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Calcium Bioavailability from Diets Based on White Cheese Containing Probiotics or Synbiotics in Short-Time Study in Rats

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Abstract: One of the postulated effects of pro- and prebiotics is their beneficial influence on mineral absorption from a diet. In the present study, calcium absorption from diets containing white cheese with the potentially probiotic *Lactobacillus plantarum* strain with a prebiotic addition such as inulin HPX (high performance for high temperature processes) and maltodextrin was investigated. 2.5% of each prebiotic was added to the cheese preparation. The bioavailability of minerals was expressed as apparent absorption and retention indexes. The highest calcium absorption (mg 5 day^{-1}) was noted in group receiving probiotic cheese, however there was no significant difference between this and the group receiving control diet without pro- and prebiotics. Maltodextrin addition to the diet caused a negative effect on Ca balance. Both, apparent absorption (%, mg 5 days^{-1}) and retention (mg 5 days^{-1}) indexes in this group were significantly lower ($P < 0.05$) compared to other groups. The apparent retention (mg 5 days^{-1}) of calcium was significantly higher ($P < 0.05$) in rats receiving probiotic cheese. Feeding rats with probiotic cheese, containing *Lactobacillus plantarum* strain, resulted in increased calcium retention compared to control and synbiotic diets.

Key words: Bioavailability, calcium, probiotics, prebiotcs, rats, cheese, pro- and synbiotics and calcium bioavailability

INTRODUCTION

Probiotics are defined as viable microorganisms that exhibit a beneficial effect on host health (Mattila-Sandholm *et al*., 2002). The many beneficial effects of probiotics include: re-establishment of balanced intestinal microflora, reduction of faecal enzymes, reduction of lactose intolerance and serum cholesterol level, enhancing the immune system and improvement of calcium absorption (Ziemer and Gibson, 1998). Also prebiotics exert health-promoting effects. According to the definition, prebiotics are non-digestible food ingredients that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon. Prebiotics are resistant to digestion in the upper part of the intestinal tract and are subsequently fermented in the colon by beneficial components of the indigenous gut flora (Roberfroid, 2002). The most important postulated health effects exerted by prebiotics are protection against colon cancer, improving host resistance to pathogens, increasing calcium absorption, lowering blood lipid and stimulating immune system (Manning and Gibson, 2004). Inulins are carbohydrates belonging to prebiotics In the food industry, inulins are used not only because of their health-promoting properties but also for their functional features. They are water soluble and in food products they increase viscosity, thus improving sensory properties (mouthfeel), provide a high moisture-retaining capacity, prevent excessive drying and inhibit starch retrogradation (Crittenden and Playne, 1996). They can be used in a wide range of food products such as: beverages and fermented milks, dairy products, health drink, bakery products, infant formulae, cereals, cakes, desserts (Voragen, 1998).

Prebiotics can be added to food products containing probiotic cultures and, in this case, the product may be described as synbiotic. Synbiotic products have been shown to confer benefits of pre- and probiotics and, moreover, prebiotics are thought to increase the survivability of probiotic strains in the gut and promote their proliferation (Holzapfel and Schillinger, 1998).

It is known that dairy products are one of the best sources of minerals, especially calcium (Weaver, 1998). There are numerous data concerning the prebiotic influence on the calcium bioavailability in rats (Lopez *et al*., 2000; Roberfroid *et al*., 2002), volunteers (Griffin *et al*., 2003; Dahl *et al*., 2005; Bosscher *et al*., 2006) and in vitro studies (Bosscher *et al*., 2003), but there are no studies on synbiotic influence on calcium absorption. There is few studies in which as prebiotic carrier a real food products, containing relative low doses, are used.

Apart from prebiotics, carbohydrates which found a wide range of applications in the food industry, according to their functional properties are maltodextrins. Maltodextrins are partially hydrolized starch products. They are used as texture provider, fat replacer, freeze-control agent or film former (Marchal *et al*., 1999).

The aim of the study was to investigate the influence of diet consisted on probiotic or synbiotic white cheese on calcium absorption in rats. The effect of inulin HPX, in a low dose reasonable from technological point of view, or maltodextrin, being often used as functional additive in food products, on calcium bioavailability in rats was studied.

