Effects of Yogurt Supplemented with Brewer’s Yeast Cell Wall on Intestinal Environment and Defecation in Healthy Female Adults

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Yogurt supplemented with brewer’s yeast cell wall (BYC) was administered to 24 constipated female volunteers, and the effect on the stool frequencies, fecal quantities, fecal characteristics, and fecal microflora were examined. Yogurt contained 6 g of BYC per 200 ml, and the other batch without BYC served as control diet. The volunteers ingested BYC-yogurt (BYC-Y) or control yogurt (CONT) every day for one week in a crossover experiment. Stool frequencies and fecal quantities of the subjects during the entire period of BYC-Y intake were found to be significantly higher than those under the CONT diet. It was observed that the proportion of Bifidobacterium in the fecal microflora significantly increased (p < 0.05), and those of Clostridium perfringens and Streptococcus significantly decreased (p < 0.05) with the ingestion of BYC-Y. The fecal water content and levels of short-chain fatty acids (SCFAs) in feces were also higher in subjects with BYC-Y intake than in CONT diet. Furthermore, because of the fermentation of SCFAs, BYC-Y administration significantly lowered fecal pH. A similar effect was observed with CONT intake; however, it was less evident than in the BYC-Y intake. These results indicate that the BYC-Y intake is effective to prevent the constipation. Furthermore, these findings suggest that the synergistic effects of beneficial bacteria in yogurt and BYC in improving the intestinal environment and bowel movement. These effects of BYC are related to such properties as fermentation, water-holding capacity, and swelling force in the large intestine.

Key words: brewer’s yeast cell wall; yogurt; intestinal environment; defecation; female adults

INTRODUCTION

Fermented milk is considered to be a health-promoting food because of its nutritional value and the presence of more than 10⁷ viable lactic acid bacteria/ml in it (12, 14, 19). Bifidobacteria are common anaerobes in the human intestinal microflora, which can contribute to human health and longevity (4, 13, 31). The belief that yogurt has special nutritional value and therapeutic effects has persisted for many years. In particular, the ingestion of yogurt is effective in improving the intestinal environment, fecal characteristics, and bowel movement. Viable microbial cultures such as yogurt are currently called “probiotics,” as suggested by Havenaar et al. (11). They defined probiotics as “mono- or mixed cultures of live microorganism which, when administered to animals or humans, beneficially affect the host by improving the properties of indigenous microflora.” On the other hand, the indigestible foodstuff is thought to contribute much to improve fecal characteristics and stool frequency and to alleviate constipation. Indigestible foodstuffs are called “prebiotics” and are defined as follows: those 1) that cannot be digested in the upper intestinal tract, 2) that enforce the growth of autochthonous microorganisms in the bowel, and 3) that stimulate bowel movement (22). In previous studies, indigestible oligosaccharides have been found to have beneficial effects on the intestinal environment and defecation (30, 32).

The cell wall of yeast is composed mainly of polysaccharides and their complexes with proteins (7). The principal building units of cell wall polysaccharides in yeast are glucose and mannose, followed by galactose, xylose, N-acetyl-D-glucosamine, uronic acids, and other minor components (7). Especially, brewer’s yeast cell wall (BYC) is a substance that can be regarded as a foodstuff. BYC consists mainly of water-insoluble dietary fibers, such as β-glucan and α-mannan, and of indigestible protein bound to the constitutive α-mannan. BYC largely contains indigestible polysaccharides and proteins, with more than 70% indigestible components. Several means of extracting BYC are available.
in the literature; however, we developed a technique that could maximize the BYC yield during its preparation, as given under Materials and Methods. Since BYC has been found to contain a large amount of dietary fibers and resistant proteins, the authors thought of utilizing these advantages to improve the fecal characteristics, defecation, and fecal microflora of constipated individuals. Thus with the composition of BYC, it may be considered as one of the prebiotics. In a previous study, it was reported that BYC alleviated constipation induced by the consumption of loperamide in rats at a dose of 5% BYC in their diet (26). BYC is considered to be a very safe foodstuff, since its source material, brewer’s yeast, has been ingested by humans as a nutrition and health food for many years. Furthermore, the safety of BYC in healthy volunteers was certified under physician’s control (25). The yogurt supplemented BYC (BYC-Y) is considered to be both a probiotic and a prebiotic; therefore, it is thought to have synergistic effects that improve the intestinal environment and bowel movement. Thus a previous study made an investigation on the preventive effect of BYC-Y on the improvement of defecation in slightly constipated female students, and the study provided evidence that BYC-Y intake effectively increased stool frequency and fecal quantity. The effects are significantly higher than ingestion of the control yogurt not supplemented with BYC (24). However, the effects associated with the influence of BYC-Y or BYC on fecal microflora have never been shown.

