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EVALUATION OF THE IMPACT OF DIET SUPPLEMENTATION WITH CALCIUM, MAGNESIUM, ZINC AND CHROMIUM ON CONCENTRATION OF SELECTED INDICES OF PROTEIN METABOLISM IN FEMALE RATS

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ABSTRACT

An animal model study was undertaken in order to determine the effect of modifying diet composition and its supplementation with calcium, magnesium, zinc and chromium on protein metabolism. The study was conducted on 36 female rats aged 5–6 months, kept in individual cages Group I received basal feed mixture, Groups II and III – modified feed mixture. For drinking the animals from Groups I and II received tap water, Group III – an aqueous solution of mineral components (270 mg Ca^{2+}; 60 mg Mg^{2+}; 3.96 mg Zn^{2+} and 0.726 mg Cr^{3+} per 1 kg of feed mixture).

Diet, in which whole grains of cereals were substituted for wheat flour and saccharose and supplemented with Ca, Mg, Zn and Cr, caused a reduced intake of feed and proteins, which could be the reason of lower protein content in muscles of the animals. Modification of diet composition affected the increase in the concentration of α_1-globulins in blood, which might indicate an ongoing inflammatory process, whilst the supplementation with mineral components made no difference in this respect. The supplementation with Ca, Mg, Zn, Cr did not restore the values of selected markers of protein metabolism determined in plasma of the animals fed the modified feed mixtures to the levels noted in the animals receiving basal diet.

Key words: Female rats, mineral supplementation, protein metabolism.

INTRODUCTION

During the last few years a big interest in diet supplements containing such ingredients as calcium, magnesium, zinc and chromium, which take essential part in metabolism of carbohydrates and lipids, has been observed. These supplements are now widely advertised especially as preparations, which are supposed to assist people with diabetes in glycaemia regulation and make losing weight easier by influencing lower food consumption and intensification of metabolic processes [2, 26, 34]. Application of nutritive components supplements creates a risk of antagonistic interaction between respective elements like: calcium to zinc, and zinc and chromium to magnesium21]. And what is more, the level of absorption of Ca, Mg, and Zn (25–75%) from alimentary tract is higher than that of Cr (<25%) [9]. Disproportion in consumption and absorption may result in changes of mineral concentration both in organism and its functioning. The results of the research concerning justification of applying the supplements to men and animals are not univocal.

The assumption of this research was based on findings achieved by Friedrich and Sawicka [11] in a study with model animals as to the effect of diet composition modification and its supplementation with calcium, magnesium, zinc and chromium on the anti-oxidative status, which demonstrated the enhancement of free-radical reactions in animals supplemented with the above-mentioned elements Friedrich and Serwotka [12] demonstrated also that in the case of female rats a change in diet composition and its supplementation with calcium, magnesium, zinc and chromium resulted in a decreased feed intake, which was accompanied by increased body weight gains, significantly increased accumulation of peripheral-organ fatty tissue, and changes in its fatty acids composition.

As to the protein metabolism, calcium has influence on synthesis and antibodies release, on building and regulatory protein [40], magnesium takes part, among others, in amino acid metabolism into active forms and their aggregation with t-RNA, zinc (Zn) plays an important role in many metabolic processes, such as DNA, RNA, and protein synthesis [6], whereas chromium influences incorporation of amino acids into heart proteins and their uptake by tissue in rats [5].

This is the reason why it should be checked on the animal model whether under the changed diet content, in which full components (full wheat and corn grains) are isocalorifically exchanged for white flour and saccharose and its supplementation with calcium, magnesium, zinc and chromium, there are changes in protein metabolism in rats.

