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Value of Wool Composition in Assessing the Pool of Chemical Elements in Rabbits and Rats

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Abstract

Elemental composition of animal wool and human hair is being increasingly used in clinical toxicology for diagnosing diseases. In our research, we studied the informational value of wool composition in evaluating the pool of chemical elements in the rabbits and rats in case of unbalanced nutrition. Two 4-8-weeks long experiments were conducted with Wistar rabbits and rats. We studied the content of 20 chemical elements in the organs and tissues. The elemental composition was determined using the method of atomic-emission and mass spectrometry (AES-ISP and MS-ISP). The experiment showed increase in the content of a number of elements (Ca, K, Mg, Na, P) in animals when there is a shortage in the diet. For calcium, the element rearrangement from "depot" into the internal organs, with increasing (decreasing) concentrations in the wool, was observed. Veracious correlations between the content of the element in the wool and increase in its pool in the organism when there is unbalanced mineral diet were found. In the example of rabbits, veracious correlation has been shown between the composition of wool and the size of its pool for 15 elements (As, Co, Cr, Cu, Fe, I, K, Mg, Na, P, Pb, Se, Sr, V, Zn). The elemental composition of animals' wool may be used for characterizing pools of chemical elements in the organism in case of insufficient mineral diet.

Keywords

Elemental composition; Wool; Diet; Laboratory animals

Introduction

Recently, animal wool and human hair, as an alternative to samples of blood, urine, and biopsy material, have been widely used in clinical toxicology and chemistry [1-3]. The elemental analysis of human hair is widely used in diagnosing oncological diseases [4]; pathologies caused by intoxication with heavy metals [5]; metabolic syndromes [6,7]; thyroid diseases [8], etc.

The elemental composition of animal wool is studied in the course of ecological assessment of territories [9,10]; in diagnosing diseases of dairy cows [11], etc.

However, lack of information and the differences in the study methods often lead to different findings about the information content of wool (hair) in assessing the elemental status of the organism and the characteristics of metabolism [12].

However, literature indicates shortcomings of using human hair and animal wool as a means for assessing the state of nutrition and metabolism in the organism [13,14]. The absence of objective information does not make it possible to use the indicators of wool content in diagnosing and correcting elementoses and detecting other diseases in animals. In this regard, our research was focused on studying the informativity of the elemental composition of wool in assessing the metabolism and the size of element pools in the organisms of rabbits in the conditions of various mineral supply.

Materials and Methods

Animals

Experiment 1 was made on rabbits (Oryctolagus cuniculus), $m = 1{,}600\text{-}1{,}650$ g. At the age of 3 months, all animals were weighed and placed in equal conditions. On the basis of the data of individual weighing and forage requirements, at the age of 4 months, two groups of animals were formed using the method of analogous pairs ($n = 30$): control (I) and experimental (II). The duration of the accounting period was 4 weeks.

Experiment 2 was made on Wistar rats. For the research, 40 2-month-old males that weighed 111.8 ± 1.5 g were selected. After 2 weeks of the preparatory period, the animals were divided into two groups ($n = 20$): control (I) and experimental (II). The duration of the accounting period was 8 weeks.

The experimental research involved keeping animals in equal conditions in accordance with the existing density, temperature, and humidity standards.

Ethics statement

The experimental research on animals was conducted according to the instructions recommended by the Russian Regulations, 1987 (Order No. 755 on 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)".

The animals were placed in the attestation vivarium at the Institute of Bioelementology of the Orenburg State University. The vivarium was duly equipped and was run by competent personnel. The veterinary requirements were met.

Rations and forage

Experiment 1. The animals in group 1 were fed with a balanced diet including 100 g. of Sudan grass hay; 12 g. of mineral premix; 50 g. of wheat; 50 g. of barley grains; and 0.5 g. of salt.

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The animals in group 2 received a diet deficient in minerals that included 100 g. of wheat straws; 0.5 g. of salt; 17 g. of soy concentrate; 100 g. of starch; 10 g. of vegetable oil (refined); and vitamins (A, D, C, E, PP, B1, B2, B6, BC, B12). Redistilled water was used. In calculating the rations, the norms and rations of feeding agricultural animals were used [15].

