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ORIGINAL COMMUNICATION

Comparison of digestibility and breath hydrogen gas excretion of fructo-oligosaccharide, galactosyl-sucrose, and isomalto-oligosaccharide in healthy human subjects

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Objectives: To clarify the difference of digestibility in the small intestine among fructo-oligosaccharide (FOS), galactosyl-sucrose (GS), and isomalto-oligosaccharide (IMO) using breath hydrogen test.
Design: The first step: screening test of breath hydrogen excretion and FOS tolerance test to select the subjects. The second step: breath hydrogen test of three kinds of oligosaccharides, carried out using precautionary regulations. The ingestion order was 10 g of FOS, GS, and IMO, with increases, at 1-week interval, up to 20 g, respectively. Breath gas was collected before, at 20 min intervals from 40 to 120 min after, and at 30 min intervals from 120 min to 7 h after ingestion of test substance.
Setting: Laboratory of Public Health Nutrition, Department of Nutrition and Health Sciences, Siebold University of Nagasaki, Nagasaki, Japan.
Subjects: A total of nine males (average: age 25.7 ± 3.5 y, weight 61.9 ± 8.8 kg, height 170.0 ± 6.0 cm) and 29 females (average: 23.1 ± 7.2 y, 52.9 ± 5.3 kg, 157.5 ± 5.1 cm) from the University of Tokyo and Siebold University of Nagasaki.
Main outcome measures: Breath hydrogen excretion from end-expiratory gas.
Result: Breath hydrogen of FOS was more remarkably excreted than that of GS; that of IMO was slight; and that of AUC (10 g) was significantly different. FOS was 9768 ± 3253 ppm, GS was 3662 ± 2632 ppm, and IMO was 831 ± 1154 ppm. A dose dependence was observed at doses between 10 and 20 g of FOS and GS, and the initial time of 20 g was earlier than that of 10 g.
Conclusions: FOS was not hydrolyzed, GS was slightly hydrolyzed, and IMO was readily hydrolyzed by small intestinal enzymes. H_2 gas reflected fermentability in the large intestine.
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Introduction

Physiologically functional oligosaccharides have been actively developed and are already in use in processing foods with beneficial health effects. Nondigestible oligosaccharides, which escape digestion and absorption in the small intestine, are completely fermented by microbes in the large intestine, and are metabolized to short-chain fatty acids, carbon dioxide, hydrogen, methane, and components of microbes.

During the process of fermentation, hydrogen gas is generated (Calloway, 1966; Levitt, 1969; Michael & Levitt, 1971), and is removed from the intestine through eructation, lumen-to-blood diffusion with subsequent expiration

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through the lungs, and flatus (McIver *et al*, 1926; Blair *et al*, 1947; Stanley & Oscar, 1963; Levitt, 1969; Barr *et al*, 1984). Colonic fermentation is the only source of hydrogen production (Calloway *et al*, 1966; Levitt, 1969; Muir *et al*, 1995). Therefore, the breath hydrogen detected following administration of oligosaccharides may indicate digestibility or nondigestibility in the small intestine. Since the amount of breath hydrogen excretion depends on the amount of oligosaccharides that reach the large intestine, and may also reflect the fermentability by microbes in the large intestine, breath hydrogen test is able to become an indicator of digestibility (Calloway, 1966). In addition, the rise in breath hydrogen excretion may represent the mouth-to-cecum transit time for the head of the oligosaccharides load as it passes through the gastrointestinal tract (Bond & Levitt, 1975; Levitt *et al*, 1987; Hirakawa *et al*, 1988; Miller *et al*, 1997). Also, the amount of breath hydrogen excretion may relate to the dosage of oral administration of nondigestible oligosaccharides.

Fructo-oligosaccharide (FOS), galactosyl-sucrose (GS), and isomalto-oligosaccharide (IMO) have already been included in the Food for Specified Health Uses as prebiotics, which improve intestinal microflora by oral ingestion. However, the digestibility greatly differs among these oligosaccharides, based on experimental results using rat small intestinal enzymes. FOS is nondigestible (Oku *et al*, 1984); GS is hardly hydrolyzed (Fujita *et al*, 1991); and IMO is partly digested (Kohmoto *et al*, 1992). Further, the relation between physiological functionality and digestibility of these oligosaccharides has not yet been clarified in the case of humans.

