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Change of Elemental Composition in Muscular Tissue and Hair under Food Stress

Svetlana Viktorovna Notova[1], Ainagul Habidullovna Duskaeva[2], Sergey Alexandrovich Miroshnikov[3], Galimzhan Kalihanovich Duskaev[4] and Elena Anatolyevna Sizova[5]

[1]Orenburg State University, Russia, 460018, Orenburg, 13 Pobedy Pr. and All-Russian Research Institute of Beef Cattle Breeding, Russia, 460000, Orenburg, 29, 9 Yanvarya St.;
[2]Orenburg State Univeristy, Russia 460018, Orenburg, 29, 9 Yanvarya St.
[3]All-Russian Research Institute of Beef Cattle Breeding, Russia, 460000, Orenburg, 29, 9 Yanvarya St.;
[4]All-Russian Research Institute of Beef Cattle Breeding, Russia, 460000, Orenburg, 29, 9 Yanvarya St.;
[5]Orenburg State University, Russia, 460018, Orenburg 13 Pobedy Pr. and All-Russian Research Institute of Beef Cattle Breeding, Russia, 460000, Orenburg, 29, 9 Yanvarya St.;

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Article presents the results of research on composition of elements in skeletal muscles and hair of lab rats given the influence of food factors. Research was conducted on male Wistar rats from 2-month age (n=51). Within the record period animals were divided into 3 groups depending on the consumed diet. Animals of the control group (Ê) were fed with full-value semisynthetic diet within 60 days (basic diet (BD). Animals of the I test group consumed semisynthetic diet I (SD I), consisting of mixture of major feed (50%), instant food (IF) (50%) and water, animals of the II group – semisynthetic diet II (SD II), consisting of mixture of major feed, instant food and carbonated soft drink (each 50%). Elements of the researched biological substrates and food products were analyzed by atomic emission and mass spectroscopy with inductive-connected argon plasma using devices Optima 2000 DV and ELAN 9000 (PerkinElmer, USA). It was established that in the experimental conditions the elemental composition of hair changed in accordance with changes in pool of the most elements in muscular tissue. It was found out that concentrations of phosphorus, potassium, sodium, iron and cobalt increased in hair (1.1 - 8.7 times) and calcium, zinc and manganese increased (1.3 – 1.4 times) with decrease in common pool of elements in muscular tissue.

Key words: macroelements; microelements, food stress, rats, skeletal muscles, hair.

Nutrient sources can provide important minerals that are necessary for prevention and elimination of mineral deficiency. These sources are cost-effective, safe, available and convenient. At the same time (Salau and Hasan, 2014) it was proven in the course of study that the food can be an effective source of elements when you choose appropriate products.

* To whom all correspondence should be addressed.

The priority elements (iodine, iron and zinc) were listed in the addition to the previously selected elements (selenium, copper, calcium and magnesium) in various joint researches and publications WHO / FAO (WHO, 1996; WHO / UNU / UNICEF, 2001; Yip, 2001; FAO / WHO, 2004). Different supplements can be the sources of these essential elements. However, sometimes supplements have some side effects or toxicity when they are administered in excess or not tolerated by the human body. In this regard, there is a need to study the influence of feeding (certain foods) on the elemental composition of the body.

The mineral deficiency and diseases caused by it constitute approximately 11% of the total global disease burden and take the main position in health care (Lancet, 2008). Countries can lose up to 3% of GDP per year because of deficiency of iron, iodine, zinc (WHO, 1996).

The most common diseases are related to deficiency of iron (Sagin *et al*, 2002; Foo *et al*, 2004; Norhaizan and Faizadatul, 2009), calcium, phosphorus, magnesium (Lai, *et al*, 2008; Norhaizan and Faizadatul, 2009).

It is necessary to take into account that not only parents can be influenced by nutrition, but also a similar predisposition may arise in the offspring (Ong and Muhlhausle, 2011; Bayol *et al*, 2009). The influence of nutrition and active consumption of carbonated soft drinks raise concerns about the health (Milei *et al*., 2011; Nseir *et al*., 2010; Malik *et al*., 2006).

It is well known (Skalny, 2003) that it is possible to study the element deficiency based on the elemental composition of hair (wool) (www.microelements.ru). Element analysis of human hair is used when cancer (Czerny *et al*., 2014), pathologies caused by intoxication by heavy metals (Grabeklis *et al*., 2011); metabolic syndromes (Park *et al*., 2009); thyroid disease (Momèiloviæ *et al*., 2014) are diagnosed.

At the same time, there are not enough data on the comparison of the elemental composition of hair and body tissues. Thus, a significant increase of K, Na, P, Cr, Mn, Ni, Si, Sr was observed in the hair of laboratory animals regardless of their diet and during physical exercises. At the same time, the concentration of Ca, Cu, Fe, V, Pb and Al decreased (Alidzhanova *et al*, 2009). Elemental composition of body, hair and thyroid changed after the artificial change in thyroid status (Notova *et al*, 2014).

