Oral Intake of Slowly Digestible α-Glucan Such as Resistant Maltodextrin Leads to Increased Secretion of Glucagon-Like Peptide-2 in Rats and Helps Thicken Their Ileal Mucosae

Tomoya Goto¹, Tomoki Umeda², Shingo Hino³, Tatsuya Morita³ and Naomichi Nishimura¹,*

¹Department of Applied Life Sciences, Faculty of Agriculture, Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka 422–8529, Japan
²Graduate School of Integrated Science and Technology, Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka 422–8529, Japan
³College of Agriculture, Academic Institute, Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka 422–8529, Japan

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Summary To investigate whether the oral intake of slowly digestible α-glucan (SDG) could have a trophic (i.e., thickening) effect on their ileal mucosae, for 10 d, rats were given control (non-SDG), 10% isomaltodextrin (IMD) or 10% resistant maltodextrin (RMD) diets. In addition, experimental rat groups were further divided into two groups each and their diets either had or had not 1% sodium carboxymethylcellulose (CMC) added as a thickening agent. In the jejunum and the ilea, compared with control rats, the villus length and the mucosal thickness, but not the crypt depth, were significantly greater in the RMD-fed rats, with the trophic effect being weaker in the IMD-fed rats than in the RMD-fed rats. The colonic crypt depth was significantly greater in SDG groups than in the control group. The concentration of plasma glucagon-like peptide (GLP)-2 in the portal veins of the RMD group but not the IMD group was significantly higher than in the control group, with no effect of CMC supplementation on its concentration. The concentrations of cecal short-chain fatty acids did not significantly increase with SDG supplementation except for propionate concentration of the IMD-supplemented rats, compared with those in the control rats. We concluded that SDGs, especially RMD, thickened the mucosae of the rat distal small intestines. In particular, this effect of RMD but not IMD could have resulted from increased glucose available as a secretagogue of the trophic hormone GLP-2, in the ileum.

Key Words slowly digestible α-glucan, trophic effect, glucagon-like peptide-2, ileum, rats

The surface of the lumen in the small intestine is covered with the epithelial monolayer, which acts as a barrier blocking the invasion of foreign substances such as antigens and bacteria, whilst allowing the absorption of nutrients. The epithelial integrity in the small intestine must be maintained because the epithelial mucosa is the ultimate intestinal barrier. It has been shown that total parenteral nutrition, fasting and starvation provoke atrophy of the small intestinal mucosa in rodents (1–3). This evidence indicates that nutrients such as carbohydrates and fats are likely crucial factors for the maintenance of the small intestinal mucosa. However, easily digestible nutrients such as rapid digestible starch, fats and proteins are almost completely digested and absorbed in the proximal small intestine, i.e., the duodenum and jejunum, leaving little nutrients available to the distal small intestine, the ileum. This phenomenon likely results in the mucosa being thinner towards the distal end of the small intestine, causing a decreased barrier function in this intestinal region. Although the bacterial density in the small intestine is lower when compared with that in the large intestine, the total bacteria count is larger in the ileum, being approximately $10^8$–$10^9$ cfu/mL (4). Therefore, helping the ileal mucosa to thicken could become an important strategy to improve its barrier function in the ileum.

The factors considered to be important for the thickening of the intestinal mucosa are: 1) the supply of nutrients (5), 2) the physical stimulus (6) and 3) the stimulation of gut hormone production (5). Nutrients such as carbohydrates and amino acids greatly promote mucosal growth (7–9). High viscosity in the digesta due to the presence of viscous polysaccharides is also necessary to stimulate the thickness of the small intestinal mucosa (10). In addition, previous work showed that intravenous administration of glucagon-like peptide (GLP)-2, a gut hormone, induced a trophic effect on the distal small intestine and the colon (11). A diet meeting these criteria could possibly have a trophic effect on the ileal mucosa and thus contribute to the development of a healthy barrier function in the lower small intestine.

Slowly digestible/absorbable carbohydrates, or lente carbohydrates, are recommended in diets for diabetics...
because they help lower the glycemic response. The digestion and absorption of \textit{lente} carbohydrates are slow and they are extended towards the distal small intestine, possibly rendering these carbohydrates as vehicles of glucose for the distal small intestine. GLP-1 is a proglucagon-derived gut hormone that induces pancreatic secretion of insulin. The secretion of this hormone could increase by the delivery of glucose to the ileum, which is rich in L cells, because these cells produce and secrete GLP-1 when stimulated with glucose (12).

