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BIOAVAILABILITY OF CALCIUM AND PHOSPHORUS FROM DIETS CONTAINING WHITE CHEESES SUPPLEMENTED WITH PREBIOTICS IN RATS

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The intake of food products containing prebiotics has a beneficial effect on the functioning of the gastrointestinal tract and may also contribute to increased mineral absorption from a diet. In the food industry prebiotics (inulins and maltodextrins) are also used because of their functional properties such as stabilizing emulsions. A study was undertaken to investigate the effect of inulin HPX and maltodextrin, applied in the production of white soft cheese as stabilizers, at a dose of 2.5%, on the bioavailability of calcium and phosphorus in rats. In the study, Wistar rats (n = 6 per group) were fed diets composed of cheese without prebiotic (control) or cheeses containing either 2.5% of inulin or maltodextrin, for 10 days. The bioavailability of the minerals was expressed by means of apparent absorption (A) and retention (R) coefficients (mg/5 days,%). The apparent Ca absorption and retention (mg/5 days) were the highest in the control group ($p < 0.05$), whereas the A (%) and R (%) coefficients did not differ significantly between the groups. Also, the apparent absorption of P (mg/5 days) was the highest ($p<0.05$) in the control group and no differences in A (%), R (mg/5 days), R (%) coefficients were noted among the groups. Inulin HPX and maltodextrin used in white cheese production do not increase Ca nor P bioavailability, which suggests that the technologically-justified 2.5% dose is too low to exert a positive effect on mineral balance.

INTRODUCTION

The application of dietary fiber in food technology is gaining an increasing interest due to its multiple functional properties, including its capacity for gelling, stabilizing emulsions and increasing the viscosity of food products. Dietary fibers that are not digested in the small intestine and reach the colon intact, where they stimulate the growth or activity of a limited number of bacteria (thus exerting a positive influence on human health), have been defined as prebiotics [Crittenden & Playne, 1996; Roberfroid *et al.*, 1998; Losada & Olleros, 2002; Śliżewska & Libudzisz, 2002; Zduńczyk, 2002]. The best recognized carbohydrate prebiotics include fructo-oligosaccharides, galacto-oligosaccharides, xylo-oligosaccharides, isomalto-oligosaccharides, soybean oligosaccharides, and lactulose [Ziemer & Gibson, 1998].

Amongst the above-mentioned oligosaccharides, of high interest are fructo-oligosaccharides (FOS) which occur in such plants as: chicory, garlic, onion, artichoke, and asparagus, yet in too low concentrations to evoke a favorable effect on intestinal microflora. On an industrial scale, they can be produced enzymatically [Roberfroid, 2000]. Fructo-oligosaccharides are polymers of D-fructose linked with a β-(2→1) bond with α-(1→2) bonds of the terminal molecule of glucose [Roberfroid & Delzenne, 1998; Flamm *et al.*, 2001]. Substances with a degree of polymerization ranging from 2 to 60 are classified as inulin, whereas those whose degree of polymerization is lower than 10 are classified as oligofructose [Bosscher *et al.*, 2003].

Carbohydrates that possess prebiotic potential include also maltodextrins. They are water-soluble polysaccharides, not displaying a sweet taste, built of D-glucose linked with α(1→4) bonds, less commonly with α(1→6) glycoside ones. The degree of polymerization (DP) of maltodextrins is diversified and ranges from 3 to 20 glucose molecules. Usually, they are obtained in the pathway of partial hydrolysis of starch with the use of amylolytic enzymes [Voragen, 1998; Fortuna & Sobolewska, 2002; Krzyżaniak *et al.*, 2003].

The intake of food products containing dietary fiber with a prebiotic potential results in a variety of advantages: stimulation of the intestinal motility, suppressed absorption of fat and cholesterol, increased volume and water absorption of intestinal content, as well as increased absorption of calcium, magnesium and iron. A combination of those effects exerts a beneficial influence on health, reducing the risk of the incidence of intestinal disorders (constipation, diarrhea), diseases of the circulatory system and colonic carcinoma [Blaut, 2002; Losada & Olleros, 2002].

Currently, great effort is being put into elaboration of technologies in which additives to raw material not only affect the sensory and physicochemical properties of products, but also have a beneficial effect on the health of consumers. A great deal of studies concerning prebiotic influence on mineral absorption and retention were performed on animal models with the use of a standard diet supplemented with 0-10% of prebiotics. In the present study, as a major component of animals diet use was made of soft white cheese, produced

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in a dairy plant in Poland, without or with 2.5% of prebiotic. The relatively low dose of prebiotics was chosen on the basis of the preliminary studies which suggested that the most appropriate sensory properties possessed products containing 2.5% of inulin or maltodextrin. The study was undertaken, therefore, to determine the effect of HPX inulin and maltodextrin, applied in the production of white soft cheese, on the bioavailability of calcium and phosphorus from a diet in rats.