Experiment 2. The animals in group I were fed with balanced diet that included 3.7 g. of sunflower seeds; 10.3 g. of oat; 4.0 g. of wheat bread; 2.5 g. of millet porridge; 2.0 g. of cottage cheese (5% fat); 0.5 g. of fish flour; 4.0 g. of beef; 8 g. of carrot; 8.0 g. of green salad; 0.1 g. of fish tallow; 0.1 g. of yeast; and 0.15 g. of salt.

The animals in group 2 received a diet deficient in minerals that included 30 g. of boiled polished rice; 1 g. of saccharose; 1.25 g. of soy concentrate; 1 g. of vegetable oil (refined); and vitamins (A, D, C, E, PP, B1, B2, B6, BC, B12). Redistilled water was used for watering.

Sampling

Samples of wool were taken at the end of the experiments. The wool was taken from the withers of rabbits and from the backs of rats. The locations for taking wool samples had been shaven at the beginning of the experiment. This was done to avoid sampling the wool that had grown before the accounting period.

The animals were slaughtered at the end of the experiments to study the tissues. The used method of euthanasia was decapitation under pentobarbital anesthesia. The weight of wool, skin, and flesh of the carcass, internal organs, gastrointestinal tract, internal fat, blood, etc., was determined individually for each animal. Preparation of samples included grinding and homogenization of animal biosubstrates.

Elemental analysis

The elemental composition of the forage and the animal biological substrates (muscles, internal organs, bones gastrointestinal tract, skin, wool, blood, etc.) was tested for the content of 20 chemical elements (Al, As, Ca, Cd, Co, Cr, Cu, Fe, I, K, Mg, Mn, Na, Ni, P, Pb, Se, Sr, V, Zn). The methods of atomic emission and mass spectrometry (AES-ISP and MS-ISP) were applied with the use of equipment Elan 9000 (Perkin Elmer, USA) and Optima 2000 V (Perkin Elmer, USA). The analysis of samples was performed in the laboratory of the Independent Inspection Services “Center of Biotic Medicine” (Registration Certificate of ISO 9001: 2000, Number 4017-5.04.06).

The size of the pool of chemical elements in the organism was determined by summing up the weights of the elements in separate organs and tissues.

Statistical analysis

The statistical analysis of the results was performed with the use of statistical software package Statistica 10. Statistical comparison of the results was performed with the use of the Mann-Whitney U and Student’s criterion. The parameter $p < 0.05$ was used as the limit of significance.

Results

The mineral composition of the rations

The elemental composition of the used rations is shown in Table 1.

The content of the chemical elements in the diet of rabbits in group I was higher than the content in group II by all assessed elements, except for cadmium, iron, lead, and selenium.

	Experiment			
	I		II	
	Group			
Element	I	II	I	II
	Macroelements, g			
Ca	1.40 ± 0.13	0.76 ± 0.005	1.43 ± 0.08	0.094 ± 0.0018
K	2.91 ± 0.61	2.48 ± 1.1	0.55 ± 0.021	0.25 ± 0.038
Mg	1.12 ± 0.098	0.63 ± 65	2.21 ± 0.077 0.15 ± 0.044	
Na	1.15 ± 0.10	0.88 ± 0.05	0.67 ± 0.15	0.35 ± 0.09
P	0.82 ± 0.232	0.55 ± 0.163	0.75 ± 0.037	0.07 ± 0.005
	Microelements, mg			
Al	39.8 ± 2.9	48.3 ± 1.9	43.2 ± 1.71	1.17 ± 0.09
As	0.01 ± 0.003	0.009 ± 0.0008	0.378 ± 0.052	0.045 ± 0.001
Cd	0.05 ± 0.007	0.23 ± 0.019	0.099 ± 0.005 0.0022 ± 0.0001	
Co	0.087 ± 0.009	0.076 ± 0.0056	0.3 ± 0.028	0.006 ± 0.0001
Cr	0.15 ± 0.017	0.066 ± 0.009	0.453 ± 0.051	0.05 ± 0.001
Cu	1.5 ± 0.001	0.6 ± 0.003	9.98 ± 0.15	1.6 ± 0.02
Fe	43 ± 12.3	74.3 ± 26.1	131.4 ± 7.1	7.36 ± 0.05
I	0.7 ± 0.064	0.1 ± 0.013	0.38 ± 0.05	0.04 ± 0.093
Mn	12.8 ± 0.8	6.6 ± 0.56	126.1 ± 5.08	3.78 ± 0.27
Ni	0.5 ± 0.04	0.57 ± 0.032	1.99 ± 0.27	0.159 ± 0.05
Pb	0.16 ± 0.001	0.58 ± 0.003	0.07 ± 0.008	0.0053 ± 0.0001
Se	0.02 ± 0.001	0.03 ± 0.0009	0.483 ± 0.005	0.039 ± 0.003
Sr	1.66 ± 0.13	0.2 ± 0.018	27.8 ± 2.11	0.89 ± 0.13
3.4 ± 1,179.9 ± 3,126.3 ± 0.82 V	24.5 ± 1.9	21.3 ± 1.1	0.31 ± 0.05	0.011 ± 0.007
18.0 ± 1.3 Zn				

