Basic nutritional investigation

Unhydrolyzed and hydrolyzed konjac glucomannans modulated cecal and fecal microflora in Balb/c mice

Hsiao-Ling Chen, Ph.D., R.D.a*, Ying-Hsin Fan, M.S.a, Mei-En Chen, M.S.a, and You Chan, Ph.D.b

a School of Nutrition, Institute of Nutritional Science, Chung Shan Medical University, Taichung, Taiwan, Republic of China
b Department of Microbiology and Immunology, Chung Shan Medical University, Taichung, Taiwan, Republic of China

Manuscript received September 24, 2004; accepted February 2, 2005.

Abstract

Objective: The prebiotic role of intact konjac glucomannan (KGM) is contradictory. Short-chain glucomannan may cause a greater or faster effect on colonic microflora compared with KGM. Therefore, time-course and dose-dependent studies were conducted to examine and compare effects of unhydrolyzed KGM with those of acid-hydrolyzed glucomannan (KH) on cecal and fecal microflora. Short-chain fatty acid concentrations in cecal content were also determined.

Methods: Seven-week-old male Balb/c mice were fed 5% (w/w) cellulose and KGM or KH diets for 2 or 4 wk in a time-course study. Cecal total anaerobes, bifidobacteria, Clostridium perfringens and Escherichia coli counts, and short-chain fatty acid concentrations were determined. In a subsequent dose-dependent study, Balb/c mice were fed AIN-93 fiber-free diets supplemented with 2.5%, 5%, or 7.5% of KGM or KH for 4 wk. Anaerobes, bifidobacteria, C. perfringens, and E. coli were enumerated in the cecal content and feces.

Results: KGM and KH significantly increased cecal anaerobes and bifidobacteria counts at weeks 2 and 4, respectively, compared with cellulose. In contrast, KGM and KH significantly decreased cecal C. perfringens counts only at week 4. Acetate and propionate concentrations in cecal contents were increased by KGM and KH diets at weeks 2 and 4, respectively. In the dose-dependent study, KH increased cecal bifidobacteria counts only at the 2.5% level but increased fecal bifidobacteria count and suppressed C. perfringens counts at each dose level as compared with KGM.

Conclusion: Hydrolyzed glucomannan exerts a greater prebiotic effect than does KGM in Balb/c mice. © 2005 Elsevier Inc. All rights reserved.

Keywords: Glucomannan; Hydrolysate; Anaerobes; Bifidobacteria; Clostridium perfringens; Short-chain fatty acid

Introduction

Intestinal microflora pattern has been associated with human health [1,2]. Beneficial flora including the genera of Bifidobacterium or Lactobacillus promote the colonic environment by decreasing pathogenic bacteria and carcinogenic material and improving immunity [1,3]. Conversely, Clostridium perfringens and some pathogenic Escherichia coli strains are considered detrimental [3]. Therefore, incorporation of prebiotics, food ingredients that selectively stimulate the growth and activity of beneficial bacteria, into an ordinary diet is recommended [4].

Konjac glucomannan (KGM), derived from the tuber of Amorphophallus konjac C. Koch, has been consumed traditionally in Asia for centuries. It has proved to be an effective adjunct to manage hyperglycemia and hypercholesterolemia [5,6]. Supplementation of this dietary fiber also improves bowel habits [7,8]. However, the prebiotic effect of KGM has been contradictory [9,10]. Mizutani and Mitsuoka [9] reported that feeding mice a konjac-rich diet (10% [w/w]) for 10 mo increases fecal bifidobacteria, whereas the study of Fujiwara et al. [10] did not support this observation. Therefore, the role of KGM on colonic microflora ecology remained to be further studied.
The high viscosity of KGM limits its application in food and the downsizing of the glucomannan polymer may expand its application. The physiologic effect of glucomannan hydrolysate (KH) has never been studied. Because hydrolyzed chicory inulin has been shown to exert greater bifidogenic effects than unhydrolyzed chicory inulin [11], KH may promote the modulatory effects of intact KGM on the colonic microflora ecology.

