In Vitro Fermentation Pattern of D-Tagatose Is Affected by Adaptation of the Microbiota from the Gastrointestinal Tract of Pigs

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ABSTRACT Knowledge of the fermentation pattern of D-tagatose is important for the assessment of energy value and compliance of D-tagatose. In vitro fermentation experiments with pig intestinal contents and bacteria harvested from the gastrointestinal tract of pigs were used to investigate the degradation of D-tagatose and the formation of fermentation products. Two groups of eight pigs were fed either a control diet containing 150 g/kg sucrose or a diet which had 100 g/kg of the sucrose replaced by D-tagatose. After 18 d the pigs were killed and the gastrointestinal contents collected for in vitro studies. No microbial fermentation of D-tagatose occurred in the stomach or in the small intestine, whereas the sugar was fermented in the cecum and colon. Formate, acetate, propionate, butyrate, valerate, caproate and some heptanoate were produced by the microbial fermentation of D-tagatose by gut microbiota. Hydrogen and methane were also produced. The population of D-tagatose-degrading bacteria in fecal samples and the capacity of bacteria from the hindgut to degrade D-tagatose were higher in the pigs adapted to D-tagatose compared with unadapted pigs. In unadapted pigs, the major fermentation product from D-tagatose was acetic acid. Much more butyric and valeric acids were produced from D-tagatose by bacterial slurries of tagatose-adapted pigs compared with unadapted pigs; this was especially the case for samples from the colon. We conclude that D-tagatose is not fermented in the upper gastrointestinal tract, and the ability of the large intestinal microbiota to ferment D-tagatose is dependent on adaptation.


KEY WORDS: • sweetener • sugar • fermentation • adaptation • pigs • D-tagatose

D-tagatose is a stereoisomer of D-fructose and is considered a low-energy full-bulk sweetener, which is an alternative to sucrose (Levin et al. 1995). However, the metabolism of D-tagatose is unclear. Earlier results in rats have shown that 43% of intravenously administered carbon of 14C-labeled D-tagatose was excreted in urine and 37% in expired (Levin et al. 1995, Saunders et al. 1999). However, when administered orally, only 5–6% was excreted in the urine, 49–68% was excreted in expiration and 11–29% with feces. Gastrointestinal adaptation to D-tagatose seems to be important for metabolism because the extent of urinary and fecal excretion was dependent on whether the rats were adapted to D-tagatose in the diet. We previously found that <26% of D-tagatose was absorbed in the proximal two-thirds of the small intestine (SI) of pigs fed a D-tagatose-containing diet for 20 d (Lærke and Jensen 1999). However, no D-tagatose could be detected in feces, which suggests that the malabsorbed D-tagatose was fully fermented in the large intestine. Thus to assess the energy value and compliance of diets containing D-tagatose, it is of great interest to study the capability of unadapted and adapted gut microbiota to degrade D-tagatose.

The aims of this investigation were as follows: 1) to study the influence of D-tagatose on the short chain fatty acids (SCFA) and lactic acid production in the gastrointestinal tract determined by in vitro incubations of gut contents from control pigs or pigs adapted to D-tagatose, 2) to estimate the degradation of D-tagatose and formation of fermentation products from D-tagatose during in vitro incubation of D-tagatose, with slurries of bacteria harvested from various regions of the gastrointestinal tract and 3) to investigate the population size of D-tagatose-degrading bacteria in feces from control pigs or pigs adapted to ingestion of D-tagatose.

MATERIALS AND METHODS

Animals and feeding. At 7-d intervals, two groups of eight (2 × 4 littermates and 4 × 2 littermates) to 75-d-old castrated male Landrace × Yorkshire pigs, obtained from the swine herd at the Danish Institute of Agricultural Sciences, Foulum, Denmark, were given a low-fiber basal diet containing 150 g/kg sucrose (control diet) or 50 g/kg sucrose + 100 g/kg of D-tagatose (tagatose diet) for 18 d. 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

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2 d on 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 to d 17). The pigs were fed twice a day at 0700 and 1500 h. The feed intake was restricted to a total amount of 40 g/kg body weight × d. For details about the experimental diets see Lærke and Jensen (1999). The protocol used in this experiment complied with the Danish Ministry of Justice, law no. 726 (December 9, 1993) concerning animal experimentation and care of experimental animals.

