Effects of Brewer's Yeast Cell Wall on Constipation and Defecation in Experimentally Constipated Rats

Tomohiko Nakamura, Kazue Agata, Mai Mizutani, and Hisakazu Ino

1 Applied Bioresearch Center, Corporate Research and Development Department, Kirin Brewery Co., Ltd., Miyahara Machi 3, Takasaki, Gunma 370-1295, Japan
2 Showa Women's University, I-7 Taishido, Setagaya-ku, Tokyo 154-0004, Japan

Received August 2, 2000; Accepted December 4, 2000

Brewer's yeast cell wall (BYC) was tested on constipated male Sprague-Dawley rats that had been induced by loperamide (2 mg/kg of body weight). The preventive effect of BYC on constipation was examined and compared with that of a non-fiber diet (NF) as the control. The dose-response of BYC and the effect on defecation by constipated experimental rats were also compared with the characteristics of cellulose diet (CE) group which served as a control. Defecation was observed to be greater by the rats fed with BYC than by those fed with NF or CE. The fecal water content and level of volatile fatty acids (VFA) in the cecal contents were likewise higher in the rats fed with BYC. These results indicate that the administration of BYC was effective for improving defecation and other parameters related to defecation. These favorable effects of BYC supplemented to the diet are attributed to the fermentation ability, water holding capacity and swelling force in the large intestine.

Key words: yeast cell wall; constipation; defecation; dietary fiber; rat

The cell wall of yeast is composed mainly of polysaccharides and their complexes with proteins. The principal building units of the cell wall polysaccharides in yeast are glucose and mannose, with galactose, xylose, N-acetyl-D-glucosamine and uronic acids as the other minor components. The proteins in yeast exist as covalent complexes with mannans. Brewer's yeast cell wall (BYC) is considered to be an improved foodstuff component consisting mainly of water-insoluble dietary fibers such as β-glucan and α-mannan, and of indigestible protein bound to the constitutive α-mannan. BYC is made up of largely indigestible polysaccharides and proteins compared with brewer's yeast, and contains more than 60% indigestible components.

Previous studies have shown that not only indigestible polysaccharides but also resistant proteins are associated with fermentation in the large intestine and contribute to normalization of the intestinal environment. Hence, since BYC contains a large amount of dietary fibers and resistant proteins, it can effectively improve the fecal characteristics, defecation frequency and constipation.

In addition to the fermentative ability of the indigestible components, it has been reported that the swelling capacity of dietary fiber in the intestinal tract affects defecation. Furthermore, the swelling capacity, which is measured as the settling volume (SV) in an artificial intestinal phase (a 1/15M phosphate buffer at pH 6.8) has been found to be closely related to the fecal water content, defecation frequency, and even to the improvement of diarrhea.

Yeasts have been used for food since ancient times and for the production of many kinds of fermented food such as bread, beer and wine. Dried yeast has recently been widely used for producing healthy and nutritious foods, and is included in the list of the Japanese pharmacopoeia. Moreover, extracts of yeast have been used as organic ingredients of seasonings and other food components. Yeast extracts are composed of nucleic acids, amino acids and such peptides as inosinic acid and glutamic acid. Yeast extracts are already produced commercially for industrial use. The cell wall of yeast has also been used for food and is recognized as one of the food additives in Japan. Studies on the effects of brewer's yeast on different aspects related to the bowel environment have been reported. However, the effect of BYC on defecation, bowel environment and preventing constipation have not been adequately established.

In this study, the preventive effect of BYC on constipation induced by loperamide administration was investigated. To evaluate the effect of BYC on constipation, diets containing non-fibers were administered as the control. In addition, the dose-response relationship between BYC and its effect on the defe-
cation of constipated rats was compared with that of a cellulose diet (CE) as the control. An investigation was also performed on the increment of SV in BYC as it affects the environment of the large intestine and the fecal characteristics.

