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RESEARCH ARTICLE

# The content of essential and toxic elements in the hair of the mane of the trotter horses depending on their speed

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**Abstract**
A study on the Russian trotting breeds was conducted to assess the impact of horses' sporting results and the degree of accumulation of chemical elements in the hair. In the first phase of the research, the elemental composition of the mane hair of trotter horses ($n = 215$) was studied. Based on these studies, percentile intervals for the distribution of concentrations of chemical elements in the hair have been established, and the values of 25 and 75 percentile adopted as a "physiological standard" have been defined. In the second stage of the research into clinically healthy Russian trotting breeds ($n = 56$), it was estimated that the sporting results were dependent on the elemental status defined by the hair. The elemental composition of the hair was defined by 25 chemical elements using atomic emission and mass spectrometry. It is established that the mane hair is closely related to the sporting results of trotter horses. Thus, in animal wool with the highest sporting achievements, there were reliably less I, Cr, Co, Li, V, Al, Pb, and Cd, and reliably more Si than the low ones. Differences in individual elements exceeded 200%. As sporting performance diminished, the number of elements within the standard increased. For example, for mares with average speed, there were deviations from the physiological standard by 6 elements (P, Fe, Mn, I, Co, Si), with the low one by 13 elements (P, Fe, Cu, Mn, I, Co, Si, K, Cr, Ni, V, Al, Pb). A comparative estimate of the mineralization of the horses' mane measured by the sum of the amount of substances showed that there was a negative correlation between the accumulation of toxic elements and the speed ($r = -0.59$). On the basis of the above, a conclusion is reached on the future use of the mane hair to assess the speed qualities of trotter horses.

**Keywords** Horse · Mare · Russian trotter breed · Elemental status · Sporting achievements · Toxic elements

## Introduction

Muscle and physical stress in sports involve an increase in the need for macro- and microelements (Speich et al. 2001; Hazar et al. 2012). Vital elements are necessary for a broad spectrum of metabolic and physiological processes in the body of athletes; they participate in muscle contraction, oxygen transport, conduction of nerve impulses, immune functions, and others (Volpe and Nguyen 2013; Otag et al. 2014). The insufficiency of vital chemical elements can lead not only to a decline in sporting results but also to the development of pathologies (Burke 1999; Purcell 2013).

Vital chemical elements are increasingly associated with sporting results in equestrian sports (Wijnberg et al. 2002; Haggett et al. 2010).

Together with the obvious dependence of the sporting results on the nutritional balance by the essential elements, the physiological status and performance of the athletes can be determined by the intake and the pool of the toxic elements in the body. This includes, in particular, data from scientific research demonstrating the dependence of morphofunctional features on the level of toxic elements (Goyer 1993; Hill et al. 2015).

However, there are only a few studies in the literature that assess the exchange of heavy metals among athletes. Thus, the

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fact of their higher elimination in people with high physical loads compared with less mobile ones is described (Llerena et al. 2012).

In order to control the level of the chemical elements in the body, the elemental analysis of the various biosubstrates is used, including the saliva (Horvat et al. 1997; Nabatov et al. 2017); blood (Garland et al. 1993); nails (Xun et al. 2011; Martens et al. 2015); and hair (Rodrigues et al. 2008). It seems to be a sufficiently informative non-invasive screening method.

It is recognized that multielemental hair analysis is a highly informative non-invasive screening method for assessing the elemental status (Drasch and Roider 2002; Rodrigues et al. 2008).

Correlations have been established between the concentration of elements in biosubstrates and such important signs of athletic performance as endurance (Louis et al. 2010); physical performance (Lukaski 2004); and stress resistance (Jeukendrup and Gleeson 2010); they allow the use of elemental analysis for the donozological diagnosis of the state of health and purposeful correction of the imbalance of the elements.

The purpose of our research was to study the sporting results of trotter horses from the level in the system of toxic chemical elements assessed by the elemental composition of the horses' hair, depending on their speed qualities.

## Materials and methods

### Object of the research

The studies were performed on the Russian trotter horses; their living mass at the time of the hair sampling was 462.3 ± 5.98 kg, the age—3–4 years.

