Caecal fermentation and energy accumulation in the rat fed on indigestible oligosaccharides

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The energetic contribution from, and effects on the gastrointestinal tract of, indigestible oligosaccharides in growing rats were compared with those of sucrose (S). S and two types of oligosaccharides, fructo-oligosaccharide (Fru) and 6′-galacto-oligosaccharide (Gal) were added to a basal diet at a level of 100 g/kg. The basal diet was given either *ad libitum* (group B) or at a level approximately 90 % of the *ad libitum* intakes of the Fru and Gal groups (group BR). During a 50 d feeding period, feed intake, digestibilities of nutrients, and digesta retention times using liquid (Co-EDTA) and particulate (Cr-cell-wall constituents) markers were measured. The carcass and the contents of the stomach and caecum were sampled on the last day of the experimental period. There was no significant difference in feed intake between groups other than BR. Addition of Fru and Gal to the basal diet resulted in increased crude ash digestibility and decreased crude protein and fat digestibilities. Mean retention times of digesta markers were increased by addition of Fru and Gal to the diet, and this was associated with enlargement of the caecum. Concentrations and amounts of total organic acids in the caecum were higher in groups Fru and Gal than the other groups. The amount of energy accumulated in the carcass of rats in the Gal group was significantly ($P < 0{\cdot}05$) lower than that of rats fed on S but not Fru. Contributions to energy accumulation tended to be different between Fru and Gal; these were associated with differences in composition of caecal organic acids and of fatty acids in body fat.

Oligosaccharides: Caecum: Fermentation: Energy contribution

Unabsorbable carbohydrates (non-starch oligo- and polysaccharides) escape digestion in the rat small intestine and reach the large intestine, where they are fermented mainly to short-chain fatty acids (SCFA) and lactic acid. SCFA are readily absorbed (Hagen & Robinson, 1953) and utilized as a source of energy in epithelial cells of the large intestine (Henning & Hird, 1972; Roediger, 1980) and in the liver (Demigné *et al.* 1986; Schumann *et al.* 1991).

Fructo-oligosaccharides and galacto-oligosaccharides are classified as indigestible oligosaccharides because of their resistance to digestion in the small intestine. These oligosaccharides are metabolized mainly to SCFA by intestinal micro-organisms, and the SCFA are absorbed and further utilized in body tissues (Hosoya *et al.* 1988; Matsumoto *et al.* 1993).

In the present study we estimated the contribution to energy accumulation of these indigestible oligosaccharides, which enter the intermediary metabolism of the animal via microbial fermentation in the large intestine. The energy supplied by each oligosaccharide can be expected to vary, depending on its fermentability and the metabolic pathways involved in the intermediary metabolism of each organic acid absorbed. Therefore, the estimation of the energy contribution of indigestible oligosaccharides must be based on biological assay.

The purpose of the present study was to determine the relative energy contribution of indigestible but fermentable oligosaccharides by measuring energy accumulation in rats fed on different oligosaccharides. Energy contributions were compared with that of the easily digestible disaccharide sucrose, which is commonly used as a sweetening agent.

Materials and methods

Animals and feeding

Male growing Wistar rats with an initial mean body mass of 70 g were used. The rats were randomly divided into five groups of seven individuals. They were kept individually in wire-mesh cages in an air-conditioned room maintained at 23 ± 1° with a constant light–dark cycle (14 h light : 10 h dark). At the beginning of the experiment, two rats, which were the heaviest and the lightest among the rats used for the feeding trial, were killed by exsanguination under

Abbreviations: Fru, fructo-oligosaccharide mixture; Gal, 6′-galacto-oligosaccharide mixture; S, sucrose; SCFA, short-chain fatty acids.

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Table 1. Composition of the basal diet (g/kg) on an air-dry basis

Ingredients	
Wheat starch	483
Milk casein	250
Cellulose powder	100
Soyabean oil	80
Mineral mix*	60
Vitamin mix†	20
DL-Methionine	4
Choline bitartrate	3
Analysis	
DM	909
Crude protein (N × 6·25)	227
Crude fat	82
Crude ash	41
Energy (kJ/g)	18·6

* Composition (g/kg mixture): 145·6 $CaHPO_4 \cdot 2H_2O$, 257·2 KH_2PO_4, 93·5 $NaH_2PO_4 \cdot H_2O$, 46·6 NaCl, 350·9 Ca-lactate, 31·4 Fe-citrate, 71·7 $MgSO_4$, 1·1 $ZnCO_3$, 1·2 $MnSO_4 \cdot 6H_2O$, 0·3 $CuSO_4 \cdot 5H_2O$, 0·1 KI.