Materials and Methods

Materials. BYC was produced from brewer’s yeast slurry provided by the Kirin Brewery Shiga factory (Shiga, Japan). The slurry was diluted with water, mechanically stirred, and the extra proteins were removed by sieving. The slurry was made alkaline with NaOH to the desired pH value (pH 8–pH 12) and then centrifuged at 2000–5000 rpm and at 10°C for 10 min to separate the residue from the supernatant. The residue was cooled and homogenized at 500–950 kgf/cm² with a high pressure homogeniser (APV). The resulting homogenized slurry was quickly heated to the desired temperature (30–50°C), some enzymes were added (Alcalase, Flavourzyme and Neutrase purchased from Novo Nordisk) and incubated at 40–65°C (pH 7.0–8.0). The mixture was quickly heated to the desired temperature (70–90°C) to inactivate the enzymes, before being centrifuged at 2000–5000 rpm and 40–65°C for 10 min to separate the residue from the supernatant. The residue was recovered after being homogenized at 500–950 kgf/cm² and washed twice with distilled water. The mixture was again centrifuged. The residue was recovered by lyophilization and this served as the dietary fiber-rich fraction which was sterilized by heating at 90°C for 30 min. BYC is considered to be the indigestible polysaccharides and the resistant proteins as covalent complexes. The chemical composition of BYC is shown in Table 1.

All the dietary components were purchased from Oriental Yeast Co. (Tokyo, Japan), while all the reagents were purchased from Wako Pure Chemical Industries Co. (Osaka, Japan). Cellulose, wheat bran, corn fiber and beet fiber used in the experiment were purchased from Nabelin Co. (Nagano, Japan). The cellulose used in Experiment 2 was a refined type of material for food application (crystalline form).

Methods. Experiments 1 and 2 in this study were approved by the Ethics Committee of Kirin Brewery Co., Ltd. The animals were maintained in accordance with the guidelines of this Committee for the care and use of laboratory animals.

Experiment 1. The effect of BYC on constipation that had been induced by loperamide in rats was investigated, using a non-fiber group (NF) as the control. The methods used in this experiment were similar to those reported by Kanauchi et al. Three-week-old male rats of the Sprague-Dawley strain (purchased from Charles River Japan Co., Yokohama, Japan) were housed in individual stainless steel cages with wire screen bottoms in a room kept at 22±1°C, with lighting provided from 8:00 a.m. to 8:00 p.m. daily. The rats were allowed free access to their respective diets and to drinking water. After adaptation to the laboratory diet (CE-2, Nihon Clea Co., Tokyo, Japan) for seven days, the rats were divided into two groups (n=10), each group being fed either with the NF- or BYC-containing diet for 15 consecutive days. The detailed composition of the experimental diets is shown in Table 2. On day 14, loperamide was orally administered to the rats by means of a gavage at a dose of 2 mg/kg of body weight. During the experimental period, the body weight was recorded on the 5th, 8th, 12th, 13th, 14th and 15th days, and the food intake was recorded on the 3rd, 6th, 10th, 13th, 14th and 15th days before replenishing the diet. Feces excreted during the 12th, 13th and 14th days of feeding on the experimental diets with no loperamide administration, and on the final day (15th day) of the loperamide administration period, were collected. The number of feces were counted and all were stored at −80°C. After lyophilization, the dry weight of the feces was determined. On day 15, the rats were anesthetized with urethane, and the ceca were isolated. Fresh feces in the rectum were collected and the fecal water content was measured by lyophilization. The concentration of volatile fatty acids (VFA) in the cecal contents was analyzed by gas chromatography.
Experiment 2. The second experiment involved an examination of the dose-response relationship between the percentage of BYC in the diet and the effect on defecation by the constipated experimental rat model induced by loperamide. The CE group was used as the control. The methods used for this experiment were similar to those of experiment 1, except for the control diet and the method of loperamide administration. Three-week-old male rats of the Sprague-Dawley strain were used as the experimental animals (purchased from Charles River Japan Co., Yokohama, Japan). After their adaptation to the laboratory diet (CE-2, Nihon Clea Co., Tokyo, Japan) for seven days, the rats were divided into six groups (n = 10), each group being fed either with the CE- or BYC-containing diet for 11 days. The test diet contained BYC, wherein the protein and dietary fiber contents were adjusted to the same levels as those in the CE diet by changing the levels of casein and cellulose. The six groups of rats were fed on the corresponding diets as follows: cellulose equivalent to dietary fiber contained in 2% BYC (CE2); cellulose equivalent to dietary fiber contained in 3% BYC (CE3); cellulose equivalent to dietary fiber contained in 5% BYC (CE5); 2% BYC (BYC2); 3% BYC (BYC3); and 5% BYC (BYC5). This equivalence was according to the dietary fiber level measured by the Prosky method. The detailed composition of each experimental diet is shown in Table 3. On day 11, loperamide was added to both diets, and the rats were subsequently fed with the respective diets for another three days. During the experimental period, the body weight was recorded on the 5th, 8th, 11th, 12th, 13th and 14th days, and the food intake was recorded on the 3rd, 6th, 9th, 12th, 13th and 14th days before replenishing the diet. Feces excreted on the final three days (12, 13 and 14) of loperamide administration were collected. The number of feces was counted and the wet weight was determined. The excreted feces were collected each day on the 12th, 13th and 14th days for determination of the apparent fecal wet weight. On day 14, the rats were anesthetized with urethane, and the ceca were isolated. Fresh feces in the rectum were collected, and the fecal water content was measured by lyophilization. The concentration of VFA in the cecal contents was measured by gas chromatography.

