Effects of Partially Hydrolyzed Guar Gum Intake on Human Intestinal Microflora and Its Metabolism

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The growth responses of a variety of human intestinal bacteria to partially hydrolyzed guar gum (PHGG) were investigated in vitro and in vivo. In an in vitro experiment, PHGG moderately enhanced growth of some bacterial strains including Bacteroides ovatus, Clostridium coccoide, C. butyricum, and Peptostreptococcus productus.

Effects of PHGG intake (7 g/volunteer, 3 times per day, for 14 days) on fecal microflora, bacterial metabolites, and pH were investigated using nine healthy human volunteers. The count of Bifidobacterium spp. and the percentage of these species in the total count increased significantly during the PHGG intake periods. Among the acid-forming bacteria, Lactobacillus spp. also increased. The fecal pH and fecal bacterial metabolites such as β-glucuronidase activity, putrefactive products, and ammonia content were significantly decreased by PHGG intake. Two weeks after the end of PHGG intake, the bacterial counts and their biological manifestations appeared to return to the former state.

Guar gum, a water-soluble dietary fiber derived from the seeds of Cyanopsis tetragonolobus, has been used as a stabilizer and a thickener in various fields of food processing. It is known that guar gum is effective to reduce serum cholesterol in normal and hyperlipidemic subjects.1,2 On the other hand, partially hydrolyzed guar gum prepared by microbial β-endo-mannanase to reduce the high viscosity of original guar gum, has been used for food processing and its physiological effects were investigated in comparison with untreated guar gum.3,4

Many papers5–7 have reported on the influence of guar gum on intestinal bacteria and enzymes in the human colon, and it has been found that guar gum is enzymatically degraded to low molecular weight galactomannan and then used by intestinal bacteria as a carbon source.

This paper describes in vitro and in vivo experiments on the effects of partially hydrolyzed guar gum on the composition of microflora and their activities of human intestinal bacteria.

Materials and Methods

PHGG used. A commercial PHGG preparation, namely ‘Sunfiber’ (Taiyo Kagaku Co., Ltd., Japan) was used in this study. The PHGG was prepared by treatment of guar gum with β-endo-galacto-mannanase from a strain of Aspergillus niger8 and its average molecular mass measured by HPLC was 20,000 Da. The total dietary fiber content of the PHGG was 85% measured by the method of AOAC. A PHGG beverage was prepared by dissolving Sunfiber at 7% (w/v) in water and used in an in vitro experiment.

Bacteria and culture conditions in vitro experiments. The bacterial strains used are listed in Table 1. These strains were supplied from the RIKEN culture collection, unless otherwise noted. Stock cultures of all the strains were done routinely using EIGLE agar stored at −80°C and when necessary, they were subcultured on BL and EG agar (Eiken Chemical Co., Ltd., Tokyo, Japan) together with 5% horse blood, for bifidobacteria and other bacteria, respectively. All the plates were incubated at 37°C for 2 days in the 100% CO2 atmosphere. The microorganisms that appeared were then cultivated in EGF broth (pH 7.2) in an atmosphere of 80% N2, 15% CO2, and 5% H2. All the cultures were checked for possible contamination at the end of the growth cycle of each bacterium.

Microbiological assay. For measurement of bacterial growth, Mitsuoka’s method was used. 9 To examine the bifidus factor(s) derived from non-carbon sources we used Peptone Yeast Extract Fidts Solution (PYF) broth (pH 7.2). The bacterial cells grown in EGF broth were sedimented at 3000rpm for 10 min, washed three times with 5 ml of sterile physiological saline (0.85% NaCl, 0.1% l-cysteine-HCl, and 0.1% sodium thioglycolate), and finally suspended in 5 ml of reduced physiological saline. One-tenth portions of the suspension were inoculated onto the agar containing the media described above. Filter-sterilized PHGG was added at 0.5% concentration to the media for a total of 5 ml. The culture was incubated anaerobically at 37°C for 48 h and bacterial growth was monitored by examining the change in pH. The results of growth response were classified as follows: the strongest response, pH 4.5–5.0; moderate, pH 5.1–5.5; weak, pH 5.6–6.0 on PYF broth; and no response –. Each assay was done in triplicate experiments.

