

# Failure of D-psicose absorbed in the small intestine to metabolize into energy and its low large intestinal fermentability in humans

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## Abstract

Experiments with rats have produced data on the metabolism and energy value of D-psicose; however, no such data have been obtained in humans. The authors assessed the availability of D-psicose absorbed in the small intestine by measuring carbohydrate energy expenditure (CEE) by indirect calorimetry. They measured the urinary excretion rate by quantifying D-psicose in urine for 48 hours. To examine D-psicose fermentation in the large intestine, the authors measured breath hydrogen gas and fermentability using 35 strains of intestinal bacteria. Six healthy subjects participated in the CEE test, and 14 participated in breath hydrogen gas and urine tests. D-Psicose fermentation subsequent to an 8-week adaptation period was also assessed by measuring hydrogen gas in 8 subjects. D-Psicose absorbed in the small intestine was not metabolized into energy, unlike glucose, because CEE did not increase within 3 hours of D-psicose ingestion (0.35 g/kg body weight [BW]). The accumulated D-psicose urinary excretion rates were around 70% for 0.34, 0.17, and 0.08 g/kg BW of ingested D-psicose. Low D-psicose fermentability was observed in intestinal bacteria and breath hydrogen gas tests, in which fructooligosaccharide (0.34, 0.17, and 0.08 g/kg BW) was used as a positive control because its available energy is known to be 8.4 kJ/g. Based on the results of the plot of breath hydrogen concentration vs calories ingested, the energy value of D-psicose was expected to be less than 1.6 kJ/g. Incremental D-psicose fermentability subsequent to an adaptation period was not observed.

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## 1. Introduction

Some rare sugars are sweet and hard to metabolize and have the potential to serve as low-energy sweeteners. Research on D-tagatose and erythritol is more advanced than that on other rare sugars; and the reported energy values are 6.3 and 1.7 kJ/g, respectively [1,2]. D-Psicose (D-ribo-2-hexulose; Chemical Abstracts Service (CAS) registration no., 551-68-8; molecular formula, C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>; molecular weight, 180.156), which is a C-3 epimer of D-

fructose, has an estimated effective available energy value of zero, based on an increase in rat body weight (BW) [3]. The Japanese Ministry of Health, Labor, and Welfare requires that energy assessments be based on data showing degradability by fermentation and digestion-absorption studies in humans for the purpose of calculating carbohydrate energy value, rather than on increase in BW. Similar assessment would be required for the energy value of D-psicose when using it as a low-calorie saccharine ingredient.

Several experiments on the absorption, distribution, metabolism, and excretion of D-psicose in rats have been reported [4,5]. Urinary excretion ranged from 15% to 25% in a study in which D-psicose was orally administered, suggesting that D-psicose is likely to be absorbed by the small intestine. Another study reported that about 98% of intravenously administered D-psicose is excreted in the urine within 6 hours. This indicates that D-psicose absorbed in the small intestine may pass into the bloodstream and be

None of the authors had any personal or financial conflicts of interest.

The study protocol and implementation complied with the Declaration of Helsinki (approval in 1964, revision in 2004) under the approval of the ethical committee of Matsutani Chemical Industry. Written consent was obtained from the subjects before the study.

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excreted in the urine, without being significantly metabolized. For fermentation of the D-psicose that escaped absorption in the small intestine and was transferred to the large intestine, it was reported that continuous administration of D-psicose increased short-chain fatty acids within the appendix, suggesting that D-psicose is fermented in the appendix to a certain extent. Around 10% of orally administered D-psicose is excreted.

As described, experiments in rats have assessed the calorie value and metabolism of D-psicose; however, there are no studies involving humans. The purpose of the present study was to clarify whether D-psicose absorbed in the small intestine is metabolized into energy and whether D-psicose that escapes absorption in the small intestine and is transferred to the large intestine is fermented by intestinal bacteria in humans. The authors conducted carbohydrate energy expenditure (CEE) tests by indirect calorimetry, measurements of D-psicose excretion rates in urine, and fermentability tests to estimate D-psicose bioavailability in humans. They conducted breath hydrogen excretion tests, which reflect the degree of fermentation [6,7], and performed in vitro fermentation tests with 35 typical intestinal bacteria strains. If D-psicose ingestion produces marked increases in breath hydrogen excretion, D-psicose may be fermentable in the large intestine. The authors used fructooligosaccharide (FOS) as a positive control to calculate the available energy from D-psicose because it is known to be a typically indigestible carbohydrate fermented by intestinal bacteria and has an available energy of approximately 8.4 kJ/g [8].

## 2. Methods

### 2.1. Subjects

Healthy male and female volunteers were recruited from Hyogo prefecture in Japan. Subjects were excluded if they were being treated for diabetes, had any notable systemic disease that would hinder performance of the study, had hepatic or renal function disorders, or were proven ineligible by a physician. Finally, 21 subjects participated in the experiments. Table 1 summarizes their background for studies 1, 2, 3, and 4.

Table 1  
Background of the subjects for studies 1 to 4

|             | Subjects of study 1           | Subjects of studies 2 and 3   | Subjects of study 4           |
|-------------|-------------------------------|-------------------------------|-------------------------------|
|             | Male, n = 3;<br>female, n = 3 | Male, n = 7;<br>female, n = 7 | Male, n = 5;<br>female, n = 3 |
| Age (y)     | 33.3 ± 3.7                    | 34.1 ± 3.1                    | 36.1 ± 8.0                    |
| Weight (kg) | 57.7 ± 10.2                   | 59.5 ± 11.9                   | 61.8 ± 11.6                   |
| Height (cm) | 166.7 ± 9.9                   | 165.4 ± 9.3                   | 169.1 ± 10.5                  |
| BMI         | 20.6 ± 2.2                    | 21.6 ± 2.4                    | 21.4 ± 2.3                    |

Data are expressed as mean ± SD. BMI indicates body mass index.

### 2.2. Ethics

The study protocol and its implementation complied with the Declaration of Helsinki (approval in 1964, revision in 2004) and were approved by the ethics committee of Matsutani Chemical Industry. Subjects were fully informed of the objective of the study, test methods, expected adverse reactions, and other related matters. Written consent was obtained from all participants before the study.

### 2.3. Protocol

Subjects were instructed to avoid excessive drinking, overeating, and excessive exercise and to follow their regular daily routine during the experimental period. The experimental methods used in this human trial have been referred to in a previous report [9].