Our previous study using cecectomized rats given antibiotics demonstrated that the administration of \textit{lente} carbohydrates such as slowly digestible \(\alpha\)-glucans (SDGs) enhanced the secretion of GLP-1 in the distal small intestine (1.3). Since GLP-2 is another product of proglucagon-processing in L cells and co-secreted with GLP-1, SDGs such as resistant maltodextrin (RMD) and isomaltooligosaccharide (IMD) could act as secretagogues of both hormones in the distal small intestine. By carrying glucose to the ileum, which would increase GLP-2 secretion, \textit{lente} carbohydrates could indirectly exert a trophic effect on the distal small intestinal mucosa that would help maintain its integrity and prevent local and systemic inflammation.

In the present work, we studied the delivery of glucose in RMD and IMD, as well as non-fermentable, viscous fiber, carboxymethylcellulose, to the ileum, and estimated whether these SDGs exerted trophic effects on the distal small intestinal mucosa and the secretion of GLP-2.

**MATERIALS AND METHODS**

**Samples.** IMD (Fibryxa\(^{8}\)), which is produced from starch using enzymes \(\alpha\)-glucosyl transferase and \(\alpha\)-amylase derived from \textit{Paenibacillus alginolyticus} PP710, was supplied by Hayashibara Co., Ltd. (Okayama, Japan). IMD is a highly branched dextrin with \(\alpha\)-glycosidic linkages (3\% \(\alpha\)-1,3 linkages, 19\% \(\alpha\)-1,4 linkages, 49\% \(\alpha\)-1,6 linkages, 7\% \(\alpha\)-1,3,6 linkages and 5\% \(\alpha\)-1,4,6 linkages; average DP 30) (14) and has 15\% digestibility, as per the AOAC2001.03 method, and 80\% digestibility in rat small intestine (1.3). Resistant maltodextrin (RMD, Fibersol-2\(^{8}\)), which is prepared by hydrolysis and transglucosidation of starch at 140–160°C with a small amount of acid, was supplied by Matsutani Chemical Industry Co., Ltd. (Hyogo, Japan). RMD is also a branched dextrin with \(\alpha\)- and \(\beta\)-glycosidic linkages (6\% \(\alpha\)-1,3 linkages, 42\% \(\alpha\)-1,4 linkages, 9\% \(\alpha\)-1,6 linkages, 2\% \(\beta\)-1,3,4 linkages and 11\% \(\beta\)-1,4,6 linkages; average DP 10) (15) and has 8.5\% digestibility, as per the AOAC 2001.03 method, and 30\% digestibility in rat small intestine (16). Sodium carboxymethylcellulose (CMC, CMC Daicel\(^{8}\) #1190; 1,700 mPa·s [1% solution]) was supplied by Daicel Corp (Tokyo, Japan).

**Animals and diets.** Thirty-six 5-wk-old Male Sprague-Dawley rats (body weight range: 130–150 g) were purchased from Japan SLC, Inc. (Haruno colony; Shizuoka, Japan) and used for the experiments. Rats were housed in individual cages with screen bottoms made of stainless steel and kept in a room maintained at 23±2°C, with 50–70\% humidity, and under 12-h light (0700 to 1900) and 12-h darkness conditions. For all experiments, rats were first acclimatized to the experimental settings for 10 d and given a previously reported basal, 25\% casein diet (13, 17) (Table 1), and water ad libitum.

The present study was approved by the Shizuoka University Animal Use Committee (approval numbers: 2019A-16). Animals were kept and cared for as per the Guidelines for the Care and Use of Laboratory Animals, issued by Shizuoka University.

