Mechanism for the Increased Defecation and Jejunum Mucosal Protein Content in Rats by Feeding Germinated Barley Foodstuff

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We investigated the effects of germinated barley foodstuff (GBF) on the fecal excretion and jejunal mucosal protein content in male Sprague-Dawley rats fed on various diets with the same protein and dietary fiber levels. Under these experimental conditions, GBF was confirmed to induce greater fecal output compared with commercial water-soluble or -insoluble dietary fibers.

While the dietary fiber extracted from GBF increased the fecal output and mucosal protein content, the protein fraction of GBF degraded to the peptide form did not increase the fecal output or mucosal protein content. Increased mucosal protein and fecal output were thus found to require the presence of the dietary fiber fraction or possibly the protein fraction bound tightly to the dietary fiber of GBF. GBF feeding increased the volatile fatty acids concentration in the cecum, indicating that GBF may be efficiently fermented in the intestinal tract.

Key words: jejunum mucosa; dietary fiber; germination; volatile fatty acids; rat

In our previous study,1) we reported the production of germinated barley foodstuff (GBF) containing Glu-rich protein and dietary fiber from Brewer’s spent grain (BSG) by a completely mechanical process involving pulverizing and sieving. We have previously evaluated the physiological effects of GBF on defecation and jejunal mucosa. Since supplementation of the basal diet with some Glu-containing materials did not affect the defecation or mucosal protein content, these effects must have been related to the combination of protein with dietary fiber, and not to the content of Glu itself. Similarly, such purified dietary fiber sources as cellulose, hemicellulose, and lignin, as well as a mixture of these, when added to the basal diet, did not affect defecation or the mucosal protein content. We concluded that the purified dietary fiber source contained in GBF was not itself responsible for the physiological effects we observed.

Some differences in physiological effects have been reported between purified dietary fiber and crude plant dietary fiber.2-5) Many differences in these effects between water-soluble and -insoluble dietary fibers have also been reported.69) The present studies were designed to investigate these effects in view of the difference in the dietary fiber source and chemical composition from those of GBF.

We first clarified the effect of the form of GBF containing water-insoluble or -soluble dietary fiber on defecation and jejunal mucosa. We then attempted to identify the active ingredient of GBF and the mechanism for the physiological effects of GBF. We next separated the protein fraction and dietary fiber fraction from GBF by enzymatic techniques and fed these fractions to rats, in order to clarify the interaction between dietary fiber and protein. If the active ingredient of GBF could be identified, a small amount of it could be used to increase fecal output without injuring the gastrointestinal tract.

We finally evaluated the extent of utilization of GBF in the gastrointestinal tract. The mechanisms by which GBF increased the fecal output and jejunal mucosal protein content are discussed in this report.

Materials and Methods

Materials

Experiment 1. Two types of water-soluble dietary fibers were used, these being polydextrose (PD) purchased from Pfizer Co. (Tokyo, Japan) and synthesized from glucose and citric acid, and pectin (PC) purchased from Wako Pure Chemical Industries Co. (Osaka, Japan) and isolated from apples. Except for corn oil (Ajinomoto Co., Tokyo) and corn starch (Nacalai Tesque Co., Tokyo), all other dietary components and reagents were purchased from Oriental Yeast Co. (Tokyo) and Wako Pure Chemical Industries Co.

Experiment 2. Wheat bran (WB) purchased from Nippon Seiun Co. (Tokyo) was used as a representative water-insoluble fiber. BSG was obtained from Kirin’s Toride brewery plant (Ibaraki, Japan) and beer husk (BH) was obtained as a by-product of GBF production as described in detail in our previous study.1) The other dietary components used are described in Experiment 1.

Experiment 3. Pepsin and pancreatin were purchased from Sigma Co. (St. Louis, MO, U.S.A.) and ALCALASE from Novo Industry Co. (Tokyo). All other reagents were purchased from Wako Pure Chemical Industries Co.

Experiment 4. The Dietary components and experimental reagents were prepared in the same way as that for Experiments 1 to 3.