**Sampling during the experiment.** Fecal samples were taken directly from the anus in the morning when the pigs were fed the standard diet (d −3) and after 1, 8 and 15 d of consuming the experimental diets for determination of total anaerobic bacteria, D-tagatose–degrading bacteria, dry matter and pH.

**Collection 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 segments by ligatures as previously described (Lærke and Jensen 1999). Samples from the stomach, the distal third of the small intestine (distal SI), the cecum and the mid third of the colon (mid colon) were used for in vitro incubations.

**In vitro fermentation of unfractionated gut contents.** The rate of production of SCFA and lactic acid in the gastrointestinal tract of pigs on the two diets was determined by incubation of a 20 g/100 g slurry of gastrointestinal contents from the stomach, distal SI, cecum or mid colon in 100 mmol/L Na-phosphate buffer (pH 6.5). The incubations were essentially carried out as described by Jensen and Jensen (1993), with an incubation time of 2 h in serum bottles. Samples were taken for analysis after 0, 15, 30, 60 and 120 min.

In the contents of the stomach and the distal SI, a linear increase in lactic and acetic acid production was detected up to 1 h of incubation, followed by a steeper increase in concentration (presumably due to bacterial multiplication). With the contents from the cecum and large intestine, constant SCFA production was detected during the first 30 min (cecum) to 1 h (mid colon). After this time, the SCFA production leveled off. As a consequence, the rate of microbial fermentation and the composition of fermentation products after in vitro fermentation of D-tagatose were determined from D-tagatose was therefore calculated from the concentration of D-tagatose determined after 4 h of incubation.

**Calculations.** The energy in D-tagatose recovered as SCFA after microbial fermentation (E) was calculated according to the following formula:

\[ E = a \cdot \sum \frac{m_i}{100} \cdot H_i \]

Here \( a \) is the mean rate of SCFA production per gram of degraded D-tagatose for bacteria in the cecum or colon, respectively, \( m_i \) is the molar proportion (mol/100 mol) of the specific SCFA and \( H_i \) is the heat of combustion of the same SCFA. The values used for heat of combustion were as follows: D-tagatose, 15.6 kJ/g; formic acid, 255 kJ/mol; acetic acid, 877 kJ/mol; propionic acid, 1533 kJ/mol; butyric acid, 2185 kJ/mol; valeric acid, 2758 kJ/mol; caproic acid, 3501 kJ/mol; and heptanoic acid, 4137 kJ/mol (Chemical Rubber Company 1983).

**Statistical analysis.** The effect of diet in a given intestinal segment, including data obtained from in vitro incubations, was tested using a simple ANOVA based on the following general linear model (GLM):

\[ Y_{di} = \mu + \alpha_d + \beta_i + \epsilon_{di} \]

Here \( Y_{di} \) is the dependent variable, \( \mu \) is the overall mean, \( \alpha_d \) is the effect of diet, \( d = 1, 2 \) and \( \epsilon_{di} \sim N(0, \sigma^2) \) represents the unexplained random error (Snedecor and Cochran 1973).

Data concerning the effect of diet on the development over time were analyzed using multivariate ANOVA with diet as the between animal effect and time (d 1, d 8 and d 15) as the within animal effect according to the following model:

\[ Y_{dij} = \mu + \alpha_d + \beta_i + \alpha\beta_{di} + \epsilon_{dij} \]

Here \( \alpha_d \) denotes the effect of diet, \( d = 1, 2 \), \( \beta_i \) is the effect of time, \( i = 1, 8, 15 \), \( \alpha\beta_{di} \) is the interaction between diet and segment and \( i \) refers to an individual pig. The variance component \( \sigma^2 \) accounts for the repeated measurements that were made on the same individual, thereby rendering these observations correlated, whereas the term \( \epsilon_{dij} \sim N(0, \sigma^2) \) represents the unexplained random error (SAS Institute 1989).

The effect of litter and series was tested and found not to be significant, therefore series and litter were not included in the statis-
tical analyses presented here. The analyses were performed with SAS for Windows version 6.12 (SAS Institute, Cary, NC).

Statistical analysis of bacterial counts was performed after logarithmic conversion of the data. Values are means ± SEM.