Subjects and diets in vivo experiment. Nine healthy male volunteers from 22 to 39 years old were allowed to eat popular Japanese diets for six weeks. They were made free from antibiotics and other medicines before and during the experiment. The experimental period was divided into four consecutive periods: Control A (first two weeks), Test 1 (subsequent 3rd week), Test 2 (4th week), and Control B (last 6th week), PHGG beverages (100 ml/volunteer, 3 times per day after meals) were administered only during the period of Tests 1 and 2. This experiment was done according to the proposals of the Helsinki Declaration updated in Tokyo in 1975.

Collection of feces. Feces from volunteers were collected twice on days 12 and 14 for Control A, on the 19th and 21st days for Test 1, on the 26th and 28th days for Test 2, and on the 40th and 42nd days for Control B. Collected feces were kept at 4°C and anaerobically transported to our laboratory, and the fecal weight, the fecal microflora, fecal pH, and fecal enzyme activities were measured within 3 h after collection. The remainder of feces was frozen at −80°C for analysis of putrefactive products and volatile fatty acids.
<table>
<thead>
<tr>
<th>Media</th>
<th>Main enumerated microorganisms</th>
<th>Dilutions to be plated*</th>
<th>Incubation time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic culture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TS blood agar</td>
<td>Predominant aerobes</td>
<td>10^7 – 10^9</td>
<td>2</td>
</tr>
<tr>
<td>DHL agar</td>
<td>Enterobacteriaceae</td>
<td>10^1 – 10^3</td>
<td>1</td>
</tr>
<tr>
<td>TATAC agar</td>
<td>Streptococcus spp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEES agar</td>
<td>Staphylococcus spp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAC agar</td>
<td>Pseudomonas aeruginosa</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>P agar</td>
<td>Yeasts and molds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaerobic culture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M10 agar</td>
<td>Predominant anaerobes</td>
<td>10^7 – 10^9</td>
<td>3</td>
</tr>
<tr>
<td>EG agar</td>
<td>Predominant anaerobes</td>
<td>10^7 – 10^9</td>
<td>2</td>
</tr>
<tr>
<td>NGBT agar</td>
<td>Bacteroidaceae</td>
<td>10^1 – 10^3</td>
<td>2</td>
</tr>
<tr>
<td>BS agar</td>
<td>Bifidobacterium spp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ES agar</td>
<td>Eubacterium spp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VS agar</td>
<td>Veillonella spp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBS agar</td>
<td>Lactobacillus spp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NN agar</td>
<td>Clostridium perfringens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CW agar</td>
<td>Clostridium perfringens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC agar</td>
<td>Clostridium spp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC agar</td>
<td>Clostridium spp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC agar</td>
<td>Clostridium spp.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* A sample (0.05 ml) of each dilution was plated on respective medium.

Microflora analysis: The method of Mitsuoka et al. was used for fecal microflora analysis. The culture media and methods for isolation and identification of each bacterium are presented in Table I. Bacterial colonies on the respective medium were counted and identified according to their colonial and cellular morphologies (for Gram stain, spore formation, and aerobic growth). The bacterial cell counts per gram of wet feces were expressed by their numbers in log_{10}. Frequency of occurrence was calculated as the number of feces having the bacteria per total fecal samples. Percentage composition was presented as % of the bacterium against total bacterial cell counts.

Biochemical analysis: The pH of feces was measured directly by inserting a glass electrode into the feces.

Volatile fatty acids (acetic, propionic, iso-butyric, n-butyric, n-valeric, iso-caproic, and n-caproic acids), and lactic acid were measured by a slightly modified method of Miwa et al. using high pressure liquid chromatography (HPLC). One gram of fecal sample was homogenized in a solution containing 2 ml of 10% sodium tungstate, 2 ml of 1 M sodium hydroxide, and 1 M of 15 mM 2-ethylbutyric acid (internal standard) and then centrifuged for 10 min at 3000 rpm. The volatile fatty acids and lactic acid in the supernatant were converted to their respective 2-nitrohydroxynaphthalene using 1-ethyl-3-(3-dimethyl amino propyl) carbodiimide hydrochloride for HPLC analysis. A Waters 600E high pressure liquid chromatograph with a Waters 490E multilengthwave detector operating at 400 nm and a Waters 741 data module were used. A YMCPack FA column (250 x 6.0 mm i.d., YMC Co., Ltd., Japan) was maintained at 55 C and eluted with acetonitrile methanol water (18:12:70 to 42:28:30) of a linear gradient system for 40 min at a flow rate of 1.2 ml/min.

