Stimulation of Butyrate Production in the Large Intestine of Weaning Piglets by Dietary Fructooligosaccharides and Its Influence on the Histological Variables of the Large Intestinal Mucosa

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Summary Fructooligosaccharides (FOS) reach the large intestine and are fermented into short-chain fatty acids (SCFA), lactate, and carbon dioxide. As the major energy source for the epithelial cells of the large intestine, *n*-butyrate stimulates the proliferation of cells as well as mineral and water absorption from the lumen. We examined the effect of dietary FOS supplementation on luminal SCFA production and its influence on the morphometrical variables of mucosa of the large intestine in commercially available pigs. Six weaning piglets were used. After 7 d of adaptation, three pigs were given a test diet containing FOS (10%) ad libitum for 10 d. The other three remained on the basal diet and were used as controls. At the end of the experiment, their large intestines were removed, and the cecum, gyri centripetales, gyri centrifugales, and rectum were separated. The contents of each portion were collected and measured for SCFA concentration, pH, and moisture. A micrometer was used to measure the crypt depth. The numbers of epithelial and mitotic cells in the crypt columns were also counted. The concentration of SCFA was significantly higher in piglets fed FOS than in the controls. The concentration of *n*-butyrate was markedly stimulated by FOS. The number of epithelial, mitotic, and mucin-containing cells was higher in piglets fed FOS than in the controls. Accordingly, the crypt depth was larger in the FOS-fed piglets. The luminal *n*-butyrate concentration showed a significantly positive correlation with the crypt depth and the number of epithelial, mitotic, and mucin-containing cells.

Key Words n-butyrate, epithelial cell, fructooligosaccharides, mucosa, piglet

Fructooligosaccharides (FOS), one of the most widely used prebiotics, reach the large intestine and are fermented by the resident microbiota into lactate, shortchain fatty acids (SCFA), and carbon dioxide (1). Many investigators have reported that they are beneficial to human health (1–3). The beneficial effect of FOS is believed to depend on the selective increases in lactic acid bacteria, such as *Bifidobacteria* and *Lactobacilli* (1, 4-6).

On the other hand, FOS increases *n*-butyrate concentration in the hindgut of rats (7, 8) and humans (9). *n*-Butyrate is the major energy source for the epithelial cells of the large intestine (10, 11) and stimulates mucus release (12) and epithelial cell proliferation (13, 14) as well as mineral and water absorption from the lumen (15, 16). Therefore, the effect of FOS on hindgut physiology should be realized through an increase in luminal *n*-butyrate concentration.

The rat is typically used to assess the effects of prebiotics or dietary fiber on epithelial cell proliferation in vivo (17-20). However, the effect of FOS on epithelial cell proliferation in the large intestine is so far inconsis-

tent in the rat model (21, 22). One reason for the inconsistency could be lactate accumulation induced by FOS (8, 21, 22) because luminal lactate accumulation leads to diarrhea (23, 24) and damages the hindgut mucosa with low luminal pH (25). The hindgut microbiota of commercially available rats are artificially constructed and are so simple that they often cause abnormal fermentation, which is characterized by hyperlactate production in the hindgut when indigestible oligosaccharides are fed (24, 26). They usually lack the predominant acid-utilizing groups of bacteria (24, 27, 28). Experiments with pigs may have advantages over those with rats in this context because typical commercial pigs do not have artificially controlled simple intestinal microbiota; therefore, an examination of indigestible oligosaccharides can be conducted under conventional conditions. Indeed, abnormal fermentation in the hindgut has not been reported so far when resistant starch or soybean curd residues were tested (29, Maekawa and Ushida. unpublished), which, in turn, often leads to abnormal fermentation in the rat (26, Maekawa andUshida, unpublished).

In the present study, we examined the effects of dietary FOS supplementation on luminal SCFA production and its effect on the hindgut mucosa of weaning

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piglets. We have found significant stimulation of *n*butyrate production concomitant with a significant increase in epithelial cell proliferation in the hindgut of pigs.