Table 1: Chemical composition in daily rations

Element	Experiment			
	I		II	
	Group			
	I	II	I	II
	Macroelements, g			
Ca	24.0 ± 1.50	14.5 ± 1.33^a	2.55 ± 0.10	0.95 ± 0.04^b
K	4.00 ± 0.12	2.85 ± 0.14^a	1.41 ± 0.03	0.66 ± 0.05^b
Mg 0.54 ± 0.044	0.35 ± 0.047^a	0.15 ± 0.005	0.10 ± 0.005^b	
Na	1.75 ± 0.03	1.60 ± 0.12	0.41 ± 0.01	0.23 ± 0.006^b
P	7.33 ± 0.344	3.92 ± 0.74^a	2.37 ± 0.079	1.68 ± 0.035^b
	Microelements, mg			
Al	4.40 ± 0.37	8.81 ± 0.29^a	1.39 ± 0.314	0.92 ± 0.33
As	0.009 ± 0.0002	0.010 ± 0.0010	0.029 ± 0.006 0.021 ± 0.005	
Cd	0.007 ± 0.0009	0.009 ± 0.0014	0.0037 ± 0.00026	0.001 ± 0.0004
Co	0.070 ± 0.0095	0.036 ± 0.0052	0.007 ± 0.0006	0.005 ± 0.0005
Cr	0.21 ± 0.009	0.28 ± 0.061	0.10 ± 0.002	0.02 ± 0.0005^b
Cu	2.23 ± 0.208	1.55 ± 0.229	0.41 ± 0.018	0.29 ± 0.005
Fe	51.8 ± 3.41	57.3 ± 0.58	22.0 ± 1.23	10.4 ± 0.94^b
I	0.55 ± 0.137	0.19 ± 0.029	0.02 ± 0.0003	0.016 ± 0.013
Mn	2.70 ± 0.319	1.25 ± 0.106	0.29 ± 0.006	0.05 ± 0.005^b
Ni	0.78 ± 0.14	0.55 ± 0.17	0.18 ± 0.018	0.13 ± 0.003
Pb	0.079 ± 0.0098	0.105 ± 0.0155	0.026 ± 0.0002	0.015 ± 0.0025
Se	0.144 ± 0.0020	0.162 ± 0.0194	0.137 ± 0.0087 0.061 ± 0.008^b	
Sr	29.9 ± 2.10	17.4 ± 2.81^a	6.01 ± 0.029	4.92 ± 0.077^b
V	0.014 ± 0.0009	0.026 ± 0.0010^a	0.012 ± 0.009	0.007 ± 0.003^b
Zn	51.5 ± 2.87	34.7 ± 5.00^a	11.9 ± 0.38	6.84 ± 0.33

Table 2: The size of the pool of chemical elements in the organism of animals (at the end of the experiments)

Changes in the live weight

At the beginning of the experiment, the live weight of rabbits in group I was 1,616.4 ± 85.4 g, in group II—1,602.8 ± 47.6 g; at the end of the experiment the live weight in group I was 1,923.1 ± 105.6 g, and in group II—1,583.1 ± 50.3 g.

At the beginning of the experiment, the live weight of rats in group I was 111.9 ± 2.7 g, in group II—115 ± 2.5 g; at the end of the experiment the live weight in group I was 255.8 ± 3.2 g, and in group II—292.0 ± 4.1 g.

The size of pools of chemical elements in the organisms of animals

In the course of the studies, changes in the content of chemical elements in animals have been described (Table 2).

The data is expressed as the mean ± SD. Characters a and b indicate the statistically significant difference between the groups ($p < 0.05$) in experiments I and II.

