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ORIGINAL PAPER

THE EFFECT OF DIETS INCLUDING GLUTEN -FREE BREAD ENRICHED WITH NATURAL ADDITIVES ON THE CONTENT OF CALCIUM AND MAGNESIUM IN RATS WITH DEFICIENCIES OF THESE ELEMENTS*

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Abstract

Celiac disease and gluten intolerance affect a growing proportion of the population. The most important minerals for proper growth are calcium and magnesium. These minerals are often deficient. Bread is the most popular type of food, and therefore this product was enriched in this study. The aim was to assess the influence of a diet including gluten-free breads enriched with milk and seeds on the content of calcium and magnesium in the organs (liver, spleen, kidney, femur and heart) and blood of 48 male Wistar rats, after a deficiency of the selected minerals had been induced by a 40-day nutritional intervention. After the intervention, the rats were euthanized, the organs were collected and their mineral content was measured. The aim was to design breads with a high content of Ca and Mg. The number of additives influenced the content of minerals in the diet. There was no statistical difference in the content of calcium and magnesium in the blood of rats in any group. Significant differences in the content of calcium were observed in the not pancreas but spleen and liver of rats fed the all types of bread and thenumber of additives. Significant differences in the content of magnesium were observed in the femur of rats fed all types of breads and the number of additives. No influence was found on the general nutritional parameters and blood con-tent of calcium and magnesium. However, the addition of enriched gluten-free breads to a diet was found to influence the content of calcium and magnesium in selected organs.

Keywords: calcium deficiency, magnesium deficiency, gluten-free bread, enriched diet, rats.

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INTRODUCTION

Celiac disease and gluten intolerance affect a growing proportion of the population. Now it affects around 1% of the population worldwide. The most common age at diagnosis is between 35 and 55 years. Statistically, the disease affects female patients twice as often (Catassi et al. 2010, Lionetti, Catassi 2014). The disease causes the destruction of intestinal villi and reduced absorption of nutrients, including minerals (Barera et al. 2000, Tovoli 2015, Leonard et al. 2016, 2017). Currently, more measures are being taken to improve people's nutritional status through the scientific development of new food products with increasing health-promoting properties, or to counteract deficiencies of elements in a diet (Reguła et al. 2010, 2016, 2018, Pasqualone et al. 2010, Świeca et al. 2015). For people with celiac disease, the most important products include gluten-free bread and grain products. These products are the most widely consumed ones, and are the basic source of carbohydrates in the diet (Suliburska et al. 2013).

The most important minerals for proper growth are calcium and magnesium. Calcium is a basic element which builds bones and teeth. It can exist in the body in a free form, bound with protein or as a salt. Its most important biological roles are the activation of enzymes, building hormones and nerve impulse transmission. Calcium is necessary for correct cardiac function and a healthy vascular system. Calcium deficiency can negatively influence bone quality and cause demineralization and deformation. It has been shown that chronic deficiencies of this element can lead to high blood pressure (Bianchi, Bardella 2008).

Magnesium is the most important intracellular cation in the body. It is responsible for enzyme activation (Iannello, Belfiore 2001). It is necessary for the synthesis of nucleic acids and protein, and plays a very important role in homeostasis and blood pressure. Magnesium deficiency can cause disorders in the vascular, nervous and muscular systems (Matsuzaki et al. 2005).

There is significant risk connected with mineral deficiencies, and they negatively impact upon human health. Attempts have been made to enrich products with deficient elements. For this purpose, product which are commonly consumed by each group of people are chosen (Regula et al. 2018). The aim of this study was to make an assessment of nutritional parameters and mineral metabolism of rats fed a diet with the addition of enriched gluten-free breads. Gluten-free breads were enriched with additives which had high nutritional value. Rice flour is a source of fiber, potassium, calcium, zinc and magnesium. It has an important role in hormonal balance and ageing (Irakli et al. 2019). Buckwheat flour is a good source of protein with a high content of lysine and arginine. A high content of magnesium has a positive effect on the cardiovascular system (Zieliński et al. 2019). Poppy seed and flax are a good source of vitamins B, calcium and magnesium, which play an important role in the immune and nervous systems (Ghafoor et al. 2019, Pająk et al. 2019). Hazelnuts are a good source of potassium and polyunsaturated fatty acid, which regulate blood pressure and the level of LDL cholesterol (Fang et al. 2018). Pumpkin seeds have a high content of zinc and polyunsaturated fatty acid, which influence the nervous system and have a hypoglycemic effect (Bouazzaoui, Mulengi 2018). Sunflower seeds with their high content of phytosterols influence the cardiovascular system and decrease LDL cholesterol (Kvashin et al. 2018). Amaranth flour with its high content of calcium, magnesium, iron influence the LDL cholesterol level and the cardiovascular system (Horstmann et al. 2019). Soy flour with its high content of copper, zinc and iron have an influence on the nervous system and the cardiovascular system, and produce a hypoglycemic effect (Huang et al. 2019).

MATERIALS AND METHODS

Breads

Gluten-free breads had rice flour or buckwheat flour as the basic ingredient. The recipe also included corn starch, potato starch, pectin, yeast, sugar, salt, rapeseed oil, linseed oil and olive oil. Its composition was formulated to provide a high content of calcium and magnesium. Milk bread contained poppy seeds and whole milk powder. Breads with seeds contained poppy seeds, whole milk powder, amaranth flour, flax, sunflower seeds, pumpkin seeds, hazelnuts and egg yolk. The percentage composition of the breads is shown in Table 1.