#### 2.3.1. Carbohydrate availability of D-psicose in the small intestine (study 1)

Six subjects participated in the study 1 trial. They ingested 3 kinds of test samples containing starch hydrolysate (TK16 [trade name], Matsutani Chemical Industry), D-psicose (Rare Sugar Research Center, Kagawa University, Kagawa, Japan), or water alone at intervals of at least 1 week. Ingestion doses of starch hydrolysate and D-psicose were adjusted to 0.35 g/kg BW in a 100-mL solution, corresponding to 20 g of ingestion for subjects of average weight. These subjects consumed an alcohol-free evening meal before 9:00 PM on the day before ingestion and were not permitted to consume any food or drink other than water from the end of the evening meal to the completion of measurements. Shortly after urination and ingestion of a test sample, respiratory exchange was measured, with the subjects lying down, using a Vmax 29 (SensorMedics, Yorba Linda, CA) for 180 minutes. Their urine was collected at the end of measurement. Data from minute-by-minute measurements were averaged for 10 minutes. Standard oxygen (26%) and carbon dioxide (4%) gas accurate to within 0.1% was used for calibration. Nitrogen in urine was measured using the Dumas (combustion) method in an NC-80 nitrogen analyzer (Sumitomo Chemical Industry, Osaka, Japan). Carbohydrate energy expenditure was calculated using the Weir method, as follows: CEE (in kilojoules per minute) =  $(71.80 \times V_{CO_2}) - (50.8 \times V_{O_2}) - (44.4 \times UN)$ , where  $V_{CO_2}$  = carbon dioxide production (in liters per minute),  $V_{O_2}$  = oxygen consumption (in liters per minute), and UN = nitrogen excretion (in grams per minute) [10].

#### 2.3.2. D-Psicose excretion in urine (study 2) and D-psicose fermentability in the large intestine (study 3)

Fourteen subjects participated in studies 2 and 3. Two kinds of test samples (D-psicose or FOS [Meiji Food Material, Tokyo, Japan] as a positive control) were provided in the following doses: 0.33 g/kg BW corresponding to 20 g of ingestion for subjects of average weight (P20, D-psicose ingestion; F20, FOS ingestion), 0.17 g/kg BW corresponding

to 10 g of ingestion (P10, D-psicose ingestion; F10, FOS ingestion), and 0.08 g/kg BW corresponding to 5 g of ingestion (P5, D-psicose ingestion; F5, FOS ingestion). Noningestion was used as a negative control. Each measurement was randomly performed at intervals of at least 1 week. A time schedule for collecting breath hydrogen gas and urine as well as experimental meals is presented in Table 2. The subjects consumed an alcohol-free evening meal before 9:00 PM on the day before ingestion. They were given the following dinner menu, which did not include dietary fiber and lactic acid bacteria that influence intestinal bacteria: (1) wheat noodles and rice, (2) bowl of eel and rice, (3) baked ox tongue and rice, or (4) spaghetti with cod roe sauce. They were not allowed to consume any food or drink other than water, green tea, and restricted items from the end of the evening meal to the completion of measurement. To avoid feeling hungry, the subjects ingested experimental meals 4 and 8 hours after test sample ingestion. Both meals (2352 kJ) comprised an egg, tuna fish canned in oil, 2 pieces of processed cheese, and a soft drink; the meals contained 34 g fat, 29 g protein, and 34 g carbohydrates and were easy to digest, thus not forming hydrogen gas. End-expiratory gas was collected using a special collection bag equipped with a valve to stop backward flow after removal of dead-space gas. After initial collection of expiratory gas, subjects ingested a test sample; and end-expiratory gas was collected at 1-hour intervals for 10 hours. These end-expiratory gas samples were measured using a simple gas chromatograph (TGA-2000H breath analyzer; Laboratory for Expiration Biochemistry Nourishment Metabolism, Nara, Japan). The subjects' urine was collected using a urine collection instrument, which is used for precise proportional sampling of 1/50 of the daily (24 hours) volume of urine (Urinemate P; Sumitomo Bakelite, Tokyo, Japan), for 48 hours—divided into 3 parts (1, 0–12 hours; 2, 12–24 hours; and 3, 24–48 hours)—immediately after urination and D-psicose ingestion. The subjects were permitted to eat and drink normally once the breath hydrogen gas was collected. After desalination of urine, urinary excretion of D-psicose was assayed by high-performance liquid chromatography (column, CK08EC [Tosoh, Tokyo, Japan]; detection, differential refractometer;

column temperature, 80°C; flow rate, 0.4 mL/min; mobile phase, distilled water; sample volume, 10 µL; internal reference, 1% L-sorbose).

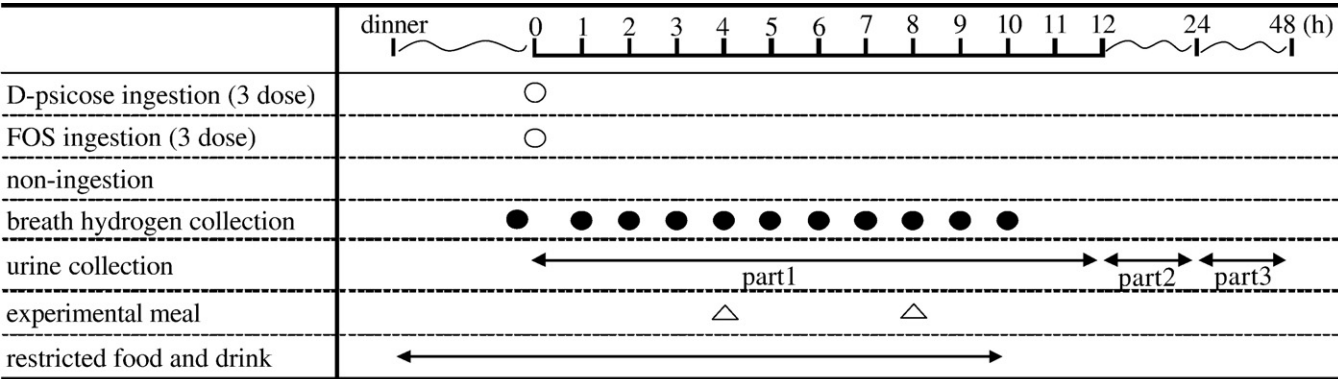
2.3.3. D-Psicose fermentability in the large intestine after an adaptation period (study 4)

Eight subjects participated in study 4. They ingested 5 g of D-psicose 3 times a day—at breakfast, lunch, and dinner—for 8 weeks. End-expiratory gas collection was conducted on the first and last day of the ingestion period in which the subjects ingested 15 g D-psicose before collection. End-expiratory gas was also collected as a blank before the ingestion period, without D-psicose ingestion. Experimental conditions and a time schedule for collecting breath hydrogen gas and experimental meals were identical to those in study 3, except that the breath hydrogen gas was collected on 8 occasions after ingestion of 15 g D-psicose. Results were expressed by subtracting the blank values from the values before and after an adaptation period.