† Composition (mg/kg mixture): 1000 retinol acetate, 2·5 cholecalciferol, 1200 thiamin hydrochloride, 4000 riboflavin, 800 pyridoxine hydrochloride, 0·5 cyanocobalamin, 30 000 ascorbic acid, 5000 tocopherol acetate, 5200 menadione, 20 D-biotin, 200 pteroylmonoglutamic acid, 5000 calcium pantothenate, 50 000 *p*-aminobenzoic acid, 60 000 nicotinic acid, 6000 inositol, 200 000 choline chloride, 730 577 cellulose powder.

anaesthesia. Their energy contents were analysed to calculate the initial energy contents of the rats fed on the experimental diets in order to estimate energy accumulation in the body after the feeding trial.

The composition of the basal diet is shown in Table 1. The basal diet contained no oligosaccharides. Sucrose (S) and two types of oligosaccharides, fructo-oligosaccharide (a mixture (g/g) of 0·445 1-kestose, 0·442 nystose and 0·073 1^F-β-fructofuranosyl nystose, 0·035 sucrose, 0·005 glucose and 0·011 moisture; Meiji Seika Kaisha Ltd., Tokyo, Japan) (Fru), and 6′-galacto-oligosaccharide (a mixture (g/g) of 0·48 trisaccharides including 6′-galactosyllactose, 0·37 tetrasaccharides, 0·13 penta- and hexasaccharides, 0·01 disaccharides and under 0·05 moisture; Yakult Honsha Co. Ltd., Tokyo, Japan) (Gal) (Matusumoto *et al.* 1993) were added at a level of 100 g/kg basal diet to make three different diets. Three groups of rats (groups S, Fru and Gal) were fed on the experimental diets for 50 d. Two groups (groups B and BR) of rats were offered the basal diet. The amount of the basal diet offered to group BR was restricted to 90 % of the mean intake of groups Fru and Gal. The rats of group S consumed more than the other groups (B, Fru and Gal); therefore the food intake of group S was restricted to the mean intake of group Fru and Gal on the previous day plus 2 g. Water was provided *ad libitum* throughout. During the experimental period, food residues were collected every day and subtracted from the amount offered to calculate daily intake. Body weights were recorded twice weekly. Faeces were collected daily for a period of 7 d from day 18 of the experimental period in order to estimate digestibility.

Markers for fluid and particulate digesta were mixed with wheat starch cake and given to four rats of each group on day 31 and to the other three rats of each group on day 38 of the experimental period.

The rats were fasted for 2 h before they were given the markers, to encourage rapid ingestion of the markers. Faecal samples were collected every 2 h for the first 14 h, every 4 h for the next 24 h and every 8 h for a further 40 h after a dose.

On the last day of the experimental period, all animals were killed by exsanguination under diethyl ether anaesthesia, and then the stomach and caecum were removed, chilled and stored at −20°.

Markers

Cr-mordanted Italian ryegrass (*Lolium multiflorum* L.) cell-wall constituents and Co-EDTA were used to estimate retention times of particulate and fluid digesta respectively. Cr-cell-wall constituents prepared using the methods of Udén *et al.* (1980) were ground to a coarse particle size and passed through a 20 mesh screen. The resulting particles were then passed through a 40 mesh screen and those which remained on the screen were used in the experiment. The diameter of the particles was in the range 0·38–0·84 mm and the maximum length was shorter than 5 mm. This means that a small proportion of the particle marker was coarser than the food materials used. In another experiment, we found no difference in transit time and mean retention time between a larger particle marker, greater than 0·5 mm and smaller than 0·84 mm, that was prepared through the same procedures as those in the present experiment, and a smaller particle marker (less than 0·5 mm) in rats (E Sakaguchi, C Sakoda and Y Toramaru, unpublished results). This suggests that a small amount of such coarse material would not bias the data of digesta movement.

Analytical methods

The pooled faecal samples from the digestion trials were oven-dried at 60° and ground to under 1 mm. The ground faecal samples and foods were analysed in duplicate for moisture, N, diethyl-ether extract and crude ash (Association of Official Analytical Chemists, 1975), and for gross energy by bomb calorimetry.