This study compared the modulatory effects of KGM and KH in Balb/c mice in time-course and dose-dependent studies. Effects of cellulose, KGM, and KH on total anaerobes, bifidobacteria, C. perfringens and E. coli counts in cecal content, and cecal short-chain fatty acid concentrations were determined after 2 and 4 wk of feeding. The second study compared the effectiveness of KGM with that of KH in the lower gut. The dose-related effects of KGM and KH on microflora ecology in cecum content and in feces were determined.

Materials and methods

Preparation and characterization of KH

Konjac glucomannan (Fukar Co., Taipei, Taiwan) was partly hydrolyzed in 0.2 N HCl (25 g/L) after being boiled for 20 min in a round-bottom flask with a reflux condenser. The KH solution was neutralized with NaOH before it was lyophilized and incorporated into the experimental diets. To examine the extent of acid hydrolysis of the glucomannan polymer, the average degree of polymerization (DP) of KH was calculated as the ratio of total sugar content (milligrams per gram) to reducing sugar (milligrams per gram). Determinations of total sugar and reducing sugar followed the methods described by Dubious et al. [12] and the Somogyi-Nelson method [13,14], respectively. In addition, to examine whether the KH preparation contained any glucose, glucose in KH was determined with a commercial kit (Sigma, St. Louis, MO, USA).

Experimental design

Time-course experiment.

Seven-week-old male Balb/c mice (National Laboratory Animal Breeding and Research Center, Taipei, Taiwan) were housed in solid-bottom plastic cages with wood shavings for bedding in a room maintained on a 12-h light/12-h dark cycle (8:00 AM to 8:00 PM), 24 ± 1°C, and 50% humidity until they were singly housed in metabolic cages during the final 5-d period of the 2- or 4-wk study. All animals were allowed free access to water and food during the study. Animal care followed guidelines of the National Research Council and was approved by the institutional animal care and use committee in Chung Shan Medical University [15]. After a 10-d adaptation period, mice were randomly assigned to one of six groups (n = 8/group) to compare effects of duration of feeding (2 or 4 wk) and test fiber (cellulose, KGM, or KH) on cecal microflora. The diet was composed of 200.0 g/kg of casein, 529.5 g/kg of corn starch, 100.0 g/kg of sucrose, 70.0 g/kg of corn oil, 35 g/kg of AIN-93G mineral mix, 1.0 g/kg of AIN-93 vitamin mix, 3.0 g/kg of l-cystine, 2.5 g/kg of choline bitartrate, 0.014 g/kg of butylated hydroxytoluene, and 50 g/kg of test fiber. The powder diet was mixed with an equal weight of distilled water and made into pellets. Thus, the weight of feed could be measured correctly without the complication of spillage. Food intake was weighed everyday and body weight was measured twice a week. Mice were housed individually in metabolic cages on the final 5 d of each experimental period to avoid the interference of coprophagy and consumption of wood bedding. Mice were killed after 16 h of fasting, and ceci were collected within 2 min on day 14 or 28 for the 2- or 4-wk study, respectively. A portion of the cecal content was immediately collected into 10 mL of anaerobic solution (0.1 M phosphate buffer, pH7.0, 2 g/L of gelatin, 0.5 g/L of cysteine, and 0.002 g/L of resazurin) in an anaerobic chamber, weighed, homogenized by a stomacher for 2 min, and diluted serially with anaerobic solution. Cecal contents were immediately processed for plate culturing of total anaerobes, bifidobacteria, C. perfringens, and E. coli. The other portion was stored at −20°C for analysis of short-chain fatty acid.

Dose-dependent experiment.

Seven-week-old male Balb/c mice were randomly allocated to consume one of six AIN-93-modified diets (n = 8/group) that contained 25, 50, or 75 g/kg of KGM or KH as the sole dietary fiber for 4 wk. The powder diet was made into pellets as described previously. Animals received the same care as for the time-course study. Fresh feces were collected into the anaerobic solution immediately after mice defecated on day 28. Mice were then killed with CO2 inhalation. Cecal contents were collected in the same manner as described for the time-course study. Feces and cecal content were enumerated for the same bacteria as those described for the time-course study.

