POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES
Pol. J. Food Nutr. Sci. 2003, Vol. 12/53, SI 2, pp. 64-68

SELECTION OF THE PROBIOTIC STRAINS OF LACTIC ACID BACTERIA STIMULATED BY FRUCTANS IN THE PRESENCE OF CALCIUM

Anna Majkowska, Maria Bielecka, Elżbieta Biedrzycka*

Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland

Key words: probiotics, synbiotics, fructooligosaccharides, calcium

The effect of fructooligosaccharides (FOS, WAKO, Japan) and fructan mix (Raftilose Synergy 1, Orafti, Belgium) on the growth and acidifying activity of *Lactobacillus* (7 strains) and *Bifidobacterium* (13 strains) in the presence of calcium lactate or carbonate (8 g/L) was studied in comparison to the control which did not contain calcium.

The calcium compounds supplemented to the media containing FOS or Raftilose Synergy 1 did not affect the growth of *Lactobacillus*, though, in the presence of Raftilose Synergy 1, the growth of strains was weaker in comparison to the FOS. The pH level in *Lactobacillus* cultures containing FOS and calcium compounds, especially calcium carbonate, was higher than in the control. Growth and acidifying activity of *Bifidobacterium* strains in the media containing FOS or Raftilose Synergy 1 and calcium compounds were comparable to the control.

The studies allowed the development of synbiotics for the improvement of calcium bioavailability from diet supplemented with the mineral nutrient.

INTRODUCTION

The significance of dietary calcium for bone metabolism and health can be stated without any doubt. Chronic deficiency of calcium, due to either insufficient supply or impaired intestinal absorption, can be an important cause of reduction of bone mass followed by osteoporosis. The results of up-to-date studies indicate some undigested oligo- and polysaccharides belonging to β-fructan group, oligofructose and inulin, as dietary factors improving calcium bioavailability [Coudray & Fairweather-Tait, 1998; Griffin *et al*., 2002; Scholz-Ahrens & Schrezenmeir, 2002]. Fructans, undigested in the upper parts of the gastrointestinal tract, reach the colon and are utilised as substrates by some groups of intestinal microflora, mainly by bifidobacteria. The products of fermentation are short-chain fatty acids which acidify the lumen, facilitating absorption of calcium [Scholz-Ahrens *et al*., 2001; Greger, 1999]. It was proved that fructooligosaccharides are utilised by bifidobacteria, moreover, they preferentially stimulate their growth [Bouhnik *et al*., 1996; Gibson *et al*., 1995; Bielecka *et al*., 2002]. Combining of properly selected probiotic strains of bifidobacteria and fructans stimulating their growth in synergistic sets, named synbiotics, may bring further improvement of mineral bioavailability from calcium-fortified diets.

Aiming at the improvement of dietary calcium absorption with synbiotics, the studies focused on the selection of the probiotic strains of lactic acid bacteria stimulated by fructans in the presence of calcium compounds selected for supplementation of calcium-fortified foods leading to development of synergistic sets.

MATERIAL AND METHODS

Bacterial strains. Seven *Lactobacillus* and 13 *Bifidobacterium* strains, previously isolated, classified and characterised due to their probiotic features as well as technological abilities on our own effort were used [Bielecka *et al*., 2000a; b]. They were selected from a few dozen strains able to utilise fructooligosaccharides as stimulated by fructooligosaccharides [Bielecka *et al*., 2002].

Fructans. In the previous studies, the better growth of intestinal bifidobacteria utilising the low-polymerised fructooligosaccharides and some low-polymerised inulins was shown [Biedrzycka & Bielecka, 2002]. Therefore, fructooligosaccharides (FOS, Wako Pure, Japan) of the degree of polymerisation (DP) 2-4 as well as a combination of inulin (DP 2-60) enriched with oligofructose (DP 2-8) (Raftilose Synergy 1, Orafti, Belgium) were studied as substrates for fermentation.