To determine concentrations of Cr and Co for the calculation of retention times of digesta, faecal samples were oven-dried at 60° and ashed at 550° for 5 h. The ashed samples were treated according to the method described by Williams *et al.* (1962). Analysis of Cr and Co in the treated sample was made by atomic absorption spectroscopy (atomic absorption spectrophotometer AA-80; Nippon Jarrell-Ash, Kyoto, Japan).

Each carcass was homogenized and analysed for moisture, N, crude ash and gross energy by the same methods used for the faeces. The fatty acid composition of fat extracted from the homogenized carcass by chloroform–methanol (2 : 1, v/v) was estimated by HPLC (L-6000 Hitachi, Ltd., Tokyo, Japan) using a Cica-Merk Hiber Lichrospher 100 RP-18 (5 mm) column (4 × 250 mm; Kanto Chemical Corporation Inc., Tokyo, Japan) after esterification with 9-anthryl diazomethane as follows. A mixture of 2·0–2·5 g of the carcass and 30 ml chloroform–methanol (2 : 1, v/v) was homogenized, then filtered through a glass filter. The filtrate was added to 10 ml saline (9 g NaCl/l) and mixed gently. The upper phase was evaporated to dryness over a water bath. The fat so obtained was dissolved in 0·5 ml n-hexane and 2·0 ml ethanol at 70°, then added to 3 ml 9-anthryl diazomethane (1 g/l) in a solvent of methanol–tetrahydrofuran (tetra methylene

oxide) (1 : 1, v/v). The mixture was allowed to stand for 1 h, then methanol–ethanol–n-hexane (1 : 1 : 1, by vol.) was added, to a final volume of 10 ml. This solution was used for analysis of fatty acids. Acetonitrile was used as the eluent in the HPLC, at a flow rate of 1·5 ml/min at ambient temperature.

The concentration of organic acids in caecal contents was determined by the method described by Kikuchi & Yajima (1992) with HPLC (ICA-3000; Toa Electronics Co. Ltd., Tokyo, Japan).

Calculations

Energy accumulation in the body was estimated by subtracting energy content in the body at the beginning of the experiment from the energy content in the body at the end of the experiment. The energy content at the beginning of the experiment was calculated by multiplication of mean energy concentration (kJ/g body weight) of two rats and body weight (g) at the beginning of the experiment.

Rats can be considered to have two mixing pools of digesta, stomach and caecum. However, concentrations of Cr and Co reached a peak value rapidly after their first appearance in the faeces, then declined, following a straight line on a semilog scale, suggesting that single compartment analysis could be used to calculate mean retention times of digesta markers. Therefore, single exponential regression equations were fitted statistically to time-course declines of faecal concentrations of Cr and Co. The turnover time of each marker was estimated from the decline in faecal concentration of marker by the function (Brandt & Thacker, 1958):

$$Y = Y_0 \times e^{-kt},$$

where Y is the concentration of Cr or Co in faeces at time t, Y_0 is the constant depending on the level of Cr and Co given, k is the rate constant and t is the time interval after feeding of the markers (h). Turnover time was calculated as the reciprocal of the rate constant (k) of the exponential curve fitted to the time-course excretion values of the markers after faecal marker concentration reached a maximum. Total mean retention time in the gastrointestinal tract was calculated as the sum of the reciprocal of k and transit time, the time of first appearance of the marker after a dose. Mean retention time of digesta was calculated also by the total collection method (Coombe & Kay, 1965).

The contribution of each oligosaccharide to energy accumulation in the body was estimated using the following equations:

$$EA_{S,Fru,Gal} = ETA_{S,Fru,Gal} - ARE_{BR} \times I_{Basal},$$

$$ARE_{S,Fru,Gal} = (EA_{S,Fru,Gal}/I_{S,Fru,Gal}) \times 100,$$

where $EA_{S,Fru,Gal}$ is the energy accumulation from S, Fru or Gal; $ETA_{S,Fru,Gal}$ is the total amount of energy accumulated in the rat carcass of each group; ARE_{BR} is the energy accumulation rate ((amount of energy accumulated)/(total food intake)) in group BR rats; I_{Basal} is the intake of the basal diet ((total feed intake) × 100/110) of each group fed with a sugar or oligosaccharide; $ARE_{S,Fru,Gal}$ is the energy accumulation rate of each sugar or oligosaccharide and $I_{S,Fru,Gal}$ is the intake of each sugar or oligosaccharide ((total feed intake) × 10/100).

