D-Tagatose Has Low Small Intestinal Digestibility but High Large Intestinal Fermentability in Pigs[1,2,3]

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ABSTRACT The digestibility of D-tagatose, its effect on the digestibility of macronutrients and the metabolic response of the microbiota of the gastrointestinal tract to the ingestion of this carbohydrate were studied in pigs. Eight pigs were fed a low fiber diet comprising 15% sucrose (control group). Another eight pigs were fed a similar diet except that 100 g sucrose per kg diet was replaced by D-tagatose (test group). After 18 d, the pigs were killed and the gastrointestinal contents removed for analysis. The digestibility of D-tagatose was 25.8 ± 5.6% in the distal third of the small intestine. The small intestinal digestibilities of dry matter (86.9 ± 1.3 vs. 92.9 ± 0.9%), gross energy (74.4 ± 1.6 vs. 80.7 ± 1.8%) and sucrose (90.4 ± 2.5 vs. 98.0 ± 0.5%) were lower ($P < 0.05$) in the pigs fed D-tagatose. Digestibilities of starch, protein and fat did not differ between groups. D-Tagatose, sucrose and starch were fully digested in the large intestine. The fecal digestibilities of energy, dry matter and fat did not differ between the two groups, whereas D-tagatose reduced the fecal digestibility of protein (91.1 ± 0.6 vs. 93.5 ± 0.7%, $P < 0.05$). D-Tagatose served as a substrate for the microbiota in the cecum and proximal colon as indicated by a reduced pH, and a greater ATP concentration, adenylate energy charge (AEC) ratio and concentration of short-chain fatty acids. In particular, the increase in the concentrations of propionate, butyrate and valerate suggests possible health benefits of this monosaccharide. J. Nutr. 129: 1002–1009, 1999.

KEY WORDS: • *sweetener* • *sugar* • *digestibility* • *fermentation* • *pigs* • *D-tagatose*

D-Tagatose is a ketohexose, a stereoisomer of fructose (Levin et al. 1995), patented as a low energy carbohydrate sweetener and bulking agent (Zehner 1988), and as an anti-hyperglycemic agent (Zehner et al. 1994). The net energy value has been estimated at slightly <0 on the basis of a study in rats (Livesey and Brown 1996). D-Tagatose may thus have a use as a sugar substitute of particular benefit because of its lower energy value. Studies in vitro have also indicated that D-tagatose can inhibit the activity of carbohydrases in the small intestine (Hertel 1997, Seri et al. 1995) with a possible further reduction of the energy value of the diet and a depression of the glycemic response (Seri et al. 1995).

A metabolism study in rats has shown that carbon of orally administered ^{14}C-labeled D-tagatose is excreted in the urine (5–6%), breath (49–68%) and feces (11–29%) with variation in the urinary and fecal excretion depending on whether the rats are adapted to dietary D-tagatose (Levin et al. 1995). However, there is little information to date on the route by which carbon from D-tagatose appears in the peripheral tissues and in the urine; however, it appears that events taking place in the gastrointestinal tract are of great importance. A study with only one ileum-fistulated pig supplemented with antibiotics to eliminate any microflora residing in the small intestine indicates that 78 ± 8% of D-tagatose could be absorbed, in contrast to lactitol, which had an ileal digestibility of 5 ± 7% (Levin et al. 1995). However, given the limited number of observations, these values should be interpreted with great caution.

We have performed an experiment with normal pigs designed to study the apparent ileal and fecal digestibility of D-tagatose, its influence on the apparent ileal and fecal digestibilities of starch, sucrose, protein, fat and energy, and changes in microbial activity [ATP concentrations and adenylate energy charge, (AEC)[6]], pH and concentration of short-chain fatty acids (SCFA) in the gut contents.

MATERIALS AND METHODS

Animals and housing. At 7-d intervals, two series of experiments were performed with eight castrated male Danish Landrace × Yorkshire pigs. The pigs were obtained from the herd at the Danish Institute of Agricultural Sciences, Foulum, Denmark. The pigs for the first series of experiments came from two sows (2 × 4 littermates) and

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[6] Abbreviations used: AEC, adenylate energy charge; Ce, cecum; LI_1, LI_2, LI_3, three equal parts of large intestine (proximal, mid- and distal including rectum); SCFA, short-chain fatty acids; SI_1, SI_2, SI_3, three equal parts of the small intestine (proximal, mid- and distal); St, stomach.

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TABLE 1

Composition of experimental diets

	Control	Tagatose
	g/kg	
Ingredient		
Sucrose	150	50
D-Tagatose	0	100
White wheat flour	480	480
Barley	100	100
Casein	120	120
Soybean oil	80	80
Wheat bran	30	30
Vitamin/mineral mixture[1]	30	30
Polyethylene glycol	8	8
Chromic oxide	2	2
	g/kg dry matter	
Chemical composition		
Starch	431.8	424.0
Sucrose	171.1	57.9
D-Tagatose	—	109.2
Protein (N × 6.25)	192.3	196.5
HCl-fat	107.4	108.6
Polyethylene glycol	9.2	10.5
Chromic oxide	2.1	2.0
	MJ/kg dry matter	
Gross energy	4884	4847

[1] Provided in mg/kg diet: $Ca_2(PO_4)_3$, 17000; K_2PO_4, 5700; NaCl, 4000; $CaCO_3$, 2290; $FeSO_4 \cdot 7H_2O$, 212; ZnO, 85; Mn_3O_4, 31; $CuSO_4 \cdot 5H_2O$, 68; KI, 0.2; $Na_2SeO_3 \cdot 5H_2O$, 0.6; retinol acetate, 1.3; cholecalciferol, 0.02; dl-α-tocopherol, 51; menadione, 1.9; riboflavin, 3.4; pyridoxine, 2.8; D-pantothenic acid, 9.3; niacin, 19; biotin, 0.05; cyanocobalamin, 0.02.

for the second series from four sows (4 × 2 littermates). The pigs in series 1 were 67–69 d old and weighed 26.1 ± 1.1 kg. The pigs in series 2 were 62–75 d old and weighed 24.6 ± 1.9 kg. The pigs were housed individually in 4 m^2 pens with concrete floors.