MATERIALS AND METHODS

Upon the approval of the Local Ethical Commission (Approval no 7/2005), the study was conducted on 36 female rats of SPRD/MolLod strain, aged 5-6 months with initial body mass of 219.2 ±13.2 g, kept in individual cages. Rats were obtained from the animal husbandry of Chair and Department of Toxicology, Poznań University of Medical Sciences, Poland. After one-week conditioning under vivarium conditions (temp. 21–22°C, relative air humidity 55–60%, light/dark cycle 12/12 h), the animals were divided into three equal groups (n=12) that were fed *ad libitum* with pelleted feed mixtures produced from the same components, except for the differentiating ones, by the Feed Mixtures and Concentrates Production Plant in Kcynia. Group I received basal feed mixture (Labofeed B), that met the requirements stipulated for Reeves et al. [28] and contained whole grains of wheat and maize. Groups II and III were administered a modified feed mixture in which 83.5% of wheat present in the basal diet was substituted for wheat flour (type 500) and 50% of maize – for saccharose (Table 1).

Table 1. Component composition of compound feeding stuffs used in the experiment

Item	Basic fodder	Modified fodder
Ingredients [%]		
wheat	36.4	6
corn grain	20	10
wheat bran	20	20
dry whey	3.0	3.0
fodder salt	0.3	0.3
soy-bean grain 48%	17	17
fodder chalk	1.5	1.5
phosphate 2-CA	0.8	0.8
premix LRM	1	1
wheat flour (type 500)	–	30.4
saccharose	–	10
Nutrient composition [%]		
total protein	18.1	17.7
crude fat	2.1	2.2
total carbohydrates within crude fiber within digested carbohydrates	65.80 2.91 62.90	66.10 2.73 63.40
total ash	6.1	5.7
dry matter	92.1	91.7
Brutto energy [kcal × g^{-1}] [kJ×g^{-1}]	 3.83 16.0	 3.84 16.1
Metabolic energy [kcal × g^{-1}] [kJ×g^{-1}]	 3.43 14.3	 3.44 14.4

The contribution of other components in feed mixtures was identical. The substitution of dietary components made while composing the modified feed mixture was aimed at mirroring, to some extent, contemporarily observed nutritional mistakes in humans i.e. increasing contribution of saccharose in the calorific value of a food ration and increased contents of refined carbohydrates in diet. The prepared feed mixtures were subjected to a chemical analysis to determine the contents of total nitrogen (with Kjeldahl's method) expressed per protein content, crude fat (with Soxhlet's method), dry matter (with gravimetric method), and ash (with gravimetric method) [4]. The crude fiber content was determined as well (in an ANKOM 220 apparatus) at the Research Institute of Animal Production National Laboratory for Feedingstuffs (PB-02/PS). The content of carbohydrates was calculated from a difference between dry matter and the sum of other solid dietary components. The gross and metabolic energy contents were calculated with the generally applied energy equivalents [10]. The feeds mixtures were prepared maintaining the isocalorifical and isoprotein balance (Table 1).

The content of Ca, Mg, Zn (Table 2) in mixtures were assayed on Atomic Absorption Spectrometry methods at the Research Institute of Animal Production National Laboratory for Feedingstuffs [25], while Cr was assayed by wet digestion in

concentrated HNO_3 in microwave oven MDS 2000 and determined by using atomic emission spectrometry induction coupled plasma (ICP-AES) in the apparatus Jobin Yvan JY-24 type.

Table 2. Contents of selected minerals in 100 g diet

Trait	Basic fodder	Modified fodder	Minerals content difference	
				[%]
Calcium [mg·100 g feed]	1370	1280	90	6.6
Magnesium [mg·100 g feed]	200	180	20	10.0
Zinc [mg·100 g feed]	10.3	8.98	1.32	12.8
Chromium [mg·100 g feed]	0.451	0.209	0.242	53.7

For drinking, the animals from Groups I and II were receiving settled tap water. In turn, rats from Group III, in the period of enhanced activity, were administered 50 cm^3 of an aqueous solution of mineral components. Doses of the administered components (270 mg Ca^{2+}; 60 mg Mg^{2+}; 3.96 mg Zn^{2+} and 0.726 mg Cr^{3+} per 1 kg of feed mixture), calculated in respect of daily feed intake by animals, exceeded three times the difference between contents of those components in the basal diet and the modified feed mixture (Table 2), which to some extent simulated the mode of supplementation in humans. The minerals used in the study originated from commercially-available pharmaceutical preparations: *calcium dobesilate, magnesium hydroaspartate, chromium polynicotinate, zinc gluconate*. Once the solution of minerals was administered, the animals were provided with pure, settled tap water. After one-week conditioning period, the experiment spanned for 6 weeks during which feed intake in all groups as well as minerals intake in the supplemented group were monitored systematically.