**Experimental design and sampling.** To determine if glucose released from IMD and RMD had trophic effects on the ileal mucosa, we examined the thickening of ileal mucosae in rats that were given IMD and RMD, and compared it with that of rats not given these SDGs (control). Following the acclimatization period and based on comparable body weight, rats were divided in 6 groups, as follows. Two groups (\(n=6\), each) were categorized as control (slowly digestible \(\alpha\)-glucan-free diet) and the diet of one group had 1\% CMC added as a thickening agent, whereas the other was given a CMC-free diet. Similarly, two rat groups (\(n=6\), each) were given 10\% IMD diets, but only one of these rat groups had 1\% CMC added to their diet. Lastly, two more rat groups were created and given 10\% RMD diets. Again, only one

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Basal</th>
<th>SDG</th>
<th>CMC</th>
<th>SDG+CMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein(^{2})</td>
<td>250</td>
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<tr>
<td>Maize starch(^{3})</td>
<td>482.5</td>
<td>382.5</td>
<td>472.5</td>
<td>372.5</td>
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<tr>
<td>Sucrose(^{4})</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Soybean oil(^{5})</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Mineral mix(^{6})</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Vitamin mix(^{6})</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Choline bitartrate(^{7})</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
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<tr>
<td>Cellulose(^{8})</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Slowly digestible (\alpha)-glucana</td>
<td>—</td>
<td>100</td>
<td>—</td>
<td>100</td>
</tr>
<tr>
<td>Carboxymethylcellulose(^{10})</td>
<td>—</td>
<td>10</td>
<td>—</td>
<td>10</td>
</tr>
</tbody>
</table>

1 CMC, carboxymethylcellulose; SDG, slowly digestible \(\alpha\)-glucan.
2 Acid casein was purchased from Meggle Japan Co. Ltd. (Tokyo, Japan).
3 Supplied by Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan).
4 Supplied by Nippon Beet Sugar Manufacturing Co. Ltd. (Obihiro, Japan).
5 Purchased from Ajinomoto Co. Inc. (Tokyo, Japan).
6 Mineral and vitamin mixtures were identical to AIN-93G-MX and AIN-93-VX, respectively. These mixtures were purchased from Oriental Yeast Co. Ltd. (Tokyo, Japan).
7 Purchased from FUJIFILM Wako Pure Chemical Corporation (Tokyo, Japan).
8 Purchased from Oriental Yeast Co. Ltd. (Tokyo, Japan).
9 Resistant maltodextrin and isomaltooligosaccharide were used as SDG.
10 Supplied by Daicel Corp (Tokyo, Japan).
Table 2. The length of the small intestine, and structural parameters of the jejunal, ileal and colonic mucosae of rats given control, 10% isomaltodextrin or 10% resistant maltodextrin diets, with or without 1% sodium carboxymethylcellulose added as a thickening agent.

<table>
<thead>
<tr>
<th></th>
<th>CMC (-)</th>
<th>CMC (+)</th>
<th>Factor SDG</th>
<th>Two-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>IMD</td>
<td>RMD</td>
<td>Control</td>
</tr>
<tr>
<td>Small intestine length, m</td>
<td>1.14±0.02</td>
<td>1.12±0.03</td>
<td>1.17±0.02</td>
<td>1.19±0.01</td>
</tr>
<tr>
<td>Jejunum</td>
<td>Villus height, µm</td>
<td>448±15</td>
<td>517±21</td>
<td>560±18</td>
</tr>
<tr>
<td></td>
<td>Crypt depth, µm</td>
<td>128±6</td>
<td>139±8</td>
<td>154±7</td>
</tr>
<tr>
<td></td>
<td>Villus height/crypt depth</td>
<td>3.52±0.16</td>
<td>3.76±0.11</td>
<td>3.67±0.20</td>
</tr>
<tr>
<td></td>
<td>Mucosal thickness, µm</td>
<td>576±18</td>
<td>656±28</td>
<td>714±20</td>
</tr>
<tr>
<td></td>
<td>Submucosa, µm</td>
<td>97.9±4.6</td>
<td>103±7</td>
<td>109±6</td>
</tr>
<tr>
<td>Ileum</td>
<td>Villus height, µm</td>
<td>336±12</td>
<td>369±17</td>
<td>389±17</td>
</tr>
<tr>
<td></td>
<td>Crypt depth, µm</td>
<td>88.7±4.2</td>
<td>96.5±6.0</td>
<td>101±4</td>
</tr>
<tr>
<td></td>
<td>Villus height/crypt depth</td>
<td>3.81±0.15</td>
<td>3.84±0.10</td>
<td>3.89±0.18</td>
</tr>
<tr>
<td></td>
<td>Mucosal thickness, µm</td>
<td>425±14</td>
<td>466±22</td>
<td>490±19</td>
</tr>
<tr>
<td></td>
<td>Submucosa, µm</td>
<td>77.7±5.5</td>
<td>89.0±5.1</td>
<td>85.0±5.6</td>
</tr>
<tr>
<td>Colon</td>
<td>Crypt depth, µm</td>
<td>158±4</td>
<td>168±7</td>
<td>167±3</td>
</tr>
<tr>
<td></td>
<td>Submucosa, µm</td>
<td>24.4±0.7a</td>
<td>22.2±0.8abc</td>
<td>20.2±0.6bc</td>
</tr>
</tbody>
</table>

