Assessment of regional differences of chemical concentration in mane hair of the Thoroughbred

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Abstract. The study is conducted on Thoroughbred stallions. The first stage covered the study of the elemental composition (20 elements) of stallion hair (n=148) raised in the southeast of Russia (Stavropol Territory, Krasnodar Krai, Volgograd and Rostov regions). On the basis of obtained results 10, 25, 75, 90 percentile intervals of concentration of the main essential and toxic elements in hair were established. At the second stage, the stallions raised on the farms of Stavropol Territory (n=29) and Krasnodar Krai (n=32), as well as Rostov (n=24) and Volgograd (n=28) regions were examined. Hair samples were selected from mane area in the first cervical vertebra projection. Proximal part of hair (15 mm long) was selected from the hair root for analysis. The elemental analysis of samples was made via inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively coupled mass spectrometry (ICP-MS). It is established that the hair of stallions raised in Stavropol Territory had the highest concentration of Al, As, Sn and Hg at a reliable difference (p<0.05) with similar indicators for species from Krasnodar Krai. The typical feature of animals raised in Volgograd Region was the maximum concentration of Cd, Pb and Sr fixed against the background of the minimum Cr, I, Mn and Se values. All studied micro-populations were characterized by the excess of established norms of toxic metals. The most considerable deviations (2.5-6.9 times) of aluminum, mercury and tin were typical for animals raised in Stavropol Territory. The species from Krasnodar Krai had lower concentration of zinc. The assessment of the frequency of deviations of the element status from the norm showed that the zinc content in the hair of 48.3% of examined animals from Stavropol Territory and 50.0% of species from Rostov Region made more than 75%. A large number of species from Krasnodar Krai was characterized by the lack of iodine (46.9%) and selenium (34.4%) against the background of the minimum values of cadmium (50.0%) and mercury (100%).

1. Introduction

The need for complex study aimed at systems to assess the level of chemical elements intake in humans and animals is caused by the biological role of the latter ones in metabolism, reproduction processes, cellular respiration, neutralization of toxic substances, as well as the influence on hematogenesis, oxidation-reduction processes, permeability of vessels, tissues, etc. [1, 2, 3, 4].

One of the methods of mass screening and control over the influence of environmental factors on the organism of animals and humans is the multielement analysis of hair [5-8].

The study showed that the element structure of hair is quite informative to assess the exchange pools of essential [9-11] and toxic chemical elements in the organism of horses [12-14].

At the same time the element status of an organism can be defined under the influence of some factors [15, 16], including environmental condition [17, 18].

In the territory of Russia there are several regions that differ in the content of chemical elements or their compounds in soils, waters and sedimentary deposits thus causing biogeochemical endemic diseases of plants, animals and humans. Modern industrial development makes a tangible contribution to the change of animal habitat and large industrial enterprises form various chemical anomalies [19]. The change of the content of chemical elements in environmental objects leads to their change in bio-substrates of horses raised close to industrial-scale production [20], busy highways [21, 22], large megalopolises, in regions contaminated with wastewaters [23], etc. Adverse changes can affect the state of health and result in the decrease of conventional resistance of an organism, functional changes of various physiological systems and as a result have a negatively influence on productivity of farm animals, which is the case with sports horses [24, 25].

Thus, the study of the elemental status of horses raised in certain biogeochemical provinces seems quite interesting in terms of control over the state of health by identifying and eliminating elements typical for the province.

2. Materials and methods

2.1 Subject of the study

The study is conducted on Thoroughbred stallions raised in the southeast of Russia (Stavropol Territory, Krasnodar Krai, Volgograd and Rostov regions). Age: 3-7 years. Hair color: black. Sampling period: 2017-2018. The animals were kept and pilot studies were conducted according to instructions and guidelines of the Decree of the USSR Ministry of Health No. 701 of 27 July 1978 *On Amendments into the Decree of the USSR Ministry of Health No. 755 of 12.08.77* and *The Guide for Care and Use of Laboratory Animals* (National Academy Press, Washington, D.C. 1996). During the study the authors took all efforts to minimize the suffering of animals and to reduce the number of used samples.