In this study, the preventive effect of BYC-Y on constipation and the improvement of fecal microflora in slightly constipated female adults were studied. To evaluate the effects of BYC-Y, yogurt without added BYC was administered as the control diet.

**MATERIALS AND METHODS**

*Sample preparation.* In our previous study (26), we reported the method for the production of brewer’s yeast cell wall (BYC), which consisted of polysaccharides and resistant proteins, from brewer’s yeast by a process involving alkalinization, enzymatic treatments, subsequent homogenization, and washing. BYC is sterilized by heating at 90°C for 30 min. Its chemical composition is given in Table 1. For the manufacture of yogurt (in liquid form for drinking), two strains of lactic acid bacteria were used as starters for fermentation. *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were inoculated in fresh milk, skim milk, sugar, whey protein concentrate, flavoring extract, bicarbonate of soda, and BYC. The detailed composition of BYC-Y is shown in Table 2. Standard yogurt used as the control diet was prepared by using the same amount of starters and ingredients, but BYC was not added.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>CONTa</th>
<th>BYC-Yb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td>15.95</td>
<td>15.95</td>
</tr>
<tr>
<td>Sugar</td>
<td>7.14</td>
<td>7.14</td>
</tr>
<tr>
<td>Skim milk</td>
<td>6.97</td>
<td>6.97</td>
</tr>
<tr>
<td>Flavor</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Bicarbonate of soda</td>
<td>0.16</td>
<td>0.16</td>
</tr>
<tr>
<td>WPCc</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Starterd</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td>BYC</td>
<td>0.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Water</td>
<td>72.20</td>
<td>69.20</td>
</tr>
</tbody>
</table>

*a* Control yogurt.  
*b* Yogurt supplemented with brewer’s yeast cell wall (BYC).  
*c* Whey protein concentrate.  
*d* Starter: *Streptococcus thermophilus, Lactobacillus bulgaricus.*
(CONT) was conducted with 24 slightly constipated female subjects (average age 22 years). All subjects were given 200 ml each of CONT or that of BYC-Y containing 6 g of BYC at a time. After a preliminary assessment of usual stool condition for 7 days, subjects were divided into 2 groups, and each group consisted of 12 subjects. Group 1 was administered BYC-Y and Group 2 CONT every day for 7 days. After interval period of one week, Group 1 was administered with CONT and Group 2 with BYC-Y every day for 7 days. The subjects were instructed to control their food intake throughout the experimental period. That is, we instructed them to avoid eating probiotic products, fermented milk, oligosaccharide products, dietary fiber-rich foods, and natto (soybeans fermented with Bacillus subtilis).