At the end of the experiment the organisms of rabbits in group II, as compared to group I, contained significantly less: zinc—by 48.4% ($p < 0.01$), calcium—by 65.5% ($p < 0.01$), magnesium—by 53% ($p < 0.05$), phosphorus—1.87 times ($p < 0.01$), potassium—by 40.4% ($p < 0.05$), strontium—by 71% ($p < 0.001$), and more: vanadium—by 86% ($p < 0.01$), aluminum—2 times ($p < 0.001$).

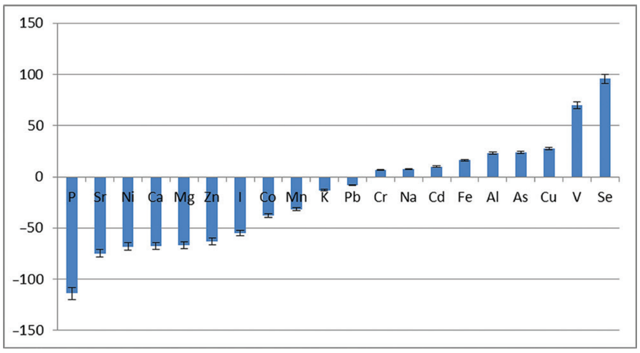

Figure 1: The difference in the content of chemical elements in the skeleton of rabbits in group II, as compared to group I, %

The studies of rats revealed more than one fact of a larger pool of chemical elements in animals of group II, as compared to that in group I. Assessment of the difference in the total content of chemical elements in the skeleton of rabbits revealed identity with the common pool of chemical elements in the organism (Figure 1).

The experiment with rats revealed increase in the content of strontium—by 22.1% ($p < 0.001$), selenium—32.5% ($p < 0.001$) in the skeleton of animals in group II, as compared to group I (Figure 2).

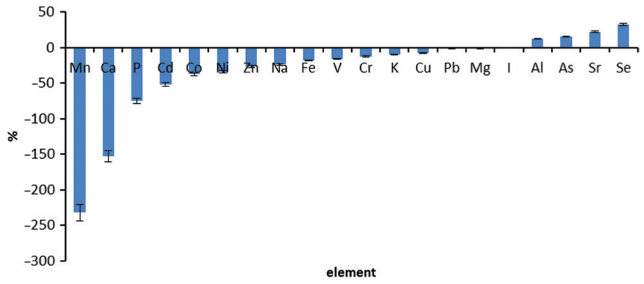

Figure 2: The difference in the content of the chemical elements in the skeleton of rats in group II, as compared to group I, %

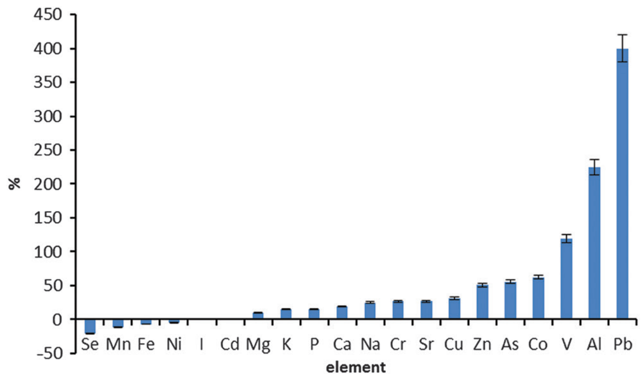

Figure 3: The difference in the content of chemical elements in the liver of rats in group II, as compared to group I, %