MATERIAL AND METHODS

Diets and animals

The experiment was carried out on 18 standardized white Wistar rats, obtained from the Department of Biological Analysis of Food, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences in Olsztyn, which were divided into 3 groups of 6 rats each (A, B, C groups). The initial body weight of the animals reached *ca*. 91-98 g. The animals were housed in individual metabolic metal-free cages in a room at 22°C and 65% relative humidity, with a 12-h light-dark cycle. Metabolic cages enabled separate collection of urine and feces. A balance experiment was carried out that involved a 5-day adaptation period and a 5-day proper experimental period. In the proper experimental period, food intake was recorded and feces were collected daily. Diets and water were provided *ad libitum*.

Three diets were prepared: A – control, with soft cheese without prebiotics, B – experimental with white cheese containing 2.5% of HPX inulin (polymerization degree DP≥23, Orafti, Belgium), and C – experimental with white cheese containing 2.5% of maltodextrin (dextrose equivalent DE=16.2, Pepes Sp. z o.o., Poland). Cheese used in the study was produced in a dairy plant from milk containing 13% of fat with commercial mesophilic lactic culture and rennet. After coagulation, curd was cut, subjected to thermization and next centrifugation in order to establish moisture content at a level of 63-65%. After cooling, 2.5% of inulin and maltodextrin were added. The cheese was stirred, packed in 2 kg containers and cooled to 6-8°C.

The approximate composition of the diets was as follows: protein – 10% (N x 6.38), vitamins – 1%, minerals – 3%, potato starch – 5%, corn starch – as a constituent supplementing the composition to 100 g of dry matter of the diet (Table 1). Vitamins per 1 g of the mixture included: A 2 000 IU, D_3 200 IU, E 10 UI, K 0.5 mg, choline chloride 200 mg, paraaminobenzoic acid 10.0 mg, inositol 10.0 mg, niacin 4.0 mg, calcium panthothenian 4.0 mg, B_2 0.8 mg, thiamine 0.5 mg, B_6 0.5 mg, folic acid 0.2 mg, biotin 0.04 mg, B_{12} 0.003 mg, glucose as a constituent supplementing the composition to 1 g [AOAC, 1975]. The mineral mixture supplied the following (g/kg): K_2HPO_4 81.0, K_2SO_4 68.0, NaCl 30.0, $CaCO_3$ 21.0, $NaHPO_4$ 21.4, MgO 18.0, corn starch 735.0, microelements 18.0, microelements mixture (g/100 g): ferric citrate (16.7% Fe) 31.0, $ZnCO_3$ (56% Zn) 4.5, $MnCO_3$ (44.4% Mn) 23.4, $CuCO_3$ (55.5% Cu) 1.85, KI 0.04, citric acid 39.21, (modified from mineral mixture [NRC, 1978]). Fat content of white cheeses was taken into account while balancing diets. The diet covered animals' requirements for energy, but contained a low level of dietary calcium, in order to demonstrate the impact of a prebiotic on calcium absorption from white cheeses.

TABLE 1. Diet composition in a feeding experiment on rats.

Constituent	Diets		
	A	B	C
White cheese (%)	61.9	70.8	69.3
Vitamins (%)	1.0	1.0	1.0
Minerals (%)	3.0	3.0	3.0
Potato starch (%)	5.0	5.0	5.0
Corn starch (%)	29.1	20.2	21.7
Ca (mg/g)	0.9825	0.7861	0.7653
P (mg/g)	1.176	1.051	1.054

Chemical analyses

Cheese used in the study was analysed for moisture, crude protein, fat and ash [AOAC, 1990]. The physicochemical composition of white cheese was presented in Table 2.

Quantitative determination of calcium in the diet, feces and urine of the rats was carried out with the method of flame atomic absorption spectrometry (Unicam 393, Solar). In order to determine calcium content, samples were diluted with lanthanum chloride, and measurements were taken under the following conditions: wavelength of 422.7 nm, slit width of 0.5 nm, lamp power of 80%, and time of 4.0 s [Whiteside & Miner, 1984]. To assay the content of phosphorus in white soft cheeses, diets, feces and urine, the colorimetric method was used [PN-76R-64781, 1976].

Calculations and statistics

The bioavailability of calcium and phosphorus was expressed with the use of coefficients of apparent absorption (A) and retention (R). The absorption coefficient was calculated as the difference between the quantity of minerals intaken and those excreted with feces. The retention coefficient was calculated as the difference between the quantity of minerals absorbed with a diet and excreted with feces and urine and expressed as (mg/5 days) and (%). The most distant results, which were outside the range of ±2 standard deviations around the group mean, were rejected. The results were presented as means±standard deviation. A statistical analysis of the results obtained was carried out with Duncan's test (Statistica 6.0, StatSoft. Inc.) at a significance level of $p<0.05$ to determine significant differences between group means [Winer *et al*., 1991].