Diets and feeding. Two experimental diets were used for the experiments (control and tagatose diet in the following). The composition is given in **Table 1**. Chromic oxide was used as a digestibility marker. Because D-tagatose is soluble in water, polyethylene glycol 4000 (PEG) was also tested as a digestibility marker but was not found useful. For each series, four pigs were given the same experimental diet, with littermates fed different experimental diets. The average weight of the pigs was balanced between the groups. The pigs had an adaptation period of 2 d during which they were fed a traditional Danish pig's diet (d-4 and d-3), followed by 2 d of consuming a 1:1 mixture of the standard feed and the experimental diet (d-2 and d-1). Subsequently, the pigs were fed the experimental diet for 18 d (d 0–17). The pigs were fed twice a day at 0700 and 1500 h. The feed intake was restricted to 40 g/(kg body weight · d).

Collection of feces. For collection of feces, a plastic bag was mounted on the rump of the pigs for three consecutive days just before slaughter (d 14–16). The bags were changed twice a day at feeding, and the collected feces were frozen to −18°C. The feces samples from each day were pooled for each pig and freeze-dried for further analysis.

Collections of samples after slaughter. On d 17, the pigs were killed 3 h after the morning meal with a lethal injection of pentobarbital sodium (200 g/L). Immediately after slaughter, the gastrointestinal tract was removed and divided into eight segments by ligatures, essentially as described by Clemens et al. (1975). The segments were as follows: the stomach, three equal parts of the small intestine (SI_1, SI_2, SI_3), the cecum and three equal parts of the large intestine including the rectum (LI_1, LI_2, LI_3). The luminal contents of each segment were carefully collected by gently squeezing the material out of the gut segment. The collected material was weighed and a sample taken for analysis.

Analyses. The concentrations of ATP, SCFA and dry matter and pH were determined in wet intestinal contents (digesta). All other chemical analyses were conducted on freeze-dried samples.

The concentration of ATP and the AEC ratio were determined by the luciferin-luciferase method as described by Jensen and Jørgensen (1994). The concentration and composition of SCFA and the concentration of lactate were determined as described by Jensen et al. (1995). Luminal pH was determined with a combined pH-electrode (GK 2401C, Radiometer, Copenhagen, Denmark).

Gross energy was analyzed by a LECO AC 300 automated calorimeter system (LECO, St. Joseph, MI) and crude protein by the Kjeldahl-method as N × 6.25 (AOAC 1990). HCl-fat was analyzed by extraction in diethyl ether after hydrolysis with 3 mol/L HCl (Stoldt 1952). Chromic oxide was determined by the method of Schurch et al. (1950).

Without prior extraction of low-molecular-weight sugars (which are not hydrolyzed to liberate glucose in the analytical procedure), starch was determined by gelatinization and simultaneous hydrolysis with thermostable α-amylase (EC 3.2.1.1; Termamyl®, Novo Nordisk A/S, Copenhagen, Denmark) for 1 h followed by a 2-h incubation with β-glucanase-free amyloglucosidase (EC 3.2.1.3; cat. no. 1202 367, Boehringer Mannheim, Mannheim, Germany); the resulting glucose monomers were quantified with a glucose oxidase reagent (EC 1.1.3.4; cat. no. K-GLUC, Megazyme International Ireland, Wicklow, Ireland) as described by Bach Knudsen and Hessov (1995). Free glucose from enzymatic degradation of sucrose was considered negligible because this degradation takes place on the brush border and not in the intestinal lumen.

Sucrose and D-tagatose in freeze-dried samples were determined essentially as described by Johansen et al. (1996). Briefly, the sample (125–250 mg) was extracted with 5 mL 50% (v/v) methanol containing 1 g/L sorbitol (internal standard) under constant mixing in a heating block (50°C) and subsequently centrifuged at 2000 × *g* for 10 min. The supernatant was filtered by using a Waters Sep-Pak C_{18} (Waters Corporation, Milford, MA) filter with a Vac Elut SPS 24 (Analytichem International, Harbor City, CA). Before filtration of the sample, the Sep-Pak filter was pretreated with 1 volume 100% methanol, 3 volumes water and 1 volume 50% (v/v) methanol. A volume of 1.5 mL of the filtrate was evaporated overnight in a vacuum centrifuge at 50°C and redissolved in 1.5 mL HPLC grade water. Before injection on the HPLC column, the sample was filtered using a 0.2 μm Sartorius filter (Sartorius AG, Goettingen, Germany). The sugars were separated on a calcium-based resin column (Aminex HPX-87C, BioRad Laboratories, Herkules, CA), kept at 85°C, by injection of 25 μL filtrate using a Waters HPLC LC Module1 (Waters Corporation) at a flow rate of 0.6 mL/min and a Waters 410 RI detector (Waters Corporation), internal temperature 45°C, sensitivity 64. The samples were calibrated against a standard mixture (4 g/L). Preliminary experiments showed linearity in the range 0.05–8.0 g/L (corresponding to a detection limit of 0.3 g D-tagatose/kg dry matter). D-Tagatose and sorbitol were quantified on the basis of peak area. Sucrose was quantified on the basis of peak height because of insufficient baseline separation from other components.

Calculations. The digestibility in the distal third of the small intestine (SI_3) and in feces was calculated as follows:

$$\left[1 - \frac{M_D}{C_D} \cdot \frac{C_{I/F}}{M_{I/F}}\right] \cdot 100$$

where M_D and $M_{I/F}$ are the concentrations of marker in the diet (*D*), and intestine (*I*) or feces (*F*). C_D and $C_{I/F}$ are the corresponding concentrations of the feed component, for which the digestibility is to be calculated.

Statistical analysis. Two kinds of analyses were conducted. One concerned comparison of treatment effects (i.e., diets) in a given intestinal segment. This was accomplished using a simple ANOVA based on the following general linear model (GLM):

$$Y_{di} = \mu + \alpha_d + \epsilon_{di}$$

TABLE 2

P-values for multivariate analysis of amounts of digesta, dry matter, dry matter concentration, pH, adenylate energy charge (AEC) and concentration of short-chain fatty acids (SCFA) in upper gut (stomach and three equal segments of the small intestine) and the lower gut (cecum and three equal segments of the colon) of pigs fed D-tagatose and control diets