MATERIALS AND METHODS

Animals and diet. Six 40 d-old crossbred (Landrace \times Large white \times Duroc) castrated male piglets weighing approximately 12 kg were obtained from a commercial pig farm. The pigs were individually housed in metabolic cages placed in a temperature-conditioned room (25°C) and adapted for a commercial diet for weaning piglets (Standard Diet Swine No. 1, SDS No. 1; Nippon Formula Feed, Yokohama, Japan). The diet was free from intestinal flora modifiers, such as antibiotics and probiotics. The nutrient composition of this diet (g/ kg) expressed on a dry-matter basis (906 g/kg) was as follows: crude protein, 220 g; crude fat, 46 g; crude fiber, 9 g; and crude ash, 63 g (23). The diet and water were given ad libitum for 7 d.

After this adaptation period, three piglets were randomly selected and given the SDS No. 1 supplemented with 10% (w/w) fructooligosaccharides (FOS, Meiji Seika Kaisya, Ltd., Tokyo, Japan). The other three piglets were maintained on the SDS No. 1 diet and served as controls. Two were fed ad libitum for 10 d, and the other one, for 11 d.

Sampling and analytical procedures. The animals were slaughtered by exsanguination under general anesthesia with an intravascular injection of ketamine HCl (Ketalar 50; Sankyo, Tokyo, Japan) at the end of the experiment. After a midline incision, the entire intestine was immediately removed and separated into the cecum, gyri centripetales, gyri centrifugales, and rectum as reported previously (30). A longitudinal section of each segment of the intestine was incised, and the luminal contents were carefully collected so as not to injure the mucosa. Each segment of the intestine was flushed with 10% (v/v) neutralized formalin solution to remove residual digesta and fixed in 10% neutralized formalin solution. Collected luminal contents were subjected to organic acid analyses and determination of pH and moisture contents.

Since the daily fluctuation of succinate and lactate concentrations in the hindgut was particularly large (Sakata, personal communication), the slaughter schedule was determined in order to minimize the difference of each sampling time relative to feeding. On day 10, one control pig and one FOS-fed pig were killed at 10:00 h. After sampling treatments, another set of pigs was killed at 11:00 h. On the next day, the remaining set of pigs was killed at 10:00 h.

Organic acids (SCFA, succinate, lactate, and formate) in the large intestine were analyzed by ion-exclusion HPLC as described elsewhere (31). The moisture content was determined by lyophilization. The pH was measured with a compact pH meter (twin B-211, Horiba, Kyoto, Japan).

The fixed intestine was further cut into cross-sections approximately 10 mm in length. The positions of the

segments were as follows: the longitudinal middle portion for the cecum; 200 mm below the ceco-colonic junction for the gyri centripetales; 100 mm below the flexura centralis for the gyri centrifugales; and 100 mm below the portion adjacent to the right kidney for the rectum (*30*). These cross-sectioned tissue samples were embedded in paraffin. Cross sections of 3 μ m thick were prepared and stained with hematoxylin and eosin (HE) and Alcian green (AG) counter-stained with hematoxylin. Staining with AG was easier and more successful than that using Alcian blue pH 2.5, as reported previously (*32*, *33*).

Twenty well-oriented crypts were randomly selected, and the absolute length of the axial crypts was measured with an eye-piece micrometer on HE-stained preparations at $100 \times$ magnification. The number of columnar epithelial cells, mucin-containing cells, and mitotic cells per longitudinal section of the left side of the crypt column was also counted at $400 \times$ magnification on AG-stained preparations. The mitotic zone and mitotic index (%) were measured as described by Ichikawa and Sakata (34). The number of crypts per unit length (mm) of luminal circumference (crypt density) was counted according to Ichikawa and Sakata (34).