Body weight of the animals was measured once a week. The animals were fasted 12 h before the end of the experiment. Next, they were anaesthetized with Ketanest (Pfizer Ireland Pharmaceuticals) and blood samples were collected from their hearts. Having centrifuged the coagulate, the resultant blood serum was determined for concentration of total protein with the buret method [29] using a Marcel Media Bio spectrophotometer; concentrations of protein fractions (albumins, α_1-, α_2-, β- and γ-globulins) with the method of electrophoretic separation in chambers on agarose gel by Cormay Diagnostics and the read out made with DT-93 densitometer (Cormay); concentration of urea and creatinine with the kinetic method using bio-tests by BioSystems company onto a Marcel Media Bio spectrophotometer; activity of asparagine aminotransferase AST EC 2.6.1.2), alanine aminotransferase (ALT EC. 2.6.1.1) and γ-glutamyltranspeptidase (γ-GT, EC 2.3.2.2) with the kinetic method using biotests by BioSystems onto a Marcel Media Bio spectrophotometer. The prepared muscles (*m. quadriceps femoris, m. semimembranosus, m. adduktor femoris, m. superficialis gluteus*)and livers of the animals were assayed for the content of total protein with the Kiejdahl's method [4], on a Kjeltec 2100 apparatus by Foss Tecator and fat with Soxhlet method on Soxtec HT6 Foss Tecator apparatus.

The feed conversion ratio (FRC) was calculated as total amount of feed fed during the period of 42 days by rats divided by total weight gain. The protein efficiency ratio (PER) was calculated as weight gain over a period of 42 days divided by protein consumed [33]. The food conversion efficiency (FCE) defined as body-weight gain x 100/food intake were calculated according to the methods described by Johnson and Gee [16]. Results are shown as means ± standard deviation. Statistical significance was done by one-way analysis of variance (ANOVA) using the Statistica 9.0. When the ANOVA indicated significant difference among the means, the differences were further evaluated using the Duncan's multiple range tests. The difference was considered significant when $p \leq 0.05$ and $p \leq 0.01$ [35].

RESULTS

The analysis of the effect of the changes in diet composition and its supplementation with selected minerals on the feed intake demonstrated the lowest feed intake in the group of animals supplemented with minerals (Table 3). The values reported for that group were statistically significantly lower than those noted in the group fed the basal diet. The observed differences in feed intake were also reflected in a significantly lower protein intake However, one ascertained higher value of PER coefficient which indicates that protein sources in the diet could better provide essential amino acid requirement of the animals, had better nutrition value and were better utilized. The animals from groups fed the modified feed mixtures were, in contrast, characterized by greater body weight gains, when expressed per 100 g of ingested feed mixture, but still statistically significant differences were observed only between the groups fed the basal diet and the non-supplemented modified feed mixture. No difference was observed in the value of FRC.

