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ORIGINAL PAPER

SELENIUM STATUS IN SERUM AND TESTICLES OF MALE RATS FED A DIET CONTAINING SUCROSE AND SUPPLEMENTED WITH CHOSEN B GROUP VITAMINS*

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Abstract

The aim of this research was to evaluate, on an animal model, the influence of a change in a diet composition and dietary supplementation (complementary or excessive) with vitamins B_1, B_2, B_6 and niacin on the selenium content and antioxidant enzymatic defence of the testicular tissue. Forty-four male Wistar rats were divided into four equal groups; the control group (I) was fed Basic Diet (BD), while groups II-IV were given Modified Diet (MD), in which 83.5% of wheat was replaced with wheat flour and 50% of maize was replaced with sucrose. Animals from groups I-II received only water to drink, while group III was given an aqueous solution of vitamins supplementing the deficiency created by the dietary modification (MD + Adequate Supplementation: B1-0.94, B2-0.48, B6-0.5, niacin-1.9 mg); group IV received a solution in order to supplement both the deficiency resulting from the change in a diet and the recommended prophylactic dose of vitamins (MD + Excessive Supplementation: B1-3.1, B2-2.3, B6-2.4, niacin-6.65 mg). Blood serum was examined to determine glucose and selenium concentrations. The activity of GPx, CAT, SOD, GST and selenium content were measured in the testicular tissue. The change in a diet composition made by replacing full grains with wheat flour and sucrose and dietary supplementation with B_1, B_2, B_6 vitamins and niacin with varying amounts of feed ($p \leq 0.01$), selenium ($p \leq 0.01$) and liquids ($p \leq 0.01$) did not significantly influence the body gain of the animals, glucose concentration in blood or the selenium content and activity of antioxidant defence enzymes in testicles. A significant ($p \leq 0.05$) decrease in the selenium concentration in the blood of animals supplemented excessive amounts of the above vitamins, with a lack of significant differences in the selenium content in testicles, co-occurred with the highest activity of antioxidant enzymes.

Keywords: testicular tissue, blood, antioxidant defence, Se, vitamin.

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INTRODUCTION

The contemporary human diet is characterized by a significant contribution of processed products, purified and rich in simple sugars, as well as reduced levels of B-group vitamins and many other important trace elements, e.g. selenium. Moreover, a growing intake of monosaccharides in food entails an increased demand for B-group vitamins, necessary *inter alia* for hydrocarbon metabolism. These vitamins are also a key co-factor in response pathways for the regulation of transport and delivery of Se to many tissues (Combs et al. 2011). In the long run, having a diet rich in sources of simple sugars results in hyperglycaemia, which induces autoxidation of glucose, glycation of proteins and activation of polyol metabolism. Consequently, more numerous reactive oxygen species (ROS) are formed and oxidative stress is induced (Sies et al. 2005). A change in the redox equilibrium can lead to many undesired changes in cellular metabolism (Osawa et al. 2005). Cells contain a multi-component system of antioxidation defence, which consists of specialized enzymes and low molecular antioxidants like vitamins, peptides and trace elements. Vitamins B_1, B_2, B_6 and niacin have been found to influence the body's antioxidant status, as they exert anti- or pro-oxidant activity, depending on the applied dose and duration of supplementation (Higashi-Okai et al. 2006, Hsu et al. 2015). Vitamins also have an indirect influence on the activity of some enzymes that belong to the antioxidant defence system, e.g. glutathione peroxidase (GPx). This peroxidase, which is a selenoprotein, contains a selenium atom conjugated with cysteine (SeCys) in its active center. Earlier findings in rats showed that vitamin B6 is involved in the utilization of Se for GPx synthesis (Beilstein et al. 1989, Yin et al. 1991). The influence of an unbalanced diet rich in mono-sugars varies, depending on the tissues and organs affected. Hyperglycaemia can especially damage the testicular function and lower fertility. The process of spermatogenesis also requires a constant supply of high amounts of energy, which can be a reason for abundant ROS formation. Prolonged exposure to a high concentration of reactive oxygen species can result in the depletion of endo- and exogenous antioxidants, and can destroy the cellular redox homeostasis. In mice, extended oxidative stress, which can arise from Se deficiency, reduced the activity of gluthatione peroxidases (GPx) and phospholipid hydroperoxide glutathione peroxidase (PHGPx), which influenced spermatogenesis and delayed the sperm maturation process (Kaur, Bansal 2004).