2.3.4. D-Psicose fermentability by intestinal bacteria (study 5)

D-Psicose fermentability was assessed using 35 typical intestinal bacteria strains (6 *Bacteroides* strains, 7 *Bifidobacterium* strains, 5 *Clostridium* strains, 5 *Lactobacillus* strains, and 12 other strains) by Calpis (Tokyo, Japan). Initial cultivation was performed using a Gifu anaerobic medium (GAM) bouillon medium (Nissui Pharmaceutical, Tokyo, Japan) containing 0.4% Fildes solution and 0.15% agar at 37°C for 24 hours. These culture solutions (0.03 mL) were added to a 1.5-mL peptone-yeast-Fildes (PYF) solution containing 0.5% D-glucose or 0.5% D-psicose, or just the PYF solution. The PYF medium (pH 7.2) contained 10.0 g trypticase, 5.0 g yeast extract, 40.0 mL Fildes solution, 40.0 mL salt solution (0.2 g anhydrous CaCl<sub>2</sub>, 0.2 g MgSO<sub>4</sub>, 1.0 g K<sub>2</sub>HPO<sub>4</sub>, 1.0 g KH<sub>2</sub>PO<sub>4</sub>, 10.0 g NaHCO<sub>3</sub>, 2.0 g NaCl, and 1000.0 mL distilled water), 0.5 g L-cysteine hydrochloride hydrate, and 920.0 mL distilled water. The pH of the solutions was measured after they were anaerobically cultivated under a mixed gas consisting of 10% CO<sub>2</sub>, 10% H<sub>2</sub>, and 80% N<sub>2</sub> at 37°C for 96 hours. Fermentation was evaluated based on a decrease in pH: (–)

Table 2  
Time schedule of breath hydrogen and urine collection for studies 2 and 3



$\text{pH} = 6.0, (\pm) 5.5 = \text{pH} < 6.0, (+) 5.0 = \text{pH} < 5.5, (++) 4.5 = \text{pH} < 5.0, (+++) \text{pH} < 4.5$ .

#### 2.4. Statistical analysis

Male and female subject data were combined, and the data were calculated as mean  $\pm$  standard deviation (SD). The normality of the data was first assessed using the Shapiro-Wilks test and normal probability plot. Normally distributed variables were compared by the Dunnett multiple comparison test and the paired Student *t* test; and for nonnormally distributed variables, Wilcoxon signed rank test was used.  $P < .05$  was considered statistically significant, and analysis was performed with SPSS 13.0 J (SPSS, Chicago, IL). After subtracting water values from starch hydrolysate and D-psicose values, the area under the curve (AUC) of CEE was calculated by accumulating the triangulated and trapezoidal areas under the CEE vs time curves from 30 minutes after ingestion of a test substance, at which time subjects felt relaxed, to 180 minutes. The average respiratory quotient (RQ;  $\text{VCO}_2/\text{VO}_2$ ) was also calculated 180 minutes after ingesting a test substance. Hydrogen gas excretion results were expressed by subtracting negative control values from D-psicose and FOS values. The AUC of hydrogen gas excretion was calculated by accumulating the triangulated and trapezoidal areas under the hydrogen concentration vs time curves for 10 hours after ingestion of test substances.

### 3. Results

All subjects completed the experiments successfully.

#### 3.1. Carbohydrate availability of D-psicose in the small intestine (study 1)

After starch hydrolysate ingestion, CEE and RQ began to increase at around 30 minutes, reached a peak at about 60 minutes, and maintained the peak level until 90 minutes (Fig. 1A, B). In contrast, there was no increase in CEE and RQ after D-psicose or water ingestion. Average RQ after starch hydrolysate ingestion was 0.97; and that after D-psicose ingestion was 0.89, which was the same as that after water ingestion. The normality of the data was presumed except for a few points. Because the number of samples was limited ( $n = 6$ ), the Shapiro-Wilks test did not show the normality at a few points. The Dunnett multiple comparison test was accordingly used in this analysis. There were several significant differences in CEE and RQ between starch hydrolysate and water ingestion 30 minutes after sample ingestion. In Fig. 1C, the normality of the data was presumed; and accordingly, paired *t* test was used. The AUCs of CEE from starch hydrolysate and D-psicose ingestion were  $2.33 \pm 0.95$  and  $0.02 \pm 0.62$  kJ/min per kilogram BW, respectively; and there was a significant difference between the 2 sample ingestions ( $P < .01$ ).

