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THE INFLUENCE OF SYNBIOTICS ON MAGNESIUM BIOAVAILABILITY FROM DIETS IN RATS

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Abstract

Products containing pro- and prebiotics are known as synbiotics. The benefits of pro- and prebiotics on the host include: normalization of the microbial balance in the gastrointestinal tract, increase of mineral bioavailability, reduction of cholesterol level in blood and prevention of gastrointestinal disorders. The aim of the work was to compare the apparent absorption and retention indexes in rats fed diets containing probiotic or synbiotic soft cheeses. As a probiotic, the strain *Lactobacillus plantarum* 14 was used, whereas as prebiotics inulin HPX and maltodextrin were used. For 10 days, the animals were fed diets consisting of 61-81% of soft cheese with probiotic (A diet), probiotic and 2,5% of inulin HPX (B diet) and probiotic and 2.5% of maltodextrin (C diet). On the basis of the magnesium concentration in the diets and the urine and faeces excreted during the last 5 days of the experiment, the apparent absorption (A) and retention (R) indexes (%, mg 5 days^{-1}) were calculated. The apparent absorption indexes obtained did not differ statistically among the groups, although the highest value of apparent absorption (A%) was obtained in group B. The apparent retention indexes in group A were significantly higher (p < 0.05) compared to groups B and C. On the other hand, in B and C groups increased faecal mass was detected, but the inulin influence was stronger than that of maltodextrin. Although the short-term supplementation of rat diets with inulin HPX and maltodextrin did not increase magnesium absorption and retention, their use in probiotic products is reasonable because of the beneficial physiological effects.

Key words: probiotics, prebiotics, magnesium, absorption, retention, rats, soft cheese.

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WPŁYW SYNBIOTYKÓW NA BIODOSTĘPNOŚĆ MAGNEZU Z DIETY U SZCZURÓW

Abstrakt

Jako produkty synbiotyczne są określane wyroby zawierające jednocześnie probiotyki i prebiotyki. Korzystny wpływ pro- i prebiotyków na organizm obejmuje m.in. normalizację składu mikroflory przewodu pokarmowego, zwiększanie biodostępności składników mineralnych, obniżanie poziomu cholesterolu we krwi oraz zapobieganie występowaniu zaburzeń jelitowych. Celem pracy było porównanie wpływu diety zawierającej probiotyczny i synbiotyczne serki twarogowe na absorpcję i retencję magnezu u szczurów. Zastosowanym szczepem probiotycznym był *Lactobacillus plantarum* 14, a prebiotykami inulina HPX oraz maltodekstryna średniosckukrzona. Zwierzętom przez 10 dni podawano diety, w skład których wchodził serek twarogowy, w ilości 61-81%, zawierający: probiotyk (dieta A), probiotyk i 2,5% inuliny HPX (dieta B) lub probiotyk i 2,5% maltodekstryny (dieta C). Na podstawie zawartości magnezu w diecie, kale i moczu wydalonego w czasie ostatnich 5 dni eksperymentu, wyznaczono współczynniki absorpcji (A) i retencji (R) pozornej (%, mg 5 dni^{-1}). Uzyskane współczynniki absorpcji nie różniły się znacząco między grupami zwierząt, chociaż najwyższy (A%) odnotowano w grupie B, natomiast wartości współczynników retencji pozornej w grupie A były istotnie wyższe (p < 0,05) w porównaniu z grupami B i C. W grupach przyjmujących dietę synbiotyczną obserwowano zwiększenie masy kału w porównaniu z grupą kontrolną A, przy czym działanie inuliny HPX było silniejsze niż maltodekstryny. Chociaż krótkotrwała suplementacja diety szczurów inuliną HPX i maltodekstryną średnioscukrzoną nie przyczyniła się do wzrostu absorpcji i retencji magnezu, to stosowanie tych prebiotyków łącznie ze szczepem probiotycznym jest uzasadnione ze względu na korzystne efekty fizjologiczne.

Słowa kluczowe: probiotyki, prebiotyki, magnez, absorpcja, retencja, szczury, ser twarogowy.

