



# HOKKAIDO UNIVERSITY

Title	Ingestion of difructose anhydride III, a non-digestible disaccharide, prevents gastrectomy-induced iron malabsorption and anemia in rats
Author(s)	Shiga, Kazuki; Nishimukai, Megumi; Tomita, Fusao et al.
Citation	Nutrition, 22(7-8), 786-793 <a href="https://doi.org/10.1016/j.nut.2005.12.013">https://doi.org/10.1016/j.nut.2005.12.013</a>
Issue Date	2006-07
Doc URL	<a href="https://hdl.handle.net/2115/14569">https://hdl.handle.net/2115/14569</a>
Type	journal article
File Information	NUTRITION.pdf



**Title**

Ingestion of Difructose Anhydride III, a Nondigestible Disaccharide, Prevents Gastrectomy-Induced Iron Malabsorption and Anemia in Rats

**Authors**

Kazuki Shiga, PhD, Megumi Nishimukai, PhD, Fusao Tomita, PhD, Hiroshi Hara, PhD

**Institution**

From the Northern Advancement Center for Scientific and Technology, and the Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, and the Department of Nutrition, School of Nursing and Nutrition, Tenshi College, Sapporo, Japan

**Corresponding Author**

Hiroshi Hara

Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University,

Kita-9, Nishi-9, Kita-ku, Sapporo 060-8589, Japan

Tel: +81-11-706-3352 Fax: +81-11-706-2504

E-mail:hara@chem.agr.hokudai.ac.jp

**Running title**

Difructose Anhydride III and Postgastrectomy Anemia

**ABSTRACT**

**OBJECTIVE:** Total gastrectomy produces iron malabsorption and anemia, and several nondigestible carbohydrates promote mineral absorption. In this study, we examined the effects of feeding difructose anhydride III (DFAIII), a nondigestible disaccharide, on gastrectomy-induced iron malabsorption and anemia in rats in comparison with those of feeding fructooligosaccharides (FOS).

**METHODS:** Sham-operated and totally gastrectomized male Sprague-Dawley rats were fed the control, DFAIII (30 g/kg), or FOS (30 g/kg) diet for 4 wk. Feces and tail blood were collected at 2 and 4 wk to evaluate body iron status and iron absorption.

**RESULTS:** Gastrectomy severely reduced net iron absorption, hemoglobin concentration, and hematocrit in the control dietary group. The reduced absorption in gastrectomized rats was restored to the sham control level by feeding the DFAIII or FOS diets. Iron absorption in sham rats was higher in the FOS and DFAIII groups than in the control group. Hemoglobin concentration and hematocrit in gastrectomized rats fed the DFAIII diet, but not the FOS diet, returned to levels comparable to those in the sham rats fed the control diet. Feeding DFAIII increased short-chain fatty acids pools and decreased the pH in cecal contents. These parameters for cecal fermentation correlated with iron absorption.

**CONCLUSIONS:** DFAIII feeding restores gastrectomy-induced iron malabsorption, resulting in the complete prevention of iron-deficient anemia in rats. Cecal fermentation of DFAIII may contribute to the improvement in these gastrectomy-induced defects. Feeding with low level of FOS did not fully improve postgastrectomy anemia.

**KEY WORDS:** difructose anhydride III, gastrectomy, iron absorption, anemia

## INTRODUCTION

Anemia is a common complication in patients after gastric resection.<sup>1,2</sup> It has been reported that iron<sup>3</sup> and/or vitamin B-12<sup>4</sup> deficiencies after gastrectomy contribute to this anemia in humans. In the early stages after gastrectomy, iron-deficient anemia occurs<sup>2</sup> because gastric acid plays an important role in the small intestinal iron absorption through solubilization of dietary insoluble iron salts.<sup>5</sup> Recently, total gastrectomy has been shown to induce iron malabsorption and anemia also in rats,<sup>6,7</sup> indicating that iron deficiency is a leading factor in gastrectomy-induced anemia.

Nondigestible carbohydrates such as dietary fibers, oligosaccharides and resistant starch have various physiologic functions,<sup>8,9</sup> and the promotive effects of several nondigestible carbohydrates on mineral absorption have been well examined.<sup>10-14</sup> We previously reported that difructose anhydride III (DFAIII), a nondigestible disaccharide, enhances intestinal calcium absorption in both in vivo<sup>15-18</sup> and in vitro<sup>19-21</sup> studies. The mechanisms for the promotion of calcium absorption are 1) enhancement of paracellular calcium transport stimulated by intact DFAIII and 2) promotion of calcium absorption in the large intestine through the increase in fermentation of DFAIII by intestinal bacteria; DFAIII is a unique disaccharide that promotes calcium absorption through two mechanisms both in the small and large intestines. In in vivo balance studies, the promotive effect of DFAIII on calcium absorption was higher than that of other saccharides such as fructooligosaccharides (FOS) and raffinose.<sup>15</sup> In contrast with calcium absorption, there has been only a single report showing the effect of DFAIII on iron absorption and that report also showed that the promotive effect of DFAIII is greater than that of FOS.<sup>22</sup> The mechanisms for the promotion of iron absorption by DFAIII have not yet been clarified. Sakai et al.<sup>23</sup> have reported that FOS improves gastrectomy-induced anemia, and suggested involvement in the intestinal fermentation of FOS.