Calcium compounds. On the basis of the previously obtained results [Majkowska *et al*., 2002], calcium lactate and carbonate were selected for calcium fortification of the media. Despite different amount of active calcium in both compounds, 13% and 40%, respectively, the concentration of 8 g/L was previously selected as the maximal dose not inhibiting the growth of *Lactobacillus* and *Bifidobacterium* strains [Majkowska *et al*., 2002].

Model of experiments. The ability of the strains to utilise the fructans in the presence of calcium was studied in the basic nutrient media: MRS [IDF Standard, 1991] for *Lactobacillus*, and modified Garche's [Rasic, 1990] (with bactocasitone re-

* Author's address for correspondence: Anna Majkowska, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, ul. Tuwima 10, 10-747, Olsztyn, Poland; tel.: (48 89) 523 46 03; fax: (48 89) 524 01 24; e-mail: anmaj@pan.olsztyn.pl

TABLE 1. Growth and acidifying activity of *Lactobacillus* strains in medium containing FOS and calcium compounds.

Lactobacillus strains	Lactose		FOS control			FOS + calcium lactate			FOS + calcium carbonate		
	pH	cfu/mL	pH	cfu/mL	mfc[1)]	pH	cfu/mL	mfc	pH	cfu/mL	mfc
L. acidophilus Bs	4.10[2)]	2.8x10^8	4.17	8.7x10^8	3.1x	4.35*	9.5x10^8	3.4x	4.44*	1.1x10^9	3.9x
L. acidophilus K1	3.82	2.9x10^8	4.26	1.1x10^9	3.8x	4.44	9.6x10^8	3.1x	4.61**	1.2x10^9	4.1x
L. acidophilus K2	4.03	1.7x10^8	4.35	5.8x10^8	3.4x	4.46	5.9x10^8	3.5x	4.65**	5.0x10^8	2.9x
L. acidophilus 5	3.94	2.0x10^8	3.95	2.7x10^8	1.4x	4.01	2.6x10^8	1.3x	4.02	3.8x10^8	1.9x
L. acidophilus 145	3.89	2.7x10^8	4.39	3.0x10^8	1.1x	4.48	3.3x10^8	1.2x	4.96**	3.8x10^8	1.4x
L. acidophilus ACD-1	4.09	2.5x10^8	4.18	4.6x10^8	1.8x	4.37*	5.3x10^8	2.1x	4.43*	5.5x10^8	2.0x
L. rhamnosus 8/4	4.04	1.5x10^8	4.21	2.9x10^8	1.9x	4.40*	3.3x10^8	2.2x	4.55**	4.3x10^8*	2.9x

[1)] Multiplication factor of count in FOS-containing medium in comparison to lactose; [2)] The results are average from triplicates. Conditions of incubation: 37°C/8-14 h finished at the beginning of stationary phase; * difference significant at $p<0.05$ in relation to the control; ** difference significant at $p<0.01$ in relation to the control.

TABLE 2. Growth and acidifying activity of *Lactobacillus* strains in medium containing Raftilose Synergy 1 and calcium compounds.

Lactobacillus strains	Lactose		Raftilose Synergy 1 control			Raftilose Synergy 1 + calcium lactate			Raftilose Synergy 1 + calcium carbonate		
	pH	cfu/mL	pH	cfu/mL	mfc[1)]	pH	cfu/mL	mfc	pH	cfu/mL	mfc
L. acidophilus Bs	4.10[2)]	2.8x10^8	4.31	5.0x10^8	1.8x	4.27	5.5x10^8	1.9x	4.38	6.1x10^8	2.2x
L. acidophilus K1	3.82	2.9x10^8	4.32	1.9x10^8		4.29	3.2x10^8*	1.1x	4.41	4.6x10^8**	1.6x
L. acidophilus K2	4.03	1.7x10^8	4.45	1.2x10^8		4.43	1.3x10^8		4.50	2.0x10^8	1.2x
L. acidophilus 5	3.94	2.0x10^8	4.31	2.8x10^8	1.4x	4.21	3.6x10^8	1.8x	4.26	4.0x10^8	2.0x
L. acidophilus 145	3.89	2.7x10^8	4.19	3.6x10^8	1.3x	4.18	3.4x10^8	1.3x	4.26	3.9x10^8	1.4x
L. acidophilus ACD-1	4.09	2.5x10^8	4.88	1.9x10^8		4.77	2.3x10^8		4.83	2.2x10^8	
L. rhamnosus 8/4	4.04	1.5x10^8	4.71	1.7x10^8	1.1x	4.70	1.3x10^8		4.74	1.1x10^8	