Values are means ± SE, n=6, except for factor SDG (n=12). A two-way ANOVA was used to assess the effects of SDG and viscosity, and the interactions between SDG and viscosity. When the interaction was significant, further multiple comparisons were performed with the Tukey-Kramer post hoc test. When the effect of SDG was significant and the interaction was not significant, the data were arranged by the factor SDG and analyzed by Tukey-Kramer post hoc test. Values in a row not sharing a common superscript letters indicate significance (p<0.05). The symbol (−) indicated that no CMC was added as a thickening agent to the diets. The symbol (+) indicated that 1% CMC was added as a thickening agent to the diets. CMC, sodium carboxymethylcellulose; IMD, isomaltodextrin; RMD, resistant maltodextrin; SDG, slowly digestible α-glucan.
of these groups had 1% CMC added as the thickening agent. All rats were given the diets for 10 d. Treatment diets were formulated by replacing 100 and 10 g of maize starch per kg of the basal diet with equal amounts of SDGs (IMD or RMD) and CMC, respectively.

At the end of the experimental period and to laparatomy, all rats were deprived of food for 3.5 h and anesthetized via inhalation of 2% isoflurane. Under anesthesia, the abdomens of rats were longitudinally incised, and the portal veins were localized. One milliliter of blood was quickly collected from these veins into microtubes containing heparin (10 units/mL; Ajinomoto, Inc., Japan), aprotinin (500 kallikrein inhibitor units/mL; FUJIFILM Wako Pure Chemical Corporation) and DPP-IV inhibitor (50 μmol/mL, Millipore). Plasma was separated from blood by centrifugation and frozen at −80˚C until further analysis. It was also visually and palpably confirmed that the stomachs and small intestines of all rats were full of digesta.

After blood collection, rats were euthanized by exsanguination. The small intestines (i.e., jejunum and ileum), the ceca and the colons were removed from the rat carcasses. The small intestine was removed at the ligament of Treitz and the ileocecal valve. The total length of the small intestine was determined by vertical suspension without an attached weight. Rat intestines were sampled as follows. First, 10 cm were measured from the ligament of Treitz to the jejunum, where 2-cm segments were then excised as jejunal samples. Similarly, 70 cm were measured from the ligament of Treitz, past the jejunum and to the ileum, where 2-cm segments were then excised as ileal samples. Finally, large intestines were sampled by measuring 2 cm from the ileo-cecal valve to the colon where 1-cm segments were excised. A representative illustration of the intestinal sampling procedure can be found in Fig. S1 (Supplemental Online Material).

The intestinal segments were cut longitudinally and washed out with cold saline to remove the digesta. Cut segments were fixed with 4% paraformaldehyde in 0.1 mol/L of phosphate buffer at pH 7.4.

Evaluation of mucosal thickness in the gut. Fixed tissues were dehydrated first with ethanol then with xylene, and embedded in paraffin. Four 4-μm sections were sliced at 200-μm intervals from each embedded sample and stained with hematoxylin-eosin. Stained samples were observed using an Olympus BX41 microscope (Tokyo, Japan). The length of villi and the depth of crypts in the jejunal, ileal and colonic sections of all rats were measured. Five microscopic fields per section were randomly selected, and one villus and one crypt with full length per field were evaluated by two blind observers.