Statistical analysis

The data were analysed as a single factorial design and tested for statistical difference by Duncan's multiple range test (Duncan, 1955).

Results

Food intake and weight gain (Table 2)

Mean values of actual food intake (offered minus refusals and spillage) during the 50 d experimental period were almost the same in the groups of rats which were fed *ad libitum* (groups B, S, Fru, Gal). The rats which were given restricted amounts of the basal diet (group BR) consumed 89 % of that consumed by group B.

Weight gains are listed not only as gross but also as net

Table 2. Feed intake, weight gain and feed efficiency in rats fed on diets containing different oligosaccharides*
(Mean values with their pooled standard errors)

	Diet					
	B (n 7)	BR (n 7)	S (n 6)	Fru (n 7)	Gal (n 7)	SEM
Feed intake (g/50 d)	1063·4[a]	944·1[b]	1051·8[a]	1030·6[a]	1015·8[a]	25·5
Weight gain (g/50 d)						
Gross†	368·6	327·5	372·2	354·7	346·4	15·2
Net‡	364·3[a]	323·0[b]	368·1[a]	344·2[a]	337·7[ab]	15·1
Feed efficiency§ (%)						
Gross†	0·346	0·347	0·354	0·344	0·341	0·010
Net‡	0·342	0·342	0·350	0·334	0·332	0·010

B, basal diet offered *ad libitum*; BR, basal diet restricted to approximately 90 % of the intake of group B; S, diet containing 100 g sucrose/kg; Fru, diet containing 100 g fructo-oligosaccharide/kg; Gal, diet containing 100 g galacto-oligosaccharide/kg.
[a,b] Mean values within a row not sharing a common superscript letter were significantly different, $P < 0·05$.
* For details of diets and procedures, see Table 1 and pp. 469–471.
† Calculation based on whole-body weight gain.
‡ Calculation based on weight gain of carcass without the contents of the stomach and caecum, after bleeding.
§ Weight gain : feed intake.

Table 3. Apparent digestibilities (g/g), in rats, of diets containing different oligosaccharides*
(Mean values with their pooled standard errors)

	Diet					
	B (*n* 7)	BR (*n* 7)	S (*n* 6)	Fru (*n* 7)	Gal (*n* 7)	SEM
DM	0·848[a]	0·855[b]	0·865[c]	0·852[a]	0·851[a]	0·002
Organic matter	0·854[a]	0·861[b]	0·871[c]	0·856[a]	0·855[a]	0·002
Crude protein	0·940[a]	0·950[b]	0·944[a]	0·909[c]	0·915[c]	0·002
Crude fat	0·973[ab]	0·978[a]	0·968[bc]	0·960[d]	0·966[cd]	0·003
ADF	0·040	0·045	0·046	0·042	0·032	0·016
NDF	0·110[a]	0·150[b]	0·132[ab]	0·113[a]	0·127[ab]	0·014
Crude ash	0·725[a]	0·743[ab]	0·737[ab]	0·751[b]	0·756[b]	0·009
Gross energy	0·873[a]	0·880[b]	0·886[c]	0·869[a]	0·872[a]	0·002

B, basal diet offered *ad libitum*; BR, basal diet restricted to approximately 90 % of the intake of group B; S, diet containing 100 g sucrose/kg; Fru, diet containing 100 g fructo-oligosaccharide/kg; Gal, diet containing 100 g galacto-oligosaccharide/kg; ADF, acid-detergent fibre (Van Soest, 1963); NDF, neutral-detergent fibre (Van Soest & Wine, 1967).
[a,b,c,d] Mean values within a row not sharing a common superscript letter were significantly different, $P < 0·05$.
* For details of diets and procedures, see Table 1 and pp. 469–471.

values because of the variance of the weight of caecal contents between groups. The weights (g) of caecal tissue and caecal contents were 0·6 and 3·1 for group B, 0·5 and 3·2 for group BR, 0·6 and 3·0 for group S, 1·3 and 8·2 for group Fru and 1·2 and 7·0 for group Gal. Both tissue and content weights of groups Fru and Gal were significantly ($P < 0·05$) higher than those of the other groups.