The animal maintenance and experimental studies were performed in accordance with the instructions and recommendations of the Russian Regulations, 1987 (Order No. 755 dated 12.08.1977, the USSR Ministry of Health) and "The Guide for Care and Use of Laboratory Animals" (National Academy Press Washington, D.C. 1996). During the research, the efforts had been made to minimize animal suffering and reduce the number of samples used.

### Experimental design

The first stage of the research was to examine the elemental composition of the mane hair of the trotter horses ($n = 215$) bred in one of Russia's regions, the Bryansk Oblast. Based on these studies, percentile intervals were established for the distribution of chemical concentrations in the hair; the values of 25 and 75 percentile adopted on the recommendation (Skalnaya et al. 2003) had been defined as the "physiological standard."

In the second stage of the research into clinically healthy Russian trotter horses ($n = 56$) bred in Russia, it was stated that the sporting results were dependent on the elemental status defined by the hair. For this purpose, depending on the speed of passage of 1600 m (the rules of test of pedigree horses of trotting breeds on racetracks of the Russian Federation 2009), they were divided into III groups: I—up to 25 percentile ($n = 13$) with the speed of 120.8 ± 1.96 s; II—within the range of 25–75 percentile ($n = 29$) with the speed of 127.1 ± 1.84 s; and III—above 75 percentile ($n = 14$) with the speed of 138.1 ± 1.76 s (Fig. 1).

### Hair sampling

Sampling was carried out in August 2017. The methodology for the selection of biosubstrates was the selection of hair samples from the mane of animals formed in the same period of time, which was achieved by cutting the hair at a distance of 1 cm from the skin, with the subsequent trimming of the hair and preservation of a 4-cm long hair corresponding to the June–July formation period. The total weight of the sampled hair was not less than 1 mg.

### Elemental status assessment

The elemental composition of the hair was defined by atomic emission and mass spectrometry (AES and MS) at the Test Laboratory of the ANO "Center for Biotic Medicine", Moscow (Registration Certificate of ISO 9001: 2000, Number 4017 – 5.04.06). The biosubstrates were ashed using the MD-2000 microwave decomposition system (USA). The content of elements in the resulting ash was estimated using the Elan 9000 mass spectrometer (Perkin Elmer, USA) and an Optima 2000 V atomic emission spectrometer (Perkin Elmer, USA). Elemental composition of biosubstrates was studied by

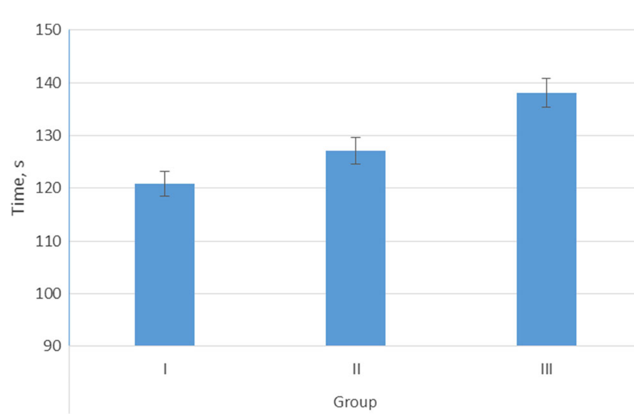



**Fig. 1** The speed of mares of the Russian trotting breed at a distance of 1600 m (s)

25 indicators (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).

## Statistical processing

The criterion of Shapiro-Wilk was used to test the hypothesis on the normality of the quantitative features distribution. The median (Me) was used in the calculation of averages and as a measure of central tendency. The distribution law of the numerical values studied was different from the normal one, so the reliability of the differences was checked using the Mann-Whitney $U$ test. In all the statistical analysis procedures, the level of significance achieved ($p$) was calculated, and the critical level of importance in the study was adopted by less than or equal to 0.05. The Statistica 10.0 ("Stat Soft Inc.", USA) was used to process data.

## Results

An examination of the elemental composition of the mane hair of the sports horses made it possible to set the following characteristics (Table 1).

Comparative assessment of the chemical composition of hair of Russian trotters revealed a significant difference in the concentrations of elements depending on the sports results. Thus, in animal wool with the highest sporting achievements—group I—there were reliably lower amounts of I, Cr, Co, Li, V, Al, Pb, and Cd, and reliably more Si as compared to the group III. The differences in individual elements exceeded 200% (Fig. 2).