The aim of this study was to examine the effects of feeding DFAIII on gastrectomy-induced iron malabsorption and anemia in rats in comparison with those of feeding FOS. We also evaluated the role of intestinal fermentation in the DFAIII-mediated effects by measuring cecal organic acids pools and pH because the cecum substantially contributes to large intestinal fermentation in rats.<sup>13</sup>

## **MATERIALS AND METHODS**

### ***Test material***

Difuctose anhydride III (DFAIII; Nippon Beet Sugar Manufacturing, Tokyo, Japan) is a nondigestible disaccharide consisting of two fructose residues with two glycoside bonds (Figure 1), and is prepared from inulin with *Arthrobacter* sp. H65-7 inulin fructotransferase (Inulinase II; EC 2.4.1.93).<sup>24</sup> Recently, a procedure was established for the mass production of DFAIII.<sup>25</sup> Fructooligosaccharides (FOS) is a mixture of 42% 1-kestose, 46% nystose and 9% 1F--fructofuranosylnystose (Meiologo-P<sup>®</sup>, Meiji Seika Kaisha, Tokyo, Japan).<sup>26</sup>

### ***Animals and diets***

Male Sprague-Dawley rats (4 wk old; Clea Japan, Tokyo, Japan) were housed in individual stainless steel cages (17.5 cm × 25 cm × 17 cm) in a room with controlled temperature (22 ± 2°C), relative humidity (40-60%) and a 12-h light-and-dark cycle (light 0800-2000 h). Rats were fed the stock diet<sup>27</sup> shown in TABLE I for an acclimation period of 4-5 d, and were then divided into two groups using a randomized block design based on body weight. After food deprivation for 24 h, the rats in one group were subjected to total gastrectomy;<sup>28, 29</sup> the stomach was removed after ligation of several vessels that supplied blood to the stomach, and an end-to-side anastomosis was carried out between the cut edge of the esophagus and the upper jejunum 8 cm distal from the ligament of Treitz (Gastrectomized group). The rats in the other group were subjected to laparotomy (Sham-operated group). Both operations were performed under the same anesthetic procedure (Nembutal: sodium pentobarbital, 40 mg/kg

body weight, Abbott Laboratories, North Chicago, IL). All rats were deprived of food and water for 24 h after the operations, and were then fed cow's milk for 2-3 d.

The rats in each operation group (Sham-operated group,  $n = 18$ ; Gastrectomized group,  $n = 32$ ) were divided into three subgroups on the basis of body weight, hemoglobin concentration and hematocrit after the postoperative recovery period. The rats in the first (Sham-operated group,  $n = 6$ ; Gastrectomized group,  $n = 11$ ) and second (Sham-operated group,  $n = 6$ ; Gastrectomized group,  $n = 11$ ) subgroups were fed the test diet containing fructooligosaccharides (FOS diet, 30 g FOS/kg diet) and difructose anhydride III (DFAIII diet, 30 g DFAIII/kg diet), respectively (TABLE I). The rats in the last subgroup (Sham-operated group,  $n = 6$ ; Gastrectomized group,  $n = 10$ ) were fed the test diet without FOS or DFAIII (Control diet). The three test diets were prepared according to the AIN-93G formulation.<sup>27</sup> All rats were fed the assigned test diets for 28 d starting immediately after the recovery period. On d 1 and 2 of the test feeding period, the rats were given 5 and 10 g of diet/d, respectively; thereafter, they were given 15 g of diet/d for the remaining 26 d. We observed previously that totally gastrectomized rats could not consume > 15 g of diet/d (unpublished data). Therefore, all rats including sham rats in this study were given 15 g of diet/d to adjust iron intake among all groups. Rats were allowed free access to deionized water throughout the test period. Vitamin B-12 (0.5 mg/kg body weight, Wako Pure Chemical Industries, Tokyo, Japan) was supplied subcutaneously every 2 wk to prevent pernicious anemia. Body weight and food intake were measured every day. Food intake was corrected with spilled portions of diet carefully collected and weighed. Tail blood was collected before and 14 and 28 d after consumption of test diets to measure the hemoglobin concentration and hematocrit. Feces were collected for 5 consecutive days from d 9 (1st period) and from d 23 (2nd period) of the feeding period and were freeze-dried to evaluate net absorption of iron. It has been reported that coprophagy does not affect iron absorption from a ferric compound in rats.<sup>30</sup> We also

sampled the feces via a stainless wire mesh set under the cages to minimize coprophagy. On the last day of the experiment, all rats were killed under pentobarbital anesthesia (Nembutal: sodium pentobarbital, 50 mg/kg body weight, Abbott Laboratories). The cecum was removed together with its contents, and the contents were collected, weighed, frozen immediately with liquid nitrogen and stored at  $-40^{\circ}\text{C}$  until subsequent analyses. The cecal wall was washed with saline, and weighed. The weight of the cecal contents was evaluated as the difference in weight between the cecum with and without contents.

This study was approved by the Hokkaido University Animal Committee, and animals were maintained in accordance with the guidelines for the care and use of laboratory animals of Hokkaido University.

