The elemental composition of various biosubstrates of productive horses

T V Kalashnikova, N V Blohina, I S Gavrilicheva

All-Russian Research Institute for Horse breeding, Divovo, Ryazan Region, 391105, Russia

E-mail: t_kalash81@mail.ru

Abstract. The study of the elemental composition of biosubstrates of horses of heavy breeds in the system blood-milk-wool was undertaken. The average values of the exchange pool of 6 toxic (aluminum, arsenic, cadmium, mercury, lead, tin) and 13 essential elements (calcium, potassium, manganese, sodium, phosphorus, cobalt, chromium, copper, iron, iodine, magnesium, selenium and zinc) in the body of lactating horses were determined. Comparison of the concentration of toxic elements in various horse nutrient depots showed a significant positive correlation with individual essential nutrients.

1. Introduction
Studies of the exchange pool of chemical elements are increasingly used in animal husbandry in the detection and correction of metabolic disorders [2, 3, 5]. Blood and milk are complex polydisperse systems of the body. Blood composition is a reliable indicator of the state of the body in real time. Most of the essential elements are deposited in the milk to provide the offspring with full nutrition [4]. A relatively new highly informative biosubstrate for the assessment of prolonged metabolic changes in the body is the hair (hair) of animals. It can be stored for a long time and is suitable for mass screening [9]. Sampling does not injure the animal and does not require special skills from researchers. Metabolic processes in the organism of horses at the level of different systems occur not at the same time. The structure of the hair, blood and milk contains and accumulates toxic elements. Entering the body from the external environment, even in negligible quantities, they can lead to severe metabolic disorders, leading to various diseases and death of the animal [10, 14].

Based on this, the aim of our study was to study the concentrations of 6 toxic elements (aluminum, arsenic, cadmium, mercury, lead, tin) in the body of horses - milk producers. Also, we studied the features of inter-element interaction of antagonistic groups of the nutrient exchange pool for the comparative assessment of the informative value of the cumulative and polydisperse fluids of the body of the horse.

2. Materials and methods

The studies were conducted in the period from November 2017 to August 2018 on the basis of the experimental farm of All-Russian Research Institute for Horse breeding donor. Samples of biosubstrates were taken from clinically healthy mares of Heavy Draught breeds of different ages: hair, blood, milk. In total, 145 samples of hair, 123 samples of blood and 96 samples of milk from 23 horses were studied. Sampling was carried out at intervals of 1 time per month. The samples were taken at the same time according to the developed technique [8]. The samples were investigated using atomic emission (Optima
2000DV, PerkinElmerCorp) and mass spectral (NEXION 300D, PerkinElmerCorp) analysis with inductively coupled plasma. Multielement analysis was performed in the laboratory of "Center of Biotic medicine", Moscow (Registration Certificate of ISO 9001: 2000, Number 4017 – 5.04.06). The content of 25 chemical elements was determined in the samples [11]. The study used data on the content of substrates of 6 toxic chemical elements (aluminum, arsenic, mercury, lead, cadmium, tin) and 13 essential elements (calcium, potassium, manganese, sodium, phosphorus, cobalt, chromium, copper, iron, iodine, magnesium, selenium and zinc). The average content of each chemical element in wool, blood and milk of the sample was determined. We compared the average readings in different biosubstrates between themselves and between groups of elements, determined the correlation between toxic and essential elements. Statistical data processing was carried out using non-parametric methods in the software Microsoft Excel 2010 and STATISTICA 8.