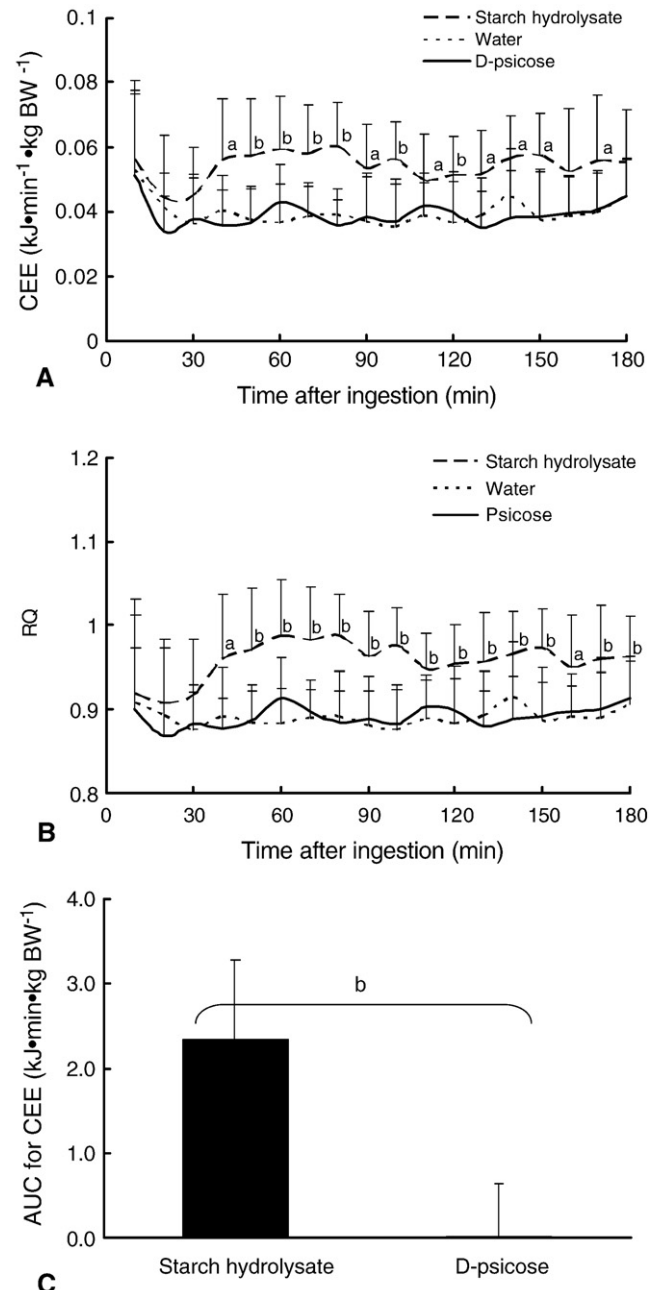


Fig. 1. Oxidative utilization of D-psicose absorbed in the small intestine. Data are expressed as mean  $\pm$  SD. There were significant differences in CEE (A) and RQ (B) between starch hydrolysate and water, and D-psicose and water, and in the AUC of CEE (C) between D-psicose and starch hydrolysate at <sup>a</sup> $P < .05$  and <sup>b</sup> $P < .01$ , respectively, by Dunnett multiple comparison test (A, B) and paired Student *t* test (C).

#### 3.2. D-Psicose excretion in urine (study 2)

Fig. 2 shows the D-psicose excretion rates in urine collected for 48 hours (1, 0–12 hours; 2, 12–24 hours; 3, 24–48 hours) after D-psicose ingestion. Excretion rates for the first 12 hours were  $54.4\% \pm 10.5\%$  for P20,  $62.9\% \pm 10.0\%$  for P10, and  $62.7\% \pm 11.2\%$  for P5; those for the next

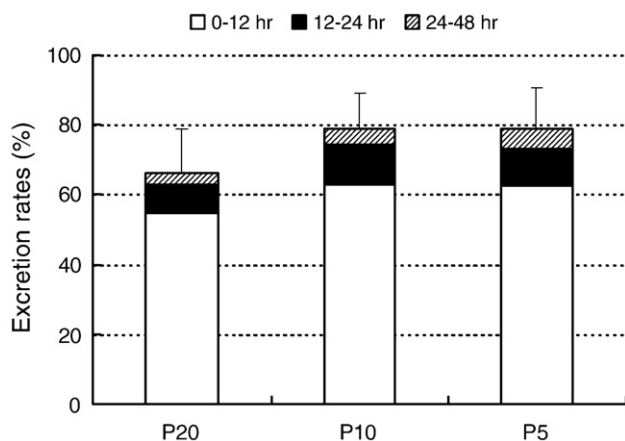


Fig. 2. Excretion rates of D-psicose in human urine for 48 hours. Data are expressed as mean  $\pm$  SD. P20, 0.33 g/kg BW D-psicose ingestion; P10, 0.17 g/kg BW D-psicose ingestion; P5, 0.08 g/kg BW D-psicose ingestion.

12 hours were  $8.4\% \pm 2.7\%$  for P20,  $11.4\% \pm 2.9\%$  for P10, and  $10.4\% \pm 3.7\%$  for P5; and those for the last 24 hours were  $3.4\% \pm 1.0\%$  for P20,  $4.3\% \pm 1.1\%$  for P10, and  $5.7\% \pm 1.9\%$  for P5. The cumulative excretion rates for 48 hours were  $66.2\% \pm 12.6\%$  for P20,  $78.6\% \pm 10.6\%$  for P10, and  $78.8\% \pm 11.7\%$  for P5.

### 3.3. D-Psicose fermentability in the large intestine (study 3)

As shown in Fig. 3, after FOS ingestion, breath hydrogen gas excretion began to increase at around 1 hour, reaching a peak at about 3 or 4 hours (Fig. 3A). Gradual declines in breath hydrogen gas excretion were recognized until the end of measurement. Because the data were nonnormally distributed, the statistical difference was determined by Wilcoxon signed rank test. There were significant differences in hydrogen excretion between F20 and P20, F10 and P10, and F5 and P5. There was a dose dependency in the amount of excreted hydrogen gas, as shown in a previous report [11,12]. The AUC of FOS was  $506.9 \pm 437.5$  ppm/h for F20,  $291.0 \pm 227.5$  ppm/h for F10, and  $148 \pm 151.1$  ppm/h for F5 (Fig. 3B). In contrast, a low concentration of breath hydrogen gas was excreted after D-psicose ingestion. The AUC of D-psicose was  $56.0 \pm 91.0$  ppm/h for P20,  $26.7 \pm 33.7$  ppm/h for P10, and  $25.3 \pm 37.6$  ppm/h for P5. The AUC of breath hydrogen gas excretion after D-psicose ingestion (P20 and P10) differed significantly from that after FOS ingestion (F20 and F10) ( $P < .01$ ). A graph was plotted with AUC (parts per million per hour) of FOS along the vertical axis and the energy taken (in kilojoules) along the horizontal axis (Fig. 3C). The graph was a straight line with a slope of 3.1 ( $R^2 = 0.99$ ). D-Psicose energy values were calculated using a graph plotted for FOS based on the results (AUC) of P20, 56.0 ppm; P10, 26.7 ppm; and P5, 25.3 ppm as follows: P20, 0.89 kJ/g; P10, 0.85 kJ/g; and P5, 1.61 kJ/g.

