Effects of Lactitol-Oligosaccharides on the Intestinal Microflora in Rats

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Summary Lactitol-oligosaccharide (LO) was prepared from lactitol by a transgalactosylation reaction catalyzed by Aspergillus oryzae β-galactosidase. The utilization of LO by human intestinal bacteria, the digestion of LO by rat jejunum mucosal homogenates and the effects of LO on the intestinal microflora in rats were compared with those of lactitol. 1) LO was utilized in vitro by Bifidobacterium, but lactitol was not utilized. 2) Neither LO nor lactitol were digested by rat jejunum mucosal homogenates. 3) A significant increase in the fecal counts of Bifidobacterium was observed in the LO diets. 4) The concentration of organic acids in feces and cecal contents significantly increased in the LO diets. 5) The concentration of fecal putrefactive products significantly decreased in the LO and lactitol diets. These findings suggest that LO is effective for improving intestinal conditions.

Key Words lactitol-oligosaccharide, lactitol, transgalactosylation, β-galactosidase, Aspergillus oryzae, Bifidobacterium, organic acid, microorganism, intestinal conditions, rat

Balances of intestinal bacterial flora are intimately related to human health and a predominance of Bifidobacterium is particularly considered to be important (1–3). It is well known that diseases and aging causes a decrease or the disappearance of Bifidobacterium in intestines (4, 5). Following this, various researches have been carried out to increase bifidobacteria in the human intestine. Recently, galactosyl-lactose (6), galactooligosaccharide (7,8), fructooligosaccharide (9,10), isomaltoligosaccharide (11), xylooligosaccharide (12), and soybean oligosaccharide (13, 14) have been reported as selective growth factor of bifidobacteria.

On the other hand, lactitol produced by lactose reduction is recommended as a low-caloric sweetener (15,16), and as a medicine in the treatment of hepatic encephalopathy (17,18). These applications are possible due to the fact that lactitol is not hydrolyzed and thus is not absorbed in the small intestines (19,20).

Oligosaccharide containing a lactitol unit were prepared from a corresponding
galactosyllactose, formed from lactose by transgalactosylation catalyzed by \( \beta \)-galactosidase, by reduction with sodium borohydride. We have developed a new sweetener, lactitol-oligosaccharides, first synthesized from lactitol by transgalactosylation catalyzed with \( \beta \)-galactosidase (21), but their physiological properties have not been reported.

In the present study, we investigated the utilization of lactitol-oligosaccharide (LO) by human intestinal bacteria in vitro, and the effects of LO administration on rat intestinal conditions.

**METHODS**

*Preparation of lactitol-oligosaccharide (LO).* The LO was prepared from lactitol by *Aspergillus oryzae* \( \beta \)-galactosidase according to the method reported previously (21). The LO was purified from the reaction mixture by charcoal column chromatography. After purification, HPLC analysis data indicated the purity to be 95\%. The structure and composition of LO (21) are summarized in Table 1.

*Utilization of carbohydrates by human intestinal bacteria.* The strains used in this utilization test are shown in Table 4. The carbohydrates tested were LO, lactitol, and glucose. Five milliliters of Peptone Yeast Extract Fildes solution (PYF) Broth, with 0.5\% of various test carbohydrates added, were inoculated with

| Structures | Composition (%) |
|------------|-----------------|
| \( \beta \)-D-Gal-(1\( \rightarrow \)4)-\( \beta \)-D-Gal-(1\( \rightarrow \)4)-D-Glcol | 3.5 |
| \( \beta \)-D-Gal-(1\( \rightarrow \)3)-\( \beta \)-D-Gal-(1\( \rightarrow \)4)-D-Glcol | 5.6 |
| \( \beta \)-D-Gal | 1 |
| ↓ | 3.0 |
| 5 | |
| \( \beta \)-D-Gal-(1\( \rightarrow \)4)-D-Glcol | |
| \( \beta \)-D-Gal-(1\( \rightarrow \)6)-\( \beta \)-D-Gal-(1\( \rightarrow \)4)-D-Glcol | 23.0 |
| \( \beta \)-D-Gal | 1 |
| ↓ | 40.9 |
| 6 | |
| \( \beta \)-D-Gal-(1\( \rightarrow \)4)-D-Glcol | |
| \( \beta \)-D-Gal | 1 |
| ↓ | 24.0 |
| 1 | |
| \( \beta \)-D-Gal-(1\( \rightarrow \)4)-D-Glcol | |

1Gal, galactose; Glcol, glucitol.