### ***Analytical methods***

Freeze-dried feces were ground to a fine powder, and the powdered feces (~1.5 g) were ashed at temperatures elevated linearly to  $550^{\circ}\text{C}$  for 6 h, and then at  $550^{\circ}\text{C}$  for 18 h with an electric furnace (EYELA TMF-3200, Tokyo Rikakikai, Tokyo, Japan). The ashed samples were treated with 5.49 mol/L HCl at  $200^{\circ}\text{C}$  for 30 min and dissolved in 0.82 mol/L HCl. Iron concentrations in the ashed solutions were measured by atomic absorption spectrometry (Shimadzu AA-6400F, Shimadzu Seisakusyo, Kyoto, Japan) after suitable dilution. The amount of iron in the test diets was determined in the same manner. We performed recovery tests to confirm the accuracy of the above-mentioned method, and the recovery of iron was  $105 \pm 5.1\%$  ( $n = 5$ , CV = 5.7%).

Hemoglobin concentration was evaluated using a commercial assay kit (Hemoglobin B-test, Wako Pure Chemical Industries, Osaka, Japan). Hematocrits were determined after centrifugation ( $15,000 \times g$ ) of blood (Centrifuge Hematocrit MC-201, Hitachi, Tokyo, Japan). Serum iron concentrations and unsaturated iron binding capacities (UIBC) were determined by assay kits (Fe C-test and UIBC-test, Wako Pure Chemical Industries). The

total iron-binding capacities (TIBC) and transferrin saturations (Tf) were calculated from the values of serum iron and UIBC.

The cecal contents were diluted with 4 volumes of deionized water and homogenized by a Teflon homogenizer. The pH of these homogenates was measured with a semiconducting electrode (ISFET pH sensor 0010-15C, HORIBA, Kyoto, Japan) as the pH of cecal contents. The amount of total iron in the homogenates was determined by the above-mentioned atomic absorption spectrometry after dry-ashing with an electric furnace. Soluble iron in the supernatant obtained upon centrifugation ( $30,000 \times g$  for 20 min at  $4^{\circ}\text{C}$ ) of the homogenate was determined by atomic absorption spectrometry after deproteinizing with 0.5 mol/L perchloric acid. Concentrations of organic acids (acetic, propionic, butyric, succinic and lactic acids) in the homogenate of cecal contents were measured after sample preparation by the procedure described previously<sup>14, 31</sup> using a HPLC (LC-10ADvp, Shimadzu Seisakusyo, Kyoto, Japan) equipped with two Shim-pack SCR-102H columns (8 mm i.d.  $\times$  30 cm long, Shimadzu Seisakusyo) and an electroconductibility detector (CDD-6A, Shimadzu Seisakusyo).

#### ***Calculations and statistical analyses***

Net absorption of iron was calculated by the following formula: Net Fe absorption (%) =  $100 \times (\text{total Fe intake} - \text{fecal Fe excretion}) / \text{Fe intake}$ .

Data were analyzed by two- or three-way ANOVA for the two or three factors (operation, diet and time) and their interactions. Duncan's multiple range test<sup>32</sup> was used to determine whether mean values were significantly different between groups ( $P < 0.05$ ). Correlation coefficients for the relationships between net iron absorption and several cecal parameters were calculated by the least squares method.<sup>33</sup> These statistical analyses were done using the General Linear Models procedure of SAS (SAS Version 6.07, SAS Institute Inc., Cary, NC).

## RESULTS

Initial body weight and body weight gain were lower in gastrectomized rats than in sham rats (TABLE II). Sham rats completely consumed each given diet (15 g/d); however, gastrectomized rats did not. Food intake in the control and FOS groups was slightly but significantly lower in gastrectomized rats than in sham rats, but not in the DFAlII group.

Net iron absorption was much lower in the control group of gastrectomized rats than in the same dietary group of sham rats in the 1st (Figure 2A) and 2nd (Figure 2B) periods. In gastrectomized rats, the iron absorption was higher in the FOS and DFAlII groups than in the control group, and the iron absorption in the FOS and DFAlII groups was similar to that in the control group of sham rats both in the 1st and 2nd periods. In sham rats, the absorption in the DFAlII group in the 1st period and in the FOS and DFAlII groups in the 2nd period was higher than that in the control group, respectively.

Hemoglobin concentrations and hematocrits at 2 and 4 wk after the start of the feeding period were lower in the control group of gastrectomized rats than in the other five groups (Figure 3A, 3B). Hemoglobin concentrations of gastrectomized rats at 2 wk in the FOS and DFAlII groups were similarly higher than those in the control group, but lower than those in the three sham groups. At 4 wk, the concentration of the DFAlII group, but not of the FOS group, tended to be increased and comparable to the values of the three sham groups. The hematocrit value at 4 wk in the DFAlII group of gastrectomized rats was very similar to that in the control group of sham rats. Hematocrit of the FOS group was still lower than the values for the sham rats and also the value for the DFAlII group of gastrectomized rats. Serum iron concentrations in the control diet groups were much lower in gastrectomized rats than in sham rats (TABLE III). In gastrectomized rats, the serum iron concentrations were higher in the FOS and DFAlII groups than in the control group; however, there were no differences among the two oligosaccharide groups. Tf was lower and UIBC was higher in gastrectomized rats

than in sham rats (data not shown). In gastrectomized rats, the Tf was higher and UIBC was lower in the FOS and DFAlII groups than in the control group.