MATERIALS AND METHODS

24 standardized white male Wistar rats, obtained from the Department of Biological Food Evaluation of the Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences in Olsztyn, with initial body weights ranging from 91-98g, were housed in metabolic cages to collect and separate faeces and urine. The animals were housed in individual metabolic metal-free cages in a room at 22°C and 65% relative humidity, with a 12-hour light-dark cycle. The food intake was recorded daily and feces were collected in 2 periods (4^{th} and 10^{th} day of the experiment). The food and water were provided ad libitum. The experimental protocol was approved by the Local Animal Ethics Committee of University of Warmia and Mazury in Olsztyn, Poland.

The animals were divided into 4 groups of 6 rats each, each receiving control or experimental diets. The animals received diets composed of white soft cheeses without or with additives (pro- and prebiotics) and supplements such as vitamins, minerals, potato and corn starch. Each diet contained 10% protein (N x 6.38), 1% vitamins (AOAC, 1975), 3% minerals without Ca (with modification NRC, 1978). In the diet preparation, fat content in cheeses was considered. Control diet contained 61.9% of cheese without pre- or probiotic, diet A, 60.8% of probiotic cheese with *Lactobacillus plantarum*, whereas diet B 81.1% of cheeses with *L. plantarum* and inulin HPX (Orafti, Belgium) and diet C 69.6% of cheeses with *L. plantarum* and maltodextrin (Pepes S.A., Poland). The number of live *Lactobacillus plantarum*, possessing probiotic properties, previously isolated from gastrointestinal tract of infants, possessing probiotic properties (Modzelewska *et al*., 2003), in every cheese was at the level of 10^7 cfu g^{-1}. The adaptation period of the trial lasted 5 days and during the following 5 days excrements and uneaten food were collected.

In the diets, the content of phosphorus was determined with the colorimetric method (Zegarska, 2000) and the content of calcium by flame atomic absorption spectrometry (Unicam 9393 Solar). For Ca, assaying samples were diluted with lanthanum (Whiteside and Miner, 1984) and measured at 422.7nm.

The bioavailability of calcium was expressed as the apparent absorption index (A) and the apparent retention index (R). Apparent mineral absorption was calculated as dietary mineral intake minus faecal mineral excretion and apparent mineral retention as dietary mineral intake minus faecal and urinary excretion. Results are expressed as means for the balance period and group±standard deviation (SD).

Statistical analysis was perform using Statistica 6.0 (StatSoft, Inc.) Duncan's test at a significance level of $P < 0.05$.

RESULTS

Physicochemical properties of white cheeses such as: dry matter, protein, fat, ash, calcium and phosphorus content, were at a similar lever in all cheeses regardless of the additions (data not shown). In addition, the calcium: phosphorus ratio, crucial from a nutritional point of view, was similar in the cheeses and was close to value 1.

Table 1 summarizes the results obtained for calcium absorption and retention in rats fed with probiotic or synbiotic white cheeses. The highest calcium absorption (mg $5day^{-1}$) was noted in group A, however there was no significant difference between this and control group. The feeding of cheese containing *L. plantarum* and inulin HPX resulted in a statistically insignificant increase in the relative apparent absorption (A%), which was an effect of reducing the amount of calcium excretion with faeces. On the other hand, maltodextrin addition to the diet caused a negative effect on Ca balance. Both, apparent absorption (%, mg 5 $days^{-1}$) and retention (mg 5 $days^{-1}$) indexes in this group were significantly lower ($P < 0.05$) compared to other groups. The apparent retention (mg 5 $days^{-1}$) of calcium was significantly higher ($P < 0.05$) in rats receiving probiotic cheese (diet A) then in other groups whereas the highest apparent retention index (%) were noted in groups A and B.

DISCUSSION

Calcium availability depends not only on the source of the mineral but other factors play important roles, such as age, intestinal mucosal mass and transit time and the size of ingested load of the mineral (Nzeusseu *et al*., 2006). In the present study relatively high the apparent absorption and retention indexes were noted in all groups, probably due to the fact that diets contained white cheese, which is a good calcium source. Delisle *et al.* (1995) found that calcium from cheese was best absorbed by young rats compared to other dairy products such as evaporated milk, yoghurt and skim milk powder.

