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HAIR MACRO- AND MICROELEMENT LEVELS AS ESTIMATES OF MINERAL STATUS IN HORSES OF STUD AND LOCAL BREEDS FROM DIFFERENT RUSSIAN REGIONS

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Abstract

The optimal balance of chemical elements plays a vital role in the functioning of animal body. In horses, such balance is especially important because of specific hard work, in some cases on the verge of physiological limits. Besides, sometimes under the traditional technology of horse herd the rearing conditions may be extremal, for example, when horses are outdoors around the clock in severe winters of the northern Yakutia at extreme subzero Arctic temperatures. Estimate of mineral profile of a horse is the most important marker of physiological well-being, viability, productivity and adaptability to stresses. The purpose of this study was to identify levels of micro- and macroelements in hair of horses (*Equus ferus caballus*) of various genetic groups that are bred and used in various technological conditions in different regions of the Russian Federation. Totally, we used 198 hair samples of horses of stud breeds (Russian Trotter, Thoughbred, Purebred Arabian, Purebred Akhal-Teke) and local (indigenous) Bashkir, Kabardin, Mezenskaya, Vyatskaya breeds, Tuva and Yakut horses. The stud horses were kept at stables and the other in herds on pastures in 11 regions of the Russian Federation. In hair samples, 5 macroelements (Ca, K, Mg, Na, P) and 8 essential microelements (Co, Cr, Cu, Fe, I, Mn, Se, Z) were determined. Genetic determination of the content of macro- and microelements in the hair were evaluated in the Thoroughbred horse, Purebred Arabian and Purebred Akhal-Teke breeds (Stavropol Krai) kept under identical climatic conditions and common technology. Genetic profile was assessed for 16 autosomal DNA microsatellite loci, and the relationship between the level of homozygosity and distribution of chemical elements was evaluated in each of these breeds. All the studied groups significantly differed in hair chemical elements depending on genetic group (breeds), areal and rearing. Animals from the Republic of Sakha (Yakutia) had a deficit for 11 out of 13 chemical elements analyzed when compared to the average indices for all surveyed regions The Republic of Bashkortostan and the Republic of Kabardino-Balkaria shared the second place for the deficit of macro- and microelements, the Republic of Kalmykia and Stavropol Krai were the third and followed by Arhangelsk, Briansk and Lipetsk regions. The most favorable situation was in the Republic of Tyva and Krasnodar Krai where the surplus was registered for 12 and 11 elements respectively and some of them amounted up to 220 % compared to the average for the country. The advantage of stables keeping of horses, which provides the best balance of chemical elements in the body by regulation of nutrients in the diet, was observed. Hair mineral levels in horses of different origin correlated to their genetically homogeneous groups. Raising the level of hair macro- and microelements and an increase in heterozygosity of the three purebred breeds, as detected by 16 DNA microsatellite loci polymorphism, was found out to correspond. The Akhal-Teke breed that is distinguished by a greater heterozygosity was the most well-balanced for macro- and microelements. The micronutrient level database for horses of various breeds and breed groups form different Russian regions is of interest for the studying factors determining mineral metabolism in animals that should facilitate the innovative development of horse breeding.

Keywords: *Equus ferus caballus*, horses, trotting and race breeds, herd breeds, indigenous breeds, microelements, hair level of microelement, area, rearing, microsatellite DNA, genetic studies

Macroelements and essential microelements are of vital importance in the metabolism. Stable pool of chemical elements is one of the key factors for normal functioning of every living system [1, 2]. Deviations in the chemical elements content, due to the action of exogenous and endogenous factors, lead to disturbances up to developing pathologies [3]. At elementosis, a shortage or excess of minerals causes significant harm to livestock by reducing productivity and fertility, slowing growth down, lowering the immune status, and increasing mortality of animals [4].