	Upper gut			Lower gut		
Parameter	Diet	Segment	D x S[1]	Diet	Segment	D x S
Digesta	0.0001	0.0001	0.0013	0.9606	0.0002	0.1106
Dry matter (DM)	0.1126	0.0001	0.3594	0.2804	0.0001	0.6438
DM concentration	0.0001	0.0001	0.0158	0.4145	0.0001	0.0353
pH	0.3639	0.0001	0.7336	0.0001	0.2488	0.4647
ATP	0.0004	0.0002	0.0054	0.0031	0.0001	0.0241
AEC	0.0025	0.0001	0.0182	0.0157	0.0001	0.0086
Formate	0.3851	0.0001	0.1410	0.1530	0.9576	0.6526
Lactate	0.0219	0.0001	0.0004	1.0000	1.0000	1.0000
Acetate	0.0001	0.0001	0.0001	0.8199	0.2299	0.0497
Propionate	0.2298	0.1860	0.4074	0.0090	0.0554	0.5188
Butyrate	0.9024	0.1576	0.5983	0.0578	0.0447	0.3732
Valerate	1.0000	1.0000	1.0000	0.0002	0.0349	0.1241
Isobutyrate	1.0000	1.0000	1.0000	0.6479	0.0018	0.4700
Isovalerate	1.0000	1.0000	1.0000	0.7174	0.0008	0.6500
Total SCFA	0.0142	0.0001	0.0004	0.0118	0.0555	0.0687

[1] D x S, diet-segment interaction.

where Y_{di} is the dependent variable, μ is the overall mean, α_d is the effect of diet, d= 1,2, and $\epsilon_{di} \sim N(0,\sigma^2)$ represents the unexplained random error.

The effect of treatment over a range of segments was analyzed using multivariate ANOVA with diet as the between-animal effect and segment as the within-animal effect by using the following general linear model:

$$Y_{dsi} = \mu + \alpha_d + \beta_s + \alpha\beta_{ds} + \gamma_i + \epsilon_{dsi}$$

where α_d denotes the effect of diet, d=1,2, β_s is the effect of segment, s= 1,. . .4, $\alpha\beta_{ds}$ is the interaction between diet and segment, and i refers to an individual pig. The variance component $\gamma_i \sim N(0,\tau^2)$ accounts for the fact that the repeated measurements were made on the same individual, thereby rendering these observations correlated, whereas the term $\epsilon_{dsi} \sim N(0,\sigma^2)$ represents the unexplained random error (SAS Institute 1989).

Because the digestive processes in the upper and lower gut are quite different, the stomach and small intestine were considered as one compartment (upper gut), the cecum and colon as another (lower gut), and the compartments were analyzed separately.

The experimental diets were balanced between the two series of experiments; thus series were not included in the statistical analyses. The analyses were performed with SAS for Windows version 6.12 (SAS Institute, Cary, NC).

In tables and figures, significance of difference ($P < 0.05$) refers to univariate analysis. SEM values were calculated for each group separately. When effects of the dietary treatment in a specific segment are referred to, probability levels stated in the text refer to the univariate analysis.

RESULTS

P-values for the results of multivariate analyses of parameters in the upper and lower gut are given in **Table 2**. Except for some of the SCFA, which were present in very low amounts, all variables were significantly different ($P < 0.05$) among segments in the upper gut. In the lower gut, there were significant differences among the segments, except for the pH and concentrations of formate, lactate, acetate, propionate and total SCFA. Statistically significant effects of the diet ($P < 0.05$), demonstrated an overall difference between the two diet groups, although the effect of diet was not significant in all segments when analyzed separately in the univariate analyses. Significant interactions ($P < 0.05$) between the diet and segment (D × S) indicate that the differences between one segment and another along the gastrointestinal tract followed a different pattern for pigs fed the two experimental diets.

Amount of digesta and dry matter in the gastrointestinal tract at slaughter. Significantly larger amounts of digesta were collected from the stomach ($P = 0.04$), SI_1 ($P = 0.02$), SI_2 and SI_3 ($P = 0.0001$) of tagatose-fed pigs compared with pigs fed the control diet (**Table 3**). In the large intestine, there was also a significantly larger amount of digesta in LI_1 of the tagatose group than the control group ($P = 0.03$), but there was no overall significant effect of diet in the multivariate analysis of the amount of digesta in the lower gut (Table 2).

The amount of dry matter was significantly higher in SI_3 of the tagatose-fed pigs compared with pigs fed the control diet ($P = 0.006$, Table 3), but no overall effect of diet was detected in the multivariate analysis of the upper gut. There was no significant effect of experimental diet on the amount of dry matter collected from the lower gut.

The total amount of digesta present in the upper gut was significantly higher in the tagatose group than in the control group (2540 g vs. 1675 g, $P = 0.0001$), whereas no significant difference was seen in the amount of dry matter collected from the pigs in the control group (380 g) and the tagatose group (416 g, $P = 0.10$).

The dry matter concentration (g/kg digesta) in the upper gut was lower for the tagatose group in the stomach, SI_2 and SI_3 (Table 3), which led to an overall significant effect of diet and an interaction between segment and diet in the multivariate analysis (Table 2). In addition to a greater reduction of dry matter concentration from the stomach to SI_1, the dry matter concentration decreased further from SI_1 to SI_2 in the pigs fed the tagatose diet. In contrast, in the control group, the dry matter concentration in the small intestine increased through passage of the small intestine. No overall effect of diet was seen in the dry matter concentration in the lower gut, but there was an interaction between diet and segment (Table 2).

TABLE 3

Digesta and dry matter contents and dry matter concentration in different segments of the gastrointestinal tract of pigs fed the control or the tagatose diet[1]

	Digesta		Dry Matter			
	Control	Tagatose	Control	Tagatose	Control	Tagatose
	g				*g/kg*	
Stomach	1249 ± 59	1500 ± 96*	315 ± 13	331 ± 18	253 ± 5	222 ± 7**
SI_1[2]	34 ± 12	93 ± 18*	5 ± 2	10 ± 2	141 ± 27	105 ± 9
SI_2	195 ± 26	401 ± 28***	28 ± 3	30 ± 2	147 ± 11	78 ± 6***
SI_3	197 ± 20	547 ± 39***	32 ± 2	45 ± 3**	168 ± 8	82 ± 3***
Cecum	88 ± 25	145 ± 37	19 ± 7	21 ± 5	184 ± 38	153 ± 14
LI_1	150 ± 11	197 ± 16*	40 ± 4	40 ± 3	265 ± 22	214 ± 18
LI_2	96 ± 8	78 ± 11	27 ± 3	22 ± 4	292 ± 15	295 ± 35
LI_3	71 ± 7	58 ± 15	23 ± 3	20 ± 6	327 ± 12	333 ± 10

[1] Values are means ± SEM, $n = 8$ except $n = 7$ for the dry matter content and concentration in SI_1 of the control group.
[2] SI_1, SI_2, SI_3, proximal, mid-, and distal third of the small intestine: LI_1, LI_2, LI_3, proximal, mid-, and distal third of the colon.
* Significantly different from control ($P < 0.05$); ** significantly different from control ($P < 0.01$); *** significantly different from control ($P < 0.001$).

