79)  | 542.0 (379.4-704.6) |
Reference intervals of concentrations of essential and toxic elements in blood serum were calculated by a similar method (table 2).

**Table 2.** Reference intervals of essential and toxic elements concentrations in blood serum of Arabian purebred stallions, μg/g [7].

| Element | Percentile 2.5 (90 % CI) | Percentile 97.5 (90 % CI) |
|---------|--------------------------|---------------------------|
| **Macronutrients** | | |
| Ca | 106.2(74.2-137.8) | 161.3(112.7-209.3) |
| K | 89.19(62.43-115.9) | 187.1(130.9-243.1) |
| Mg | 18.16(12.71-23.61) | 27.05(18.94-35.17) |
| P | 78.32(54.82-101.8) | 153.1(107.1-198.9) |
| **Essential elements** | | |
| Co | 0.0003(0.0002-0.0004) | 0.0015 (0.001-0.002) |
| Cr | 0.0003(0.0002-0.0004) | 0.0101(0.0071-0.0131) |
| Cu | 0.700(0.490-0.910) | 1.46(1.02-1.90) |
| Fe | 1.35(0.945-1.76) | 3.51(2.46-4.56) |
| Mn | 0.0005(0.0004-0.0007) | 0.0043(0.0031-0.0056) |
| I | 0.007(0.005-0.009) | 0.019(0.013-0.024) |
We calculated the reference intervals using the proposed method on the basis of recommendations given by M.G. Skalnaya and others in 2003. Also, we considered the chemical elements concentration in hair in the interval from the 10th to the 25th percentile and from the 75th to the 90th percentile as a condition before the manifestation of diselementosis; concentrations less than the 10th and more than 90th percentiles were stated as conditions associated with the clinical manifestation of syndromes and symptoms characteristic of elementoses (tables 3-4).

**Table 3.** Reference intervals of essential and toxic elements concentrations in stallions’ mane hair of Arabian purebred horses, μg/g [8].

| Element | m±std | Percentile |
|---------|-------|------------|
|         |       | 10  | 25  | 75  | 90  |
| **Macronutrients** | | | | |
| Ca      | 916.8±367.4 | 496.5 | 687.2 | 1107.8 | 1373.2 |
| K       | 671.4±461.8 | 272.5 | 314.6 | 781.5 | 1282.4 |
| Mg      | 478.5±251.9 | 129.0 | 334.1 | 636.5 | 869.2 |
| Na      | 202.0±111.5 | 102.4 | 141.5 | 250.2 | 353.7 |
| P       | 540.3±158.4 | 391.0 | 442.9 | 646.9 | 724.3 |
| **Essential elements** | | | | |
| Co      | 0.041±0.045 | 0.009 | 0.014 | 0.040 | 0.109 |
| Cr      | 0.247±0.297 | 0.042 | 0.060 | 0.388 | 0.653 |
| Cu      | 5.78±1.51   | 4.410 | 5.05  | 6.670 | 7.35 |
| Fe      | 84.0±95.47  | 23.26 | 32.57 | 71.19 | 279.5 |
| I       | 0.076±0.086 | 0.013 | 0.029 | 0.103 | 0.172 |
| Mn      | 1.57±1.62   | 0.558 | 0.734 | 1.480 | 3.66 |
| Se      | 0.451±0.112 | 0.338 | 0.420 | 0.530 | 0.567 |
| Zn      | 140.6±31.86 | 117.5 | 129.1 | 160.3 | 174.8 |
| **Conditionally essential elements** | | | | |
| B       | 7.25±8.18   | 0.6200 | 1.80  | 12.39 | 21.20 |
| Element | m±std | 10 | 25 | 75 | 90 |
|---------|-------|----|----|----|----|
| **Macronutrients** | | | | | |
| Ca      | 142.7±11.55 | 130.5 | 137.3 | 151.4 | 156.5 |
| K       | 139.6±23.76 | 108.5 | 124.6 | 157.7 | 167.5 |
| Mg      | 21.33±2.13  | 18.72 | 19.43 | 22.37 | 24.09 |
| P       | 108.9±19.78 | 86.03 | 93.90 | 121.8 | 137.6 |
| **Essential elements** | | | | | |
| Co      | 0.0009±0.000 | 0.0006 | 0.0007 | 0.0010 | 0.0013 |
| Cr      | 0.0023±0.003 | 0.0003 | 0.0003 | 0.0015 | 0.0073 |
| Cu      | 0.991±0.187  | 0.760  | 0.811  | 1.12   | 1.21  |
| Fe      | 2.14±0.510   | 1.60   | 1.80   | 2.38   | 2.93  |
| I       | 0.012±0.003  | 0.008  | 0.010  | 0.013  | 0.016 |
| Mn      | 0.0011±0.001 | 0.0006 | 0.0007 | 0.0010 | 0.0014 |
| Se      | 0.163±0.023  | 0.129  | 0.152  | 0.181  | 0.193 |
| Zn      | 0.742±0.101  | 0.593  | 0.683  | 0.810  | 0.855 |
| **Conditionally essential elements** | | | | | |
| B       | 0.075±0.020  | 0.046  | 0.058  | 0.086  | 0.308 |
| Li      | 0.137±0.104  | 0.053  | 0.059  | 0.207  | 0.094 |
| Ni      | 0.003±0.002  | 0.001  | 0.002  | 0.003  | 0.006 |
| V       | 0.001±0.002  | 0.000  | 0.000  | 0.0001 | 0.005 |
| **Toxic elements** | | | | | |
| Al      | 0.016±0.0044 | 0.0097 | 0.014  | 0.018  | 0.021 |
| As      | 0.0020±0.0010 | 0.0014 | 0.0014 | 0.0016 | 0.0041 |
| Cd      | 0.0001±0.0004 | 0.0000 | 0.0000 | 0.0001 | 0.0001 |
| Hg      | 0.0003±0.00029 | 0.0002 | 0.0002 | 0.0002 | 0.0009 |
| Pb      | 0.0004±0.0005 | 0.0001 | 0.0002 | 0.0004 | 0.0008 |
| Sn      | 0.0001±0.0004 | 0.0000 | 0.0000 | 0.0001 | 0.0001 |