Radical intensification of technologies at robotized megafarms and big commercial farm complexes raises the relevance of the research focuses on the elemental status of farm animals and poultry [5]. Obviously, objective tests are required to control providing an optimal pool of nutrients. Recently, in this regard, the attention of researchers is attracted to the so-called accumulating biomaterials, i.e. skin epithelium and horny layer, hair, and bone tissue [6]. Unlike buffer systems (blood, urine, lymph), they reflect the long-term intake (within a month or more) of microelements in the body. Animal hair, meanwhile, serves as the most informative biomaterial, which recreates the elemental status of organism, as a whole [7, 8]. The constancy of hair chemical composition is provided by the keratin coat which prevents penetration of external contaminants and the loss of internal components [9]. The advantages of using hair to assess the elemental status of farm animals are safe and easy sample collection, including mass screening, which are simply to transport and store for a long time without the use of special equipment [10].

The importance of these data is due to a diversified uses of horses by people, from meat and dairy breeding to olympic sports, and it is especially relevant in Russia because of wide geographic spreading of hours breeds in contrasting natural and climatic zones [11].

This paper is the first to characterize the pool of basic macro- and microelements in Russian landrace and thoroughbred horse breeds under various breeding technologies in contrasting climatic conditions It was revealed that in the Republic of Sakha (Yakutia), animals suffer from deficit in 11 out of the 13 studied chemical elements, while the most prosperous regions are the Republic of Tyva and the Krasnodar Territory. The advantage of stable keeping was shown. Our findings showed rise of mineral concentrations in hairs of three purebred breeds as their heterozygosity increases.

Our aim was to reveal, based on the analysis of mane hair, the peculiarities of elemental state of horses (*Equus ferus caballus*) from heterogeneous genetic groups, with view to regional conditions of animal keeping and breeding.

Techniques. The survey (2017) involved adult horses from 11 Russian regions (the Republic of Yakutia, Tyva, Bashkiria, Kalmykia, Dagestan, Kabardino-Balkaria, Stavropol and Krasnodar, Arkhangelsk, Lipetsk, Bryansk regions). The stud breeds were Russian Trotter, Thoughbred, purebred Arabian, purebred Akhal-Teke, also local (indigenous) Bashkir, Kabardin, Mezenskaya, Vyatskaya breeds and Tuva and Yakut horses were involved. Horses were grouped according to the breed, the region of their origin, rearing and use, and also given the technology of their keeping. In each group, the average concentration of microelements in the main hair was determined [12]. The group indicators were compared with each other and with the sample average [13].

Mane hairs were collected as per the instructions and recommendations of the Order of the Ministry of Health of the USSR of July 27, 1978 No. 701 "Additions to the Order of the Ministry of Health of the USSR of 12.08.77 № 755" and "The Guide for Care and Use of Laboratory Animals" (National Academy Press, Washington, DC, 1996). In the samples ($n = 198$), after digestion using a microwave SW4 (Berghof, Germany), the macro- and microelements were measured by atomic emission (ICP-AES) and inductively coupled plasma-mass spectrometry (ICP-MS) (a mass spectrometer Elan 9000 and an atomic emission spectrometer Optima 2000 V, Perkin Elmer, USA).

The amount of macro- and microelements in the mane hair, as depended on a genotype, was studied in Thoroughbred horse, purebred Arabian horse and purebred Akhal-Teke, with stabling in identical climatic conditions [14] (Tersky horse-breeding farm No. 169 and Stavropol stud farm No. 170, Stavropol Krai). Genotyping was performed with 16 autosomal microsatellite loci: AHT4, AHT5, ASB17, ASB2, ASB23, CA425, HMS1, HMS2, HMS3, HMS6, HMS7, HTG10, HTG4, HTG6, HTG7, and VHL20. DNA was extracted from hair follicles using the commercial ExtraGene™ DNA Prep 200 kit (Isogen Laboratory, Moscow). Separation and detection of amplification products was performed on a genetic analyzer AB 3130 (Applied Biosystems, USA). The degree of homozygosity was determined for each breed (the experimental samples included 319 purebred Arabian horse, 359 Thoroughbred horses and 141 Akhal-Teke horses).