[1)]Multiplication factor of count in FOS-containing medium in comparison to lactose; [2)] The results are average from triplicates. Conditions of incubation: 37°C/8-14 h finished at the beginning of stationary phase; * difference significant at $p<0.05$ in relation to the control; ** difference significant at $p<0.01$ in relation to the control.

placed by Peptobak, BTL, Lódź, Poland, and without lithium chloride) for *Bifidobacterium*. The media were deprived of saccharides and supplemented with 1% (w/v) of FOS or Raftilose Synergy 1 and/or fortified with calcium lactate or calcium carbonate (as described above). The growth of the strains was controlled using the media containing lactose. The media were inoculated with active bacterial cultures in the amount of 10^5-10^6 cfu/mL, and incubated at 37°C/8-14 h for *Lactobacillus*, or 37°C/24 h under anaerobic conditions (pyrogallol stoppers) for *Bifidobacterium* strains. The growth and acidifying activity of the strains fermenting fructans in the presence of mineral supply were evaluated in comparison to the results for the control medium containing fructans but not calcium compounds. The experiments were carried out in triplicates.

Determinations. At the end of fermentation, the pH level was measured using an HI 9025 microcomputer pH-meter (Hanna Instruments, USA) and bacterial counts were determined by plating method. The analyses were carried out under the following conditions: MRS agar medium (pH 6.4 $\pm$0.1), a double-layer technique, and incubation at 37°C/48 h were used for determination of *Lactobacillus* count, and modified Garche's agar medium, incubation at 37°C/48 h under anaerobic conditions (Anaerobic System, Oxoid, with Gas Pak CO_2+H_2, Linegal Chemicals GmbH, Poland) - for *Bifidobacterium*.

RESULTS

In the nutrient medium containing lactose, *Lactobacillus* counts ranged from 1.5x10^8 to 2.9x10^8 cfu/mL, and acidifying activity – from 3.82 to 4.10, whereas in the medium with FOS or Raftilose Synergy 1, they ranged from 2.7x10^8 to 1.1x10^9 cfu/mL and from 1.2x10^8 to 5.0x10^8 cfu/mL, respectively, and pH level ranged from 3.95 to 4.39 and from 4.19 to 4.88, respectively (Tables 1 and 2). In comparison to lactose, FOS stimulated 1.1-3.8 times the growth of all *Lactobacillus* strains studied, whereas Raftilose Synergy 1 stimulated 1.1-1.8 times the growth of 4 out of 7 strains, however the growth of other strains was not significantly lower in comparison to lactose ($p>0.05$). Stimulation of growth was accompanied by significantly higher pH values in the majority of cultures containing FOS ($p\leq0.05$, $p\leq0.01$) and in all cultures containing Raftilose Synergy 1 ($p\leq0.05$, $p\leq0.01$).

Lactobacillus counts in the medium containing FOS and supplemented with calcium lactate or carbonate, 2.6x10^8-9.6x10^8 cfu/mL or 3.8x10^8-1.2x10^9 cfu/mL, respectively, were not significantly different from the control ($p>0.05$), except for one strain of *L. rhamnosus* 8/4 growing better in the presence of calcium carbonate ($p\leq0.05$). Similarly, *Lactobacillus* counts in the medium containing Raftilose Synergy 1 and calcium lactate or carbonate, 1.3x10^8-5.5x10^8

TABLE 3. Growth and acidifying activity of *Bifidobacterium* strains in medium containing FOS and calcium compounds.

