Influence of deficient nutrition on trace element status and antioxidant defense system

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Abstract. The aim of research was to study the effect of unbalanced nutrition on rat’s liver and hair element composition and antioxidant defense system in the experiment. The study was conducted on male Wistar rats. The experimental group was on mineral deficient diet, the control group received a standard diet. The elemental composition of the liver and hair was determined by atomic emission and mass spectrometry with inductively coupled argon plasma. Blood biochemical parameters were determined spectrometrically using a Clima MC-15 A/O Unimed analyzer. The level of malondialdehyde and the activity of glutathione peroxidase in the liver of animals were determined by standard methods using ELISA kits. Results. A decrease in the content of copper, zinc, and selenium was found in liver tissue by 1.5, 1.14, and 3.2 times, respectively. Significant increase of lead and aluminum was established. A similar changes were observed in the elemental composition of hair. GPx activity decreased in 1.4 times and MDA level increased in the liver of animals of the experimental group. An increase in ALT, AST and total protein was noted on mineral-deficient diet.

1. Introduction
Currently, the need for research of the optimization of diets in agriculture remains an urgent task. One of the important aspects of the diet is its micronutrient composition. A trace element’s deficient nutrition can lead to various metabolic disorders [1]. On the other hand, an excessive content of trace elements in the composition of the feed can lead to toxic effects and a decrease in productivity, as well as to environmental pollution through animal excretion with metal salts [2].

When studying the metabolic effects of the diet consumed on the body, many researchers rely not only on the indicators of the elemental composition of various tissues of the body, but also on hematological indicators and markers of antioxidant status. [3, 4]. Liver tissue is considered to be sufficiently informative material for assessing the elemental and antioxidant status of the body, since the liver takes the most active part in metabolic processes [5, 6]. The aim of the investigation was to study the effect of an unbalanced nutrition on the liver and hair element composition and antioxidant defense system in the experiment.
2. Materials and methods

2.1 Animals

For experimental studies, two groups of adult male Wistar rats (n = 20) were formed, (n=20, weight 210-240 g). The animals were housed at room temperature (22-25°C) and 60-70% humidity, 12 hr light-12 hr dark cycle. The animals were supplied with water ad libitum. All procedures involving animals and their care were performed in accordance with the protocol approved by the Institutional Animal Care and Use Committee of the Federal Research Center for Biological Systems and Agrotechnologies of the Russian Academy of Sciences, Orenburg, Russian Federation and complied with Directive of the European Parliament and the Council of the European Union 2010/63 / EU.

2.2 Experimental design

The experimental group consumed micronutrient deficiency nutrition, the control group received standard diet for two months. At the preliminary stage of the experiment, elemental analysis of rations was carried out. Figure 1 shows the composition of the deficient diet as a percentage of a balanced diet.

![Figure 1. The content of essential elements in the diet of the experimental group in comparison with the control, %](image-url)

The functional state of laboratory animals was assessed by an integral indicator, which included dynamics of body weight (weekly), the volume of daily food and fluid intake, changes in external signs and the degree of activity of laboratory animals.

2.3. Biochemical analysis

Animals were later sacrificed under anaesthesia and liver was homogenized for the determination of glutathione peroxidase (GPx) and malondialdehyde (MDA) levels. The supernatant absorbance was measured spectrophotometrically at 530 nm. Serum biochemical parameters assessing liver functions (alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total protein) were determined spectrophotometrically using commercial kits by biochemical analyzer (Clima MC-15 A / O Unimed.) The concentration of GPx and MDA, were measured using ELISA kit (Cloud-Clone Corp, Houston, TX, USA), according to the manufacturer’s instructions.

2.4 Trace elements analysis

Samples of hair and liver were used as biosubstrates to study rat’s element status. The analysis of samples was carried out on 25 chemical elements in the laboratory of the ANO "Center for Biotic Medicine" (registration number in the state register - Ross. RU 0001. 513118 dated May 29, 2003; Registration Certificate of ISO 9001: 2000, Number 4017-5.04.06) atomic emission and mass spectrometry with inductively coupled argon plasma on Optima 2000 DV and ELAN 9000 devices (Perkin Elmer, USA).
2.5 Statistical analysis
Statistical data analysis was performed using the program "Statistica 10.0". Parameters of descriptive statistics for quantitative indicators are given in the form of a median (Me) and interquartile range (25th; 75th percentile - Q1; Q3). Since \( n \) does not exceed 30, the Mann-Whitney U-test was used to assess the significance of the similarity/difference between two independent samples. The difference was considered significant at \( p < 0.05 \).

3. Result and discussion
The assessment of biochemical parameters of liver function was done. In the blood serum of experimental animals ALT and AST activity significant increased by a factor of 3.5 and 1.5 times (Table 1). Also an increase of total protein concentration (1.4-fold) was observed. Such changes in biochemical markers could characterize liver dysfunction.

