Comparison of the Effects of Longer Chain Inulins with Different Degrees of Polymerization on Colonic Fermentation in a Mixed Culture of Swine Fecal Bacteria

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(Received October 2, 2013)

Summary The effects of inulin with three different degrees of polymerization (DP) on microbial dietary fiber fermentation were compared in an in vitro simulator of the porcine large bowel. The three inulin isolates had a DP of 15 (from chicory grown in Hokkaido, Japan), and 10 and 24 (from Orafti, Belgium), while cellulose was used as a control. The Lactobacillus level in the DP 10 group at 48 h and bifidobacteria levels in the DP 15 and DP 24 groups at 24 and 48 h were significantly higher than in the carbohydrate-free and cellulose groups. The SCFA concentrations in all the inulin groups were significantly higher than in the carbohydrate-free and cellulose groups at 24 and 48 h. Ammonia nitrogen concentrations in all the inulin groups were significantly lower than those in the carbohydrate-free and cellulose groups at 24 and 48 h. The three different inulin types were fermentable by gut microbiota as indicated by substantial increases in SCFA. In particular, inulin DP 15 exhibited a clear potential to be used as a prebiotic with significant increases in Lactobacillus and Bifidobacterium populations, and concomitantly propionate and butyrate productions than cellulose at the early incubation time. There was a negative correlation between SCFA and ammonia nitrogen concentrations. These results indicate DP 15 product has similar potential as a prebiotic to DP 10 or DP 24 product and showed substantial equivalence to DP 10 and DP 24 products.

Key Words inulin, in vitro batch fermentation ecosystem, Lactobacillus, Bifidobacterium, SCFA

The complex human large bowel microbiome is critical for health and its growth and metabolic activity is highly dependent on substrate availability (1, 2). Quantitatively, the major carbon and energy sources are nondigestible dietary carbohydrates (fiber), undigested proteins and host mucopolysaccharides (3, 4). The main fiber carbohydrates are non starch polysaccharides (NSP) and resistant starches (RS) which are fermented to short-chain fatty acids (SCFAs) leading to a lowering of pH, which is thought to protect against the absorption of cytotoxic and genotoxic bacterial products. Of the three major SCFAs, acetate, propionate and butyrate, the last is thought to be especially important for the maintenance of optimal colonic function and protection against serious large bowel disease in the long term.

Recently, inulin-type fructans (β-(2,1)-linked fructosyl residues) have attracted industrial and scientific attention. These fiber components are produced mostly from chicory roots and are fermented extensively by the large bowel microbiome. Fructans are classified to their degree of polymerization (DP) as oligofructose (average DP of 4, ranging from 2 to 8) or longer chain inulins (average DP≥10, ranging from 2 to 60). The latter are subdivided into granulated inulin (GR; average DP of 10, ranging from 2 to 60), and high-performance inulin (HP; average DP of 24, ranging from 10 to 60) as well as Synergy 1, a specific 1 : 1 mixture of oligofructose and the HP-inulin. It has been established that both the rate and end products of gut microbial fermentation of fructans are affected profoundly by DP (5, 6). Short-chain saccharides are likely to be fermented more rapidly than longer ones (7, 8). Previously we have shown in rats that an inulin-type fructan (inulin-Tokachi, average DP of 15, range 2–60) was fermented throughout the colon more slowly than inulin-GR (average DP of 10) but faster than inulin-HP (average DP of 24) (9). This difference translated to in differences in the number of colonic microbial species and hypolipidemic response (9).

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Comparison of the colonic effects of inulin products with different DP is difficult in humans as fermentation patterns differ along the large bowel and fecal SCFAs do not always reflect those within the gut. In vitro batch fermentation ecosystems with pure culture or mixed culture enable key questions to be answered effectively (10–12). The ability of \textit{Bifidobacterium} strains to ferment oligofructose is well known (13), but very little is known about the relationship between DP and fermentability by mixed cultures. The aim of this study is to compare the effect of three longer-chain inulins with different degrees of polymerization (average DPs of 10, 15 and 24) on fermentation and the microbial community structure using a mixed culture of swine fecal bacteria. The pig feces have been shown to be a good model inoculum for human fiber metabolism (14), in terms of the composition of the colonic flora (15).