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0.1 ml (10^8 c.f.u./ml) of the test organisms. The inoculated media were incubated anaerobically at 37°C for 24 h by the steel wool method. Utilization of substrates by the bacteria was evaluated by measuring the absorbance at 660 nm and pH value. The absorbance was scored in the following manner: +++, >1.5; ++, 1.49–1.0; +, 0.99–0.5; ±, 0.49–0.21; −, <0.2.

*Hydrolysis of carbohydrates by rat jejunum mucosal homogenates.* Male Sprague-Dawley rats weighing about 300 g were used in this test. The small intestines were slit open and washed with ice-cold physiological saline. The mucosal in small intestines was scraped with a glass slide and homogenized in five volumes of saline solution. The homogenates were used as an enzyme solution for the test. The hydrolysis of carbohydrates in the jejunal mucosa was determined by the method of Harju (19, 20). The enzyme solution was added to 0.1 M maleate buffer (pH 6.0) containing 14.6 mM substrates, and the solution was incubated at 37°C. The amount of galactose released from substrates were determined by enzymatically with Boehringer-Manheim's test kit. Protein concentration of the jejunal mucosal homogenate was determined by the method of Lowry.

*Animals and diets.* Twenty-eight male Sprague-Dawley rats, with an initial mean weight of 50 g, were purchased from Charles River Inc. After a 1 week period of adaptation to the control diet (Table 2), the rats were randomly assigned to three diet groups with six rats in each group. They were fed on the experimental diet described in Table 2 for 3 weeks. Diets and tap water were provided ad libitum. Body weight and food intake were recorded daily.

*Analysis of fecal microflora.* Fresh fecal specimens were collected before and at the 1st, 2nd, and 3rd weeks after administration of experimental diets. The method for bacterial analysis of fecal microflora was essentially the same as that of Mitsuoka et al. (22). The used medium and culture conditions are shown in Table 3. The results are expressed as the log_{10} of the number of bacteria per gram wet weight of fecal material.

*Analysis of fecal organic acids.* Fecal organic acids were determined in the sample used in fecal microflora analysis. The sample was centrifuged for 15 min at

| Component           | Diets          |
|---------------------|----------------|
|                     | Control | LO^1  | Lactitol |
| Casein              | 22      | 22    | 22       |
| Lard                | 9       | 9     | 9        |
| Corn oil            | 1       | 1     | 1        |
| Salt mixture^2      | 4       | 4     | 4        |
| Vitamin mixture^2   | 1       | 1     | 1        |
| Test sugar          | —       | 5     | 5        |
| α-Corn starch       | 63      | 58    | 58       |

^1 LO, lactitol-oligosaccharides.  ^2 Prepared according to Harper’s formulation.
Table 3. Culture media and methods.

| Media          | Organisms enumerated usually | Dilution to be plated | Incubation time (days) |
|----------------|------------------------------|-----------------------|------------------------|
| Aerobic incubation |                              |                       |                        |
| TS bloods agar  | Aerobese                     | $10^{-5}$,$10^{-6}$,$10^{-7}$ | 1                      |
| DHL agar       | Enterobacteriaceae           | $10^{-1}$,$10^{-2}$,$10^{-3}$ | 1                      |
| TATAc agar     | Streptococcus                | $10^{-1}$,$10^{-2}$,$10^{-3}$ | 2                      |
| PEES agar      | Staphylococcus               | $10^{-1}$,$10^{-2}$,$10^{-3}$ | 2                      |
| P agar         | Yeasts and molds             | $10^{-1}$,$10^{-2}$,$10^{-3}$ | 2                      |
| Anaerobic incubation | Steel wool jar replaced air with CO₂ |              |                        |
| EG agar        | Anaerobes                    | $10^{-6}$,$10^{-7}$,$10^{-8}$ | 3                      |
| BL agar        | Anaerobes                    | $10^{-6}$,$10^{-7}$,$10^{-8}$ | 3                      |
| BS agar        | Bifidobacterium              | $10^{-1}$,$10^{-2}$,$10^{-3}$ | 3                      |
| ES agar        | Eubacterium                  | $10^{-1}$,$10^{-2}$,$10^{-3}$ | 3                      |
| NBGT agar      | Bacteroidaceae               | $10^{-1}$,$10^{-2}$,$10^{-3}$ | 3                      |
| LBS agar       | Lactobacillus                | $10^{-1}$,$10^{-2}$,$10^{-3}$ | 3                      |
| NN agar        | Clostridium perfringens      | $10^{-1}$,$10^{-2}$,$10^{-3}$ | 3                      |