Experiment 3. This particular experiment investigated the swelling capacity of BYC which is believed to have influence on the fecal water content, defecation frequency, and further alleviation of diarrhea. The swelling capacity of BYC was determined by methods similar to those used by Middleton et al., (1) Takeda et al., (2) and Nakamura et al. (3) The swelling capacity was measured in terms of SV in an artificial intestinal phase (a 1/15M phosphate buffer at pH 6.8), an increment in SV indicating an increase in the water-holding capacity and swelling force in the large intestine.

Approximately 50 ml of water was added to a 100-ml media bottle containing 1.0 g of dietary fiber. This was mechanically stirred in a water bath while air was removed for 1 min and then ultrasonicated in the water bath for 5 min. The mixture was immediately transferred to a 100-ml volumetric cylinder, and topped up with distilled water to the 100-ml mark. The SV value of dietary fiber in the cylinder was determined after the mixture had been allowed to settle for 20 min. Cellulose, wheat bran, corn fiber and beet fiber served as the controls.

Statistical analyses. Statistical comparisons between the two groups were made by using Student’s t-test. In all statistical analyses, an associated probability (p value) of < 5% has been considered as significant.

Table 3. Composition of the Experimental Diets (Experiment 2)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>CE2&lt;sup&gt;a&lt;/sup&gt;</th>
<th>BYC2&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CE3</th>
<th>BYC3</th>
<th>CE5</th>
<th>BYC5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casel</td>
<td>15.0</td>
<td>14.6</td>
<td>15.0</td>
<td>14.4</td>
<td>15.0</td>
<td>14.0</td>
</tr>
<tr>
<td>Vitamin mixture&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Mineral mixture&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Brewer's yeast cell wall</td>
<td></td>
<td>2.0</td>
<td></td>
<td>3.0</td>
<td></td>
<td>5.0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>1.2</td>
<td></td>
<td>1.8</td>
<td>3.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loperamide&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.01</td>
<td></td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5.0</td>
<td></td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>74.1</td>
<td></td>
<td>73.7</td>
<td>72.9</td>
<td>72.3</td>
<td>71.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Based on the AIN-93 vitamin mixture protocol.<sup>20</sup>
<sup>b</sup> Based on the AIN-93G mineral mixture protocol.<sup>21</sup>
<sup>c</sup> Volume of loperamide was estimated as (0.20 kg of body weight × 10 mg of loperamide/kg of body weight)/0.02 kg of food intake = 450 mg of loperamide/kg of diet.