The aim of this research was to evaluate the influence of a change in a diet and type of dietary supplementation with vitamins B_1, B_2, B_6 and niacin on the selenium status and enzymatic antioxidant defence activity of testicular tissues.

MATERIAL AND METHODS

The research was conducted on 44 male Wistar rats, 5 months old and with initial weight of 428.7 ± 50.9 g, which were placed in individual cages. The animals were divided into four equinumerous feeding groups (11 animals in each), which were fed *ad libitum* with granulated feed produced by the Morawski Feed Production Plant in Kcynia (Poland). For six weeks, the experimental group I received Basic Diet (Labofeed B) that contained, *inter alia*, whole grains of wheat and maize. Groups II – IV received Modified Diet (MD), in which 83.5% of wheat from the basic feed was substituted by wheat flour (type 500), and 50% of maize was replaced by sucrose (Table 1).

Animals from groups I and II received settled tap water to drink, while animals from group III and IV, in periods of increased activity, received 25 cm^3 of aqueous solution of synthetic vitamins: B_1 (*Thiamini hydrochloridum*), B_2 (*Riboflavinum*), B_6 (*Pyridoxinum hydrochloricum*), niacin (*Nicotinamidum*), which came from widely available pharmaceuticals. Animals from group III (MD+AS, Modified Diet + Adequate Supplementation) received the following amounts of vitamins in 25 ml of aqueous solution (freely available): B_1 – 0.94 mg, B_2 – 0.48 mg, B_6 – 0.5 mg, niacin – 1.9 mg. Animals from group IV (MD+ES, Modified Diet + Excessive Supplementation) received B_1 – 3.1 mg, B_2 – 2.3 mg, B_6 – 2.4 mg and niacin 6.65 mg. Vitamins were administered in the amounts individually calculated for each supplementation method, so that supplementation in group III (MD+AS) would make up for the deficiency created by the changed dietary ingredients. Supplementation in group IV (MD+ES) addressed both the deficiency caused by differences in the vitamin content between Basic Diet and Modified Diet and the recommended prophylactic dose of these vitamins, so that the vitamin dose exceeded RDA standards. The total vitamin dose administered in group IV was approximately 2-4 times as high as the RDA (B_1 – 2.3 times, B_2 – 3.8 times, B_6 – 3.8 times, niacin – 2.5 times), which to some extent imitated consequences of synthetic vitamin supplementation in humans. However, these were not toxic amounts for animals (EFSA, 2006). Having drunk the vitamin solution, the animals additionally received some clear, settled tap water. Feed Conversion Ratio (FCR) was calculated according to the formula: FCR % = total feed intake (g)/final body weight (g) (Johnson, Gee 1986). The research was conducted with the approval from the Local Commission of Ethics (no 8/2015).

Determination of the chemical and nutritional composition of feed

The chemical composition of the prepared feeds was determined (Table 1), and the analyses included: total Kjeldahl nitrogen using a Kjeltec 2100 system from Foss Tecator (Hillerød, Denmark), converted into the protein content; crude fat with the Soxhlet method using a Soxtec HT6 system from Foss Tecator (Hillerød, Denmark); dry weight with the gravimetric method

Table 1

Ingredients and nutrient composition in 100 g of the diets

Specification	Basic diet (BD)	Modified diet (MD)
	Ingredients	
Wheat grain (g)	36.4	6.0
Maize grain (g)	20.0	10.0
Wheat bran (g)	20.0	20.0
Dry whey (g)	3.0	3.0
NaCl (g)	0.27	0.27
Soyabean meal 48% (g)*	17.0	17.0
Fodder chalk (g)**	2.0	2.0
Phosphate 1-CA (g)***	0.27	0.27
Mineral and vitamin mix (g)#	0.8	0.8
Wheat flour type 500 (Gg)	-	30.4
Sucrose (g)	-	10.0
	Nutrient composition	
Total protein (g)	19.1	17.8
Crude fat (g)	3.08	4.18
Carbohydrates (g)	62.3	63.8
Crude fibre (g)	2.91	2.73
Dry weight (g)	91.4	91.2
Total ash (g)	6.94	6.47
Thiamine (mg)	2.5	0.62
Riboflavin (mg)	2.1	1.14
Pyridoxine (mg)	2.35	1.35
Niacin (mg)	8.6	4.8
Selenium (mg)	0.000255	0.000204
Gross energy (kJ g^{-1})	16.6	16.9
Metabolizable energy (kJ g^{-1})	14.8	15.2