Table 1

Composition of breads (%)

Components	R	B	RM	BM	RMS	BMS
Buckwheat flour	0.000	33.00	0.000	30.00	0.000	26.00
Rice flour	33.00	0.000	30.00	0.000	26.00	0.000
Corn starch	17.00	17.00	14.00	14.00	12.00	12.00
Potato starch	33.00	33.00	29.00	29.00	20.00	20.00
Pectin	4.000	4.000	4.000	4.000	4.000	4.000
Yeast	4.000	4.000	4.000	4.000	4.000	4.000
Sugar	5.000	5.000	4.000	4.000	3.000	3.000
Salt	1.000	1.000	1.000	1.000	1.000	1.000
Mixed of oil	3.000	3.000	3.000	3.000	2.000	2.000
Whole milk powder	0.000	0.000	4.000	4.000	4.000	4.000
Mix of seeds	0.000	0.000	7.000	7.000	23.00	23.00
Egg yolk	0.000	0.000	0.000	0.000	1.000	1.000

R – rice bread, B – buckwheat bread, RM – rice bread with milk, BM – buckwheat bread with milk, RMS – rice bread with milk and seeds, BMS – buckwheat bread with milk and seeds

Animals, diet and experiment

The research was carried out on 48 male Wistar rats aged 6 weeks, with an initial body mass of 266 g ± 37.9. Animal care and handling for the experimental study were approved by the Local Regulatory Committee for animal studies (Approval number 888/11). The rats used in this experiment were housed in a thermostatically controlled room (22°C ± 2), in a 12 h light/dark cycle (lights on at 8:00), at humidity 55-65%, with unlimited access to distilled water (*ad libitum*). The animals were fed a modified, semisynthetic diet AIN93M (REEVES 1997). In order to induce deficiencies, 42 out of 48 rats were fed diets without Ca and Mg in the mineral mix for 30 days. The composition of the experimental diets is shown in Table 2 and the nutritional

Table 2

Composition of experimental diets (g kg^{-1})

Component	AIN control diet	AIN deficient in Ca and Mg	Without additves	M	MS
Caseine[1]	200.0	200.0	140.0	140.0	140.0
Rapeseed oil [8]	70.00	70.00	49.00	49.00	49.00
Saccharose[8]	100.0	100.0	70.00	70.00	70.00
Wheat starch[2]	530.0	530.0	371.0	371.0	371.0
Breads addition	0.000	0.000	300.0	300.0	300.0
Potato starch[3]	50.00	50.00	35.00	35.00	35.00
L-cystine[4]	3.000	3.000	2.100	2.100	2.100
Mineral mix[5]	35.00	35.00	24.50	24.50	24.50
Vitamin mix[6]	10.00	10.00	7.000	7.000	7.000
Choline[7]	2.000	2.000	1.400	1.400	1.400

M – diet with milk, MS – diet with milk and seeds;
[1] Manufacture of dairy products in Murowana Goslina, [2] "Celiko" Poznan, [3] Manufacture of potato in Iława, [4,7] Sigma-Aldrich (Germany), [5] AIN-93-MX (REEVES 1997), [6] AIN-93-VX (REEVES 1997), [8] Commercial food products

value of the diets with gluten-free breads in Table 3. Total fat was determined by the Soxhlet extraction method and the protein content was assayed by the Kjeldahl total nitrogen method. Carbohydrates constituted the difference of 100 and the sum of water, ash, protein and fat. The gross and metabolic energy were calculated using commonly applied energy equivalents.

After 30 days, 12 rats were chosen and sacrified (6 from the deficiency group and 6 from the control group) to establish whether a deficiency had been induced. The rats were euthanized with a mixture of CO_2 (70%) and air (30%). Then, blood from the left heart chamber and organs (liver, spleen, heart, kidney and left femur) were collected to measure Ca and Mg levels. The remaining animals were then divided into six groups. They were fed AIN93M diets with 70% added gluten-free breads. The nutritional

Table 3

Nutritional value of diets with gluten-free breads (ingredients in 100 g)