Plasma analysis. The concentration of plasma GLP-2 was determined using commercial kits (Rat GLP-2 EIA kit, Yanaihara Institute Inc., Shizuoka, Japan).

Determination of cecal organic acid. Cecal organic acids (acetate, propionate, n-butyrate and succinate) were measured using an HPLC system (LC-10A, Shimadzu, Kyoto, Japan) equipped with a Shim-pack SCR-102H column (8 mm i.d.×30 cm; Shimadzu) and an electroconductivity detector, as previously reported (18).

Statistical analysis. To determine the adequate sam-
With respect to the concentrations of organic acids, acetate significantly lowered in SDG-supplemented rats, whereas the increase in the ilea did not reach statistical significance. In contrast, both the height of villi and the thickness of the mucosae, but not the depth of crypts, were significantly greater in the jejuna and had no CMC added to their diets (Table 2). The height of villi and the thickness of the mucosae between the ileal samples of rat groups (Table 2; Fig. 1). Likewise, no significant differences were observed in the thickness of the small intestinal submucosae between the ileal samples of rats. However, the depth of crypts was greater in the colonic samples of RMD- and IMD-supplemented rats than in those of rats not given CMC, although no differences in these structural parameters were observed between the jejunal samples of rat groups (Table 2; Fig. 1). Likewise, no significant differences were observed in the thickness of the small intestinal submucosae between the ileal samples of rats. However, the depth of crypts was greater in the colonic samples of RMD- and IMD-supplemented rats than in those of rats not given SDGs, independently of whether CMC was added to their diets or not.

### RESULTS

Whilst food intake of RMD-fed rats was significantly lower, neither body weight gains nor food efficiency significantly differed between experimental groups (Supplemental Online Material, Table S1). Soft feces were observed up to 3 d after the start of the experiment in rats given RMD, but not in IMD. The small intestines of CMC-supplemented rats were significantly \( p=0.0011 \) longer than those of rats who had no CMC added to their diets (Table 2). The height of villi and the thickness of the mucosae, but not the depth of crypts, were significantly greater in the jejuna and the ilea of RMD-supplemented groups when compared with rats not given SDGs, and these parameters of IMD supplemented groups showed a significant increase in the ilea, whereas the increase in the ilea did not reach statistical significance. In contrast, both the height of villi and the depth of crypts were greater in the ileal samples of CMC-supplemented rats than in those of rats not given CMC, although no differences in these structural parameters were observed between the jejunal samples of rat groups (Table 2; Fig. 1). Likewise, no significant differences were observed in the thickness of the small intestinal submucosae between the ileal samples of rats. However, the depth of crypts was greater in the colonic samples of RMD- and IMD-supplemented rats than in those of rats not given SDGs (Table 2).

The weight of cecal tissues of rats having SDGs or CMC was greater than those of unsupplemented rats, but only those being supplemented with SDGs had a greater weight of their cecal contents (Table 3). Cecal pH was significantly lower in samples of SDG-supplemented rats than in rats not given SDGs, independently of whether CMC was added to their diets or not.

### Table 3: Weight of cecal tissue and cecal content, and the concentrations of cecal organic acids of rats given control, 10% isomaltooltriose or 10% resistant maltodextrin diets, with or without 1% sodium carboxymethylcellulose added as a thickening agent.

<table>
<thead>
<tr>
<th>Factor SDG</th>
<th>IMD</th>
<th>RMD</th>
<th>CMC (+)</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cecum Tissue G</td>
<td>0.68±0.03</td>
<td>0.89±0.05</td>
<td>0.89±0.05</td>
<td>0.89±0.05</td>
</tr>
<tr>
<td>Cecal content G</td>
<td>0.43±0.03</td>
<td>0.67±0.03</td>
<td>0.67±0.03</td>
<td>0.67±0.03</td>
</tr>
<tr>
<td>Organic acid</td>
<td>2.5±31.5</td>
<td>3.2±75.1</td>
<td>3.2±75.1</td>
<td>3.2±75.1</td>
</tr>
<tr>
<td>Propionate, mmol/g</td>
<td>3.1±0.3</td>
<td>3.8±0.3</td>
<td>3.8±0.3</td>
<td>3.8±0.3</td>
</tr>
<tr>
<td>n-Butyrate, mmol/g</td>
<td>3.4±0.4</td>
<td>3.4±0.4</td>
<td>3.4±0.4</td>
<td>3.4±0.4</td>
</tr>
<tr>
<td>Succinate, mmol/g</td>
<td>4.3±0.3</td>
<td>4.3±0.3</td>
<td>4.3±0.3</td>
<td>4.3±0.3</td>
</tr>
</tbody>
</table>