The ammonia content in the supernatant of fecal suspension was measured using an Ammonia-Test Kit Wako (Wako Pure Chemical Industries, Ltd., Japan). The measurement of fecal putrefactive products (phenol, p-creosol, 4-ethylphenol, indole, and skatole) was done by our method as follows: each fecal sample (0.5 g) was steam-distilled and the distillate was directly analyzed by HPLC (solvent = 50% acetonitrile, column = reversed phase column, and internal standard = p-iso-prophenylphenol).

Activities of β-glucuronidase, β-galactosidase, nitroreductase, and azoreductase attributed to fecal bacteria were measured by a slight modification of the methods of Goldin et al. and β-glucuronidase and β-glucosidase activities were measured under aerobic conditions using p-nitrophenyl-β-d-glucuronide and p-nitrophenyl-β-d-glucopyranoside, respectively, as the substrate. Nitroreductase and azoreductase were measured under anaerobic conditions using p-nitrobenzoic acid and Sunset Yellow FCF (Tokyo Kasei Kogyo Co., Ltd.), respectively, as the specific substrate. Fecal microfloral urease activity was measured as follows: the sample feces were suspended to be 100 mg/ml in 0.1 M potassium phosphate buffer (pH 7.0) and homogenized. The reaction mixture for urease assay contained 0.1 M potassium phosphate buffer (pH 7.0), 14C-urea (7.4 KBq/ml, 5 mCi) and fecal homogenate (100 μl). After an hour incubation at 37 C, the radioactivity of liberated 14CO2 was measured by Iwai and Taguchi by trapping onto β-phenylglyoxaline detection. The radioactivity was measured using a Packard Tri-Carb Liquid Scintillation Analyzer Model 1500 (Packer Co., Ltd.). Fecal microbial tryptophanase activity was assayed by the following method: the sample feces were suspended to be 100 mg/ml in 0.1 M potassium phosphate buffer (pH 7.0), l-[side chain-3,14C]-tryptophan (22.2 KBq/ml, 10.8 μM), complete reaction mixture (reported by Demoss and Moser and fecal homogenate (200 μl)). After incubation of reaction mixture at 37 C for 1 h, 1 ml of 60% perchloric acid was added to the reaction mixture and then centrifuged at 10,000 rpm for 3 min. The supernatant was put on Dowex 50WX4 4 H+ column chromatography (12 x 50 mm) and eluted with deionized water. The radioactivity of each eluted fraction was measured with a liquid scintillation analyzer described above. The reagents for these enzyme activities were used as follows: 14C-Urea (37 KBq/ml, 2.0 GBq/mmol) and l-[side chain-3,14C]-tryptophan (0.74 MBq/ml, 2.0 GBq/mmol) from NEN Research Products.

Statistical analysis: A Student’s t-test was used to analyze all the parameters, except for the frequency of occurrence of microflora analysis, which was done using Fisher’s exact probability test.

Results

Growth responses

The results of the in vitro experiment of the effects of PHGG on human intestinal bacteria are shown in Table II. Bacteroides ovatus, Clostridium coccoidei, C. butyricum, and Peptostreptococcus productus showed moderate growth response, and seven strains of Bacteroides including B. thetaotaomicron and B. distasonis, two clostridia including C. paraputrificum and C. ramosus, Peptostreptococcus sp., and Eubacterium tortuosum showed a slight growth-response in PYF medium with 0.5% PHGG. Other microorganisms such as bifidobacteria, lactobacilli, and E. coli showed negative growth on media with PHGG as a carbon source.

Fecal flora

The changes of fecal microflora and frequency of occurrence of each bacterium by PHGG intake are given in Table III. Lactic-acid-forming bacteria including Bifidobacterium spp. and Lactobacillus spp. were particularly increased by PHGG intake. Their counts (in log_{10}) increased from 9.91 to 10.30–10.34 for Bifidobacterium spp. and from 4.07 to 4.89–5.00 for Lactobacillus spp., during Tests 1 and 2, respectively. The results of change in Bifidobacterium spp. in Tests 1 and 2 period indicate that at least two weeks of continuous intake of PHGG were necessary to promote the bifidobacterial growth. In contrast, other bacteria including Clostridium spp., Clostridium,