The breath hydrogen test is a convenient, noninvasive, and widely available methodology for use in diagnosis of bacterial overgrowth and malabsorption of carbohydrate (Barr *et al*, 1978). A few studies have found dose dependence of breath hydrogen excretion owing to the oral ingestion of nondigestible olgosaccharide (Rhodes *et al*, 1979; Martin *et al*, 1985; Wursch *et al*, 1989; Hertzler *et al*, 1997; Ulla *et al*, 1999). In this study, we investigated whether breath hydrogen excretion is associated with structural features of oligosaccharides, and whether it is related to the dose, with the aim of determining the utilization or bioavailability of these oligosaccharides in humans. The objectives of this study were to clarify the differences in digestibility and absorbability among FOS, GS, and IMO, using breath hydrogen test in healthy humans, and to discuss comparatively the physiological functionality among three kinds of oligosaccharides with different structural components.

Materials and methods

Materials

FOS (purity: more than 97%) was kindly provided by Meiji Seika Kaisha, Ltd (Japan), GS (purity: more than 99%) was from Bio Research Corporation of Yokohama (Japan), and IMO (purity: more than 90.8%) was from Showa Sangyo Co., Ltd (Japan) (Table 1). All the chemicals were of analytical grade or of the best grade available.

Subjects

A total of 38 adults (nine males and 29 females) participated in this study. None of the subjects were taking antibiotics or laxatives at least 2 weeks prior to the experiment, had any history of gastrointestinal or pulmonary diseases, or had any carbohydrate malabsorption or disaccharidase deficiency. All of them were confirmed by screening test to be hydrogen gas producers. Subject characteristics are shown in Table 2.

In order to avoid the transitory diarrhea caused by high osmotic pressure, subjects with resistance to diarrhea were selected. All subjects showed low lactase activity in the breath hydrogen gas experiment by lactose ingestion. The first administration level (a single dose of 10 g) was the smallest amount of the test substance, which was then increased stepwise, at intervals of 5 or 10 g. The subjects who did not experience diarrhea in response to ingestion of more than 30 g of FOS were selected to participate in this tolerance test.

Table 1 Composition of FOS, GS, and IMO

FOS			*GS*		*IMO*	
DP-1	Fructose+glucose	1.7%	Fructose+glucose	0.3%	Glucose	3.8%
DP-2	Sucrose	1.3	Sucrose+lactose	0.43	Maltose	4.5
			Isomaltose	22.8		
			Nigerose+cojibiose	13.1		
DP-3	Kestose (GF_2)	36.9	GS	99.27	Maltotriose	0.9
					Panose	11.6
					Isomaltotriose	16.7
DP-4	Nystose (GF_3)	50.7	—	—	Isomalto-tetraose+others	17.7
DP-5	GF_4	9.4	—	—	Isomalto-pentaose+others	7.2
≥DP-6	—	—	—	—	Isomalto-hexaose+others	1.7

DP: degree of polymerization; GF_4: fructofuranosyl-nystose.

Table 2 Characteristics of the subjects

	Male (n=*9*)	*Female* (n=*29*)
Age (y)	25.7±3.5	23.1±7.2
Weight (kg)	61.9±8.8	52.9±5.3
Height (cm)	170.0±6.0	157.5±5.1

Data are expressed as mean±s.d.

Ingestion of test substances

The different challenges were spaced at least 4–7 days to dissipate completely the influence of the test substance ingested orally. The dose level of test substance used to compare the difference in breath hydrogen excretion was a single dose of 10 and 20 g, respectively, of each test substance. The test substances were dissolved in about 150 ml of tap water, then ingested orally by the subjects within less than 1–2 min.

Experimental protocol

The first breath gas was collected before ingestion of test solution, at 20 min intervals from 40 to 120 min after ingestion, and at 30 min intervals till 7 h after ingestion. One experiment was carried out according to the given scheme (Figure 1), and gastrointestinal symptoms were reported in detail during the 7 h after test substance load. Symptoms included the occurrence of flatus, distention, borborygmus, abdominal pain, and diarrhea. Their onset time was also recorded. All of the subjects were controlled under strict regulations, as follows: (1) The subjects fasted at least 12 h overnight prior to the start of the experiments. (2) A special meal made from completely digestible ingredients (cookies and a soft drink) was given both 2 h before administration of test substance and 4 h after administration. (3) Drinking was allowed at anytime, although beverages containing nondigestible carbohydrates and sugar–alcohol were prohibited. (4) Before breath collection, the oral cavity was rinsed with tap water. (5) From the preceding evening, intake of subjects was restricted in order to minimize fermentation of retained carbohydrates in the colon. (6) On the experimental day, all eating except for the special meal mentioned above, smoking, sleeping, and exercise with hyperventilation were not allowed until after the last breath collection. (7) Subjects were in a sitting posture during the experiment in a day.