2.2 Design of experiment

The first stage covered the study of the elemental structure of hair of Thoroughbred stallions (n=148) raised in the southeast of Russia (Stavropol Territory and Krasnodar Krai, Volgograd and Rostov regions).

On the basis of obtained results 10, 25, 75, 90 percentile intervals of concentration of the main essential and toxic elements in hair were established. At the second stage, the stallions raised on the farms of Stavropol Territory (n=29) and Krasnodar Krai (n=32), as well as Rostov (n=24) and Volgograd (n=28) regions were examined. The content of chemical elements in hair was compared to 25 and 75 percentile intervals of “physiologically normal state” received during the first stage.

2.3 Sampling and analysis

Hair samples were selected from mane area in the first cervical vertebra projection. Proximal part of hair (15 mm long) was selected from the hair root for analysis. The elemental analysis of samples was made via ICP-AES and ICP-MS methods.

2.4 Statistical analysis

The reliability of differences was checked via the Mann-Whitney U test. The significance value (P) was accepted as lower or equal 0.05. Statistica 10.0 application program package (StatSoft, Inc., USA) was used for data processing.
3. Results
Table 1 shows the content of main essential and toxic elements in the mane hair of Thoroughbred stallions.

Table 1. Concentration and reference intervals of main essential and toxic minerals in the mane hair of Thoroughbred stallions raised in the southeast of Russia (Stavropol Territory, Krasnodar Krai, Volgograd and Rostov regions), μg/g

| Element | M± STD | Percentile 10 | Percentile 25 | Percentile 75 | Percentile 90 | Minimum | Maximum |
|---------|--------|----------------|---------------|---------------|---------------|---------|---------|
| Co      | 0.019±0.018 | 0.009          | 0.010         | 0.018         | 0.037         | 0.008   | 0.120   |
| Cr      | 0.119±0.090  | 0.043          | 0.064         | 0.132         | 0.210         | 0.021   | 0.52    |
| Cu      | 5.70±0.611   | 4.96           | 5.30          | 6.04          | 6.49          | 4.42    | 8.54    |
| Fe      | 33.69±41.04  | 13.24          | 15.88         | 30.7          | 62.94         | 10.12   | 261     |
| I       | 1.13±1.78    | 0.108          | 0.172         | 1.24          | 2.71          | 0.056   | 9.19    |
| Mn      | 2.07±3.54    | 0.86           | 1.03          | 2.11          | 2.96          | 0.568   | 34.6    |
| Se      | 0.493±0.119  | 0.359          | 0.405         | 0.561         | 0.631         | 0.271   | 0.873   |
| Zn      | 129.3±14.68  | 112            | 121.0         | 138           | 149.0         | 97.22   | 173     |

| Element | M± STD | Percentile 10 | Percentile 25 | Percentile 75 | Percentile 90 | Minimum | Maximum |
|---------|--------|----------------|---------------|---------------|---------------|---------|---------|
| B       | 2.47±2.36 | 0.623          | 1.33          | 2.98          | 3.72          | 0.112   | 18.64   |
| Li      | 0.066±0.058 | 0.018          | 0.028         | 0.085         | 0.131         | 0.010   | 0.299   |
| Ni      | 0.162±0.088 | 0.088          | 0.108         | 0.187         | 0.26          | 0.052   | 0.54    |
| Si      | 13.53±8.16  | 3.51           | 6.92          | 19.28         | 25.05         | 0.670   | 33.8    |
| V       | 0.045±0.067 | 0.010          | 0.015         | 0.038         | 0.115         | 0.006   | 0.404   |