\section*{MATERIALS AND METHODS}

\subsection*{Preparation of inulin.} Three types of inulin from chicory roots were used: Orafti GR (average DP of 10, ranging from 2 to 60; Beneo-Orafti, Tienen, Belgium), Orafti HP (average DP of 24, ranging from 10 to 60; Beneo-Orafti), and inulin-Tokachi (average DP of 15, ranging from 2 to 60; Nippon Beet Sugar Manufacturing Co., Ltd., Tokyo, Japan), which is composed of both short- and long-chain inulins (Fig. 1) (16).

\subsection*{Fecal samples and in vitro fermentation.} The carbohydrates were incubated in an inoculum prepared from fresh feces of three sows from the herd of the Field Center of Animal Science and Agriculture in Obihiro University of Agriculture and Veterinary Medicine that were fed a diet free of antibiotics. The feces were mixed with a buffer solution composed of salts and minerals to give a slurry (17). In brief the incubation system consisted of gently stirred pH-controlled small-scale fermenters (220 mL working volume) which were filled with basal nutrient broth (Difco, Sparks, MD) and gassed overnight with CO$_2$ (Fig. 2). Test substrates (inulin with different DPs obtained from chicory roots, DP 10, DP 15, DP 24, and cellulose as a control) were dissolved in this medium to a final concentration of 30 g/L, and each vessel was additionally inoculated with 20 g/L fecal slurry. A vessel without any substrate was used as the negative control. The temperature was kept at 37°C at the lower pH limit of 5.2. Samples (1 mL) were taken at 0, 12, 24, and 48 h for fecal flora, SCFAs, and ammonia nitrogen analyses.

\subsection*{Bacterial analyses.} Coliform bacteria from the fermenters were inoculated and grown for 2 d on EMB agar (Eiken Chemical Co. Ltd., Tokyo, Japan) plates at 37°C. Anaerobes, \textit{Lactobacillus}, \textit{Bifidobacterium} and \textit{Clostridium} from the fermenters were incubated for 5 d on GAM agar (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan), Rogosa agar (Merck KGaA, Daemstadt, Germany), BL agar (Eiken Pharmaceutical Co. Ltd., Tokyo, Japan), and Clostridia Count agar (Nissui Pharmaceutical Co. Ltd.) at 37°C by the gaspak method according to the procedure of Mitsuoka and colleagues (18–20).

\subsection*{SCFA analyses.} Aliquots from the fermenters were taken out into desalting water in vials without exposure to air and the suspensions were deproteinized with perchloric acid to form sodium salts of the SCFAs which were measured by the bromothymol blue (BTB) post-column method using an HPLC system (Shimadzu LC-10AD, Kyoto, Japan) with an RSpak KC-811 column (8.0 mm×300 mm, Shodex, Tokyo, Japan). Condi-
tions were as follows: eluent and flow rate, 3 mM HClO₄ with 0.7 mL/min; column temperature, 60°C; reaction reagent and flow rate, 0.2 mM BTB and 15 mM Na₂HPO₄ with 1.2 mL/min; and detector wavelength, 455 nm. The injection volume was 10 µL.

Ammonia nitrogen analyses. Ammonia nitrogen concentrations in the fermenters were measured using a commercially available kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan) according to the manufacturer’s instructions.

Statistics. All data are presented as mean and standard deviation (SD). Significant differences among treatment groups were determined by ANOVA with the Tukey test. Analyses were performed using Enterprise 3.0 software (SAS Institute, Cary, NC).