Apparent digestibility of D-tagatose and nutrients. The apparent digestibility of dry matter in the distal third of the small intestine was lower for the tagatose diet than the control diet ($P = 0.01$). This was reflected by a lower digestibility of gross energy ($P = 0.02$) (**Table 4**). There were no differences between groups in the small intestinal digestibilities of protein, fat or starch, but there was a lower digestibility of sucrose ($P = 0.009$) in pigs fed the tagatose diet. The digestibility of D-tagatose in SI_3 was 25.8 ± 5.6%, whereas no D-tagatose was recovered from feces (Table 4).

TABLE 4

Apparent ileal and fecal digestibilities of dry matter, gross energy, protein, fat, starch, sucrose and D-tagatose in pigs fed either the control or the tagatose diet

	Diet group	
	Control	Tagatose
	% of intake	
SI_3[1,2]		
Dry matter	92.9 ± 0.9	86.9 ± 1.3**
Gross energy	80.7 ± 1.8	74.4 ± 1.6*
Protein	87.2 ± 3.3	85.9 ± 1.1
Fat	81.5 ± 1.0	82.5 ± 2.0
Starch	92.1 ± 2.6	92.8 ± 1.6
Sucrose	98.0 ± 0.5	90.4 ± 2.5*
D-Tagatose	ND	25.8 ± 5.6
Fecal[3]		
Dry matter	92.9 ± 0.2	92.7 ± 0.2
Gross energy	93.0 ± 0.3	92.5 ± 0.3
Protein	93.5 ± 0.7	91.1 ± 0.6*
Fat	90.3 ± 0.7	91.1 ± 0.6
Starch	100.0 ± 0.0	99.9 ± 0.0
Sucrose	100.0 ± 0.0	100.0 ± 0.0
D-Tagatose	ND	100.0 ± 0.0

[1] SI_3, distal small intestine.
[2] Values are means ± SEM, $n = 8$.
[3] Values are means ± SEM, $n = 8$ for the control group and $n = 7$ for the tagatose group.
[4] ND, not determined.
* Significantly different from control ($P < 0.05$); ** significantly different from control ($P < 0.01$).

The apparent fecal digestibilities of dry matter, gross energy, fat, starch and sucrose were not significantly different between the two groups, but the apparent fecal digestibility of protein was significantly reduced in pigs fed the tagatose diet compared with the control diet ($P = 0.03$).

pH of the gut content at slaughter. There was no difference between diet groups in the pH of the stomach or small intestinal contents (**Fig. 1**). The pH increased from 4.6 in the stomach to 6.1 in SI_1 and 7.1 in SI_3 with significant differences among segments (Table 2). Along the large intestine, there were no significant changes in pH; overall, however, the tagatose diet resulted in a lower pH than did the control diet in the lower gut (Table 2). This difference was significant in the cecum ($P = 0.03$) and in LI_1 ($P = 0.004$).

Microbial activity in the gut contents at slaughter. The microbial activity expressed by the concentration of ATP (Fig. 1) was generally very low in the stomach and the two first segments of the small intestine (<0.4 mg/kg digesta), but increased subsequently in SI_3 to 1.3 mg/kg digesta in pigs fed the tagatose diet and 4.2 mg/kg digesta in those fed the control diet ($P = 0.0006$). The microbial activity increased in the cecum to 17.1 mg/kg digesta without any difference between the two diet groups. However, in the following segment (LI_1), there was a reduction in the microbial activity to 6.8 mg/kg digesta for the pigs fed the control diet, whereas the activity remained high (16.8 mg/kg digesta) in pigs fed the tagatose diet ($P = 0.0007$). There was no difference between groups in segments LI_2 and LI_3 but overall, the activity was higher in the lower gut of the tagatose-fed pigs compared with those fed the control diet (Table 2).

The higher specific activity (mg/kg digesta) in the distal small intestine of the pigs fed the control diet did not reflect a higher total activity in this part of the small intestine. When accounting for the different amounts of digesta collected from the segment, the total amounts of ATP in the stomach and small intestine were not significantly different for pigs fed the two diets (**Table 5**). The total microbial activity, on the other hand, was considerably higher in the cecum ($P = 0.03$) and colon ($P = 0.003$) of the tagatose-fed pigs compared with pigs fed the control diet.

AEC increased appreciably from 0.10–0.17 in the stomach, SI_1 and SI_2 to 0.41 for the tagatose group and 0.68 for the control group in SI_3 (Fig. 1). The difference between the diet

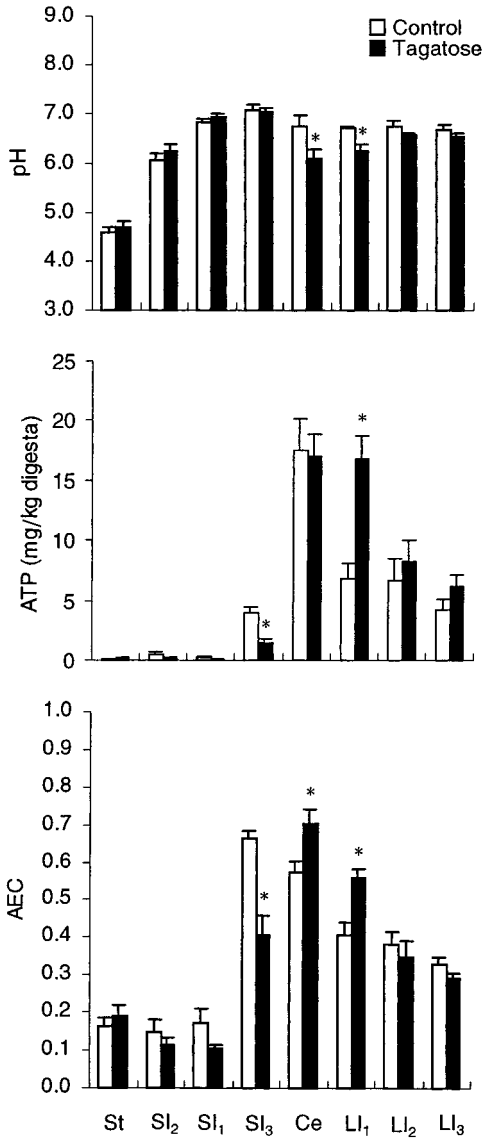

FIGURE 1 Luminal pH, concentration of ATP and adenylate energy charge (AEC) in digesta from different segments of the gastrointestinal tract of pigs fed either the control or the tagatose diet. Values are means ± SEM, $n = 8$. For ATP and AEC in SI_1, $n = 5$ in the control group, $n = 7$ in the tagatose group and $n = 7$ for Ce in the control group. *Significantly different from the control group ($P < 0.05$) in univariate analysis. St, stomach; SI_1, SI_2, SI_3, proximal, mid-, and distal third of the small intestine; Ce, cecum; LI_1, LI_2, LI_3 proximal, mid-, and distal third of the colon.