Enumeration of intestinal microflora

Brucella blood agar (Merck) [16], bifidobacteria iodoacetate medium-25 [17], and modified differential clostridial agar (Merck) supplemented with polymyxinB sulfate (8.5 mg/L) [18] were used to selectively identify anaerobes, bifidobacteria, and C. perfringens, respectively, in an anaerobic chamber (H2/CO2/N2, 10/10/80). Eschericia coli was selectively identified using eosin methylene blue agar (Merck) [19]. Plates were inoculated in triplicate and incubated at 37°C for 3 d. To confirm the identity of colonies, polymerase chain reactions were performed with specific primers as described by Wang et al. [20].
Effects were considered statistically significant at \( P < 0.05 \). Results were analyzed by two-way ANOVA to determine the effect of duration of feeding (duration), fiber source (fiber), and their interaction. Differences at \( P < 0.05 \) were considered significant for main effects and their interaction and those at \( P \geq 0.05 \) were considered not significant.

### Analysis of cecal short-chain fatty acids

Cecal contents (~0.04 g/mouse) were extracted with ether and analyzed in a gas chromatograph (QC-14B, Shimadzu, Japan) fitted with a glass capillary column (0.25 mm, 30 m, Stabilwax-DA, Restek Corp., Bellefonte, PA, USA) and a flame ionization detector as described previously [21]. Each sample was analyzed in triplicate.

#### Statistical analysis

Data were expressed as means ± standard deviation. All statistical analyses were conducted with SPSS 10.0 for Windows (SPSS Inc., Chicago, IL, USA). Bacterial colony counts were logarithmically treated to fit a normal distribution, and the normality of the data was confirmed. For the time-course study, two-way analysis of variance was used to determine the effects of duration of feeding (2 or 4 wk), test fiber (cellulose, KGM, or KH), and interactions of these two factors. Differences across fiber groups at each time point were also compared by Tukey’s test [22]. For the dose-dependent study, effects of dose (2.5%, 5%, and 7.5%), test fiber (KGM and KH), and interactions between these two factors were analyzed by two-way analysis of variance. Effects of KH and KGM were compared by Student’s t test. Effects were considered statistically significant at \( P < 0.05 \).

#### Results

The average DP of KH was 12 ± 1 with triplicate analysis. The KH preparation did not contain glucose.

In the time-course experiment, mice were healthy throughout the study. Daily weight gain was unaffected by diet (0.2 g/d per rat, pooled standard deviation 0.1 g). Daily food intake was also similar across groups, regardless of feeding duration and the type of dietary fiber (3.7 g/d per rat, pooled standard deviation 0.4 g). KGM and KH increased anaerobes and bifidobacteria counts (colony-forming units per gram of wet mass) at 2 and 4 wk as compared with cellulose (Table 1). Total anaerobe counts increased similarly in mice fed the KGM and KH diets at 2 wk as compared with mice fed the cellulose diet. At 4 wk, the KH group had the largest cecal anaerobe count, with two- and four-fold levels compared with the KGM and cellulose groups, respectively. KGM and KH increased cecal bifidobacteria counts to similar extents and approximately an eight-fold level compared with the cellulose group after 2 wk of feeding. However, the bifidogenic effect of KH was more pronounced than that of KGM after 4 wk of feeding. KGM and KH suppressed cecal \textit{C. perfringens} by 55.5% and 59.6%, respectively, as compared with cellulose only after 4 wk of feeding. In contrast, \textit{E. coli} in the cecal content decreased with the KGM and KH diets only at 2 wk.

Acetate was the major short-chain fatty acid in the cecal content in all experimental groups (Table 2). Cecal acetate concentrations of the KGM and KH groups were 1.5- and almost four-fold, respectively, that in the cellulose group at 2 wk. Further, cecal acetate concentrations of the KGM and KH groups were approximately three-fold and almost five-fold, respectively, that in cellulose group at 4 wk. The KGM and KH diets increased cecal propionate concentrations by 40% and approximately four-fold, respectively, compared with that in the cellulose group at 2 wk. KGM and KH diets further increased cecal propionate concentrations to almost three-fold that in the cellulose group at 4 wk. Cecal i-butyrate concentration was increased by KH at 2 and 4 wk. In contrast, the KH diet significantly decreased n-butyrate concentrations at 2 and 4 wk compared with the cellulose diet.