INTRODUCTION

Studies into the acquisition of new probiotic cultures and their application in the food production process have been underway for years. Probiotics, live cultures of bacteria and fungi (Sanders, Klaenhammer 2001, Holzapfel, Schillinger 2002), are applied both in food of animal origin, including fermented dairy drinks, ripening cheeses, white fresh cheeses, fermented sausages, as well as in food of plant origin. Such products are sought by consumers aware of the positive effect of probiotic bacteria on the human body. These effects include: normalization of intestinal microflora, prevention or attenuation of disorders and diseases of the alimentary tract, and strengthening the immune system. Probiotic bacteria synthesize B group vitamins, folic and nicotinic acids, increase the availability of proteins as well as the absorption of the minerals Ca, Cu, Fe, Mn, P and Zn, and are likely to contribute to a reduction in the blood level of cholesterol (Defecińska, Libudzisz 2000, Kaur et al. 2002). Similar effects in terms of normalizing the composition of alimentary tract microflora, increasing bioavailability of minerals, preventing intestinal disorders and reducing cholesterol level in blood are produced by prebiotics (BLAUT 2002, LOSADA, OLLEROS 2002). They are the dietary components which escape digestion in the small intestine, are transferred intact to the colon, where they are utilized by probiotic microflora. Prebiotics are substrates that selectively stimulate the development of a given species or strain of probiotic bacteria, thus exerting a beneficial effect on the health status of the host (ŚLIŻEWSKA, LIBUDZISZ 2002, ZDUŃCZYK 2002, CUMMINGS et al. 2004). Carbohydrates that display characteristics of prebiotic substances include inulins and maltodextrin, widely applied in the food industry due to their functional properties, i.e. gel-forming ability, stabilization and concentration of emulsions and providing foodstuffs with attractive sensory attributes (VORAGEN 1998, FORTUNA, SOBOLEWSKA 2000, JAKUBCZYK, KOSIKOWSKA 2000, POLAK 2001, KŁĘBUKOWSKA et al. 2002, KRZYŻANIAK et al. 2003, SKOWRONEK, FIEDUREK 2003).

Other food products that also contain prebiotics are referred to as synbiotics. Since the concept of synbiotics is relatively new, there are few reports of interactions between pro- and prebiotics. Bearing in mind their properties, prebiotics should positively affect the growth and survivability of probiotics. By adapting its metabolism to a specified substrate (prebiotic), a probiotic strain has a greater chance for colonizing the gastrointestinal tract owing to increased ability to compete with the existing microflora (FOOKS et al. 1999, SAARELA et al. 2000, PUPPONEN-PIMIA et al. 2003). A combined application of probiotics and prebiotics should, therefore, increase the efficacy of their action onto the host's body.

The research was aimed at comparing the absorption and retention of magnesium in rats administered a prebiotic diet containing soft cheese produced with addition of *Lactobacillus plantarum* strain as well as a probiotic-containing diet additionally supplemented with prebiotics, inulin HPX and medium-saccharified maltodextrin.

MATERIAL AND METHODS

Experiments were carried out on 18 standardized white experimental rats of Wistar strain, obtained from the Department of Biological Analysis of Food, Institute of Animal Reproduction and Research of the Polish Academy of Sciences in Olsztyn. Initial body weight of the animals ranged from *ca* 91 to 98 g. They were divided into 3 experimental groups, 6 rats each, and kept in single metabolic cages, which enabled separate collection of urine and faeces.

Experimental diets were prepared based on soft cheeses, produced in a dairy plant, containing inulin HPX (Orafti, Belgium), medium-saccharified maltodextrin (Pepes Sp. z o.o., Poland) and a potentially probiotic strain *Lactobacillus plantarum* 14. The basic composition of the diets was as follows: protein – 10% (N x 6.38), vitamins – 1% (AOAC 1975), mineral salts – 3% (NRC 1976), potato starch – 5%, and maize starch – supplementing diet composition to 100 g of dry matter of diet. Fat content of the soft cheese was taken into account while balancing the diets. Three diets were prepared in the study: A – probiotic diet (control): with soft cheese containing *L. plantarum* as well as synbiotic diets containing, apart from the probiotic culture, a prebiotic: inulin HPX (diet B) or medium-saccharified maltodextrin (diet C). The addition of prebiotics to the soft cheeses reached 2.5%. In order to obtain a similar dry matter content in all products, 2.5% of skimmed milk powder was added to the probiotic soft cheese. The contribution of particular components in the experimental diets is presented in Table 1, whereas the physicochemical characteristics of soft cheeses used in the study can be found in Table 2. The count of *L. plantarum* 14 strain in the products was 10^7cfu g^{-1}.

Table 1
Tabela 1

Compositions of diet used in the feeding trial (g 100 g^{-1} of d.m.)
Udział poszczególnych komponentów w dietach sporządzonych do doświadczenia żywieniowego (g 100 g^{-1} s.s.)