Ingredients	Type of bread	Additives		
		without	M	MS
Metabolic energy (kcal) Metabolic energy (KJ)	R	368.4 ± 1.800^{aA} 1543 ± 7.542^{aA}	383.6 ± 2.300^{aB} 1607 ± 9.637^{aB}	388.8 ± 3.600^{aB} 1629 ± 15.08^{aB}
	B	368.6 ± 2.000^{aA} 1544 ± 8.380^{aA}	380.7 ± 3.400^{aB} 1595 ± 14.24^{aB}	391.4 ± 2.500^{aB} 1639 ± 10.47^{aB}
Gross energy (kcal) Gross energy (KJ)	R	411.5 ± 2.010^{aA} 1723 ± 8.424^{aA}	428.4 ± 2.569^{aB} 1795 ± 10.76^{aB}	434.2 ± 4.021^{aB} 1819 ± 16.84^{aB}
	B	411.7 ± 2.234^{aA} 1724 ± 15.90^{aA}	425.2 ± 3.7978^{aB} $1781 \pm 11{,}69^{aB}$	437.1 ± 2.792^{aB} $1830 \pm 9{,}360^{aB}$
Protein (g)	R	11.81 ± 0.400^{aA}	12.80 ± 0.400^{aB}	13.60 ± 0.600^{aB}
	B	12.50 ± 0.500^{aA}	13.40 ± 0.200^{aB}	14.10 ± 0.400^{aB}
Fat (g)	R	2.750 ± 0.800^{aA}	4.550 ± 0.300^{aB}	5.890 ± 0.400^{aC}
	B	3.021 ± 0.700^{aA}	4.452 ± 0.600^{aB}	6.610 ± 0.300^{aC}
Carbohydrates (g)	R	71.78 ± 1.000^{aA}	70.52 ± 1.100^{aA}	68.10 ± 1.700^{aA}
	B	70.55 ± 1.200^{aA}	69.51 ± 1.600^{aA}	66.60 ± 2.100^{aA}
Calcium (mg)	R	190.2 ± 3.100^{aA}	335.2 ± 2.600^{aB}	340.5 ± 3.800^{aB}
	B	195.0 ± 2.400^{aA}	334.9 ± 3.200^{aB}	331.6 ± 4.600^{aB}
Magnesium (mg)	R	26.40 ± 0.900^{aA}	55.55 ± 1.200^{aB}	74.40 ± 3.200^{aC}
	B	73.80 ± 1.400^{bA}	95.56 ± 2.300^{bB}	94.31 ± 2.300^{bB}

Superscript lowercase letters (*a*, *b*, *c*) indicate statistical differences between the type of bread (between the lines) $p < 0.05$. Superscript uppercase letters (*A*, *B*, *C*) indicate statistical differences between the additives used (between columns) $p < 0.05$;
R – rice bread, B – buckwheat bread, M – bread with milk, MS – bread with milk and seeds

intervention lasted 40 days, then the rats were sacrified with a mixture of CO_2 (70%) and air (30%). Blood was then collected from the left heart chamber and organs (liver, spleen, heart, kidney and left femur). In this biological material, the levels of calcium and magnesium were determined. The study design is shown in Figure 1. Throughout the period of intervention, the amount of food consumed and food residue left over were recorded. The body weight of the rats was measured once weekly. The food efficiency ratio (FER) was calculated as the growth weight after consuming 100 g of diet.

The tissue samples were dried to dry mass and then wet mineralized in a microwave system with nitric acid (HNO_3, supra pure, 65%, Mars X5, CEM, USA). After mineralization, the samples were quantitatively transferred to PP vials, using deionized water. The calcium and magnesium concentrations in the mineralized tissue samples were determined by the flame atomic spectrometry method (spectrometer AAS-3, Zeiss, Germany), and serum Ca and Mg were assayed by colorimetric methods. The parameters

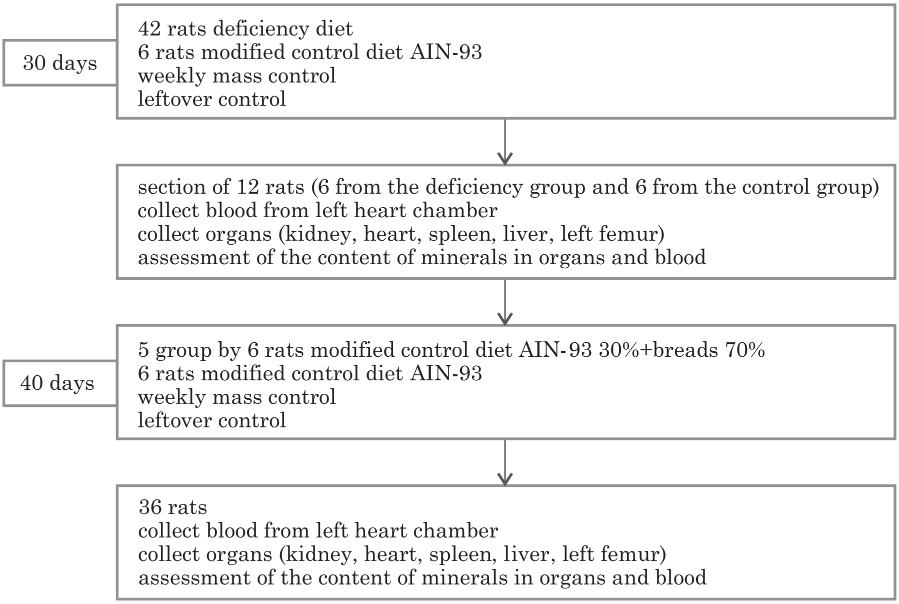

Fig. 1. Study design

used were: wavelength 422.7 nm for Ca and 285.2 nm for Mg, width of slits 0.5 nm for Ca and 0.2 nm for Mg, air flow 400 l min^{-1} for Ca and Mg, acetylene flow 80 l min^{-1} for Ca and 60 l min^{-1} for Mg, lamp current 7.5 mA for Ca and 3.0 mA for Mg, sensitivity 0.01 µg cm^{-3} for Ca and 0.004 µg cm^{-3} for Mg, slotted burner 10 cm for Ca and Mg (SULIBURSKA et al. 2013, 2015). The calcium in the rats' blood was marked with 5-nitro-5'-metylo-BAPTA in an alkaline medium and with EDTA. The change in absorbance is directly proportional to the calcium concentration and is measured by photometry (BOURGUIGNON et al. 2014). Test Ref. 05061482190 produced by Roche Diagnostics GmBH, Mannheim, Germany, was employed. Magnesium in the rats' blood was marked by the colorimetric method with chlorophosphonazo III (CPZIII) in an alkaline medium and with EDTA. The difference in absorbance between the magnesium-CPZ III complex and the EDTA treated complex is the absorbance due to magnesium alone (GUNDERSEN 2017). Test Ref. 20737593322 produced by Roche Diagnostics GmBH, Mannheim, Germany, was employed.