groups in SI_3 was significant ($P = 0.0003$); in the multivariate analysis, it gave overall significant effects of diet ($P = 0.003$) along with significant differences between segments and an interaction between diet and segment ($P = 0.02$, Table 2). The tagatose diet resulted in higher AEC in the cecum ($P = 0.03$) and LI_1 ($P = 0.003$) compared with the control diet. The AEC then declined through the large intestine for both dietary groups, and in LI_2, there was no difference between the two groups (Fig. 1). Thus, in the multivariate analysis of AEC in the lower gut, there were significant differences between segments, diets, and an interaction between segment and diet (Table 2).

Concentration of lactate and short-chain fatty acids in gut contents at slaughter. There was a notable increase in the concentration of lactate from the stomach (0.6 mmol/kg digesta) through the small intestine to 11.2 mmol/kg digesta in SI_3 of the pigs fed the control diet (**Fig. 2**). In the pigs fed the tagatose diet, lactate increased from 0.9 in the stomach to only 3.1 mmol/kg digesta in SI_1, with a further small increment through the remainder of the small intestine to 3.8 mmol/kg digesta in SI_3. This gave a significantly lower lactate concentration in SI_2 ($P = 0.02$) and SI_3 ($P = 0.005$) of the tagatose-fed pigs compared with the pigs fed the control diet, and there was a significant interaction between segment and diet in the multivariate analysis ($P = 0.0004$, Table 2). However, the differences in concentration did not reflect differences in lac-

TABLE 5

Total microbial activity in different parts of the gastrointestinal tract of pigs fed either the control or the tagatose diet[1]

	ATP	
	Control	Tagatose
	μg	
Stomach	174 ± 42	253 ± 48
Small intestine	886 ± 157	783 ± 213
Cecum	426 ± 101	2510 ± 864*
Colon	1985 ± 396	4282 ± 504**

[1] Values are means ± SEM, $n = 8$.
* Significantly different from control ($P < 0.05$); ** significantly different from control ($P < 0.01$).

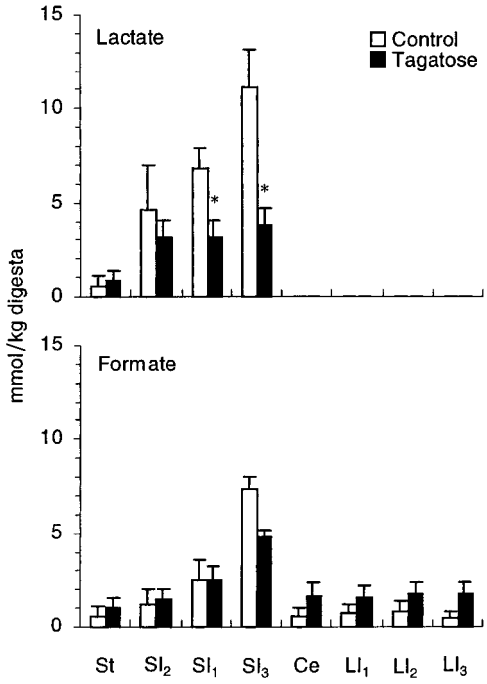

FIGURE 2 Concentrations of lactate and formate in digesta from different segments of the gastrointestinal tract of pigs fed either the control or the tagatose diet. Values are means ± SEM, $n = 8$. In SI_1, $n = 7$ for the control group, and in LI_3, $n = 7$ for the tagatose group. *Significantly different from the control group ($P < 0.05$) in univariate analysis. St, stomach; SI_1, SI_2, SI_3, proximal, mid-, and distal third of the small intestine; Ce, cecum; LI_1, LI_2, LI_3 proximal, mid-, and distal third of the colon.

tate production because the amount of digesta in the tagatose-fed group was higher than that in the control group, especially in SI_3. Therefore, there was no significant difference between the two diet groups in the total amount of lactate in the distal third of the small intestine ($P = 0.7$).

The concentration of formate increased from 1.0 and 0.6 mmol/kg digesta in the stomach to 4.8 and 7.4 mmol/kg digesta in SI_3 for the tagatose and control diet groups, respectively (Fig. 2). There was no significant effect of diet on the level of formate in the upper gut, but a significant difference between the segments (Table 2). No lactate was detected in the cecum and colon, and the level of formate was similar to that of the stomach and almost constant throughout the large intestine. The apparent higher mean concentration of formate in the large intestine in the tagatose-fed group (1.7 mmol/kg digesta) than in the control group (0.6 mmol/kg digesta) was not significant ($P = 0.15$).

There was no acetate in the stomach, SI_1 or SI_2, but the concentrations in SI_3 were 1.9 mmol/kg digesta in pigs fed the tagatose diet and 7.0 mmol/kg digesta in those fed the control diet ($P = 0.0001$, **Fig. 3**). This gave overall significant effects of diet, segment and an interaction between diet and segment in the multivariate analysis (Table 2). Propionate and butyrate were present in very low concentrations, and valerate was totally absent from the upper gut of the pigs in both dietary groups (Fig. 3).

For all unbranched acids except lactic and formic acids, the concentration increased dramatically in the lower gut (**Figs.** 3 and **4**). The concentration of acetate increased in the cecum to 41.1 mmol/kg digesta in the control group and 32.5 mmol/kg digesta in the tagatose-fed group ($P = 0.03$, Fig. 3). For the control group, the concentration decreased subsequently through the large intestine to 33.5 mmol/kg digesta in the LI_3. For the tagatose-fed group, there was a slight increase to a maximal concentration in LI_2 (39.0 mmol/kg digesta). However, the concentration in the cecum was the only case in which there was a significant difference between the two diet groups ($P = 0.02$). In the multivariate analysis, this led to a significant interaction between segment and diet, but no significant main effects of segment or diet (Table 2).