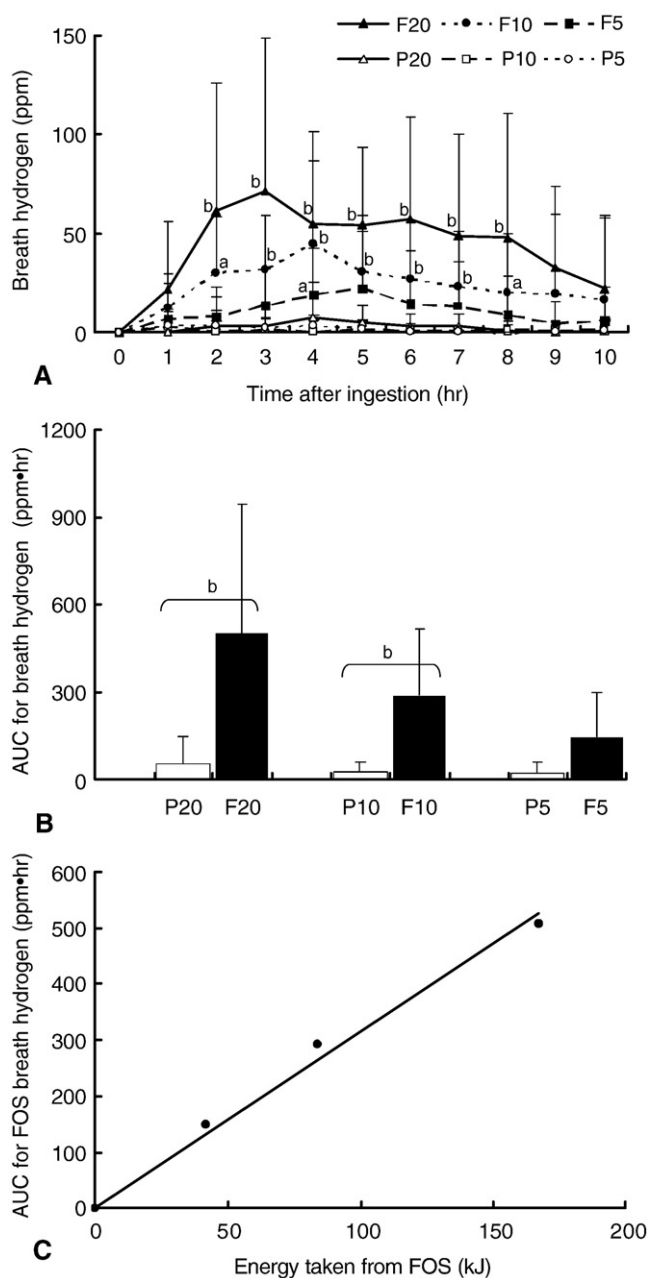


Fig. 3. Profiles of breath hydrogen excretion after oral ingestion of FOS and D-psicose. Data are expressed as mean  $\pm$  SD. P20, 0.33 g/kg BW D-psicose ingestion; P10, 0.17 g/kg BW D-psicose ingestion; P5, 0.08 g/kg BW D-psicose ingestion; F20, 0.33 g/kg BW FOS ingestion; F10, 0.17 g/kg BW FOS ingestion; and F5, 0.08 g/kg BW FOS ingestion. There were significant differences in hydrogen excretion (A) and its AUC (B) between F20 and P20, F10 and P10, and F5 and P5 at  $^aP < .05$  and  $^bP < .01$ , respectively, by Wilcoxon signed rank test. An AUC plot of FOS vs energy taken from FOS (C).

### 3.4. D-Psicose fermentability in the large intestine after an adaptation period (study 4)

Fig. 4 shows time-dependent variations in breath hydrogen gas excretion. Breath hydrogen gas excretion before and after the adaptation period increased to  $6.6 \pm$

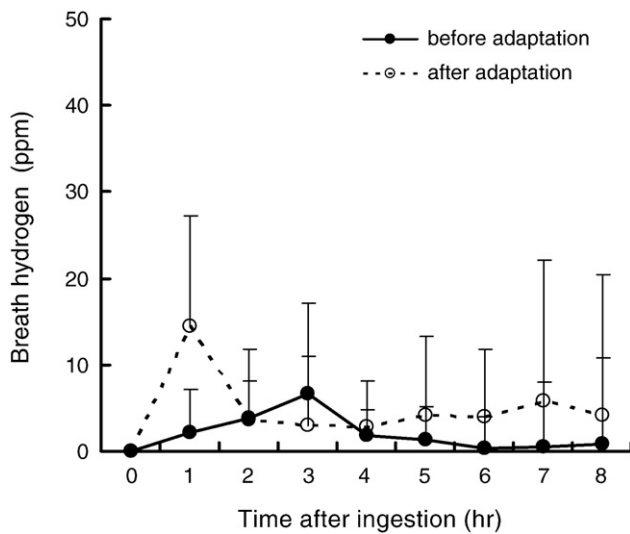


Fig. 4. Comparison of breath hydrogen excretion for 8 hours after oral ingestion of 15 g D-psicose before and after an 8-week adaptation period. Data are expressed as mean  $\pm$  SD. There was no significant difference in the breath hydrogen excretion after D-psicose ingestion before and after the adaptation period by Wilcoxon signed rank test.

10.5 ppm 3 hours after ingestion and to  $14.5 \pm 12.8$  ppm 1 hour after ingestion. Marked increases in breath hydrogen gas excretion before and after the adaptation period were not recognized until the end of measurement. Because the data were nonnormally distributed, the statistical difference was determined by Wilcoxon signed rank test. There was no significant difference in breath hydrogen excretion after D-psicose ingestion before and after the adaptation period.

### 3.5. D-Psicose fermentability by intestinal bacteria (study 5)

Table 3 shows the pH and estimate of D-psicose fermentability by intestinal bacteria. D-Psicose fermentability was not recognized in 31 of 35 strains, whereas the other 4 strains showed low fermentability.

## 4. Discussion

It has been specifically deemed reasonable to use the following formula to calculate the energy value of indigestible nonabsorbable sugars according to fermentation-based degradation: the energy value of indigestible nonabsorbable sugar (in kilojoules per gram) = [available carbohydrate ratio (ACR)  $\times$  16.7 (in kilojoules per gram)] + [fermentable carbohydrate ratio (FCR)  $\times$  8.4 (in kilojoules per gram)]. Available carbohydrate ratio is the metabolism ratio after digestion and absorption in the small intestine, and FCR is the fermentation and degradation ratio in the large intestine by intestinal bacteria. To estimate ACR and FCR, researchers are currently conducting studies in which ACR and FCR are estimated for overall assessment [13]. The authors calculated the approximate ACR and FCR values from the absorption capacity and metabolism of absorbed