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In the text, there seems to be a mix-up between tables and supplementary notes. The content appears to be a mix of data presentation and methodological details, typical of a scientific journal article. The text is not entirely clear due to the mix of data and text, but it appears to describe a study involving the analysis of microflora in fecal samples using various methods such as culture media, biochemical analysis, and growth responses. The tables (Table I and Table II) are not provided, but the description suggests an extensive analysis of the effects of PHGG on human intestinal bacteria, focusing on changes in bacterial populations and growth responses.
Table II. Growth Responses of Various Strains of Intestinal Bacteria to PHGG

<table>
<thead>
<tr>
<th>Strain</th>
<th>Response</th>
<th>Strain</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. infantis S-12 and I-10-5</td>
<td>–</td>
<td>Eubacterium lentum 515</td>
<td>–</td>
</tr>
<tr>
<td>B. breve aS-1, bs-46 and I-53-8w</td>
<td>–</td>
<td>E. limosum E-1, UPI-1939 and ATCC-8486</td>
<td>–</td>
</tr>
<tr>
<td>B. longum A-E194b, M-101-2, Kb-5-6 and bs-3</td>
<td>–</td>
<td>E. multiforme ATCC-25552</td>
<td>–</td>
</tr>
<tr>
<td>Lactobacillus acidophilus IF-164, ATCC-4356 and Omf1</td>
<td>–</td>
<td>E. niitrogenes ATCC-25547</td>
<td>–</td>
</tr>
<tr>
<td>L. salivarius ATCC-11741 and ATCC-11742</td>
<td>–</td>
<td>E. rontosus ATCC-25548 (+)</td>
<td>–</td>
</tr>
<tr>
<td>L. casei ATCC-7469 and IFO-3425</td>
<td>–</td>
<td>Peptostreptococcus anaerobius X-36</td>
<td>–</td>
</tr>
<tr>
<td>Bacteroides fragilis 3676 and M-601</td>
<td>–</td>
<td>P. productus M-601</td>
<td>+</td>
</tr>
<tr>
<td>B. ovatus ATCC8434</td>
<td>+</td>
<td>P. productus R-299-2A</td>
<td>–</td>
</tr>
<tr>
<td>B. distasonis S-601, M-602, M-603</td>
<td>–</td>
<td>P. prevotii ATCC-9321</td>
<td>–</td>
</tr>
<tr>
<td>B. melaninogenicus 485 and ATCC-25845T</td>
<td>–</td>
<td>P. acarayolycytis VPI-5045A</td>
<td>–</td>
</tr>
<tr>
<td>Clostridium bifurcans B-1 and B-4</td>
<td>–</td>
<td>Mitsuokella multiaciada NCTC-10934</td>
<td>–</td>
</tr>
<tr>
<td>C. cocoides B-2</td>
<td>+</td>
<td>Megasphaera hypermegas Do34-6-11a</td>
<td>–</td>
</tr>
<tr>
<td>C. paraputrificum B-34 and VPI-6372 (+)</td>
<td>–</td>
<td>Streptococcus faecalis IFO-3971</td>
<td>–</td>
</tr>
<tr>
<td>C. difficile ATCC-9689</td>
<td>–</td>
<td>Propionibacterium acnes ATCC-11829</td>
<td>–</td>
</tr>
<tr>
<td>C. ramosum C-00 and ATCC-25582</td>
<td>–</td>
<td>Exserohilum coll O-601 and M-602</td>
<td>–</td>
</tr>
<tr>
<td>C. perfringens C-01 and ATCC-13124</td>
<td>–</td>
<td>Fusobacterium variium P-103-112</td>
<td>–</td>
</tr>
<tr>
<td>C. butyricum ATCC-14823</td>
<td>+</td>
<td>F. hiacus P S-64-476</td>
<td>–</td>
</tr>
</tbody>
</table>

Responses were scored as +, pH 4.5-5.0; +, 5.1-5.5; (+), 5.6-6.0; --, >6.1.