Table 4. Reference intervals of essential and toxic elements concentrations in stallions’ blood serum of Arabian purebred horses, µg/g [8].
As a result of a comparative analysis, it was found that the concentration of chemical elements in hair is significantly higher than those established for blood serum (table 5).

**Table 5.** Correlation coefficients of the main toxic and essential elements in hair and blood serum of Arabian purebred stallions.

|                   | Mane hair | Blood |
|-------------------|-----------|-------|
| **Element**       | **Al**    | **Cd**| **Co** | **Cr** | **Cu** | **Fe** | **Hg** | **I** | **Mn** | **Pb** | **Se** | **Sr** | **Zn** |
| Al                | -0.1      | -0.1  | -0.2  | 0.0    | 0.2    | -0.1  | 0.0    | 0.2  | -0.2  | 0.0    | 0.0    | -0.1  | 0.2    |
| Cd                | -0.2      | 0.3   | -0.1  | 0.0    | -0.2  | 0.1   | 0.2    | -0.2 | -0.2  | 0.1    | 0.1    | -0.1  |       |
| Co                | 0.1       | -0.2  | 0.0   | 0.0    | 0.1   | 0.1   | -0.4*  | 0.3  | 0.1   | 0.2    | 0.5*   | 0.0    | 0.2    |
| Cr                | 0.2       | 0.3   | 0.2   | 0.4*   | 0.2   | 0.2   | 0.1    | 0.1  | 0.1   | 0.1    | 0.0    | 0.1    | 0.1    |
| Cu                | -0.1      | 0.0   | -0.1  | -0.1   | 0.2   | -0.2  | -0.1   | 0.0  | -0.3  | -0.2   | 0.0    | -0.3   | 0.0    |
| Fe                | -0.2      | -0.3  | -0.3  | -0.1   | -0.2  | -0.3  | 0.0    | -0.2 | -0.2  | 0.2    | 0.1    | -0.3   | -0.3   |
| Hg                | 0.3       | 0.4   | 0.3   | 0.4*   | 0.1   | 0.3   | 0.3    | 0.0  | 0.3   | 0.1    | 0.1    | 0.2    | 0.0    |
| I                 | -0.1      | 0.0   | -0.2  | 0.0    | -0.1  | 0.0   | 0.1    | -0.1 | 0.1   | 0.2    | 0.4*   | -0.2   |       |
| Mn                | 0.0       | -0.1  | -0.2  | 0.0    | -0.2  | 0.3   | 0.1    | -0.2 | -0.2  | 0.0    | -0.1   | 0.1    | 0.1    |
| Pb                | -0.1      | 0.2   | 0.1   | 0.0    | 0.0   | 0.2   | 0.3    | 0.0  | 0.0   | -0.1   | 0.0    | 0.2    |       |
| Se                | -0.1      | -0.2  | -0.1  | -0.2   | 0.1   | -0.2  | -0.2   | -0.2 | -0.2  | -0.0   | -0.5*  | 0.0    |       |
| Sr                | 0.4       | 0.3   | 0.3   | 0.5*   | 0.3   | 0.4*  | 0.1    | 0.1  | 0.3   | 0.2    | 0.1    | 0.2    | 0.2    |
| Zn                | -0.3      | -0.2  | -0.2  | -0.4*  | 0.1   | -0.2  | 0.0    | 0.2  | -0.2  | 0.0    | 0.1    | -0.1   | 0.1    |