The net weight gain of group BR was significantly ($P < 0·05$) smaller than those of the other groups. The rats of groups Fru and Gal gained slightly less weight than those of groups B and S, but the difference was not statistically significant.

Digestibility (Table 3)

Apparent digestibilities of DM, organic matter and gross energy were higher in group S than in the other four groups, and higher in group BR than in group B. Digestibility of crude protein was lower in groups Fru and Gal than the other three groups, and was also higher in group BR than in group B. The digestibility of crude ash was higher in groups Fru and Gal than in group B.

Digesta retention time (Table 4)

Mean retention times of digesta markers were estimated by both compartmental analysis and total collection. Addition of fructo- and galacto-oligosaccharide (groups Fru and Gal) resulted in longer retention times of digesta markers. However, there was no significant difference in transit time between any of the groups. The fluid digesta marker passed through the intestine faster than the particle digesta marker.

Table 4. Transit time (TT, h) and mean retention time (MRT, h) of digesta markers (Cr and Co) in rats fed on diets containing different oligosaccharides†
(Mean values with their pooled standard errors)

		Diet					
		B (*n* 7)	BR (*n* 7)	S (*n* 6)	Fru (*n* 7)	Gal (*n* 7)	SEM
Cr	1/*k*‡	11·4[a]	14·2[ab]	13·3[ab]	22·1[c]	18·3[bc]	2·3
	TT§	10·3*	9·3*	10·9*	12·7*	11·3*	1·4
	MRT‖	21·7[a]	23·5[a]	24·2a*	34·8[b]	29·6[ab]	2·6
	$\sum x_i t_i / \sum x_i$¶	21·9[ab]	24·1[ab]	21·1[a]	26·9[b]	26·0[ab]	2·4
Co	1/*k*	12·4[a]	15·4[a]	13·6[a]	25·3[b]	21·7[b]	2·2
	TT	5·0	3·6	4·4	4·4	5·6	1·2
	MRT	17·4[a]	19·0[ab]	18·0[a]	29·7[b]	27·3[b]	2·5
	$\sum x_i t_i / \sum x_i$	22·6[a]	24·1[ab]	22·0[a]	28·6[b]	27·1[ab]	2·4

B, basal diet offered *ad libitum*; BR, basal diet restricted to approximately 90 % of the intake of group B; S, diet containing 100 g sucrose/kg diet; Fru, diet containing 100 g fructo-oligosaccharide/kg; Gal, diet containing 100 g galacto-oligosaccharide/kg; Cr, particle marker (Cr-mordanted cell-wall constituents); Co, liquid marker, Co-EDTA.
[a,b,c] Mean values within a row not sharing a common superscript letter were significantly different, $P < 0·05$.
Mean values were significantly different from those for Co, *$P < 0·05$.
† For details of diets and procedures, see Table 1 and pp. 469–471.
‡ Turnover time (h) of the marker in the whole digestive tract (*k* is the rate constant which is the dilution rate (per h) of the marker in the digestive tract).
§ Time interval between feeding and first appearance of the marker in the faeces.
‖ Sum of 1/*k* and TT.
¶ MRT calculated using the method described by Coombe & Kay (1965), where x_i is the amount of marker excreted in the i-th collection period and t_i is the collection time (h) after marker dose.

Table 5. Body composition and energy accumulation (kJ) in rats fed on diets containing different oligosaccharides*
(Mean values with their pooled standard errors)

	Diet					
	B (*n* 7)	BR (*n* 7)	S (*n* 6)	Fru (*n* 7)	Gal (*n* 7)	SEM
Body composition						
Moisture (g/g)	0·608	0·620	0·615	0·612	0·623	0·010
Crude protein (g/g)	0·202	0·199	0·198	0·200	0·200	0·006
Crude fat (g/g)	0·141	0·132	0·134	0·147	0·136	0·012
Crude ash (g/g)	0·031	0·029	0·028	0·031	0·029	0·002
Gross energy (kJ/g)	10·46	10·17	10·71	10·71	10·08	0·33
Body energy (kJ)						
Total amount	4248^{a}	3763^{b}	4411^{ab}	4221^{a}	3893^{a}	211
Accumulation during 50 d†	3557^{ab}	3038^{c}	3684^{a}	3488^{ac}	3164^{bc}	207
Energy consumed during 50 d	19754^{a}	17538^{b}	19220^{a}	18714^{ab}	18829^{a}	532
Energy efficiency‡	0·179	0·173	0·192	0·186	0·168	0·008
Contribution of oligosaccharide to energy accumulation (kJ/g)§			6·95^{a}	5·69^{ab}	2·86^{b}	1·38