Table 3. Effects of diet composition and minerals (Ca, Mg, Zn, Cr) supplementation on fodder and protein consumption, body weight gain and protein content in muscles and liver at female rats (x ±SD, n=36)

Trait	Basic fodder	Modified fodder (Mf)	Mf + supplement.
Feed consumption [g]	$711.0^{a} \pm 69.6$	$672.0^{ab} \pm 46.9$	$661.0^{b} \pm 46.8$
Feed consumption [g × g body weight^{-1}]	$2.70^{A} \pm 0.19$	$2.59^{AB} \pm 0.11$	$2.52^{B} \pm 0.08$
Feed consumption[g × 24h^{-1}]	$16.9^{a} \pm 1.7$	$16.0^{ab} \pm 1.1$	$15.7^{b} \pm 1.1$
Total calcium intake [mg × 24h^{-1}]	$225.3^{B} \pm 22.0$	$204.7^{C} \pm 14.3$	$243.8^{A} \pm 17.3$
Total magnesium intake [mg × 24h^{-1}]	$33.9^{B} \pm 3.3$	$28.8^{C} \pm 2.0$	$37.7^{A} \pm 2.7$

Total zinc intake [mg × 24h^{-1}]	$1.73^{Ba} \pm 0.2$	$1.44^{Bb} \pm 0.1$	$7.6^{A} \pm 0.5$
Total chromium intake [mg × 24h^{-1}]	$0.076^{B} \pm 0.007$	$0.033^{C} \pm 0.002$	$0.15^{A} \pm 0.01$
FRC [g feed consumed × g live weight gain^{-1}]	2.67 ± 0.30	2.59 ± 0.23	2.54 ± 0.28
FCE [g body weight gain ·100 g feed^{-1}]	$4.75^{b} \pm 1.3$	$5.88^{a} \pm 1.2$	$5.39^{ab} \pm 1.4$
Protein consumption [g·100 g body weight^{-1}]	$48.8^{A} \pm 3.5$	$46.8^{AB} \pm 1.9$	$45.5^{B} \pm 1.5$
PER [g weight gain × g protein consumed^{-1}]	$2.63^{b} \pm 0.7$	$3.32^{a} \pm 0.7$	$3.04^{ab} \pm 0.8$
Protein [%] Muscle Liver	 $22.1^{A} \pm 0.5$ 18.2 ± 0.6	 $21.1^{Ba} \pm 0.3$ 18.1 ± 0.4	 $20.8^{Bb} \pm 0.3$ 18.4 ± 0.7
^{ABC} – Mean values marked by different capital letters differ significantly at p =0.01 ^{ab} – Mean values marked by different small letters differ significantly at p =0.05.			

In the process of analyzing the achieved results a significantly lower protein content was noted in muscles of the rats fed the non-supplemented modified feed mixture compared to those fed the basal diet and the applied supplementation with selected mineral components even intensified this effect (Table 3). The content of protein in liver of the investigated animals was alike and comparable in all feeding groups.

The modification of diet composition was found to trigger an increase in glucose concentration in blood serum of the animals, the changes were however not statistically significant (Table 4). In contrast, significant in this respect turned out to be the effect of the applied minerals. The animals receiving the supplemented feed mixtures were characterized by a lower concentration of glucose in blood serum as compared to those fed the non-supplemented modified feed mixture. This concentration was comparable to that determined for the rats fed the basal diet. The applied change in diet composition, despite no significant effect on the concentration of total protein, caused a significant increase in the concentration of α_1-globulins, which remained unaffected by the applied supplementation with Ca, Mg, Zn and Cr (Table 4).

Table 4. Effects of diet composition and minerals supplementation (Ca, Mg, Zn, Cr) on serum concentration of glucose and chosen indicators of protein transmutation at female rats (x ±SD, n=36)