RESULTS

pH and bacteria levels

The pH of the fermenters was lowered to 5.2 at 12 h and stayed at that value until 48 h in the DP 10, DP 15 and DP 24 incubations but that of the carbohydrate-free and cellulose remained at pH 6.2 throughout the incubation time (Fig. 3). Figure 4 shows the anaerobe, coliform, Lactobacillus, and Bifidobacterium levels during incubation with the three different inulin types. There was no significant difference in anaerobe levels among the groups at 48 h but anaerobe levels in the DP 15 group were significantly (p<0.05) higher than that in the cellulose group at 12 h. Coliform levels for the three inulin types were significantly (p<0.05) lower than those in the carbohydrate-free and cellulose groups at 48 h. The coliform level of DP 10, in particular, decreased significantly (p<0.05). On the other hand, Lactobacillus and Bifidobacterium levels in the DP 10, DP 15 and DP 24 groups tended to be higher than those in the carbohydrate-free and cellulose groups throughout the incubation time (Fig. 4). In particular, the DP 15 group exhibited significant increases in Lactobacillus and Bifidobacterium populations compared to the cellulose group.

![Fig. 3. pH during in vitro fermentation at 0, 12, 24, and 48 h using no carbohydrate (.), cellulose (■), inulin DP 10 (▲), inulin DP 15 (○) and inulin DP 24 (●). Each value represents the mean and standard deviation (n=3). Mean values (a, b, c) with unlike letters at the same time point are significantly different (p<0.05), as determined by ANOVA with Tukey’s test.](image)

![Fig. 4. Bacterial populations during in vitro fermentation at 0, 12, 24, and 48 h using no carbohydrate (.), cellulose (■), inulin DP 10 (▲), inulin DP 15 (○) and inulin DP 24 (●). Each value represents the mean and standard deviation (n=3). Mean values (a, b, c) with unlike letters at the same time point are significantly different (p<0.05), as determined by ANOVA with Tukey’s test.](image)
Inulin and In vitro Fermentation

Inulin and In Vitro Fermentation

The Lactobacillus level in the DP 10 group at 12 h. The *Bifidobacterium* level in the DP 15 and DP 24 groups at 24 and 48 h were also significantly (**p** < 0.05) higher than in the carbohydrate-free and cellulose groups.

SCFA concentrations in the fermenters

Figure 5 shows the values for SCFAs produced during incubation with the three different inulin types. At 12 h, the acetic acid concentration in the DP 10 group had increased significantly (**p** < 0.05) compared to the carbohydrate-free and cellulose groups. However, there was no significant difference in the acetic acid concentrations between the DP 10, carbohydrate-free and cellulose groups at 48 h. On the other hand, the acetic acid concentration in the DP 24 group had increased significantly (**p** < 0.05) compared to the carbohydrate-free and cellulose groups at 48 h. The propionic acid concentrations in the DP 10 and DP 15 groups were significantly (**p** < 0.05) higher than in the carbohydrate-free and cellulose groups throughout the incubation time. However, there was no significant difference in the propionic acid concentrations between the DP 24, carbohydrate-free and cellulose groups at 12 h or 48 h. The butyric acid concentrations in the DP 10 and DP 15 groups were significantly (**p** < 0.05) higher than in the carbohydrate-free and cellulose groups at 12 h, and those in all the inulin groups were significantly (**p** < 0.05) higher than in the carbohydrate-free and cellulose groups at 24 h and 48 h. The changing pattern of the total SCFA concentration in all the inulin groups was similar to the pattern of the propionic acid concentration.

Ammonia nitrogen concentrations in the fermenters

The ammonia nitrogen concentration in the DP 10 group was significantly (**p** < 0.05) lower than those in the carbohydrate-free and cellulose groups at 12 h, and the ammonia nitrogen concentrations in the DP 15 and DP 24 groups were also significantly lower than in the carbohydrate-free group at 12 h (Fig. 6). The ammonia

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**Fig. 5.** Short-chain fatty acids concentrations during in vitro fermentation at 0, 12, 24, and 48 h using no carbohydrate (■), cellulose (□), inulin DP 10 (△), inulin DP 15 (■) and inulin DP 24 (■). Each value represents the mean and standard deviation (**n** = 3). Mean values (a, b, c) with unlike letters at the same time point are significantly different (**p** < 0.05), as determined by ANOVA with Tukey’s test.