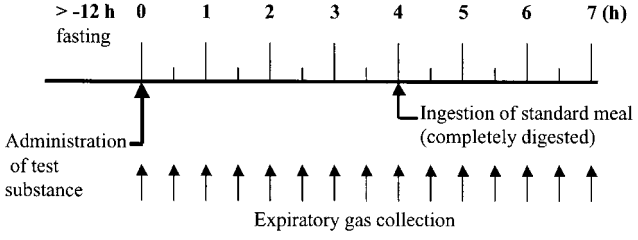

Figure 1 Experimental protocol of breath hydrogen test of oligosaccharides.

Analysis of breath gas

End-expiratory gas (750 ml) without dead space air was collected from the mouth using a commercial sampling bag (Teramecs Co. Ltd, Kyoto, Japan). Hydrogen and methane concentrations were measured simultaneously in the same gas sample on the compact gas chromatography Breath Gas Analyzer TGA2000 (Teramecs Co. Ltd, Japan).

Calculations and statistics

It was considered that initial sustained rising time of gas excretion was at least 5 ppm above basal level. The triangulated and trapezoidal areas under the hydrogen and methane concentration *vs* time curves (AUC) were accumulated for 7 h after administration. Results were expressed as means±s.d., and a *P*-value of less than 0.05 was considered significant with ANOVA or paired Student's *t*-test, using SPSS for Japan ver. 10.0 (SPSS Inc., Japan).

Ethics

The experimental protocol was approved by the respective ethics committees of the Faculty of Medicine, University of Tokyo, and that of Siebold University of Nagasaki. Each subject gave his or her informed written consent to participate in these experiments. All studies were conducted in the Laboratory of Biomedical Chemistry of the Faculty of Medicine, University of Tokyo, and the Laboratory of Public Health Nutrition, Siebold University of Nagasaki.

Results

Profiles and initial time of breath hydrogen excretion

The profiles of breath hydrogen excretion were significantly different among FOS, GS, and IMO as shown in Figure 2, when 10 g of these three oligosaccharides was orally ingested. The breath hydrogen gas after FOS ingestion was more remarkably excreted than that of GS, and breath hydrogen gas excretion after ingesting IMO was slight.

The initial time at which breath hydrogen excretion started to increase was the earliest in the case of ingestion of FOSs; increase began at 100 min after ingestion The initial time of GS occurred at 180 min after ingestion. This time was clearly slower than that of FOS. Notably, breath hydrogen excretion was not detectable after the ingestion of IMO.

Areas under the curves for 7 h (AUC) were significantly different among these three oligosaccharides, as shown in Table 3. AUC of 10 g of FOS orally ingested was 9768±3253 ppm, that of the same dose of GS was

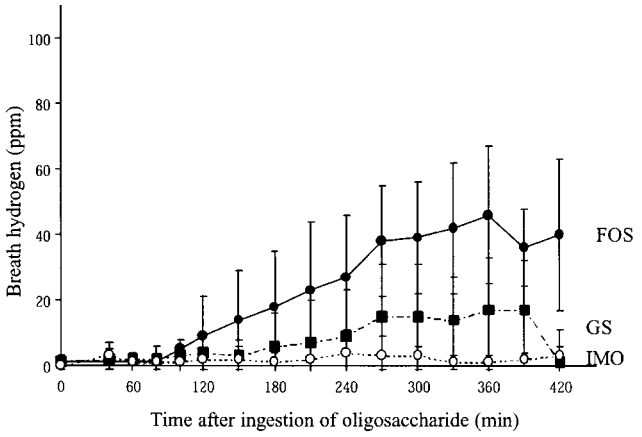

Figure 2 Profiles of breath hydrogen excretion by ingestion of three kinds of oligosaccharides. Data are expressed as mean ± s.d. of 8–13 subjects. There is a significant difference, from the AUC, of hydrogen excretion among FOS, GS, and IMO by 10 g of administration.