Relative weights (g/100 g body wt) of cecal contents and cecal wall of sham rats were similarly higher in the FOS and DFAlII groups than in the control group (TABLE IV); however, the contents weight in gastrectomized rats was higher in the DFAlII group than in the FOS group. The pH of cecal contents in the FOS and DFAlII groups was lower than that in the control group both in sham and gastrectomized rats. Total and soluble iron pools in the cecal contents were similarly higher in the FOS and DFAlII groups than in the control group both in sham and gastrectomized rats (data not shown). Pools of acetic, propionic, butyric, and total short-chain fatty acids (SCFA) (the sum of acetic, propionic, and butyric acids) in cecal contents were higher in the DFAlII group than in the control group both in sham and gastrectomized rats except for butyric acid in sham rats (Figure 4). The propionic acid pool in sham rats and the butyric and total SCFA pools in gastrectomized rats were higher in the FOS group than in the control group.

## **DISCUSSION**

We found that total gastrectomy induces iron malabsorption (Figure 2) and anemia (Figure 3), and that the feeding of DFAlII, a nondigestible disaccharide, prevents these gastrectomy-induced defects in rats. We previously showed that ingestion of DFAlII ameliorates tannic acid-induced iron malabsorption, resulting in an improvement in iron-deficient anemia.<sup>22</sup> Feeding of DFAlII largely increased iron absorption also in sham rats (Figure 2), which agrees with the results of a previous study.<sup>22</sup> These findings indicate that DFAlII stimulates not only calcium absorption<sup>15-18</sup> but also iron absorption in rats.

Iron absorption was severely reduced by total gastrectomy (Figure 2), which agrees with our previous studies.<sup>34</sup> However, the etiology of iron malabsorption following gastrectomy has not been fully clarified. It has been reported that gastric mucin is involved in the

integrin-mobilferrin pathway, which is one of the proposed iron transport pathways in the small intestine,<sup>35</sup> suggesting that the lack of the gastric mucin is responsible for the gastrectomy-induced iron malabsorption. In contrast, many researchers have shown that solubilization of dietary iron salts by gastric acid is an important step in intestinal iron absorption.<sup>5,36</sup> In this study, the iron source in the test diets was ferric citrate, an iron salt that dissolves very slowly in water.<sup>37</sup> We propose another possible mechanism that ingested ferric citrates might flow into the small intestine without sufficient solubilization in gastrectomized rats, and thus iron absorption is reduced in the small intestine.

DFAIII or FOS feeding perfectly prevented iron malabsorption induced by total gastrectomy (Figure 2). We have already suggested that cecal fermentation of FOS is involved in the amelioration of iron malabsorption after gastrectomy.<sup>23</sup> However, the mechanism for enhancement of iron absorption by DFAII and the part of the intestine is involved in the DFA III effects remain unclear. In the present study, feeding DFAIII increased the weights of the cecal contents and cecal wall, pools of organic acids and soluble iron in cecal contents with decreasing the pH of cecal contents (TABLE IV, Figure 4). In gastrectomized rats fed the control or DFAIII diet, the iron absorption in the 2nd period was correlated with many cecal fermentation variables such as soluble iron pool, propionic acid pool and cecal wall weight (TABLE V). These results suggest that the preventive effect of DFAIII on gastrectomy-induced iron malabsorption occurs in the large intestine. Possibly, the solubilization of iron salts and the enlargement of the area for iron absorption induced by cecal fermentation of DFAIII contribute to the effect.

In the present study, total gastrectomy caused anemia, and this was completely prevented by the improvement in gastrectomy-induced iron malabsorption as a result of DFAIII feeding (Figure 2, 3). Gastrectomized rats received vitamin B-12 subcutaneously to prevent pernicious anemia.<sup>38</sup> These results indicate that the postgastrectomy anemia observed in this

study was iron-deficient anemia. In gastrectomized rats fed the control or DFAIII diet, the hematocrit at 4 wk was positively correlated with net iron absorption and cecal variables [ $n = 21$ ,  $r = 0.594$ ,  $P < 0.01$  (Fe absorption),  $r = 0.600$ ,  $P < 0.01$  (Soluble Fe pool),  $r = 0.469$ ,  $P < 0.05$  (Total SCFA pool) and  $r = 0.569$ ,  $P < 0.01$  (Cecal wall weight)], suggesting that the preventive effect of feeding DFAIII on postgastrectomy anemia depends on the increase in iron absorption resulting from cecal fermentation of DFAIII. In contrast, FOS feeding did not perfectly prevent postgastrectomy anemia; the hematocrit in gastrectomized rats fed the FOS diet did not recover to levels equivalent to those in sham rats fed the control diet (Figure 3). As described above, ingestion of FOS completely improved gastrectomy-induced iron malabsorption (Figure 2), and previous results showed that FOS feeding completely prevents anemia in totally gastrectomized rats.<sup>6</sup> This may be due to differences in the dose of FOS, i.e., a 75 g of FOS/kg diet used in the previous work, but only a 30 g of FOS/kg diet in the present study. The present results on the comparison of DFAIII with FOS suggest that DFAIII might prevent gastrectomy-induced anemia through improvement in not only intestinal iron absorption but also other iron-related metabolism.

Our findings may be beneficial for patients with postgastrectomy anemia. We have already confirmed that repeated ingestion of DFAIII (5 g/d) for 12 d induces no serious adverse events in healthy humans.<sup>39</sup> However, further evidence is necessary to adapt to the clinical treatment of human. In the next development of the study, we are planning to examine the effects of feeding DFAIII on intestinal iron absorption in healthy humans prior to the adaptation for patients with postgastrectomy anemia. Additionally, in this study, fermentability of DFAIII was estimated by measuring organic acids pools and pH in the cecum because large intestinal fermentation in rats mainly occurs in the cecum.<sup>13</sup> In contrast, the fermentation in humans occurs in the colon. In further studies, fermentability of DFAIII must be evaluated also in humans.