The analytical methods used to acquire data from chemical analyses of the respective dietary samples were as described in our previous study.1

Methods

Experiment 1. In this experiment, we compared the physiological effects of GBF with those of water-soluble dietary fibers. Male Sprague-Dawley rats weighing about 50 g each were purchased from Charles River Japan (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 fed ad libitum with a commercial diet (CE2; Nihon Clea Co., Tokyo, Japan) for 7 days during the...
acclimatization period, and were then randomly allocated to 4 bodyweight-matched diet groups (n = 6–8 per group) receiving cellulose (CE), GBF, PD, or PC. Subsequently, each group was fed on the experimental diet for 14 days. The rats were allowed free access to the respective diets and to drinking water. During the experimental period, the food intake and body weight were measured every 3 days. Feces excreted during the final 3 days (on days 12, 13, and 14) of the experimental period were collected after the number of feces had been counted, and stored at −80°C. After lyophilization, the fecal dry weight was measured. On day 14, the rats were anesthetized with urethane, and the small intestines were excised.

Experiment 1. In this experiment, we compared the physiological effects of dietary GBF with various water-insoluble dietary fibers. The preparation of GBF and of the BH fraction has been described in the previous report.19 After acclimatization, the rats were assigned to CE, GBF, BSG, BH, and wheat bran (WB) groups of 6–8 rats each. The other experimental conditions were those described in Experiment 1.

We then isolated the protein fraction of GBF. In the previous study,1,10,11,14 since extracting the protein in GBF in its intact form proved very difficult, GBF was suspended in an alkali solution and hydrolyzed by alkaline-protease. We thus obtained the peptide form of the protein fraction of GBF by modification of the routine method.15 Five hundred grams of GBF was suspended in 5000 ml of an alkali solution (adjusted to pH 9.5 with 0.1N NaOH) and heated in an autoclave (121°C, 10 min). The pH value was then adjusted and dietary fiber fraction from GBF (GBF-DF) by chemical and enzymatic methods to examine the active ingredient of GBF, using a modified sequential gastric and intestinal digestive method for the later.12 One hundred grams of GBF was suspended in 1600 ml of an enzyme solution (0.087% pepsin and 0.03% NaCl in 0.069% HCl), and 1200 ml of 0.1M NaHCO3 (final pH value of 2.1). This solution was incubated at 37°C for 4 h. After the incubation, the reaction was stopped by soaking the hydrolyzed GBF. Further digestion was performed by adding 500 ml of an enzyme solution (0.217% pancreatic in a 0.5% Tris-HCl buffer) and 2000 ml of 0.01N HCl to the partially digested GBF (final pH value of 7.4), with 10 drops of tolene also being added as a preservative. This suspended solution was incubated at 37°C for 24 h. The approximate recovery of GBF-DF was 60% of whole GBF on a weight basis.

Statistical analysis. All data, except for the fecal water content, are expressed as the mean ± SE. The fecal water content is presented as the mean of pooled samples from five rats each in the CE and GBF groups. The homogeneity of variance was initially evaluated by using Bartlett's test, and significant differences were evaluated with the Newman–Kuels test. The analytical results of the concentration of VFA in the cecum content was evaluated by Student's t-test, a value of p < 0.05 being considered significant.

Results

The chemical composition of each dietary supplement used is shown in Table I, and the compositions of the diets in Experiment 1, 2, and 4 are shown in Table II.

Experiment 1

As shown in Table III, the GBF group had the greatest body weight gain of all the dietary groups, while the weight gains in the PC and PD groups were significantly smaller than that in the CE group. The rats in the PD and PC groups developed diarrhea, and the body weight gain was thus retarded. The food intake by the GBF group was significantly greater than that by the CE group, and this is presumed to have accounted for the increased body weight gain. The food intake by the other groups was the same as that by the CE group. As the animals in the PD and PC groups developed diarrhea, we could not obtain any formed feces at all from the PD group, and only partially from the PC group. The GBF group showed the greatest fecal excretion of all the groups in this experiment, and the jejunal mucosal protein content of the GBF group was significantly greater than that of each of the CE, PD, and PC groups. The jejunal mucosal content of animals in the PC group was significantly lower than that in the CE group.

Experiment 2

The body weight gain, food intake, number of feces, fecal dry weight, and jejunal mucosal protein content are shown in Table IV. No significant differences in body weight gain were found among the dietary groups in this experiment, in contrast with the results from Experiment 1. The number of feces from the BH group was significantly higher than that from the CE group, although this difference was not as marked as that between the CE and GBF groups.