Statistics was analysed using Microsoft Excel 2010 and Statistica 8 software (StatSoft, Inc., USA). While mathematical processing, the mean values (M), their standard errors (±SEM) and coefficients of variation (*Cv*) were calculated. Statistical significance of differences was assessed by Student's *t*-test at $p \leq 0.05$.

Results. In this report, we have analized the data on the five macroelements (calcium, potassium, magnesium, sodium, and phosphorus) and eight essential microelements (cobalt, chromium, copper, iron, iodine, manganese, selenium, and zinc). Also we have identified differences in this indicator between the investigated groups from different regions (Table 1). The highest calcium content was found in the horses from the Krasnodar Krai. Calcium participates in the formation of the bone tissue, it is important for the functioning of muscle tissue, heart, nervous system, skin. Hypercalcemia often leads to zinc and phosphorus deficiency in the body. The main causes of calcium deficiency are its inadequate intake with a diet, excess of phosphor, zinc, cobalt, magnesium, iron, potassium and sodium, as well as soft drinking water and shortage of vitamin D [15]. The minimum calcium amount was found in native horses from Yakutia and Bashkiria.

A high phosphorus level was observed in purebred riding horses from Dagestan. Phosphorus plays an important role in the brain function, cardiac and skeletal muscles. It also participates in transmembrane transport of the substances necessary for enzyme activity [16]. The lowest phosphorus content was pointed out for Vyatskaya breed horses from Lipetsk region and also for Yakut and Kabardino-Balkarian native horses.

The maximum concentration of potassium was found in horses of the Krasnodar Krai, whereas its minimum content was characteristic of native horses of Yakutia, Kabardino-Balkaria and Bashkiria. The highest magnesium level was in Mezen horses from Arkhangelsk region, the least among was detected in Yakutskaya breed. Herd horses from Tyva were characterized by significantly higher manganese and sodium contents compared to horses from other regions (see Table 1).

1. Chemical elements in mane hair (μg/g) of native and purebred horses from different Russian regions ($M \pm m$, 2017)

Region	Macroelements					Essential microelements							
	Ca	K	Mg	Na	P	Co	Cr	Cu	Fe	I	Mn	Se	Zn
1	883.16±48.21	385.73±62.55	259.88±13.90	185.68±25.88	361.87±9.89	0.04±0.01	0.49±0.05	5.39±0.16	82.28±15.34	0.42±0.06	5.10±1.18	0.08±0.01	125.37±3.51
2	1827.21±103.75	1518.95±360.78	471.84±51.96	1210.47±388.76	512.58±26.40	0.16±0.02	0.66±0.06	5.88±0.24	321.94±41.26	0.37±0.09	19.02±2.05	0.12±0.01	140.94±4.59
3	1064.05±66.84	560.25±84.61	314.05±22.09	261.89±63.43	430.40±14.65	0.09±0.01	0.80±0.14	5.83±0.22	161.28±20.93	0.29±0.06	5.55±0.57	0.22±0.02	142.80±4.33
4	1271.5±247.81	952.00±147.36	369.85±78.12	324.83±51.98	456.47±86.35	0.10±0.02	0.42±0.09	4.08±0.69	232.13±49.88	0.35±0.07	7.32±1.67	0.46±0.09	124.99±21.99
5	1479.75±215.77	918.50±70.44	474.25±43.80	282.50±48.42	700.00±43.64	0.09±0.04	0.27±0.10	5.49±0.20	180.74±78.28	1.38±0.81	5.34±2.25	0.53±0.023	124.50±7.66
6	1467.35±41.59	429.35±53.96	407.35±20.60	142.16±25.78	370.60±10.77	0.08±0.01	0.20±0.02	5.99±0.18	115.08±15.66	0.20±0.02	8.18±1.71	0.29±0.02	150.35±3.24
7	1243.89±86.65	878.92±105.48	534.37±48.25	311.81±45.48	557.63±20.86	0.07±0.29	0.29±0.04	5.94±0.19	164.18±23.07	0.34±0.06	4.86±0.95	0.51±0.02	136.88±3.16
8	2388.33±424.15	2953.17±1063.06	526.50±88.18	551.98±316.94	519.00±69.23	0.16±0.05	0.31±0.09	6.54±1.35	189.57±62.06	0.87±0.19	14.68±4.00	0.32±0.07	127.17±6.46
9	1420.05±108.09	1338.40±152.02	566.25±49.00	399.85±64.34	436.70±23.06	0.04±0.01	0.25±0.01	5.00±0.16	47.21±5.85	0.32±0.06	12.68±3.17	0.15±0.01	140.05±5.36
10	1369.60±93.74	1199.90±738.29	373.05±56.59	581.68±367.28	344.80±8.88	0.14±0.02	0.42±0.05	4.97±0.12	239.36±31.26	0.27±0.03	8.24±1.46	0.29±0.01	106.19±2.67
11	1291.46±57.24	709.53±99.95	376.87±20.00	170.08±27.83	575.40±27.93	0.12±0.01	0.34±0.04	6.26±0.13	442.53±61.40	0.40±0.13	12.95±1.65	0.11±0.01	130.53±3.86