In conclusion, ingestion of DFAIII completely prevents postgastrectomy iron malabsorption and anemia in rats, and cecal fermentation of DFAIII may be involved in improving these gastrectomy-induced complications.

**REFERENCES**

1. Bradley EL, Isaacs J. Postresectional anemia. A preventable complication of total gastrectomy. *Arch Surg* 1976;111:844
2. Tovey FI, Hobsley M. Post-gastrectomy patients need to be followed up for 20-30 years. *World J Gastroenterol* 2000;6:45
3. Fischermann K, Harly S, Worning H, Zacho A. Pancreatic function and the absorption of fat, iron, vitamin B12, and calcium after total gastrectomy for gastric cancer. *Gut* 1967;8:260
4. Adachi S, Kawamoto T, Otsuka M, Todoroki T, Fukao K. Enteral vitamin B12 supplements reverse postgastrectomy B12 deficiency. *Ann Surg* 2000;232:199
5. Bezwoda W, Charlton R, Bothwell T, Torrance J, Mayet F. The importance of gastric hydrochloric acid in the absorption of nonheme food iron. *J Lab Clin Med* 1978;92:108
6. Ohta A, Ohtsuki M, Uehara M, et al. Dietary fructooligosaccharides prevent postgastrectomy anemia and osteopenia in rats. *J Nutr* 1998;128:485
7. Ohta A, Sakai K, Takasaki M, et al. Dietary heme iron does not prevent postgastrectomy anemia but fructooligosaccharides improve bioavailability of heme iron in rats. *Int J Vitam Nutr Res* 1999;69:348
8. Schneeman BO. Fiber, inulin and oligofructose: similarities and differences. *J Nutr* 1999;129:1424S
9. Greger JL. Nondigestible carbohydrates and mineral bioavailability. *J Nutr* 1999;129:1434S
10. Rémésy C, Levrat MA, Gamet L, Demigné C. Cecal fermentations in rats fed oligosaccharides (inulin) are modulated by dietary calcium level. *Am J Physiol* 1993;264:G855
11. Younes H, Demigné C, Rémésy C. Acidic fermentation in the caecum increases absorption of calcium and magnesium in the large intestine of the rat. *Br J Nutr* 1996;75:301

12. Ohta A, Ohtuki M, Takizawa T, et al. Effects of fructooligosaccharides on the absorption of magnesium and calcium by cecectomized rats. *Int J Vitam Nutr Res* 1994;64:316
13. Hara H, Nagata M, Ohta A, Kasai T. Increases in calcium absorption with ingestion of soluble dietary fibre, guar-gum hydrolysate, depend on the caecum in partially nephrectomized and normal rats. *Br J Nutr* 1996;76:773
14. Hayashi K, Hara H, Asvarujanon P, Aoyama Y, Luangpituksa P. Ingestion of insoluble dietary fibre increased zinc and iron absorption and restored growth rate and zinc absorption suppressed by dietary phytate in rats. *Br J Nutr* 2001;86:443
15. Suzuki T, Hara H, Kasai T, Tomita F. Effects of difructose anhydride III on calcium absorption in small and large intestines of rats. *Biosci Biotechnol Biochem* 1998;62:837
16. Mitamura R, Hara H, Aoyama Y, Chiji H. Supplemental feeding of difructose anhydride III restores calcium absorption impaired by ovariectomy in rats. *J Nutr* 2002;132:3387
17. Shiga K, Hara H, Okano G, et al. Ingestion of difructose anhydride III and voluntary running exercise independently increase femoral and tibial bone mineral density and bone strength with increasing calcium absorption in rats. *J Nutr* 2003;133:4207
18. Shigematsu N, Okuhara Y, Shiomi T, Tomita F, Hara H. Effect of difructose anhydride III on calcium absorption in humans. *Biosci Biotechnol Biochem* 2004;68:1011
19. Mineo H, Hara H, Kikuchi H, Sakurai H, Tomita F. Various indigestible saccharides enhance net calcium transport from the epithelium of the small and large intestine of rats in vitro. *J Nutr* 2001;131:3243
20. Mineo H, Hara H, Shigematsu N, Okuhara Y, Tomita F. Melibiose, difructose anhydride III and difructose anhydride IV enhance net calcium absorption in rat small and large intestinal epithelium by increasing the passage of tight junctions in vitro. *J Nutr* 2002;132:3394
21. Suzuki T, Hara H. Various nondigestible saccharides open a paracellular calcium transport

pathway with the induction of intracellular calcium signaling in human intestinal Caco-2 cells.

J Nutr 2004;134:1935

22. Afsana K, Shiga K, Ishizuka S, Hara H. Ingestion of an indigestible saccharide, difructose anhydride III, partially prevents the tannic acid-induced suppression of iron absorption in rats.