Table III. Effects of PHGG on Intestinal Microflora Counts1,2

<table>
<thead>
<tr>
<th>Microflora</th>
<th>Control A</th>
<th>Test 1</th>
<th>Test 2</th>
<th>Control B</th>
<th>Pooled SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10.80</td>
<td>10.94</td>
<td>10.95</td>
<td>10.90</td>
<td>0.44</td>
</tr>
<tr>
<td>Bacteroides spp.</td>
<td>10.35 (100)</td>
<td>10.49 (100)</td>
<td>10.65 (100)</td>
<td>10.52 (100)</td>
<td>0.58</td>
</tr>
<tr>
<td>Eubacterium spp.</td>
<td>10.16 (100)</td>
<td>10.27 (100)</td>
<td>10.25 (100)</td>
<td>10.33 (100)</td>
<td>0.64</td>
</tr>
<tr>
<td>Peptococcaceae</td>
<td>9.52 (100)</td>
<td>9.72 (100)</td>
<td>9.45 (100)</td>
<td>9.40 (100)</td>
<td>0.68</td>
</tr>
<tr>
<td>Bifidobacterium spp.</td>
<td>9.91R (100)</td>
<td>10.30R (100)</td>
<td>10.34R (100)</td>
<td>10.05R (100)</td>
<td>0.70</td>
</tr>
<tr>
<td>Veillonella spp.</td>
<td>6.47 (78)</td>
<td>7.40 (67)</td>
<td>6.39 (38)</td>
<td>7.43 (56)</td>
<td>3.61</td>
</tr>
<tr>
<td>Megasphaera spp.</td>
<td>8.42 (22)</td>
<td>8.60 (17)</td>
<td>nd (0)</td>
<td>nd (0)</td>
<td>–</td>
</tr>
<tr>
<td>Clostridium spp.</td>
<td>8.49 (100)</td>
<td>8.13 (100)</td>
<td>7.77 (88)</td>
<td>8.45 (100)</td>
<td>2.14</td>
</tr>
<tr>
<td>C. perfringens</td>
<td>5.14 (89)</td>
<td>4.30 (67)</td>
<td>4.75 (63)</td>
<td>6.75 (78)</td>
<td>3.27</td>
</tr>
<tr>
<td>Lactobacillus spp.</td>
<td>4.07 (67)</td>
<td>4.89 (89)</td>
<td>5.00 (100)</td>
<td>4.74 (83)</td>
<td>2.80</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>8.61 (100)</td>
<td>7.55 (100)</td>
<td>7.96 (100)</td>
<td>8.13 (100)</td>
<td>2.38</td>
</tr>
<tr>
<td>Streptococcaceae</td>
<td>7.61 (100)</td>
<td>7.25 (100)</td>
<td>6.86 (100)</td>
<td>7.62 (100)</td>
<td>2.33</td>
</tr>
<tr>
<td>Micrococcaceae</td>
<td>3.34 (44)</td>
<td>2.91 (17)</td>
<td>3.13 (25)</td>
<td>3.03 (33)</td>
<td>1.45</td>
</tr>
<tr>
<td>Bacillus spp.</td>
<td>3.18 (33)</td>
<td>3.20 (61)</td>
<td>2.94 (56)</td>
<td>3.09 (72)</td>
<td>1.20</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>2.60 (33)</td>
<td>3.51 (28)</td>
<td>2.57 (31)</td>
<td>3.40 (44)</td>
<td>2.31</td>
</tr>
<tr>
<td>Corynebacterium spp.</td>
<td>2.60 (6)</td>
<td>nd (0)</td>
<td>nd (0)</td>
<td>nd (0)</td>
<td>–</td>
</tr>
<tr>
<td>Yeasts</td>
<td>3.40 (56)</td>
<td>3.67 (50)</td>
<td>3.51 (81)</td>
<td>3.56 (56)</td>
<td>2.05</td>
</tr>
<tr>
<td>Molds</td>
<td>nd (0)</td>
<td>nd (0)</td>
<td>nd (0)</td>
<td>nd (0)</td>
<td>–</td>
</tr>
</tbody>
</table>

1 Mean of log10 bacteria/gram wet feces, with frequency of occurrence (number of feces having the bacteria/total samples) in the brackets.
2 Means of counts and frequency of occurrence for each microflora in Control A, Test 1, Test 2 and Control B were compared. Means having at least one similar letter were not significantly different at p<0.01.

perfringens, Enterobacteriaceae, and Streptococcaceae were rather decreased to some extent.

The frequency of occurrence of Lactobacillus spp. significantly varied as shown in Table III in which it was 67% in Control A while it was 89% and 100% in Tests 1 and 2, respectively. Those of Veillonella spp., Clostridium perfringens, and Micrococcaceae decreased during the test periods (no significant differences), while those of other bacteria were not affected by PHGG.