#### Table 1

Viable bacterial counts in cecal content of Balb/c mice fed C, KGM, or KH diets for 2 or 4 wk*

<table>
<thead>
<tr>
<th>Group</th>
<th>Total anaerobes</th>
<th>Bifidobacteria</th>
<th>\textit{Clostridium perfringens}</th>
<th>\textit{Escherichia coli}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( 2 \text{ wk} )</td>
<td>( 4 \text{ wk} )</td>
<td>( 2 \text{ wk} )</td>
<td>( 4 \text{ wk} )</td>
</tr>
<tr>
<td>C</td>
<td>8.2 ± 0.1(^a)</td>
<td>8.3 ± 0.1(^a)</td>
<td>7.6 ± 0.1(^a)</td>
<td>6.2 ± 0.1(^a)</td>
</tr>
<tr>
<td>KGM</td>
<td>8.5 ± 0.1(^b)</td>
<td>8.6 ± 0.1(^b)</td>
<td>7.6 ± 0.1(^a)</td>
<td>5.0 ± 0.1(^a)</td>
</tr>
<tr>
<td>KH</td>
<td>8.5 ± 0.1(^b)</td>
<td>8.9 ± 0.1(^c)</td>
<td>7.5 ± 0.1(^a)</td>
<td>5.3 ± 0.1(^b)</td>
</tr>
</tbody>
</table>

ANOVA, analysis of variance; C, cellulose; KGM, konjac glucomannan; KH, acid-hydrolyzed glucomannan; NS, not significant.

* Values are in \( \log_{10} \) colony-forming units per gram of wet weight of cecal content and are expressed as means ± standard deviation (\( n = 8/\text{group} \)). Differences between fiber groups at each time point were analyzed by Tukey’s test. Values within each column with different superscripts are significantly different (\( P < 0.05 \)). Results were analyzed by two-way ANOVA to determine the effect of duration of feeding (duration), fiber source (fiber), and their interaction. Differences at \( P < 0.05 \) were considered significant for main effects and their interaction and those at \( P \geq 0.05 \) were considered not significant.
In the dose-related experiment, animals were healthy throughout the study. Daily weight gain increased as the level of KGM or KH increased (Table 3). In contrast, food intake decreased as the level of KGM or KH increased. Diet efficiency increased as the level of KGM or KH increased. Although KH tended to cause greater diet efficiency than did KGM, the effect was not significant at any fiber level.

The effect of dose was significant for every fecal flora investigated in this study (Table 4). The main effect of fiber was significant for all bacteria investigated in this study except for E. coli. KH consistently increased cecal anaerobe counts at each dose level and did not influence the E. coli counts at any level compared with KGM.

The effect of dose was significant for every fecal flora investigated in this study (Table 5). KH caused larger fecal total anaerobe counts at the 2.5% and 5% levels compared with KGM. KH was more bifidogenic than KGM at each fiber level. Fecal C. perfringens counts were lower in the KH group than in the KGM group at every level, whereas E. coli counts were lower in KH groups only at the 5% and 7.5% levels.

### Table 2
Wet weight and short-chain fatty acid concentrations of cecal contents in Balb/c mice fed C, KGM, or KH diets for 2 or 4 wk*

<table>
<thead>
<tr>
<th>Group</th>
<th>Wet weight (g/mouse)</th>
<th>Acetate (μmol/g wet cecal content)</th>
<th>Propionate (μmol/g wet cecal content)</th>
<th>i-Butyrate (μmol/g wet cecal content)</th>
<th>n-Butyrate (μmol/g wet cecal content)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.05 ± 0.01</td>
<td>22.1 ± 9.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.7 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.6 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.2 ± 2.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>KGM</td>
<td>0.06 ± 0.01</td>
<td>33.2 ± 17.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.8 ± 2.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.9 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.7 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>KH</td>
<td>0.07 ± 0.01</td>
<td>75.6 ± 53.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.2 ± 8.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.7 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.4 ± 3.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>4 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.06 ± 0.02</td>
<td>27.1 ± 17.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.6 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.6 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.9 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>KGM</td>
<td>0.06 ± 0.02</td>
<td>81.2 ± 26.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.3 ± 3.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.7 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.7 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>KH</td>
<td>0.05 ± 0.03</td>
<td>130.2 ± 42.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.7 ± 2.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.9 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.6 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

ANOVA
- Duration: NS
- Fiber: NS
- Duration × fiber: NS

ANOVA, analysis of variance; C, cellulose; KGM, konjac glucomannan; KH, acid-hydrolyzed glucomannan; NS, not significant.