1 An aliquot (0.05 ml) of each dilution was plated on each medium. TS, trypticase soy; DHL, deoxycholate hydrogen sulfide lactose; TATAc, tetrazolium-azidothallium-acridine orange-crystal violet; PEES, phenylethyl alcohol-egg yolk-Staphylococcus 110; P, potato dextrose; EG, eggerth-gagnon; BL, glucose-blood liver; BS, Bifidobacterium-selective; ES, Eubacterium-selective; NBGT, Neomycin-brilliant green-taurocholate-blood; LBS, Lactobacillus-selective; NN, Neomycin-Nagler.

11,000 × g. After the addition of pyroglutamic acid as an internal standard, organic acids were determined using a carboxylic acid analyzer (model S-14, Tokyo Rika, Tokyo, Japan).

**Analysis of cecal contents.** After 17 h fasting, the rats were anesthetized with diethyl ether and the cecums were removed. They were homogenized with nine volumes of distilled water, and organic acids were analyzed using a carboxylic acid analyzer. Cecal pH values were determined with a flat glass electrode.

**Analysis of fecal putrefactive products (indol, skatole, and p-cresol).** Fecal putrefactive products were analyzed by HPLC using the method of Nagasawa (23).

**Statistical analysis.** Group means were compared by one-way analysis of variance. Means were analyzed for significant differences ($p < 0.05$) by Tukey's multiple comparison test.

**RESULTS**

**Utilization of LO by the human intestinal bacteria**

The results of utilization tests in vitro of LO, lactitol, and glucose are shown in Table 4. LO was utilized at the same level as that of glucose, but lactitol was found to be not utilized by Bifidobacterium. LO was utilized by some of Clostridium species, but the extent of utilization was much lower than that of lactitol. Other

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Table 4. Utilization of three sugars by human intestinal bacteria.

| Bacterial species                      | LO  | Lacol | Glc  |
|----------------------------------------|-----|-------|------|
| *Bifidobacterium adolescentis* (ATCC-15703) | +++ | + +   | +++  |
| *bifidum* (ATCC-29521)                 | +++ | ±     | +    |
| *brevae* (ATCC-15698)                  | +++ | +     | +++  |
| *infantis* (ATCC-15697)                | +++ | +     | +++  |
| *longum* (ATCC-15707)                  | +   | –     | +++  |
| *Lactobacillus acidophilus* (ATCC-314)  | –   | ±     | +++  |
| *casei* (ATCC-4646)                    | –   | +++++ | +++  |
| *Peptococcus prevotii* (ATCC-9321)     | –   | –     | +    |
| *Streptococcus faecalis* (ATCC-14506)  | –   | ±     | +    |
| *Bacteroides distasonis* (SBT-3177)¹  | ±   | +     | +    |
| *fragilis* (SBT-3181)¹                 | ±   | ±     | +    |
| *Clostridium bifodorum* (ATCC-19299)   | –   | –     | +    |
| *butyricum* (JCM-1391)                 | +   | +     | +    |
| *clostridioforme* (JCM-1291)           | +   | +     | +++  |
| *paraputrificum* (ATCC-25780)         | ±   | +     | +    |
| *perfringens* (ATCC-13124)            | +   | +     | +    |
| *ramosum* (JCM-1298)                  | +   | +     | +++  |

LO, lactitol-oligosaccharide; Lacol, lactitol; Glc, glucose. Evaluation of bacteria growth (O.D. 660nm): ++++, >1.5; ++, 1.00–1.49; +, 0.50–0.99; ±, 0.2–0.49; –, <0.19. ¹Snow Brand Typeculture Collection.

human intestinal bacteria, including *Lactobacillus, Streptococcus, Bacteroides,* and *Escherichia coli* did not utilize LO. The LO, which is the result of linking a galactose to lactitol, shows different utilization by bifidobacteria compared to that of lactitol.

