Digestibility of Dietary Fiber in Brown Alga, Kombu, by Rats

Takeshi Suzuki,*1 Kiyonori Nakai,*1,2 Yumiko Yoshie,*1 Takaaki Shirai,*1 and Toshiyuki Hirano*1
(Received January 19, 1993)

Digestibility of the edible brown alga kombu was studied by feeding rats for 28 days. Food intake of both rats fed with basal and kombu diets was statistically similar. Body weights of kombu groups were significantly lighter, but their fecal weights were heavier, when compared with their counterparts with diets excluding kombu. There were no major differences in the weights of liver, pancreas, and kidney, but cecum, small intestine, and large intestine of rats fed with kombu were significantly heavier than those without kombu. With the kombu diet, in the early feeding period protein digestibility was significantly lower than in the control, but the digestibility of kombu groups recovered to the level of the control in the late feeding period. Fat digestibility in the kombu diet was significantly higher than that in the basal diet. Digestibility of insoluble and total dietary fibers in the kombu diet tended to increase in the feeding period. Alginate digestibility significantly increased, and the molecular weight of alginate was down by 60%. Since the molar ratio of mannuronic and guluronic acids in alginate decreased significantly in the feeding period, mannuronic acid may be more decomposable in comparison with guluronic acid in rats.

It used to be believed that dietary fiber itself had no nutrient value and was not even absorbed into the human body. However, most dietary fiber sources are not energy (calorie) free, and the energy contributed by dietary fiber results from the action of large bowel microflora.1) Bacteria recovered from feces show a variety of enzymes which break down polysaccharides, and the energy content of dietary fiber was influenced by factors such as types of bowel flora, diets eaten, and environmental conditions. Soluble dietary fibers are generally the most digestible while insoluble dietary fibers are the least digestible.12) The energy contents (cal/g) of bran of some grain and carbohydrates were estimated using the rat model.13) As for humans, approximately 80% of the ingested cellulose and 96% of the ingested hemicellulose were digested in normal subjects with intact intestinal tracts.14)

In Japan, kombu (for the most part of genus Laminaria) is an important marine alga which has been used as food since ancient times, and it occupies the majority of kelp foods at present. The energy of seaweed does not appear in the Standard Tables of Food Composition in Japan.15) The digestibility of kombu has been studied by some researchers.5-7) However, the digestibility of alginate and dietary fiber have not been evaluated.

Therefore the present work was designed to study the digestibility of protein and fat as well as dietary fiber and alginate of kombu in rats fed on diets containing kombu. The effect of kombu diet on the growth and digestive organs of rats was also examined.

Materials and Methods

Animals and Diets

Male Wister rats (Cler Japan Inc., Tokyo) were obtained at the age of 3 weeks and housed individually in stainless steel, wire mesh-bottomed cages, in a room with a constant temperature of 22±1°C and a 12 h daily light-dark cycle. The rats were given ad libitum access to food (a stock diet, CE-II, Cler Japan Inc., Tokyo) and water for 4 weeks to acclimate them to laboratory conditions.

Dried brown alga, naga-kombu Laminaria angustata f. longissima, which was harvested in...
Hokkaido, was obtained from Tokyo Central Wholesale Market. The kombu was pulverized by a grinder and passed through a 30-mesh screen. A kombu diet was made by diluting the stock diet (CE-II, Cler Japan Inc., Tokyo) with the powdered kombu to a content of 20%, and a basal diet was made by diluting the stock diet with corn starch to the same content.

The rats were randomly distributed into two groups, the basal and kombu diets, with six animals each. Food and water were allowed ad libitum for 28 days. The food intake, fecal output, and body weight of the rats were measured daily. Feces were collected during the first four days (from Day 2 to Day 5) and the last four days (from Day 25 to Day 28) of the feeding period, and freeze-dried.

Digestive Organs

Each animal was anesthetized with ether and the abdominal cavity was opened. The digestive organs, such as liver, pancreas, kidney, cecum, small intestine, and large intestine including contents, were quickly removed and weighed.

Digestibility

Protein and fat contents of the diets and the freeze-dried feces were measured by the Kjeldahl procedure and the Soxhlet method, as usual. Dietary fiber in the diets and the freeze-dried feces were determined by an enzymatic-gravimetric method, but this method was modified here by using pancreatin.