Bifidobacterium strains	Lactose		FOS control			FOS + calcium lactate			FOS + calcium carbonate		
	pH	cfu/mL	pH	cfu/mL	mfc[1)]	pH	cfu/mL	mfc	pH	cfu/mL	mfc
B. animalis Bi30	4.61[2)]	$4.8x10^8$	4.97	$8.2x10^8$	1.7x	4.95	$9.2x10^8$	1.9x	5.03	$9.2x10^8$	1.9x
B. animalis Bi11	4.67	$2.3x10^8$	4.96	$1.1x10^9$	4.8x	4.91	$1.0x10^9$	4.4x	4.98	$9.6x10^8$	4.2x
B. animalis Bi60	4.75	$6.7x10^8$	4.85	$6.8x10^8$	1.0x	4.82	$5.9x10^8$		4.90	$7.4x10^8$	1.1x
B. animalis K	4.62	$2.8x10^8$	4.95	$3.7x10^8$	1.3x	4.91	$3.6x10^8$	1.3x	5.02	$3.8x10^8$	1.4x
B. animalis G	4.72	$4.7x10^8$	4.99	$9.0x10^8$	1.9x	4.88	$8.3x10^8$	1.8x	4.96	$8.8x10^8$	1.9x
B. animalis J38	4.65	$5.5x10^8$	4.88	$1.0x10^9$	1.8x	4.87	$8.3x10^8$	1.5x	4.83	$1.1x10^9$	2.0x
B. animalis PS46	4.81	$2.9x10^8$	4.96	$3.0x10^8$	1.0x	4.95	$2.5x10^8$		5.03	$1.8x10^8$	
B. animalis KSp4	4.76	$2.5x10^8$	4.91	$2.8x10^8$	1.1x	4.90	$3.0x10^8$	1.2x	4.99	$3.0x10^8$	1.2x
B. animalis KD10	4.59	$4.1x10^8$	4.96	$9.2x10^8$	2.2x	4.92	$8.7x10^8$	2.1x	5.02	$9.3x10^8$	2.3x
B. longum KN29.1	4.24	$3.9x10^8$	4.77	$1.2x10^9$	3.1x	4.64	$9.0x10^8$	2.3x	4.88	$9.2x10^8$	2.4x
B. longum KN4	4.23	$5.0x10^8$	4.76	$1.1x10^9$	2.2x	4.72	$9.3x10^8$	1.9x	4.95	$9.0x10^8$	1.8x
B. adolescentis KD11	4.72	$3.7x10^8$	5.01	$7.9x10^8$	2.1x	4.96	$6.1x10^8$	1.7x	5.12	$8.2x10^8$	2.2x
B. sp. KP9	4.69	$2.1x10^8$	4.86	$6.3x10^8$	3.0x	4.80	$5.5x10^8$	2.6x	4.94	$7.5x10^8$	3.6x

[1)] Multiplication factor of count in FOS-containing medium in comparison to lactose; [2)] The results are average from triplicates. Conditions of incubation: 37°C/24 h under anaerobic conditions.

TABLE 4. Growth and acidifying activity of *Bifidobacterium* strains in medium containing Raftilose Synergy 1and calcium compounds.