**Fig. 6.** Ammonia nitrogen concentrations during in vitro fermentation at 0, 12, 24, and 48 h using no carbohydrate (■), cellulose (□), inulin DP 10 (△), inulin DP 15 (■) and inulin DP 24 (■). Each value represents the mean and standard deviation (**n** = 3). Mean values (a, b, c) with unlike letters at the same time point are significantly different (**p** < 0.05), as determined by ANOVA with Tukey’s test.
nitrogen concentrations in all inulin groups were significantly \( p<0.05 \) lower than in the carbohydrate-free and cellulose groups at 24 h and 48 h (Fig. 6).

**DISCUSSION**

In this study, we have shown that in our in vitro model, all longer chain inulins had a positive influence on the intestinal flora. SCFA and ammonia concentrations. In agreement with reports that both the rate and end products of fermentation of inulins are affected strongly by DP (5, 6), the fermentation patterns of DP 10 and DP 24 inulin isolates were differentially exhibited by swine gut microbiota during the incubation period, while DP 15 product had similar potential as a prebiotic to DP 10 or DP 24 product and showed substantial equivalence to DP 10 and DP 24 products. It might be considered as a wider distribution of the fructose dimer to that of its monomer, like that of Synergy 1, a combination of oligofructose and inulin-HP.

SCFAs exert a number of general actions on the large bowel that include lowering colonic pH, and increasing electrolyte and fluid absorption, which assists in the prevention of diarrhea (21). In this study, during the supplementation period the fermentative capacity of the microbes was increased and led to a general rise in SCFAs. The strongest increases were observed with the DP 10 and DP 15 groups, indicating high carbohydrate fermentation at 12 h. Notably, butyric and propionic acids in the DP 10 and DP 15 groups showed the highest rate of increase at 12 h. However, the total SCFAs among the three inulin groups reached similar levels at 48 h. It might be possible that longer-chain inulin was fermented more slowly (but steadily) by the microflora. Stewart and others also reported that short-chain inulin is fermented rapidly compared with longer-chain inulin (22). This is corroborated by our previous study, which also showed similar findings in animals fed DP 10, DP 15, and DP 24 inulins, where most of the low molecular weight inulin led to lower cecal SCFA concentrations than high molecular weight inulin (9). However, accumulated data from animal and in vitro studies suggest that there is considerable variation between inulin-type fructans in the rate of fermentation and the SCFAs produced. Both low and high molecular weight inulins have been reported to increase cecal SCFA levels in rats (6) but this increase is not observed in pigs (23). A previous study in pigs failed to show these changes with fructooligosaccharide, suggesting that there was a channeling of substrate to bacterial species other than probiotic ones (24). Furthermore, it is noteworthy that in this mixed culture system there were strong increases in both butyrate and propionate as well as acetate, unlike the data from animal experiments. This might be due simply to the fact that butyric and propionic acids were not absorbed and remained in the jar-fermenter, thereby steadily producing and accumulating. Similarly, some inulin-type fructans were shown to increase butyrate and propionate productions in vitro fermentation by swine fecal microflora (25). So it is possible that it is a species difference in the response to inulin supplementation.

Ito and colleagues reported that cecal Lactobacillus counts were higher for DP 4, DP 8, and DP 16, whereas those of Bifidobacterium were higher for DP 8, DP 16, and DP 23 when rats consumed inulin-type fructans (6). Regmi and others reported that some starches with low in vitro digestion rates enhanced post-ileal nutrient flow and microbial fermentation, and selectively promoted Bifidobacterium spp. in the distal gut (26). The present study showed a tendency for inulin with higher DP (DP 24=DP 15>DP 20) to support the growth of Bifidobacterium preferentially. However, that previous study demonstrated that bifidobacteria preferred oligofructose (short-chain inulin-type fructan) as a substrate for growth. Not all bifidobacteria can ferment inulin (average DP of 25) as shown in a mixed culture using Bifidobacterium strains from human feces (13). It might be possible that bifidobacteria from pigs are dissimilar to those which predominate in the human colon. In fact, it has been reported that animal strains of Bifidobacterium can metabolize inulin better than human ones (27, 28).