**Hydrolyzation of LO by rat jejunum homogenate**

The results of hydrolyzation experiments of LO, lactitol, and lactose with rat jejunum homogenate are shown in Fig. 1. LO and lactitol were not hydrolyzed by rat jejunum homogenate while lactose was hydrolyzed with time.

**Body weight and food intake**

The body weights, food intake, and food efficiency of the 3 groups were not significantly different (Table 5).

**Change of fecal flora**

The results of the analysis of rat fecal flora are shown in Table 6. The counts of *Bifidobacterium* increased significantly during administration of LO diets, compared with control and lactitol diets. The total bacterial counts increased significantly in the 3rd week after administration of LO diets. Other fecal flora did not show significant changes.
Fig. 1. Hydrolysis of lactitol-oligosaccharide (LO) solution (14.6 mM), lactitol solution (14.6 mM) and lactose solution (14.6 mM) by rat jejunal mucosal homogenates. —■—, LO; —□—, lactitol; —○—, lactose.

Table 5. Effects of LO and lactitol feeding on body weight, food intake, and food efficiency.

|                  | Control  | LO       | Lacol    |
|------------------|----------|----------|----------|
| Initial weight (g) | 181 ± 19 | 185 ± 6  | 184 ± 10 |
| Final weight (g)  | 333 ± 40 | 341 ± 14 | 344 ± 36 |
| Weight gain (g)   | 152 ± 23 | 156 ± 14 | 160 ± 31 |
| Food intake (g)   | 439 ± 46 | 438 ± 28 | 439 ± 53 |
| Food efficiency   | 0.34 ± 0.02 | 0.36 ± 0.02 | 0.36 ± 0.03 |

Values are means ± SE (n = 6). LO, lactitol-oligosaccharides; Lacol, lactitol.

Changes of fecal organic acids

The results of the analysis of rat fecal organic acids are shown in Table 7. The concentration of fecal total organic acids increased significantly in the LO diets in the 1st and 2nd weeks, compared with the control and lactitol diets. The greatest increase was observed in acetic acid. An increase in butyric acid was also observed. However, after the 3rd week, the concentration of fecal organic acids in the LO diets decreased to the same level as that of the control and lactitol diets. Subsequently, to determine this cause, organic acids in the cecal contents were analyzed.

Changes of organic acids and pH in cecal contents

The results of the analysis of organic acids and pH in the rat cecal contents are shown in Table 8. Acetic acid, lactic acid, propionic acid, and total organic acids increased 2- to 3-folds in the LO diets, compared to that of control and lactitol diets. The cecal pH decreased significantly in the LO diets.
Table 6. The influence of LO and lactitol intake on the rat fecal microflora.