Bifidobacterium strains	Lactose		Raftilose Synergy 1 control			Raftilose Synergy 1 + calcium lactate			Raftilose Synergy 1 + calcium carbonate		
	pH	cfu/mL	pH	cfu/mL	mfc[1)]	pH	cfu/mL	mfc	pH	cfu/mL	mfc
B. animalis Bi30	4.61[2)]	$4.8x10^8$	5.01	$9.2x10^8$	1.9x	4.99	$9.5x10^8$	2.0x	5.09	$9.9x10^8$	2.1x
B. animalis Bi11	4.67	$2.3x10^8$	4.96	$8.6x10^8$	3.7x	4.93	$8.0x10^8$	3.5x	5.02	$8.8x10^8$	3.8x
B. animalis Bi60	4.75	$6.7x10^8$	4.89	$7.0x10^8$	1.0x	4.85	$7.1x10^8$	1.1x	4.94	$9.0x10^8$	1.3x
B. animalis K	4.62	$2.8x10^8$	4.99	$4.2x10^8$	1.0x	4.97	$3.3x10^8$	1.2x	5.06	$3.8x10^8$	1.4x
B. animalis G	4.72	$4.7x10^8$	5.06	$8.0x10^8$	1.7x	4.94	$7.4x10^8$	1.6x	4.99	$7.9x10^8$	1.7x
B. animalis J38	4.65	$5.5x10^8$	4.89	$8.8x10^8$	1.6x	4.85	$8.3x10^8$	1.5x	4.89	$9.8x10^8$	1.8x
B. animalis PS46	4.81	$2.9x10^8$	4.98	$3.5x10^8$	1.2x	4.93	$3.2x10^8$	1.1x	5.06	$3.8x10^8$	1.3x
B. animalis KSp4	4.76	$2.5x10^8$	4.94	$4.8x10^8$	1.9x	4.90	$3.5x10^8$	1.4x	5.04	$3.8x10^8$	1.5x
B. animalis KD10	4.59	$4.1x10^8$	4.99	$7.9x10^8$	1.9x	4.96	$6.4x10^8$	1.6x	5.08	$8.6x10^8$	2.1x
B. longum KN29.1	4.24	$3.9x10^8$	4.81	$9.0x10^8$	2.3x	4.84	$9.2x10^8$	2.4x	4.94	$9.5x10^8$	2.5x
B. longum KN4	4.23	$5.0x10^8$	4.86	$7.8x10^8$	1.6x	4.82	$7.3x10^8$	1.5x	4.98	$7.1x10^8$	1.4x
B. adolescentis KD11	4.72	$3.7x10^8$	5.07	$6.9x10^8$	1.9x	4.98	$4.9x10^8$	1.3x	5.15	$7.9x10^8$	2.1x
B. sp. KP9	4.69	$2.1x10^8$	4.89	$2.6x10^8$	1.2x	4.82	$2.2x10^8$	1.1x	4.99	$3.9x10^8$*	1.9x

[1)] Multiplication factor of count in FOS-containing medium in comparison to lactose; [2)] The results are average from triplicates. Conditions of incubation: 37°C/24 under anaerobic conditions; * difference significant at $p<0.05$ in relation to the control.

cfu/mL or $1.1x10^8$-$6.1x10^8$ cfu/mL, respectively, were not significantly different from the control ($p>0.05$), except for one strain of *L. acidophilus* K1 growing better in the presence of calcium lactate ($p\leq0.05$) and calcium carbonate ($p\leq0.01$). In the medium containing FOS and calcium lactate or calcium carbonate, the pH level ranged from 4.01 to 4.48 or from 4.02 to 4.96, respectively, and in 3 out of 7 cultures with calcium lactate and in almost all cultures with calcium carbonate, it was significantly higher than in the control ($p\leq0.05$, $p\leq0.01$). The results indicate a significant role of calcium carbonate in maintaining higher pH during growth of *Lactobacillus* strains in the MRS medium containing FOS. In the medium containing Raftilose Synergy 1 and calcium lactate or calcium carbonate, the pH level ranged from 4.18 to 4.77 or from 4.26 to 4.83, respectively, and was comparable to the control (Tables 1 and 2).

Bifidobacterium counts in medium containing lactose, from $2.1x10^8$ to $6.7x10^8$ cfu/mL, were on the same level or lower than in those containing FOS or Raftilose Synergy 1, from $2.8x10^8$ to $1.2x10^9$ cfu/mL or from $2.6x10^8$ to $9.2x10^8$ cfu/mL, respectively (Table 3 and 4), by 1.0-4.8 or 1.0-3.7 times, respectively. They were much less different between FOS and Raftilose Synergy 1 than those of *Lactobacillus*. The pH level after *Bifidobacterium* fermentation of lactose, 4.23-4.81, was lower than after fermentation of FOS or Raftilose Synergy 1, 4.76-5.01 or 4.81-5.07, respectively.