Apparent digestibility of protein, fat, dietary fiber, and alginate was calculated as follows:

\[
\text{Digestibility} = 100 \times \frac{(\text{intake} - \text{output})}{\text{intake}}
\]

Alginate

Alginate in the kombu diet and the freeze-dried feces of rats fed this diet was extracted with sodium carbonate and the extract was dialyzed against distilled water. The molecular weight of the extracted sodium alginate in the feces was determined by HPLC (Shimadzu LC-6A, Shimadzu Co., Kyoto) equipped with a reflective detector (Shimadzu RID-6A). A gel permeable column (Asahipak GFA-7 MF 7.6 × 300 mm Asahi Kasei Co., Tokyo) was used, and elution was done with 50 mM NaNO₃ at 0.5 ml/min: a calibration curve was prepared by measuring a pullulan standard (Shodex standard P-82, Showa Denko Co., Tokyo).

The alginate in the feces was hydrolyzed by sulfuric acid. Guluronic and mannuronic acids were determined by HPLC using an ion exchange column.

Statistical Analysis

Values are presented as the mean and standard error. Significant differences between groups were determined by Student’s t-test or a Welch test. Results were considered statistically significant at \( p<0.05 \) or lower, as shown in a previous paper.

Results and Discussion

Food Intake and Growth

The food intake, fecal weight, and body weight of rats during the experimental period are shown in Figs. 1, 2, and 3, respectively. There was no significant difference in food intake between rats fed with basal and kombu diets, and total food intakes were 514 ± 8 and 525 ± 11 g, respectively, for the 28 days of the feeding period (Fig. 1).

Fecal wet weight of rats fed with kombu (154 ± 4 g) was significantly increased when compared...
Table 1. Weights of digestive organs in rats (Mean±S.E.)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Basal</th>
<th>Kombu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver (g)</td>
<td>12.91±0.4a</td>
<td>11.80±0.4a</td>
</tr>
<tr>
<td>% of body weight</td>
<td>4.88±0.2a</td>
<td>5.03±0.1a</td>
</tr>
<tr>
<td>Pancreas (g)</td>
<td>0.58±0.1a</td>
<td>0.70±0.1a</td>
</tr>
<tr>
<td>% of body weight</td>
<td>0.33±0.0a</td>
<td>0.29±0.0a</td>
</tr>
<tr>
<td>Kidney (g)</td>
<td>2.78±0.2a</td>
<td>2.75±0.1a</td>
</tr>
<tr>
<td>% of body weight</td>
<td>1.05±0.1a</td>
<td>1.18±0.1a</td>
</tr>
<tr>
<td>Cecum (g)</td>
<td>5.39±0.2a</td>
<td>8.79±0.4b</td>
</tr>
<tr>
<td>% of body weight</td>
<td>2.03±0.1a</td>
<td>3.76±0.2b</td>
</tr>
<tr>
<td>Small intestine (g)</td>
<td>10.07±1.6b</td>
<td>14.69±0.5b</td>
</tr>
<tr>
<td>% of body weight</td>
<td>3.73±0.5b</td>
<td>6.26±0.2b</td>
</tr>
<tr>
<td>Large intestine (g)</td>
<td>4.28±0.2a</td>
<td>6.67±0.2b</td>
</tr>
<tr>
<td>% of body weight</td>
<td>1.67±0.1a</td>
<td>2.86±0.1b</td>
</tr>
</tbody>
</table>

Horizontal values not sharing the same superscript letters are significantly different (p<0.05).

Digestive Organs

The wet weight of liver, pancreas, kidney, cecum, small intestine, and large intestine including contents, and the percentage of the weight of these digestive organs to body weight are shown in Table 1. There were no major differences in the wet weight or the percentage of liver, pancreas, and kidney between the two groups of rats. With kombu feeding, the wet weight and the percentage of cecum, small intestine, and large intestine were significantly higher than in basal.

When rats were given pullulan and glucomannan, i.e. soluble dietary fiber, an increase in cecum weight was recognized, and that of the large intestine increased in the case of cellulose, i.e. insoluble dietary fiber. As shown in the previous paper, alginate diets produced significantly lighter liver and spleen but heavier cecum, small intestine, and large intestine when compared with diets excluding alginate. Therefore, alginate in kombu may partly influence the digestive organs in this experiment.

Digestibility of Protein and Fat

Apparent digestibility and daily intake and output of protein and fat during the first four days (from Day 2 to Day 5) and the last four days (from Day 25 to Day 28) of the feeding period are summarized in Table 2. The daily output of protein in the kombu diet (655±7 g) was significantly higher than that in the basal diet (506±7 g), but the daily intake in the two groups was similar (2903±72 and 2885±46 g, respectively).
in the first four days. Therefore, protein digestibility in rats fed with kombu (77.4±0.6%) was significantly lower than that without kombu (82.4±0.4%). The digestibility of the kombu groups recovered to more than 82% in the last four days, and this was the same for the animals without kombu. This result suggested that the animals gradually adapted themselves to the kombu diet from the first to the last feeding period (from Day 5 to Day 25, approximately 3 weeks).