| Organisms          | Groups     | 0 week    | 1 week    | 2 week    | 3 week    |
|--------------------|------------|-----------|-----------|-----------|-----------|
| Enterobacteriaceae | Control    | 7.9±0.4   | 7.3±0.7   | 7.4±1.0   | 7.1±0.2   |
|                    | LO         | 7.9±0.4   | 8.2±0.3   | 8.0±0.7   | 7.3±1.0   |
|                    | Lacol      | 7.9±0.4   | 8.1±0.2   | 7.9±0.5   | 7.7±0.7   |
| Streptococcus      | Control    | 7.7±1.1   | 7.0±0.8   | 7.7±1.2   | 7.3±0.8   |
|                    | LO         | 7.7±1.1   | 6.4±1.1   | 7.1±1.2   | 7.3±2.4   |
|                    | Lacol      | 7.7±1.1   | 6.1±0.6   | 6.6±1.1   | 7.3±0.8   |
| Staphylococcus     | Control    | 4.5±1.6   | 3.6±1.4   | 2.6±0.2   | 3.4±0.6   |
|                    | LO         | 4.5±1.6   | 4.4±1.5   | 2.5       | 3.2±0.6   |
|                    | Lacol      | 4.5±1.6   | 2.9±0.6   | 2.6       | 3.6±1.2   |
| Lactobacillus      | Control    | 9.2±0.8   | 9.5±1.0   | 9.8±0.5   | 9.7±0.4   |
|                    | LO         | 9.2±0.8   | 9.2±0.3   | 9.8±0.3   | 9.6±0.5   |
|                    | Lacol      | 9.2±0.8   | 9.5±0.5   | 9.6±0.4   | 9.3±0.3   |
| Bifidobacterium    | Control    | 8.9±0.5   | 9.2±0.3   | 8.9±1.1   | 9.1±0.8   |
|                    | LO         | 8.9±0.5   | 9.7±0.2<sup>a,b</sup> | 9.9±0.1<sup>a,b</sup> | 9.7±0.4<sup>b</sup> |
|                    | Lacol      | 8.9±0.5   | 9.1±0.2   | 9.0±0.4   | 8.4±0.9   |
| Eubacterium        | Control    | 9.6±0.5   | 9.5±0.7   | 10.0±0.4  | 9.3±0.5   |
|                    | LO         | 9.6±0.5   | 9.9±0.5   | 10.1±0.4  | 9.8±0.3   |
|                    | Lacol      | 9.6±0.5   | 10.0±0.6  | 10.2±0.3  | 9.6±0.5   |
| Bacteroidaceae     | Control    | 9.3±0.5   | 9.2±0.5   | 9.3±0.6   | 8.5±0.7   |
|                    | LO         | 9.3±0.5   | 9.3±0.5   | 9.6±0.4   | 9.2±0.3<sup>a</sup> |
|                    | Lacol      | 9.3±0.5   | 9.3±0.5   | 9.5±0.5   | 9.3±0.3<sup>a</sup> |
| Peptococcaceae     | Control    | 8.7±1.0   | 8.5±1.0   | 8.8±0.8   | 8.7±1.2   |
|                    | LO         | 8.7±1.0   | 9.4±0.5<sup>a</sup> | 10.0±0.4<sup>a</sup> | 10.0±0.2<sup>a</sup> |
|                    | Lacol      | 8.7±1.0   | 9.5±0.5<sup>a</sup> | 9.6±0.3<sup>a</sup> | 9.3±0.4   |
| Clostridium perfringens | Control | 0        | 7.9±0.2   | 8.0±0.8   | 8.0±0.2   |
|                    | LO         | 0        | 1.0       | 1.0       | 0         |
|                    | Lacol      | 0        | 0         | 0         | 0         |
| Total bacteria     | Control    | 9.9±0.4   | 10.0±0.7  | 10.3±0.2  | 10.0±0.4  |
|                    | LO         | 9.9±0.4   | 10.3±0.3  | 10.6±0.2  | 10.4±0.3<sup>a,b</sup> |
|                    | Lacol      | 9.9±0.4   | 10.3±0.3  | 10.4±0.3  | 10.0±0.1  |

Values are mean±SE of log bacteria counts per gram of wet feces (n=6). LO, lactitol-oligosaccharides; Lacol, lactitol. *Significantly different from Control group (p<0.05). †Significantly different from Lactitol group (p<0.05).

**Fecal putrefactive products**

The results of the analysis of fecal putrefactive products are shown in Table 9. A significant decrease of fecal putrefactive products was observed on the administration of LO and lactitol.

**DISCUSSION**

For the administration of oligosaccharide to have growth promoting effects on bifidobacteria, the following two conditions must be fulfilled (24):
Table 7. The influence of LO and lactitol intake on fecal organic acids.