The present study showed a tendency for inulin with lower DP (DP 10>DP 15>DP 24) to stimulate the growth of lactobacilli relatively strongly. Our results were in accordance with those of previous studies showing that dietary inulin with lower DP predominantly increased the proportion of Lactobacillus in healthy rodents (6, 29, 30). Conversely, Vos and others showed that Synergy 1 stimulated the growth of Lactobacillus more strongly than oligosaccharide or inulin in animal models (31). In the case of Lactobacillus in vitro, we hypothesized that Lactobacillus might have a relatively low potential to metabolize inulin with higher DP. On the other hand, coliform levels with the three inulin types were significantly lower than in the carbohydrate-free and cellulose groups in this study. Recently, it was reported that genes encoding an ATP-binding-cassette-type carbohydrate transporter present in certain bifidobacteria contribute to protecting mice against death induced by Escherichia coli O157 : H7 (32). However, De Boever and others reported that the coliform level was not influenced by soy germ supplementation (33). The reason for the difference between our results and theirs (31) remains unclear; though it might be due simply to the type of supplementation.

Ammonia nitrogen concentration in the DP 10 group was significantly lower than that in the carbohydrate-free and cellulose groups at 12 h and lower in all the inulin groups than in the carbohydrate-free and cellulose groups at 24 h and 48 h. Ammonia can alter the morphology and intermediary metabolism of intestinal cells, increase DNA synthesis, and promote tumorigenesis (34). Hence, an increase in the ammonia concentration is considered to be potentially harmful to the host. Our data showed that inulin with lower DP decreased the ammonia product in the early incubation time. Ammonia nitrogen correlated negatively with the total SCFA concentrations at 24 h and 48 h of incubation (24 h: \( n=15, r=-0.894, p<0.001 \), and 48 h: \( n=15, r=-0.955, p<0.001 \)). This might be due to a decrease
in the growth of harmful ammonia-producing bacteria and an increase in the growth of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium*. On the other hand, though, there was no significant difference in indoles or skatoles compound concentrations among the groups. Those of the three inulin groups tended to be lower (p<0.1) than in the carbohydrate-free and cellulose groups during the supplementation period (data not shown). Many putrefactive compounds such as sulfur-containing compounds, indoles, aliphatic amines and phenols generated by bacterial fermentation in the colon are responsible for the malodor of flatus and feces (35). Furthermore, phenolic and indolic compounds are related to a variety of disease states in humans including initiation of cancer, malabsorption, and anemia (36). Our data revealed that during the addition of the three types of inulin the amount of odor tended to decrease.

In conclusion, the three different longer-chain inulins were fermentable by swine gut microbiota in the jar-fermenter, as measured by increased SCFA production while there was a negative correlation between SCFA and ammonia nitrogen concentrations. In particular, inulin DP 15 might have a clear potential to be used as a prebiotic with significant increases in microflora populations in parallel with increases in SCFAs. Our data suggest that a swine batch fermenter is a useful means of assessing the fermentability of fiber sources with potential health benefit.

**Acknowledgments**

This research was supported by a grant from the Regional Innovation Cluster Program (City Area Type) of the Ministry of Education, Culture, Sports, Science, and Technology of Japan. We wish to thank Dr. David Christl SU, Gibson GR, Cummings JH. 1992. Role of dietary sulphate in the regulation of methanogenesis in the human large intestine. *Digest Absorp Metabolism* **33**: 69–79.