B, basal diet offered *ad libitum*; BR, basal diet restricted to approximately 90 % of the intake of group B; S, diet containing 100 g sucrose/kg; Fru, diet containing 100 g fructo-oligosaccharide/kg; Gal, diet containing 100 g galacto-oligosaccharide/kg.
^{a,b,c} Mean values within a row not sharing a common superscript letter were significantly different, $P < 0·05$.
* For details of diets and procedures, see Table 1 and pp. 469–471.
† Difference between energy content of the body at the beginning of the experiment and energy content of the body at the end of the experiment.
‡ Energy accumulated : energy consumed.
§ Energy accumulation from oligosaccharides : oligosaccharides consumed. For details of calculation, see p. 471.

Body composition and energy accumulation (Table 5)

Concentrations of moisture, crude protein, crude fat, crude ash and gross energy in the body were similar for all groups.

Rats in group BR accumulated less energy in the carcass than group B rats due to their lower energy consumption. The amount of energy accumulated in group S was significantly ($P < 0·05$) higher than that of group Gal, but not significantly higher than that of group Fru.

The estimated contributions of the sugar or oligosaccharide to energy accumulation were similar for groups S and Fru, and both were higher than group Gal.

Caecal organic acids (Table 6)

Total organic acid concentration was higher in groups Fru and Gal than in the other three groups. There were also much higher amounts of organic acids in the caecum because of both the higher concentration and the larger caecal volume of rats from groups Fru and Gal compared with the other groups. Succinic acid and lactic acid were produced much more in groups Fru and Gal than in the other groups. It is noticeable that the concentrations of each organic acid were different for groups Fru and Gal; acetic acid was much higher in group Gal, but succinic and

Table 6. Concentration (μmol/g contents) and total amount (μmol) of organic acids in the caecal digesta of rats fed on diets containing different oligosaccharides*
(Mean values with their pooled standard errors)

	Diet					
	B (*n* 7)	BR (*n* 7)	S (*n* 6)	Fru (*n* 7)	Gal (*n* 7)	SEM
Concentration						
Succinic acid	0·8^{a}	0·8^{a}	0·9^{a}	13·1^{b}	4·3^{c}	1·0
Lactic acid	4·5^{a}	4·6^{a}	6·9^{ab}	13·7^{bc}	20·1^{c}	4·0
Formic acid	0·8^{a}	0·7^{a}	0·9^{a}	2·0^{ab}	5·0^{b}	0·9
Acetic acid	41·0^{ab}	35·3^{b}	46·9^{a}	33·9^{b}	79·2^{c}	4·9
Propionic acid	12·9^{ac}	11·2^{ac}	14·6^{a}	19·6^{b}	9·2^{c}	2·2
Isobutyric acid	1·0^{a}	0·9^{a}	1·2^{a}	0·4^{b}	0·1^{b}	0·2
Butyric acid	7·9^{a}	7·4^{a}	8·5^{a}	40·0^{b}	19·2^{c}	2·7
Isovaleric acid	1·5^{a}	1·3^{a}	1·5^{a}	0·7^{b}	0·4^{b}	0·2
Valeric acid	1·2	0·9	1·2	0·6	trace	0·3
Total acids	71·5^{ab}	63·1^{a}	82·5^{b}	123·9^{c}	137·4^{c}	9·9
Acetic acid : propionic acid	3·2^{a}	3·2^{a}	3·2^{a}	2·0^{a}	8·8^{b}	0·6
Total amount						
Total acids	221·9^{a}	195·4^{a}	241·2^{a}	1005·9^{b}	969·5^{b}	81·8
Total fatty acids	205·6^{a}	179·1^{a}	218·1^{a}	788·3^{b}	803·9^{b}	68·6

B, basal diet offered *ad libitum*; BR, basal diet restricted to approximately 90 % of the intake of group B; S, diet containing 100 g sucrose/kg; Fru, diet containing 100 g fructo-oligosaccharide/kg; Gal, diet containing 100 g galacto-oligosaccharide/kg.
^{a,b,c} Mean values within a row not sharing a common superscript letter were significantly different, $P < 0·05$.
* For details of diets and procedures, see Table 1 and pp. 469–471.