| Organic acids  | Groups    | 0 week | 1 week | 2 week | 3 week |
|----------------|-----------|--------|--------|--------|--------|
| Lactic acid    | Control   | 1.1±0.6| 1.2±0.5| 0.8±0.2| 0.9±0.4|
|                | LO        | 1.1±0.6| 1.0±0.8| 0.9±0.9| 0.4±0.3|
|                | Lacol     | 1.1±0.6| 0.7±0.5| 0.5±0.4| 0.6±0.6|
| Acetic acid    | Control   | 3.0±0.9| 3.6±0.5| 3.7±0.6| 3.5±0.9|
|                | LO        | 3.0±0.9| 4.4±0.5<sup>a,b</sup> | 4.6±0.5<sup>a</sup> | 3.3±1.1|
|                | Lacol     | 3.0±0.9| 2.3±0.9| 3.2±0.7<sup>a</sup> | 2.1±1.4|
| Propionic acid | Control   | 0.8±0.4| 0.9±0.2| 1.2±0.3| 0.7±0.3|
|                | LO        | 0.8±0.4| 1.1±0.3| 1.4±0.7| 1.1±0.5<sup>b</sup>|
|                | Lacol     | 0.8±0.4| 0.6±0.2| 1.1±0.3| 0.5±0.2|
| Butyric acid   | Control   | 1.1±0.6| 1.0±0.3| 1.1±0.5| 0.5±0.3|
|                | LO        | 1.1±0.6| 2.1±0.6<sup>a</sup> | 3.0±0.4<sup>a</sup> | 1.2±0.4<sup>a</sup>|
|                | Lacol     | 1.1±0.6| 2.1±0.6<sup>a</sup> | 2.9±1.7<sup>a</sup> | 1.3±0.7<sup>a</sup>|
| Total organic  | Control   | 6.0±0.9| 6.6±0.4| 6.8±0.7| 5.5±1.3|
| acids          | LO        | 6.0±0.9| 8.6±1.3<sup>a,b</sup> | 10.0±0.6<sup>a,b</sup> | 6.0±1.4|
|                | Lacol     | 6.0±0.9| 5.6±1.2| 7.6±2.1| 4.5±1.9|

Values are means±SE (mg/g wet feces (n=6)). LO, lactitol-oligosaccharides; Lacol, lactitol. *Significantly different from Control group (p<0.05). <sup>b</sup>Significantly different from lactitol group (p<0.05).

Table 8. The influence of LO and lactitol intake on the concentration of organic acids and pH in rat cecal content.

| Organic acids  | Control  | LO     | Lacol  |
|----------------|----------|--------|--------|
| Lactic acid    | 0.3±0.1  | 0.9±0.4<sup>a</sup> | 0.4±0.1|
| Acetic acid    | 5.3±0.2  | 12.1±4.6<sup>a,b</sup> | 4.4±1.4|
| Malic acid     | n.d.     | 0.7±0.6<sup>a</sup> | 0.4±0.2|
| Propionic acid | 1.9±0.8  | 4.2±1.4<sup>a,b</sup> | 2.1±0.5|
| Succinic acid  | n.d.     | 0.7±0.8<sup>a</sup> | 0.1±0.2|
| Butyric acid   | 0.6±0.5  | 2.4±1.4<sup>a,b</sup> | 1.0±0.3|
| Total          | 8.1±3.8  | 21.0±8.2<sup>a,b</sup> | 8.4±2.1|
| Cecal pH       | 7.5±0.1  | 6.8±0.1<sup>a,b</sup> | 7.2±0.2|

Values are means±SE (mg/cecal contents (n=6)). LO, lactitol-oligosaccharides; Lacol, lactitol. *Significantly different from Control group (p<0.05). <sup>b</sup>Significantly different from Lactitol group (p<0.05).

1) The oligosaccharide is not digested by endogenous enzymes. 2) The oligosaccharide is selectively utilized by bifidobacteria.

To test for the bifidobacteria growth-promoting effect of oligosaccharide, the above two conditions must be examined by experiments in vitro, and only then could the in vivo experiments be carried out.

In this study, a series of the above experiments were carried out to examine the bifidobacteria growth-promoting effect of lactitol-oligosaccharide (LO) which is
enzymatically synthesized from lactitol.

It is well known that lactitol is neither hydrolyzed nor absorbed in the small intestines of humans and animals (19, 20, 25). We studied the hydrolyzation of LO using rat jejunal mucosal homogenates. Neither LO nor lactitol were hydrolyzed in rat jejunal mucosal homogenates. Therefore, ingested LO can be expected to pass through the small intestine without being digested by endogenous enzyme and thus is fermented by the bacteria in the large intestine.