The objective of this research was to study the influence of food stress on elemental composition of muscular tissue and hair of lab animals.

MATERIALS AND METHODS

Experimental research was carried out in experimental biological clinic (vivarium) of the Trace Element Institute of the Orenburg State University. Animal experiments were carried out in accordance with 1985 International Guiding Principles for Biomedical Research Involving Animals, with permission of the Committee on the Ethics of the Orenburg State University (Protocol # 12 as of 22.01.2007).

Animals and rations

Research was carried on male Wistar rats starting from the age of 2 months (n=45). During the record period the animals were divided into 3 groups. The first experimental group (I) consumed a semisynthetic diet I (SD I) consisting of a mixture of major feed (50%), instant food (IF) (50%) and water; the second group (II) - a semisynthetic diet II (SD II) consisting of a mixture of major feed, IF and carbonated soft drinks (50%). The control group (C) received balanced semisynthetic diet (BD) containing 58% corn starch, 25% casein, 5% unrefined sunflower oil, 5% of lard, 4% salt mixture, 1% vitamin mixture, 2% of microcrystalline cellulose. IF mixture consisted of noodles, hot dog and porridge in equal proportion. Nutritional value of instant noodles (per 100 g): protein – 4.8 g, carbohydrates - 30 g, fat - 2 g, calories – 270 kcal; porridge: protein – 10.0 g, fat – 5.0 g, carbohydrates 70.0 g, calories – 350 kcal; hot dog: protein – 10.6 g, fat - 15.0 g, carbohydrates - 27.5 g, calories – 247 kcal.

Contents of carbonated soft drinks: purified carbonated water, sugar, color, phosphoric acid, flavor, caffeine (carbohydrate - 10.6 g, sodium - 10.0 mg, potassium - 1.0 mg, calcium 4.0 mg, magnesium - 1.0 mg, phosphorus - 15 mg).

The products were purchased in a retail network. According to the results of laboratory tests (test center, All-Russian Research Institute of Beef Cattle Breeding, accreditation certificate No.ROSS RU 0001.21 PF 59) they met the requirements for food products.

Biological substrates

The following biological substrates were used to study elemental status: instant food (general diet, semisynthetic diet), hair (sample collection was taken from side and upper body in the end of research), muscular tissue (after decapitation - skeletal musculature was separated to get an average sample of muscle tissue, then it was ground, homogenized and dried to constant weight). Three samples were taken from each animal; all in all, 135 measurements were recorded (n_i).

Analysis

Duration of the experiment was 60 days. The animals were weighed weekly before feeding and watering. Element analysis of the researched bio-substrates and feed of animals (major feed, IF) was carried out in the lab of ANO "Centre for Biotic Medicine", Moscow, Russia (accreditation certificate ROSS RU.0001. 513118 as of May 29, 2003; Registration Certificate of ISO 9001:2000, Number 4017-5.04.06) with a atomic emission and mass spectrometry with inductively-coupled argon plasma using Optima 2000 DV and ELAN 9000 equipment (PerkinElmer, USA).

Statistical Analysis

Statistical analysis using Student t-test was performed with IBM program "SPSS Statistics Version 20", calculation of mean value (M), mean square deviation (σ), standard deviation of error (m). Level of significance was considered probably true at $p<0.05$. Student t-test (Platonov, 2000) and the Wilcoxon test were used for statistical analysis.

RESULTS AND DISCUSSION

Analysis of mineral composition of major and semisynthetic diets used in the experiment attests that the content of chemical elements in them is significantly different (Table 1).

So, semisynthetic diet differed by significantly higher (36.7 times) content of Na and K (4.1 times), lower level of Mg (1.5 times) against a background of practically constant level of Ca and Ð. Among essential and conventionally essential elements in semisynthetic diet lower content of Cu (1,3 times), Fe (1.5 times), Co (1.8 times), Zn (1.6 times), Mn (2 times) and higher levels of Cr (6.6 times), I (3 times), V (1.9 times) was recorded. Higher content of such toxic elements

Table 1. Content of chemical elements in diets of the experimental animals, mg/kg

Element	General diet (GD)	(SD I, II)	Element	General diet(GD)	(SD I, II)
Ca	1390	1486	Co	0.31	0.17
K	551	2257.5	Zn	81.5	51.7
Mg	2179	1468.5	Mn	136.2	67.8
Na	688	25263	V	0.38	0.73
P	7501	6040	Ni	1.5	1.14
Cr	0.35	2.3	Se	0.51	0.5
Cu	8.2	6.3	Cd	0.09	0.19
Fe	130	88.2	Pb	0.07	0.09
I	0.31	0.96	Sr	28.5	18.5
As	0.21	0.39	Al	42.4	25.9