The protein and dietary fiber contents in both diets were adjusted to the same levels.
To examine the dose-response relationship between the BYC-ingestion level and defeation in Experiment 2, all data were analyzed by one-way or two-way ANOVA. Fisher's least significant difference test was used to determine whether mean values were significantly different at $p<0.05$.

**Results**

_Effect of BYC on body weight gain, food intake and fecal characteristics_

There were no significant differences in the body weight gain and food intake between the NF- and BYC-supplemented diet groups. The food intake by these two groups decreased slightly after the administration of loperamide (day 14). This decrease in food intake resulted in a slight retardation of the body weight gain by the rats.

Figure 1 presents the number of feces and fecal dry weight for normal rats for three consecutive days (9, 10 and 11) within the experimental period. The number of feces and fecal dry weight were found to be significantly higher in the rats fed with BYC than in those fed with NF.

Figure 2 gives the number of feces and fecal dry weight in the constipated-model rats for three consecutive days (12, 13 and 14), and the fecal water content and cecal VFA concentration in these rats at the end of the experimental period (day 15). The number of feces, the fecal dry weight and the water content (%) of the feces in the rectum were observed to be significantly higher in the rats fed with BYC than in those fed with NF. These findings signify that BYC effectively increased the fecal frequency, fecal volume and the fecal water content. In addition, BYC increased the cecal acetate, propionate and butyrate contents, their concentrations being found to be significantly higher in the rats fed with BYC than in those fed with NF. BYC is thus presumed to have been used efficiently by the rats, as exhibited by the large number of intestinal microflora. It is assumed that the cecal pH level was also lowered during BYC administration to the constipated rats, although this aspect was not examined.

Experiment 2. Effect of BYC concentration on the body weight gain, food intake and fecal characteristics

A statistical analysis of the data obtained revealed no significant differences in the body weight gain and food intake between the two groups at different BYC concentrations. The food intake by all groups slightly decreased after the administration of loperamide (day 12), although the rats in all groups had immediately recovered by days 13 and 14. This decrease in food intake resulted in a slight retardation of the body weight gain.

The number of feces and fecal wet weight on days 12, 13 and 14 in the constipated-model rats are

---

Fig. 1. Comparison between the Non-fiber Diet (NF) Group and the Brewer's Yeast Cell Wall Diet (BYC) Group on the Number of Feces (A) and Fecal Dry Weight (B) in Normal Rats for 3 Consecutive Days (days 9, 10 and 11) of Feeding (Experiment 1). *$p<0.05$ compared with the NF group. Student's $t$-test was used for the statistical analysis.

Fig. 2. Comparison between the Non-fiber Diet (NF) Group and the Brewer's Yeast Cell Wall Diet (BYC) Group on the Number of Feces (A), Fecal Dry Weight (B), Fecal Water Content (C) and Volatile Fatty Acid Concentration (D) in the Constipated-model Rats during the Last Three Days (days 12, 13 and 14) of the Experimental Period (Experiment 1). *$p<0.05$ compared with the NF group. Student's $t$-test was used for the statistical analysis.
Fig. 3. Comparison between the Cellulose Diet (CE) Group and the Brewer’s Yeast Cell Wall Diet (BYC) Group on the Number of Feces (A), Fecal Wet Weight (B) and Fecal Water Content (C) of Rats Fed with Cellulose (CE) and Brewer’s Yeast Cell Wall (BYC) at Different Concentrations (Experiment 2).

Each value is the mean for 10 rats (n = 10). The number of feces and fecal wet weight were measured during the last 3 days, while the fecal water content was determined on day 14 of the experimental period.