Values are means±SE; 6 except for factor SDG (n=12). A two-way ANOVA was used to assess the effects of SDG and viscosity, and the interactions between SDG and viscosity. When the interaction was significant, further multiple comparisons were performed with the Steel-Dwass test. If sample variances were still unequal after log-transformation, the Steel-Dwass test was used instead. Apart from the power analysis, all statistical analyses were carried out using SAS JMP software (version 13.2.1; Tokyo, Japan). Values obtained from the experiments were the means±standard errors, and the means were statistically significant when \( p<0.05 \).
when compared with those not being supplemented with SDGs. Propionate concentration of the IMD and RMD groups was significantly higher and lower than in the control group, respectively. In addition, only in rats given CMC, the concentration of n-butyrate was significantly lower in IMD-supplemented groups than in the control groups. By contrast, compared with that of the control groups, the concentration of succinate was significantly higher only in rats supplemented with IMD when the diets were CMC-free. However, when CMC was added to diets, the concentration of succinate increased in both IMD- and RMD-supplemented rats (Table 3). Finally, the concentration of portal GLP-2 was significantly higher in the RMD-supplemented group than in the control and IMD groups, with CMC having no effect on the concentration of this hormone (Fig. 2).

DISCUSSION

Theoretically, dietary carbohydrates that could be digested and absorbed more slowly such as SDGs, would help extend the digestion and absorption areas towards the distal small intestine (19). Such phenomenon could also help deliver glucose to the lower part of the small intestine, which would likely provide the L-cell-rich ileal mucosa with energy and help promote the secretion of gut hormones such as GLP-1 (20). A previous study at these premises demonstrated that supplementation with IMD increased the glucose supply to the distal small intestine in antibiotic-administered, cecectomized rats, which resulted in an increased concentration of GLP-1 in their portal veins (13). Elsewhere, RMD was also previously reported to promote the secretion of GLP-1 in entire, non-antibiotic-administered rats (21).

In the present study, it was expected to find significantly higher concentrations of portal GLP-2 in SDG-fed rats because trophic hormone GLP-2 is produced from proglucagon in and secreted from the intestinal L cells along with GLP-1 (22). Also expected was SDG supplementation exerting trophic effects on the ileal mucosa. Therefore, it is reasonable to theorize that the delivery of glucose in RMD to the distal small intestine would have well promoted the secretion of GLP-2, which then stimulated the thickening of the ileal mucosa.

Contrary of our expectations, the ability of IMD to thicken the distal small intestinal mucosa was weaker than that of RMD, with an increased portal GLP-2 concentration not being observed in the IMD-supplemented group. The difference between IMD and RMD effects is perhaps due to their differential digestibilities in the small intestine (RMD, 30% (16); IMD, 80% (13)). Differential digestibilities could affect the amount of glucose delivered to the distal small intestine, in turn resulting in differential ileal GLP-2 secretions and trophic effects. Therefore, SDGs such as RMD, which could release relatively more glucose in the distal small intestine, would stimulate the secretion of GLP-2 from the L-cells, contributing to the thickening of mucosa. Because according to Said and Kaunitz (23), GLP-1 also have trophic effect on the intestinal epithelium, GLP-1 may be partially involved in the effect of SDGs.