The concentration of propionate ranged from 31.1 mmol/kg digesta in the cecum to 20.8 mmol/kg digesta in LI_3 for pigs fed the control diet, and from 34.5 to 27.1 mmol/kg digesta for those fed the tagatose diet, with significant differences between dietary groups in LI_1 and LI_2 (Fig. 3). This gave an overall significantly higher concentration in pigs fed the tagatose diet ($P = 0.009$), whereas the differences between segments were not significant ($P = 0.055$, Table 2).

The concentration of butyrate was 9.7–11.9 mmol/kg digesta in the cecum, and D-tagatose induced an increase of the concentration to 13.2 mmol/kg digesta in LI_1, followed by a reduction in the following segments. In contrast, in pigs fed the control diet, there was a slight reduction in the concentration of butyrate along the large intestine to 7.7 mmol/kg digesta in LI_3. There was only a significant difference between the diet groups in LI_1 ($P = 0.03$), but no overall effect of diet in the lower gut ($P = 0.06$, Table 2).

There was a >1.3 times higher concentration of valerate in the cecum and colon of tagatose-fed pigs compared with pigs fed the control diet ($P = 0.0002$, Fig. 3). In addition, there was a small but significant difference between the segments of the lower gut ($P = 0.03$).

The branched-chain SCFA, which are produced from fermentation of protein, also increased in the lower gut (Fig. 4). For the tagatose-fed pigs, the concentration of isobutyrate increased from 0.63 mmol/kg digesta in the cecum to 1.63 mmol/kg digesta in LI_1 and further to 2.56 mmol/kg digesta in LI_3. For the control pigs, the concentration changed from 1.04 mmol/kg digesta in the cecum to 1.99 mmol/kg digesta in LI_1 and 2.19 mmol/kg digesta in LI_3. Similar results were obtained for isovalerate whose concentration increased from 0.53 mmol/kg digesta in the cecum of tagatose-fed pigs to 2.33 mmol/kg digesta in LI_3. For the pigs fed the control diet, the corresponding values were 0.86 and 2.06 mmol/kg digesta. There was no significant difference between the diet groups, but there were significant differences among segments (Table 2).

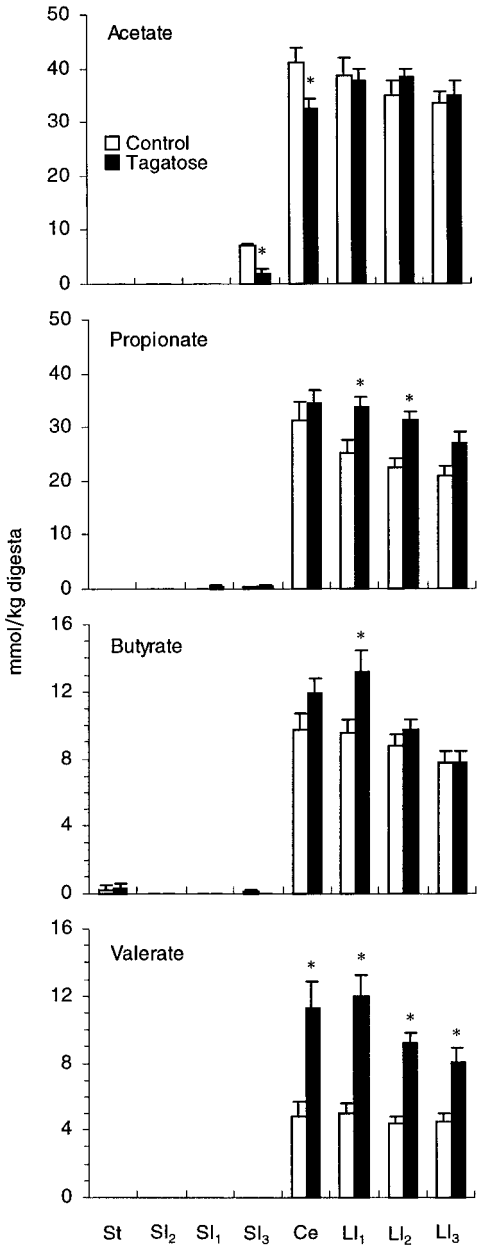

FIGURE 3 Concentrations of acetate, propionate, butyrate and valerate in digesta from different segments of the gastrointestinal tract of pigs fed either the control or the tagatose diet. Values are means ± SEM, $n = 8$. In SI_1, $n = 7$ for the control group, and in LI_3, $n = 7$ for the tagatose group. *Significantly different from the control group ($P < 0.05$) in univariate analysis. St, stomach; SI_1, SI_2, SI_3, proximal, mid-, and distal third of the small intestine; Ce, cecum; LI_1, LI_2, LI_3 proximal, mid-, and distal third of the colon.

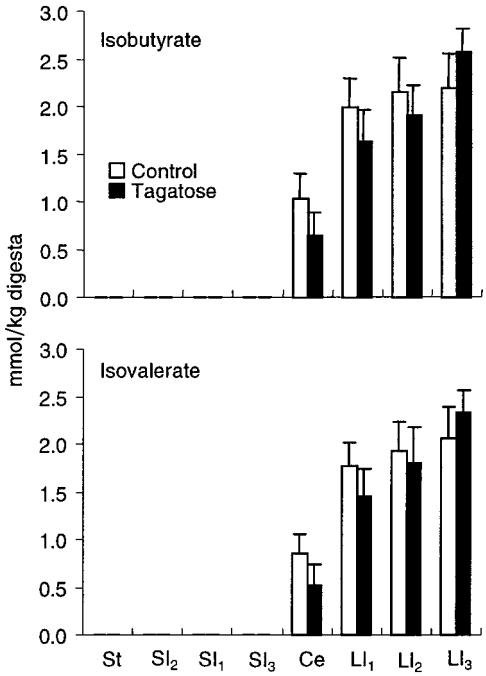

FIGURE 4 Concentrations of isobutyrate and isovalerate in digesta from different segments of the gastrointestinal tract of pigs fed either the control or the tagatose diet, Values are means ± SEM, $n = 8$. In SI_1, $n = 7$ for the control group, and in LI_3, $n = 7$ for the tagatose group. St, stomach; SI_1, SI_2, SI_3, proximal, mid-, and distal third of the small intestine; Ce, cecum; LI_1, LI_2, LI_3 proximal, mid-, and distal third of the colon.