*p < 0.05 compared with the CE group with the same concentration of dietary fiber. Student’s t-test was used for the statistical analysis.

Presented in Figs. 3(A) and 3(B), respectively. The number of feces and fecal wet weight were significantly higher in the rats fed with the highest concentration of BYC (BYC5) than in those fed with the control diet at the same concentration (CE5). However, no significant differences were apparent in the number of feces and fecal wet weight between the two groups fed at the lower concentrations (CE2 and BYC2; CE3 and BYC3). No particular relationship was found between the level of BYC ingestion and the number of feces, there being no significant difference between the BYC2-fed and BYC3-fed groups (p = 0.130). On the contrary, the number of feces in the BYC5 group was significantly higher than in the BYC3 group (p = 0.005). Furthermore, the fecal wet weight in the BYC3 group was higher than that in the BYC2 group (p = 0.016), and the fecal wet weight in BYC3 group was eventually significantly lower than in BYC5 group (p = 0.005), indicating an evident relationship between the BYC-ingestion level and fecal wet weight.

Figure 3(C) shows the fecal water content (%) in constipated-model rats on day 14. The fecal water content in the rectum was significantly higher in the rats fed with BYC than in those fed with CE. On the other hand, no relationship was apparent between the level of BYC-ingestion and the fecal water content. While the results indicate a higher fecal water content in the BYC3 group than in the BYC2 group (p = 0.072), the content in the BYC3 group was not significantly lower than that in the BYC5 group (p = 0.17). These findings show that the number of feces and fecal wet weight were highly dose-responsive to the BYC-ingestion level.

Figure 4 shows the VFA concentrations at different BYC levels in the cecal contents on day 14 of the experimental period. The VFA concentration (μmol/g of cecal content) in the cecal contents was found to be significantly higher in the rats fed with BYC than in those fed with CE. Thus, BYC might have been efficiently used in the system as indicated by the large number of intestinal microflora. The butyrate and propionate concentrations in the rats fed with BYC2 were significantly higher than those in the rats fed with CE2. Moreover, the butyrate, propionate and acetate concentrations in the rats fed with BYC3 or BYC5 were significantly higher than in those fed with the equivalent CE3 or CE5. These results reveal that the level of BYC ingestion was not in any way related to the VFA concentration. It is possible that 2% BYC in the diet provided adequate response for the fermentation of VFA by cecal bacteria.

Experiment 3. Effect of the source of dietary fiber on the settling volume

The mean values for the settling volume (SV) measured in the artificial intestinal phase (a 1/15M phosphate buffer at pH 6.8) range from 4.0 ml/g to 50 ml/g depending on the kind of dietary fiber as follows: cellulose, 6.0 ml/g; wheat bran, 4.0 ml/g; corn fiber, 6.0 ml/g; beet fiber, 13.0 ml/g; BYC, 50.0 ml/g. The SV value was lowest for wheat bran and highest for BYC, this value correlating very well with the water-holding capacity and swelling force in the intestinal tract. It may be deduced, therefore, that the increment of SV in BYC affected the fecal water content and defecation, and consequently improved the intestinal environment.

Discussion

The preventive effect of dietary fiber on constipation induced by loperamide in rats has recently been reported by Kanauchi et al. Loperamide is used as a
potent internal medicine for functional bowel disorders like diarrhea. Loperamide is also known to inhibit intestinal fluid secretion and stop diarrhea by disturbing the intestinal motility,\textsuperscript{17} and thus promotes constipation.