**Fecal analysis.** Whole freshly evacuated feces were collected from subjects at the end of each week of the experimental period. The samples were immediately kept under anaerobic conditions and stored at 4°C until ready for analysis. The fecal weight and pH were measured, and an analysis of fecal microflora was performed within 12 hr after excretion. The samples for an analysis of short-chain fatty acids (SCFAs) were frozen at −20°C immediately after weighing, and the frozen samples were thawed before analysis. The method for bacteriological analysis of the fecal microflora was basically identical to that reported by Mitsuoka et al. (21). After the fecal sample was mixed, serial dilutions were made by using anaerobic diluent, and 0.05 ml aliquots of the appropriate dilutions were spread onto 3 nonselective agar plates (modified Egggerth-Gagnon [EG] agar and glucose-blood-liver [BL] agar for anaerobes, and Trypticase soy [TS] agar with 5% blood for aerobes), as well as 7 selective agar plates (media for bifidobacteria [BS], eubacteria [ES], bacteroides [Bacteroides], lactobacilli [LBS], clostridia [Clostridia], enterobacteria [DHL], and Streptococcus [TS]). Plates for anaerobes (EG, BL, BS, ES, LBS, Clostridia, and Bacteroides) were incubated at 37°C for 3 days in an anaerobic jar filled with an atmosphere of oxygen-free CO2. Conversely, plates for aerobes (TS and DHL) were aerobically incubated at 37°C for 2 days. After incubation, the plates were examined for the growth of bacterial colonies. The identification of 7 bacterial groups was based on colony appearance and cell morphologies, Gram-reaction, spore formation, and aerobic growth. For every bacterial species identified, the colony count per gram of wet feces was calculated and expressed into logarithmic equivalent. Fecal water content was measured by lyophilization, as described previously (16). The concentration of SCFAs in feces was analyzed by gas chromatography (15).

**Fecal characteristics and defecation frequency.** An evaluation on the effect of BYC-Y for the improvement of defecation was made possible by requesting the volunteers to answer a questionnaire. Subjects recorded the occurrence of their stool frequency, fecal characteristics (quantity and consistency), evacuation condition, and feeling after evacuation. The physical condition of each subject was properly monitored every day. The subjects were also asked to record the intake of supplements for constipation, such as yogurt or dietary fiber, and the intake of medicines, such as antibiotics. Fecal quantity was measured as the number of equivalents to the amount of one styrene foam stick (22 x 15 cm). All data were collected and analyzed. During the experimental period, 11 subjects withdrew from participation; thus their data were excluded for analyses of fecal frequency and fecal quantity. Furthermore, 3 subjects dropped out of the collection of feces; thus the analyses of fecal microflora, fecal pH, and fecal water content were conducted in accordance with the data of 10 subjects.

**Statistical analysis.** All data were expressed as mean ± SEM. Statistical comparisons of fecal quantity and stool frequency were made by using the Wilcoxon signed rank-sum test. Statistical analyses of fecal water content, fecal pH, fecal microflora, and fecal SCFA concentrations were made by using the Student’s t-test. In statistical analysis, an associated probability (p-value) of < 5% was considered significant.

**RESULTS**

Stool frequency and fecal quantity are shown in Table 3. The stool frequency of the subjects under BYC-Y diet is significantly higher than those given with the CONT and nontreated diet (p < 0.01). The fecal quantity of the subjects under the BYC-Y diet is also significantly higher than those given with the CONT and nontreated diet (p < 0.01).

The changes in fecal microflora as affected by BYC-Y consumption are given in Table 4. On the other hand, the changes in the relative percentages of dominant bacterial groups in the fecal microflora are presented in Table 5. The number of Bifidobacterium significantly increased (p = 0.025) and that of Clostridium perfringens significantly decreased (p = 0.017) during the BYC-Y intake period compared with those during the nontreated period. Furthermore, the number of Streptococcus significantly decreased (p = 0.043) during BYC-Y intake period compared with the nontreated...
period. There was no significant difference observed in the number of each bacterial group between the non-treated period and the CONT intake period. With regard to the percentage distribution of isolated bacteria, no significant differences were found among treatments in all bacterial groups. However, the percentage of Bifidobacterium in BYC-Y diet was higher but not significantly different from the nontreated and CONT diets. On the other hand, Bacteroidaceae, Clostridium perfringens, and total aerobic bacteria in the BYC-Y diet exhibited lower percentages, but not significantly different, in comparison with the other two groups. The number, the percentage distribution, and the occurrence frequency of Clostridium perfringens departed from the usual range because the number of Clostridium perfringens from a subject was deviate.