In order to determine the effect of the additive and type of bread on nutrition and morphological parameters, the analysis of variance was applied, while the significance of intergroup differences was assessed using the Tukey's test. All differences were considered to be statistically significant at a 5% probability level. Data were analyzed with Statistica 13.0 software.

RESULTS

The designed gluten-free breads had different ingredients and amounts of additives. The aim was to design breads with a high content of Ca and Mg. The results of the nutritional values and analyses of the minerals are shown in Table 3. When the amount of additives increased, the nutritional value of diets with rice bread and buckwheat bread also increased. The diet with bread without additives had a significantly lower calorie content than the other diets. The same was true of protein. When the amount of additives was higher, the amount of protein was also higher in both rice bread and buckwheat bread.

A higher content of fat was found when the amount of additives was higher. This difference was statistically significant in diets with rice breads and buckwheat breads, which is associated with the high content of fat in nuts and seeds added to the breads. When the amount of additives was higher, the content of protein and fat was higher and the content of carbohydrates was lower. Additives such as whole milk powder and seeds influenced the content of calcium in the breads. The content of this element in the diet including breads without additives was significantly lower than in the ones with enriched breads. There was no statistically significant difference in the content of calcium between the types of breads. When the amount of additives was higher, the content of magnesium was also higher. The type of breads and the amount of additives influenced the content of magnesium in the breads. Buckwheat breads had a higher content of magnesium than rice breads.

Throughout the entire period of nutritional intervention, parameters such as weight and consumed diet were measured. These results are shown in Table 4. When the amount of additives was higher, the consumed diet was also higher in diets with both rice breads and buckwheat breads. This difference was not statistically significant. Initial weight was not statistically significant in any group. A significantly higher weight grain and FER were observed in rats on the diet with additives. The type of diet did not influence organ mass in any group. The type of breads had no statistically significant influence on any general nutrition parameters.

Total consumption of calcium and magnesium by the rats was calculated based on nutritional values and consumed diets for the whole period of the experiment. It was calculated also per 100 g body weight. These results are shown in Table 5. Significantly higher consumption of calcium was observed for diets with gluten-free bread with additives, for both types of bread. When the amount of additives was higher, the consumption of calcium was higher with rice breads. Rats on the diet with buckwheat breads had a significantly higher consumption of magnesium than rats on the diet with rice breads. Also, the amount of additives had a statistically significant influence on the consumption of magnesium.

Table 4

General nutritional parameters of rats on diets with gluten-free breads

Ingredients	Type of bread	Additives		
		without	M	MS
Consumed diet (g)	R	997.5 ± 14.40^{aA}	1060 ± 59.10^{aA}	1077 ± 71.80^{aA}
	B	1027 ± 46.50^{aA}	1068 ± 68.30^{aA}	1077 ± 71.80^{aA}
Initial weight (g)	R	243.7 ± 32.70^{aA}	269.1 ± 43.00^{aA}	269.8 ± 38.70^{aA}
	B	268.6 ± 43.50^{aA}	269.0 ± 40.90^{aA}	268.1 ± 38.50^{aA}
Weight gain (g)	R	63.25 ± 14.90^{aA}	90.30 ± 12.80^{bA}	86.80 ± 16.40^{bA}
	B	60.80 ± 10.30^{aA}	84.80 ± 13.20^{bA}	89.10 ± 18.10^{bA}
FER*	R	6.328 ± 1.400^{abA}	8.514 ± 1.600^{bA}	8.000 ± 1.500^{abA}
	B	5.916 ± 1.000^{aA}	7.910 ± 1.000^{bA}	8.212 ± 1.400^{bA}
Femur mass (g)	R	0.743 ± 0.070^{aA}	0.763 ± 0.080^{aA}	0.767 ± 0.070^{aA}
	B	0.787 ± 0.070^{aA}	0.792 ± 0.060^{aA}	0.770 ± 0.070^{aA}
Liver mass (g)	R	9.496 ± 1.310^{aA}	10.84 ± 1.220^{aA}	10.39 ± 1.580^{aA}
	B	9.940 ± 1.150^{aA}	10.22 ± 0.930^{aA}	11.24 ± 1.460^{aA}
Kidney mass (g)	R	1.984 ± 0.390^{aA}	2.290 ± 0.220^{aA}	2.240 ± 0.270^{aA}
	B	2.250 ± 0.110^{aA}	2.210 ± 0.120^{aA}	2.194 ± 0.300^{aA}
Spleen mass (g)	R	0.504 ± 0.070^{aA}	0.505 ± 0.010^{aA}	0.527 ± 0.060^{aA}
	B	0.497 ± 0.040^{aA}	0.519 ± 0.030^{aA}	0.539 ± 0.030^{aA}
Heart mass (g)	R	0.965 ± 0.130^{aA}	1.071 ± 0.100^{aA}	1.040 ± 0.100^{aA}
	B	1.028 ± 0.120^{aA}	1.030 ± 0.080^{aA}	1.060 ± 0.140^{aA}