In the large intestine, the production of short-chain fatty acids (SCFAs) is enhanced by digesta rich in nondigestible saccharides (24). The large intestine is also densely populated with L-cells (12), which secrete GLP-1 (25, 26) and GLP-2 (27) when SCFAs are readily available. Sakata reported that colonic butyrate exerted an indirect trophic effect on the ileal mucosa of rats (28), which may have increased the secretion of GLP-2. In addition, SCFA supplementation, in particular butyrate, to the rat colon, increases the DNA content and the weight of the jejunal and ileal mucosae (29). These findings seem to indicate that colonic SCFAs promote the secretion of GLP-2, which results in trophic effects on the jejunum and the ileum. In previous work, similar morphological changes were observed in rats given basal enteral nutrients supplemented with 1.4% RMD (30). Hashizume and Okuma hypothesized that the observed changes were a consequence of SCFAs being produced from the colonic fermentation of RMD, because the evidence available at the time suggested that RMD was barely digested in the small intestine and was
mostly fermented to SCFAs in the large intestine (30). It is now known that RMD and IMD are partially digested in the small intestine and afterwards their residues reach the large intestine, where they are utilized as substrates for fermentation (31, 32). In the present study, although the oral intake of RMD helped increase the concentration of portal GLP-2 and thicken the jejunal and ileal mucosae, the concentrations of cecal SCFAs did not increase in the RMD-supplemented group, which agreed with previous studies (16, 21, 32). Nonetheless, we were able to confirm that a high fermentability of RMD and IMD in the large intestine, resulting in an increased concentration of cecal succinate (itself, an intermediate product of propionate fermentation), was detected in rats supplemented with these saccharides. Therefore, it was concluded that SCFAs derived from the colonic fermentation would not have been responsible for the increased concentration of portal GLP-2 and that instead, the thickening of the jejunal and ileal mucosae resulted from the delivery of glucose in SDGs to the ileum.

As viscosity is a function of shear stress and shear rate, highly viscous fluids can cause physical stimulation of the gastrointestinal epithelium when the digesta moves down aided by the peristaltic mechanism. The aforementioned stimulation likely promotes the growth of the intestinal epithelia by gut hormones such as GLP-2 highly secreted (6). Unlike non-fermentable and highly viscous carbohydrates, those that are fermentable and highly viscous are reported to elevate plasma enteroglucagon (10). In the present study, no increase in GLP-2 secretion was observed upon addition of CMC, a non-fermentable and highly viscous polysaccharide, to the diets. Therefore, the physical stress caused to the intestinal epithelia by viscosity alone would not have promoted the secretion of GLP-2. However, the thickness of the mucosae increased in the jejunum and ileum of CMC-supplemented rats, suggesting that the physical stress induced this mucosal thickening of mucosae in the small intestines via stimulation by factors other than GLP-2. Although the viscosity of the small intestinal contents was not measured in this study, it should be added that the viscosity of the small intestinal contents may not have increased enough to affect the GLP-2 concentration.

There are certain anatomical and physiological differences between the gastrointestinal tracts of rats and humans. These differences can affect the digestibility and absorbability of nutrients. Although the transit time of digesta through the small intestine is similar in rats and humans, the surface area along the length of the small intestine is smaller in rats than in humans (33). Therefore, it is likely that SDGs are more rapidly digested in and absorbed from the upper small intestine in humans than in rats. Moreover, although enteral nutrition is considered to be more beneficial to the mucosal integrity than total parenteral nutrition, unlike rodents, studies on humans failed to show an atrophy of the small intestinal mucosal with total parenteral nutrition (34), even when enteral feeding was denied for 21 d (35). The present work seemed to indicate that SDGs could reinforce the integrity of the distal small intestinal mucosa. However, it is recommended that future studies be conducted to clarify if the results of the present study can be extrapolated to humans.

In conclusion, in the present study it was shown that SDGs, especially RMD, thickened the mucosa of the rat distal small intestine. This phenomenon could be attributed to an increased availability of glucose as a secretagogue of trophic hormone GLP-2 in the ileum. These results could be proven evidence that SDGs such as RMD are crucial nutrients to reinforce the barrier function of the distal small intestine. For example, for patients subject to enteral feeding, fortification of their nutrition with SDGs could result in an increased amount of glucose available for their ilea, improving their barrier functions by thickening their mucosae.

**Authorship**

NN designed the research; TG and TU conducted the research; TG, TU, SH, TM and NN analyzed the data; NN wrote the manuscript and was the primary responsible for the final content. All authors were involved in designing the study, reviewing and interpreting the results, and drafting the manuscript. In addition, all authors read and approved the final document. This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

**Disclosure of state of COI**

The authors have no conflict of interest associated with the present study.

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**Supporting information**

Supplemental online material is available on J-STAGE.

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