The cell wall of yeast is known to be composed mainly of polysaccharides and proteins such as \( \beta \)-glucan, \( \alpha \)-mannan, and mannoprotein. This composition is basically of water-insoluble and indigestible dietary fibers. Mannoprotein in BYC is considered to be a resistant protein. Most of the proteins in yeast, when treated with some enzymes and washed with water after their homogenization under high pressure, are thought to be completely deprived of their mannan structure by centrifugation. The residual protein portion in BYC obtained by precipitation contains substances with indigestible properties. The proteins in yeast removed by the process of centrifugation are liberated from the polysaccharide portions of the yeast mannoprotein supernatant fractions as amino acids or peptides. Due to this indigestible property, it is presumed that BYC has favorable effect on defecation and furthermore helps in preventing constipation.

This study has focused on the effects of BYC on defecation and preventing constipation in experimental rats. Furthermore, the dose-response relationship between the BYC-ingestion level and defecation was examined. In the normal rats, the BYC-supplemented diet increased the number of feces and fecal dry weight. In the constipated model, BYC not only increased the number of feces and fecal dry weight, but also markedly improved the fecal water content. In addition, BYC was found to increase the cecal acetate, propionate and butyrate contents. Based on the data obtained, BYC is considered to have been efficiently used, as was evident from the large number of intestinal microflora.

A dose-response relationship between BYC and defecation by the constipated experimental rats was identified from the number of feces and their consistency indicated by the fecal wet weight. The fecal water content was not markedly increased and this did not depend on the percentage of BYC in the diet. When BYC was given at 2\% and 3\% concentrations, constipation was not adequately improved. The results reveal that while the inclusion of 5\% BYC in the diet significantly increased the number of feces and fecal weight, 2\% BYC in the diet could make an acceptable adjustment to the fecal water content and increase the cecal VFA concentration.

BYC was proved to have the highest SV value among the dietary fibers tested in this study. It may thus be concluded that the fecal water content was improved due to the high water-holding capacity of BYC. Apart from the fecal water content, the fecal frequency and fecal volume were likewise affected by the water-holding capacity and swelling force in the large intestine. This swelling force serves as a physical stimulus to defecation.

BYC is effectively utilized by the microorganisms present in the large intestine and may thus induce a change in the intestinal microflora, resulting in an increase in beneficial microorganisms in the large intestine. VFA, and especially butyrate when fermented by intestinal microflora, becomes a major energy source for intestinal epithelial cells, and stimulates the activity of defecation.\textsuperscript{18-20} Furthermore, butyrate has been shown to be an effective compound for ulcerative colitis,\textsuperscript{21} and it was claimed that butyrate enemas inhibit mucosal damage in human ulcerative colitis.\textsuperscript{22} Like butyrate, dietary fiber as a "prebiotic" has also been effective in curing ulcerative colitis. The effect of dietary fiber on dextran sulfate sodium-induced colitis in rats has recently been studied.\textsuperscript{23} This study showed that 2\% BYC in the diet could significantly increase the cecal butyrate concentration, signifying the usefulness of BYC as a substance to improve the intestinal microflora. Hence, the preventive effect of BYC on constipation may be explained not only by the physical stimulation of intestinal mucosa, but also by the production of VFA from fermentable dietary fiber. In Experiment 2, crystalline cellulose, which is thought difficult to be utilized by intestinal microflora, was used as a control, and it is assumed that one of the reasons for the remarkable effect of VFA production from BYC ingestion is that BYC was more easily utilized by intestinal microflora than the control. A comparison with other dietary fibers of the effect on intestinal microflora remains to be investigated.

The detailed mechanism for the effect of BYC on improving or stabilizing the intestinal system must be
studied. Furthermore, future studies should be made on the effect of BYC in inhibiting ulcerative colitis.

Acknowledgments

We thank Kirin Brewery Shiga factory for the financial support in this endeavor, and extend our sincere thank to engineers at the factory for their assistance and cooperation. We also thank Dr. Osamu Kanauchi, Dr. Yoshiharu Shirasu and Dr. Katsumi Nakamura (Kirin Brewery Co., Ltd.) for their helpful advice and encouragement in this study. We also acknowledge the considerable cooperation of Dr. Erlinda I. Dizon in this study.

References