As a result of a comparative analysis, it was found that the concentration of chemical elements in mane hair is significantly higher than those established for blood serum (figure 1).

**Figure 1.** Multiplicity of deviations of chemical elements concentrations in mane hair relative to blood serum in Arab purebred stallions.
4. Discussion of the results
The article states that reference intervals were calculated in accordance with the American Society of Veterinary Clinical Pathology guidelines for quality assurance and laboratory standards [7]. However, according to the practical results accumulated in medicine, a range of chemical elements levels can also be calculated as an interval between the 25th and 75th percentiles obtained from the representative sample [8]. The efficiency of this calculation algorithm was confirmed in earlier studies, which established a relationship between the level of sports achievements and the correspondence of the concentrations of toxic elements in the mane hair with the reference intervals established within the 25th and 75th percentiles [5].

The study showed that the average values of the essential elements concentrations (Cu, Mn, Se, Zn) correspond to the previously obtained data [9-11], while the content of toxic elements in mane hair of stallions in this study was 2.8-20.9 times lower than with the results given in the same sources.

The observed difference between the obtained data may arise due to the different conditions of the biogeochemical provinces of animal breeding [12-14]. In particular, the reliably low content of toxic metals in the mane hair of horses in this study could be a consequence of the relatively low background environmental pollution by industrial enterprises located in the region [15-17]. Thus, it was found that the content of mercury in the hair of residents of the North Caucasus was significantly lower compared to residents of other regions of Russia [18].

Also, a comparison of laboratory data from different studies can be difficult due to differences in sampling methods [19], methods of analysis [20,21], hair color [22,23], seasons [19], etc.

It should be noted that mane hair was characterized by a much higher content of chemical elements in comparison with blood serum; it indicates a higher information content of the elemental composition of wool in comparison with the blood serum of animals. However, any reliable connections were not found between the content of elements in mane hair and blood serum in our study. This is due to the fact that hair analysis reflects changes in the balance of elements over a long period preceding the analysis. Blood reflects changes in the balance of elements for a much shorter period of time [24, 25].

5. Conclusion
The calculated reference intervals of chemical elements concentrations in hair and blood serum can be used for the evaluation of the elemental status of Arabian purebreed stallions bred in the North Caucasus.