Trait	Basic fodder	Modified fodder (Mf)	Mf + supplement.
Glucose [mmol × l^{-1}]	$6.89^{ab} \pm 1.2$	$7.94^{a} \pm 2.0$	$6.57^{b} \pm 2.5$
Total protein [g × l^{-1}]	62.5 ± 3.4	65.4 ± 4.2	62.9 ± 6.8
Albumin [g × l^{-1}] [%]	 29.7 ± 1.9 48.0 ± 2.0	 30.9 ± 2.2 47.3 ± 2.3	 29.1 ± 3.0 47.4 ± 3.1
α_1-globulin [g × l^{-1}] [%]	 $10.9^{B} \pm 0.7$ $17.6^{B} \pm 1.0$	 $12.8^{A} \pm 1.3$ $19.5^{A} \pm 1.3$	 $12.2^{A} \pm 0.6$ $20.0^{A} \pm 0.6$
α_2-globulin [g × l^{-1}] [%]	 2.9 ± 0.3 4.7 ± 0.6	 3.1 ± 0.9 4.8 ± 1.1	 2.8 ± 0.3 4.6 ± 0.4
β-globulin [g×l^{-1}] [%]	 10.9 ± 1.6 17.5 ± 2.5	 11.5 ± 1.1 17.5 ± 1.2	 10.4 ± 1.1 17.0 ± 1.8
γ-globulin [g × l^{-1}] [%]	 7.0 ± 0.7 11.4 ± 1.0	 7.2 ± 0.9 11.0 ± 1.3	 6.8 ± 0.8 11.0 ± 1.2
Albumin/Globulin Ratio	0.93 ± 0.1	0.90 ± 0.1	0.91 ± 0.1
g-GT [U × l^{-1}]	$11.0^{b} \pm 3.2$	$10.4^{b} \pm 2.2$	$14.5^{a} \pm 5.9$
AST [U × l^{-1}]	$48.1^{Bc} \pm 4.0$	$57.6^{b} \pm 8.2$	$66.6^{Aa} \pm 12.6$
ALT [U × l^{-1}]	$27.7^{a} \pm 7.6$	$21.8^{Bb} \pm 4.8$	$30.4^{A} \pm 7.4$
Urea [µmol × l^{-1}]	$7.66^{A} \pm 0.9$	$3.49^{C} \pm 0.5$	$5.92^{B} \pm 1.0$
Creatinine [µmol × l^{-1}]	$71.2^{ab} \pm 2.9$	$69.1^{b} \pm 4.5$	$73.6^{a} \pm 2.5$
^{ABC} – Mean values marked by different capital letters differ significantly at p ≤0.01 ^{abc} – Mean values marked by different small letters differ significantly at p ≤0.05.			

Furthermore, the modification of diet composition was observed to influence an increase in the activity of asparagine

aminotransferase, a decrease in the activity of alanine aminotransferase, and a decrease in urea concentration in blood serum of the rats examined (Table 4). In turn, the supplementation of this diet with selected minerals evoked the enhancement in the activities of γ-glutamyltranspeptidase, asparagine and alanine aminotransferase as well as increase in the concentrations of urea and creatinine in blood serum of the animals under research (Table 4).

DISCUSSION

The analysis of the results achieved demonstrated that the applied experimental factors, through their impact on feed intake, affected a decreased protein intake by the animals fed the modified feed mixtures. Taking into consideration physiological mechanisms regulating food intake, it is known that one of many factors having influence on the amount of taken feed by animals is its energy value [1]. In the conducted experiment the animals received *at libitum* isocalorific feed and the mineral ingredients added to drinking water did not change the energy value of the feed which eliminated influence of this factor on the amount of taken feed. Increased intake of the feed by animals from the group under research can be explained by rats ability to compensate lower nutritive density of this diet [31] being the result of higher inclusion of raw fiber in this feed in comparison to modified feed.

As the result of lower protein consumption in feed a lower content of protein in animals' muscles in the animals fed with modified feed with or without supplements could be observed in order to maintain protein homeostasis in liver and blood plasma. In the case of non-supplemented modified feed mixture, this effect could be ascribed to a lower content of zinc in the modified feed mixtures containing wheat flour and saccharose characterized by a lower content of zinc compared to that of the constituents removed from the feed. As demonstrated by Roth [30], a lower zinc supply in diet was accompanied by a lower intake of feed and protein by experimental rats, but also by the enhanced activity of ALT in plasma, which indicated an increased rate of transamination of amino acids originating from both feed protein and endogenous protein, in the liver. Many investigations have demonstrated suppressed synthesis of proteins, assayed *in vivo* or *in vitro,* in liver, muscles, thymus or bones of rats as effect by zinc deficiency in diet. Taking into account that amongst the supplemented minerals, zinc is the key component of the effective protein utilization from diet, improvement could be expected in this respect in the supplemented animals. However, the simultaneous supplementation with calcium could have affected the reduced absorption of zinc.