* FER (food efficiency ratio) = weight gain (g)/food intake (g) × 100;
Superscript lowercase letters (*a*, *b*, *c*) indicate statistical differences between the type of bread (between the lines) $p < 0.05$. Superscript uppercase letters (*A*, *B*, *C*) indicate statistical differences between the additives used (between columns) $p < 0.05$;
R – rice bread, B – buckwheat bread, M – bread with milk, MS – bread with milk and seeds

Table 5

Consumption of Ca and Mg by rats on diets with gluten-free breads

Ingredients	Type of bread	Additives		
		without	M	MS
Consumption of Ca (mg)	R	1616 ± 164.2^{aA}	2985 ± 195.4^{bB}	2842 ± 347.2^{bB}
	B	1664 ± 191.8^{aA}	2959 ± 268.9^{bB}	2802 ± 416.4^{bB}
Consumption of Ca (mg kg^{-1} body weight)	R	1618 ± 141.5^{aA}	2815 ± 69.40^{cB}	2631 ± 158.5^{bB}
	B	1618 ± 142.8^{aA}	2766 ± 92.23^{bcB}	2591 ± 234.1^{bB}
Consumption of Mg (mg)	R	224.6 ± 22.80^{aA}	495 ± 32.40^{bB}	621.1 ± 75.80^{bcB}
	B	636.8 ± 73.40^{bcA}	845.3 ± 76.80^{cB}	796.9 ± 118.4^{cB}
Consumption of Mg (mg kg^{-1} body weight)	R	225.0 ± 19.67^{aA}	466.9 ± 11.50^{bB}	575.1 ± 34.65^{cB}
	B	619.3 ± 54.67^{cA}	790.2 ± 26.35^{eB}	737.0 ± 66.57^{dB}

Explanations see Table 3

In Table 6, the content of calcium and magnesium in the blood of rats on diets containing gluten-free breads is shown. There were no statistically significant differences in the content of these minerals in any group.

In Table 7, the content of calcium and magnesium in the organs and femur is shown. Diets with gluten-free breads were found to have no influence

Table 6

Content of Ca and Mg in the serum of rats fed diets based on gluten-free breads

Ingredients	Type of bread	Additives		
		without	M	MS
Content of Ca in blood (mmol dm^{-3})	R	2.925 ± 0.050^{aA}	3.070 ± 0.100^{aA}	2.970 ± 0.080^{aA}
	B	2.977 ± 0.180^{aA}	3.003 ± 0.130^{aA}	2.985 ± 0.030^{aA}
Content of Mg in blood (mmol dm^{-3})	R	1.148 ± 0.120^{aA}	1.197 ± 0.120^{aA}	1.254 ± 0.080^{aA}
	B	1.250 ± 0.210^{aA}	0.825 ± 0.080^{aA}	1.283 ± 0.160^{aA}

Explanations see Table 3

Table 7

Content of Ca and Mg in the organs of rats fed diets based on gluten-free breads

Organs	Type of bread	Additives		
		without	M	MS
Heart Ca (mg kg^{-1})	R	18.14 ± 7.800^{aA}	11.07 ± 4.600^{aA}	9.97 ± 1.400^{aA}
	B	17.41 ± 3.900^{aA}	13.21 ± 4.100^{aA}	10.10 ± 3.500^{aA}
Spleen Ca (mg kg^{-1})	R	56.62 ± 27.50^{aA}	224.9 ± 122.9^{bB}	100.6 ± 46.30^{abA}
	B	75.24 ± 42.90^{aA}	181.0 ± 41.50^{bB}	64.63 ± 14.50^{aA}
Kidney Ca (mg kg^{-1})	R	113.9 ± 18.60^{aA}	89.47 ± 35.00^{aA}	119.7 ± 29.40^{aA}
	B	147.1 ± 84.80^{aA}	155.5 ± 89.20^{aA}	90.85 ± 20.20^{aA}
Femur Ca (g kg^{-1})	R	154.8 ± 12.90^{aA}	168.8 ± 3.500^{aA}	165.7 ± 2.600^{aA}
	B	162.8 ± 4.500^{aA}	165.9 ± 6.200^{aA}	168.7 ± 7.600^{aA}
Liver Ca (mg kg^{-1})	R	45.65 ± 12.50^{aB}	22.71 ± 5.900^{aA}	29.31 ± 12.60^{aAB}
	B	39.55 ± 12.10^{aB}	32.84 ± 14.40^{aA}	35.97 ± 13.60^{aAB}
Femur Mg (g kg^{-1})	R	3.087 ± 0.300^{aA}	3.292 ± 0.100^{abAB}	3.402 ± 0.100^{abB}
	B	3.320 ± 0.200^{abA}	3.390 ± 0.100^{abAB}	3.530 ± 0.100^{bB}
Kidney Mg (mg kg^{-1})	R	225.8 ± 18.60^{aA}	224.1 ± 11.80^{aA}	230.4 ± 5.400^{aA}
	B	228.9 ± 8.800^{aA}	217.3 ± 14.90^{aA}	235.0 ± 48.50^{aA}
Spleen Mg (mg kg^{-1})	R	256.1 ± 10.10^{aA}	246.4 ± 24.80^{aA}	248.6 ± 11.40^{aA}
	B	260.2 ± 11.70^{aA}	241.5 ± 14.80^{aA}	248.0 ± 12.00^{aA}
Heart Mg (mg kg^{-1})	R	186.6 ± 15.60^{aA}	176.0 ± 11.90^{aA}	170.7 ± 8.900^{aA}
	B	180.5 ± 10.30^{aA}	176.1 ± 7.800^{aA}	172.2 ± 9.900^{aA}
Liver Mg (mg kg^{-1})	R	249.5 ± 35.60^{bB}	183.4 ± 20.80^{aA}	200.5 ± 17.30^{abA}
	B	214.7 ± 28.70^{bB}	193.2 ± 29.60^{abA}	184.8 ± 31.40^{aA}