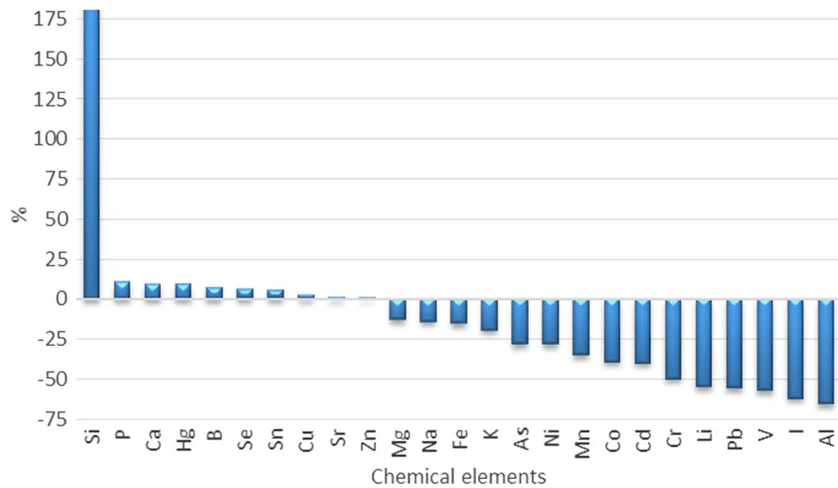
The differences were similar in the elemental composition of the mane hair of groups II and III. It was found that in animals with higher sports results, the concentration of Si

**Table 1** Concentration and percentile values of chemical elements in the mane hair of the sports horses of the Russian trotting breed, μg/g

| Element | "Physiological standard" in percentiles | | Group | | |
|---|---|---|---|---|---|
| | 25 | 75 | I | II | III |
| Macroelements | | | | | |
| Ca | 929 | 1536 | 1388.7 ± 64.2 | 1291.7 ± 52.5 | 1268.3 ± 83.1 |
| K | 307 | 988 | 813.7 ± 130.1 | 574.3 ± 109.0** | 1011.7 ± 121.4 |
| Mg | 283 | 499 | 390.7 ± 39.0 | 344.9 ± 24.3 | 448.3 ± 54.7 |
| Na | 105 | 334 | 205.7 ± 51.1 | 141.2 ± 33.2 | 239.9 ± 61.9 |
| P | 369 | 521 | 615.7 ± 54.8 | 558.7 ± 31.6 | 556.0 ± 45.6 |
| Vital microelements | | | | | |
| Zn | 119.0 | 150.0 | 124.3 ± 6.3 | 129.9 ± 5.7 | 123.3 ± 5.5 |
| Fe | 48.8 | 261.0 | 405.3 ± 78.9 | 494.0 ± 89.2 | 477.3 ± 97.1 |
| Cu | 4.94 | 6.21 | 6.41 ± 0.5 | 6.19 ± 0.2 | 6.26 ± 0.4 |
| Mn | 2.45 | 10.01 | 11.8 ± 1.6 | 11.8 ± 2.2 | 18.1 ± 2.8 |
| I | 0.129 | 0.416 | 0.200 ± 0.10* | 0.458 ± 0.16 | 0.528 ± 0.11 |
| Se | 0.109 | 0.368 | 0.119 ± 0.02 | 0.111 ± 0.01 | 0.112 ± 0.02 |
| Cr | 0.197 | 0.489 | 0.263 ± 0.06* | 0.309 ± 0.02* | 0.525 ± 0.10 |
| Co | 0.028 | 0.123 | 0.099 ± 0.01* | 0.126 ± 0.03 | 0.163 ± 0.02 |
| Conditionally vital microelements | | | | | |
| Si | 6.02 | 21.09 | 9.10 ± 1.3*** | 4.66 ± 0.3* | 3.13 ± 0.7 |
| B | 1.23 | 3.81 | 2.39 ± 0.36 | 3.11 ± 0.49 | 2.22 ± 0.20 |
| Li | 0.10 | 0.25 | 0.11 ± 0.03* | 0.13 ± 0.01** | 0.24 ± 0.04 |
| Ni | 0.18 | 0.49 | 0.42 ± 0.10 | 0.44 ± 0.03 | 0.59 ± 0.19 |
| V | 0.10 | 0.42 | 0.23 ± 0.04* | 0.32 ± 0.04 | 0.55 ± 0.12 |
| As | 0.05 | 0.12 | 0.06 ± 0.01 | 0.06 ± 0.01 | 0.08 ± 0.02 |
| The content of toxic microelements | | | | | |
| Al | 27.6 | 157.0 | 105.2 ± 17.0*** | 139.0 ± 13.1*** | 304.7 ± 18.6 |
| Sr | 2.31 | 6.15 | 5.50 ± 0.1 | 5.16 ± 0.3 | 5.44 ± 0.9 |
| Pb | 0.102 | 0.293 | 0.23 ± 0.04** | 0.27 ± 0.07 | 0.51 ± 0.09 |
| Sn | 0.015 | 0.138 | 0.03 ± 0.01 | 0.02 ± 0.03 | 0.03 ± 0.01 |
| Cd | 0.005 | 0.014 | 0.008 ± 0.001* | 0.009 ± 0.003 | 0.014 ± 0.002 |
| Hg | 0.005 | 0.013 | 0.010 ± 0.002 | 0.010 ± 0.001 | 0.009 ± 0.003 |