* Values are means ± standard deviation (n = 8/group). Differences between fiber groups at each time point were analyzed by Tukey’s test. Values within each column with different superscripts are significantly different (P < 0.05). Results were analyzed by two-way ANOVA to determine the effect of duration of feeding (duration), fiber source (fiber), and their interaction. Differences at P < 0.05 were considered significant for main effects and their interaction and those at P ≥ 0.05 were considered not significant.

### Table 3
Weight gain, food intake, and diet efficiency of Balb/c mice fed diets containing 2.5%, 5%, or 7.5% KGM or KH for 4 wk*

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight gain (g/d)</th>
<th>Food intake (g/d)</th>
<th>Diet efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5% KGM</td>
<td>0.1 ± 0.1</td>
<td>4.9 ± 0.1</td>
<td>2.6 ± 1.2</td>
</tr>
<tr>
<td>2.5% KH</td>
<td>0.2 ± 0.0</td>
<td>4.6 ± 0.1</td>
<td>3.5 ± 0.6</td>
</tr>
<tr>
<td>5.0% KGM</td>
<td>0.2 ± 0.0</td>
<td>4.7 ± 0.0</td>
<td>4.0 ± 0.8</td>
</tr>
<tr>
<td>5.0% KH</td>
<td>0.2 ± 0.0</td>
<td>4.5 ± 0.0</td>
<td>4.3 ± 1.0</td>
</tr>
<tr>
<td>7.5% KGM</td>
<td>0.2 ± 0.1</td>
<td>4.7 ± 0.1</td>
<td>4.2 ± 1.8</td>
</tr>
<tr>
<td>7.5% KH</td>
<td>0.2 ± 0.1</td>
<td>4.3 ± 0.1</td>
<td>5.4 ± 1.7</td>
</tr>
</tbody>
</table>

ANOVA
- Dose: 0.012
- Fiber: 0.0001
- Dose × fiber: NS

ANOVA, analysis of variance; KGM, konjac glucomannan; KH, acid-hydrolyzed glucomannan; NS, not significant.

* Values are means ± standard deviation (n = 8/group). Significant difference from KGM as analyzed by Student’s t test at each dose level (P < 0.05). Results were analyzed by two-way ANOVA to determine the effect of dose, fiber, and their interaction. Differences at P < 0.05 were considered significant for main effects and their interaction and those at P ≥ 0.05 were considered not significant.

### Table 4
Viable bacterial counts in cecal contents of Balb/c mice fed diets containing 2.5%, 5%, or 7.5% KGM or KH for 4 wk*

<table>
<thead>
<tr>
<th>Group</th>
<th>Total anaerobes (cfu/g)</th>
<th>Bifidobacteria (cfu/g)</th>
<th>Clostridium perfringens (cfu/g)</th>
<th>Escherichia coli (cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5% KGM</td>
<td>8.5 ± 0.1</td>
<td>6.9 ± 0.1</td>
<td>8.2 ± 0.1</td>
<td>6.4 ± 0.2</td>
</tr>
<tr>
<td>2.5% KH</td>
<td>8.8 ± 0.1</td>
<td>7.1 ± 0.1</td>
<td>8.6 ± 0.5</td>
<td>6.7 ± 0.1</td>
</tr>
<tr>
<td>5.0% KGM</td>
<td>8.6 ± 0.3</td>
<td>7.1 ± 0.1</td>
<td>7.6 ± 0.1</td>
<td>6.2 ± 0.2</td>
</tr>
<tr>
<td>5.0% KH</td>
<td>8.9 ± 0.3</td>
<td>7.2 ± 0.1</td>
<td>7.3 ± 0.1</td>
<td>6.1 ± 0.2</td>
</tr>
<tr>
<td>7.5% KGM</td>
<td>8.8 ± 0.1</td>
<td>7.1 ± 0.1</td>
<td>7.8 ± 0.1</td>
<td>6.3 ± 0.1</td>
</tr>
<tr>
<td>7.5% KH</td>
<td>9.2 ± 0.1</td>
<td>7.2 ± 0.1</td>
<td>6.9 ± 0.2</td>
<td>6.2 ± 0.1</td>
</tr>
</tbody>
</table>

ANOVA
- Dose: 0.0001
- Fiber: 0.0001
- Dose × fiber: 0.0027

ANOVA, analysis of variance; KGM, konjac glucomannan; KH, acid-hydrolyzed glucomannan; NS, not significant.