Both in the first and the last four days, fat digestibility in the kombu diet (85.6±0.4 and 84.3±0.2%) was significantly higher than that in the basal diet (83.5±0.4 and 78.1±1.6%, respectively). These differences were caused by the daily intake of fat, because the daily output of fat was the same, 70±1 and 71±1 g on Day 2 to Day 5, for basal and kombu diets, and also 128±8 and 128±4 g on Day 25 to Day 28.

There have been several studies of the effects of dietary fibers on the digestibility of protein and fat. Increasing the intake of dietary fiber resulted in a greater fecal loss of energy, and in most instances of protein and fat. Miyada et al. reported that the feeding of wheat bran, solka-floc, and pectin significantly reduced protein digestibility using gastrectomized rats. Refined corn bran showed a tendency towards decreased dietary protein efficiency in rats, and significantly reduced the food efficiency at higher levels of this fiber. The effects of viscous indigestible polysaccharides such as sodium alginate on the pancreas exocrine function were investigated in growing rats by Ikekami et al. They reported that the polysaccharides may have interfered with the digestion and absorption of nutrients, resulting in a decreased digestibility and an enlargement of digestive organs.

### Digestibility of Dietary Fiber

Apparent digestibility as well as daily intake and output of soluble, insoluble, and total dietary fibers in the first and last four days of the feeding period are presented in Table 3. Daily intake and output of all dietary fibers of each group from Day 25 to Day 28 were significantly higher than those from Day 2 to Day 5. Also the daily intake and output of the kombu group in each feeding period were significantly higher than those of the basal group. The digestibility of soluble dietary fiber in the kombu group was significantly lower than that in the control, and the digestibility of the basal diet decreased from 95.2±0.1 to 85.2±1.1% on Day 5 to Day 25, or over about 3 weeks. On the other hand, the digestibility of insoluble dietary fiber in the basal diet was essentially the same in both feeding periods (41.9±1.4 and 41.1±4.2%), but that in the kombu diet tended to increase from 39.7±1.7 to 42.0±0.8% over the 3 weeks. The digestibility of total dietary fiber in the basal diet decreased from 54.0±1.1 to 51.1±3.5%, while that in the kombu diet increased from 41.8±1.6 to 43.1±0.8%. The basal diet contains 17.5 g/100 g of insoluble and 5.1 g/100 g of soluble dietary fibers. Therefore, an increase in digestibility may be attributable to the digestion of kombu.

There are several studies on grain; for processed wheat bran, the components in rats’ digestibility were 0% for lignin, 15% for cellulose, and up to 50% for hemicellulose. Some water soluble dietary fibers such as pectin are completely degraded in the normal human colon. All polysaccharidases studied to date in human colonic
bacteria have proved to be inducible, i.e., an appreciable amount of enzyme is produced only when the organism is exposed to the polysaccharide substrate.23) The results of dietary fiber digestibility obtained in this experiment were somewhat lower than those reported by Kishi et al.,5) who showed that apparent kombu digestion was 57.6%. However, the apparent digestibility of crude fiber in kombu reported by Yanase et al.7) was 77.6%, which they calculated according to a subtraction of the value of a basal diet from that of a kombu diet. When we calculated in this manner, the digestibility of insoluble dietary fiber in kombu in our study was 33.3% (Day 2 to Day 5) and 42.9% (Day 25 to Day 28). Their result (77.6%) was too high for the digestibility of the insoluble dietary fiber in our study. Fujita and Fuwa,6) who investigated the influence of kombu on the digestive enzymes and functions of rats, found that total lactase and sucrase activities in the small intestinal mucosa and protease activity in the pancreas increased after feeding with a kombu diet. They estimated that these increases were caused by the adaptation of the rats to the kombu diets.