Explanations see Table 3

on the content of calcium in the rats' hearts. A significantly higher content of calcium was observed in the spleen of rats on the diet with rice and buckwheat breads with milk. There was no significant difference in the content of calcium in the kidney or femur for any type of bread. There was a significantly higher content of this mineral in the livers of rats on the diet with gluten-free breads without additives compared to rats on the diet with enriched gluten-free breads. The content of magnesium was statistically significant, depending on the type of bread and the amount of additives. The content of these minerals was higher when the amount of additives was higher. It was higher in buckwheat breads compared to rice breads. There was no significant difference in the content of magnesium in the kidney, spleen and heart. A significantly higher content of magnesium in the liver was observed in rats on the diet with gluten-free breads without additives. There was also a significant difference between rice breads and buckwheat breads in terms of the content of magnesium in the liver.

DISCUSSION

Enriched gluten-free breads were designed to promote the higher consumption of calcium and magnesium. Deficiencies of these minerals have been observed in people with celiac disease. These minerals have an important role in many metabolic process, so their correct level is very important in maintaining the proper condition of the body (Wójciak et al. 2017). Many attempts have been made to enrich diets with ingredients which should increase the content of macro- and micronutrients. However, even when the content of additives is high, the levels found in the organs are not sufficient. This is because the body limits the absorption and use of many elements and nutrients (Leontowicz et al. 2006). Factors which can limit the absorption of calcium and magnesium are age, pH of food content, ratio of calcium to phosphorus, fractions of fiber, consumption of protein and fat, lactose, phytates and intake of calcium and magnesium. Research has shown that insoluble fiber negatively correlates with the bioavailability of minerals, unlike soluble fiber (Heaney et al. 1988, Gralak et al. 1996). Another study suggested that the bioavailability of calcium can decrease by 10% when there is an excess of fiber (Soto et al. 2016). This can explain the lower content of these elements in the organs of rats which were fed the diet including breads enriched with seeds. Research which was carried out on human subjects in Norwich (UK) showed that casein can increase the absorption of calcium by up to 10% (Teucher et al. 2006). In this research, 14% of casein was used in the diet with breads and 20% in the diet without breads. *In vitro* studies have shown that high fat intake has a negative influence on the bioavailability of calcium (Cilla et al. 2011). This is due to the lower content of calcium in organs, which is correlated with the higher amount of additives in gluten-free breads. Seeds in breads contain fat, which is the cause of lower absorption of calcium. Calcium and magnesium compete for absorption, which leads to a high content of calcium in the diet but a low content in the femur (Creedon, Cashman 2001). Research in this area is as yet inconclusive. However, it has been shown that higher intake of calcium results in lower absorption of magnesium (Matsuzaki et al. 2005). Another study has shown that the content of magnesium is not correlated with higher calcium intake (Roncero-Ramos et al. 2013). Increase in the calcium content in rats' liver can be a signal of pancreatitis, but this area is still not well understood. Some researchers have suggested the need for more research (Li et al. 2014). Less is known about the mechanisms responsible for increasing the magnesium content in bone, but this can involve the endocrine system (Castiglioni et al. 2013). An experiment carried out on rats in Tokyo showed higher absorption of calcium by rats with magnesium deficiencies compared to the control group. Clinical tests have shown that low calcium levels might influence low magnesium levels in the blood (Yamamoto et al. 2011). The ambiguity of these results makes further research necessary.

It is also necessary to monitor the additives used in diets and finished products in order to obtain a composition of additives which is a good source of calcium and magnesium with high bioavailability.

CONCLUSION

The higher content of calcium and magnesium in enriched gluten-free breads did not influence the general nutrition parameters or the content of these minerals in the blood of rats. This diet had some influence on the content of calcium and magnesium in selected organs of the rats.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The study was approved by the Local Ethics Commission in Poznan, Poland (Approval number 888/11).

REFERENCES

Barera G., Mora S., Brambilla P., Ricotti A., Menni L., Beccio S., Bianchi C. 2000. *Body composition in children with celiac disease and the effects of a gluten-free diet: a prospective case-control study*. Am. J. Clin. Nutr., 72: 71-75 https://doi.org/10.1093/ajcn/72.1.71

BIANCHI M.-L., BARDELLA M.T. 2008. *Bone in celiac disease*. Osteoporos. Int. 19: 1705-1716. https://doi.org/10.1007/s00198-008-0624-0

BOUAZZAOUI N., MULENGI J.K. 2018. *Fatty acids and mineral composition of melon (Cucumis* melo*) and pumpkin (Cucurbita moschata) seeds*. J. Herbs. Spices Med. Plants, 24: 315-322. https://doi.org/10.1080/10496475.2018.1485125

BOURGUIGNON C., DUPUY A.M., COSTE T., MICHEL F., CRISTOL J.P. 2014. *Evaluation of NM-BAPTA method for plasma total calcium measurement on Cobas 8000®*. Clin. Biochem., 47: 636-639. https://doi.org/10.1016/J.CLINBIOCHEM.2013.12.027