Bifidobacterium counts in the medium containing FOS and calcium lactate or calcium carbonate ranged from $2.5x10^8$ to $1.0x10^9$ cfu/mL or from $1.8x10^8$ to $1.1x10^9$ cfu/mL, respectively, and were not significantly different from the control ($p>0.05$). In those cultures, pH level ranged from 4.64 to 4.96 or from 4.83 to 5.12, respectively, and was not significantly different from the control ($p>0.05$). Similarly, *Bifidobacterium* counts in the medium containing Raftilose Synergy 1 and calcium lactate or carbonate ranged from $2.2x10^8$ to $9.5x10^8$ cfu/mL or from $3.8x10^8$ to $9.9x10^8$ cfu/mL, respectively, and were not significantly different from the control ($p>0.05$), except for one strain growing on calcium carbonate. In those cultures, the pH level ranged from 4.82 to 4.99 or from 4.89 to 5.15, respectively, and was not significantly different from the control ($p>0.05$) (Tables 3 and 4).

DISCUSSION

Due to the tremendous progress in medicine and subsequent prolongation of average human life span, osteoporosis became a significant problem of ageing Western populations. Some data indicate that about 30% of post menopausal women suffer from osteoporosis. With age, bone metabolism is characterised by deficient bone formation, which makes progressive bone structure destruction impossible to repair. However, their strength is determined much earlier, till 26 years of life [Teegarden *et al*., 1995]. Therefore, adequate calcium intake in youth is essential for prevention of osteoporosis, though, the importance of high calcium intake at various stages of life is now accepted [Danone, 2001]. Another matter is that the degree of calcium absorption may differ from 5 to 80% [Dawson-Hugnes, 1996], opening additional opportunity for diet manipulation. In the literature, some optimistic premises about beneficial influence of oligofructose and inulin on the absorption of mineral compounds, especially of calcium and magnesium, can be found [Scholz-Ahrens & Schrezenmeir, 2002]. Griffin *et al*. [2002] studied the effect of Raftilose P95 (oligofructose) and Raftilose Synergy 1, administered for 3 weeks to girls at or near menarche, on calcium absorption. The results showed higher (by 18%) calcium absorption in group receiving Raftilose Synergy 1 and a lack of differences between placebo- and oligofructose groups. The effects seemed to be related to the fermentation by some groups of the intestinal flora (mainly bifidobacteria) [Scholz-Ahrens *et al*., 2001]. The products of fermentation were short-chain fatty and organic acids which contributed to a reduced luminal pH in the large intestine, which in turn was associated with an increased amount of soluble calcium, and increased absorption [Cashman, 2002].

CONCLUSIONS

Unfortunately, the review of literature does not answer definitely some basic questions on the nature and amount of calcium compounds used for supplementation as well as their impact on microflora in the aspect of food production and utilisation by human intestinal bacteria. The first aspect concerns growth of technological cultures (*e.g.* lactic acid bacteria), additional cultures (including probiotic bacteria), and contaminating organisms, during technological processes of production and storage of fortified food. The latter is directed into beneficial lactic acid bacteria and bifidobacteria, opportunistic bacteria and pathogens of the gastrointestinal tract. The results of the presented study indicate that, when applied in a dose of 8 g/L, calcium compounds selected for supplementation of diets (calcium lactate and calcium carbonate) had no negative effect on the growth of the selected probiotic strains of *Lactobacillus* and *Bifidobacterium*. Moreover, the tendency of stimulation of bacterial growth by fructooligosaccharides or inulin enriched in oligofructose in the presence of calcium compounds was maintained. The results indicate that the synergistic sets can be developed using the strains of *L. acidophilus* Bs, K1 and FOS, *L. acidophilus* Bs and Raftilose Synergy 1, *B. animalis* Bi 30, Bi11, J38, *B. longum* KN29.1, KN4 and FOS or Raftilose Synergy 1.