Element	Experiment			
	I		II	
	Group			
	I	II	I	II
	Macroelements			
Ca	1,128 ± 19	1,443 ± 53^a	414 ± 7	285 ± 15^b
K 1,534 ± 47	1,947 ± 87^a	3,896 ± 241	2,000 ± 140^b	
Mg	235 ± 33.4	448 ± 59^a	159 ± 5	87 ± 12^b
Na	752 ± 49	1,056 ± 47^a	585 ± 157	3,482 ± 297^b
P	358 ± 19	492 ± 37^a	485 ± 61	1,117 ± 204^b
	Microelements			
Al	21.4 ± 2.3	136.0 ± 3.0^a	5.39 ± 0.31	6.18 ± 0.47
As	0.042 ± 0.010	0.133 ± 0.030	0.110 ± 0.02 0.180 ± 0.005	
Cd	0.010 ± 0.006	0.031 ± 0.007	0.014 ± 0.004	0.012 ± 0.001
Co	0.163 ± 0.040	0.215 ± 0.047	0.005 ± 0.0006	0.006 ± 0.0009
Cr	0.518 ± 0.057	3.55 ± 0.247^a	0.73 ± 0.02	0.95 ± 0.04^b
Cu	9.9 ± 0.64	11.9 ± 1.45	5.71 ± 0.59	5.34 ± 0.18
Fe	53.3 ± 14.3	220.3 ± 19.5^a	57.19 ± 11.01	31.89 ± 4.8
I	2.5 ± 0.4	1.35 ± 0.23	0.19 ± 0.02	0.33 ± 0.06
Mn	3.10 ± 0.48	7.11 ± 0.14^a	0.54 ± 0.09	0.43 ± 0.15
Pb	0.405 ± 0.034	1.566 ± 0.062^a	0.21 ± 0.07	0.32 ± 0.18
Ni	0.411 ± 0.180	2.147 ± 0.057^a	0.01 ± 0.0001	0.03 ± 0.03^b
Se	0.21 ± 0.027	0.22 ± 0.032	0.72 ± 0.13	0.55 ± 0.17
Sr	3.55 ± 0.21	4.79 ± 0.30	0.63 ± 0.08	0.7 ± 0.02
V	0.067 ± 0.040	0.396 ± 0.073^a	0.2 ± 0.01	0.25 ± 0.02
Zn	234 ± 15	315 ± 20	178 ± 11.5	133 ± 16

Table 3: The content of chemical elements in wool, mg/kg

In the liver of rats in group II, the content of 14 out of 20 elements significantly increased. The most significant increase was noted for lead—4 times ($p < 0.001$), aluminum—2.3 times ($p < 0.001$), cobalt—62.5% ($p < 0.01$), etc. (Figure 3).

The content of chemical elements in the wool of rabbits and rats

The research has revealed the higher content of chromium—6.85 times ($p < 0.001$), iron—4.1 times ($p < 0.001$), manganese—2.2 times ($p < 0.01$), nickel—5.2 times ($p < 0.001$), vanadium—5.9 times ($p < 0.01$), aluminum—6.3 times ($p < 0.01$), and lead—3.9 times ($p < 0.01$) in the wool of rabbits in group II grown during the experiment, as compared to group I (Table 3).

The data is expressed as the mean ± SD. Characters a and b indicate the statistically significant difference between the groups ($p < 0.05$) in experiments I and II.

The wool of rats of group II contained more chemical elements, as compared to group I: sodium—5.95 times ($p < 0.001$), phosphorus—2.3 times ($p < 0.001$), chromium—by 30.1% ($p < 0.05$), and nickel—3 times ($p < 0.001$).

Informational value of wool composition in evaluating the pool of elements

Group I of rabbits revealed the presence of only six elements, whose concentration in the wool veraciously correlated with the size of the pool of element in the organism, including vanadium ($r = 0.78$), iodine ($r = 0.88$), cobalt ($r = -0.85$), phosphorus ($r = 0.80$), aluminum ($r = 0.89$), and manganese ($r = -0.71$). Group II of rabbits revealed veracious correlation between the composition of wool and the size of its pool for 15 elements (As, Co, Cr, Cu, Fe, I, K, Mg, Na, P, Pb, Se, Sr, V, Zn) out of 20. Examination of the correlation of wool with the size of individual pools of chemical elements in the internal organs, gastrointestinal tract, muscles, skeleton, blood, and skin also confirmed an increase in the number of veracious correlations in the background of unbalanced feeding. The highest number of correlations was observed in group II when comparing the composition of wool and skin by 14 elements (Al, As, Ca, Cd, Co, Cr, Cu, K, Mn, Ni, Pb, Sr, V, Zn).

In the experiment, rats confirmed the dependence of the number of correlations in wool composition with the pool of elements in the organism of animals against the background of balanced mineral nutrition. Group II of rabbits revealed veracious correlation between the composition of wool and the size of its pool for 13 elements (Ca, Cd, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Se, Sr, V, Zn) out of 20.

Discussion

The study of the elemental composition of wool (hair) is being increasingly used in cattle breeding for diagnosing diseases in dairy cows [16], in diagnosing elementoses [17], and in medicine for diagnosing and treating human diseases [18,19].

The theory of human elementoses has gone a long way in recent years, from the development of analytical methods for primary research and creating databases, to stating reference and centile values of the element composition in human biosubstrates [20,21]. The practical significance of the new technologies is confirmed by the number of visits to the health centers that use new approaches to diagnosing and correcting of elementoses (http://en.microelements.ru/).