N o t e. Republic of Yakutia (n = 30), 2 — Republic of Tuva (n = 19), 3 — Republic of Bashkiria (n = 20), 4 — Republic of Kalmykia (n = 6), 5 — Republic of Dagestan (n = 4), 6 — Republic of Kabardino-Balkaria (n = 20), 7 — Stavropol Krai (n = 38), 8 — Krasnodar Krai (n = 6), 9 — Arkhangelsk Province (n = 20), 10 — Lipetsk Province (n = 20), 11 — Bryansk Province (n = 15).

Among the groups, there were significant differences in the hair cobalt content: the highest amount was in horses from Tyva and the Krasnodar Krai, the least amount was in Mezen horses from Arkhangelsk region and native horses from Yakutia. Herd horses of native breeds from Bashkiria, Tyga and Yakutia were leaders in chromium content, while the least amount was characteristic of the animals from Kabardino-Balkaria. The Russian Trotter horses from Bryansk region were superior in the iron content, and the minimum amount was found in animals of local Mezen breed. The increased quantity of iodine has been detected in stud horses from Dagestan and the Krasnodar Krai, Kabardian horses showed lower iodine level. Excess selenium was found in Thoroughbred, Arabs and Akhal-Teke horses from Dagestan, the Stavropol Krai and Kalmykia, the selenium content was low in herd horses of native breeds from Yakutia and Tyva, as well as horses of Russian trotting breed from the Bryansk region.

To estimate total deficit (surplus) of all studied macro- and microelements in horses from different regions, a percentage of the regional average was calculated for each element (Table 2). This indicator makes it possible to compare the influence of various external factors on the hair elemental composition [13]. However, adding positive or negative deviations from the average even more apparently differentiates these regions in terms of the content of the most important chemical elements in the horse mane hair.

2. Deficit (–) and surplus of chemical elements in the mane hair of native and purebred horses from different Russian regions vs. the average per the country (%) (2017)

Регион	Macroelements					Essential microelements							
	Ca	K	Mg	Na	P	Co	Cr	Cu	Fe	I	Mn	Se	Zn
1	–32.16	–57.92	–38.2	–51.12	–21.92	–54.98	20.94	–5.02	–53.77	17.31	–40.06	–69.7	–7.37
2	40.36	65.7	12.21	218.63	10.59	80.07	62.89	3.62	80.9	3.34	123.53	-54.55	4.13
3	–18.26	–38.89	–25.31	–31.06	–7.14	1.29	97.45	2.74	–9.38	–19	–34.77	–16.67	5.51
4	–2.32	3.85	–12.04	–14.5	–1.51	12.55	3.66	–28.1	30.43	–2.24	–13.97	74.23	–7.65
5	13.67	0.19	12.78	–25.64	51.03	1.29	–33.36	–3.26	1.56	285.44	–37.24	100.75	–8.01
6	12.72	–53.16	–3.13	–62.58	–20.04	–9.96	–50.64	5.56	–35.34	–44.14	–3.87	9.84	11.08
7	–4.45	–4.12	27.08	–17.92	20.31	–21.22	–28.43	4.67	–7.75	–5.04	–42.88	93.17	1.13
8	83.47	222.15	25.21	45.3	11.98	80.07	–23.49	15.25	6.52	142.99	72.53	21.21	–6.04
9	9.09	46.00	34.66	5.25	–5.78	–52.73	–38.3	–11.89	–73.47	–10.62	49.02	–43.18	3.47
10	5.21	30.89	–11.28	53.11	–25.61	57.56	3.66	–12.42	34.5	–24.59	–3.16	9.84	–21.54
11	–0.79	–22.60	–10.38	–55.23	24.15	35.05	–16.08	10.31	148.66	11.72	52.19	–58.34	–3.56