The percentage composition of Bifidobacterium spp. in total bacterial cell counts (Fig. 1) increased significantly from 14.7% in Control A to 31.7% in Test 1 and 24.8% in Test 2 and then returned to the original value during the subsequent control period. On the other hand, the growth of other bacteria except Eubacterium spp. were not affected by PHGG intake. The percentage of Eubacterium spp. decreased during the test periods, but the rate of decrease was too small to signify.
Fig. 1. Percentage Composition of Each Intestinal Microflora in Total Counts.
Significant difference: *p<0.05

Table IV. Effects of PHGG Intake on Fecal pH, Organic Acids, Ammonia, and Putrefactive Products Contents

<table>
<thead>
<tr>
<th></th>
<th>Control A</th>
<th>Test 1</th>
<th>Test 2</th>
<th>Control B</th>
<th>Pooled SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.14</td>
<td>5.87</td>
<td>5.77</td>
<td>6.16</td>
<td>1.03</td>
</tr>
<tr>
<td>Organic acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Lactic acid</td>
<td>0.244</td>
<td>0.208</td>
<td>0.259</td>
<td>0.263</td>
<td>0.227</td>
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<tr>
<td>Acetic acid</td>
<td>16.936</td>
<td>18.607</td>
<td>19.629</td>
<td>17.446</td>
<td>15.363</td>
</tr>
<tr>
<td>Proponic acid</td>
<td>5.843</td>
<td>4.885</td>
<td>5.831</td>
<td>6.150</td>
<td>4.911</td>
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<tr>
<td>iso-Butyric acid</td>
<td>0.380</td>
<td>0.521*</td>
<td>0.352</td>
<td>0.518</td>
<td>0.421</td>
</tr>
<tr>
<td>n-Butyric acid</td>
<td>5.882</td>
<td>5.733</td>
<td>5.232</td>
<td>5.313</td>
<td>5.662</td>
</tr>
<tr>
<td>iso-Valeric acid</td>
<td>0.379</td>
<td>0.427</td>
<td>0.409</td>
<td>0.495</td>
<td>0.414</td>
</tr>
<tr>
<td>n-Valeric acid</td>
<td>1.015</td>
<td>0.993</td>
<td>0.501</td>
<td>0.873</td>
<td>1.560</td>
</tr>
<tr>
<td>Total acid</td>
<td>30.656</td>
<td>31.528</td>
<td>32.995</td>
<td>32.959</td>
<td>23.742</td>
</tr>
<tr>
<td>Ammonia (μg/g dry feces)</td>
<td>1062.8</td>
<td>734.9**</td>
<td>760.4*</td>
<td>1264.8</td>
<td>690.8</td>
</tr>
<tr>
<td>Putrefactive products (μg/g dry feces)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-Cresol</td>
<td>189.74</td>
<td>156.63</td>
<td>167.76</td>
<td>181.21</td>
<td>196.44</td>
</tr>
<tr>
<td>Indole</td>
<td>72.10</td>
<td>51.84</td>
<td>35.67**</td>
<td>70.63</td>
<td>78.28</td>
</tr>
<tr>
<td>Total*</td>
<td>203.86</td>
<td>155.60</td>
<td>126.57</td>
<td>213.16</td>
<td>271.40</td>
</tr>
</tbody>
</table>

* Total includes p-cresol, indole, phenol, 4-ethylphenol, and skatole. Significant difference: *p<0.05, **p<0.01.

As shown in Table III, Fig. 1, it is clear that intake of PHGG increases the viable counts, frequency of occurrence and percentage of acid-forming bacteria, such as bifidobacteria and lactobacilli, among the intestinal microorganisms.

Fecal bacterial metabolites
The relationship between PHGG and fecal bacterial metabolites including pH, various organic acids, ammonia content, and putrefactive products and activities of some enzymes, was investigated. In the controls A and B the average pH of the feces was 6.15. However, as shown in Table IV, the pH during the period of PHGG intake was 5.77 in Test 2. The contents of volatile fatty acids and lactic acid of feces during this period not changed. PHGG intake resulted in a significant decrease in contents of fecal ammonia and indole. It should be noted that the most detectable reduction in the indole contents was more pronounced in Test 2. In parallel, it was also observed that intake of PHGG significantly affected the activity of fecal bacterial β-glucuronidase activity, but it has no effect on other enzyme activities examined (Table V). Two weeks after ceasing the intake of PHGG, pH, contents of ammonia, indole, and β-glucuronidase activity of feces returned to the values observed before PHGG intake.