Subsequently, in vitro utilization tests showed that LO was utilized by Bifidobacterium spp. used to a degree comparable to glucose. Other human intestinal bacteria, including Lactobacillus, Peptococcus, Bacteroides, Clostridium, and E. coli, did not utilize LO. This was confirmed by the pH of the cultures. Culture media containing LO had a pH value of less than 4.5 after assimilation by Bifidobacterium spp. On the other hand, lactitol was utilized by bifidobacteria to a lesser extent than LO, and was also utilized by L. casei and Clostridium spp. Culture media containing lactitol had a pH in the range between 5.0 to 5.5 after assimilation by Bifidobacterium, except for Bifidobacterium adolescentis. LO was found to be selectively utilized by human intestinal bifidobacteria, compared with lactitol. Furthermore, LO diets resulted in selective increases of bifidobacteria in the rat fecal microflora.

From the above results, LO was found to promote the growth of bifidobacteria by the in vitro and in vivo test. Thus, LO can contribute to the maintenance of good bacterial conditions in digestive tracts, as bifidobacteria is considered beneficial to the equilibrium of the intestinal microflora (26, 27).

The LO, which is composed of galactose linked to lactitol, shows different utilization by bifidobacteria compared with that of lactitol.

Lactitol is known to form intermolecular hydrogen bonding between ring oxygen of the galactose and hydroxyl groups of the glucitol (28). As shown in Table 1, three out of six structures of LO (70% equivalent of total LO) has galactose linked to glucitol residue of lactitol. It can therefore be presumed that LO does not form intermolecular hydrogen bonding. In other words, compared with lactitol, LO has a less rigid structure. This difference in molecular structure may

### Table 9. The influence of LO and lactitol intake on rat fecal putrefactive products.

| Groups | 0 week | 1 week | 2 week | 3 week |
|--------|--------|--------|--------|--------|
| Indole |        |        |        |        |
| Control | 11.0 ± 3.7 | 10.6 ± 2.7 | 8.9 ± 3.8 | 10.1 ± 3.8 |
| LO     | 11.0 ± 3.7 | 2.7 ± 1.9* | 1.4 ± 1.1* | 4.4 ± 3.6* |
| Lacol  | 11.0 ± 3.7 | 2.5 ± 2.0* | 3.1 ± 2.2* | 4.4 ± 2.6* |
| p-Cresol |        |        |        |        |
| Control | 22.5 ± 8.0 | 24.3 ± 7.9 | 28.5 ± 9.0 | 24.4 ± 7.1 |
| LO     | 22.5 ± 8.0 | 0.7 ± 1.1* | 2.7 ± 2.8* | 8.7 ± 4.6* |
| Lacol  | 22.5 ± 8.0 | 0.5 ± 1.1* | 4.7 ± 4.1* | 7.4 ± 4.1* |

Values are means ± SE (μg/g wet feces (n = 6)). LO, lactitol-oligosaccharides; Lacol, lactitol. *Significantly different from Control group (p < 0.05).
result in different utilization by human intestinal bifidobacteria.

The concentration of fecal acetic acid and total organic acids increased with LO administration until the 2nd week, but acetic acid and total organic acids did not increase at 3rd week. LO is presumed to be rapidly fermented by intestinal bacteria including bifidobacteria, and a large amount of organic acid is rapidly absorbed from the rat gut (29). After completion of the experiments, the pH and the concentration of organic acids in rat cecal contents were measured. Organic acids in the LO diet group significantly increased, compared with the control and lactitol groups. The LO diet rats also exhibited a lower pH of cecal contents, (6.8 ± 0.2) compared to the control group (7.5 ± 0.1). This result indicated that greater acidity of the feces and cecal contents were consistent with increase in Bifidobacterium population.

It has been hypothesized that alkaline conditions in the colon and cecum increase the risk of colon cancer (30, 31). Acidification of colonic contents could, in turn, inhibit bile acid degradation (32). This hypothesis has been supported by the findings that lactulose lowered fecal pH and subsequently suppressed 1,2-dimethylhydrazine (DMH)-induced colon tumor in rats (33). LO may act in a similar manner to these findings in the colon. Furthermore, the fecal putrefactive products are suppressed in LO diets. This indicated that bacterial proteolysis (34) and subsequent deamination (35), respectively, were suppressed by LO diets.

In conclusion, LO is effective for improving intestinal conditions by increasing bifidobacteria, organic acids and decreasing putrefactive products.

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