J Nutr 2003;133:3553

23. Sakai K, Ohta A, Shiga K, et al. The cecum and dietary short-chain fructooligosaccharides are involved in preventing postgastrectomy anemia in rats. J Nutr 2000;130:1608

24. Uchiyama T. Action of *Arthrobacter ureafaciens* inulinase II on several oligofructans and bacterial levans. Biochim Biophys Acta 1975;397:153

25. Saito K, Tomita F. Difructose anhydrides: their mass-production and physiological functions. Biosci Biotechnol Biochem 2000;64:1321

26. Hidaka H, Hirayama M, Sumi N. A fructooligosaccharide-producing enzyme from *Aspergillus niger* ATCC 20611. Agric Biol Chem 1988;52:1181

27. Reeves PG, Nielsen FH, Fahey GJ. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr 1993;123:1939

28. Lambert R. Gastrectomy in the rat. In: *Surgery of the digestive system in the rat*. Springfield, IL: Charles C. Thomas Publisher, 1965:419

29. Shiga K, Hara H, Takahashi T, et al. Ingestion of water-soluble soybean fiber improves gastrectomy-induced calcium malabsorption and osteopenia in rats. Nutrition 2002;18:636

30. Zhang D, Hendricks DG, Mahoney AW. Effect of coprophagy on bioavailability of iron from plant foods fed to anemic rats. Plant Foods Hum Nutr 1992;42:97

31. Hoshi S, Sakata T, Mikuni K, Hashimoto H, Kimura S. Galactosylsucrose and xylosylfructoside alter digestive tract size and concentrations of cecal organic acids in rats fed diets containing cholesterol and cholic acid. J Nutr 1994;124:52

32. Duncan DB. Multiple range and multiple F test. *Biometrics* 1955;11:1
33. Ichihara K. Correlation coefficient. In: *Statistics for Bioscience: Practical Technique and Theory*. Tokyo, Japan: Nankoudou, 1991:224
34. Shiga K, Hara H, Okano G, Aoyama Y. Ingestion of water-soluble soybean fiber prevents gastrectomy-induced iron malabsorption, anemia and impairment of voluntary running exercise performance in rats. *J Nutr* 2003;133:1120
35. Conrad ME, Umbreit JN, Moore EG. A role for mucin in the absorption of inorganic iron and other metal cations. A study in rats. *Gastroenterology* 1991;100:129
36. Skikne BS, Lynch SR, Cook JD. Role of gastric acid in food iron absorption. *Gastroenterology* 1981;81:1068
37. Merck Research Laboratories. Ferric Citrate. In: O'Neil MJ, Smith A, Heckelman PE, Budavari S, eds. *The Merck Index*, 13th ed. Whitehouse Station, NJ: Merck Research Laboratories Division of Merck & Company, 2001:712
38. Castle WB, Townsend WC. Observations on the etiologic relationship of achylia gastrica to pernicious anemia. II. The effect of the administration to patients with pernicious anemia of beef muscle after incubation with normal human gastric juice. *Am J Med Sci* 1929;178:764
39. Tamura A, Shiomi T, Tamaki N, Shigematsu N, Tomita F, Hara H. Comparative effect of repeated ingestion of difructose anhydride III and palatinose on the induction of gastrointestinal symptoms in humans. *Biosci Biotechnol Biochem* 2004;68:1882

### Figure legends

FIG. 1. Structural formula of difructose anhydride III (DFAIII) produced from inulin with *Arthrobacter* sp. H65-7 inulaseII (EC 2.4.1.93.).

FIG. 2. Net iron absorption in sham-operated and gastrectomized rats fed the control, fructooligosaccharides (FOS) or difructose anhydride III (DFAIII) diets in the 1st period (d 9-13 of the feeding period, A) and 2nd period (d 23-27 of the feeding period, B). Net iron absorption was much lower in the control group of gastrectomized rats than in the same dietary group of sham rats. However, the iron absorption in the FOS and DFAIII groups of the gastrectomized rats was comparable to that in the control group of sham rats. Each value represents a mean  $\pm$  SEM,  $n = 6-11$ . Means in a panel without a common letter differ,  $P < 0.05$ .  $P$ -values estimated by two-way ANOVA were:  $<0.001$  for operation,  $<0.001$  for diet and  $0.395$  for operation X diet (A), and  $<0.001$  for operation,  $<0.001$  for diet and  $0.907$  for operation X diet (B).

FIG. 3. Hemoglobin concentrations (A) and hematocrit (B) in sham-operated and gastrectomized rats fed the control, fructooligosaccharides (FOS) or difructose anhydride III (DFAIII) diets at 0, 2 and 4 wk after the start of the feeding period. Hemoglobin concentration and hematocrit at 2 wk were lower in gastrectomized rats than in sham rats. However, these hematological variables at 4 wk in the DFAIII group of the gastrectomized rats were comparable to the values of the three sham groups. Each value represents a mean  $\pm$  SEM,  $n = 6-11$ . Means in a period without a common letter differ,  $P < 0.05$ . #Significantly different from the value at wk 0 in each group,  $P < 0.05$ .  $P$ -values estimated by three-way ANOVA were:  $<0.001$  for operation,  $0.001$  for diet,  $<0.001$  for time,  $0.071$  for operation X diet,  $0.003$  for diet X time,  $<0.001$  for operation X time and  $0.317$  for operation X diet X time

(A), and  $<0.001$  for operation, 0.001 for diet,  $<0.001$  for time, 0.191 for operation X diet, 0.017 for diet X time,  $<0.001$  for operation X time and 0.109 for operation X diet X time (B).