REFERENCES

1. Biedrzycka E., Bielecka M., Antagonistic activity of probiotic strains of bifidobacteria against food-borne pathogen – *Salmonella.* Pol. J. Food Nutr. Sci., 2002, 11/52, SI 1, 32-38.
2. Bielecka M., Biedrzycka E., Biedrzycka El., Isolation and identification of bifidobacterial strains. Medical Science Monitor, Intern. Med. J. Experim. Clinical Res., 2000a, 6 (Suppl. 3), 123.
3. Bielecka M., Biedrzycka E., Majkowska A., Selection of probiotics and prebiotics for synbiotics and confirmation of their *in vivo* effectiveness. Food Res. Intern., 2002, 35,139-144.
4. Bielecka M., Biedrzycka E., Majkowska A., Selection of bifidobacterial strains capable for colonisation of gastrointestinal tract. Medical Science Monitor, Intern. Med. J. Experim. Clinical Res., 2000b, 6 (Suppl. 3), 123.
5. Bouhnik Y., Flourie B., Ouarne F., Andrieux C., Bisetti N., Briet F., Rambaud J.C., Effects of *Bifidobacterium* sp. fermented milk ingested with or witout inulin on colonic bifidobactria and enzymatic activities in healthy humans. Eur. J. Clin. Nutr., 1996, 50, 269-273.
6. Cashman K., Prebiotics and calcium bioavailability. 2002, *In*: Probiotics and Prebiotics: Where are we going? (ed. G.W. Tannock) Caister Academic Press, Wymondham, England, Chapter 6, pp. 149-174.
7. Coudray C., Fairweather-Tait S.J., Do oligosaccharides affect the intestinal absorption of calcium in humans? Am. J. Clin. Nutr., 1998, 68, 921-923.
8. Danone World Newsletter, 2001, Fermented milk and successful ageing. 22, pp. 1-14.
9. Dawson-Hugnes B., Calcium and vitamin D nutritional needs of elderly women. Am. J.Clin. Nutr., Suppl., 1996, 1165S-1167S.
10. Gibson G.R., Beatty E.R., Wang X., Cummings J.H., Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. Gastroenterology, 1995, 108, 975-982.
11. Greger J.L., Nondigestible carbohydrates and mineral bioavailability. J. Nutr., 1999, 129, 1434S-1435S.
12. Griffin I.J., Davila P.M., Abrams S.A., Non-digestible oligosaccharides and calcium absorption in girls with ad-

equate calcium intakes. Brit. J. Nutr., 2002, 87, (Suppl. 2), S187-S191.
13. IDF Standard 146:1991.
14. Majkowska A. Bielecka M., Biedrzycka E., Majewska M., Effect of food supplementation with calcium on growth and acidifying activity of probiotyc strains of *Lactobacillus* and *Bifidobacterium*. Pol. J. Food Nutr. Sci., 2002, 11/52 (SI 2), 49-53.
15. Rasic J.Lj., Culture media for detection and enumeration of bifidobacteria in fermented milk products. Bull. IDF, 1990, 252, 24-34.
16. Scholz-Ahrens K.E, Schaafsma G., van den Heuvel E.G.H.M., Schrezenmeir J., Effects of prebiotics on mineral metabolism. Am. J. Clin. Nutr., 2001, 73, 459S-464S.
17. Scholz-Ahrens K.E., Schrezenmeir J., Inulin, oligofructose and mineral metabolism – experimental data and mechanism. Brit. J. Nutr., 2002, 87 (SI 2), S179-S186.
18. Teegarden D., Proulx W.R., Martin B.R., Zhao J., McCabe G.P., Lyle R.M., Peacock M., Slemenda C., Johnston C.C., Weaver C.M., Peak bone mass in young women. J. Bone Mineral. Res., 1995, 10, 711-715.