Specification Wyszczególnienie	Diets Diety		
	A	B	C
Cheese Ser	60.8	81.1	69.6
Vitamins Witaminy	1.0	1.0	1.0
Mineral salts Sole mineralne	3.0	3.0	3.0
Potato starch Skrobia ziemniaczana	5.0	5.0	5.0
Corn starch Skrobia kukurydziana	30.3	9.9	21.4

Diets containing soft cheese with: A – probiotic strain *L. plantarum*, B – probiotic strain *L. plantarum* and 2.5% of inulin HPX, C – probiotic strain *L. plantarum* and 2.5% of maltodextrin.
Diety zawierające serek z: A – probiotycznym szczepem *L. plantarum*, B – probiotycznym szczepem *L. plantarum* i 2,5% inuliny HPX, C – probiotycznym szczepem *L. plantarum* i 2,5% maltodekstryny średnioscukrzonej.

A balanced experiment was conducted, including a 5-day preliminary period and a 5-day experimental period. Diet intake was monitored each day by collection of leftovers and samples of faeces and urine were collected for analyses.

Table 2
Tabela 2

Physicochemical composition of white cheeses
Charakterystyka fizykochemiczna serków twarogowych

Specification Wyszczególnienie	Cheese Ser		
	A	B	C
Dry weight Sucha masa (%)	35.2	35.4	36.0
Protein Białko ogółem (%)	5.8	4.4	5.2
Fat Tłuszcz (%)	24.5	25.0	26.0
Ash Popiół ogółem (%)	0.7	0.5	0.5
Magnesium Magnez (mg g^{-1})	96.4	69.4	70.5

Soft cheese with: A – probiotic strain *L. plantarum*, B – probiotic strain *L. plantarum* and 2.5% of inulin HPX, C – probiotic strain *L. plantarum* and 2.5% of maltodextrin
Serek z: A – probiotycznym szczepem *L. plantarum*, B – probiotycznym szczepem *L. plantarum* i 2,5% inuliny HPX, C – probiotycznym szczepem *L. plantarum* i 2,5% maltodekstryny średnioscukrzonej

A quantitative analysis of magnesium in the diet, faeces and urine of rats was carried out by means of flame atomic absorption spectrophotometry (Unicam 393, Solar). Measurements were performed at a wavelength of 285.2 nm.

Bioavailability of magnesium was expressed by means of coefficients of apparent absorption (A) and apparent retention (R). The first was calculated from the difference between the quantity of the mineral absorbed with diet and its quantity excreted with faeces, whereas the latter was computed as the difference between the quantity of the mineral absorbed with the diet and that excreted with faeces and urine. The values obtained were expressed in mg 5 days^{-1} and in percentage units. The results were presented as mean values ± standard deviation.

Statistical analysis of the results was carried out with Duncan's test (Statistica 6.0, StatSoft. Inc.) at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Soft cheeses produced with addition of potentially probiotic bacteria and prebiotics were observed to differ in magnesium content (Table 2).

The highest content of this mineral was determined in probiotic cheeses (96.4 mg g^{-1}), whereas the lowest one was in synbiotic products (69.4 mg g^{-1} and 70.5 mg g^{-1}). The intake of magnesium was at a similar level in all groups (Table 3), though its lowest level was observed in group B. Coefficients of apparent absorption (mg 5 $days^{-1}$, %) did not differ significantly between the groups of animals, yet the lowest value of A (%) was recorded in the group of rats administered a diet with synbiotic soft cheese containing inulin HPX (Table 3). In turn, values of apparent retention coefficients (mg 5 $days^{-1}$ and %) determined in the control group, fed a diet with the probiotic soft cheese, were significantly higher ($p < 0.05$) as compared to the groups administered synbiotic soft cheeses.