Statistical analyses. All statistical analyses were performed using Statcel (35) and StatLight (Yukms Co., Ltd., Tokyo, Japan), which consists of add-on applications of Microsoft Excel (ver 5.0, Microsoft, Seattle, WA, USA). We conducted a randomized blocked design 2way ANOVA (2 experimental groups × large intestinal segments) to detect the effect of the fructooligosaccharide supplementation on the digesta and morphometrical variables. When an interaction effect or large intestinal factor was significant, Dunn's post-hoc test was used. The correlation between the digesta parameters and morphometrical variables was also analyzed. Differences were considered significant at p < 0.05.

Chemicals. Chemicals were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) or Nacalai Tesque, Inc. (Kyoto, Japan) unless otherwise stated.

Ethics. The experimental animals were handled in accordance with the guideline for studies with laboratory animals of the Experimental Animal Committee of Kyoto Prefectural University.

RESULTS

Water contents, pH, and organic acid concentrations of the hindgut digesta (Table 1)

The water content of digesta was significantly higher in FOS-fed pigs than in control pigs (p < 0.05). Water content varied among the segments of the large intestine. The highest values were in the cecum, followed by the gyri centripetales, the gyri centrifugales, and the rectum.

Digesta pH did not vary significantly among the treatment groups or among the segments of the large intestine.