3662 ± 2632 ppm, and that of the same dose of IMO was 831 ± 1154 ppm.

These results support that FOS is not hydrolyzed by intestinal enzymes, and demonstrate that almost all FOSs reached the large intestine, where they were fermented by intestinal microbes. The fact that AUC of GS was smaller than that of FOS indicates that GS is partly digested in the small intestine, and the nondetectability of breath hydrogen excretion after IMO ingestion suggests that IMO is mostly digested.

Dose–response of breath hydrogen excretion

When the administration dose was increased from 10 to 20 g, AUC for breath hydrogen excretion of FOS was significantly increased from 9768 ± 3253 to 16 356 ± 4008 ppm, as shown in Table 3. Also, AUC for breath hydrogen excretion of GS was significantly increased from 3662 ± 2632 to 9820 ± 4271 ppm by increment of dosage from 10 to 20 g. Dose–response for breath hydrogen excretion was observed in the ingestion of FOS and GS, respectively. However, there was no significant difference in breath hydrogen excretion between ingestion of 10 and 20 g of IMO. The result demonstrates that almost all of IMO ingested was spontaneously digested by enzymes in the small intestine and could not arrive at the large intestine where it is fermented by intestinal microbes.

Table 3 Difference from AUC for breath hydrogen excretion among three kinds of oligosaccharides

	Administered dose (g)		
	10	*20*	*40*
Fructo-oligosaccharide	9768 ± 3253[a]	16356 ± 4008[a,b]	—
Galactosyl-sucrose	3662 ± 2632[a]	9820 ± 4271[a,b]	—
Isomalto-oligosaccharide	831 ± 1154[a]	1154 ± 1028[a]	2440 ± 20

AUC is the areas under the curve for 420 min *vs* hydrogen excretion (ppm). Data are expressed as mean ± s.d.
[a]Significant difference among FOS, GS, and IMO by ANOVA.
[b]Significant difference from 10 g dose of the same oligosaccharide by paired Student's *t*-test.

Abdominal symptoms

All of the subjects who ingested 10 g of FOS had abdominal symptoms 30–60 min after administration, and in the case of ingestion of 20 g, these abdominal symptoms were more severe. All of the subjects experienced distention and borborygmus, and some of them experienced flatus after ingestion of 20 g of FOS. When the subjects ingested 10 g of GS, which is partially hydrolyzed, they had no abdominal symptoms. However, when administration of GS was increased to 20 g, some subjects experienced distention or borborygmus. In contrast, IMO did not cause any abdominal symptoms after ingestion of 10 or 20 g.

These results demonstrate that FOS is heavily fermented and greatly produces gas in the colon, GS moderately produces gas by fermentation, and IMO hardly reaches the large intestine and does not produce gas.

Discussion

Mammalian cells do not produce hydrogen. The hydrogen is produced when nondigestible and/or nonabsorbable saccharide traverses the upper gastrointestinal tract and is metabolized by colonic bacteria (Calloway *et al*, 1966; Muir *et al*, 1995). Although more than 50% of hydrogen that is produced by fermentation in the large intestine is eliminated with flatus, the remaining hydrogen is absorbed into the bloodstream by diffusion and excreted in expiratory gas throughout the lung. Therefore, the difference in breath hydrogen excretion after the same dosage of several oligosaccharides indicates that digestibility, fermentability, and structural components differ among oligosaccharides.

Breath hydrogen gas excretion was highly variable from subject to subject, and the quantitative variability of hydrogen gas production by colonic bacteria seemed to be very large among subjects, when the same amount of test substances was ingested. However, the pattern, not quantity, of breath hydrogen gas excretion was similar among subjects. Therefore, the average of breath hydrogen excretion from each subject appears to demonstrate the digestibility and fermentability of each oligosaccharide ingested.