**REFERENCES**

1) Gibson GR, Beatty EH, Wang X, Cummings JH. 1995. Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. *Gastroenterology* **106**: 975–982.
2) Christl SU, Gibson GR, Cummings JH. 1992. Role of dietary sulphate in the regulation of methanogenesis in the human large intestine. *Gut* **33**: 1234–1238.
3) Macfarlane GT, Cummings JH. 1991. The colonic flora, fermentation and large bowel digestive function. In: *The Large Intestine: Physiology, Pathophysiology and Disease* (Phillips SF, Pemberton JH, Shorter RG, eds), p 51–92. Raven Press, New York.
4) Cummings JH, Macfarlane GT. 1991. The control and consequences of bacterial fermentation in the human colon—a review. *J Appl Bacteriol* **70**: 443–459.
5) Wada T, Sugatani J, Terada E, Ohguchi M, Miwa M. 2005. Physicochemical characterization and biological effects of inulin enzymatically synthesized from sucrose. *J Agric Food Chem* **53**: 1246–1253.
6) Ito H, Takemura N, Sonoyama K, Kawagishi H, Topping DL, Conlon MA, Morita T. 2011. Degree of polymerization of inulin type fructans differentially affects number of lactic acid bacteria, intestinal immune functions, and immunoglobulin A secretion in the rat cecum. *J Agric Food Chem* **59**: 5771–5778.
7) Kok N, Roberfroid M, Delzenne N. 1996. Dietary oligofructose modifies the impact of fructose on hepatic triacylglycerol metabolism. *Metabolism* **45**: 1547–1550.
8) Kok NN, Taper HS, Delzenne NM. 1998. Oligofructose modulates lipid metabolism alterations induced by a fat diet in rats. *J Appl Toxicol* **18**: 47–53.
9) Han KH, Tsuchihiro H, Nakamura Y, Shimada K, Ohba K, Aritsuka T, Uchino H, Kikuchi H, Fukushima M. 2013. Inulin-type fructan with different degree of polymerization improves lipid metabolism but not glucose metabolism in rats fed a high-fat diet under energy restriction. *Dig Dis Sci* **58**: 2177–2186.
10) Kedia G, Vázquez JA, Charalampopoulos D, Pundiella SS. 2009. In vitro fermentation of oat bran obtained by debarring with a mixed culture of human fecal bacteria. *Curr Microbiol* **58**: 338–342.
11) Suzuki T, SatoT, Kominumi M. 1994. A dense cell retention culture system using a stirred ceramic membrane reactor system. *Biotecnol Bioeng* **144**: 1186–1192.
12) Chang HN, Lee WG, Kim BS. 1994. Cell retention culture with an internal filter module: continuous ethanol fermentation. *Biotecnol Bioeng* **41**: 677–681.
13) Rossi M, Corradini C, Amaretti A, Nicolini M, Pompei A, Zanoni S, Matteuzzi D. 2005. In vitro fermentation of fructooligosaccharides and inulin by bifidobacteria: a comparative study of pure and fecal cultures. *Appl Environ Microbiol* **71**: 6150–6158.
14) Williams BA, Mikkelsen D, Le Pauh L, Gidley MJ. 2011. In vitro fermentation kinetics and end-products of cereal arabinoxylans and (1,3; 1,4)-β-glucans by porcine feces. *J Cereal Sci* **53**: 53–58.
15) Miller ER, Ulrey DE. 1987. The pig as a model for human nutrition. *Ann Rev Nutr* **7**: 361–382.
16) Fukushima M, Nakamura Y, Lee S, Tsuchihiro H, Kobayashi Y, Kawakami S, Okada T, Shimada K, Han KH. 2010. Trends in functional sugar research—focus on prebiotic effects of functional sugar. *Digest Absorp* **33**: 202–215 (in Japanese).
17) Menke K, Steingass H. 1988. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. *Anim Res Develop* **28**: 7–55.
18) Mitsuoka T, Ohko K, Benno Y, Suzuki K, Namba K. 1976. The faecal flora of man. *Zentralbl Bakteriol Bakteriol Orig A* **234**: 219–233.
19) Mitsuoka T, Sega T, Yamamoto S. 1964. A new selective medium for Bacteroides. *Zentralbl Bakteriol Bakteriol Orig A* **195**: 69–79.
20) Mitsuoka T, Sega T, Yamamoto S. 1965. Improved methodology of qualitative and quantitative analysis of the intestinal flora of man and animals. *Zentralbl Bakteriol Bakteriol Orig A* **195**: 455–469.
21) Topping DL, Clifton PM. 2001. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiol Rev* **81**: 1031–1064.
22) Stewart ML, Timm DA, Slavin JL. 2008. Fructooligosaccharides exhibit more rapid fermentation than long-chain inulin in an in vitro fermentation system. *Nutr Res* **28**: 329–344.
23) Loh G, Eberhard M, Brunner RM, Hennig U, Kuhla S, Kleessen B, Metges CC. 2006. Inulin alters the intestinal microbiota and short-chain fatty acid concentrations in...
growing pigs regardless of their basal diet. J Nutr 136: 1198–1202.