3. Results and discussion

The data obtained during the experiment indicate significant differences between the studied groups of horses biosubstrates in the content of a number of toxic and essential elements. On average, toxic elements in different environments are distributed inhomogeneously

| element | hair from the mane | blood | milk |
|---------|-------------------|-------|------|
| Al      | 22.76±1.345       | 11.31±1.273 | 8.807±1.56 |
| As      | 0.027±0.003       | 0.016±0.003 | 0.020±0.001 |
| Cd      | 0.007±0.001       | 0.004±0.0006 | 0.006±0.001 |
| Pb      | 0.079±0.005       | 0.076±0.002 | 0.069±0.003 |
| Sn      | 0.018±0.003       | 0.009±0.0001 | 0.008±0.001 |
| Hg      | 0.002±0.0007      | 0.002±0.0001 | 0.003±0.0001 |

It was found that in high concentrations, compared with polydisperse media, toxic elements accumulated in the hair of horses, which is probably due to the active excretion of harmful substances from vital environments of the body, including the hair depot. In the blood, the concentration of this group of elements is twice lower, except for mercury and lead. This fact is consistent with previous studies, when it was found that horses from the Ryazan region of Russia are characterized by an increased concentration of lead in the hair depot compared to the horse populations from other biogeochronological provinces of the Russian Federation [6]. In milk there is a higher concentration of arsenic and mercury than that in the blood. Arsenic is one of the highly toxic elements that slows down the absorption of zinc, vitamins A and E [7]. Cadmium is found in the minimum concentration in the blood and in the maximum concentration in the hair. Cadmium, coming from the outside, accumulates intracellularly, binding to the cytoplasmic and nuclear material, blocks sulfhydryl groups of proteins, displacing copper, zinc, calcium, iron and selenium, has a pronounced antagonism with zinc and is able to replace zinc in the structure of enzymes, thereby disrupting their functioning. Tin, as a normal part of the body, has an effect on the activity of flavin enzymes, is able to enhance the growth processes. In our study, the concentration of tin is also the highest in the composition of the mane. The toxic effect of tin is equivalent to that of lead [10]. In the available literature there are no data on maximum allowable concentrations of toxic trace elements in the body of the horse, so more research will be required to identify metabolic disorders associated with intoxication. However, it is obvious that the accumulation of toxic metals in the body is undesirable, because under the influence of the latter there is an inhibition of the exchange of vital chemical elements, therefore the less they are contained in any environment of the body, the better.

In determining the average concentrations of vital elements there is their more uniform distribution in their hair, blood and milk (table 2).
Table 2. Average concentrations of vital elements in different biosubstrates of horses (µg/g)

| element | hair from the mane | blood      | milk       |
|---------|-------------------|------------|------------|
| Ca      | 706.66±21.23      | 474.26±37.52 | 565.36±23.4 |
| K       | 1073.69±121.2     | 1012.54±98.76 | 965.63±75.34 |
| Mg      | 158.14±17.23      | 109.27±9.89  | 143.74±9.87 |
| Na      | 657.55±45.12      | 923.70±87.45 | 726.93±45.2 |
| P       | 386.57±31.2       | 333.19±13.43 | 399.05±2.78 |
| Co      | 0.02±0.001        | 0.02±0.001  | 0.01±0.0009 |
| Cr      | 0.07±0.002        | 0.03±0.001  | 0.04±0.001  |
| Cu      | 2.72±0.07         | 1.95±0.09   | 2.62±0.089  |
| Fe      | 125.20±1.23       | 259.38±15.34 | 230.04±1.27 |
| I       | 0.32±0.027        | 0.22±0.023  | 0.06±0.005  |
| Mn      | 1.84±0.032        | 1.29±0.056  | 1.23±0.034  |
| Se      | 0.35±0.018        | 0.36±0.012  | 0.45±0.004  |
| Zn      | 67.02±3.56        | 39.28±1.35  | 56.77±1.99  |

This nature of distribution is associated with the constant expenditure of these nutrients for the needs of living systems, while in higher concentrations they are deposited in wool, which can serve as a criterion for the provision of the body with the necessary macro- and microelements [12]. However, selenium, sodium and iron are least deposited in the hair and are actively involved in liquid media as components of enzymes, salts and proteins. Salts of potassium and sodium are contained in milk in the form of a well-dissociating chlorides, phosphates and nitrates. They provide a salt balance, that is, a certain ratio between calcium ions and anions of phosphoric and citric acids, contributing to dissolution. In milk, the elements are associated with the shells of lipid globules (Fe, Cu), casein and whey proteins (I, Se, Zn, Al), are part of enzymes (Fe, Mo, Mn, Zn, Se), vitamins (Co). Magnesium accumulates most of all in the hair and milk, it plays an important role in the development of the newborn’s immunity, increases its resistance to intestinal diseases, improves growth and development, and is also necessary for the normal functioning of the stomach microflora [13].