Table 6 presents the fecal pH and water content of feces from subjects under the different diets prepared in the study. The fecal pH of subjects given the BYC-Y diet was significantly decreased in comparison with

<table>
<thead>
<tr>
<th>Table 3. Effect of yogurt supplemented with brewer’s yeast cell wall (BYC) on stool frequency and fecal quantity in female adults.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-treated</strong> (N)</td>
</tr>
<tr>
<td>Stool frequency</td>
</tr>
<tr>
<td>Fecal quantity</td>
</tr>
</tbody>
</table>

Wilcoxon signed rank-sum test (n = 13)

<table>
<thead>
<tr>
<th>N : C</th>
<th>N : B</th>
<th>C : B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stool frequency</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Fecal quantity</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

Table 4. Influence of yogurt supplemented with brewer’s yeast cell wall (BYC) on the number of fecal microflora.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Non-treated</th>
<th>CONT</th>
<th>BYC-Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacteriaceae</td>
<td>7.2 ± 0.4 (100)</td>
<td>6.8 ± 0.3 (100)</td>
<td>6.9 ± 0.2 (100)</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>6.8 ± 0.4 (100)</td>
<td>6.7 ± 0.3 (90)</td>
<td>6.0 ± 0.3 (90)</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>6.0 ± 0.6 (20)</td>
<td>7.3 ± 0.8 (70)</td>
<td>6.7 ± 0.5 (60)</td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td>9.6 ± 0.1 (90)</td>
<td>9.8 ± 0.2 (100)</td>
<td>9.8 ± 0.1 (100)</td>
</tr>
<tr>
<td>Eubacterium</td>
<td>9.0 ± 0.2 (80)</td>
<td>9.0 ± 0.3 (100)</td>
<td>9.1 ± 0.2 (100)</td>
</tr>
<tr>
<td>Bacteroidaceae</td>
<td>9.8 ± 0.1 (90)</td>
<td>10.1 ± 0.1 (100)</td>
<td>9.8 ± 0.2 (100)</td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td>8.2 ± 0.3 (90)</td>
<td>7.0 ± 0.4 (90)</td>
<td>6.7 ± 0.4 (90)</td>
</tr>
</tbody>
</table>

Volunteers (n = 10) consumed 200 ml of yogurt supplemented with BYC or control yogurt every day for 7 days.

| | | | |
|---|---|---|
| Total | 7.6 ± 0.3 | 7.2 ± 0.3 | 7.0 ± 0.2 |
| Total anaerobic bacteria | 9.9 ± 0.2 | 10.4 ± 0.1 | 10.1 ± 0.2 |

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a Number of times/week.
b Number of styrene foam stick (21 x 15 cm)/week.
c Not administered with CONT or BYC-Y.
d Control yogurt.
e Yogurt supplemented with BYC.
f Values are expressed as mean ± SEM.
g N, non-treated; C, CONT; B, BYC-Y.

Mean values are significantly different: * p < 0.01.
Table 5. Influence of yogurt supplemented with brewer’s yeast cell wall (BYC) on the percentage distribution of fecal microflora.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Nontreateda</th>
<th>CONTb</th>
<th>BYC-Yc</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bifidobacterium</em></td>
<td>35.6 ± 6.1d</td>
<td>35.0 ± 7.8</td>
<td>46.7 ± 8.7</td>
</tr>
<tr>
<td><em>Eubacterium</em></td>
<td>11.8 ± 4.7</td>
<td>8.2 ± 3.4</td>
<td>9.9 ± 3.1</td>
</tr>
<tr>
<td><em>Bacteroidaceae</em></td>
<td>49.5 ± 6.6</td>
<td>53.4 ± 6.5</td>
<td>43.1 ± 7.0</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>5.8 ± 3.7</td>
<td>0.7 ± 0.6</td>
<td>0.1 ± 0.04</td>
</tr>
<tr>
<td>Total aerobic bacteria</td>
<td>8.7 ± 7.0</td>
<td>0.2 ± 0.06</td>
<td>0.1 ± 0.04</td>
</tr>
</tbody>
</table>