Table 2. Average values of macroelement content in skeletal muscles of the experimental animals, mg/kg

Statistical parameters	Ca	P	K	Mg	Na
Control (GD) n=15					
M	159	1042.1	302	224	623
m	1726.7	906	429	39.0	111.7
I group (SD I) n=15					
M	337.9	2353.6	845.6	604.2	1977.5
m	2918	936	325	26	126
p_K	0.02	0.04	0.03	0.05	0.04
II group (SD II) n=15					
M	362.6	2374.7	785.2	604.5	2287.3
m	3702	1507	338	52	204
$p_{k;\,I}$	0.05; 0.08	0.04; 0.68	0.03; 0.22	0.04; 0.89	0.02; 0.08

Note hereinafter 0.02-0.05 - statistically significant variations (p_K – in comparison with control group; p_I – in comparison with the I experimental group) as Cd (2 times) and Pb (1.3 times) was recorded in semisynthetic diet.

Significant changes in the elemental status of muscular tissue of experimental animals were demonstrated during the assessment of the elemental composition of body tissues (Table 2, 3).

Addition to the basic diet resulted in a significant increase of the following macronutrients in muscular tissue of experimental animals: calcium (2 and 2.3 times), phosphorus (2.3 and 2.7 times), potassium (2.8 and 2.6 times), magnesium (3.2 and 2.7 times) ($P < 0.05$).

Influence of the studied food mixtures on the elemental composition of skeletal muscles of rats has not yet been studied, that is why the obtained results could not be compared with the published data. At the same time it was previously noted (Prescha *et al*., 2014) that concentration of calcium and iron in skeletal muscles of rats decreased after the addition of cellulose and pectin to the diets containing chromium. Low calcium levels can adversely affect muscle contractility (Moe, 2005). Calcium increased in bones and liver after the increase of calcium carbonate in the diet of rats by 0.5-1.25% (Shackelford *et al*., 1994). Moreover, concentration of phosphorus, zinc and magnesium increased in liver, and iron concentration decreased. In this study, the difference in calcium content between control and experimental groups was 0.7%.

Sodium content in I and II experimental groups increased significantly, its level exceeded the control value 3.2 and 3.7 times, respectively. It

Table 3. Average values of essential elements content in skeletal muscles of the experimental animals, mg/kg

Statistical parameters	Zn	Mn	Cu	Fe	Co	Se	I
Control (GD) n=15							
M	17.9	0.31	0.93	24.0	0.005	0.31	0.03
m	7.1	0.12	0.12	3.2	0.005	0.11	0.023
I group (SD I) n=15							
M	14.2	0.11	2.4	34.1	0.01	0.23	0.02
m	1.5	0.015	0.026	5.2	0.01	0.061	0.064
p_K	0.05	0.04	0.05	0.03	0.04	0.02	0.10
II group (SD II) n=15							
M	15.7	0.14	2.5	23.7	0.01	0.25	0.03
m	6.5	0.015	0.144	4.5	0.005	0.051	0.04
$p_{K; I}$	0.02;	0.04;	0.04;	0.89;	0.03;	0.04;	0.14;
	0.02	0.05	0.13	0.10	0.45	0.11	0.08

Table 4. Average values of macroelement content in wool of the experimental animals, mg/kg

Statistical parameters	Ca	P	K	Mg	Na
Control (GD) n=15					
M	498	285	1656	163	371
m	19.8	29.3	53.3	9.2	39
I group (SD I) n=15					
M	468	533	9143	150	2975
m	31.6	36.4	331	11.4	172
p_K	0.04	0.04	0.04	0.22	0.04
II group (SD II) n=15					
M	328	556	9286	151	3275
m	41.5	72	506	12.4	1003
p_{KI}	0.04;	0.04;	0.04;	0.23;	0.04;
	0.04	0.46	0.34	0.89	0.46

can be explained, first of all, by a high content of this element in synthetic diets and in the carbonated beverage.

It is known from literature sources that carbonated mineral water, rich with sodium promotes the reduction of risk of cardiovascular diseases and metabolic syndrome (Schoppen *et al*., 2004).

When 50% of drinking water was replaced by the carbonated beverage in the diet, it had no significant influence on the content of macroelements in muscular tissue. At the same time, it is observed (Lo *et al*, 2008) that the consumption of carbonated sugary drinks in greater volume may increase the risk of bone fractures, caries development and promote obesity.