Table 3

D-Psicose fermentability by various intestinal bacteria

| Bacterial strain                                        | Control |          | D-Glucose |          | D-Psicose |          |
|---------------------------------------------------------|---------|----------|-----------|----------|-----------|----------|
|                                                         | pH      | Judgment | pH        | Judgment | pH        | Judgment |
| <i>Bacteroides distasonis</i> GAI# 5462 <sup>T</sup>    | 6.9     | –        | 5.3       | +        | 6.9       | –        |
| <i>Bacteroides fragilis</i> GAI# 5524 <sup>T</sup>      | 7.0     | –        | 4.9       | ++       | 6.7       | –        |
| <i>Bacteroides ovatus</i> JCM 5824 <sup>T</sup>         | 6.1     | –        | 5.0       | ++       | 6.1       | –        |
| <i>Bacteroides thetaiotaomicron</i> GAI# 5628           | 6.0     | –        | 5.2       | +        | 5.9       | $\pm$    |
| <i>Bacteroides uniformis</i> GAI# 5466 <sup>T</sup>     | 6.0     | $\pm$    | 5.3       | +        | 6.0       | $\pm$    |
| <i>Bacteroides vulgatus</i> GAI# 5460 <sup>T</sup>      | 7.2     | –        | 5.1       | +        | 6.4       | –        |
| <i>Bifidobacterium adolescentis</i> CIFL N0042          | 7.0     | –        | 4.3       | +++      | 6.7       | –        |
| <i>Bifidobacterium</i> CIFL N0077 <sup>T</sup>          | 7.0     | –        | 4.2       | +++      | 6.8       | –        |
| <i>Bifidobacterium bifidum</i> CIFL N0067               | 7.0     | –        | 4.2       | +++      | 6.7       | –        |
| <i>Bifidobacterium breve</i> CIFL N0078 <sup>T</sup>    | 6.9     | –        | 4.2       | +++      | 6.6       | –        |
| <i>Bifidobacterium dentium</i> CIFL N0121 <sup>T</sup>  | 6.6     | –        | 4.2       | +++      | 5.7       | $\pm$    |
| <i>Bifidobacterium infantis</i> CIFL N0050 <sup>T</sup> | 6.9     | –        | 4.2       | +++      | 6.7       | –        |
| <i>Bifidobacterium longum</i> CIFL N0051 <sup>T</sup>   | 6.9     | –        | 4.2       | +++      | 6.7       | –        |
| <i>Clostridium butyricum</i> GAI# 7503 <sup>T</sup>     | 6.6     | –        | 4.6       | ++       | 6.4       | –        |
| <i>Clostridium clostridioforme</i> GAI# 5458            | 7.1     | –        | 5.3       | +        | 7.2       | –        |
| <i>Clostridium difficile</i> CIFL N0013 <sup>T</sup>    | 7.4     | –        | 5.9       | $\pm$    | 7.4       | –        |
| <i>Clostridium paraputrificum</i> CIFL N0019            | 6.6     | –        | 5.2       | +        | 6.5       | –        |
| <i>Clostridium perfringens</i> GAI# 5526 <sup>T</sup>   | 7.2     | –        | 5.5       | +        | 7.2       | –        |
| <i>Collinsella aerofaciens</i> CIFL N0070               | 7.3     | –        | 4.8       | ++       | 7.3       | –        |
| <i>Eggerthella lenta</i> CIFL N0059                     | 7.3     | –        | 7.2       | –        | 7.4       | –        |
| <i>Eubacterium limosum</i> CIFL N0104 <sup>T</sup>      | 6.7     | –        | 4.9       | ++       | 6.6       | –        |
| <i>Mitsuokella multiaida</i> CIFL N0111 <sup>T</sup>    | 6.4     | –        | 4.3       | +++      | 6.0       | –        |
| <i>Propionibacterium acnes</i> GAI# 5468 <sup>T</sup>   | 7.2     | –        | 4.8       | ++       | 7.1       | –        |
| <i>Ruminococcus productus</i> JCM 1471 <sup>T</sup>     | 6.1     | –        | 5.1       | +        | 5.8       | $\pm$    |
| <i>Veillonella parvula</i> GAI# 5602 <sup>T</sup>       | 7.6     | –        | 7.2       | –        | 7.4       | –        |
| <i>Enterococcus faecalis</i> CIFL A0013                 | 6.6     | –        | 4.2       | +++      | 6.3       | –        |
| <i>Escherichia coli</i> CIFL A0008                      | 7.3     | –        | 4.9       | ++       | 7.4       | –        |
| <i>Klebsiella pneumoniae</i> CIFL A0003 <sup>T</sup>    | 7.2     | –        | 4.9       | ++       | 7.1       | –        |

(continued on next page)

Table 3 (continued)

| Bacterial strain                             | Control |          | D-Glucose |          | D-Psicose |          |
|----------------------------------------------|---------|----------|-----------|----------|-----------|----------|
|                                              | pH      | Judgment | pH        | Judgment | pH        | Judgment |
| <i>Lactobacillus acidophilus</i> CIFL A0038  | 6.6     | –        | 4.0       | +++      | 6.4       | –        |
| <i>L. casei</i> CIFL A0039 <sup>T</sup>      | 6.6     | –        | 3.9       | +++      | 6.2       | –        |
| <i>L. fermentum</i> JCM 1173 <sup>T</sup>    | 7.3     | –        | 4.7       | ++       | 7.3       | –        |
| <i>L. gasseri</i> JCM 1131 <sup>T</sup>      | 6.6     | –        | 3.9       | +++      | 6.3       | –        |
| <i>L. salivarius</i> CIFL A0041 <sup>T</sup> | 7.0     | –        | 4.9       | ++       | 6.5       | –        |
| <i>Serratia marcescens</i> CIFL A0007        | 6.7     | –        | 5.2       | +        | 6.9       | –        |
| <i>Staphylococcus aureus</i> CIFL A0012      | 6.8     | –        | 4.8       | ++       | 6.7       | –        |

Fermentation was evaluated based on a decrease in pH: (–) pH = 6.0, (±) 5.5 = pH < 6.0, (+) 5.0 = pH < 5.5, (++) 4.5 = pH < 5.0, (+++) pH < 4.5.

D-psicose in the small intestine and the fermenting capacity in the large intestine, respectively. They estimated the D-psicose energy value based on the results.