CASTIGLIONI S., CAZZANIGA A., ALBISETTI W., MAIER J.A.M. 2013. *Magnesium and osteoporosis: current state of knowledge and future research directions*. Nutrients, 5: 3022-3033. https://doi.org/10.3390/nu5083022

CATASSI C., KRYSZAK D., BHATTI B., STURGEON C., HELZLSOUER K., CLIPP S.L., GELFOND D., PUPPA E., SFERRUZZA A., FASANO A. 2010. *Natural history of celiac disease autoimmunity in a USA cohort followed since 1974*. Ann. Med., 42: 530-538. https://doi.org/10.3109/07853890.2010.514285

CILLA A., LAGARDA M.J., ALEGRÍA A., DE ANCOS B., CANO M.P., SÁNCHEZ-MORENO C., PLAZA L., BARBERÁ R. 2011. *Effect of processing and food matrix on calcium and phosphorous bioavailability from milk-based fruit beverages in Caco-2 cells*. Food Res. Int., 44: 3030-3038. https://doi.org/10.1016/J.FOODRES.2011.07.018

CREEDON A., CASHMAN K.D. 2001. *The effect of calcium intake on bone composition and bone resorption in the young growing rat*. Br. J. Nutr., 86: 453-459.

FANG L., REN D., WANG Z., LIU C., WANG J., MIN W. 2018. *Protective role of hazelnut peptides on oxidative stress injury in human umbilical vein endothelial cells*. J. Food Biochem., 43, e12722. https://doi.org/10.1111/jfbc.12722

GHAFOOR K., ÖZCAN M.M., AL-JUHAIMI F., BABIKER E.E., FADIMU G. J. 2019. *Changes in quality, bioactive compounds, fatty acids, tocopherols, and phenolic composition in oven- and microwave-roasted poppy seeds and oil*. LWT, 99: 490-496. https://doi.org/10.1016/J.LWT.2018.10.017

GRALAK M.A., LEONTOWICZ M., MORAWIEC M., BARTNIKOWSKA E., KULASEK G.W. 1996. *Comparison of the influence of dietary fibre sources with different proportions of soluble and insoluble fibre on Ca, Mg, Fe, Zn, Mn and Cu apparent absorption in rats*. Arch. Tierernahr., 49: 293-299.

GUNDERSEN. 2017. *Standard Operating Procedure References Detail INTENDED USE, Magnesium Integra 400*.

HEANEY R.P., WEAVER C.M., RECKER R.R. 1988. *Calcium absorbability from spinach*. Am. J. Clin. Nutr., 47: 707-709. https://doi.org/10.1093/ajcn/47.4.707

HORSTMANN S.W., ATZLER J.J., HEITMANN M., ZANNINI E., LYNCH K.M., ARENDT E.K. 2019. *A comparative study of gluten-free sprouts in the gluten-free bread-making process*. Eur. Food Res. Technol., 245: 617-629. https://doi.org/10.1007/s00217-018-3185-2

HUANG J., YOKOYAMA W.H., KIM Y. 2019. *Soy noodles processed from soy flour or tofu affects antioxidant content, lipid accumulation in 3T3-L1 cells, and plasma lipids in hamsters*. J. Food Process. Preserv., 43, e13871. https://doi.org/10.1111/jfpp.13871

IANNELLO S., BELFIORE F. 2001. *Hypomagnesemia. A review of pathophysiological, clinical and therapeutical aspects*. Panminerva Med., 43: 177-209.

IRAKLI M., MYGDALIA A., CHATZOPOULOU P., KATSANTONIS D. 2019. *Impact of the combination of sourdough fermentation and hop extract addition on baking properties, antioxidant capacity and phenolics bioaccessibility of rice bran-enhanced bread*. Food Chem., 285: 231-239. https://doi.org/10.1016/J.FOODCHEM.2019.01.145

KVASHIN A.A., NESHCHADIM N.N., YABLONSKAY E.K., GORPINCHENKO K.N. 2018. *Crop yield and the quality of sunflower seeds in the use of fertilizers and growth regulation substances*. Helia, 41: 227-239. https://doi.org/10.1515/helia-2017-0014

LEONARD M.M., FOGLE R., ASCH A., KATZ A. 2016. *Screening for Celiac Disease in a Pediatric Primary Care Setting*. Clin. Pediatr. (Phila)., 55: 214-218 https://doi.org/10.1177/0009922815593879

LEONARD M.M., SAPONE A., CATASSI C., FASANO A. 2017. *Celiac Disease and Nonceliac Gluten Sensitivity*. JAMA, 318: 647-656. https://doi.org/10.1001/jama.2017.9730

LEONTOWICZ H., LEONTOWICZ M., STRYCZEK A., GORINSTEIN S. 2006. *Effect of apple pulp on calcium, magnesium and manganese content in organs of rats fed atherogenic diets*. Pol. J. Food Nutr. Sci., 15: 55-58.