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$ as compared to the group III

Fig. 2 Elemental profile of horses of group I relative to group III, %




was increased in mane hair by 49.1% ($P \leq 0.05$), with reduced concentration of potassium by 43.2% ($P \leq 0.05$), lithium by 46.1% ($P \leq 0.05$), chromium by 41.1% ($P \leq 0.05$), and aluminum by 54.4% ($P \leq 0.001$) (Fig. 3).

In general, the elemental status of horses, established by the composition of the hair, with the highest sports results (group I) for the largest number of elements corresponded to the physiological standard determined by us in the range from 25 to 75 percentile. The exceptions were only P, Fe, and Mn, the concentrations of which the animal hairs of the group I exceeded the values of 75 percentile. As the sporting performance decreased, the number of elements outside the range 25–75 percentile increased. So, the mares of the Russian trotting breed from group II were characterized by the differences in the physiological standard by 6 elements: P, Fe, Mn, I, Co, Si, from group III by 13 elements: P, Fe, Cu, Mn, I, Co, Si, K, Cr, Ni, V, Al, and Pb.

Individual evaluation of the elemental status of horses with the highest athletic achievements also demonstrated the importance of the compliance to the physiological standard. As an example, we give the element profile of the "Virtuoznaya" mare born in 2013 (Fig. 4).

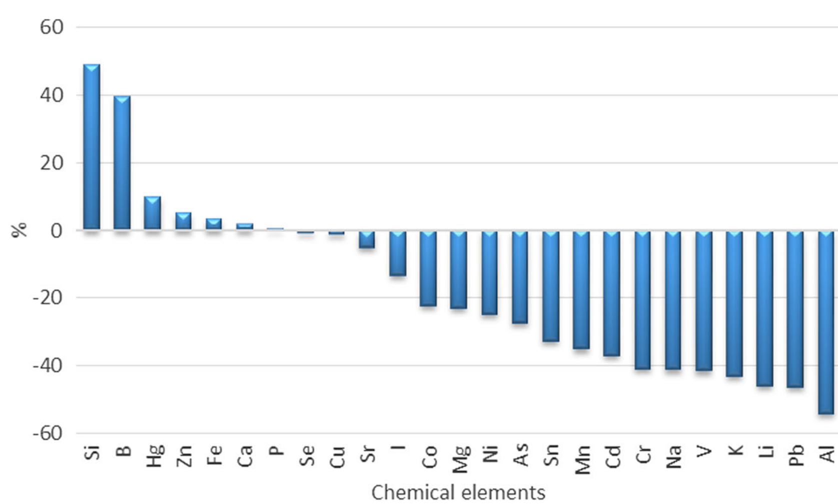
A comparative evaluation of the results of the elemental composition of the mares' hair of Russian trotting breeds revealed a significant difference in the mineralization of this biosubstrate evaluated in terms of the sum of an amount of substances' index (Table 2).