In this study, breath gas was collected until 7 h after the ingestion of three kinds of oligosaccharides, and then the concentration of breath hydrogen was determined. As shown in Figures 2 and 3, breath hydrogen excretion was still actively maintained up to 7 h after the ingestion of test

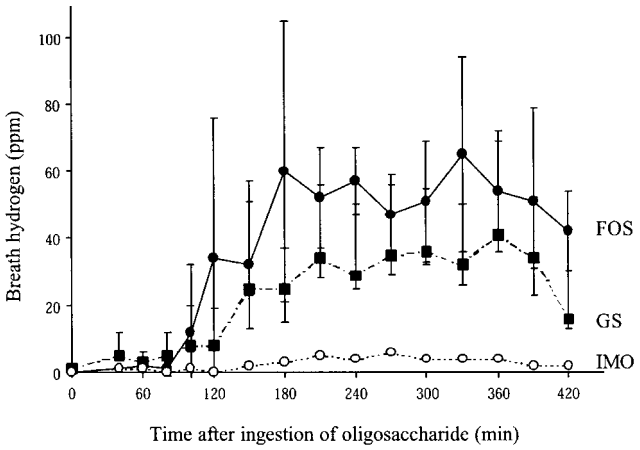

Figure 3 Comparison of breath hydrogen excretion by ingestion of three kinds of oligosaccharides. Data are expressed as mean ± s.d. of 8–13 subjects. There is a significant difference, from the AUC, of hydrogen excretion by 20 g of oligosaccharides administration.

substances. Although reasonable results were obtained in this study, the period of breath gas collection should be planned more than 7 h in order to compare the total excretion of breath hydrogen after the ingestion of three oligosaccharides. However, it was very difficult to impose restrictions on the subjects who are repeatedly requested as volunteers in the series of same experiment during long periods. The special meal of cookies and a soft drink, neither of which produces hydrogen, was given to subjects 2 h before and 4 h after the ingestion of test substance to keep the subjects from feeling hungry during the course of the present study.

Among three oligosaccharides, breath hydrogen excretion of FOS was the highest, and the second highest was that of GS, whereas IMO was hardly detected. These results seem to reflect the relative digestibility of three oligosaccharides. FOS, which has α 1–2 β bond unit of two molecules of fructose, cannot be hydrolyzed in *in vitro* assay using small intestinal enzymes of rat. In addition, it has been demonstrated that orally ingested FOS escapes digestion in the small intestine and is metabolized to carbon dioxide via short-chain fatty acids produced by colonic microbes (Tokunaga *et al*, 1986, 1989). Breath hydrogen gas excretion of GS was lower than that of FOS. GS, a trisaccharide in which galactose is bound to the glucose residue of sucrose by a β-1,4 linkage, is very slowly hydrolyzed by both sucrase and lactase of rat small intestine in *in vitro* assay. The hydrolyzing activity was situated between those of lactitol and isomaltitol in the product assay using glucose oxidase (Oku, 1997). The remnant that is not hydrolyzed in the small intestine reaches the colon, where it is fermented by colonic microbes. Therefore, it is considered that breath hydrogen excretion of GS would be lower than that of FOS.

From the results of luminal clearance from rat jejunum loops, Kaneko *et al* (1995) demonstrate that IMO is slowly digested (Kaneko *et al*, 1995). However, panose and isomaltotriose, which are the main components of IMO, are readily hydrolyzed in *in vitro* assay using rat small intestinal enzymes (Oku, 1997). Therefore, it is reasonable that breath hydrogen excretion of IMO was negligible, and far less than those of FOS and GS. As all hydrogen produced is not exhausted via flatus. Ingestion of a sufficient amount of IMO may cause the excretion of hydrogen to breath gas via the bloodstream.

Breath hydrogen excretion after the ingestion of FOS began to be detected at about 60 min, and reached a peak about 5–6 h after ingestion (Figures 2 and 3). If breath hydrogen is produced only in the large intestine, the initial time, 60 min, of breath hydrogen excretion indicates the period of transit to the large intestine. However, this period appears to be too short. FOS, which was directly administered to the stomach, takes about 3–4 h to reach the large intestine in rats (Tokunaga *et al*, 1989). The initial time of breath carbon dioxide excretion is about 1–2 h after oral ingestion of FOS (Hosoya *et al*, 1988) or maltitol (Oku *et al*, 1991) in humans. The distribution of intestinal bacteria in the gastrointestinal tract may differ between rats and humans. Probably, a portion of the oligosaccharides must be fermented by microbes during transit in the small intestine, and the majority should be actively fermented after arrival at the large intestine. The peak of breath hydrogen excretion, at 3–4 h, appears to demonstrate the maximal fermentation in the large intestine.