LI J., ZHOU R., ZHANG J., LI Z.-F. 2014. *Calcium signaling of pancreatic acinar cells in the pathogenesis of pancreatitis*. World J. Gastroenterol., 20: 16146-16152. https://doi.org/10.3748/wjg.v20.i43.16146

LIONETTI E., CATASSI C. 2014. *Co-localization of gluten consumption and HLA-DQ2 and -DQ8 genotypes, a clue to the history of celiac disease*. Dig. Liver Dis., 46: 1057-1063. https://doi.org/10.1016/j.dld.2014.08.002

MATSUZAKI H., KATSUMANTA S.I., UEHARA M., SUZUKI K., NAKAMURA K. 2005. *Effects of high calcium intake on bone metabolism in magnesium-deficient rats*. Magnes. Res., 18: 97-102.

PAJĄK P., SOCHA R., BRONIEK J., KRÓLIKOWSKA K., FORTUNA T. 2019. *Antioxidant properties, phenolic and mineral composition of germinated chia, golden flax, evening primrose, phacelia and fenugreek*. Food Chem., 275: 69-76. https://doi.org/10.1016/J.FOODCHEM.2018.09.081

PASQUALONE A., CAPONIO F., SUMMO C., PARADISO V.M., BOTTEGA G., PAGANI M.A. 2010. *Gluten-Free Bread Making Trials from Cassava (Manihot esculenta* Crantz*) flour and sensory evaluation of the final product*. Int. J. Food Prop., 13: 562-573. https://doi.org/10.1080/10942910802713172

REEVES P.G. 1997. *Components of the AIN-93 diets as improvements in the AIN-76A diet*. J. Nutr., 127: 838-841. https://doi.org/10.1093/jn/127.5.838S

REGULA J., CERBA A., SULIBURSKA J., TINKOV A.A. 2018. *In vitro bioavailability of calcium, magnesium, iron, zinc, and copper from gluten-free breads supplemented with natural additives*. Biol. Trace Elem. Res., 182: 140-146. https://doi.org/10.1007/s12011-017-1065-4

REGUŁA J., KREJPCIO Z., STANIEK H. 2010. *Bioavailability of iron from cereal products enriched with dried shittake mushrooms (Lentinula edodes) as determined by iron regeneration efficacy method in female rats*. J. Med. Food, 13: 1189-1194. https://doi.org/10.1089/jmf.2009.0200

REGUŁA J., KREJPCIO Z., STANIEK H. 2016. *Iron bioavailability from cereal products enriched with Pleurotus ostreatus mushrooms in rats with induced anaemia*. Ann. Agric. Environ. Med., 23: 310-314. https://doi.org/10.5604/12321966.1203896

RONCERO-RAMOS I., PASTORIZA S., PILAR NAVARRO M., DELGADO-ANDRADE C. 2013. *Consumption of model maillard reaction products has no significant impact on Ca and Mg retention or on tissue distribution in rats*. Int. J. Vitam. Nutr. Res., 83: 246-253. https://doi.org/10.1024/0300-9831/a000167

SOTO A.M., GARCIA M.L., SELGAS M.D. 2016. *Technological and sensory properties of calcium-enriched dry fermented sausages: A study of the calcium bioavailability*. J. Food Qual., 39: 476-486. https://doi.org/10.1111/jfq.12223

SULIBURSKA J., KREJPCIO Z., REGUŁA J., GROCHOWICZ A. 2013. *Evaluation of the content and the potential bioavailability of minerals from gluten-free products*. Acta Sci. Pol. Technol. Aliment., 12: 75-80.

SULIBURSKA J., KRÓL E., STANIEK H., WÓJCIAK R.W., REGUŁA J., MARCINEK K., KREJPCIO Z. 2015. *Calcium, magnesium, iron, zinc, and copper status in women aged 18-70 years determined by hair analysis*. Probl Hig Epidemiol, 96: 444-447. (in Polish)

ŚWIECA M., REGUŁA J., SULIBURSKA J., ZŁOTEK U., GAWLIK-DZIKI U. 2015. *Effects of gluten-free breads, with varying functional supplements, on the biochemical parameters and antioxidant status of rat serum*. Food Chem., 182: 268-274. https://doi.org/10.1016/j.foodchem.2015.03.014

Teucher B., Majsak-Newman G., Dainty J.R., McDonagh D., FitzGerald R.J., Fairweather-Tait S.J. 2006. *Calcium absorption is not increased by caseinophosphopeptides*. Am. J. Clin. Nutr., 84: 162-166. https://doi.org/10.1093/ajcn/84.1.162

Tovoli F. 2015. *Clinical and diagnostic aspects of gluten related disorders*. World J. Clin. Cases, 3: 275. https://doi.org/10.12998/wjcc.v3.i3.275

Wójciak R. W., Staniek H., Mojs E., Król E., Marcinek K., Krejpcio Z. 2017. *The effect of different forms of food deprivation on calcium and magnesium concentrations in the serum, brain and femoral bone of female wistar rats*. J. Elem., 22: 1005-1014. https://doi.org/10.5601/jelem.2016.21.2.1149

Yamamoto M., Yamaguchi T., Yamauchi M., Yano S., Sugimoto T. 2011. *Acute-onset hypomagnesemia-induced hypocalcemia caused by the refractoriness of bones and renal tubules to parathyroid hormone*. J. Bone Miner. Metab., 29: 752-755. https://doi.org/10.1007/s00774-011-0275-7

Zieliński H., Szawara-Nowak D., Bączek N., Wronkowska M. 2019. *Effect of liquid-state fermentation on the antioxidant and functional properties of raw and roasted buckwheat flours*. Food Chem., 271: 291-297. https://doi.org/10.1016/J.FOODCHEM.2018.07.182