| Element | M± STD | Percentile 10 | Percentile 25 | Percentile 75 | Percentile 90 | Minimum | Maximum |
|---------|--------|----------------|---------------|---------------|---------------|---------|---------|
| Al      | 16.51±26.61 | 3.10           | 4.50          | 13.51         | 40.78         | 2.31    | 150     |
| As      | 0.023±0.020 | 0.010          | 0.012         | 0.025         | 0.038         | 0.005   | 0.132   |
| Cd      | 0.011±0.016 | 0.002          | 0.003         | 0.011         | 0.025         | 0.001   | 0.102   |
| Pb      | 0.078±0.100 | 0.029          | 0.036         | 0.081         | 0.119         | 0.016   | 0.796   |
| Sn      | 0.102±0.523 | 0.006          | 0.010         | 0.032         | 0.125         | 0.003   | 4.99    |
| Hg      | 0.004±0.005 | 0.002          | 0.002         | 0.002         | 0.009         | 0       | 0.024   |
| Sr      | 3.36±1.82   | 1.63           | 2.11          | 4.22          | 6.07          | 0.819   | 10.58   |

Data provided as average ± STD

Comparative assessment of elemental composition of the mane hair of stallions raised in the southeast of Russia (Stavropol Territory, Krasnodar Krai, Volgograd and Rostov regions) revealed some significant differences between the studied groups (Table 2).
To be honest, the obtained data were surprising. The concentration of Al, As, Se, and Hg in the mane hair of Thoroughbred stallions from the southeast of Russia (Stavropol Territory, Krasnodar Krai, Volgograd and Rostov regions) was higher than in comparable indicators for similar species from the neighboring regions (Table 2).

### Table 2. Concentration of main essential and toxic minerals in the mane hair of Thoroughbred stallions raised in the southeast of Russia (Stavropol Territory, Krasnodar Krai, Volgograd and Rostov regions), $\mu g/g$

| Element | Stavropol Territory | Krasnodar Krai | Rostov Region | Volgograd Region |
|---------|---------------------|----------------|--------------|-----------------|
| Co      | 0.023±0.023         | 0.014±0.006$^a$| 0.017±0.010  | 0.020±0.026     |
| Cr      | 0.130±0.115         | 0.104±0.085    | 0.110±0.048  | 0.132±0.076     |
| Cu      | 5.69±0.542          | 5.58±0.521     | 5.95±0.493$^b$| 5.71±0.881      |
| Fe      | 50.1±66.39          | 25.5±15.38$^a$ | 35.1±26.47   | 20.8±11.18$^c$ |
| I       | 1.15±1.34           | 1.10±2.26      | 1.04±1.53    | 1.33±1.73       |
| Mn      | 2.08±1.11           | 1.26±0.586$^a$ | 1.96±0.985$^b$| 3.58±7.79       |
| Se      | 0.501±0.154         | 0.481±0.115    | 0.441±0.059  | 0.541±0.080$^c$ |
| Zn      | 132.7±17.35         | 115.4±14.39    | 131.2±10.30  | 127.3±13.03     |

### Vital microelements
- B 2.96±3.47, Li 0.070±0.067, Ni 0.164±0.098, Si 10.60±6.75, V 0.067±0.101
- Co, Cr, Cu, Fe, I, Mn, Ni, Si, V, Zn

### Conditionally vital microelements
- Al 27.27±40.69, As 0.030±0.032, Cd 0.011±0.009, Pb 0.080±0.101, Sn 0.221±0.921, Hg 0.005±0.006, Sr 3.71±2.00
- Al, As, Cd, Pb, Sn, Hg, Sr

### Content of toxic microelements
- Al 27.27±40.69, As 0.030±0.032, Cd 0.011±0.009, Pb 0.080±0.101, Sn 0.221±0.921, Hg 0.005±0.006, Sr 3.71±2.00
- Al, As, Cd, Pb, Sn, Hg, Sr

Data provided as average ± STD
$^a$p≤0.05 - I in relation to II, III, IV
$^b$p≤0.05 - II in relation to III, IV
$^c$p≤0.05 - III in relation to IV

Thus, the hair of stallions raised in Stavropol Territory was characterized by the maximum concentration of Al, As, Sn and Hg, which is higher in comparison to similar indicators for species from Krasnodar Krai by 154.8 (p≤0.01), 66.7% (p≤0.05), 325.0% and 150.0% (p≤0.01). The animals raised in Volgograd Region had the maximum concentration of Cd, Pb and Sr and the minimum values of Cr, I, Mn and Se.