FIG. 4. Pools of short-chain fatty acids (SCFA) and other organic acids in the cecal contents of sham-operated and gastrectomized rats fed the control, fructooligosaccharides (FOS) or difructose anhydride III (DFAIII) diets for 28 d. Total SCFA (the sum of acetic, propionic, and butyric acids) was higher in the FOS and DFAIII groups than in the control group. Each value represents a mean  $\pm$  SEM,  $n = 6-11$ . Means in a acid without a common letter differ,  $P < 0.05$ .  $P$ -values estimated by two-way ANOVA were: 0.428 for operation,  $<0.001$  for diet and 0.895 for operation X diet (Acetic acid), and 0.928 for operation,  $<0.001$  for diet and 0.411 for operation X diet (Propionic acid), and 0.036 for operation,  $<0.001$  for diet and 0.208 for operation X diet (Butyric acid) , and 0.227 for operation,  $<0.001$  for diet and 0.867 for operation X diet (Total SCFA) , and 0.350 for operation, 0.113 for diet and 0.528 for operation X diet (Succinic acid) , and 0.533 for operation, 0.918 for diet and 0.571 for operation X diet (Lactic acid).

TABLE I.

COMPOSITION OF STOCK AND TEST DIETS			
	Stock diet	Test diets	
		Control	FOS or DFAIII
	g/kg diet		
Casein*	200	200	200
Dextrin†	400	400	400
Sucrose	200	200	200
Soybean oil	70	70	70
Mineral mixture‡	35	35	35
Vitamin mixture‡	10	10	10
Choline bitartrate	2.5	2.5	2.5
L-Cystine	3.0	3.0	3.0
Tert-butylhydroquinone	0.014	0.014	0.014
Crystallized cellulose§	80	80	50
FOS   or DFAIII¶	0	0	30

\* ALACID (New Zealand Dairy Board, Wellington, New Zealand).

† TK-16 (Matsutani Chemical Industry, Hyogo, Japan).

‡ AIN-93G formulation.

§ Avicel PH102 (Asahi Chemical Industry, Tokyo, Japan).

|| Fructooligosaccharides (Meiologo-P<sup>®</sup>, Meiji Seika Kaisha, Tokyo, Japan).

¶ Difructose Anhydride III (Nippon Beet Sugar Manufacturing, Tokyo, Japan).

TABLE II.

INITIAL BODY WEIGHT, BODY WEIGHT GAIN AND FOOD INTAKE OF SHAM-OPERATED AND GASTRECTOMIZED RATS FED THE CONTROL, FRUCTOOLIGOSACCHARIDES (FOS) OR DIFRUCTOSE ANHYDRIDE III (DFAIII) DIETS FOR 28 D*				
	<i>n</i>	Initial body weight (g)	Body weight gain (g/d)	Food intake (g/d)†
Sham				
Control	6	131 ± 3.8 <sup>a</sup>	4.25 ± 0.160 <sup>a</sup>	15.0 ± 0.001 <sup>a</sup>
FOS	6	132 ± 3.2 <sup>a</sup>	4.48 ± 0.101 <sup>a</sup>	15.0 ± 0.001 <sup>a</sup>
DFAIII	6	132 ± 2.9 <sup>a</sup>	4.31 ± 0.151 <sup>a</sup>	15.0 ± 0.001 <sup>a</sup>
Gastrectomy				
Control	10	123 ± 1.8 <sup>b</sup>	3.42 ± 0.155 <sup>b</sup>	14.1 ± 0.330 <sup>b</sup>
FOS	11	121 ± 2.6 <sup>b</sup>	3.50 ± 0.139 <sup>b</sup>	14.0 ± 0.229 <sup>b</sup>
DFAIII	11	122 ± 1.8 <sup>b</sup>	3.57 ± 0.106 <sup>b</sup>	14.5 ± 0.159 <sup>ab</sup>
ANOVA <i>P</i> values				
Operation		<0.001	<0.001	<0.001
Diet		0.969	0.518	0.532
Operation X Diet		0.810	0.700	0.532

\* Each value represents a mean ± SEM. Values in a column not sharing a superscript letter differ, *P* < 0.05.

† Means after consumption of 15 g of diet per day.

TABLE III.

SERUM IRON CONCENTRATION OF SHAM-OPERATED AND GASTRECTOMIZED RATS FED THE CONTROL, FRUCTOOLIGOSACCHARIDES (FOS) OR DIFRUCTOSE ANHYDRIDE III (DFAIII) DIETS FOR 28 D*		
	<i>n</i>	Serum Fe ( $\mu\text{mol/L}$ )
Sham		
Control	6	36.4 $\pm$ 7.44 <sup>a</sup>
FOS	6	29.7 $\pm$ 3.29 <sup>a</sup>
DFAIII	6	36.6 $\pm$ 2.23 <sup>a</sup>
Gastrectomy		
Control	10	8.55 $\pm$ 0.541 <sup>c</sup>
FOS	11	21.1 $\pm$ 3.35 <sup>b</sup>
DFAIII	11	18.1 $\pm$ 1.80 <sup>b</sup>
ANOVA <i>P</i> values		
Operation		<0.001
Diet		0.346
Operation X Diet		0.023

\* Each value represents a mean  $\pm$  SEM. Values not sharing a superscript letter differ,  $P < 0.05$ .

TABLE IV.