The lesser the hair of the horse's mane contained toxic elements, the higher was their speed. Thus, while the hair of animals of group III contained about 11.8 μmol/g of toxic elements, in group II, this value was 2.2 times lower ($P \leq 0.001$), and in group I, it was 2.9 times lower ($P \leq 0.001$).

## Discussions

The purpose of our research was to evaluate the sporting results of trotting horses, depending on the size of the exchange pool of vital and toxic elements in the body. To do this, we selected the mane hair of the animals. The

Fig. 3 Elemental profile of horses of group II relative to group III, %



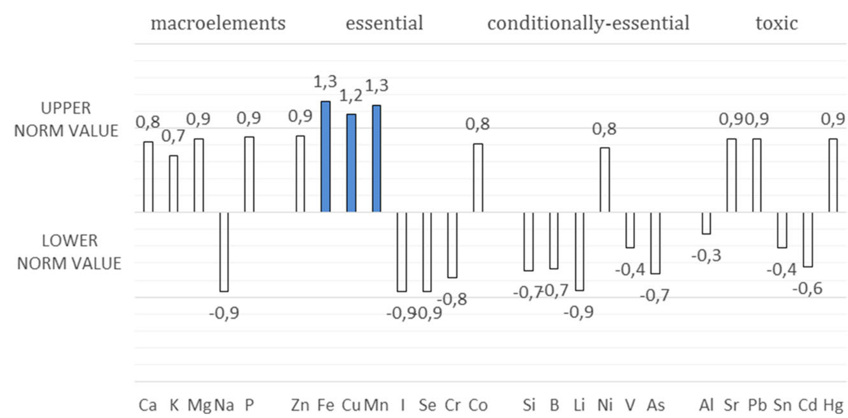



**Fig. 4** The multiplicity of the differences in the elemental composition of the mane hair of the champion of the "Virtuoznaya" Russian trotting breed (born in 2013, the speed at 1600 m is 120.4 s), and the "physiological standard" (25–75 percentile) of the chemical elements in the hair of sports horses (defined by the Dr. Scalny method)

basis for this was the results of studies on the use of elemental hair composition in medicine (Park et al. 2013) and in animal husbandry (Christodoulopoulos et al. 2003; Cygan-Szczegielniak et al. 2014; Zhao et al. 2015). Moreover, the most impressive results on the problem have been achieved in medicine. Medical elementology in recent years has evolved from the development of analytical methods of research and the initial formation of databases to the establishment of reference and centile values of the elemental composition of human biosubstrates and the large-scale use of new knowledge in practice (Skalny 2003). The practical significance of new technologies is confirmed by the number of calls to medical centers using new approaches to the diagnosis and correction of elementhosis (http://en.microelements.ru/).

We have found in our research that differences in the elemental composition of the mane hair of animals depend on the sporting results. The high "speed" of horses was associated with relatively less content of a number of chemical elements in the hair. Thus, in the hair of the mane of the animals with the highest sporting results, the level of I, Cr, Co, Li, V, Al, Pb, and Cd was reduced compared to low-performance animals. The most significant decrease occurred in the content of toxic elements. As the sports results improved in the animals in the 1600 m run from 138.1 ± 1.76 s to 127.1 ± 1.84 and 120.8 ± 1.96 s, we noted a decrease in the total content of the toxic elements (Al, Sr, Pb, Sn, Cd, Hg) from 11.78 ± 0.67 μmol/g to 5.41 ± 0.51 and 4.11 ± 0.77 μmol/g. There was a negative correlation between the accumulation of toxic elements in the mane hair and the speed of horses ($r = -0.59$).

This generally does not contradict the earlier studies (Martínez et al. 2011). Toxic elements are vital antagonists, which manifest itself during the digestion and transport of microelements (Watts 1988; Ranganathan et al. 2011), and directly during metabolism in the body tissues (Alfrey et al. 1986; Banks and Kastin 1989).

One of the evidences of the antagonistic interactions of toxic and vital elements in our case could be the level of iodine in the hair of horses. Thus, while the content of iodine in the hair of the mane of the animals of group III was 0.528 μg/g, its level in group II was lower by 15.3%, and in group I by 2.64 times. This was noted against a background of increasing concentrations of toxic elements in the hair. Earlier, the increase in iodine content in hair with increasing levels of toxic elements was noted in studies (Skalny 2005), which the author explained by the development of the "pre-deficiency" stage.