n-Butyrate concentration was statistically higher in

Table 1. Water content, p	H, and organic acid conce	entrations of	f hindgut digest:	a in the fru	ctooligosaccha	ride-fed piglet	ts or controls. ¹			
Dietary fructooligosaccharide	Large intestine (abbreviation)	Abbrevi- ations	Water content %	Hd	Total SCFA mmol/kg	Lactate mmol/kg	Acetate mmol/kg	Propionate mmol/kg	<i>n</i> -Butyrate mmol/kg	<i>n</i> -Valerate mmol/kg
FOS	Cecum (C) Gyri centripetales (GP) Gyri centrifugales (GF) Rectum (R)	FC FGF FGF	$\begin{array}{c} 86.9\pm5.1\\ 82.9\pm3.3\\ 74.8\pm0.3\\ 74.4\pm0.6\end{array}$	$\begin{array}{c} 6.1\pm0.6\\ 6.1\pm0.6\\ 6.5\pm0.1\\ 6.6\pm0.2\\ \end{array}$	$144.2\pm15.2\\147.5\pm38.4\\99.1\pm15.1\\67.1\pm15.2$	$\begin{array}{c} 11.6\pm14.\\ 10.0\pm3.1\\ 5.7\pm3.3\\ 4.9\pm1.6\end{array}$	3 68.0±31.5 71.3±33.3 46.3±9.0 34.4±9.3	32.7 ± 6.1 32.2 ± 7.4 21.3 ± 7.4 14.2 ± 6.2	$\begin{array}{c} 28.8 \pm 10.1 \\ 35.0 \pm 11.4 \\ 19.2 \pm 5.6 \\ 10.5 \pm 1.3 \end{array}$	9.8 ± 8.7 3.1 ± 2.7 6.3 ± 5.0 2.5 ± 1.9
Control	Cecum (C) Gyri centripetales (GP) Gyri centripetales (GF) Rectum (R)	CG CGP CGF	$\begin{array}{c} 79.0\pm1.8\\ 73.1\pm0.6\\ 69.1\pm1.2\\ 65.2\pm2.9\end{array}$	$\begin{array}{c} 6.5\pm0.6\\ 6.9\pm0.4\\ 6.8\pm0.3\\ 6.6\pm0.3\\ 6.6\pm0.3\end{array}$	84.6 ± 15.6 96.0±36.5 68.3±43.1 59.4±35.1	$\begin{array}{c} 13.4\pm10.4\\ 4.3\pm4.3\\ 4.2\pm5.3\\ 1.4\pm1.7\end{array}$	$\begin{array}{c} 4 & 44.3\pm11.1\\ 50.0\pm14.7\\ 33.4\pm17.1\\ 30.7\pm19.1 \end{array}$	$\begin{array}{c} 27.0\pm0.6\\ 27.7\pm20.8\\ 21.0\pm22.7\\ 15.4\pm13.9\end{array}$	$\begin{array}{c} 9.1{\pm}1.5\\ 10.2{\pm}4.3\\ 6.8{\pm}4.3\\ 5.0{\pm}2.0\end{array}$	$\begin{array}{c} 1.8 \pm 1.5 \\ 3.0 \pm 0.8 \\ 1.8 \pm 1.1 \\ 2.7 \pm 1.1 \end{array}$
Randomized block-design 2-way ANOVA <i>p</i> value ²	FOS Large intes Interaction	tine	<0.001 <0.001 0.65	$\begin{array}{c} 0.06\\ 0.43\\ 0.44\end{array}$	0.005 0.007 0.38	$\begin{array}{c} 0.68 \\ 0.14 \\ 0.93 \end{array}$	0.09 0.10 0.83	0.55 0.03 0.90	<0.001 <0.001 <0.001 0.01	0.06 0.46 0.20
Results of post-hoc multiple comparisons ³	Large intes Interaction	tine	C>GP>(GF, R) NS	NS^4 NS	(C, GP)>R NS	SN SN	NS NS	(C, GP)>R NS	NS (FC, FGP)>(CC, CGF, FR, CR), FGP)	NS SGP, SFGF
¹ Values represent means ² Randomized block-desig ³ The difference was cons ⁴ NS, not significant.	±SD, $n=3$. m 2-way ANOVA was don idered significant at $p<0$.	le for the val 05 by Dunn	ues (FOS×large 's multiple comp	: intestinal parison.	segments) obta	ined from the	sampling time	Ś		
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Dietary fructooligosacchario	le Large intestine (al	breviation)	Crypt dep µm	th Cryp	t density Epi /mm	thelial cell <i>n</i>	Mitotic cell n	Mitotic zone	Mitotic index %	Mucin-containing cell <i>n</i>
FOS	Cecum (C) Gyri centripeta Gyri centrifuga Rectum (R)	lles (GP) lles (GF)	433.4±4 388.2±74 367.5±87 395.3±13	1.3 12 4.9 13 4.7 13 36.0 13	.1+1.3 .1+1.6 .8+2.2 .6+0.8 8	3.8 ± 11.8 3.1 ± 20.0 9.5 ± 19.3 7.3 ± 27.4	2.7 ± 0.3 1.7 ± 0.0 1.5 ± 0.5 1.5 ± 0.5	39.9 ± 6.2 35.7 ± 10.3 31.6 ± 13.6 31.9 ± 13.1	2.9 ± 0.7 1.8 ± 0.3 1.8 ± 0.5 1.8 ± 0.5 1.8 ± 0.7	38.1 ± 12.3 41.0 ± 11.2 41.6 ± 10.5 44.0 ± 14.5
Control	Cecum (C) Gyri centripeta Gyri centrifuga Rectum (R)	des (GP) des (GF)	$\begin{array}{c} 312.8\pm5\\ 270.7\pm1\\ 260.4\pm3\\ 323.6\pm5\end{array}$	1.8 15 5.8 17 5.4 15 8.4 15 1.0 18	.3±3.1 7 .0±3.0 6 .0±3.3 5 .5±3.7 6	5.7 ± 5.5 4.2 ± 6.1 8.7 ± 4.1 9.4 ± 9.3	$\begin{array}{c} 1.2\pm0.5\\ 0.8\pm0.3\\ 0.4\pm0.1\\ 0.5\pm0.1\end{array}$	25.6 ± 4.6 18.4 ± 4.7 16.0 ± 2.8 21.5 ± 3.7	$\begin{array}{c} 1.5\pm0.6\\ 1.2\pm0.4\\ 0.7\pm0.2\\ 0.7\pm0.1\\ \end{array}$	24.9 ± 4.3 29.4 ± 1.9 29.7 ± 1.7 29.6 ± 2.3
Randomized block-design 2-way ANOVA <i>p</i> value ²	FOS Large intestine Interaction		0.00 0.36 0.90	1	0.002 0.29 0.50	<0.001 0.24 0.86	< 0.001 < < 0.001 < < 0.41 < 0.41	$< 0.001 \\ 0.09 \\ 0.76 $	$< 0.001 \\ 0.01 \\ 0.56$	0.001 0.65 0.99
Results of post-hoc multiple comparisons ³	Large intestine Interaction		NS ⁴ NS		NS NS	NS NS	C>(GP, GF, R) NS	NSN	C>(GP, GF, R) NS	NS NS
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¹ Microscopic determination was performed on AG-stained preparations with appropriate magnification using an eye-piece micrometer. See text for details. Values represent means±SD, n=3 (20 determinations×3 pigs). ² Randomized block-design 2-way ANOVA was done for the values (FOS×large intestinal segments) obtained from the sampling times. ³ The difference was considered significant at p<0.05 by Dunn's multiple comparison.