Explanations:
* soyabean meal 48%: extracted. content 48% crude protein and 7% fibre;
** fodder chalk: content per kg: (g) Ca 350, Mg 3.20; mg: Na 10.00, P 15.00;
*** phosphate 1-CA: additive contains min. 22% P and 15% Ca;
# vitamin and mineral Premix LRM content per kg: (IU) A 1500000, vit. D_3 100000; mg: vit. E 8000; vit. K 300, vit. B_1 1200, vit. B_2 1200, vit. B_6 1000, vit. B_{12} 8, Se 100, Fe 16000, Mn 4500, Zn 6000, Cu 1300, I 100, Co 200

by drying a sample at 105°C for 12 h in a SUP-4M laboratory dryer (Wawa-Med, Warsaw Poland); and ash with the gravimetric method by burning a sample in a FCE 7SHM muffle furnace Czylok (Jastrzębie Zdrój, Poland) at 550°C for 10 h. The National Feed Laboratory in Szczecin, National Rese-

arch Institute of Animal Production (Cracow, Poland) determined the content of the following components: vitamins B_1, B_2, B_6 and niacin, by the HPLC method on an Agilent 1200SL system (Agilent Technologies, Santa Clara, USA), crude fiber with the gravimetric method (PB-02/PS) on an ANKOM 220 Fiber Analyzer (ANKOM Technology, Macedon, USA) and iron with atomic emission spectroscopy using an ICP-AES (Horiba Jobin Yvan, Edison, USA). The content of carbohydrates was calculated from the difference between the dry weight content and the aggregated content of other solid ingredients. Gross energy and metabolic energy were calculated with the use of widely used energy equivalents (FAO. Food Energy 2003).

Glucose level assay in blood serum

The animals were fasted 12 h before the end of the experiment. Next, they were anaesthetized with Ketanest (Pfizer Ireland Pharmaceuticals, Dublin, Ireland) and blood samples were collected from their hearts. Once the coagulate was centrifuged, the resultant blood serum was examined for glucose concentration (GL) – by the Trinder's enzymatic colorimetric method (Trinder 1969), using BioSystems bio-tests (Barcelona, Spain) and a Marcel Media Bio spectrophotometer (Marcel, Zielonka, Poland).

Total selenium content in serum and testicles assay

The selenium content in analyzed tissues was determined as described earlier by Grzebuła and Witkowski (1977). To determine the selenium content in serum, a sample was wet mineralized in concentrated HNO_3 and $HClO_4$, and later using 9% HCl. Namely, samples of testicular tissues (~1 g) were digested in HNO_3 at 230°C for 180 min and in $HClO_4$ at 310°C for 20 min. In the next step, HCl (9%) was added to the mineralized samples to reduce selenates (Se VI) to selenites (Se IV). Then, selenium was derivatized with 2,3-diaminonaphtalene under conditions of controlled pH (pH 1–2) with the formation of a selenodiazole complex. This complex was extracted into cyclohexane. EDTA and hydroxylamine hydrochlorine were used as masking agents. Finally, the selenium content was determined spectrofluorometrically using a Shimadzu RF-5001 PC spectrofluorophotometer at an emission wavelength of 518 nm and an excitation wavelength of 378 nm. In parallel to the experimental samples, blank samples (reagent) were also measured. The accuracy of the analytical method was verified based on reference material BCR–185R from the European Commission Joint Research Centre Institute for Reference Materials and Measurements (LGC Standards GmbH, Wesel, Germany). The Se concentration was 97.4±1.5% of the reference value.

Antioxidant enzymes assay in testicles tissue

Samples of testicular tissue (≈ 0.1 g) were homogenized in a mechanical homogenizer on ice with 10 volumes of 50 mmol dm^{-3} phosphate buffer pH

7.1, with 1 mmol dm^{-3} EDTA. Homogenates were centrifuged for 15 min at 21 000 g-force at 4°C. Supernatants were collected and stored until analysis at -80°C.

