Note

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Effects of Palatinose and Its Condensate Intake on Human Fecal Microflora

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Abstract Effects of the intake of a candy containing palatinose and palatinose condensates, in daily dosage of 12 or 24 g for 10 days, on microflora, pH, and water contents in feces were investigated in eight healthy volunteers. The number of bifidobacteria and percentage of the microorganisms to total bacteria were higher during than before or after a period of the candy intake. Remarkable changes of fecal pH and water contents were not observed during a period of the intake.

Key words: palatinose; palatinose condensates; fecal microflora; bifidobacteria

Palatinose (6-O-α-D-glucopyranosyl-D-fructofuranose) is used in various food products as a non-cariogenic nutritive sweetener (*3*, *10*). A mixture of palatinose and its condensates is obtained by heating palatinose-melt under suitable conditions (*9*). The condensates are classified as hetero-oligosaccharides composed of glucose- and fructose-residues (*9*). Palatinose is digestible (*1*, *12*), but digestibility of the condensates is not clear. It is therefore important to examine whether a palatinose condensate intake is effective in modifying intestinal microflora or not.

The present study was undertaken to determine the effects of palatinose and its condensate intake on microflora, pH, and water contents of feces in men.

The subjects were eight healthy adult male volunteers ranging from 25 to 52 years old. None of them had been on antibiotic treatment or other therapy at least for 4 weeks prior to the first collection of fecal samples.

A candy prepared specially for this experiment was stamped out in 4 g per piece, and composed of 75.4% palatinose, 20.4% palatinose condensates, 1.0% monosaccharides, and 3.0% moisture. The palatinose condensates included 2.8% tri-, 13.8% tetra-, 3.0% hexa-, and 0.8% octa-saccharides.

Table 1. Effects of palatinose and its condensate intake on human fecal microflora

Fecal flora	Before the intake		12 g/day intake		24 g/day intake		After the intake	
	Day 7	Day 10	Day 7	Day 10	Day 7	Day 10	Day 7	Day 10
Bacteroides	10.8±0.1[a]	10.7±0.2	10.7±0.2	10.9±0.1	10.8±0.1	10.9±0.1	10.7±0.3	10.8±0.2
	(8/8)[b]	(8/8)	(8/8)	(7/7)	(8/8)	(8/8)	(8/8)	(8/8)
Eubacteria	9.9±0.4	9.9±0.3	9.9±0.4	10.1±0.2	9.8±0.4	9.8±0.6	9.4±0.9	10.1±0.2
	(8/8)	(8/8)	(8/8)	(7/7)	(8/8)	(8/8)	(8/8)	(8/8)
Anaerobic cocci	9.8±0.5	9.8±0.1	9.3±0.4	9.5±0.5	9.7±0.3	9.5±0.2	9.0±0.2	9.7±0.3
	(8/8)	(7/8)	(7/8)	(7/7)	(7/8)	(7/8)	(6/8)	(4/8)
Bifidobacteria	9.7±0.3	9.4±0.3	9.6±0.4	9.9±0.4[d]	9.9±0.4[d,e]	10.0±0.3[d,e]	9.4±0.5	9.8±0.2
	(8/8) [5.9][c]	(8/8) [5.5]	(8/8) [10.5]	(7/7) [10.2]	(8/8) [14.2]	(8/8) [11.1]	(8/8) [6.5]	(8/8) [8.9]
Veillonellae	6.2±1.2	5.3±1.9	5.5±1.9	4.6±0.9	5.1±1.2	4.5±1.6	5.4±1.6	4.0±0.6
	(7/8)	(6/8)	(6/8)	(6/7)	(6/8)	(6/8)	(3/8)	(5/8)
Megasphaerae	8.8	7.6	8.3	8.8±0.2	9.3	8.4	8.3	9.1
	(1/8)	(1/8)	(1/8)	(2/7)	(1/8)	(1/8)	(1/8)	(1/8)
Clostridia,								
lecitinase +	4.7±0.4	N.D.[f]	5.1±2.0	5.8±1.1	4.0±1.0	4.6±2.2	5.6±0.6	4.3±0.0
	(4/8)	(0/8)	(3/8)	(3/7)	(4/8)	(2/8)	(3/8)	(2/8)
lecitinase −	7.2±1.3	7.1±0.9	6.1±2.2	6.8±1.8	7.5	7.6±1.1	7.5±0.8	7.2±1.5
	(3/8)	(4/8)	(7/8)	(3/7)	(1/8)	(4/8)	(6/8)	(5/8)