N o t e. Republic of Yakutia (n = 30), 2 — Republic of Tuva (n = 19), 3 — Republic of Bashkiria (n = 20), 4 — Republic of Kalmykia (n = 6), 5 — Republic of Dagestan (n = 4), 6 — Republic of Kabardino-Balkaria (n = 20), 7 — Stavropol Krai (n = 38), 8 — Krasnodar Krai (n = 6), 9 — Arkhangelsk Province (n = 20), 10 — Lipetsk Province (n = 20), 11 — Bryansk Province (n = 15).

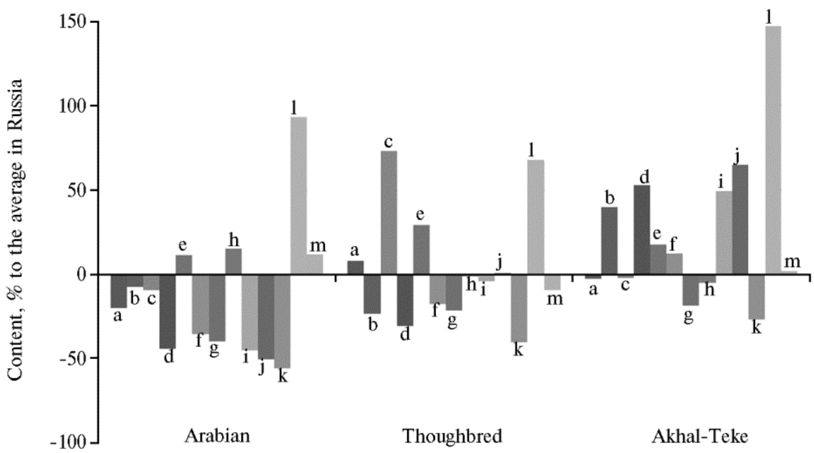
In the Republic of Sakha (Yakutia), horses had shortage of 11 out of the 13 studied chemical elements, comparing to the average for all the regions surveyed. The republics of Bashkiria and Kabardino-Balkaria shared the second place in terms of macro- and microelements shortage, the third was the Republic of Kalmykia and the Stavropol Krai, then Arkhangelsk, Bryansk and Lipetsk regions. The most prosperous were the Republic of Tuva and the Krasnodar Krai where there was a surplus for 12 and 11 elements, respectively, with some elements up to 220 % to the national average. Perhaps, the identified regional shortage of many chemical elements was associated with the biogeochemical peculiarities of a particular territory.

An explanation for the clear regional differentiation in the content of elements may be the fact that the horse hair composition is related to the technologies of keeping and feeding, which are mainly used in a particular region. So, in the Republic of Sakha (Yakutia), horses were kept outdoors all year round and used forage from under the snow in wintertime. Elemental composition of hair under herd technology was different from that of horses kept in stables on balanced diets (Table 3). In herding technology, horses consumed pasture fodder and natural water throughout the year with minimal additional coarse fodder in the winter months, so the accumulation of elements in the hair corresponded to their quantity in the environment. Animals in the stables, as a rule, received the necessary elements with feed, feed additives and water in abundance.