To determine the nature of interelement interactions, correlation coefficients between the content of toxic and essential chemical elements in various biosubstrates of horses were calculated (Table 3).

In the course of the study, the results demonstrate the relevance of elemental analysis of several biological media for the study of interelement interactions in the body of horses. A significant positive correlation in all three systems of the studied horses was noted between cobalt, chromium and the studied toxic elements. Chromium is an integral part of all organs and tissues involved in the elimination of toxins. Cobalt is a part of cyanocobalamin and takes part in the formation of red blood cells, affects the enzymatic processes necessary for normal hematopoiesis. The analysis of the composition of animal wool of the studied groups revealed a positive correlation between the content of copper, manganese, zinc in wool and toxic elements: aluminum, arsenic, lead, cadmium. Calcium and magnesium are linearly related to arsenic and cadmium. High correlation coefficients of copper and selenium with respect to arsenic and cadmium are in all systems. Zinc significantly correlates with the content of all 6 toxic elements in milk and with aluminum, arsenic and cadmium in the blood. This fact is explained by the fact that zinc is able to replace and neutralize the action of toxic metals due to the close kinetic parameters of the formation of these structures. In milk, compared with other media, most of the essential elements are linearly related to the distribution of toxic while a low negative correlation is observed among toxic elements and sodium, iron [9, 14, 16].
Table 3. Correlation coefficients between the content of toxic and essential chemical elements in various biosubstrates of horses

| element | Al  | As  | Cd  | Pb  | Sn  | Hg  |
|---------|-----|-----|-----|-----|-----|-----|
| hair    |     |     |     |     |     |     |
| Ca      | 0.47| 0.67| 0.65| 0.52| 0.49| 0.23|
| K       | -0.04| 0.15| 0.19| 0.07| 0.56| 0.05|
| Mg      | 0.44| 0.65| 0.64| 0.52| 0.56| 0.20|
| Na      | -0.34| -0.42| -0.34| -0.32| -0.06| -0.07|
| P       | 0.40| 0.46| 0.35| 0.37| 0.21| 0.18|
| Co      | 0.75| 0.75| 0.66| 0.85| 0.32| 0.13|
| Cr      | 0.82| 0.78| 0.59| 0.64| 0.45| 0.13|
| Cu      | 0.57| 0.87| 0.73| 0.66| 0.50| 0.23|
| Fe      | 0.01| -0.14| -0.14| -0.05| -0.18| -0.11|
| I       | -0.13| -0.19| -0.17| -0.13| -0.04| 0.02|
| Mn      | 0.75| 0.82| 0.91| 0.76| 0.42| 0.18|
| Se      | 0.44| 0.73| 0.68| 0.55| 0.41| 0.18|
| Zn      | 0.60| 0.88| 0.79| 0.73| 0.51| 0.22|
| blood   |     |     |     |     |     |     |
| Ca      | 0.63| 0.64| 0.89| 0.42| 0.39| 0.53|
| K       | -0.09| -0.02| -0.01| -0.08| -0.08| -0.32|
| Mg      | 0.57| 0.63| 0.74| 0.36| 0.45| 0.54|
| Na      | -0.45| -0.55| -0.43| -0.33| -0.24| -0.51|
| P       | 0.48| 0.64| 0.32| 0.35| 0.19| 0.35|
| Co      | 0.74| 0.71| 0.69| 0.87| 0.34| 0.49|
| Cr      | 0.94| 0.88| 0.69| 0.48| 0.48| 0.49|
| Cu      | 0.69| 0.91| 0.69| 0.54| 0.46| 0.54|
| Fe      | -0.27| -0.40| -0.26| -0.21| -0.43| -0.44|
| I       | -0.02| -0.10| 0.05| -0.03| -0.07| 0.51|
| Mn      | 0.72| 0.59| 0.98| 0.40| 0.29| 0.40|
| Se      | 0.51| 0.72| 0.47| 0.47| 0.18| 0.45|
| Zn      | 0.74| 0.93| 0.69| 0.58| 0.51| 0.57|
| milk    |     |     |     |     |     |     |
| Ca      | 0.73| 0.80| 0.78| 0.77| 0.57| 0.57|
| K       | -0.40| -0.50| -0.42| -0.49| -0.46| -0.43|
| Mg      | 0.65| 0.79| 0.73| 0.76| 0.61| 0.63|
| Na      | -0.56| -0.65| -0.53| -0.57| -0.54| -0.44|
| P       | 0.39| 0.50| 0.79| 0.84| -0.52| 0.76|
| Co      | 0.92| 0.90| 0.82| 0.87| 0.61| 0.58|
| Cr      | 0.84| 0.83| 0.79| 0.78| 0.61| 0.53|
| Cu      | 0.78| 0.91| 0.83| 0.84| 0.60| 0.63|
| Fe      | -0.44| -0.55| -0.45| -0.48| -0.52| -0.40|
| I       | 0.52| 0.52| 0.59| 0.55| 0.42| 0.76|
| Mn      | 0.88| 0.84| 0.91| 0.85| 0.52| 0.61|
| Se      | 0.62| 0.81| 0.67| 0.76| 0.43| 0.57|
| Zn      | 0.79| 0.94| 0.79| 0.84| 0.63| 0.65|