RESULTS

The white cheeses supplemented with prebiotics used in the study were characterised by similar proximate chemical composition, especially the contents of calcium and phosphorus. In cheeses with inulin and maltodextrin, their content accounted for 0.9 mg/g of the product, and in the control cheese – for 1.15 mg Ca/g of product and 1.12 mg P/g of product (Table 2). In all products, the calcium:phosphorus ratio was close or equal to one.

Table 3 shows results obtained for absorption and retention of calcium in rats fed a diet based on white cheeses with prebiotics (diets B and C) and without a prebiotic supplement (diet

TABLE 2. Proximate chemical composition of white cheeses used for diet's preparation in a feeding experiment on rats.

Parameters	Cheese		
	A	B	C
Dry matter (%)	35.9	34.7	35.2
Protein (%)	5.80	4.90	5.08
Fat (%)	25.0	23.8	24.8
Ash (%)	0.650	0.546	0.524
Ca (mg/g)	1.148	0.882	0.875
P (mg/g)	1.115	0.910	0.934
Ca:P	1.03	0.97	0.94

TABLE 3. Apparent Ca absorption and retention in rats fed control and prebiotic cheeses.

Parameters	Diet		
	A (n=6)	B (n=4)	C (n=4)
Diet intake (g/5 days)	87.3±9.2	83.0±14.8	88.1±6.1
Ca intake (mg/5 days)	85.8±9.1^{A*}	65.3±11.6^{B}	67.4±4.7^{B}
Absorption (mg/5 days)	77.4±7.7^{A}	56.5±8.8^{B}	62.9±4.6^{B}
Absorption (%)	90.3±3.9	87.1±7.0	93.3±3.9
Retention (mg/5 days)	72.1±6.9^{A}	52.0±8.7^{B}	60.8±4.2^{B}
Retention (%)	84.2±4.6^{AB}	80.2±6.8^{B}	90.2± 3.6^{A}

* row means with no common superscripts differ ($p<0.05$)

A). Diet intake in the control and experimental groups was at a similar level, however, statistically significant differences were observed in the intake of calcium ($p<0.05$). Its intake by animals from the control group was substantially higher and reached 85.8 mg/5 days on average, as compared to 67.4 mg/5 days reported in group C and 65.3 mg/5 days in group B. These differences resulted from a higher content of calcium in the control white cheese and, consequently, in the diet that contained it. The control group was also characterised by the highest apparent absorption and retention of calcium expressed in (mg/5 days) ($p<0.05$). The highest values of apparent absorption expressed in (%) were observed in group C, in which the rats were administered a diet with the addition of maltodextrin, although differences between that and the other groups were not statistically significant. In the case of the apparent retention coefficient expressed in (%), the highest values were reported for animals of group C, however, no statistically significant differences were observed between group C and the control. Both experimental groups were statistically different in terms of apparent retention (R%).

Results referring to the absorption and retention of phosphorus from diets are presented in Table 4. The intake of diet and phosphorus was at a similar level in all groups of animals. The highest apparent absorption of phosphorus expressed in mg/5 days was observed in the control group, whereas that expressed in%– in the group receiving white cheese with maltodextrin. In both cases, however, no statistically significant differences were noted between control animals and those administered maltodextrin-supplemented white cheese.

TABLE 4. Apparent P absorption and retention in rats fed control and prebiotic cheeses.

Parameters	Diet		
	A (n=6)	B (n=5)	C (n=5)
Diet intake (g/5 days)	87.3±9.2	85.2±13.7	86.4±6.6
P intake (mg/5 days)	102.7±10.9	89.6±14.4	91.0±6.9
Absorption (mg/5 days)	91.0±10.3^{A*}	77.3±11.8 ^{B}	81.9±6.3^{AB}
Absorption (%)	88.6±2.3^{AB}	86.4±2.5 ^{A}	90.0±1.5^{B}
Retention (mg/5 days)	54.6±5.9	47.2±8.0	51.4±4.1
Retention (%)	53.3±4.2	52.9±4.2	56.4±1.9

*row means with no common superscripts differ ($p<0.05$)

Both the apparent retention expressed in mg/5 days and that expressed in% were at a similar level in all groups of animals.

DISCUSSION

Calcium, together with phosphorus, constitute the main component of bones, thus providing an appropriate level of calcium ingested with a diet is a prerequisite for a proper calcium balance. The recommended daily intake of calcium, stipulated by the National Food and Nutrition Institute, ranges from 600 to 1200 mg/day depending on the age, sex, and physiological condition [Karczmarewicz *et al.*, 2002]. According to Karczmarewicz *et al.* [2002], in Poland, dietary allowances for calcium are met at a level of barely 30-50%, hence, the application of additives stimulating calcium absorption in food products is highly substantiated.