Long-term consumption of IF, and IF with carbonated beverages has resulted in reduction of the following essential elements in muscle tissues of body: zinc by 12.2 - 20.6% ($P < 0.05$), Mn by 48.4 - 64% ($P < 0.05$), selenium by 6.4 - 25% ($P < 0.05$) as compared with the control group (Table 3). In previous studies (Rossowska and Nakamoto, 1993) antagonistic effects of calcium and zinc in muscle tissue were observed, they were characterized by calcium decrease in homogenate and zinc increase. In our studies with respect to zinc, we observed the opposite effect - an increase of calcium and reduction of zinc. The reduction of selenium causes no decline in the quality of protein, but it is required to maintain an optimal rate of muscle cell differentiation (Ueda *et al*., 1999).

Differences in zinc concentration were registered in group I and II ($p<0.05$)

Concentration of copper and cobalt increased 2.5-2.9 times and 1.3-2.2 times, respectively, in muscle tissue of experimental groups compared with the control. The level of iodine remained virtually the same in all groups. As we can see, despite the relatively low content of copper in the diet of experimental groups, its significant accumulation in muscular tissue was registered. It can be assumed that this tissue is vulnerable to the action of this element and other tissues of the body (Kumar *et al*, 2015). Furthermore, it is known that high concentrations of cadmium in the diet can induce oxidative stress by increasing malonic aldehyde, which in its turn causes a reduction of copper in organs and tissues (Erdem *et al*, 2015). In the performed studies, copper concentration increased in muscular tissue despite the high concentration of cadmium in the experimental diets.

Researches (El-Seweidy *et al*., 2008) demonstrate that feeding with IF causes zinc reduction in tissues of rats that is connected with antioxidative processes (Esen Gursel and Tekeli. 2009). At the same time, iron concentration increases in the same tissues. A similar change was observed in our study.

After IF was added to the basic diet, the following macroelements increased significantly in hair of experimental animals: phosphorus increased 1.9 and 2.0 times, potassium - 5.5 and 5.6 times (Table 4).

Table 5. Average values of essential elements content in skeletal muscles of the experimental animals, mg/kg

Statistical parameters	Zn	Mn	Cu	Fe	Co	Se	I
Control (GD) n=15							
M	148	1.1	12.1	14	0.01	0.9	0.2
m	6.3	0.2	0.9	0.8	0.001	0.1	0.06
I group (SD I) n=15							
M	199	6	11	62	0.04	0.2	0.4
m	8.1	4.3	0.4	14	0.01	0.01	0.7
p_K	0.04	0.04	0.34	0.04	0.04	0.04	0.04
II group (SD II) n=15							
M	187	1.7	9.4	26.4	0.02	0.1	0.35
m	11	0.2	0.6	4	0.004	0.005	0.1
p_{KI}	0.04; 0.08	0.04; 0.04	0.23; 0.04	0.04; 0.04	0.04; 0.14	0.04; 0.22	0.04; 0.08

The increase of sodium was the highest; its level exceeded the control values 8.0 and 8.8 times in the I and II experimental groups respectively.

At the same time, decrease of calcium by 6-34% (P <0.05) and magnesium was observed. Previous studies demonstrate that significant increase of potassium, sodium, phosphorus and decrease of calcium and iron were observed in the hair of laboratory animals, regardless of the diet (Alidzhanova *et al*, 2009).

Consumption of IF, including its mixture with carbonated beverages caused the increase of the following essential microelements in hair: zinc 1.3 and 1.2 times (Ð<0.05), manganese 5.4 and 1.5 times (Ð<0.05), iron 4.4 and 1.9 times (Ð<0.05), cobalt 4 and 2 times, iodine 2 and 1.7 times in comparison with the control group (Table 5). Studying the influence of cadmium on organism of rats (Mou, *et al*, 2004) it has was also found that cadmium in the diet causes a loss of zinc. In our experiment, the level of cadmium in experimental diets were higher than in the control group, and since, according to some authors (Skalny A.V., Bykov A.T., 2003) the content of chemical elements in the appendages of skin reflects the trace element status of the body in whole and it is an integral indicator of mineral metabolism, increasing zinc in hair is its loss.

Among the experimental groups there were differences in the concentration of manganese, copper, iron (P <0.05). At the same time there was a decrease in copper concentration in the experimental groups by 9 - 22% and selenium by 78 - 89%. When the effect of fatty acids of different quality as part of the diet of rats was studied (de Castro Barra *et al*, 2015), a decrease of calcium and selenium in liver tissue was registered.

CONCLUSION

Thus, under the experimental conditions the elemental composition of animal hair changed in accordance with the pool size of the most elements in the muscular tissue. An increase in concentrations of phosphorus, potassium, sodium, iron and cobalt (1.1-8.7 times) was registered together with the increase of calcium, zinc, manganese (1.3 - 1.4 times) and decrease of the total pool in muscular tissue.

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