BSG, the raw material of GBF, did not result in any increase in the fecal dry weight, and in fact reduced it. The fecal dry weight from the WB group was almost the same as that from the CE group. The jejunal mucosal protein content of the GBF group was significantly greater than that of either of the CE, BSG, BH, and WB groups, while the content of each of the BSG, BH, and WB groups was significantly lower than that of the CE group.

Experiment 3

The binding of the protein fraction and dietary fiber fraction of GBF was very strong, and therefore, we could not completely isolate the protein from the dietary fiber. However, through the artificial digestion of GBF, the protein content of GBF-DF was markedly less than that of GBF itself (14.3% and 46.0%, respectively). Conversely, the dietary fiber content was lower in the production of GBF-pep. Included in these two fractions were some...
Table I. Composition of Each Dietary Supplement

<table>
<thead>
<tr>
<th></th>
<th>GBF</th>
<th>PD</th>
<th>PC</th>
<th>WB</th>
<th>BSG</th>
<th>BH</th>
<th>GBF-DF</th>
<th>GBF-pep</th>
<th>GBF-Enz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>7.8</td>
<td>5.0</td>
<td>4.5</td>
<td>3.8</td>
<td>4.0</td>
<td>6.3</td>
<td>7.0</td>
<td>5.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Protein</td>
<td>46.0</td>
<td>0.0</td>
<td>4.2</td>
<td>9.6</td>
<td>24.0</td>
<td>4.8</td>
<td>14.3</td>
<td>61.4</td>
<td>48.0</td>
</tr>
<tr>
<td>Lipids</td>
<td>10.2</td>
<td>0.1</td>
<td>0.3</td>
<td>2.7</td>
<td>10.6</td>
<td>3.9</td>
<td>5.2</td>
<td>7.0</td>
<td>10.5</td>
</tr>
<tr>
<td>Ash</td>
<td>2.0</td>
<td>0.0</td>
<td>1.0</td>
<td>4.3</td>
<td>2.4</td>
<td>2.0</td>
<td>3.0</td>
<td>4.9</td>
<td>2.4</td>
</tr>
<tr>
<td>Dietary fibera</td>
<td>34.0</td>
<td>94.9b</td>
<td>90.0b</td>
<td>79.5</td>
<td>59.0</td>
<td>83.0</td>
<td>69.5</td>
<td>21.7</td>
<td>33.1</td>
</tr>
</tbody>
</table>

This refers to neutral detergent fiber (NDF), and this value represents the sum of cellulose, hemicellulose and lignin, except for those of PD and PC. Analytical methods were as described in our previous report.1)

The dietary fiber contents of PD and PC are derived from the manufacturer's data.

Table II. Composition of the Experimental Diets (Experiments 1, 2, and 4)

<table>
<thead>
<tr>
<th></th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CEa</td>
<td>GBFb</td>
<td>PDc</td>
</tr>
<tr>
<td>Casein</td>
<td>146</td>
<td>100</td>
<td>146</td>
</tr>
<tr>
<td>Vitamin mixturee</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Mineral mixturef</td>
<td>35.0</td>
<td>35.0</td>
<td>35.0</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>30.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GFB</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polydextrose</td>
<td>30.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pectin</td>
<td>30.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BSG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GFB-pep</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>GBF-DF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GBF-Enz</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn oil</td>
<td></td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Corn starch</td>
<td></td>
<td>727</td>
<td>703</td>
</tr>
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<td></td>
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<td></td>
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</tbody>
</table>

a CE indicates the cellulose diet group, the CE group being the same in Experiments 2 and 4.
b GFB indicates the GFB diet group, and GFB group being defined in Experiments 2 and 4.
c PD, polydextrose diet group.
d PC, pectin diet group.
e BH, beer husk diet group.
f BSG, brewer's spent grain diet group.
g WB, wheat bran diet group.
ah GFB-pep, peptide fraction from GFB diet group.
i GBF-DF, indicates dietary fiber fraction from GFB diet group.
j GBF-Enz, GFB in which the protein fraction was degraded to the peptide form without separating it from the dietary fiber fraction diet group.

Prepared according to AIN 93 vitamin mixture.10)
Prepared according to AIN 93G mineral mixture.10)
In our experiments, the protein content and dietary fiber content of all the diets were adjusted to the same level (protein, 14.6%; DF, 3.0%).