However, for a number of reasons, the practice of studying the elemental composition of wool in livestock breeding has not yet been widely used, including that of the lack of objective information [22,23].

This determined the worthiness of our research. In two experiments on rabbits and Wistar rats we reproduced the conditions of the alimentary hypoelementhosis by a number of elements.

According to the obtained data, wool composition may be used for estimating the size of the pool of chemical elements in the organism, including the conditions of unbalanced mineral diet. Against the background of balanced rations, we revealed the existence of a veracious correlation between the wool composition and the size of the pool elements in the organism for only 6-7 elements of the 20 analyzed ones. Meanwhile, in groups with unbalanced diet, there was an increase in the number of elements up to 13-15, and the pools that may be described by the composition of wool. This is generally consistent with the results of the research [24], according to which the mineral composition of the hair reflects the exposition of the elements from the ration. However, along with the decreased concentration of a number of elements in the wool, when they are deficient in the ration, we detected the increased concentration of chemical elements (Ca, Mg, Na, P, etc.) in the wool, while their pool in the organism is reduced. For example, in the experiment with rabbits, the size of the total pool of calcium in animals in group II decreased to 14.5 ± 1.33 g. With that, the content of this element in the wool increased by 27.9%, as compared to group I. Previously, similar results were obtained by [25]. This fact may be explained by the phenomenon of homeostasis. It is not unexpected that it is possible to maintain constancy of the internal environment of the organism in case of calcium deficiency in the diet via removing this element from the "depot". In experiment I, we have shown a decrease in the content of calcium in the skeleton of rabbits in group II by 64.3% ($p < 0.001$). Here the weight of calcium increased in the internal organs by 28.8% ($p < 0.01$), in the blood of animals by 33.3% ($p < 0.001$), as compared to group I (Figure 3 shows a similar change in the concentration in the liver of the majority of rats in group II). Thus, the changes in the calcium metabolism were reflected in wool composition.

In this regard, the significant decrease in the concentration of calcium in the wool of rats in group II (experiment II) by 45.3% ($p < 0.01$), as compared to group I, may be explained by the exhaustion of the calcium "depot". According to our data, the calcium pool in the organism of rats of group II at the end of the experiment decreased 2.7 times. The similar decrease in experiment I was only 64%.

Speaking of the chemical elements of the pools that we failed to characterize in the course of experiment I by the composition of wool (Al, Cd, Mn, Ni), it should be noted that we failed to reach lack of aluminum, cadmium, and nickel in group II of experiment I (Table 1). Here, the toxic elements are actively involved in the metabolism against the background of essentials deficiency. This is well illustrated by the data about liver composition in animals of group II in experiment II (Figure 3). In particular, the content of lead increased 4 times ($p < 0.001$), that of aluminum 2.3 times ($p < 0.001$), etc. An important factor that changes the toxic element metabolism against the background of deficiency of essentials is the reduction of antagonistic interactions between the substances at the stage of absorption [26], due to the lack of inhibition of essential elements by ions in the intestinal absorption of toxic substances [27-29], and reduction of the competition for common transporters in the intestines. [30,31].

The question arises about the possibility of using wool for characterizing the exchange of chemical elements in case of non-deficient conditions and ways of identifying them. The explanations thereto are hidden in the algorithm based on assessing centile values of individual elements in the wool (hair).

In accordance with one of the most widely used hypotheses, the elemental composition of human hair is matched with the "norm" if its value corresponds to the interval of 25-75 centiles (the average value of the content of a particular chemical element in the population). It is proposed to consider the values in the range between 10 and 25, and between 75 and 90 centiles as the standard deviation that corresponds to the state of "predisease". The concentrations of chemical elements in the interval between 0 and 10, and between 90 and 100 centiles reflect the state of disease, and are associated with a clear clinical manifestation specific for elementoses of syndromes and symptoms [32].

Using this algorithm, it is possible to diagnose the condition of elementoses in animals, and to take measures for treatment.

Conclusion

The elemental composition of the wool of animals, e.g. rabbits and rats, may be used to characterize the pools of chemical elements in the organism in the conditions of mineral deficiency in nutrition. Increase in the concentration of macroelements and individual microelements in the wool in case of the deficiency in the ration is a consequence of their redistribution from the "depot" into internal organs and blood.

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