Germinated barley foodstuff (GBF) derived from the aleurone and scutellum fractions of germinated barley was rich in glutamine and low-lignified hemicellulose. The diarrhea caused by ceco-colecotomy could be prevented by feeding GBF to rats. GBF could also increase the protein content and sucrose activity of small intestinal mucosa in this model. This diarrhea-preventive effect of GBF would be based on the water-holding capacity and bulking force under alkaline conditions, e.g. in the small intestine.

Key words: intestines; dietary fiber; germination; diarrhea; barley

Our previous study showed that germinated barley foodstuff (GBF), which was derived from the aleurone, germ and scutellum fractions of germinated barley, could prevent the diarrhea induced by water-soluble dietary fiber in rats. GBF was concurrently able to increase the mucosal protein, DNA, and RNA contents of the small and large intestines.

GBF contains low-lignified hemicellulose, which is formed through the germination process, and has been reported to be efficiently fermented by microflora in the lower intestine. Furthermore, the production of volatile fatty acids, especially of butyric acid, was promoted by GBF feeding. It is well known that butyric acid proliferates enterocytes.

Barley has the capability for synthesizing glutamine (Gln) and utilizes Gln as its major energy source during growth, especially germination. Although Gln is known to have many physiological functions in the intestines, it has the disadvantage as an amino acid of being very easily broken down if presented in the free form. Taking these characteristics into consideration, Gln in GBF may exist very stably, since coexisting dietary fibers could protect Gln in GBF against gastric acid or digestive enzymes. Therefore, it possesses more stable characteristics and acts more effectively on mucosa than Gln in the free form.

An oral administration of GBF markedly increased the fecal water content. On the other hand, diarrhea induced by water-soluble dietary fibers (polydextrose, poly-acrylic acid sodium salt, and water soluble-hemicellulose) was attenuated by GBF feeding. We suspect that GBF can form feces in the intestinal tract against the effect of osmotic pressure. A ceco-colecotomized model generally causes severe diarrhea, which is important evidence indicating that the colon absorbs water from the intestinal contents and forms the feces. To evaluate the effect of GBF on forming feces in the lower intestinal tract, we fed ceco-colecotomized rats with a GBF-containing diet.

The process for producing GBF has already been described in our previous studies. Briefly, germinated barley was mashed and filtered in order to extract the endosperm, which is a substrate for beer, leaving brewer’s spent grain as the residue. GBF was obtained by milling and sieving the brewer’s spent grain, and is considered to be the aleurone, scutellum and germ fractions. A cellulose (CE) diet group was established as the control. The test diet contained 10% GBF, the protein and dietary fiber contents being adjusted to the same levels as those in the CE diet (14.6% protein and 3.0% dietary fiber). Detailed dietary compositions are shown in Table 1, and the chemical composition of GBF has been described in our previous study. All the dietary compo-

<table>
<thead>
<tr>
<th>Table 1. Composition of the Experimental Diets</th>
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<tr>
<td></td>
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<tr>
<td>Casein</td>
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<tr>
<td>Vitamin mixture</td>
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<tr>
<td>Mineral mixture</td>
</tr>
<tr>
<td>Choline chloride</td>
</tr>
<tr>
<td>Cellulose</td>
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<tr>
<td>GBF</td>
</tr>
<tr>
<td>Corn oil</td>
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<td>Corn starch</td>
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</tbody>
</table>

1 The vitamin mixture was prepared according to the AIN 93 vitamin mixture protocol.
2 The mineral mixture was prepared according to the AIN 93G mineral mixture protocol.
3 CE, cellulose, GBF, germinated barley foodstuff. The protein and neutral detergent fiber contents in both diets were adjusted to the same levels.

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ments were purchased from Oriental Yeast Co. (Tokyo, Japan), and all experimental reagents were purchased from Wako Pure Chemical Co. (Osaka, Japan).

Male Sprague-Dawley rats weighing about 50 g were purchased from Charles River Japan Co. (Yokohama, Japan). They were individually housed in metabolic cages in a room kept at 22 ± 1°C, with a 12-h light and dark cycle (lighting from 8:00 a.m. to 8:00 p.m.). The rats were allowed free access to the respective diets and drinking water. The animals were first fed with a laboratory diet (CE2, Nihon Clea Co., Tokyo, Japan) for 7 days during the acclimatization period. They were then divided into 2 groups (n = 10), each group being fed either the CE- or GBF-containing diet for 7 days. On day 7, the rats were fasted for 18 h for ceco-colectomy. The rats were subsequently anesthetized with urethane, and the cecum and colon were resected, the rectum and the end of ileum being immediately sutured. The length of the residual rectum was less than 3 cm. On day 1 after surgery, 5 ml of a 5% glucose solution was administered, and then on day 2 after surgery, 3 g of the CE or GBF diet was administered during the recovery period, with free access provided to drinking water. After the recovery period, the rats were allowed free access to their respective diets and drinking water for an additional 7 days. On day 9 after surgery, the feces and anal appearance of the rats fed on the respective diets were observed.

The rats were then sacrificed to remove their small intestine which was flushed with cold saline. The jejunum was sectioned off as the 15-30 cm portion below the pylorus. The jejunum (15 cm) was then weighed, and the mucosal protein content and sucrase activity were measured. Details of the analytical method have been described in our previous study.6 Data are each expressed as the mean ± SE, except for the number of diarrheal rats. Comparisons between the two groups were made by using the Mann Whitney test for nonparametric data and Student t-test for parametric data. In all statistical analyses, an associated probability (p value) of <5% is considered as being significant.