Table III. Body Weight Gain, Food Intake, Number of Feces, Fecal Weight and Mucosal Protein Content (Experiment 1)

<table>
<thead>
<tr>
<th>Groupa, b</th>
<th>Body weight gaina, c (g/14 days)</th>
<th>Food intake (g/day)</th>
<th>Number of Feces (g/3 days)</th>
<th>Weight of Feces (g/3 days)</th>
<th>Mucosal protein (mg/cm of jejuncm2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE</td>
<td>87.3 ± 4.7a</td>
<td>20.8 ± 0.9a</td>
<td>45.1 ± 3.6b</td>
<td>2.57 ± 0.15b</td>
<td>4.43 ± 0.13b</td>
</tr>
<tr>
<td>GFB</td>
<td>104 ± 3.8b</td>
<td>23.4 ± 0.8b</td>
<td>68.0 ± 1.5a</td>
<td>3.25 ± 0.12a</td>
<td>5.23 ± 0.17a</td>
</tr>
<tr>
<td>PD</td>
<td>72.7 ± 3.1a</td>
<td>20.3 ± 0.6b</td>
<td>39.7 ± 3.7b</td>
<td>1.38 ± 0.10a</td>
<td>4.06 ± 0.16b</td>
</tr>
<tr>
<td>PC</td>
<td>70.7 ± 2.3a</td>
<td>20.3 ± 0.5b</td>
<td>39.7 ± 3.7b</td>
<td>1.38 ± 0.10a</td>
<td>3.74 ± 0.20a</td>
</tr>
</tbody>
</table>

a Abbreviations are the same as those shown in the legend to Table II.
b Each value is the mean ± SE for 8 to 10 rats, and means not sharing a common superscript letter within the same column are significantly different (P < 0.05). The overall mean initial body weight was 99.8 ± 1.0 g.
c The jejunum is defined as the portion of the small intestine from 15 cm to 30 cm from the pylorus.
indivisible fractions of combined protein and dietary fiber. We degraded the GBF protein fraction in order to assess the superstructure of the protein in GBF, the peptide length of GBF-pep being recognized as a 6- to 8-amino acid residue by the TNBS method (detailed data not shown). 18)

**Experiment 4**

The body weight, food intake, number of feces, fecal dry weight, and jejunal mucosal protein content are shown in Table V. The fecal water content in the CE and GBF groups was 34.9% and 56.1%, respectively. The body weight gains of all groups were similar, there being no statistical differences. Concerning the food intake, the GBF-pep group had the lowest and the GBF-Enz group the highest, but there was no significant difference between the intake by the GBF-pep and GBF groups.

The number of feces from the GBF-pep group was significantly fewer than from the other four groups. The numbers of feces from the GBF-DF and GBF-Enz groups were significantly more than that from the CE group, and that from the GBF group was even more.

The fecal dry weight was the highest in the GBF group, and lowest in the GBF-pep group, while there were no significant differences in fecal dry weight among the CE, GBF-DF, and GBF-Enz groups. The fecal water content in the GBF group was greater than that in the CE group. The concentration of VFA in the cecum content of animals in the CE and GBF groups are shown in the Fig. The concentrations of acetic acid and butyric acid in the cecum contents of the GBF group were significantly more than those of the CE group.

**Discussion**

It has been reported that water-soluble dietary fiber has little potency for increasing fecal volume, 20 and this study revealed essentially the same results. The addition of PC as
a water-soluble dietary fiber decreased the fecal weight while PD produced diarrhea. We could not satisfactorily collect the feces from the PD and PC groups, so the fecal output from these groups could not be correctly assessed and might be stated too low. The risk of consuming dietary fiber indiscriminately has been discussed, and our results seem to contribute further evidence to support this proposition. We consider that GBF might have prevented the diarrhea induced by ingesting water-soluble dietary fiber for two reasons: the increased number of enterocytes stimulated by GBF, and the increased water-retention capacity of GBF in the intestinal tract. When water-soluble dietary fiber is being ingested, it might be advisable to take GBF simultaneously to reduce the likelihood of diarrhea and increase fecal excretion. We furthermore suggest that GBF could be orally given in order to prevent diarrhea in the future.