3. Chemical elements (μg/g) **in the mane hair of native and purebred horses from different Russian regions depending on breeding technology (2017).**

Element	In total (n = 198)	Технология содержания			
		herd (n = 69)		stable (n = 129)	
	average in Russia	average	deviation from average in Russia	average	deviation from average in Russia
Ca	1301.76	1195.55	–106.21	1354.87	+53.11
K	916.71	748.36	–168.35	1000.89	+84.18
Mg	420.50	333.95	–86.55	463.77	+43.28
Na	379.90	489.96	+110.05	324.87	–55.03
P	463.49	423.23	–40.26	483.62	+20.13
Co	0.09	0.09	0.00	0.09	0.00
Cr	0.41	0.63	+0.22	0.29	–0.11
Cu	5.67	5.65	–0.02	5.69	+0.01
Fe	177.97	171.17	–6.80	181.37	+3.40
I	0.36	0.37	+0.01	0.35	–0.01
Mn	8.51	9.06	+0.55	8.23	–0.28
Se	0.26	0.13	–0.13	0.33	+0.07
Zn	135.35	134.71	–0.64	135.67	+0.32

N o t e. Herd technology was used in Republic of Yakutia, Republic of Tuva, Republic of Bashkiria, stable technology was used in Republic of Kalmykia, Republic of Dagestan, Republic of Kabardino-Balkaria, Stavropol Krai, Krasnodar Krai, Arkhangelsk Province, and Lipetsk Province.



Chemical elements in the mane hair of purebred Arabian (*n* = 20), Thoughbred (*n* = 20) and Akhal-Teke (*n* = 20) horses: a — Ca, b — K, c — Mg, d — Na, e — P, f — Co, g — Cr, h — Cu, i — Fe, j — I, k — Mn, l — Se, m — Zn (Stavropol Krai, 2017).

To assess the dependency of metabolic processes on the genetic heterogeneity, we compared hair samples from tribal horses of three purebred breeds, i.e. purebred Arabian, Thoroughbred and Akhal-Teke, bred without crossing for several centuries (Fig.). This has resulted in a specific genetic profile of each of the breeds. The influence of other factors (geochemical province and keeping technology) on the elemental state of main hair [14] was offset by the fact that the experiment was carried out in the same territory (the Stavropol Krai) under stabling technology. A trend toward increasing in heterozygosity at 16 autosomal microsatellite DNA loci corresponded to the vector of increasing content of chemical elements in mane hair of horses in three purebred breeds compared to the average value for all regions of Russia.

The Arabian breed, the most homozygous of these breeds (0.383), had the greatest backlog in the content of the elements compared to the average value in Russia. Then there were purebred Thoroughbred horses with homozygosity of 0.339 and the most prosperous Akhal-Teke horses with homozygosity of 0.318. Probably, genetic polymorphism of a breed can be one of the factors which determine inherited intensity of the chemical element metabolism.

Data similar to ours and indicating the dependence of hair mineral composition (metabolic pool of chemical elements) of farm animals on the conditions of the biogeochemical province were obtained in other works [17-21]. There reports on a relationship between diet composition and the accumulation of chemical elements in body depots [22-25]. Data on the expressed link of the chemical elements metabolism with the horse's belonging to a genetically homogeneous group is in line with the results in laboratory animals [26].

Thus, we have found out distinctions in the mane hair levels of macro- and microelements which are related to the specific biogeochemical profiles of the territories, keeping conditions and the primary purpose of the breed use. Stabling was shown to have superiority because of ensuring the best balance of chemical elements in animal nutrition due to normalized composition of the rations, whereas at herding, animals receive chemical elements in the ratio characteristic of a particular biogeochemical province. For three purebred breeds studied, the vector of increasing amounts of hair macro- and microelements was in conformity with the vector of heterozygosity at DNA microsatellite loci. Akhal-Teke breed which is the safest in terms of element balance is also distinguished from the other two breeds, Thoroughbred and Arabian horses, by greater heterozygosity. Our research has shown the urgency of creating database on the micronutrient status of horses. This will bring to better understanding factors that determine metabolic processes in these animals, and improve horse breeding, which is original and important livestock branch.

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