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Fig. 1. Photomicrographs of the large intestine; all sections were stained with hematoxylin and eosin. A, cecum; B, gyri centripetales; C, gyri centrifugales; D, rectum; see text for photograph site of each segment. 1, fructooligosaccharide diet; 2, control diet; see text for dietary conditions. Bars represent $100 \mu m$.

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Morphological parameters	Large intestine	Water c	ontent	pF		Lact	ate	Acet	ate	Propic	onate	n-But	yrate	n-Val	erate
		r1	p^1	r	, d	r	d	r	d	r	d	r	d	r	р
Crypt depth	Cecum Gyri centripetales Gyri centrifugales Rectum	$\begin{array}{c} 0.60\\ 0.39\\ 0.42\\ 0.27\end{array}$	0.21 0.45 0.41 0.60	-0.36 -0.35 -0.27 0.70	$\begin{array}{c} 0.48\\ 0.49\\ 0.61\\ 0.12\end{array}$	$\begin{array}{c} 0.02 \\ 0.47 \\ -0.29 \\ -0.15 \end{array}$	0.96 0.34 0.58 0.77	$\begin{array}{c} 0.06 \\ 0.06 \\ 0.16 \\ 0.10 \\ -0.10 \end{array}$	$\begin{array}{c} 0.90\\ 0.91\\ 0.77\\ 0.85\end{array}$	$\begin{array}{c} 0.73\\ 0.33\\ 0.11\\ 0.02\end{array}$	$\begin{array}{c} 0.10\\ 0.53\\ 0.83\\ 0.97\end{array}$	0.86 0.97 0.83 0.35	$\begin{array}{c} 0.03\\ 0.001\\ 0.04\\ 0.50\end{array}$	$\begin{array}{c} 0.73 \\ -0.49 \\ 0.87 \\ 0.57 \end{array}$	$\begin{array}{c} 0.10\\ 0.33\\ 0.03\\ 0.03\\ 0.24\end{array}$
Crypt density	Cecum Gyri centripetales Gyri centrifugales Rectum	-0.62 -0.50 -0.33 -0.44	$\begin{array}{c} 0.19\\ 0.32\\ 0.52\\ 0.39\end{array}$	$\begin{array}{c} 0.43\\ 0.78\\ 0.83\\ 0.45\end{array}$	$\begin{array}{c} 0.39\\ 0.06\\ 0.04\\ 0.37\end{array}$	$\begin{array}{c} 0.04 \\ -0.52 \\ -0.87 \\ -0.82 \end{array}$	$\begin{array}{c} 0.94 \\ 0.29 \\ 0.02 \\ 0.05 \end{array}$	$\begin{array}{c} 0.09 \\ -0.60 \\ -0.63 \\ -0.71 \end{array}$	$\begin{array}{c} 0.87 \\ 0.21 \\ 0.18 \\ 0.11 \end{array}$	-0.48 -0.61 -0.63 -0.59	0.33 0.20 0.18 0.22	-0.64 -0.79 -0.27 -0.94	0.17 0.06 0.61 0.01	-0.52 0.00 -0.13 -0.37	$\begin{array}{c} 0.29\\ 0.99\\ 0.81\\ 0.47 \end{array}$
Epithelial cells	Cecum Gyri centripetales Gyri centrifugales Rectum	$\begin{array}{c} 0.53\\ 0.39\\ 0.40\\ 0.36\end{array}$	0.28 0.45 0.43 0.48	-0.08 -0.31 -0.22 0.69	0.88 0.55 0.68 0.13	$\begin{array}{c} 0.30\\ 0.48\\ -0.33\\ -0.09\end{array}$	0.56 0.33 0.52 0.87	-0.13 -0.01 0.10 -0.06	$\begin{array}{c} 0.81\\ 0.98\\ 0.85\\ 0.91\\ \end{array}$	0.68 0.35 0.13 0.03	$\begin{array}{c} 0.13\\ 0.50\\ 0.81\\ 0.95\end{array}$	$\begin{array}{c} 0.92\\ 0.94\\ 0.81\\ 0.44\\ 0.44\end{array}$	0.01 0.005 0.38 0.38	$ \begin{array}{c} 0.87 \\ -0.52 \\ 0.94 \\ 0.58 \end{array} $	0.03 0.29 0.005 0.23