### Table 1. Biochemical blood parameters of laboratory animals

| Indices          | Experimental group | Control group | MWU | \( p \)  |
|------------------|--------------------|---------------|-----|---------|
| Total protein g/l| 84.05 (73.6-87.4)  | 59.7 (57.2-64.3) | 0.0002* |
| ALT U/l          | 119.7 (104-129)    | 34.6 (29.4-37.5) | 0.0002* |
| AST U/l          | 100.4 (89.3-111.2) | 67.6 (63.4-76.4) | 0.0003* |

Data presented as median (25-75); MWU=\( p \) values as assessed by Mann-Whitney U-test; * - difference significant at \( p < 0.05 \)

A significant decrease in content of copper (1.5-fold), zinc (1.14-fold), and selenium (3.2-fold) was found in the liver tissue (Table 2). The content of cobalt increased by 1.5 times. At the same time accumulation of toxic elements (lead, aluminum) was revealed (Table 3).

### Table 2. Content of essential elements in the liver of laboratory animals

| Elements | Experimental Group | Control Group | MWU | \( p \)  |
|----------|--------------------|---------------|-----|---------|
| Cr       | 0.050 (0.043 -0.080) | 0.0351 (0.0304 - 0.0462) | 0.0757 |
| Cu       | 3.095 (3.06 - 3.17)  | 4.63 (3.94 - 5.59) | 0.0002* |
| Fe       | 216.58 (194.53 - 233.19) | 218.12 (207.87 - 252.58) | 0.3846 |
| I        | 0.026 (0.022 - 0.0352) | 0.0132 (0.011 - 0.021) | 0.0756 |
| As       | 0.104 (0.074 - 0.178) | 0.163 (0.134 -0.208) | 0.1041 |
| Co       | 0.018 (0.0174 - 0.019) | 0.0121 (0.0117 - 0.0123) | 0.0002* |
| Zn       | 31.27 (30.39 - 31.99) | 35.775 (33.4 - 38.24) | 0.0045* |
| Mn       | 1.44 (1.24 - 1.68)   | 1.465 (1.25 - 1.69) | 0.7052 |
| V        | 0.0099 (0.005 - 0.0204) | 0.0031 (0.003 - 0.0329) | 0.3445 |
| Ni       | 0.04 (0.03 - 0.051)  | 0.036 (0.031 - 0.039) | 0.2413 |
| Se       | 0.18 (0.144 - 0.261) | 0.59 (0.55 - 0.612) | 0.0002* |
| Li       | 0.0033 (0.0028 -0.0037) | 0.0039 (0.0035 - 0.0043) | 0.0756 |

Data presented as median (25-75); MWU=\( p \) values as assessed by Mann-Whitney U-test; * - difference significant at \( p < 0.05 \)

Our previous studies on the effect of deficient diet on the element composition of liver were consistent with the data obtained in the present paper [7].
Table 3. Content of toxic elements in the liver of laboratory animals

| Elements | Experimental group | Control group | MWU  | p     |
|----------|--------------------|---------------|------|-------|
| Cd       | 0.059 (0.044 - 0.059) | 0.061 (0.053 - 0.064) | 0.1858 |       |
| Pb       | 0.072 (0.069 - 0.076) | 0.063 (0.061 - 0.065) | 0.0005* |       |
| Sr       | 0.084 (0.076 - 0.097) | 0.092 (0.088 - 0.096) | 0.2413 |       |
| Al       | 0.107 (0.093 - 0.158) | 0.075 (0.053 - 0.082) | 0.0008* |       |
| Sn       | 0.013 (0.011 - 0.015) | 0.0095 (0.0089 - 0.0107) | 0.0810 |       |
| Hg       | 0.0076 (0.0066 - 0.0087) | 0.0038 (0.0032 - 0.0081) | 0.1210 |       |

Data presented as median (25-75); MWU = p values as assessed by Mann-Whitney U-test; *difference significant at p<0.05

Similar dynamics in rat’s hair was noted (Table 4,5). The content of a number of essential elements declined significantly: copper 1.8-fold, iron 3.1-fold, manganese 2.1-fold, selenium 1.2-fold. The content of toxic elements (aluminum, lead) was enlarged. The zinc content in animal hair slightly increased, however, such data may indicate an increased elimination of this element against the background of diselementose [8].

Table 4. Content of essential elements in the hair of laboratory animals

| Elements | Experimental group | Control group | MWU  | p     |
|----------|--------------------|---------------|------|-------|
| Cr       | 0.432 (0.373 - 0.488) | 0.404 (0.322 - 0.415) | 0.0752 |       |
| Cu       | 5.18 (4.86 - 5.35) | 9.58 (9.43 - 9.79) | 0.0000* |       |
| Fe       | 14.31 (14.23 - 14.41) | 44.44 (26.53 - 56.3) | 0.0000* |       |
| I        | 0.142 (0.126 - 0.158) | 0.239 (0.141 - 0.24) | 0.0892 |       |
| As       | 0.554 (0.499 - 0.719) | 0.404 (0.154 - 0.512) | 0.1051 |       |
| Co       | 0.0029 (0.0026 - 0.0033) | 0.0027 (0.0013 - 0.004) | 0.6842 |       |
| Zn       | 165.54 (158.45 - 190.08) | 141.24 (138.67 - 149.16) | 0.0000* |       |
| Mn       | 0.527 (0.467 - 0.575) | 1.111 (1.034 - 1.205) | 0.0000* |       |
| Se       | 0.67 (0.61 - 0.76) | 0.836 (0.793 - 0.861) | 0.0038* |       |
| Li       | 0.035 (0.033 - 0.073) | 0.067 (0.0471 - 0.08) | 0.1230 |       |