Digestibility of Alginate

Apparent digestibility and daily intake and output of alginate in the kombu group, and molecular weight and composition of alginate in the feces of rats fed with the kombu diet in the first and last four days of the feeding period (from

Table 3. Digestibility of dietary fiber

<table>
<thead>
<tr>
<th>Feeding period</th>
<th>Diet</th>
<th>Basal</th>
<th>Kombu</th>
<th>Basal</th>
<th>Kombu</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 2 to Day 5</td>
<td></td>
<td></td>
<td>Day 25 to Day 28</td>
<td></td>
</tr>
<tr>
<td>Daily intake</td>
<td>Soluble (g)</td>
<td>0.69±0.01</td>
<td>0.80±0.02</td>
<td>0.97±0.04</td>
<td>1.31±0.03</td>
</tr>
<tr>
<td></td>
<td>Insoluble (g)</td>
<td>2.35±0.04</td>
<td>2.98±0.07</td>
<td>3.29±0.13</td>
<td>4.90±0.12</td>
</tr>
<tr>
<td></td>
<td>Total (g)</td>
<td>3.04±0.05</td>
<td>3.78±0.09</td>
<td>4.26±0.17</td>
<td>6.21±0.16</td>
</tr>
<tr>
<td>Daily output</td>
<td>Soluble (g)</td>
<td>0.03±0</td>
<td>0.40±0</td>
<td>0.14±0.01</td>
<td>0.69±0.02</td>
</tr>
<tr>
<td></td>
<td>Insoluble (g)</td>
<td>1.37±0.02</td>
<td>1.79±0.02</td>
<td>1.93±0.12</td>
<td>2.85±0.10</td>
</tr>
<tr>
<td></td>
<td>Total (g)</td>
<td>1.40±0.02</td>
<td>2.19±0.02</td>
<td>2.07±0.13</td>
<td>3.54±0.12</td>
</tr>
<tr>
<td>Digestibility</td>
<td>Soluble (%)</td>
<td>95.2±0.1</td>
<td>49.9±1.4</td>
<td>85.2±1.1</td>
<td>47.7±0.7</td>
</tr>
<tr>
<td></td>
<td>Insoluble (%)</td>
<td>41.9±1.4</td>
<td>39.7±1.7</td>
<td>41.1±4.2</td>
<td>42.0±0.8</td>
</tr>
<tr>
<td></td>
<td>Total (%)</td>
<td>54.0±1.1</td>
<td>41.8±1.6</td>
<td>51.1±3.5</td>
<td>43.1±0.8</td>
</tr>
</tbody>
</table>

Horizonal values not sharing the same superscript letters are significantly different (p<0.05).

Table 4. Digestibility of alginate, and molecular weight and composition of alginate in feces of rats fed with the kombu diet

<table>
<thead>
<tr>
<th>Feeding period</th>
<th>Day 2 to Day 5</th>
<th>Day 25 to Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alginate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily intake (mg)</td>
<td>548±14*</td>
<td>901±23b</td>
</tr>
<tr>
<td>Daily output (mg)</td>
<td>352±14*</td>
<td>354±12*</td>
</tr>
<tr>
<td>Digestibility (%)</td>
<td>36±2*</td>
<td>61±1b</td>
</tr>
<tr>
<td>Alginate in feces</td>
<td>Molecular weight</td>
<td>110,000</td>
</tr>
<tr>
<td>M/G*</td>
<td>0.80±0.03</td>
<td>0.67±0.02b</td>
</tr>
</tbody>
</table>

Horizontal values not sharing the same superscript letters are significantly different (p<0.05).

* Molar ratio of mannuronic (M) and guluronic (G) acids.

Day 2 to Day 5, and from Day 25 to Day 28) are presented in Table 4. Daily intake of alginate significantly increased from 548±14 g in the first feeding period to 901±23 g in the last feeding period, but there was no difference in daily output between the two periods (352±4 and 354±12 g). Therefore, alginate digestibility increased significantly from 36±2 to 61±1% from Day 5 to Day 25, or over about 3 weeks. The molecular weight of alginate in the feces decreased from 110,000 to 44,000, which was down by 60%. The molar ratio of mannuronic and guluronic acids in alginate of the late feeding period (0.67±0.02) was significantly smaller than that of the early feeding period (0.80±0.03). This result means mannuronic acid was rather digestible in comparison with guluronic acid in rats in adaptation.
Rats have no digestive enzyme for breaking down alginates. The degree of digestibility of a single fiber source could be expected to increase with time, a situation which might occur during long-term administration of a fiber-supplemented enteral diet. Bacteria that reside in the large bowel are able to produce enzymes that hydrolyze undigested carbohydrates. Kuda et al. examined the effects of alginic acid and kombu diets on fecal microflora and reported that the intake of brown algae allowed changes in intestinal flora and pH values.

From this research, the digestibility of mannuronic and guluronic acids (components of alginates) by microflora was found to be different; the digestibility of mannuronic acid was greater than that of guluronic acid in rats.

References


Suzuki, Nakai, Yoshie, Shirai, and Hirano