Glutathione peroxidase (GPx) activity was assayed according to Pagila and Valentine (1967) using a RANSEL kit (Randox Laboratories Ltd, Crumlin, United Kingdom), adopted to a microplate reader scale. For determination of the substrate activity, cumene hydroperoxide was used. The activity was defined as an amount of GPx that oxidizes 1 nanomole of NADPH per minute per milligram of protein at 37°C and pH 7.2.

Superoxide dismutase (SOD) activity was determined by its ability to inhibit the auto-oxidation of pyrogallol, using the method described by Marklund and Marklund (1974) and modified to the microplate assay. A volume of 0.003 cm^3 10 μmol dm^{-3} bovine erythrocyte catalase solution in 50 mM TrisCl, pH 8.2 and 0.003 cm^3 of 24 mmol dm^{-3} (in 10 mmol dm^{-3} HCl) pyrogallol solution was added to 0.270 cm^3 buffer 50 mmol dm^{-3} TrisCl, pH 8.2, 1 mmol dm^{-3} EDTA. Subsequently, 0.006 cm^3 of an appropriately diluted sample was added to the reaction mixture. An increase in absorbance at 420 nm was monitored for 2 min at 15-second intervals, after a 30-second lag phase. One unit of SOD reduces by 50 percent the auto-oxidation of pyrogallol at pH 8.5 at 25°C. SOD activity was expressed as an amount of SOD arbitrary units per mg protein.

Catalase (CAT) activity was determined according to Li and Schellhorn (2007). Ten microliters of a sample, diluted in the assay buffer (50 mmol dm^{-3} – phosphate buffer pH 7.0), were added to 0.25 cm^3 5 mmol dm^{-3} solution of hydrogen peroxide in the assay buffer, and brought to 25°C on a UV-transparent microtiter plate. Immediately after adding the sample, a decrease in absorbance at 240 nm was monitored for 2 minutes, at 15-second intervals. One unit of catalase decomposes one micromole of hydrogen peroxide to oxygen and water per minute at 25°C at pH 7.0.

Glutathione S-transferase (GST) was determined according to Habig et al. (1974). Twenty microliters of a sample diluted in the assay buffer (100 mmol dm^{-3} phosphate buffer, 2 mmol dm^{-3} EDTA, pH 6.5) were transferred to 0.18 cm^3 of the assay buffer with 1 mmol dm^{-3} reduced glutathione and 2 mmol dm^{-3} 1-chloro-2,4-dinitrobenzene (CDNB). After 1 min of lag time, an increase in absorbance was monitored for 4 min, at 15-second intervals, at 25°C. One unit of the GST specific activity is defined as the amount of enzyme that catalyzes the formation of 1 micromole of CDNB-glutathione conjugates per minute per milligram of protein.

The total protein content in tissue homogenates was assayed using the Bradford method with bovine serum albumin as a standard (Breadford 1974).

Statistical analysis

The results, after checking normality of distribution with the Shapiro--Wilk test and uniformity of variation with the Laven's test, were subjected to statistical calculations (at the significance level $p \leq 0.05$ $p \leq 0.01$) using Statistica 9.0® computer program (Statsoft, Tulsa, Ok, the USA) with the use of univariate variation analysis by the Tukey test-1. For parameters at which normal distribution was ascertained, the results were verified by the Kruskal-Wallis nonparametric test and median test (Me).