WEIGHT OF THE CECAL CONTENTS AND CECAL WALL AND THE pH OF CECAL CONTENTS OF SHAM-OPERATED AND GASTRECTOMIZED RATS FED THE CONTROL, FRUCTOOLIGOSACCHARIDES (FOS) OR DIFRUCTOSE ANHYDRIDE III (DFAIII) DIETS FOR 28 D*				
	<i>n</i>	Cecal contents (wet g/100 g body weight)	Cecal wall (wet g/100 g body weight)	pH
Sham				
Control	6	0.673 ± 0.123 <sup>c</sup>	0.304 ± 0.023 <sup>b</sup>	7.54 ± 0.133 <sup>a</sup>
FOS	6	1.28 ± 0.141 <sup>b</sup>	0.490 ± 0.063 <sup>a</sup>	7.01 ± 0.149 <sup>bc</sup>
DFAIII	6	1.42 ± 0.088 <sup>b</sup>	0.504 ± 0.025 <sup>a</sup>	6.75 ± 0.165 <sup>c</sup>
Gastrectomy				
Control	10	0.746 ± 0.071 <sup>c</sup>	0.331 ± 0.014 <sup>b</sup>	7.25 ± 0.062 <sup>ab</sup>
FOS	11	1.46 ± 0.087 <sup>b</sup>	0.485 ± 0.025 <sup>a</sup>	6.79 ± 0.093 <sup>c</sup>
DFAIII	11	1.89 ± 0.092 <sup>a</sup>	0.510 ± 0.026 <sup>a</sup>	6.93 ± 0.091 <sup>c</sup>
ANOVA <i>P</i> values				
Operation		0.006	0.698	0.243
Diet		<0.001	<0.001	<0.001
Operation X Diet		0.131	0.861	0.077

\* Each value represents a mean ± SEM. Values in a column not sharing a superscript letter differ, *P* < 0.05.

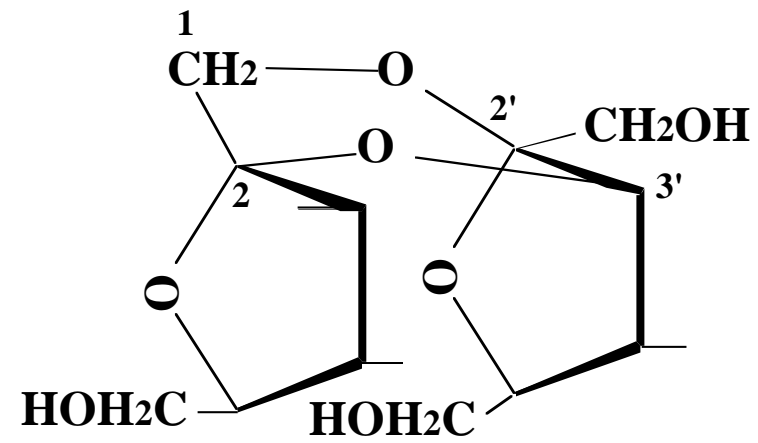
TABLE V.

CORRELATIONS BETWEEN NET IRON ABSORPTION, POOLS OF SOLUBLE IRON AND VARIOUS ORGANIC ACIDS, THE pH OF THE CECAL CONTENTS, AND CECAL WALL WEIGHT IN GASTRECTOMIZED RATS FED THE CONTROL OR DIFRUCTOSE ANHYDRIDE III (DFAIII) DIETS FOR 28 D\*

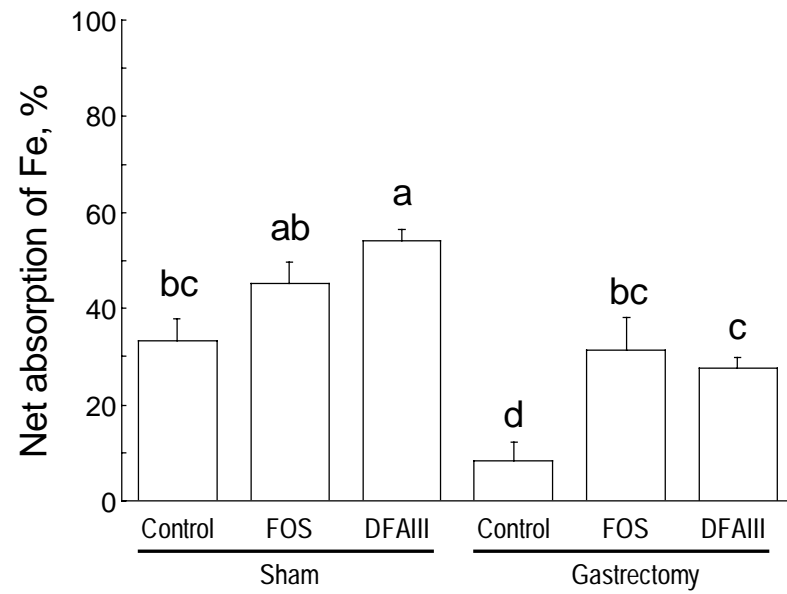
	Fe absorption
Soluble iron pool	0.694***
Total SCFA† pool	0.641**
Acetic acid pool	0.595**
Propionic acid pool	0.679***
Butyric acid pool	0.632**
Succinic acid pool	0.108
Lactic acid pool	0.327
Cecal pH	-0.507*
Cecal wall weight	0.670***

\* Significant correlation ( $n = 21$ , \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ )

† Total SCFA: sum of acetic, propionic and butyric acids.



**A**



**B**