D-Glucose is a carbohydrate that is absorbed in the small intestine, stimulates insulin secretion, and is then used quickly as energy. The energy expenditure can be measured by indirect calorimetry. Therefore, CEE is considered to increase until about 3 hours after ingestion, when part of the D-psicose is absorbed in the small intestine, metabolized, and then used as energy. As a preliminary test with rats (6 male Sprague-Dawley rats [Jcl:SD]), the authors measured respiratory exchange for 19 hours after oral ingestion of glucose, D-psicose, or water. The glucose and D-psicose dose was 2.1 g/kg BW, which was thought to be the upper limit before resulting in loose stools. The result showed a sharp peak in incremental CEE (0.054 kJ/min) at 1 hour only after glucose ingestion; the increase returned to the initial level after 2 hours, and then CEE stayed constant. There were no changes in CEE until 19 hours after ingestion of water or D-psicose. The CEE measurement in humans in this experiment also indicated the same variable trends as animal experiments. For humans, the no-observed-effect level (NOEL) for a laxative effect is 0.55 g/kg BW. The 0.35–g/kg BW dose used in the present study is considered to be appropriate (or the upper limit) as a concentration that does not produce abdominal effects or cause diarrhea [14]. These results suggest that D-psicose is not metabolized into energy when consumed by humans at a typical dose because the present study on rats and humans did not detect any increase in CEE by D-psicose absorbed through the small intestine, even at the upper limit of concentration. It has also been reported that D-psicose does not affect net blood glucose or insulin levels after ingestion [15].

With regard to urinary excretion, study 2 revealed an excretion of 66.2% in P20 at the lowest and a high excretion (of around 80%) with low doses. In reality, 20 g of D-psicose would not be consumed at one time because it is about 70% as sweet as sugar. Therefore, a large part of the ingested

D-psicose would be absorbed in the small intestine; and the remaining 20% or so would pass to the large intestine. However, because the subjects had 2 experimental meals in the first 8 hours after D-psicose ingestion and subsequently resumed their normal dietary habits, the effect of diet on absorption rates in the small intestine needs to be considered. Excretion rates of D-psicose under fasting conditions are probably different from the results of this experiment, and this needs to be explored further.

The urinary excretion rates (absorption rates in the small intestine) of D-psicose would be an appropriate value when comparing the NOEL for laxative effects because there tend to be higher indigestible carbohydrate absorption rates in the small intestine with a higher NOEL. In particular, for sorbitol, which is difficult for the small intestine to absorb, the NOEL is 0.15 to 0.17 g/kg BW for males and 0.24 to 0.3 g/kg BW for females [16]. For erythritol, which is absorbed approximately 90% in the small intestine, the NOEL is 0.66 g/kg BW for males and 0.8 g/kg BW for females. D-Psicose shows values close to erythritol: 0.5 g/kg BW for males and 0.6 g/kg BW for females [14,17].

With regard to the confirmation of hydrogen gas generation (study 3), Oku and Nakamura [8] reported that the excretion of hydrogen gas was confirmed within about 1.5 and 2.5 hours and that the level peaked 4 and 5 hours after ingestion of 20 and 5 g of FOS, respectively, and the generation continued for 8 hours. In the present study, breath hydrogen gas excretion was remarkable and continued for around 8 hours after FOS ingestion, although the concentration of hydrogen decreased after the peak. Fructooligosaccharide is therefore considered a positive control for assessing D-psicose fermentability. In this breath hydrogen test, subjects susceptible to diarrhea, as revealed in a brief interview, were excluded; therefore, ingested D-psicose did not cause diarrhea. However, 1 subject who ingested 20 g FOS experienced mild diarrhea but was not excluded on this basis. Because no subject excreted more than 10 ppm methane gas, no particular corrections to breath hydrogen concentration were made. As a result, because breath hydrogen gas is produced only by bacterial fermentation in the intestine [6–8], low breath hydrogen excretion, even when an adaptation period was set, indicates the evidence described below. The results of study 2 suggest that a large quantity of D-psicose is absorbed in the small intestine and is not transferred into the large intestine and that the small quantity of D-psicose that arrives in the large intestine is not readily fermented by intestinal bacteria. Unchanged pH in the culture (study 5) indicated that D-psicose was not fermented by typical intestinal bacteria, which complements the result of the breath hydrogen gas tests with D-psicose.

Generation of hydrogen gas in P20 was slightly higher than that in P10 and P5, indicating that slightly fermented D-psicose escaped absorption in the small intestine at the higher dose. This dose-dependent fermentation can be observed with isomaltooligosaccharide, which is more easily digestible than FOS [18].

The energy conversion factor differs from one country to another and continues to be a matter of debate. In Japan, Oku [13] recommended calculating the indigestible carbohydrate energy conversion factor in kilocalories per gram by rounding off to zero decimal places. Thus, the energy conversion factor can be estimated to be 0 kcal/g at maximum (even if 0.39 kcal/g [1.61 kJ/g] is used) when D-psicose is consumed.

There is a difference in D-psicose fermentation capacity and urinary excretion results between animal tests and the present study in humans. The contributing factors to the differences in urinary excretion between species in terms of absorption remain unknown, although excretion in our present study in humans was higher than the previously reported results in rats (15%–25%) [4,5]. With regard to fermentation capacity, short-chain fatty acids were generated in rats that consumed feed containing 10% or more of D-psicose [5]. When we maintained rats and added approximately 3% D-psicose for 3 weeks, data showing fermentation capacity, such as increased weight of the appendix and decreased hydrogen ion concentration of gut contents, were not indicated. Although the results may vary with feed administration methods and doses, D-psicose would be slightly fermented in the human large intestine at concentrations consumed daily by humans in a normal state (10 g of D-psicose [3%] to carbohydrate at 300 g/d).

However, it was reported that about 25% of the D-tagatose, an isomer of D-psicose, was absorbed and about 5% was excreted in urine [1,19]. About 20% of the D-tagatose absorbed was metabolized as CO<sub>2</sub> through the same pathway as fructose, whereas 98% or more of the D-psicose was excreted in urine when it was directly ingested into the venous bloodstream [4]. D-Tagatose fermentation is common among lactic acid bacteria, such as *Lactobacillus*, *Enterococcus*, and *Lactobacillus* strains with no D-psicose fermentation, whereas *Bacteroides*, which does not ferment D-tagatose, ferments D-psicose to some extent [20]. Furthermore, D-tagatose fermentation is affected by intestinal bacteria adaptation [21]. In this adaptation experiment after D-psicose ingestion, breath hydrogen excretion after an adaptation period did not significantly increase compared with that before the adaptation period. The duration of the adaptation period was thought to be sufficient. For example, resistant starch fermentation increased after 28-day adaptation [22]. Incremental fermentation of D-tagatose has also been recognized after an 18-day adaptation period [21]. A 15-g intake dose of indigestible sugars other than D-psicose, such as FOS, causes hydrogen excretion [8]. At present, the reason for these differences in ketose metabolism is unexplained. It is presumed that the enzyme dissimilating D-psicose was not induced for the D-psicose adaptation period. One possible reason may stem from the different reaction velocities of the enzymes, which are dependent on sugar molecule configuration or conformation. For example, some studies have reported differences in glucose-metabolizing enzyme reaction velocity of many sugars; the cleavage

rate of D-tagatose by aldolase was approximately half as high as that of D-fructose [23,24]. Fructokinase phosphorylates a large number of sugars with different reaction velocities, and the  $K_m$  value for D-psicose is lower than that of D-tagatose [25]. Furthermore, 6-phosphofructo-2-kinase catalyzes the phosphorylation of several phosphorylated sugars with different reaction velocities [26]. Another reason may be a difference in membrane permeability related to sugar transport, which is unclear and may be the next issue to elucidate D-psicose metabolism.