Table 7. Composition (mg/g total fatty acid) of the body fat of rats fed on diets containing different oligosaccharides*
(Mean values with their pooled standard errors)

	Diet					
	B (*n* 7)	BR (*n* 7)	S (*n* 6)	Fru (*n* 7)	Gal (*n* 7)	SEM
Concentration						
Linolenic acid	183·9[a]	196·3[a]	203·6[a]	282·7[b]	188·7[a]	20·8
Linoleic acid	174·2[a]	110·3[b]	114·9[b]	82·7[b]	83·2[b]	15·5
Oleic acid	255·9[a]	215·1[b]	203·4[b]	146·1[c]	195·7[b]	16·5
Myristic acid	204·7[a]	326·6[b]	314·9[b]	385·5[b]	367·9[b]	30·9
Palmitic acid	152·3[a]	126·0[a]	138·9[a]	85·9[b]	129·1[a]	11·4
Stearic acid	28·9[ab]	25·8[bc]	24·3[bc]	18·9[c]	35·5[a]	3·4
Unsaturated : saturated	1·62[a]	1·10[b]	1·11[b]	1·06[b]	0·90[b]	0·11

B, basal diet offered *ad libitum*; BR, basal diet restricted to approximately 90 % of the intake of group B; S, diet containing 100 g sucrose/kg; Fru, diet containing 100 g fructo-oligosaccharide/kg; Gal, diet containing 100 g galacto-oligosaccharide/kg.
[a,b,c] Mean values within a row not sharing a common superscript letter were significantly different, $P < 0·05$.
* For details of diets and procedures, see Table 1 and pp. 469–471.

butyric acids were significantly ($P < 0·05$) higher in group Fru.

Fatty acid composition in body fat (Table 7)

Long-chain fatty acid composition in the body (without stomach and caecum and after bleeding) was affected especially by the addition of Fru, with higher values for linolenic and lower values for oleic, palmitic and stearic acids compared with group B. In group Gal, there were significant decreases in linoleic and oleic acids and an increase in myristic acid compared with group B. Groups BR and S showed similar fatty acid compositions. The total unsaturated fatty acids : total saturated fatty acids ratio was higher in group B than the other groups; there were no significant differences between any of the other groups.

Discussion

The weights of caecal tissue and caecal contents were greater, and the concentrations and amounts of organic acids in the caecal contents were much greater in rats fed with oligosaccharides than in rats fed on diets B and S. Similar effects of the addition of poly- and oligosaccharides such as pectin, sodium alginate, gum arabic, gum xanthan (Ikegami *et al.* 1990), fructo-oligosaccharide (Tokunaga *et al.* 1986), xylo-oligosaccharides (Imaizumi *et al.* 1991) and galactosylsucrose (Yoneyama *et al.* 1992; Hoshi *et al.* 1994) to the diet of rats on increases in the size, volume and pool size of organic acids of the large intestine have been reported previously. The change in retention times of liquid and particle digesta markers in reponse to addition of Fru and Gal is connected with an enlarged caecum which retains large amounts of digesta.

Tokunaga *et al.* (1986) showed that addition of fructo-oligosaccharide to the diet shortened digesta transit time, while in the present experiment there was no difference between basal groups and supplemented groups. Tokunaga *et al.* (1986) used a basal diet containing no fibre source; in contrast, cellulose (100 g/kg) was included in the basal diet in the present experiment. This may explain the difference in the results. Moreover, a shorter transit time does not necessarily indicate a shorter retention time of digesta.

Longer mean retention times of digesta can support more complete decomposition and utilization of indigestible oligosaccharides by microbes in the large intestine. This might be expected to increase the amount of microbial N excreted in the faeces, which could explain the lower digestibility of N in rats fed with Fru and Gal.

It has been reported that addition of fructo-oligosaccharide to the diet increases the total absorption of Ca, Mg and P in rats (Ohta *et al.* 1993) and stimulates absorption of Ca and Mg from the colon and rectum in rats (Ohta *et al.* 1995). Other indigestible carbohydrates such as resistant starch (Schulz *et al.* 1993) and inulin (Rémésy *et al.* 1993) have similar effects. These findings support the increased digestibility of crude ash by addition of Fru and Gal observed in the present study.