Mitotic cells	Cecum Gyri centripetales Gyri centrifugales Rectum	0.79 0.79 0.66 0.79	0.06 0.06 0.16 0.06	-0.72 -0.77 -0.32 0.09	$\begin{array}{c} 0.11\\ 0.08\\ 0.54\\ 0.86\end{array}$	-0.23 0.15 -0.10 0.51	$\begin{array}{c} 0.65\\ 0.78\\ 0.86\\ 0.30\end{array}$	0.55 0.50 0.40 0.31	$\begin{array}{c} 0.26\\ 0.31\\ 0.43\\ 0.55\end{array}$	0.50 0.52 0.08 0.01	$\begin{array}{c} 0.31\\ 0.29\\ 0.88\\ 0.98\end{array}$	0.67 0.88 0.90 0.86	$\begin{array}{c} 0.14\\ 0.02\\ 0.01\\ 0.03\end{array}$	0.38 0.07 0.73 0.02	$\begin{array}{c} 0.46\\ 0.89\\ 0.10\\ 0.98\end{array}$
Mitotic zone	Cecum Gyri centripetales Gyri centrifugales Rectum	0.54 0.43 0.44 0.40	0.27 0.40 0.39 0.43	-0.32 -0.41 -0.39 0.58	$\begin{array}{c} 0.54\\ 0.41\\ 0.44\\ 0.44\\ 0.23\end{array}$	$\begin{array}{c} 0.18 \\ 0.52 \\ -0.13 \\ 0.06 \end{array}$	0.74 0.29 0.80 0.91	0.02 0.10 0.25 0.04	$\begin{array}{c} 0.97 \\ 0.85 \\ 0.64 \\ 0.94 \end{array}$	$\begin{array}{c} 0.78\\ 0.42\\ 0.31\\ 0.14\end{array}$	$\begin{array}{c} 0.07 \\ 0.41 \\ 0.55 \\ 0.79 \end{array}$	0.92 0.97 0.87 0.56	0.01 0.002 0.03 0.25	$\begin{array}{c} 0.81 \\ -0.45 \\ 0.98 \\ 0.65 \end{array}$	0.05 0.37 0.0003 0.16
Mitotic index	Cecum Gyri centripetales Gyri centrifugales Rectum	0.74 0.85 0.75 0.78	0.09 0.03 0.09 0.07	-0.85 -0.94 -0.36 -0.19	$\begin{array}{c} 0.03\\ 0.01\\ 0.49\\ 0.71\end{array}$	-0.39 -0.18 0.05 0.64	0.45 0.73 0.93 0.17	0.67 0.81 0.50 0.33	$0.14 \\ 0.05 \\ 0.31 \\ 0.52 \\ 0.52 \\$	$\begin{array}{c} 0.38 \\ 0.59 \\ 0.00 \\ -0.07 \end{array}$	0.45 0.21 1.00 0.90	0.47 0.56 0.83 0.78	0.34 0.24 0.04 0.07	0.15 0.55 0.50 -0.31	0.78 0.26 0.31 0.55
Mucin- containing cells	Cecum Gyri centripetales Gyri centrifugales Rectum	0.18 0.20 0.44 0.48	$\begin{array}{c} 0.73\\ 0.71\\ 0.38\\ 0.33\\ 0.33\end{array}$	$\begin{array}{c} 0.13 \\ -0.09 \\ -0.20 \\ 0.48 \end{array}$	$\begin{array}{c} 0.81 \\ 0.87 \\ 0.70 \\ 0.33 \end{array}$	$\begin{array}{c} 0.32 \\ 0.55 \\ -0.31 \\ 0.16 \end{array}$	0.54 0.25 0.55 0.76	-0.21 -0.20 0.16 0.16	0.69 0.70 0.77 0.76	$\begin{array}{c} 0.88\\ 0.11\\ 0.14\\ 0.18\\ 0.18\end{array}$	0.02 0.83 0.80 0.73	$\begin{array}{c} 0.91 \\ 0.87 \\ 0.84 \\ 0.68 \end{array}$	$\begin{array}{c} 0.01\\ 0.02\\ 0.04\\ 0.14\end{array}$	$\begin{array}{c} 0.95 \\ -0.69 \\ 0.93 \\ 0.59 \end{array}$	0.003 0.13 0.01 0.22