A useful way of preventing obesity and lifestyle-related diseases is to use a sweetener with fewer calories. There are many types of bulk sweeteners with low caloric values; but there are relatively few options because of sweetness, taste, and the upper consumption limit. D-Psicose has the potential to serve as a functional sweetener; it is sweet, has a mild taste, and has a recognized effect for improving glucose tolerance [15]. It will be necessary to clearly identify the mechanism of biochemical metabolism while considering food applications with the aim of preventing lifestyle-related diseases or offering dietary therapies.

## References

- [1] Lu Y, Levin GV, Donner TW. Tagatose, a new antidiabetic and obesity control drug. *Diabetes Obes Metab* 2008;10:109–34.
- [2] Noda K, Nakayama K, Oku T. Serum glucose and insulin levels and erythritol balance after oral administration of erythritol in healthy subjects. *Eur J Clin Nutr* 1994;48:286–92.
- [3] Matsuo T, Suzuki H, Hashiguchi M, Izumori K. D-Psicose is a rare sugar that provides no energy to growing rats. *J Nutr Sci Vitaminol* 2002;48:77–80.
- [4] Whistler RL, Singh PP, Lake WC. D-Psicose metabolism in the rat. *Carbohydr Res* 1974;34:200–2.
- [5] Matsuo T, Tanaka T, Hashiguchi M, Izumori K, Suzuki H. Metabolic effects of D-psicose in rats: studies on faecal and urinary excretion and caecal fermentation. *Asia Pac J Clin Nutr* 2003;12:225–31.
- [6] Muir JG, Lu ZX, Young GP, Cameroun-Smith D, Collier GR, O'Dea K. Resistant starch in the diet increases breath hydrogen and serum acetate in human subjects. *Am J Clin Nutr* 1995;61:792–9.
- [7] Rumessen JJ. Hydrogen and methane breath tests for evaluation of resistant carbohydrates. *Eur J Clin Nutr* 1992;46:77–90.
- [8] Oku T, Nakamura S. Evaluation of available energy of several dietary fiber materials based on the fermentability from breath hydrogen excretion in healthy human subjects. *J Jpn Assoc Diet Fiber Res* 2005;9:34–46.
- [9] Goda T, Kajiya Y, Suruga K, Tagami H, Livesey G. Availability, fermentability, and energy value of resistant maltodextrin: modeling of short-term indirect calorimetric measurements in healthy adults. *Am J Clin Nutr* 2006;83:1321–30.
- [10] Weir JBV. New methods for calculating metabolic rate with special reference to protein metabolism. *J Physiol* 1949;109:1–9.
- [11] Oku T, Nakamura S. Comparison of digestibility and breath hydrogen gas excretion of fructo-oligosaccharide, galactosyl-sucrose, and isomalto-oligosaccharide in healthy human subjects. *Eur J Clin Nutr* 2003;57:1150–6.
- [12] Nakamura S, Oku T, Ichinose M. Bioavailability of cellobiose by tolerance test and breath hydrogen excretion in humans. *Nutr* 2004;20:979–83.
- [13] Oku T. Available energy of nondigestible and/or nonabsorbable saccharides. *J Soc Nutr Diet* 1996;54:143–50.

- [14] Iida T, Kishimoto Y, Yoshikawa Y, Okuma K, Yagi K, Matsuo T, et al. Estimation of maximum non-effective level of D-psicose in causing diarrhea in human subjects. *J Adv Food Ingrid* 2007;10:15-9.
- [15] Iida T, Kishimoto Y, Yoshikawa Y, Hayashi N, Okuma K, Tohi M, et al. Acute D-psicose administration decreases the glycemic responses to an oral maltodextrin tolerance test in normal adults. *J Nutr Sci Vitaminol* 2008;54:511-4.
- [16] Koizumi N, Fujii N, Ninomiya R, Inoue Y, Kagawa T, Tsukamoto T. Study on transitory laxative effects of sorbitol and maltitol. Estimation of 50% effective dose and maximum non-effective dose. *Chemosphere* 1983;12:45-53.
- [17] Oku T, Okazaki M. Laxative threshold of sugar alcohol erythritol in human subjects. *Nutr Res* 1996;16:577-89.
- [18] Kohmoto T, Fukui F, Takaku H, Mitsuoka T. Dose-response test of isomaltooligosaccharides for increasing fecal bifidobacteria. *Agric Biol Chem* 1991;55:2157-9.
- [19] Laerke HN, Jensen BB. D-Tagatose has low small intestinal digestibility but high large intestinal fermentability in pigs. *J Nutr* 1999;129:1002-9.
- [20] Hans B, Hans A, Michael T. Fermentation of D-tagatose by human intestinal bacteria and daily lactic acid bacteria. *Microb Ecol Health Dis* 2001;13:87-95.
- [21] Laerke NH, Jensen BB, Hojsgaard S. In vitro fermentation pattern of D-tagatose is affected by adaptation of the microbiota from the gastrointestinal tract of pigs. *J Nutr* 2000;130:1772-9.
- [22] Henningsson AM, Margareta E, Nyman GL, Bjorch IME. Influences of dietary adaptation and source of resistant starch on short-chain fatty acids in the hindgut of rats. *Br J Nutr* 2003;89: 319-27.
- [23] Rognstad R. Pathway of gluconeogenesis from tagatose in rat hepatocytes. *Arch Biochem Biophys* 1982;218:488-91.
- [24] Rognstad R. Gluconeogenesis from D-tagatose by isolated rat and hamster liver cells. *FEBS Lett* 1975;52:292-4.
- [25] Raushel FM, Cleland WW. Bovine liver fructokinase: purification and kinetic properties. *Biochem* 1977;16:2169-75.
- [26] Pilkis SJ, Pilkis J, EL-Maghrabi MR, Claus TH. The sugar phosphate specificity of rat hepatic 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase. *J Biol Chem* 1985;260:7551-6.