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

Monika Modzelewska-Kapitula¹, Jan Kłobukowski², Kazimierz Kornacki³, Danuta Wiśniewska-Pantak⁴

¹Chair of Meat Technology and Chemistry, ²Chair of Human Nutrition, ³Chair of Industrial and Food Microbiology, Faculty of Food Sciences, University of Warmia and Mazury in Olsztyn

Key words: calcium, phosphorus, bioavailability, inulin, maltodextrin, rats

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; Sziwewska & 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 [Boscher 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...
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

Diet 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 metallic 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 a 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, 200 IU, E 10 IU, K 0.5 mg, choline chloride 200 mg, paraaminobenzoic acid 10.0 mg, inositol 10.0 mg, niacin 4.0 mg, calcium pantothenian 4.0 mg, B₆ 0.8 mg, thiamine 0.5 mg, B₁₂ 0.5 mg, folic acid 0.2 mg, biotin 0.04 mg, B₃ 0.003 mg, glucose as a constituent supplementing the composition to 1 g [AOAC, 1975]. The mineral mixture supplied the following (g/kg): K₂HPO₄ 81.0, K₂SO₄ 68.0, NaCl 30.0, CaCO₃ 21.0, NaHPO₄ 21.4, MgO 18.0, corn starch 735.0, microelements 18.0, microelements mixture (g/100 g): ferrie citrate (16.7% Fe) 31.0, ZnCO₃ (56% Zn) 4.5, MnCO₃ (44.4% Mn) 23.4, CuCO₃ (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.

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 & Minier, 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 intaked 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 1. Diet composition in a feeding experiment on rats.

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Diets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>White cheese (%)</td>
<td>61.9</td>
</tr>
<tr>
<td>Vitamins (%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Minerals (%)</td>
<td>3.0</td>
</tr>
<tr>
<td>Potato starch (%)</td>
<td>5.0</td>
</tr>
<tr>
<td>Corn starch (%)</td>
<td>29.1</td>
</tr>
<tr>
<td>Ca (mg/g)</td>
<td>0.9825</td>
</tr>
<tr>
<td>P (mg/g)</td>
<td>1.176</td>
</tr>
</tbody>
</table>
**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, Synergy1 and oligofructose, were used in considerably higher doses than those used in the presented study. The doses of prebiotics applied in the study examined [Griffin et al., 2003; 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 fluid content.

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>A (n=6)</th>
<th>B (n=4)</th>
<th>C (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheese</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry matter (%)</td>
<td>35.9</td>
<td>34.7</td>
<td>35.2</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>5.80</td>
<td>4.90</td>
<td>5.08</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>25.0</td>
<td>23.8</td>
<td>24.8</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>0.650</td>
<td>0.546</td>
<td>0.524</td>
</tr>
<tr>
<td>Ca (mg/g)</td>
<td>1.148</td>
<td>0.882</td>
<td>0.875</td>
</tr>
<tr>
<td>P (mg/g)</td>
<td>1.115</td>
<td>0.910</td>
<td>0.934</td>
</tr>
<tr>
<td>Ca:P</td>
<td>1.03</td>
<td>0.97</td>
<td>0.94</td>
</tr>
</tbody>
</table>

*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.

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

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

*row means with no common superscripts differ (p<0.05)
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 administered for 30 days increased Ca bioavailability in rats. However the stimulatory effect of galactooligosaccharides 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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**REFERENCES**


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