 1 r, Correlation coefficient; p, Probability.

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the FOS-fed pigs than in the control pigs in the cecum and gyri centripetales. The *n*-valerate concentration tended to be higher in the FOS-fed pigs than in the control pigs (p=0.06). The lactate, acetate, and propionate concentrations did not vary significantly between the treatment groups. Other organic acids, such as succinate, formate, iso-butyrate, and iso-valerate, were detected in amounts less than 2 mmol/kg digesta (data not shown).

Morphometric analyses (Table 2, Fig. 1)

The mucosa in the hindgut of FOS-fed pigs appeared moderately to markedly thicker than those in the controls (Fig. 1). Accordingly, the crypt depth was significantly extended by FOS supplementation. Obviously, the number of epithelial cells per crypt column was significantly greater in the FOS-fed pigs than in the controls, and the crypt density was significantly smaller in the FOS-fed pigs than in the controls. The number of mitotic cells in a crypt was significantly greater in the FOS-fed pigs than in the controls. The mitotic zone and mitotic index were also significantly greater in the FOSfed pigs than in the controls. The mitotic cell number and mitotic index were different for the segment of the large intestine. Those in the ceca were greater than the other segments of the large intestine. The number of mucin-containing cells in a crypt was significantly greater in the FOS-fed pigs than in the controls.

Correlation between luminal and morphometric variables (Table 3)

The water content and pH of the digesta were not significantly correlated with histological variables of the large intestine, with a few exceptions. Digesta pH correlated positively with the crypt density in the gyri centripetales (p=0.06) and the gyri centrifugales (p<0.05). It correlated negatively with the mitotic index in the cecum and gyri centripetales. The concentrations of lactate, acetate, and propionate were not correlated with histological variables, with several exceptions. Lactate correlated with the crypt density in the gyri centrifugales (p < 0.05) and with that in the rectum (p=0.05). Acetate correlated with the mitotic index in the gyri centripetales. Propionate correlated with the number of mucin-containing cells in the cecum. n-Butyrate correlated positively with all of the morphometrical variables, such as the crypt depth, the size of the mitotic zone, the number of epithelial cells per crypt column, the number of mitotic cells, and the number of mucin-containing cells per crypt column, depending on the portions of the large intestine (p < 0.05). There was only one exception. The crypt density in the rectum correlated negatively with n-butyrate. n-Valerate also correlated positively with most morphometrical variables, such as the crypt depth, the mitotic zone, the crypt density, and the number of mucin-containing cells per crypt column (p < 0.05), depending on the portions of the large intestine.