Under conditions of sufficient protein intake, enhancement may be observed in the metabolism of amino acids in liver, which causes an increase in the urea concentration in blood plasma of animals. However, with a lower protein intake, the organism is maintaining a protein homeostasis in blood and liver, compensating its deficits with suppressed synthesis of muscle protein or enhanced degradation of endogenous protein originating from muscles. It may, therefore, be supposed that in the reported study the enhanced degradation of systemic proteins proceeded in the animals fed the non-supplemented modified feed mixture, which was indicated by the decreased activity of ALT, the increased activity of AST, and by a lower concentration of urea in blood serum. Amino acids released in the process of muscle proteins proteolysis are transported to liver and metabolized therein. Their carbon chains are utilized for the synthesis of glucose or are included into the Krebs' cycle, while the amine residues serve for the synthesis of endogenous amino acids that maintain the protein homeostasis in blood plasma. The reduced activity of alanine aminotransferase may additionally be explained by suppressed gluconeogenesis. Alanine aminotransferase is an enzyme that participates in the metabolism of nitrogen and amino acids as well as gluconeogenesis [39]. Its major role is the conversion of alanine to glucose, and because the modified diet provided a significant content of glucose and affected a lower protein intake than in the group fed the basal diet, it diminished both urea synthesis and ALT activity in blood of this group of animals. The increased activity of AST which role is to catalyze the transfer of amine groups from any amino acid onto keto-acid, points to the enhanced catabolism of protein confirmed in this study by a significant decrease in protein content of muscles of the animals administered the modified feed mixtures.

The supplementation of the modified diet with Ca, Mg, Zn and Cr caused an increase in the concentration of urea as well as in activities of ALT, AST and γ-GT at the significantly lower protein intake by the animals from that group. The values of those parameters determined in the supplemented animals were, however, not comparable with the values noted in the control animals, and indicated the enhancement of systemic proteins degradation as well as increased metabolic rate in the case of intermediate products of this process.

The rate of glucose absorption from blood by particular tissues varies; i.e. being faster and independent of insulin concentration when absorbed by cells of liver, brain, pancreas, adrenal glands and by erythrocytes and slower, insulin-dependent when absorbed by cells of skeletal muscles and fatty tissue. Insulin increases the supply of substrate, thus stimulating the transport of glucose and amino acids to cell nucleus and facilities their intracellular utilization. Under physiological conditions, a factor stimulating insulin secretion is an increased supply of both glucose as well as amino acids, arginine, leucine, lysine and phenylalanine in particular [22], and the reduced intake of protein by the animals fed the supplemented modified feed mixture could affect its secretion to a lesser extent. In contrast, it may be presumed that the feed mixture modified through saccharose inclusion affected a greater increase in serum level of glucose than the basal diet, and that insulin secreted in response to the increased concentration of glucose in blood, might stimulate the secretion of catabolic glycocorticoids [18 37]. Under physiological conditions, glycocorticoids affect directly the reversible inhibition of insulin secretion in β-cells of pancreas [8]. In the case of rodents, this phenomenon is facilitated by local activation of the inactive form of dehydrocorticosterone to active corticosterone mediated by 11β-hydroxysteroid dehydrogenase (11β-HSD) [23], whose increased concentration in rats fed modified diet with a similar composition and supplemented with Ca, Mg, Zn and Cr for 6 weeks of experiment was demonstrated by Podlaszewska et al. [24]. Such an effect of supplementation ought to influence the increase in glucose concentration in blood serum, which was not observed in the reported study, but – as demonstrated by Ortsäter et al. [23] – the