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References
[1] Iyengar G V 1989 Elemental analysis of biological systems, biological, medical, environmental, compositional and methodological aspects Boca Ra-ton: CRC Press p 430
[2] Chyla M A and Zyrnicki W 2000 Determination of metal concentrations in animal hair by the ICP method. Comparison of various washing procedures Biological Trace Element Research 75 187-94
[3] Rodushkin I, Engström E and Baxter D C 2013 Review Isotopic analysis by ICP-MS in clinical samples Anal Bioanal Chem 405(9) 2785-97
[4] Skalnaya M G, Tinkov A A, Serebryansky E P, Demidov V A, Lobanova Y N and et al 2015 Hair concentration of essential trace elements in adult nonexposed Russian population Environ Monit Assess 187(11) 677 doi: 10.1007/s10661-015-4903-x
[5] Kalashnikov V, Zajcev A, Atroshchenko M, Kalinkova L, Kalashnikova T and et al 2018 The content of essential and toxic elements in the hair of the mane of the trotter horses depending on their speed Environmental Science and Pollution Research 22(25) 21961-7 doi: 10.1007/s11356-018-2334-2
[6] Bellinger D, Sloman J, Leviton A, Rabinowitz M, Needleman H L and Watermael C 1991 Low-level lead exposure and children's cognitive function in the preschool years Pediatrics 87(2) 219-27

[7] Friedrichs K R, Harr K E, Freeman K P, Szladovits B, Walton R M, Barnhart K F and et al ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics Vet Clin Pathol 41 441-53

[8] Skalnaya M G, Demidov V A and Skalny A V 2003 About the limits of physiological (normal) of Ca, Mg, P, Fe, Zn and Cu in human hair Trace elements in medicine 4(2) 5-10

[9] Asano R, Suzuki K, Otsuka T, Otsuka M and 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 64 607-10 https://doi.org/10.1292/jvms.64.607

[10] Asano K, Suzuki K, Chiba M, Sera K, Asano R and 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 107 135-40 https://doi.org/10.1385/BTER.107:2:135

[11] Asano K, Suzuki K, Chiba M, Sera K, Matsumoto T, Asano R and 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

[12] Gabrysziuk M, Sloniewski K, Metera E and Sakowski T 2010 Content of mineral elements in milk and hair of cows from organic farms J Elem 15 259-67

[13] Jarvis S C and Austin A R 1983 Soil and plant factor limiting the availability of copper to beef suckler herd J Agric Sci (Camb) 101 39-46

[14] Kincaid R L 2000 Assessment of traceminer al status of ruminants: a review J Anim Sci 77 1-10

[15] Tamburo E, Varrica D and Dongarra G 2015 Coverage intervals for trace elements in human scalp hair are site specific Environ Toxicol Pharmacol 39 70-6 https://doi.org/10.1016/j.etap.2014.11.005

[16] Patra R C, Swarup D, Naresh R, Kumar P, Nandi D, Shekhar P and et al Tail hair as an indicator of environmental exposure of cows to lead and cadmium in different industrial areas Environ Toxicol Saf 66 127-31 https://doi.org/10.1016/j.ecoenv.2006.01.005

[17] Kalashnikov V V, Zaitsev A M, Atroschenko M M, Miroshnikov S A, Zavyalov O A, Frolov A N et al 2019 Assessment of regional differences of chemical concentration in mane hair of the thoroughbred IOP Conference Series: Earth and Environmental Science 341 012075

[18] Skalny A V and Kiselev V F 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

[19] Jadwiga Topczewska 2012 Effects of seasons on the concentration of selected trace elements in horse hair. Journal of Central European Agriculture 13(14) 671-80

[20] Inductively coupled plasma mass spectrometry: recent trends and developments 2011 Engelhard C Anal Bioanal Chem 399(1) 213-9

[21] Rodushkin I, Engström E and Baxter DC 2013 Review Isotopic analyses by ICP-MS in clinical samples Anal Bioanal Chem 405(9) 2785-97

[22] Combs D K 1987 Hair analysis as an indicator of mineral status of livestock J Anim Sci 65 1753-8

[23] Cape L and Hintz H F 1982 Influence of month, color, age, corticosteroids, and dietary molybdenum on mineral concentration of equine hair Am J Vet Res 43 1132-6

[24] Skalnaya M G 2005 Comparative analysis of changes in the elemental composition of biosubstrates with excessive intake of Pb, Cd, As and Ni in the body Vestnik of the Orenburg State University 2 11-3.

[25] Skalny A V, Demidov V A and Skalnaya M G 2001 Evaluation of the elemental status of a population in genetic presenological diagnostics Vestnik of the Orenburg State University 3(2) 64-7