Lactobacilli	5.5±1.7 (6/8)	5.8±1.6 (6/8)	5.9±1.7 (7/8)	6.1±1.1 (7/7)	6.6±1.5 (5/8)	5.8±2.1 (8/8)	5.9±1.7 (8/8)	7.0±1.8 (6/8)
Enterobacteria	7.2±0.5 (8/8)	6.8±1.0 (8/8)	6.9±1.2 (8/8)	7.2±0.4 (7/7)	7.2±1.3 (8/8)	7.0±0.8 (8/8)	7.1±0.8 (8/8)	7.3±0.9 (8/8)
Streptococci	7.5±1.5 (8/8)	7.2±1.5 (8/8)	7.3±1.3 (8/8)	7.6±1.4 (7/7)	7.7±1.5 (8/8)	7.6±1.0 (8/8)	7.0±1.3 (8/8)	7.6±0.7 (8/8)
Staphylococci	3.3±0.8 (4/8)	2.8±0.5 (5/8)	3.4±0.9 (3/8)	3.7±1.1 (3/7)	3.3±1.0 (2/8)	3.4±1.3 (3/8)	2.7±0.1 (3/8)	3.0±0.6 (4/8)
Corynebacteria	N.D. (0/8)	5.5±1.1 (3/8)	N.D. (0/8)	N.D. (0/8)	N.D. (0/8)	N.D. (0/8)	N.D. (0/8)	N.D. (0/8)
Bacilli	2.6±0.3 (2/8)	6.3 (1/8)	N.D. (0/8)	2.3 (1/7)	3.4 (1/8)	5.1±1.2 (2/8)	4.3±2.0 (2/8)	2.3 (1/8)
Yeasts	3.4±1.0 (5/8)	3.7±0.7 (4/8)	3.3±0.7 (5/8)	3.1±0.6 (3/7)	3.1±0.5 (4/8)	2.8±0.3 (5/8)	2.9±0.2 (3/8)	3.1±0.7 (5/8)
Total bacteria	11.0±0.1	10.8±0.2	10.8±0.2	11.0±0.2	10.9±0.1	11.0±0.1	10.8±0.3	10.9±0.2

[a] Bacterial counts expressed as mean±S.D. of log N per g of feces.
[b] Frequency of occurrence expressed as number of subjects yielding the organism/number of subjects examined.
[c] Mean of individual percentages of bifidobacteria to total bacteria.
[d] Statistically significant at the $P<0.05$ level when compared with the number for Day 10 before the intake.
[e] Statistically significant at the $P<0.05$ level when compared with the number for Day 7 after the intake.
[f] Not detected.

The plan of administration consisted of 4 successive periods of 10 days. While no administration was made in the 1st and 4th periods, each of the subjects ingested one or two pieces of the candy immediately after each meal in the 2nd or 3rd period, and thus the daily dosage was 12 or 24 g, respectively.

Freshly voided fecal samples were collected from all subjects at Days 7 and 10 of each period. The method for bacterial analysis of the fecal microflora in this study was essentially the same as that of Mitsuoka et al (*7*, *8*). Eight agar plates of EG, BL, ES, NBGT, BS, VS, NN, and LBS were used for anaerobes, and five agar plates of TS, TATAC, DHL, PEES, and PDA for an aerobes. Anaerobic culture was carried out for two days using a steel-wool method filled with 100% CO_2 gas at 37°C. All agar plates for aerobes were aerobically incubated at 37°C. After incubation, fourteen bacterial groups and yeasts were identified by colonial and cellular morphologies, Gram's-reaction, spore formation, and aerobic growth. For each group, its viable count per gram of wet feces was calculated and converted into its logarithmic equivalent. Total viable count per gram of feces was calculated by aggregation of all specified counts.

Fecal pH values were measured directly by inserting a glass electrode (Cat. 6028, Horiba, Tokyo) into fecal specimens. Five g of feces was dried to constant weight by using a vacuum drying oven under heating at 70°C and aspirating at 75 cm Hg. Then, water content was determined by subtracting the constant weight from the original weight of feces.

The effect of palatinose and its condensate intake on human fecal microflora is shown in Table 1. Total bacteria were not changed significantly throughout all experimental periods. No remarkable change was also observed for all microbial groups except for bifidobacteria. Before the intake, the number of bifidobacteria was from 9.4 to 9.7 (log N/g of feces) and percentage of bifidobacteria to total bacteria was from 5.5 to 5.9. The number of bifidobacteria by Day 10 of the 12 g candy intake was significantly ($P<0.05$) higher than that by Day 10 before the intake. For the 24 g candy intake, significantly ($P<0.05$) increasing numbers (9.9 to 10.0) and higher percentages (11.1 to 14.2%) of bifidobacteria of all volunteers were observed. The number of bifidobacteria reduced again after the intake. Table 2 shows the effects of the intake on fecal pH and water contents of feces. A slight lowering of fecal pH for 24 g candy intake was not statistically significant. No remarkable change of fecal water content was found throughout all experimental periods.

Many articles concerning the beneficial effects of intestinal bifidobacteria on its host have been published (*5*, *11*). It has been also reported that decreasing or disappearance of bifidobacteria in human intestinal microflora was related to advances in age and some intestinal diseases (*5*, *6*). It has been shown that some oligosaccharides, such as fructooligosaccharides and isomalto-oligosaccharides, were effective for selective increase of bifidobacteria (*2*, *4*). The effective components of these oligosaccharides are not digested in the small intestine, but utilized by bifidobacteria in the large intestine. In the present study, fecal bifidobacteria increased with the candy intake. The condensates contained in the candy seem to

Table 2. Effects of palatinose and its condensate intake on pH and water content of feces[a]

Time	pH	Water content (%)
Before the intake		
Day 7	6.3±0.3	80.5±4.4
Day 10	6.3±0.4	80.6±7.8
12 g/day intake		
Day 7	6.2±0.3	83.2±5.8
Day 10	6.3±0.3	80.8±5.4
24 g/day intake		
Day 7	6.0±0.3	81.9±4.0
Day 10	6.0±0.3	83.2±5.8
After the intake		
Day 7	6.2±0.4	80.9±7.8
Day 10	6.5±0.6	78.8±6.7

[a] Data were expressed as mean±S.D.

be indigestible because of their complicated molecular structures. The present study suggests that palatinose condensates are effective in modifying proliferation of bifidobacteria. We intend to undertake an additional study to determine which palatinose or its condensates causes the increase of bifidobacteria.

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