Milk is the final product of the breast and (unlike blood) does not participate in the exchange as a transport system, remaining a complex polydisperse system, so the competition between antagonistic elements for bonds in protein molecules is most pronounced. Blood, being an intermediate in terms of
delivery of elements to the depositing systems, shows stable correlation between elements, high coefficients of which are duplicated in wool and milk with respect to toxic elements - except for selenium and phosphorus-cobalt, chromium, copper, manganese, zinc, calcium. As for such elements as iodine and potassium, there are no linear links between them and the content of toxic metals in biosubstrates.

The obtained data on the differences in the content of chemical elements in the studied tissues are generally consistent with the work of a number of scientists [15, 17, 18].

4. Conclusion
Thus, in the study of the level of essential and toxic elements in the blood, hair and milk of mares, the following features were noted: stable relationships of the elemental composition of all the studied biosubstrates are observed between toxic elements - aluminum, arsenic, cadmium, lead and essential - cobalt, chromium and zinc, between arsenic, cadmium and calcium, magnesium. In the blood, significant differences in the level of calcium content with aluminum, arsenic and cadmium were found, which may be due to the antagonistic interaction of elements with each other and their competition for bonds in protein molecules. In hair, blood and milk of mares, different distributions of chemical elements are noted. This is due to the interaction between biological media, and can also be caused by other factors that require additional research. It is known that the delivery of the necessary elements with blood plays an important role in the production of milk and to ensure hair growth. The difference in the heterogeneous levels of accumulation of macro-and microelements in different biosubstrates is determined by the intensity of metabolic processes in each of them and the sequence of transport of elements coming from the outside primarily into the blood and then into the milk and into the hair of mares as they grow.

The obtained information can be taken into account in the development of methods of diagnosis and correction of elements, as well as for fundamental research in the study of metabolic processes in the body of a horse.