Water-insoluble dietary fiber is well known to have potency for increasing defecation and has been reported to affect bulk, density, and hydration. However, the risk of feeding water-insoluble fiber has been recognized, as it can injure the small intestinal mucosa. For example, some dietary fibers have been reported by Cassidy et al. to increase desquamation. Although water-insoluble dietary fiber certainly increases the fecal output, mucosal desquamation might occur in some cases. In our experiment, WB feeding did not increase fecal excretion, but it did decrease the jejunal mucosal protein content. As this result was unique to the WB group, jejunal mucosal desquamation might have occurred when WB was consumed. We plan to conduct a morphological observation of the intestinal mucosa in future to clarify this effect.

The main components of GBF, the aleurone layer and scutellum, have been reported to be partly degraded and to swell in water during germination. This partial degradation of the two components is thought to be a pre-requisite of the increased fecal water content caused by GBF. BH does not swell much in water, as it is a lignified and structural protector of the inner living tissues of the grain which resists swelling. However, BH does transfer the necessary amount of water to the inner tissues. Thus, the increment in the fecal water content of animals in the GBF group is thought to have been attributable to the water retention or swelling capacity of aleurone and scutellum in the gastrointestinal tract.

BSG, the raw material of GBF, did not increase the fecal volume or mucosal protein content. This may have been due to the high content of husk with a cell wall that is not readily degraded and becomes highly lignified during germination. An increase in the fecal water content is thought to be important for increasing fecal volume, a small increment in fecal water content have been reported to significantly increase fecal output. The increased fecal water content produced by GBF is a noteworthy result of our experiment.

Although the difference in dry fecal weight between the CE group (2.93 g) and GBF group (3.68 g) was about 1.3-fold, the calculated fresh fecal weight difference was even greater:

For CE, 2.93/(1 − 0.34 [fecal water content]) = 4.44 g
For GBF, 3.68/(1 − 0.56 [fecal water content]) = 8.56 g

The difference in the fresh fecal weight between the CE and GBF groups was about 2.0-fold (8.56/4.44). This difference would be very important for patients who are experiencing problems in defecation.

The production of VFA was increased by dietary GBF, this indicating a change in intestinal fermentation. VFA, which is produced by Lactobacillus, Bifidobacterium and so on, would decrease the pH value in the lower intestine. As a result, the volume of microflora would increase and fecal output might also increase. Moreover, VFA has been reported to increase the growth of the intestinal mucosa. Sakata has also reported that VFA injected into the cecum increased the small intestinal mucosal protein content as well as that of the colon. This increase in the mucosal content of the small intestine would be concerned with VFA affecting the hormonal endocrine or nervous systems. Dietary GBF is thought to be fermented in the gastrointestinal tract, causing the production of VFA, which could consequently increase the jejunal mucosal protein content. While the fermentation of dietary fiber in the small intestine is not substantial when compared with that in the colon and cecum, it might partly increase the jejunal mucosal protein content.

The settling volume of GBF after artificial digestion was measured in our previous study, and it can be considered that GBF becomes swollen under the conditions prevailing in the intestine. Thus, GBF would be degraded by microflora. The increase in VFA produced by the GBF group might therefore be related to the increased volume of swollen GBF in the intestinal tract.

As already noted, the physiological effects of GBF can be traced to the dietary fiber fraction of GBF. Although the binding force between the protein and dietary fiber in GBF was very strong, we were able to increase the dietary fiber content of GBF from 34% to 70%. This resulting GBF-DF showed the physiological effects of increasing the mucosal protein content and the number of feces. Conversely, GBF-pep, which had a higher protein content than that of GBF, did not show these physiological effects. Furthermore, GBF-Enz, in which the superstructure of the GBF protein fraction is disrupted, but not the isolated dietary fiber fraction, demonstrated these effects. We surmise from these results that the superstructure of GBF protein might not be necessary for the physiological effects to be manifested. About 70% of GBF protein is degraded by artificial digestion, but about 30% would act as a resistant protein. Southgate has proposed that indigestible protein bound tightly to fiber can be regarded as dietary fiber.

In conclusion, dietary GBF increased fermentation in the intestinal tract and the production of VFA. At the very least, this would have been related to improved defecation: the wet fecal weight was about double that in the control group, and the fecal water content was increased in the GBF-fed rats. Accordingly, a patient with constipation might experience less pain from defecation if given GBF. The production of VFA could, in part, reinforce the jejunal mucosa. The active ingredient of GBF might be the dietary fiber fraction or a protein tightly bound to the dietary fiber, or a combination of the two. As water-soluble dietary fiber caused diarrhea, the relationship between diarrhea and dietary GBF should be studied.
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References