DISCUSSION

FOS are fermented in the large intestine by specific bacteria, such as *Bifidobacteria* and *Lactobacilli* (1, 3, 8)

into lactate and acetate (1, 7). In experiments using rats, FOS led to the accumulation of lactate in the large intestine (8, 24) and induced diarrhea (24, 36). In the present study, the concentration of lactate was not necessarily high in the FOS-fed pigs. In contrast with the results of rat experiments, the experiments using piglets indicated that dietary FOS increased *n*-butyrate and *n*valerate concentrations in the large intestine (p < 0.05and p=0.06, respectively; Table 1). The apparent contradiction between the present results and those with rats may be explained by the difference in the intestinal microbiota. Lactate as a typical intermediate product in anaerobic fermentation is converted to acetate, propionate, or *n*-butyrate by a range of acid-utilizing bacteria, such as Selenomonas ruminantium, Veillonella parvula, Desulfovibrio desulfricans, and Megasphaera elsdenii (37). The accumulation of lactate occurs in the absence of these bacteria, as observed in rumen acidosis (38). As in rumen acidosis, digesta pH has a key role in the development of lactate accumulation in the large intestine (31). *n*-Butyrate production from lactate has been demonstrated in the pig model (39, 40) and in a rat model when *M. elsdenii* was orally introduced (24). In the latter experiment, specific pathogen-free (SPF) Sprague-Dawley male rats were used, and M. elsdenii was not detected at the time of delivery from the breeder (24). In our experiment, the piglets used were purchased from a commercial pig farm where no bacterial control was conducted. In our recent experiment using pigs from the same pig farm, M. elsdenii was detected at a rate 10^3 to 10^6 cells/g by a specific real-time PCR using a light-cycler (Tagano et al., unpublished). Typically, these acid-utilizing bacteria are not predominant; however, they are indigenous members of the microbiota in the large intestine of the pigs (41, 42). Therefore, n-butyrate and n-valerate production from FOS via lactate and acetate occurred in the pigs in our study. In the large intestine of humans, these acid-utilizing bacteria were detected at a rate in excess of 10^9 cfu/g (43). The lack of these bacteria such as in the commercially available rats and, therefore, the lack of the lactate metabolism in the large intestine is misleading (24), particularly concerning the effect of FOS on the physiology of the large intestine.

FOS stimulated epithelial cell proliferation (Table 2). Increases in the morphometric variables were principally correlated with the luminal *n*-butyrate and *n*-valerate concentrations (Table 3). The role of *n*-butyrate has been repeatedly emphasized in the physiology of the large intestine; it stimulates epithelial cell proliferation (13, 14), mineral and water absorption (15, 16), and mucus secretion (12). Furthermore, it has an anti-tumorigenic effect in the large intestine (22, 44, 45). On the other hand, *n*-valerate, similar to *n*-butyrate, is a better substrate for colonocyte respiration; the maximum rate of ATP production by colonocytes from *n*-valerate (28.5 mmol/min/g) is higher than that from *n*-butyrate (16.1 mmol/min/g) (46). *n*-Valerate inhibits the proliferation of human adenocarcinoma cell lines, similarly to n-butyrate (47). Therefore, the present result indicated that stimulation by FOS of the growth of mucosa in the large intestine is explained by an increased luminal concentration of not only *n*-butyrate but also *n*-valerate. It is possible for FOS to stimulate *n*-butyrate and *n*-valerate production if lactate that utilizes *n*-butyrate producers, such as *M. elsdenii*, is present in the system; otherwise, FOS induced hyperlactate production in the lumen (24).

As shown in this experiment, the beneficial roles of FOS in the physiology of the large intestine rely on the activity of intestinal microbiota. The process is complex and includes, at least, acid-utilizing bacteria, such as *M. elsdenii* (24, 38), which produce *n*-butyrate as they consume lactate.

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