Discussion
Several papers have been published on the effects of soluble or insoluble dietary fiber on human intestinal microflora. Salyers et al. have found in in vitro experiments that several species of anaerobic bacteria isolated from human colon ferment some polysaccharides. They also have reported that the isolated bacteria classified to belong to Rumibococcus, Bifidobacterium, and Bacteroides rapidly degrade guar gum into low molecular weight fragments. Tomlin et al. showed in an experiment by the fecal incubation method that guar gum was fermented by human colonic bacteria, suggesting that guar gum was predigested by enzymes. However, the growth response of intestinal bacteria to PHGG has remained to be unraveled.

In the in vitro experiment of this work, it was shown that PHGG moderately enhanced the growth of several colonic bacterial strains as it may be used as a carbon source. However, it had no effect on the growth of bifidobacteria and lactobacilli in the in vitro test (Table II).

In the volunteer study, on the contrary, the intake of PHGG caused a considerable increase in cell counts and percentage of bifidobacteria as well as increase in frequency of occurrence of lactobacilli (Table III, Fig. 1). PHGG was not used by bifidobacteria and lactobacilli in vitro, unlike the effect of PHGG intake in the in vivo experiment. The effect of PHGG in vivo may be attributed to PHGG in human colon being consecutively degraded by certain bacteria for the degraded products to favor the growth of bifidobacteria and lactobacilli. However, it is difficult to explain this effect by in vitro experiments using
the available techniques. This should await further investigations in which change of cell counts using in vitro mixed culture and fecal incubation system might be used.

Sugawara et al.\textsuperscript{19} investigated the changes of human intestinal microflora on hemicellulose (water-insoluble arabinoxylan) derived from corn and found that a moderate decrease in cell count of Enterococcaceae occurred but with no effect on lactic-acid-forming bacteria. Corn hemicellulose and PHGG differ from each other not only in sugar composition, but also in solubilities in water. It is well known that lactic-acid-forming bacteria have beneficial effects on our healthy by amino acid production, aiding defense activity against infection,\textsuperscript{17,18} pathogen inhibition,\textsuperscript{18,19} and immunopotentiation.\textsuperscript{15,20} This paper clearly shows that PHGG intake stimulates the growth of the beneficial lactic-acid-forming bacteria among the human intestinal microflora.

In this respect, the test volunteer observed flatulence at the beginning of these experiments, but this effect gradually declined within the first week of taking PHGG, then it completely disappeared.

The fecal pH went down during PHGG intake (Table V). The vigorous growth of bifidobacteria and lactobacilli in the intestine is probably the reason for lowering the pH levels of feces.\textsuperscript{21} Low pHs in the intestine may improve intestinal conditions by reducing formation of harmful bacterial metabolites.\textsuperscript{22} However, the contents of volatile fatty acids and lactic acid in feces were not affected by intake of PHGG.

As has been known on the experiment using animals and humans, those organic acids are readily absorbed in colon to be used as energy sources. The fecal ammonia and putrefactive products (indole, p-cresol) are known to be derived from urea and protein by intestinal bacteria. It has been reported that some enzymes produced by intestinal bacteria are highly likely to be elaborated in transformation of the ingested or endogenously produced compounds to toxic, mutagenic, or carcinogenic derivatives.\textsuperscript{23,24} On the other hand, many researchers reported that dietary fiber such as pectin, hemicellulose, and wheat bran are effective to retard fecal bacterial enzyme activities and ammonia content in animal and man.\textsuperscript{16,25} In our study, significant decreases in the contents of fecal ammonia and indole with concomitant reduction in β-glucuronidase activity were observed during the PHGG intake period, and agreed with the literature cited. A linear correlation between the decreases of these parameters and fecal pH is evident from the results of Fig. 2. Besides, Vince et al.\textsuperscript{26} and Aries et al.\textsuperscript{27} reported individually that generation of ammonia by intestinal bacteria was pH dependent, and that the reduction of intestinal pH affected bacterial enzyme activities. Therefore, the PHG and bacterial metabolites in intestine are closely related to our health. The experiment on the dose response of PHGG on intestinal microflora and their metabolism is in progress and will be published elsewhere.

References