RESULTS AND DISCUSSION

The demand of an animal's organism for an indispensable amount of selenium is strictly connected to its way of life, development cycle or sex (Combs, Gerald 2015). Another pivotal factor is a diet, which should ensure appropriate amounts of essential microelements and trace elements. However, a diet may also contain components which have a negative influence on an organism, and which may result in disturbing the redox balance (Ravneet et al. 2007). The analyzed variant diets supplied to the tested rats had a diverse impact on the quantity of consumed feed, which effected, *alebit* to a varied extent, changes in selenium concentrations in the analyzed tissues. The results show that a change in the diet composition (MD) and dietary supplementation (MD+AS; MD+ES) influenced significantly the amount of feed consumed by the animals (Table 2). A significant influence of MD on higher feed consumption, both in absolute terms (Me 1055.3 g) and per 100 g of body mass (Me 250.8 g), was ascertained. Incorporating additional vitamins into the diet in amounts not exceeding the accepted standards (MD+AS) did not correct this influence. However, supplementation of the modified diet with vitamins in doses exceeding the acknowledged standards (MD+ES) resulted in a significant decrease of the feed ingestion by animals (Me 890.1; 219.9 g) to the level observed in the control group (BD) (Me 904.1; 221.9 g). A similar influence of a diet containing sucrose on increasing feed intake by animals was noticed by Chepulis and de Castro et al. (2007, 2008). The value of the feed conversion ratio (FCR) showed that the animals from group MD (2.51%) took the best advantage of the feed to achieve the highest body mass, whereas animals from MD+ES (2.20%) were the worst at it. The different amounts of consumed feed among the groups of animals under research translated into significant differences in energy consumption expressed per 100 g of body mass (Table 2). The highest consumption occurred in the group fed the modified diet (4238.4 kJ) and the lowest one was among the animals fed with feed supplemented excessively (3715.6 kJ). In this group of animals, the significantly lowest consumption of selenium was also observed (0.0045 g), compared to the animals fed with the control feed, which was due to a 20% lower amount of this element in the modified

Table 2

Effects of the diet type and B-group vitamin supplementation on feed intake and body weight gain in Wistar rats ($\bar{x}$ ± SD, Me)

Trait	BD	MD	MD+AS	MD+ES
Initial body weight (g)	393.1 ± 32.7 (406.3)	394.4 ± 29.8 (384.4)	391.3 ± 29.9 (388.6)	390.8 ± 19.9 (394.9)
Final body weight (g)	416.8 ± 34.0 (420.3)	423.3 ± 38.4 (416.2)	416.7 ± 32.7 (410.6)	414.8 ± 15.7 (420.2)
Feed intake (g)	918.5^{B} ± 39.4 (904.1)	1044.2^{A} ± 42.5 (1055.3)	998.0^{A} ± 62.1 (987.4)	907.8^{B} ± 31.7 (890.1)
Feed consumption. (g 100 g^{-1} body weight)	221.3^{Bb} ± 14.9 (221.9)	248.0^{A} ± 18.4 (250.8)	240.0^{Aa} ± 11.8 (240.5)	218.8^{B} ± 18.3 (219.9)
FCR (%)	2.21^{Bb} ± 0.2 (2.23)	2.48^{A} ± 0.2 (2.51)	2.40^{Aa} ± 0.1 (2.41)	2.19^{B} ± 0.1 (2.20)
Energy intake (kJ 100 g^{-1} body weight)	3673.9^{Bb} ± 247.0 (3700.0)	4191.2^{A} ± 310.6 (4238.4)	4056.4^{Aa} ± 198.8 (4064.6)	3698.0^{B} ± 140.2 (3715.6)
Body weight gain (g)	25.4 ± 13.7 (24.4)	28.2 ± 20.1 (26.2)	25.5 ± 8.4 (25.9)	28.6 ± 5.8 (29.4)
Body weight gain (g 100 g^{-1} feed)	2.77± 1.5 (2.52)	2.71 ± 1.8 (2.46)	2.55± 0.8 (2.59)	3.15 ± 0.6 (3.21)
Selenium intake (g 100 g^{-1} body weight)	0.056^{A} ± 0.004 (0.057)	0.051^{B} ± 0.004 (0.051)	0.049^{B} ± 0.003 (0.049)	0.045^{C} ± 0.002 (0.045)
Liquids intake (ml 100 g^{-1} body weight)	267.0^{AB} ± 25.3 (269.8)	280.2^{A} ± 23.5 (272.6)	247.3^{B} ± 19.4 (249.6)	247.4^{B} ± 9.6 (243.9)