The elemental composition of hair revealed some elements, which average content exceeds the threshold of centile intervals of physiologically optimum contents (Fig. 1, 2, 3, 4).
Figure 1. Frequency of deviations of elemental composition of mane hair of Thoroughbred stallions raised in Stavropol Territory from “physiologically normal state”

Figure 2. Frequency of deviations of elemental composition of mane hair of Thoroughbred stallions raised in Krasnodar Krai from “physiologically normal state”
Figure 3. Frequency of deviations of elemental composition of mane hair of Thoroughbred stallions raised in Rostov Region from “physiologically normal state”

It is found that in terms of the content of toxic metals, the stallions of all studies micropopulations were characterized by the excess of the established norms. The most considerable deviations (2.5-6.9 times) of aluminum, mercury and tin were typical for animals raised in Stavropol Territory, while the species from Krasnodar Krai had lower concentration of zinc (1.1 times).

The assessment of the frequency of occurrence of deviations of the elemental status from the norm showed the following results (Table 3).
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Table 3. Occurrence of deviations from the norm based on the elemental analysis of hair of Thoroughbred stallions raised in the southeast of Russia, %

| Element | Stavropol Territory | Krasnodar Krai | Rostov Region | Volgograd Region |
|---------|---------------------|----------------|---------------|------------------|
|         | <25 percentile | 75 percentile | <25 percentile | 75 percentile | <25 percentile | 75 percentile | <25 percentile | 75 percentile |
| Co      | 24.1               | 27.6           | 18.8          | 18.8             | 14.3             | 35.7           | 27.8           | 22.2           |
| Cr      | 31.0               | 34.5           | 28.1          | 15.6             | 14.3             | 14.3           | 16.7           | 33.3           |
| Cu      | 17.2               | 20.7           | 28.1          | 18.8             | 7.1              | 57.1           | 33.3           | 16.7           |
| Fe      | 34.5               | 31.0           | 18.8          | 18.8             | 7.1              | 42.9           | 33.3           | 11.1           |
| I       | 6.9                | 34.5           | 46.9          | 15.6             | 14.3             | 14.3           | 22.2           | 33.3           |
| Mn      | 10.3               | 31.0           | 40.6          | 9.4              | 14.3             | 35.7           | 27.8           | 33.3           |
| Se      | 27.6               | 31.0           | 34.4          | 21.9             | 21.4             | 7.1            | 5.6            | 33.3           |
| Zn      | 20.7               | 48.3           | 25.0          | 37.5             | 14.3             | 50.0           | 27.8           | 38.9           |
| B       | 20.7               | 34.5           | 37.5          | 9.4              | 28.6             | 14.3           | 5.6            | 44.4           |
| Li      | 34.5               | 31.0           | 31.3          | 12.5             | 7.1              | 35.7           | 11.1           | 27.8           |
| Ni      | 31.0               | 24.1           | 15.6          | 25.0             | 28.6             | 14.3           | 27.8           | 33.3           |
| Si      | 31.0               | 10.3           | 31.3          | 15.6             | 21.4             | 28.6           | 38.9           | 33.3           |
| V       | 31.0               | 27.6           | 31.3          | 15.6             | 7.1              | 35.7           | 16.7           | 27.8           |
| Al      | 20.7               | 27.6           | 31.3          | 18.8             | 14.3             | 35.7           | 27.8           | 22.2           |
| As      | 37.9               | 31.0           | 25.0          | 15.6             | 28.6             | 35.7           | 16.7           | 22.2           |
| Cd      | 17.2               | 41.4           | 50.0          | 0.0              | 7.1              | 21.4           | 5.6            | 44.4           |
| Pb      | 37.9               | 20.7           | 9.4           | 31.3             | 21.4             | 28.6           | 33.3           | 16.7           |
| Sn      | 20.7               | 31.0           | 31.3          | 12.5             | 21.4             | 21.4           | 22.2           | 38.9           |
| Hg      | 93.1               | 6.9            | 100.0         | 0.0              | 85.7             | 14.3           | 94.4           | 5.6            |
| Sr      | 20.7               | 31.0           | 31.3          | 9.4              | 28.6             | 35.7           | 16.7           | 33.3           |