It is possible that an increase in the content of silicon in the hair against the background of improved sports results also resulted from antagonistic interactions between silicon and toxic elements (Adrees et al. 2015; Greger et al. 2015). It should be noted that the content of silicon in the hair of animals with the best sporting achievements was within a certain "physiological standard." At the same time, silicon plays an important role in supporting immunity, elasticity of blood vessels, synthesis of hormones, etc.

Estimating the obtained results as a whole, it can be noted that the increase in the need of athletes in macro- and microelements is unconditional against the background of increased physical and psycho-emotional activity (Tenforde et al. 2010; McClung et al. 2014). There is probably no significant difference in the changes in the exchange of chemical elements in animals and human athletes with the similarity of muscle loads. Although in our work on trotting horses, we did not get the results confirming the data obtained for the human on the activation of metabolism of calcium, magnesium, zinc (Rankinen et al. 1998; Maughan 1999; Burke 1999;

**Table 2** Content of vital and toxic microelements in the hair of mares of Russian trotting breed, μmol/g, M ± m

| Elements | Group | | |
|---|---|---|---|
| | I | II | III |
| Vital | 9.47 ± 0.42** | 11.14 ± 0.34 | 10.87 ± 0.28 |
| Toxic | 4.11 ± 0.77*** | 5.41 ± 0.51*** | 11.78 ± 0.67 |

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$ as compared to group III

Brisswalter and Louis 2014), phosphorus, and iron (Hunt et al. 1994; Beard et al. 2003; Sandström et al. 2012) in athletes; it should be noted that this was more the result of different approaches to the problem. Obviously, in any case, a personal approach is needed, primarily in the selection and elemental analysis of the individual's biosubstrates. Only this allows us to correctly assess the features of the exchange of chemical elements in the athlete's body and draw the right conclusions.

Of fundamental importance in this regard are the results of the individual evaluation of the elemental profile of the Virtuoznaya horse (born in 2013, speed at 1600 m—120.4 s) with the best sporting results, showing the correlation between the level of sporting achievements and the correspondence of the concentrations of elements in the hair to the "physiological standards," and first of all on toxic elements. Minor differences between the above "physiological standards" and the elemental profile of the "Virtuoznaya" for iron, copper, and manganese were generally consistent with earlier data on athletes (Grantham-McGregor and Ani 1999; Braetter 2002).

It is possible to challenge the values of the "physiological norm" as the interval 25 and 75 percentile within this sample, but these figures will be supplemented in the framework of the project.

## Conclusion

The elemental composition of the mane hair is closely related to the sporting results of trotter horses.

Thus, in animal wool with the highest sporting achievements, there were reliably less I, Cr, Co, Li, V, Al, Pb, and Cd, and reliably more Si than the low ones. Differences in individual elements exceeded 200%.

In general, the elemental status of horses with high sporting results for most of the elements studied corresponded to the physiological standard we defined in the range of 25 to 75 percentile. The exception was P, Fe, and Mn, the concentration of which exceeded the values of 75 percentile. As sporting performance diminished, the number of elements within the standard increased. For example, for mares with average speed, there were deviations from the physiological standard by 6 elements (P, Fe, Mn, I, Co, Si), with the low one by 13 elements (P, Fe, Cu, Mn, I, Co, Si, K, Cr, Ni, V, Al, Pb).

A comparative estimate of the mineralization of the horses' mane measured by the sum of the amount of substances showed that there was a negative correlation between the accumulation of toxic elements and the speed ($r = -0.59$). Thus, if the hair of animals with low sporting qualities contained about 11.8 μmol/g of toxic elements (Al, Sr, Pb, Sn, Cd, Hg), in the group with averages, it was 2.2 times lower ($P \leq 0.001$), and in the group with high ones—2.9 times lower ($P \leq 0.001$). On the basis of the above, a conclusion is reached on the future use of the mane hair to assess the speed qualities of trotter horses.

It is necessary to conduct further research on the possible use of indicators of the "physiological standard" of the chemical elements' content in horsehair to enhance sporting achievements in equestrian sport.

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