Explanations: BD – Basic Diet, MD – Modified Diet, MD+AS: Modified Diet + Adequate Supplementation, MD+ES – Modified Diet + Excessive Supplementation, FCR – Feed Conversion Ratio, * ^{a}, ^{b} – statistically significant difference $p \leq 0.05$, ** A, B – statistically significant difference $p \leq 0.01$

feed. Despite the significant differences in the consumption of feed and energy between the groups of animals, they all reached the same body mass at the end of the experiment. A similar result, i.e. a lack of significant difference in the final body mass after 4 to 12 weeks of feeding male Wistar rats with a diet containing 10% - 70% of sucrose compared to the control rats has been reported by other researchers (de Castro et al. 2007, Martinez et al. 2010, Zago et al. 2010, Sheludiakowa et al. 2010). The applied supplementation of the modified diet, both complementary and excessive, did not have any influence on the final body mass. A similar result was achieved by Franca and Vianna (2010), who supplemented a diet of male Wistar strain rats with B2 vitamin in an amount of 10 mg kg^{-1} of body mass.

Animals fed the modified diet consumed significantly more liquids expressed per 100 g of body mass (276.6 cm^{3}). This was probably due to the increase in the osmolality of plasma resulting from the increased consumption of sucrose as a source of glucose and fructose. The applied vitamin

supplementation, both complementary andexcessive, resulted in a significantly lower amount of consumed liquids, even below the amounts drunk by animals fed the basic feed (BD) (249.6, 243.9 cm^3 vs 269.8 cm^3). However, in the research by FRANC and VIANNA, no connection between supplementation with vitamin B2and consumption of liquids by male Wistar rats was found.

One of the predicted effects of incorporating sucrose into a diet was an increase in the glucose concentration in blood plasma of the examined animals. However, no significant differences were found in blood plasma glucose between the groups of animals under research (Table 3). This might have

Table 3

Effects of a diet type and B-group vitamin supplementation on glucose and selenium concentrations in the blood serum, concentrations of selenium and activity of antioxidant enzymes in testicles of Wistar rats ($\overline{x}$ ± SD, Me)

Parameters	BD (a)	MD (b)	MD+AS (c)	MD+ES (d)
	in serum			
Glucose (mmol dm^{-3})	6.80 ± 1 .2 (6.92)	7.44 ± 1 .7 (6.69)	6.91 ± 1 .3 (6.67)	7.78 ± 1.2 (8.3)
Se (μg cm^{-3})	0.70^{ab} ± 0 .2 (0.63)	0.79^{a} ± 0.2 (0.69)	0.70^{ab} ± 0.2 (0.62)	0.66^{b} ± 0.1 (0.65)
In testicles				
Se (μg g^{-1})	0.59 ± 0.1 (0.60)	0.56 ± 0.1 (0.55)	0.58 ± 0.1 (0.58)	0.60 ± 0.1 (0.59)
GPx (mU mg^{-1} protein)	159.8 ± 48.6 (165.1)	156.2 ± 39 .4 (142.8)	155.0 ± 64.5 (144.0)	177.1 ± 78.5 (172.3)
GST (mU mg^{-1} protein)	532.8 ± 136.3 (563.9)	475.2 ± 200.4 (463 .7)	428.8 ± 139 .5 (408 .5)	581.2± 341.1 (475.5)
SOD (mU mg^{-1} protein)	19.10 ± 9.6 (17.2)	17.10 ± 6.9 (16 .4)	18.40 ± 7.2 (18 .3)	20.40 ± 7.5 (20.2)
CAT (mU mg^{-1} protein)	1.30 ± 0.6 (1.01)	1.10 ± 0.4 (1.00)	1.09± 0.5 (1.09)	1.21 ± 0.5 (1.25)

BD – Basic Diet, MD – Modified Diet, MD+AS – Modified Diet + Adequate Supplementation, MD+ES – Modified Diet + Excessive Supplementation,
* ^{a}, ^{b} – statistically significant difference $p \leq 0.05$, ** *A*, *B* – statistically significant difference $p \leq 0.01$

been caused by thiamin being present in the supplemented vitamins above its physiologically normal amount, which is known for its normalizing activity in this range (BABAEL-JADIDI et al. 2004, THORNALLEY 2005). Beside this, there was simultaneous dietary supplementation with niacin, which indirectly increases blood glucose by lowering the sensitivity of cells to insuline (CHANG et al. 2006). Nevertheless, the highest glucose concentration (Me 8.3 mmol dm^{-3}) was observed in the group of animals excessively supplemented with vitamins.