The initial time of breath hydrogen excretion was different among different dose levels of the same test substance, and was shorter at the higher dose level. Differences were also observed among test substances at the same dose level. The greater the amount of material that escapes digestion in the small intestine, the shorter the initial time appears to become. The initial time of ingestion of 20 g FOS was shorter than that of ingestion of 10 g. And the initial time of ingestion of 10 g was shorter in the case of FOS, which is nondigestible, than in GS, which is partially digestible. Furthermore, the initial time of breath hydrogen excretion may be affected by the fermentability of the test substance by intestinal microbes. However, the three oligosaccharides used in this experiment seem to be readily fermented by intestinal microbes; at present, the reasons for differences in fermentability among the three oligosaccharides are not clear.

In terms of abdominal symptoms, when 10 g of the test substances was ingested, FOS caused the most severe symptoms, and GS hardly caused any symptoms. These results suggest that almost all of the FOS that is orally ingested reaches the colon and is spontaneously fermented by intestinal microbes, whereas the GS that is orally ingested is partly hydrolyzed by intestinal enzymes in the small intestine.

On the other hand, the maximal permitted dosage, which does not cause transitory diarrhea with high osmotic pressure, has already been clarified for the three oligosaccharides used in this study. It is 0.34 g/kg body weight for

FOS (Hata & Nakajima, 1985); 0.6–0.8 g/kg body weight for GS, and more than 1.5 g/kg body weight for IMO (Oku & Okazaki, 1999). These maximal permitted dosages, which are closely related to digestibility, were in good reverse correlation with the amount of breath hydrogen excretion of the three oligosaccharides.

IMO consists of isomaltotriose, panose, isomaltose, and maltose, in which glucose is bound with α-1,6 or α-1-4 linkage, and glucose. Therefore, IMO ingested orally is hydrolyzed by maltase or isomaltase in the small intestine. As a result, few remnants reach the colon, and breath hydrogen excretion is hardly detected after IMO ingestion. However, increasing the administration of IMO, for example, a 40 g single dose, caused breath hydrogen excretion to increase gradually from 1 h after administration, although not by much in comparison with that after ingestion of a 20 g dose. Notably, none of the subjects had abdominal symptoms after ingestion of 10, 20, or 40 g of IMO. These findings indicate that IMO is readily digested in the human intestine.

Nondigestible oligosaccharides have some possible health benefits such as low calorie, insulin conservation, noncanogenecity, and improvement of the intestinal environment. As nondigestible oligosaccharide in the form of a prebiotics is metabolized by intestinal bacteria and produces short-chain fatty acids, the pH in the lumen of the large intestine decreases to an acidic pH (Jay *et al*, 1981; Blay *et al*, 1999). The acidic environment conduces beneficial bacteria such as *Bifidobacterium* and *Lactobacillus,* while it decreases harmful bacteria such as *Clostridum* sp (Kohmoto *et al*, 1992; Gibson *et al*, 1995; Buddington *et al*, 1996; Wolfgang *et al*, 2001). As a result, the intestinal environment is improved. It has been considered that FOS, GS, and IMO are substances of prebiotics, and they have often been used as Food for Specified Health Uses. The minimal effective dosage for improving intestinal flora is 1–3 g/day of FOS, more than 2 g/day of GS, and more than 10 g/day of IMO (Mitsuoka *et al*, 1988; Kohmoto *et al*, 1992; Ogata *et al*, 1993). However, the results obtained in this study suggest that 2 g of GS is not enough to improve the intestinal environment in healthy humans, and actually IMO cannot function as a prebiotics.

Hydrogen gas excretion was highly variable between individuals and could vary within an individual from day to day. One explanation for this is that intestinal bacteria, which are changed by eating a meal, stress, and environmental factors, etc, directly influence the production of hydrogen. Consequently, it is difficult to reproduce controlled conditions under which subjects can produce hydrogen. However, since this study was carried out with strict regulations, the results obtained offer good reliability, and constitute a useful comparison of the digestibility and fermentability of three oligosaccharides.

In conclusion, the breath hydrogen test using human subjects demonstrates that FOS is not hydrolyzed. GS is slightly hydrolyzed and IMO is readily hydrolyzed by small intestinal enzymes.

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