Data presented as median (25-75); MWU = p values as assessed by Mann-Whitney U-test; *difference significant at p<0.05

Table 5. Content of toxic elements in the hair of laboratory animals (Me (Q1-Q3))

| Elements | Experimental group | Control group | MWU  | p     |
|----------|--------------------|---------------|------|-------|
| Cd       | 0.043 (0.033 - 0.053) | 0.055 (0.049 - 0.065) | 0.0524 |       |
| Pb       | 0.202 (0.136 - 0.26) | 0.08 (0.078 - 0.081) | 0.0015* |       |
| Sr       | 0.825 (0.69 - 1.72) | 1.62 (1.02 - 1.98) | 0.0630 |       |
| Al       | 5.16 (5.01 - 5.41) | 1.32 (1.23 - 1.36) | 0.0000* |       |
| Sn       | 0.152 (0.102 - 0.173) | 0.205 (0.141 - 0.225) | 0.1230 |       |
| Hg       | 0.035 (0.027 - 0.067) | 0.0257 (0.021 - 0.039) | 0.1051 |       |

Data presented as median (25-75); MWU = p values as assessed by Mann-Whitney U-test; *difference significant at p<0.05

Changes in animals element homeostasis in various environmental conditions and different nutrition supply remains a actual topic of many studies. E. Carpene et al. presented the results of the element composition of the liver and kidneys of brown and arctic hares, while noting an increase in the concentration of copper, iron, zinc and cadmium in animals living in areas treated with pesticides [9,10]. In addition, the same authors noted different concentrations of iron, copper and aluminum in the liver of domestic pigs and wild boars, and these differences are associated with nutritional status [9]. G.P. Danezis et al. used rabbit liver tissue to determine degree of accumulation of rare earth metals in animals...
depending on the region of their habitat. [11]. In Guyot, H. et al. An extensive study of the micronutrient availability of Belgian cattle herds was carried out. The most obvious changes being obtained for Zn, Cu Se and I. In addition, adequate dietary support of micronutrients correlated with cattle health [12].

The essential elements, such as copper, zinc and selenium, are part of the active centers of many enzymes and also involved in the immune response and signaling pathways. The lack of these elements leads to physiological, morphological and functional changes in the body [4]. These trace elements are part of the cellular system of antioxidant protection, carried out by a complexes of enzymes, in particular glutathione peroxidase (GPx). Antioxidant enzyme GPx catalyzes the reduction of lipid hydroperoxides and the reduction of hydrogen peroxide to water using glutathione (GSH) as a substrate, while Se plays an important role, acting as a cofactor in the active center of this enzyme [13]. Thus, the determination of the activity of this metal enzyme could be used as a biomarker of essential elements nutritional status (14). In our study, the activity of glutathione peroxidase in animals liver of the experimental group significantly decreased by 1.4 times, while the MDA content increased by 2 times (Table 6).

Table 6. Indicators of the antioxidant status of liver tissue

| Indices   | Experimental group | Control group | MWU | p  |
|-----------|--------------------|---------------|-----|----|
| GPX µg/ml | 103.9 (91.0-112.3) | 142.3 (129.6-152.4) | 0.0000 |    |
| MDA ng/ml | 6.2 (3.9-7.1)     | 3.0 (2.4-3.4)     | 0.0005 |    |

Data presented as median (25-75); MWU=p values as assessed by Mann-Whitney U-test; difference significant at p<0.05

J. Pareja-Carrera et al. considered that levels of Mn, Se and Cu in the blood of deer were markers of a deficient micronutrient nutrition [14]. These authors also noted a decrease in the activity of glutathione peroxidase against the background of low selenium content [14], which was consistent with the data obtained in our work.

4. Conclusion
In the present paper the effect of unbalanced nutrition on the liver and hair element composition and antioxidant defense system in the experiment was studied. A decrease in the content of copper, zinc, and selenium in liver tissue by 1.5, 1.14, and 3.2 times, respectively, was found. Significant increase of lead and aluminum content was established. A similar pattern was observed in the elemental composition of animals’ hair. GPx activity in the experimental rat’s liver decreased by a factor of 1.4 times. The accumulation of MDA was determined. Mineral-deficient diet leaded to increase of ALT, AST and total protein.

5. Acknowledgments
The studies were carried out in accordance with the research plan for 2019–2020 of the Federal Research Center of Biological Systems and Agrotechnologies RAS No. 0526-2019-0001.

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