DISCUSSION

The digestibility of D-tagatose related to chromic oxide was 25.8 ± 5.6% in the distal third of the small intestine, which must be considered the maximum digestibility. However, chromic oxide is an insoluble solid phase marker, whereas the sugars (D-tagatose and sucrose) are soluble. Especially in the more proximal part of the gastrointestinal tract, there is a difference between the flow of liquids and solids with the latter having a slower rate of passage than the former (Warner 1981). For this reason, we present the digestibility results only in the distal third of the small intestine. However, if a difference in flow between the dietary component and the marker continues throughout the small intestine, the use of chromic oxide as a digestibility marker may lead to an overestimation of the digestibility. On the basis of the amount of ATP and SCFA, and the pH at slaughter, the microbial activity in the gastrointestinal tract appears to be minimal in the upper gastrointestinal tract and does not contribute to the small intestinal digestibility to any appreciable extent. Although differences were seen between diet groups in the specific microbial activity (expressed by the ATP concentration) and the concentrations of lactate, formate and acetate in the upper gut, there were no indications of a difference in the total microbial activity when the results were corrected for the "dilution" of digesta in these segments. Only the AEC ratio, expressing the relative microbial activity, indicated a lower activity in the small intestine of the pigs fed the tagatose diet. Therefore, it is plausible that most of the missing D-tagatose, relative to chromic oxide, was absorbed and not fermented by bacteria residing in the ileum of the pigs.

The small intestinal digestibility of sucrose was reduced by ~10% in the group fed the tagatose diet compared with the control group. On a quantitative basis, this reflected a malabsorption of 3.0 g sucrose/kg ingested for the control diet group compared with 4.8 g malabsorbed sucrose from the diet supplemented with D-tagatose. The presence of D-tagatose may have reduced the absorption of sucrose by inhibiting the sucrase activity in the small intestine as suggested by Seri et al. (1995). Such an inhibitory effect of D-tagatose on carbohydrases in the small intestine has not been demonstrated previously in vivo; however, in vitro results with pure enzymes indicate that D-tagatose may inhibit pig intestinal sucrase/isomaltase activity, but not the glucoamylase/maltase activity (Hertel 1997). In contrast, studies with homogenates of mucous membranes of the small intestine of rabbits have suggested that D-tagatose inhibits both enzyme complexes in a dose-dependent manner (Seri et al. 1995). In this study, there were no measurable effects of D-tagatose on the in vivo digestibility of starch, protein or fat in the distal small intestine. The lack of effect on starch digestibility is consistent with a lack of effect on maltase in vitro as demonstrated by Hertel (1997), and the lower digestibility of dry matter and energy of the tagatose diet compared with the control diet was due primarily to the low digestibility of D-tagatose itself. The difference between diets in the digestibilities of dry matter, energy and sucrose in the small intestine was totally abolished in feces as a result of microbial fermentation of the substrates in the large intestine. Starch, sucrose and D-tagatose were totally digested by the end of the large intestine. Introducing larger amounts of fermentable material into the large intestine of the tagatose-fed pigs led to a higher microbial activity and an increased excretion of fecal nitrogen. This is the reason for the lower apparent fecal digestibility of nitrogen as previously demonstrated with other easily fermentable carbohydrates (Tetens et al. 1996).

As for most other sugar substitutes, laxation is an adverse effect that may limit the use of D-tagatose in food products. In this study, inclusion of 10% D-tagatose in the diet increased markedly the amount of digesta in the small intestine, but the moisture content was also higher. Therefore, there were no differences between the groups in the amounts of dry matter collected from the segments of the upper gastrointestinal tract. These results indicate that the effect of D-tagatose on volume in the small intestine is primarily an effect on the liquid phase, presumably through a higher osmotic pressure. In the lower gut, there was no significant difference between the two diet groups in the amounts of digesta and dry matter collected. Too high an osmolarity in the gut lumen can lead to diarrhea; in this study, we observed that two pigs had loose stools temporarily 1–2 d after exposure to D-tagatose. This is in agreement with previous results in rats in which low levels (5%) of D-tagatose did not cause any adverse effects, whereas higher levels (10–20%) led to temporary signs of diarrhea (Levin et al. 1995).

The present study demonstrated that D-tagatose has strong influences on the microbial activity (ATP concentration, concentration and composition of SCFA, and pH) in the lower gut. Generally, these effects were most extensive in the cecum and the proximal part of the colon, whereas the effects in the distal part of the colon were less evident. The main effects were reduction in luminal pH, increased ATP concentration and AEC-ratio, and higher concentrations of propionate and butyrate in the cecum and colon of the tagatose-fed pigs compared with pigs fed the control diet. For the distal colon and rectum, there were significant differences between diet groups only in the concentrations of propionate, valerate and caproate. These results indicate that the ingested D-tagatose is almost completely fermented in the cecum and proximal co-

lon, whereas only small amounts reach the more distal parts of the colon.

A particularly interesting observation was the high concentration of valerate found in the large intestine of the pigs fed D-tagatose, suggesting that D-tagatose may specifically stimulate the production of this acid.

Presumably, the bacteria of the gastrointestinal tract have to adapt to fermentation of D-tagatose. Indirectly, this was shown in a rat study in which adapted rats had a 17.3% lower fecal output of ^{14}C-labeled D-tagatose (11.4%) and an 18.5% higher excretion of ^{14}C via the breath compared with unadapted rats (Levin et al. 1995). Although the ^{14}C-containing components were not identified in the study, the results clearly suggest that D-tagatose is metabolized more efficiently in adapted than unadapted rats, again suggesting that gastrointestinal microflora have to adapt to this monosaccharide. Whether similar adaptation is necessary for the microflora in the gastrointestinal tract of other monogastrics (such as pigs and humans) should be investigated.

The concentrations of SCFA found in the gut contents of the pigs in these experiments suggest that, in particular, the microbial production of C3–C5 is elevated in pigs fed D-tagatose. However, the concentrations of SCFA in the gut do not reflect the actual production because some SCFA have already been absorbed and metabolized in the gut wall or transferred to the peripheral tissues. Because butyrate is the preferred fuel for the gut tissues (Scheppach 1994), the production of butyrate may be even greater than what appears from the composition of SCFA in the gastrointestinal contents. Butyrate has been suggested as beneficial in the prevention of colon cancer (Van Munster and Nagengast 1993). Propionate and valerate have recently been shown to resemble butyrate in their ability to inhibit cell proliferation and increase alkaline phosphatase activity in human colonic adenocarcinoma cell lines (Siavoshian et al. 1997), although the effects are weaker. Thus, D-tagatose may serve as a substrate to produce high amounts of propionate, butyrate and valerate, a feature that is of interest if D-tagatose is under consideration as a functional food ingredient.