24) Bird AR, Vuaran M, Crittenden R, Hayakawa T, Playne MJ, Brown IL, Topping DL. 2009. Comparative effects of a high-amylase starch and a fructooligosaccharide on fecal bifidobacteria numbers and short-chain fatty acids in pigs fed Bifidobacterium animalis. Dig Dis Sci 54: 947–954.

25) Smiricky-Tjardes MR, Flickinger EA, Grieshop CM, Bauer LL, Murphy MR, Fahey GC Jr. 2003. In vitro fermentation characteristics of selected oligosaccharides by swine fecal microflora. J Anim Sci 81: 2505–2514.

26) Regmi PR, Matzler-Zebeli BU, Ganzle MG, van Kempen TA, Zijlstra RT. 2011. Starch with high amylase content and low in vitro digestibility increases intestinal nutrient flow and microbial fermentation and selectively promotes bifidobacteria in pigs. J Nutr 141: 1273–1280.

27) McKellar RC, Modler HW. 1989. Metabolism of fructooligosaccharides by Bifidobacterium spp. Appl Microbiol Biotechnol 31: 537–541.

28) McKellar RC, Modler HW, Mullin J. 1993. Characterization of growth and inulinase production by Bifidobacterium spp. on fructooligosaccharides. Bifidobact Microflora 12: 75–86.

29) Hosono A, Ozawa A, Kato R, Ohnishi Y, Nakanishi Y, Kimura T, Nakamura R. 2003. Dietary fructooligosaccharides induce immunoregulation of intestinal IgA secretion by murine Peyer’s patch cells. Biosci Biotechnol Biochem 67: 758–764.

30) Sakai K, Aramaki K, Takasaki M, Inaba H, Tokunaga T, Ohta A. 2001. Effect of dietary short-chain fructooligosaccharides on the cecal microflora in gastrectomized rats. Biosci Biotechnol Biochem 65: 264–269.

31) Vos AP, Haarman M, Buco A, Govers M, Knol J, Garsen J, Stahl B, Boehm G, M’Rabet L. 2006. A specific prebiotic oligosaccharide mixture stimulates delayed-type hypersensitivity in a murine influenza vaccination model. Int Immunopharmacol 6: 1277–1286.

32) Fukuda S, Toh H, Hase K, Oshima K, Nakanishi Y, Yoshimura K, Tobe T, Clarke JM, Topping DL, Suzuki T, Taylor TD, Itoh K, Kikuchi J, Morita H, Hattori M, Ohno H. 2011. Bifidobacteria can protect from enteropathogenic infection through production of acetate. Nature 469: 543–547.

33) De Boever P, Deplancke B, Verstraete W. 2000. Fermentation by gut microbiota cultured in a simulator of the human intestinal microbial ecosystem is improved by supplementing a soya gum powder. J Nutr 130: 2599–2606.

34) Ichikawa H, Sakata T. 1998. Stimulation of epithelial cell proliferation of isolated distal colon of rats by continuous colonic infusion of ammonia or short-chain fatty acids is nonadditive. J Nutr 128: 843–847.

35) Hussein HS, Flickinger EA, Fahey GC. 1999. Pet food applications of inulin and oligofructose. J Nutr 129: 1454S–1456S.

36) Macfarlane GT, Macfarlane S. 1997. Human colonic microbiota: ecology, physiology and metabolic potential of intestinal bacteria. Scand J Gastroenterol 32: 3–9.