In the experiment, a significant decrease in selenium was observed in the blood plasma of animals supplemented excessively with vitamins, which may be the result of both lower Se consumption and higher glucose concentration in the blood plasma. Hyperglycaemia may lead to oxidative stress, which affects the activity of such enzymes as glutathione peroxidase, in the structure of which Se plays a significant role. A decrease in the selenium concentration in blood suggests higher Se consumption for maintaining higher biosynthesis of glutathione peroxidase induced by an increase in the content of free radicals. This dependence had been found earlier in other research into the Se status in various metabolic disorders linked to inadequate nutrition (Nawarro, Alacron et al. 2000).

Selenium is present not only in blood but in all body tissues, which are therefore an Se pool that helps to maintain homeostasis of this trace element in organism (Alissa et al. 2003). In male mammals, a significant share of Se is deposed in testicles, where it is used for protecting mitochondria of spermatozoons against oxidation (Roveri et al. 2001). It is also an important trace element necessary for the proper development of testicles and motility of spermatozoons (Messaoudi et al. 2010). Maintenance of the physiologically adequate content of selenium in testicles is hormone regulated and has a priority in Se uptake from blood before other organs, especially in male rodents (Behne et al. 1988). Our analysis of the selenium content in the rat testicles did not show a significant influence of the type of a diet on the content of this trace element in these organs. A diet poor in this element may trigger mechanisms leading to the maintenance of a proper content of this element in testicles at the cost of other organs (Burk 2006). Because of the high requirement for energy, spermatozoons are very susceptible to oxidative stress. Energy centers present in testicles, i.e. mitochondria, are the source of reactive forms of oxygen significant for fertilization, although they can be harmful to gametes when present in excess. ROS are able to harm the DNA, proteins and phospholipids, rich in unsaturated fatty acids, which serve building blocks of the cell membranes of these gametes. Increased peroxidation of lipids may lead to a change in the membrane function, it can reduce the motility of spermatozoons, and it might have a significant influence on their development. Testicles, epididymises, sperm and sperm plasma contain antioxidant enzymes (Aruldhas et al. 2005, Aitken et al. 2012). Additionally, hyperglycaemia may lead to the destruction of the barrier between blood and a testicle, which may depress the systemic anti-oxidative activity of this organ in the long term (Alves et al. 2013). One of the important enzymes in the antioxidant barrier in testicles, and at the same time a selenoprotein, is phospholipid hyperoxide glutathione peroxidase (PHGPx, GPx4). One of its roles is to catalyze the reduction of lipid peroxide with the use of glutathione, thus preventing the dissemination of lipid oxidation process (Comhair, Erzurun 2005, Demir et al. 2011, El-Demerdash 2011). Apart from GPx, the cellular enzymatic antioxidant control consists of SOD, which catalyzes the conversion of superoxide radicals into hydrogen peroxide, and CAT,

which catalyzes the hydrogen peroxide conversion to water and oxygen (Mansour, Mossa 2009, Wafa et al. 2011). In our experiment, no significant differences were found in the activity of antioxidant enzymes GPx, GST, SOD, CAT in the rats' testicles under the experimental factors (Table 3). The highest activity of the antioxidant enzymes could be observed in the group fed MD+ES, which also showed the highest glucose concentration in blood serum. The activity of the antioxidant enzymes in this group of animals could be compared to that demonstrated in the control group, which may point to the stabilizing influence of the applied group B vitamins on the antioxidant status of testicles. It has been shown that in the long term (as in this six-week-long experiment) application of vitamins B1, B2, B6 and niacin which have antioxidant activity may adjunct/complement the activity of androgenous antioxidants (Mahfouz, Kummerow 2004, Voziyan, Hudson 2005, Ullegaddi et al. 2006).

CONCLUSIONS

In conclusion, a change in a diet composition such as by the replacement of full grains with wheat flour and sucrose as well as dietary supplementation with selected B-group vitamins and niacin, with varied amounts of feed, selenium and liquid intake, did not significantly influence the body gain of animals under research or the glucose concentration in blood. A significant decrease in the selenium concentration in the blood of animals supplemented in excess with vitamins mentioned above did not result in a significant difference in the selenium content of testicles. However, the activity of antioxidant defence enzymes was slightly higher in this groups of animals.

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