The reported study did not demonstrate any statistically significant effect of prebiotics on the apparent absorption and retention of calcium and phosphorus in rats. Divergent results were obtained, among others, by Roberfroid *et al.* [2002], Zafar *et al.* [2004] and Lobo *et al.* [2006]. In their investigations, however, different inulin preparations, *e.g.* Raftilose, Synegy1 and oligofructose, were used in considerably higher doses (from 5% to 10%), which might have affected the enhanced absorption of calcium. The doses of prebiotics applied in the presented study resulted from technological premises, *i.e.* obtaining desirable consistency and taste of soft cheeses. In addition, rats' diet based on white cheeses contained additional constituents, which, in turn, caused the animals to ingest prebiotics at a level lower than 2.5%.

Studies carried out with volunteers into the possibility of enhancing calcium absorption through diet supplementation with fructooligosaccharides, yielded contradictory results that depended, to a great extent, on the preparation administered, differences in methodology of analyses and the population examined [Griffin *et al.*, 2003; Dahl *et al.*, 2005].

As in the reported study, Dahl *et al.* [2005] did not obtain any increase in calcium absorption in the elderly under institutional care, as a result of applying a daily dose of 15 g of inulin in juices. The content of inulin (Frutafit Inulin IQ) in juices reached 2.4%, thus, the dose of the prebiotic was similar to that applied in soft cheeses. Dahl *et al.* [2005] observed the positive effect of inulin on the physiology of the gastrointestinal tract, *i.e.* loosening stool bulk without any increase in the frequency of defecation, as well as a diminished need for administration of enemas in the pensioners. Despite no effect on calcium metabolism, the intake of prebiotic brought tangible health advantages to the volunteers.

From the nutritional point of view, the calcium-to-phosphorus ratio in a diet is considered important since, theoretically, the low Ca:P ratio contributes to suppressed colonic absorption of calcium. In the case of adults, it refers only to persons applying a calcium-rich diet [Powel *et al.*, 1999]. Investigations have pointed to the fact that in subjects ingesting calcium doses recommended by nutritionists, the Ca:P ratio had no effect on the absorption of this element. Simultaneously, a Ca:P ratio higher than 1 in a diet has been demonstrated to increase bone mass in young women [Powel *et al.*, 1999]. In the products used in the reported study, the Ca:P ratio was close to unity, however its value appeared to be the highest in the control group. The Ca:P ratio considered as the most beneficial to a human body is that occurring in milk, *i.e.* 1.2:1 [Kłobukowski *et al.*, 2004]. Presumably, the stimulating effect of prebiotic administration could have been diminished, to some extent, by a slightly lower Ca:P ratio in experimental diets as compared to the control.

The low content of calcium in diets for rats might be the major factor determining the results obtained for apparent absorption and retention coefficients. According to Scholz-Ahrens *et al.* [2001], the effect of prebiotics on the availability of calcium is more pronounced with a higher content of this element in the diet, *i.e.* over 0.3-0.5%. In the current experiment, the content of Ca in the diet was at a considerably lower level, which might have affected the experimental results.

Also the time of prebiotic administration might affect the stimulatory effect on calcium bioavailability [Scholz-Ahrens *et al*., 2001]. Pérez-Conesa *et al*. [2006] found that infant formulas supplemented with prebiotics administrated for 30 days increased Ca bioavailability in rats. However the stimulatory effect of galactooligosacccharides was stronger during the first 10 days of feeding and then decreased. In contrast, Coudray *et al*. [2005] reported that inulin feeding increased Ca absorption in both short-term (13 days) and long-term study (36 days) in rats. Moreover, they noted that the effect of inulin on intestinal Ca absorption was correlated with both dietary Ca levels and experiment duration. In the short–term period, the effect of inulin was remarkable in the groups receiving high or low Ca levels, but in the long–term period inulin improved intestinal Ca absorption to a much greater extent in the group receiving the low Ca level.

CONCLUSIONS

In conclusion, feeding rats low calcium diets based on white cheeses containing inulin and maltodextrin did not increase Ca nor P bioavailability. Nevertheless it is worth emphasizing that the highest values of apparent absorption and retention of calcium expressed in (%) were noted in the group receiving white soft cheese with the addition of maltodextrin. Despite a lack of a statistically significant effect of the prebiotics examined on the absorption and retention of calcium and phosphorus in rats, introduction of inulin- or maltodextrin-supplemented white cheeses to a diet for humans will contribute to an increased intake of dietary fiber which, in turn, will yield a positive effect on the functioning of their gastrointestinal tract.

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