Table 3
Tabela 3

Mean values of Mg intake, apparent absorption and retention in rats fed probiotic or synbiotic cheeses
Średnie wartości spożycia, absorpcji i retencji magnezu u szczurów karmionych probiotycznym lub synbiotycznymi serkami twarogowymi

Specification Wyszczególnienie		Diet Dieta		
		A n=5	B n=6	C n=4
Mg intake Spożycie Mg	(mg 5 $days^{-1}$) (mg 5 dni^{-1})	22.8 ± 1.5 ^{A}	20.5 ± 1.1 ^{B}	22.8 ± 0.8 ^{A}
Apparent absorption Absorpcja pozorna	(mg 5 $days^{-1}$) (mg 5 dni^{-1})	19.1 ± 1.7 ^{A}	17.6 ± 1.2 ^{A}	18.6 ± 1.2 ^{A}
Apparent absorption Absorpcja pozorna	(%)	83.6 ± 3.6 ^{A}	85.8 ± 3.8 ^{A}	81.7 ± 5.0 ^{A}
Apparent retention Retencja pozorna	(mg 5 $days^{-1}$) (mg 5 dni^{-1})	6.0 ± 1.2 ^{A}	4.0 ± 0.5 ^{B}	4.7 ± 0.5 ^{B}
Apparent retention Retencja pozorna	(%)	26.1 ± 4.2 ^{A}	19.6 ± 2.9 ^{B}	20.6 ± 2.0 ^{B}

Diets containing soft cheese with: A – probiotic strain *L. plantarum*, B – probiotic strain *L. plantarum* and 2.5% of inulin HPX, C – probiotic strain *L. plantarum* and 2.5% of maltodextrin; ^{AB}row mean values without the same superscripts differ statistically ($p < 0.05$).
Diety zawierające serek z: A – probiotycznym szczepem *L. plantarum*, B – probiotycznym szczepem *L. plantarum* i 2,5% inuliny HPX, C – probiotycznym szczepem *L. plantarum* i 2,5% maltodekstryny średnioscukrzonej; ^{AB}średnie w rzędach bez wspólnych indeksów różnią się statystycznie ($p < 0,05$)

Among the physiological effects of administering a prebiotic-enriched diet to the rats increased mass of faeces was observed. In the groups fed diets with synbiotic soft cheese, the mass of faeces (group B – 2.86 g, group C – 2.24 g) was higher than in control group A (1.81 g), although the effect of inulin HPX was stronger than that of maltodextrin. Simultaneously, no increased excretion of magnesium along with the increased mass of faeces was observed in the group of animals fed a diet with inulin-containing soft cheese (Figure 1).

Issues referring to a possible increase in bioavailability of elements, i.e. calcium, phosphorus or magnesium, achieved through diet supplementation with prebiotics have already been addressed to and described. Studies on this subject enabled researchers to clarify the mechanism of prebiotic action. It has been demonstrated that microbiological fermentation of prebiotics in the colon decreases pH of intestinal digesta, which in turn causes an increase in the solubility of mineral compounds, thus enhancing

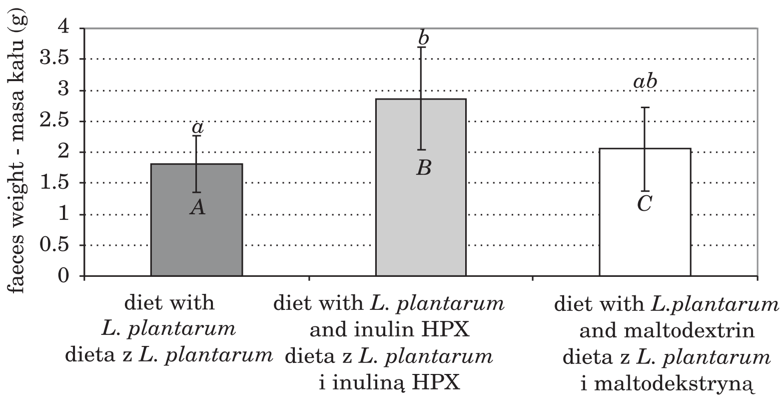

Fig. 1. The influence of the diet on faeces weight
[ab]mean values in the figure without the same superscripts differ statistically ($p < 0.05$)

Rys. 1. Wpływ diety na masę kału
[ab]średnie wartości na wykresie bez wspólnych indeksów różnią się statystycznie ($p < 0,05$)

their bioavailability (Baba et al. 1996, Scholz-Ahrens et al. 2001). Stimulation of magnesium absorption upon diet enrichment with prebiotic has already been observed in both long- and short-term studies (Lopez et al. 2000, Coudray et al. 2005) carried out on young, growing animals and on mature individuals (Rashka, Daniel 2005), which received diets that either met demands for this mineral or were magnesium-deficient (Ohta et al. 1994), and various prebiotics (Coudray et al. 2003a) at doses of 1–10%. The results obtained by Lobo et al. (2006) indicate that a 5% contribution of fructooligosaccharides (FOS) in a diet of animals lead to a decrease in the quantity of magnesium excreted with faeces as well as an increase of intestinal absorption of this element as compared to the control group. Similar observations were made in the reported study – the group of rats fed a diet with soft cheese containing *L. plantarum* strain and 2.5% of inulin HPX (B) was characterized by the lowest quantity of magnesium excreted with faeces and the highest apparent absorption (A%). Nevertheless, caution should be paid to the fact that in this group absorption