The changes in body weight gain and food intake are shown in Fig. 1. The body weight gain and food intake for both groups did not differ significantly before and after surgery. The number of rats with diarrhea, and the jejunum mucosal protein content and sucrase activity are shown in Table II. Diarrhea in the GBF-fed group was less than that in the CE-fed group, while the jejunum mucosal protein content and sucrase activity of the GBF-fed group were significantly higher than those of the CE-fed group. Figure 2 shows the appearance of the anus and feces of the rats fed on the respective diets. The GBF-fed rats did not display any diarrhea, although the CE-fed rats suffered from such severe diarrhea that we could not collect feces from the rats. Consequently, the fecal water content and its nitrogen content could not be measured. The fecal water content of normal rats without surgery fed on the CE and GBF diets was 34.9% and 56.1%, respectively.6 The fecal water content of other groups was measured as 24.7 ± 3.8% and 30.4 ± 3.2%, respectively.

Table II. Mucosal Protein Content, Sucrase Activity and Number of Rats with Diarrhea

<table>
<thead>
<tr>
<th>Group</th>
<th>Mucosal protein (mg/cm of jejunum)</th>
<th>Sucrase activity (U/cm of jejunum)</th>
<th>Diarrhea</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE</td>
<td>4.37 ± 0.35</td>
<td>0.22 ± 0.02</td>
<td>10/10</td>
</tr>
<tr>
<td>GBF</td>
<td>5.31 ± 0.22*</td>
<td>0.29 ± 0.02*</td>
<td>1/10*</td>
</tr>
</tbody>
</table>

1 Abbreviations are the same as those shown in the legend to Table I. Each value, except for the number of diarrheal rats, is the mean ± SE for 10 rats, and means with an asterisk within the same column are significantly different from the CE data (p < 0.05).

2 This value is the number of rats with diarrhea, and means with an asterisk within the same column are significantly different from the CE data (p < 0.05).

Fig. 1. Body Weight Gain and Food Intake of Rats Fed on Cellulose (CE) or Germinated Barley Foodstuff (GBF).

Each Value is the mean for 10 rats, and vertical bars represent SE. There was no significant difference between the two groups concerning the body weight gain and food intake. In this figure, "before" presents the data for rats before surgery, and "after" presents the data after surgery. Initial body weights in the CE-fed and GBF-fed groups were 88.3 ± 2.2 and 88.9 ± 1.2, respectively.

Fig. 2. Anal and Fecal Appearance of the Rats Fed on the Respective Diets.

A and B respectively show the anal appearance of the rats fed on the cellulose (CE) and germinated barley foodstuff (GBF) diets. CE-feeding caused severe diarrhea when compared with GBF-feeding. C and D respectively show the feces of rats fed on the CE and GBF diets.
content of the CE group was relatively low, compared with that of the GBF group in a normal condition. As shown in Fig. 2, the fecal appearance of the CE-fed group was like concentrated soup, while that of the GBF fed group was recognizable as of normal form.

Thus, GBF feeding was able to efficiently prevent diarrhea in ceco-collectomized rats. In our previous study, GBF also revealed the same effect on diarrhea induced with water-soluble dietary fiber which has been reported to increase the osmotic pressure in the large intestine. Therefore, GBF was suspected to form appropriate feces in the colon against the osmotic pressure. It also had potent ability as a bulging fluid in the alkaline condition in vivo and in vitro. As the pH level of the small intestine is weakly alkaline, GBF could adsorb the intestinal fluid and hold it within itself. In this way, GBF would form relatively normal feces without needing the colon. However, it is not clear whether this was due to physiological changes in the small intestine by feeding GBF and the compensatory effect of the residual rectum (less than 3 cm) by colectomizing, so more detailed studies are needed in future.

Our previous studies enabled us to propose the following physiological effects of GBF on the intestinal tract: 1) in the small intestine, Gln in GBF can be stably released to the mucosa and promote the proliferation of enterocytes; 2) in the large intestine, the dietary fiber fraction of GBF, low-lignified hemicellulose, is effectively fermented, and microflora produce the butyrate which is a good nutrient for colonic mucosa. Since the increase in mucosal protein content was accompanied by increased mucosal sucrose activity, this change in mucosal protein content would be linked with the normal epithelial cell differentiation in mucosa.

Although GBF activated the formation of feces in the small intestine, it is considered that GBF did not attenuate the nutrient absorption by this organ for the following reasons: 1) the body weight gain of the GBF- and CE-fed groups was not significantly different; 2) the food intake by these two groups was also similar. In our previous study, chitosan, which had the ability for increasing fecal output and formation, affected the nutrient absorption in the intestinal tract; therefore, the body weight gain and food intake decreased. However, in this respect, we must study the relationship between GBF feeding and the absorption of other nutrients in future.

In conclusion, we propose that GBF feeding cannot only increase mucosal proliferation, but also prevent diarrhea in the ceco-collectomized model. These results indicate that GBF is a useful foodstuff for patients with resection of the lower intestinal tract. Furthermore considering that GBF can maintain the fecal condition at a normal level, the unpleasant feeling in patients with a pretermerturine anus would be alleviated. Future studies must investigate the mechanism by which GBF acts on the intestinal tract in detail, and show how GBF forms feces in the upper intestinal tract.

References