Volunteers (n = 10) consumed 200 ml of yogurt supplemented with BYC or control yogurt every day for 7 days.

a Not administered with CONT or BYC-Y.
b Control yogurt.
c Yogurt supplemented with BYC.
d Values are expressed as mean ± SEM (n = 10).

No significant difference exists among treatments for each bacteria group.

Table 6. Effect of yogurt supplemented with brewer’s yeast cell wall (BYC) on fecal characteristics.

<table>
<thead>
<tr>
<th>pH</th>
<th>Nontreateda</th>
<th>CONTb</th>
<th>BYC-Yc</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.84 ± 0.16d</td>
<td>7.06 ± 0.08</td>
<td>6.65 ± 0.18c</td>
<td></td>
</tr>
</tbody>
</table>

Volunteers (n = 10) consumed 200 ml of yogurt supplemented with BYC or control yogurt every day for 7 days.

a Not administered with CONT or BYC-Y.
b Control yogurt.
c Yogurt supplemented with BYC.
d Values are expressed as mean ± SEM (n = 10).
e Significantly different compared with CONT (p < 0.05).
f Significantly different compared with nontreated (p < 0.05).

Fig. 1. Influence of yogurt supplemented with brewer’s yeast cell wall (BYC) on fecal short-chain fatty acids (SCFAs) concentration.

Volunteers (n = 10) consumed 200 ml of yogurt supplemented with BYC (6 g) or control yogurt every day for 7 days. CONT, control yogurt; BYC-Y, yogurt supplemented with BYC. No significant difference exists among individuals in components or total SCFA in CONT and BYC-Y.
the CONT subjects \((p = 0.016)\). Similarly, the BYC-Y intake exhibited lower fecal pH, but no significant difference was observed when compared with nontreated subjects \((p = 0.121)\). Regarding the fecal water content, BYC-Y intake was found to be significantly higher than the nontreated group \((p = 0.005)\). Moreover, although BYC-Y values were higher compared with the CONT subjects, no significant difference was noted between the two samples observed \((p = 0.14)\).

Figure 1 shows changes of the SCFA concentrations in feces from the CONT and BYC-Y diets. Results revealed no significant differences in fecal SCFA concentrations between samples from subjects administered CONT and BYC-Y. However, the total SCFAs or their individual components tended to increase after BYC-Y intake. Especially the concentration of n-butyrate in BYC-Y intake increased in comparison with the CONT at \(p < 0.10\).

**DISCUSSION**

Brewer’s yeast cell wall (BYC) consists mainly of water-insoluble dietary fibers and indigestible proteins bound to the constitutive \(\alpha\)-mannan. In our previous study, we reported the preventive effect of BYC on constipation, which was induced by loperamide in rats at a dose of 5% BYC, and we observed the dose-dependency effect on the improvement of constipation \((26)\). In the study, BYC not only increased the fecal quantity and dry weight, but it also markedly improved fecal water content. Furthermore, BYC increased cecal acetate, propionate, and butyrate concentrations. For several years, different microorganisms have been used successfully to prevent and cure diseases in animals and humans. The beneficial effect of intestinal bacteria on health is termed as probiotics, such as *Bifidobacterium*, which was first used by Lilley and Stillwell \((18)\).