Moreover, the bioavailability of calcium is influenced by certain substances present in food. Some of them-phytate, long-chain saturated fatty acids, uronic acid and cellulose-decrease calcium absorption (Delisle *et al*., 1995), whereas non-digestible dietary oligosaccharides, including inulin, fructo-oligosaccharides, lactulose and resistant starches, can increase calcium absorption

Table 1: Apparent Ca absorption and retention in rats (n = 6) fed probiotic or synbiotic cheeses

	Diet containing white cheese			
	Control	A	B	C
Diet intake, g 5days^{-1}	87.4±9.2^{A}	90.7±7.2^{A}	95.8±4.2^{A}	88.8±3.2^{A}
Ca intake, mg 5days^{-1}	85.8±9.1^{ACD}	95.2±7.6^{AC}	79.9±3.5^{AD}	68.7±2.4^{B}
Absorption, mg 5days^{-1}	77.4±7.7^{ACD}	86.1±6.1^{AC}	74.8±4.5^{AD}	58.3 ±3.7^{B}
Absorption, %	90.3±3.9^{A}	90.5±1.6^{A}	93.5±2.6^{A}	84.8±4.6^{B}
Retention, mg 5days^{-1}	72.1±6.9^{A}	82.3±7.1^{C}	72.2±4.1^{A}	55.0±2.9^{B}
Retention, %	84.2±4.5^{ABC}	86.3±1.2^{ACD}	90.3±2.5^{CD}	80.1±3.0^{AB}

$^{A, B, C, D}$Row means with no common superscripts differ (P < 0.05). Diets containing cheese: control-with no probiotic or prebiotic, A - with *L. plantarum* strain, B - with *L. plantarum* and inulin HPX (2.5%), C -*L. plantarum* and maltodextrin (2.5%)

(Zafar *et al*., 2004). Many researches observed the stimulatory effect of prebiotics used in 5-10% on calcium bioavailability (Coudray *et al*., 2003, Coudray *et al*., 2005, Raschka and Daniel, 2005a). On the contrary, in the present study no significant increase in calcium bioavailability as a result of prebiotic or probiotic supplementation was noted. Diets based on white cheeses with a inulin addition which provides appropriate sensory properties for products designed for human consumption, the animals received 2% of inulin and 1.7% of maltodextrin. Wolf *et al*. (1998) studied the influence of fructooligosaccharide on mineral bioavailability using a 1-5% prebiotic addition to the rats' diet and observed no stimulatory effect. It is probable that the only statistically significant increase in the apparent retention in the current study was caused by a low prebiotic dose.

There is a lack of reports about maltodextrin impact on Ca absorption, although in some feeding trials concerning the probiotic influence they served as non-prebiotic control (Raschka and Daniel, 2005a). Results of the present study indicated that maltodextrin addition might reduce calcium bioavailability of in low calcium diet in rats.

Another factor influencing the bioavailability of calcium from products enriched with prebiotics is the mineral content in the diet. According to Scholz-Ahrens *et al*. (2001), the prebiotic effect on calcium balance became more prominent when dietary calcium were high, ranging from 0.3-0.5%. In the current study, the calcium content in the diet was lower, which could affect the obtained results.

Although the mechanism for a mineral absorption stimulation induced by prebiotics is not fully recognized, there have been several hypotheses put forward. According to one of them, prebiotics are selectively fermented in the large intestine, which leads to an increased production of short-chain fatty acids and lowers the luminal pH (Manning and Gibson, 2004). A decrease in pH elevates the solubility of minerals and raises passive absorption (Ouwehand *et al*., 2005). Moreover, SCFA stimulate mucosa cell proliferation which results in enlargement of the absorptive surface area of the large intestine. Fermentation of prebiotics is also supposed to increase Ca absorption through its influence on the gene transcription of proteins involved in mucosal calcium binding and sequestration (Raschka and Daniel, 2005b).

As a conclusions, feeding rats with probiotic cheese, containing *Lactobacillus plantarum* strain, resulted in increased calcium retention compared to control and synbiotic diets. The results obtained in the present study indicate that the application of 2.5% of inulin HPX and maltodextrin to probiotic white cheese do not increase calcium absorption in rats fed with low calcium diet in short-term.

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