It is well established that fructo-oligosaccharides and galacto-oligosaccharides are mostly metabolized by intestinal micro-organisms and are utilized as an energy source. Only a small amount of ^{14}C was detected in faeces after oral administration of [U-^{14}C]fructo-oligosaccharides in rats (Tokunaga *et al.* 1989) and in man (Hosoya *et al.* 1988). This means that the oligosaccharides are almost totally absorbed and utilized after microbial degradation.

Amounts and concentrations of organic acids in the caecum were much higher in rats fed with Fru and Gal than those in rats fed on diets B and S. This is considered to result from the fermentation of Fru and Gal. Organic acids, mainly SCFA are readily absorbed, and can be utilized as an energy source and as materials for biosynthetic reactions in the animal's tissues.

Calculations by Roberfroid *et al.* (1993) on the basis of the pathway of fermentation by microbes and the metabolism of organic acids absorbed by the host animal, yielded estimates of the metabolizable energy of oligofructose of between 4·18 and 7·26 kJ/g. This is 25–40 % of the chemical energy of oligofructose. The estimation using [U-^{14}C]fructo-oligosaccharides showed that the energy utilization of fructo-oligosaccharides was 6·3 kJ/g in man (Hosoya *et al.* 1988).

The contributions to energy accumulation by Fru and Gal

in the present experiment were much less than that by S; the contribution by Fru was approximately 82 % of that of S, and that of Gal was approximately 41 %. These estimates are obtained by indirect means, but it is clear that the energy accumulation efficiency is different between Fru and Gal.

Concentrations of propionic acid and butyric acid were higher, but acetic acid concentration was lower, in group Fru than in group Gal. Several studies have shown that dietary propionate reduces serum cholesterol levels in rats (Chen *et al.* 1984; Illman *et al.* 1988) and pigs (Thacker *et al.* 1981), suggesting a possible effect of propionic acid on lipid metabolism. The chemical energy contents of butyric acid (24·89 kJ/g) and propionic acid (20·75 kJ/g) are much higher than that of acetic acid (14·60 kJ/g) (Pond *et al.* 1995). Furthermore, production of ATP through metabolism of propionic and butyric acids is greater than that resulting from acetic acid. In group Gal, the acetic acid : propionic acid ratio was large compared with that of group Fru. This probably explains the difference in energy accumulation efficiency between Fru and Gal.

Fru and Gal altered the fatty acid composition (unsaturated : saturated ratio) in the body. Some studies using ^{14}C-labelled fructo-oligosaccharides and plant cell-wall material showed that significant amounts of poly- and oligosaccharides ingested were fermented to organic acids in the large intestine, then the organic acids were utilized as an energy source and as materials for synthesis of fatty acids and amino acids in the rat (Hosoya *et al.* 1988; Buchanan *et al.* 1994*a*,*b*). In the liver the ^{14}C, administered as [U-^{14}C]-labelled spinach cell walls, was predominantly associated with phospholipid (Buchanan *et al.* 1994*a*,*b*). This suggests that fermentation products of the cell walls were effectively utilized to synthesize fatty acids in the liver.

Acetic acid, of which the concentration in the caecum was higher in group Gal, is the primary substrate for cholesterol and triacylglycerol synthesis. Butyric acid, of which the concentration was higher in group Fru, is another source of acetyl-CoA as well as acetate. The synthesis of saturated fatty acids begins with acetyl-CoA. The supply of acetyl-CoA was probably much larger in groups Fru and Gal than the other groups. With free access to a starch-rich diet, NADH supply would not limit the utilization of acetate and butyrate. Under the feeding conditions in the present experiment, saturated fatty acids might easily be synthesized as a result of feeding Fru and Gal. This would result in a lower unsaturated : saturated fatty acids ratio. The difference in the relative levels of these SCFA in the caecum may therefore affect fatty acid composition in the body fat. This should be investigated by a tracer experiment using ^{14}C-labelled SCFA.

In conclusion, Fru and Gal might increase the retention time of digesta in the rat. Contributions to energy accumulation of Fru and Gal are lower than those of starch or sucrose, and tend to be different, associated with differences in the composition of caecal organic acids and of fatty acids in body fat. Because the net energy value of each organic acid in the body and effects of organic acids, especially SCFA, on nutrient metabolism in the body may be different, the energetic contributions of indigestible oligosaccharides can be expected to vary depending on the extent to which they are fermented and on the relative amounts of each organic acid produced in the large intestine.

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