* Values are in log<sub>10</sub> colony-forming units per gram of wet weight of cecal content and are expressed as means ± standard deviation (n = 8/group). Significant difference from KGM as analyzed by Student’s t test at each dose level (P < 0.05). Results were analyzed by two-way ANOVA to determine the effect of dose, fiber, and their interaction and were not significant at P ≥ 0.05.
Discussion

The intrinsic chemical and physical properties of dietary fibers determine how they interact with intestinal flora. The preferred dietary fiber substrates of predominant anaerobic bacteria have been illustrated in pure culture studies [23]. Cellulose, a partly fermentable fiber, has been shown to cause the growth of Bacteroides spp., whereas soluble fibers, such as guar gum and arabinoxylans, selectively stimulate several species in the genus of Bifidobacterium [23]. This study demonstrated that KGM, a soluble fiber, specifically stimulated bifidobacteria in the cecum and further illustrated that the KH prepared by acid hydrolysis also promoted bifidobacteria in Balb/c mice. In addition, this study demonstrated that KGM and KH modulated cecal and fecal flora in dose-dependent fashions, in agreement with the physiologic effects of oligosaccharides observed in previous studies [24,25]. These beneficial effects of KGM and KH qualified them to be potential prebiotics [26].

Linear or branched oligofructoses caused greater in vitro growth rates of several Bifidobacterium spp. than did inulin [11], implying that the DP, instead of the linearity, of fructans is the key factor that decides the accessibility of fructans to bifidobacteria. Van Laere et al. [27] also found that bifidobacteria used a low DP rather than a high DP, as do carbohydrates. Our dose-response study confirmed the critical role of DP of KGM on intestinal microflora ecology, even though the structure of KH may not be identical to that of intact KGM due to acid hydrolysis.

In agreement with previous observations [28], prolonged feeding of soluble indigestible carbohydrate increased acetate concentrations in the cecum. In addition, the present study demonstrated that molar ratios of acetate increased and those of butyrate decreased as feeding duration increased in KGM- and KH- but not in cellulose-fed mice. Bifidobacteria are acetate producers but not butyrate generators [29]. Therefore, transition of cecal fatty acid profiles in mice fed the KGM and KH diets were likely to be due to increases in the bifidobacteria population.

The time-course and dose-related experiments in this study suggested that the increase in bifidobacteria was associated with decreases in C. perfringens and E. coli in cecal content. These observations agreed with the defined coculture experiments in which bifidobacteria inhibited the growth of C. perfringens and E. coli [30]. The mechanisms by which bifidobacteria exerted these effects include the increase in acidic fermentation product (mainly acetate and lactate) and secretion of antimicrobial substances [1]. The greater cecal acetate contents in the KH and KGM groups than in the cellulose group suggested that the prebiotic effects of KGM and KH were due in part to the decrease in pH. Further, an in vitro study demonstrated the konjac fluid prevents the growth of food-borne C. perfringens and E. coli [31], which implied that glucomannan per se could directly suppress colonization of these bacteria in the intestine. Therefore, KGM and KH could beneficially suppress C. perfringens and E. coli indirectly through the action of bifidobacteria or directly through their own physicochemical characteristics.

In summary, this study suggested that consuming 5% KGM or KH in the diet for 4 wk sufficiently promotes the growth of intestinal bifidobacteria and suppresses the growth of C. perfringens compared with cellulose. The bifidogenic effects of KH were greater than those of unhydrolyzed KGM as observed in the feces of Balb/c mice. The fermentation product of glucomannan and fiber likely caused the suppression of C. perfringens or E. coli in the lower gut.

References