Partial replacement of easily digestible carbohydrates with D-tagatose could have several nutritional implications. The zero net energy value calculated on the basis of a study with rats by Livesey and Brown (1996) has been suggested to stem from a thermogenic effect, but a recent study with humans has not been able to confirm this (Buemann et al. 1998). If such a thermogenic effect does not occur in humans, the zero net energy value may be questioned. However, D-tagatose may be expected to have a lower energy value than monosaccharides that are completely absorbed in the small intestine because a large part (>74%) of the D-tagatose is fermented in the large intestine. The energy obtained from microbial fermentation is generally used less efficiently than the energy obtained through direct digestion and absorption of carbohydrates in the small intestine (Livesey 1992). Furthermore, in the fermentation process, there are losses through production of heat and various gases (hydrogen, methane), and some SCFA are lost in feces. Additionally, fecal excretion of fat and nitrogen in microbial biomass may reduce the retained energy, although this was not detected in this study.

In conclusion, D-tagatose is digested and absorbed to only a small extent in the small intestine of pigs and shows some hyperosmotic effect in this part of the gastrointestinal tract. In adapted pigs, D-tagatose is completely fermented in the large intestine and contributes to the overall energy balance with a high production of short-chain fatty acids. In particular, the increases in the concentrations of propionate, butyrate and valerate draw attention toward possible specific health benefits resulting from incorporation of these monosaccharides into food products.

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LITERATURE CITED

Association of Official Analytical Chemists (1990) Nitrogen (total) in fertilizers. Kjeldahl Method, s. 18–19. Official Methods of Analysis, 15th ed. AOAC, Washington, DC.

Bach Knudsen, K. E. & Hessov, I. (1995) Recovery of inulin from Jerusalem artichoke *Helianthus tuberosus* L. in the small intestine of man. Br. J. Nutr. 74: 101–113.

Buemann, B., Toubro, S. & Astrup, A. (1998) D-Tagatose, a stereoisomer of D-fructose, increases hydrogen production in humans without affecting 24-hour energy expenditure or respiratory exchange ratio. J. Nutr. 128: 1481–1486.

Clemens, E. T., Stevens, C. E. & Southworth, M. (1975) Sites of organic acid production and pattern of digesta movement in the gastrointestinal tract of swine. J. Nutr. 105: 759–768.

Hertel, S. (1997) Interaction of Saccharase/Isomaltase and Glucoamylase/Maltase from Small Intestinal Mucosa of Pigs with Substrates and Inhibitors. (Wechselwirkungen von Saccharase/Isomaltase und Glucoamylase/Maltase aus schweinedünndarmmukosa mit substraten und inhibitoren.) Doctoral thesis, University of Hannover, Hannover, Germany.

Jensen, B. B. & Jørgensen, H. (1994) Effect of dietary fiber on microbial activity and microbial gas production in various regions of the gastrointestinal tract of pigs. Appl. Environ. Microbiol. 60: 1897–1904.

Jensen, M. T., Cox, R. P. & Jensen, B. B. (1995) Microbial production of skatole in the hind gut of pigs fed different diets and its relation to skatole deposition in backfat. Anim. Sci. 61: 293–304.

Johansen, H. N., Bach Knudsen, K. E. & Glitsø, L. V. (1996) Influence of extraction solvent and temperature on the quantitative determination of oligosaccharides from plant materials by HPLC. J. Agric. Food Chem. 44: 1470–1474.

Levin, G. V., Zehner, L. R., Saunders, J. P. & Beadle, J. R. (1995) Sugar substitutes. their energy values, bulk characteristics, and potential health benefits. Am. J. Clin. Nutr. 62 (suppl.): 1161S–1168S.

Livesey, G. (1992) The energy values of dietary fibre and sugar alcohols for man. Nutr. Res. Rev, 5: 61–84.

Livesey, G. & Brown, J. C. (1996) D-Tagatose is a bulk sweetener with zero energy determined in rats. J. Nutr. 126: 1601–1609.

SAS Institute Inc. (1989) SAS User's Guide: Statistics, Version 6, 4th ed. SAS Institute, Cary, NC.

Scheppach, W. (1994) Effects of short chain fatty acids on gut morphology and function. Gut (suppl. 1): S35–S38.

Schurch, A. F., Lloyd, L. E. & Crampton, E. W. (1950) The use of chromic oxide as an index for determining the digestibility of a diet. J. Nutr. 50: 629–636.

Seri, K., Sanai, K., Negishi, S. & Akino, T. (1995) Prophylactic and remedial preparation for diseases attendant on hyperglycemia, and wholesome food. U.S. Patent Number 5,468,734.

Siavoshian, S., Blottiere, H. M., Le Foll, E., Kaeffer, B., Cherbut, C. & Galmiche, J. P. (1997) Comparison of the effect of different short chain fatty acids on the growth and differentiation of human colonic carcinoma cell lines in vitro. Cell Biol. Int. 21: 281–287.

Stoldt, W. (1952) Vorschlag zur Vereinheitlichung der Fettbestimmung in Lebensmitteln. Fette Seifen Anstrichm. 54: 206–207.

Tetens, I., Livesey, G. & Eggum, B. O. (1996) Effects of the type and level of dietary fibre supplements on nitrogen retention and excretion patterns. Br. J. Nutr. 75: 461–469.

Van Munster, I. P. & Nagengast, F. M. (1993) The role of carbohydrate fermentation in colon cancer prevention. Scand. J. Gastroenterol. 28 (suppl. 200): 80–86.

Warner, A.C.I. (1981) Rate of passage of digesta through the gut of mammals and birds. Nutr. Abstr. Rev. B 51: 789–820.

Zehner, L. (1988) D-Tagatose as a low-caloric carbohydrate sweetener and bulking agent. U.S. Patent Number 4,786.722.

Zehner, L., Levin, G., Saunders, J, & Beadle, J. (1994) D-Tagatose as anti-hyperglycemic agent. U.S. Patent Number 4,786.722.