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References
[1] Avtsyn A P, Zhavoronkov A A, Rish M A, Strochkova L S 1991 Human trace Elements. (M.: Medicine)
[2] Agadzhanyan N A, Rocky V A 2001 Chemical elements in habitat and an ecological portrait of a man. (M. Ed. KMC)
[3] Isakova O P, Rocky A V 2018 Application of mass spectrometry with inductively coupled plasma for speciation analysis of arsenic and mercury in human hair. The Issues of biological, medical and pharmaceutical chemistry. 21(7) 36-41
[4] Zaitseva I P, Berezkina E S, Skalny A V 2016 Influence of regular sports activities on the concentration of micronutrients and mineral composition of blood. Russian physiological journal. I. M. Sechenov 102(1) 89-99
[5] Kalashnikov V V, Zaitsev A M, Kalashnikova T V, Kalinkova L V, Blokhina N V 2017 In. Research perspectives for the study of microelement composition of hair horses. Horse breeding and equestrian sport 5 21-25
[6] Kalashnikov V V, Zaitsev A M, Kalinkova L V, Kalashnikova T V, Blokhina N V, Atroshchenko M M, Zavyalov O A, Frolov A, Miroshnikov S A 2017 The concentration of toxic elements in hair of horses from different regions of the Russian Federation. Horse breeding and equestrian sport 6 20-23
[7] Kaletin G I, Pavlovskaya N A, Kalentina N A, Makarova V, Skalny A V 2009 Unidirectionality of violation of elemental status in workers in contact with arsenic compounds, and persons suffering from cancer. *Occupational medicine and industrial ecology* 10 6-13

[8] Miroshnikov S A, Zavyalov O A, Frolov A N, Kurilkin M Y, Kalashnikov V V, Zaitsev A M, Atroshchenko M 2018 Development of a method for sampling the hair of horses for research on the elemental composition. *Sirazetdinov. Livestock and feed production* 101(4) 95-101

[9] Miroshnikov S A, Zavyalov O A, Frolov A N, Kharlamov A V, Duskaev G K, Kalilina M 2017 Elemental composition of wool as a model for the study of interelement interactions in dairy cattle. *Ya. Herald of beef cattle breeding* 4(100) 96-103

[10] Skalny, A.V. 2011, Connection of the elemental status of the population of the Central Federal district with morbidity. Part 1. Toxic chemical elements: Al, As, Be, Cd, Hg, Pb, Sn. *Trace elements in medicine* 12(1)(2) 23-26

[11] Skalny A V 2003 Reference values of the concentration of chemical elements in the hair obtained by the method of ISP-NPP (ANO Center for biotic medicine). *Trace elements in medicine* 4(1) 55-56

[12] Rock A A, Skalny A A, Melikhova M V, Bonitenko E Yu, Skalny A V, Skalnaya M G, Miroshnikov S A 2016 Comparative analysis of informativeness of diagnostic biological substrates took place (serum, hair) in the determination of the elemental status of the experimental animals. *Elements in medicine* 17(1) 38-44

[13] Rocky A V, Bykov A, Serebryanskaya T I 2003 Health and ecological risk assessment of hypermetabolism the population of the metropolis (P. Orenburg)

[14] Shugaley I V, Harabagiu V A, Ilyushin M A 2012 Some aspects of the influence of aluminium and its compounds on living organisms. *Ecological chemistry* 21(3) 172-186

[15] Asano R, Suzuki K, Otsuka T, Otsuka M, Sakurai H J 2002 Concentrations of toxic metals and essential minerals in the mane hair of healthy racing horses and their relation to age. *Vet Med Sci. 64(7) 607

[16] Pozebon D, Scheffler G L, Dressler V L 2017 Elemental hair analysis: A review of procedures and applications. *Anal Chim 1(992) 1-23 (doi: 10.1016/j.aca.2017.09.017)

[17] Prejac J, Skalny A A, Grabeklis A R, Uzun S, Mimica N, Momčilović B 2018 Assessing the boron nutritional status by analyzing its cumulative frequency distribution in the hair and whole blood *Journal of Trace Elements in Medicine and Biology. 45.50-56.

[18] Solomons N W, Rosenberg I H, Cousins R J (eds) 2004 Absorption and malabsorption of mineral nutrients. *Zinc. In. Alan R Liss 125 197