Prebiotics, on the other hand, is related to probiotics and includes complex compounds, such as polysaccharide, dietary fiber, and resistant protein, which promote the growth of anaerobes and are bioconverted into SCFAs through anaerobic fermentation, thus lowering the intestinal pH and suppressing the growth of aerobic bacteria \((2, 14, 19, 20)\). SCFAs, especially butyrate, which is fermented by intestinal microorganisms, are the major energy source for intestinal epithelial cells. It stimulates the activity of bowel movements \((10, 28)\). Therefore it is assumed that yogurt, which is a probiotic, supplemented with BYC, which is thought to be a prebiotic, may greatly contribute to the improvement of defecation and increase beneficial microflora in the large intestines of humans. In all these, it is important to know whether fermented milk supplemented with prebiotic BYC will truly contribute to the improvement of beneficial intestinal microflora, the intestinal environment and bowel movements.

This study provides ample evidence that the ingestion of BYC-Y can effectively improve not only the stool frequency and fecal quantity, but also the fecal microflora and fecal characteristics \((pH\) and water content\) in female constipated adults. The stool frequency and the fecal quantity during the BYC-Y intake period were significantly higher than during other periods. Control fermented milk, on the other hand, showed only a slight improvement on all parameters described above. Increasing the number of *Bifidobacterium* and decreasing the population of the known harmful intestinal bacteria, such as *Clostridium perfringens*, *Streptococcus*, and other aerobic bacteria, could lead to a betterment of the intestinal environment. Furthermore, it has been suggested that the improvement of intestinal microflora tends to lower serum cholesterol level, reduce diarrheal incidence, stimulate the immune system, control infections, suppress tumors, and protect against colon cancer \((5, 8, 9, 17, 27)\).

These findings indicate that BYC-Y intake can remarkably improve the bowel movement and contribute in the alleviation of constipation as a result of its increasing the number of beneficial intestinal microflora and the increment of fecal SCFAs. We consider that these positive effects of BYC on the improvement of constipation could be explained by the SCFAs produced by intestinal anaerobic fermentation of BYC and by its water-holding capacity and swelling force capacity. Aside from the fecal water content, the stool frequency and fecal quantity were also affected by the water-holding capacity and swelling force in the large intestine. It has been reported that BYC has the highest swelling capacity among dietary fibers such as cellulose, wheat bran, beet fiber, and corn fiber \((23, 26)\). Furthermore, these positive effects on the alleviation of constipation are thought to be synergistically accomplished by the properties of BYC as described above and by the beneficial viable microorganisms present in yogurt.

In conclusion, the increase in beneficial bacteria, the decrease in harmful bacteria, and the increment of fecal SCFAs fermented in the large intestine were observed as a result of BYC-Y ingestion. These results suggest that BYC, which is assumed to be a prebiotic, reaches the colon undigested and stimulates the growth of beneficial microflora such as *Bifidobacterium*, which produces SCFAs, lowers pH in the intestinal tract, and
improves intestinal balance. Therefore these effects of BYC in association with its water-holding capacity and swelling force in the large intestine contribute to the improvement of constipation.

The effects of fermented milk on the improvement of fecal frequency was reported in healthy elders by Seki et al. (29), in pregnant women by Ebisawa et al. (6), and in university students by Akazawa (1). Today, female adults lead irregular lives; consequently they have lost their health and suffer from functional constipation, functional diarrhea, irritable bowel syndrome, headache, and skin diseases such as atopic dermatitis. Most young women are afflicted with chronic constipation. Therefore it is very important to certify in this study: The ingestion of only 200 ml BYC-Y (containing 6 g BYC) per day has an effect on the improvement of defecation in young woman.

The mechanism underlying the effect of BYC or BYC-Y on the improvement or stabilization of the intestinal system must be elucidated in detail. Future studies are needed to investigate their effect on the reduction of diarrhea, inflammatory bowel diseases, and irritable bowel syndrome.

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REFERENCES


(24) Nakamura T, Nishida S, Shirasu Y, Murayama T. 2000. The mechanism underlying the effect of BYC or BYC-Y on the improvement or stabilization of the intestinal system must be elucidated in detail. Future studies are needed to investigate their effect on the reduction of diarrhea, inflammatory bowel diseases, and irritable bowel syndrome.