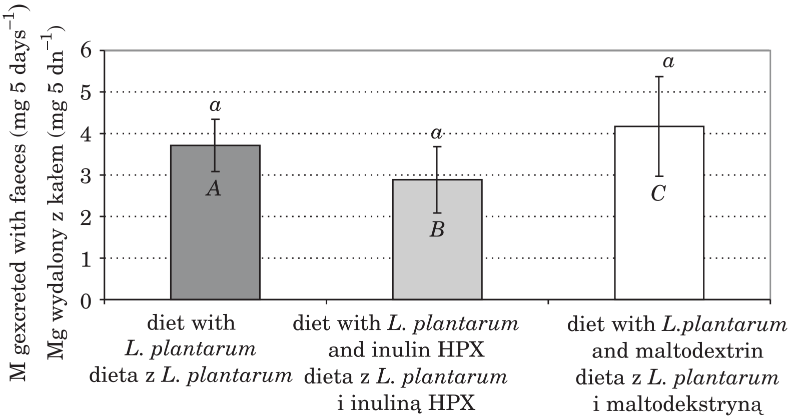

Fig. 2. The influence of the diet on amount of magnesium excreted with faeces
[ab]mean values in the figure without the same superscripts differ statistically ($p < 0.05$)

Rys. 2. Wpływ diety na ilość magnezu wydalonego z kałem
[ab]średnie wartości na wykresie bez wspólnych indeksów różnią się statystycznie ($p < 0,05$).

expressed in mg 5 days^{-1} was lower, and differences in the absorption coefficients (A%, A mg 5 days^{-1}) and quantity of magnesium excreted with faeces were not statistically significant between the groups examined.

Ohta et al. (1998) as well as Wolf et al. (1998) demonstrated the possibility of increasing magnesium absorption through diet supplementation with fructooligosaccharides (FOS) applied at concentrations of 1–10%. A dependency was reported between the content of prebiotics in a diet and their effect on magnesium absorption – increased absorption was observed along with an increasing concentration of prebiotic and, what is more, that effect occurred already at 1% FOS addition to diet (Wolf et al. 1998). Simultaneously, Wolf et al. (1998) did not found any increase in the apparent retention of magnesium in any of the experimental groups administered with 1–5% FOS, which was also confirmed in the current experiment.

In the studies by Lopez et al. (2000), Coudray et al. (2003a), Rashka, Daniel (2003), coefficients of apparent absorption of magnesium ranged from 27% (in groups fed diets without prebiotics) to 84%. In the reported study, all groups of animals were characterized by high coefficients of apparent absorption (82-86%). It was due to the administration of a diet whose major component (61-81%) were soft cheeses containing lactose. The presence of such monosaccharides as lactose or lactullose in a diet was likely to contribute to the increased permeability of cellular membranes of the intestinal epithelium, thus facilitating magnesium absorption. Furthermore, lactose – which is utilized by bacteria colonizing the colon – contributes to a decreasing pH of intestinal digesta, which additionally makes magnesium absorption easier (Coudray et al. 2003b). A correlation between apparent absorption of magnesium and lactose content of diet was also observed by DELISLE et al. (1995), who reported on the higher bioavailability of this element in a diet containing milk and milk powders, as compared to a diet with the addition of cheese – containing less lactose than milk and milk powder. The effect of lactose partly digested in the upper sections of the alimentary tract on the absorption of magnesium is, however, weaker than that of carbohydrate prebiotics undergoing fermentation already in the colon (COUDRAY et al. 2003b).

CONCLUSIONS

1. Unlike food products of plant origin, dairy products do not constitute a rich source of magnesium, yet the lactose they contain improves the bioavailability of this element.

2. The application of inulin HPX and medium-saccharified maltodextrin together with a prebiotic strain in diets for rats contributed to increased absorption and retention of magnesium, in contrast to a group fed a diet with probiotic soft cheese.

3. Supplementation of probiotic food products with prebiotics is advisable due to their beneficial physiological effects, including increasing mass of faeces observed in groups of animals fed diets containing inulin HPX and maltodextrin.

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