longstanding increase in corticosterone concentration may induce the compensative increase of insulin secretion from β-cells of pancreas and thus reduce glucose concentration in blood. The lower plasma level of glucose in the case of the supplemented animals might have also been due to the supplemented chromium which is a constituent of a glucose tolerance factor (GTF) [19] and facilitates the action of insulin by increasing the number and activating receptors for insulin [38]. In addition, chromium affects the expression of a glucose transport system located in the skeletal muscles and adipocytes (GLUT4 –*glucose transporter*). These are the mechanisms by means of which this element improves concentration of carbohydrate metabolism markers in blood of rats [32]. Studies have shown that supplementation with chromium evokes a decrease not only in the concentration of parameters of carbohydrate metabolism and lipid metabolism, but also in the activity of AST and ALT even in rats with pharmacologically-induced type 1 [20] or type 2 diabetes [7], characterized by the elevated concentrations of AST and ALT. Despite such an effect of chromium, in the reported experiment, the activities of both enzymes were observed to increase in blood of the supplemented animals. Perhaps, this was due to reduced chromium absorption as a result of the reported lower intake of protein by the animals from that group, for the availability of this component depends on the simultaneous presence of amino acids [3]. Chromium absorption could as well be diminished by both the presence of simple carbohydrates in the modified feed mixture [14] and the applied supplementation with zinc, the retention of which could additionally be enhanced by the simultaneous long-term supplementation with magnesium 17] as well as lower content of raw fiber in the modified feed.

The conducted study demonstrated also significant increase in the concentration of α_1-globulins in blood of the rats fed the modified feed mixtures, both supplemented and non-supplemented ones, as compared to the animals fed the control diet. The increased concentration of α_1-globulins in blood may be indicative of the proceeding inflammatory state, because the selected proteins of this fraction are secreted in order to inhibit proteolytic processes ongoing in muscle proteins. Anti-proteases restrict the dissemination of the acute inflammatory state by bonding into complexes with elastase which originates from neutrophil granulocytes and stimulate the secretion of pro-inflammatory cytokines. Intensifying effect of a high-saccharose or high-fructose diet poor in magnesium, which was also depleted in the modified feed mixtures used in our study on the symptoms of inflammatory processes in animals is also known. This effect is also explained by the enhancement of free-radical reactions by such a diet [15, 27]. The observed statistically significant increase in the activity of γ-GT in blood of the animals fed the supplemented modified feed mixture may also indicate a progressing inflammatory state in liver. The increased activity of γ-GT is additionally an independent predictor of impaired glucose intolerance, and is also correlated with type 2 diabetes, insulin resistance, liver steatosis and other factors of the metabolic syndrome [13, 36]. However, answering the question whether the applied supplementation of the modified diet with calcium, magnesium, zinc and chromium indeed intensifies the pro-inflammatory processes requires further extended research on markers of the pro-inflammatory processes.

In summary, it may be concluded that the modified feed mixtures administered to female rats over the 6-week experimental period caused the enhancement of catabolic processes in terms of protein metabolism as well as suppression of the synthesis of tissue proteins, and that their supplementation with selected minerals did not restore the values of the analyzed parameters to the levels observed in the animals fed the basal diet.

SUMMARY

1. Diet in which whole grains of cereals were isocalorificaly substituted for wheat flour and saccharose and further on supplemented with Ca, Mg, Zn and Cr was conducive to the reduced intake of feed and proteins, which could be the reason of the observed lower protein content in muscles of the animals under research.
2. Modification of diet composition in which whole grains of cereals were substituted for wheat flour and saccharose affected an increase in the concentration of α_1-globulins in blood, which may indicate the ongoing inflammatory process, whilst the supplementation with mineral components made no difference in this respect.
3. The supplementation with Ca, Mg, Zn, Cr did not restore the values of selected markers of protein metabolism (γ-GT, ALT, AST, urea, creatinine) determined in plasma of the animals fed the modified feed mixtures to the levels noted in the animals receiving basal diet.

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