The most indicative data were obtained for zinc, which content in the hair of 48.3% of the studied animals in Stavropol Territory and 50.0% of species in Rostov Region made over 75%. The majority of the studied animals from Krasnodar Krai was characterized by the lack of iodine (46.9%) and selenium (34.4%) against the background of minimum deviations of cadmium (50.0%) and mercury (100%) content.

4. Discussion

The analysis of literary data shows that according to us the concentration of Al, Pb and Hg in hair of horses was lower in comparison with similar studies by Asano et al. [26, 27] and Stachurska et al. [28].

Interpreting the received results regarding recommendations provided by Skalnaya M.G. et al. [7] on the expediency of 25 and 75 percentile intervals as “physiologically normal state” it is possible to conclude that in terms of the content of toxic metals the stallions of all studied micropopulations were characterized by the excess of the established norms. The last fact demonstrates the potential risk of development of pathological processes and decrease of sports results of the above animals [24].
The most considerable deviations (2.5–6.9 times) of aluminum, mercury and tin were typical for animals raised in Stavropol Territory. The excess of toxic elements in the hair of animals may be caused by the excess of these substances in soil [29]. At the same time, the increased content of toxic elements in the hair of animals was not confirmed during the assessment of the elemental status of inhabitants of this region [30].

It is possible that relatively high content of toxic elements is connected with the development of antagonism of essential elements. We noted the decrease of some essential elements (Co, Cr, I, Se and Zn) in the hair of stallions in comparison with earlier studies [28, 31, 32].

The elemental status of stallions from Stavropol Territory was characterized by zinc deficiency (Table 3). Thus, up to 50% of animals from this region were characterized by the excess of zinc in hair, while the considerable part of stallions had extremely low level of zinc. In particular, the zinc content in the hair of Ketmen stallion made 99.97 μg/g. Indigo Flame stallion – 101.0 μg/g. Earlier we described the development of deficiency in animals expressed by the excess of 75 percentile values in hair [33].

It is obvious that there is a need to adjust the elementosis of zinc, otherwise these states will be characterized by the decrease of the reproductive health of stallions [34]. This is explained by the fact that zinc is an important factor of normal functioning of a prostate gland and the reproductive system in general [35, 36]. Zinc in seminal plasma stabilizes cellular membrane and nuclear chromatin of spermatozoa [37, 38].

The difference in zinc content between the studied territories can be explained by different bioavailability of zinc due to regional variability of water parameters, including acidity [39]. Earlier study showed that excessive levels of toxic metals can also complicate Zn absorption from the environment [40]. In our experiment against the background of revealed lack of zinc at animals from Krasnodar Krai we were able to record the excess of established norms of tin and mercury by 1.1 and 1.6 times.

5. Conclusion
The elemental status of stallions raised in Stavropol Territory and Krasnodar Krai, as well as in Volgograd and Rostov regions is characterized by the excess of physiological norm regarding some toxic elements (Al, As, Cd, Pb, Sn, Hg, Sr), which may lead to deterioration of health and requires further correction of their contents in order to eliminate elements typical for studied regions.

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