RESULTS

Changes in dry matter content, pH and bacterial counts in feces during the adaptation period. The two experimental diets did not create significant differences in the dry matter content of the feces during the experimental period except at d 15, when the dry matter content was significantly lower in pigs fed the tagatose diet than in those fed the control diet (Fig. 1A). The dry matter content of feces increased from d −3 to d 1 as a consequence of changing the diet from the standard pig diet to the experimental diets. There was a further increase with time (P = 0.0001) of consuming the experimental diets, without any effect of diet (P = 0.11) or interaction between diet and time (P = 0.09).

The pH of the feces decreased 0.4 and 0.6 from d −3 to d 1 when changing from the standard pig feed to the control and tagatose diets, respectively (Fig. 1B), and was significantly lower in pigs fed the tagatose diet on d 1 and d 15, but not on d 8. Differences in the time courses for the two experimental groups led to a significant interaction between time and diet in the multivariate analysis (P = 0.03).

The shift of diet from d −3 to d 1 increased the total number of anaerobic bacteria in feces (Fig. 1C). The change in bacterial counts was evident on d 1 and no further change in numbers during the experimental period was noted (P = 0.29). Neither in the univariate analyses nor in the multivariate analysis (P = 0.47) was there any effect of dietary treatment.

The number of D-tagatose–degrading bacteria in feces from the tagatose-fed pigs was approximately ten times the value found in the feces of the pigs fed the control diet (Fig. 1D), which led to a significant effect of experimental diet in the multivariate analysis (P = 0.0001). A slight further increase in numbers was seen on d 8, resulting in a significant time effect (P = 0.046) in the experimental period (d 1 to d 15).

Production of SCFA during in vitro fermentation of un-fractionated digesta. For both dietary groups, the predominant fermentation products produced in the stomach and distal SI were lactic acid followed by acetic and formic acids. In addition, a little butyric acid was formed in the distal SI (Fig. 2).

The rate of lactic, formic and acetic acid production during incubation of digesta from the small intestine was more than twice as high with digesta of the pigs fed the control diet compared to digesta from the tagatose-fed pigs, but when correcting for differences in the volume of digesta in this particular segment (Lærke and Jensen 1999), there were no significant differences in the total productions of these acids (P > 0.35). The total SCFA and lactic acid production in the stomach, accounting for differences in digesta volumes, was 7.4 ± 2.2 and 8.2 ± 2.2 mmol/h for the control and tagatose group, respectively (P = 0.8), whereas the total production in the distal SI was 8.0 ± 1.7 mmol/h for pigs fed the control diet and 6.4 ± 1.7 mmol/h for those fed the tagatose diet (P = 0.5).

The rate of SCFA production from the cecal material of the pigs in the tagatose group consisted in decreasing order of propionic, acetic, butyric, valeric and formic acid, whereas the main acid for the control pigs was acetic acid, with lesser amounts of propionic, butyric and valeric acid. For both dietary groups, the main SCFA produced with colonic material consisted in decreasing order of acetic, propionic, butyric and valeric acid.

The rate of propionic acid production in the cecum was more than twice as high with the tagatose-fed pigs compared with the pigs fed the control diet (P = 0.006) and there was a twofold higher rate of valeric acid production (P = 0.006). Allowing for differences in digesta volume (Lærke and Jensen 1999), this led to a total production of valeric acid with the tagatose diet that was almost six times as high (1.27 ± 0.35 mmol/h) as the control diet (0.22 ± 0.35 mmol/h, P = 0.049).

Although the rate of valeric acid production with the colonic material was twice as high with the tagatose diet compared with the control diet, the total valeric acid production with
Acid; Iso-but, iso-butyric acid; Iso-val, iso-valeric acid. But, butyric acid; Val, valeric acid; Cap, caproic acid; Hep, heptanoic acid; Lac, lactic acid; For, formic acid; Ace, acetic acid; Pro, propionic acid; But, butyric, valeric and caproic acid but not for acetic acid (control group, which was significant for formic, propionic, butyric, valeric and caproic acids was higher in the latter group than in the former.

The bacteria isolated from the cecum of tagatose-fed pigs produced a significantly higher proportion of formic and valeric acid but a smaller proportion of acetic acid than bacteria obtained from the cecum of the control group (Table 1), whereas no effect of diet was seen in the proportion of propionic and butyric acid produced from d-tagatose. There was a similar picture with the bacteria from the mid colon, but the proportion of acetic acid from the control group was much higher. On the other hand, d-tagatose induced a much bigger increase in the contribution of butyric acid to the SCFA production in the colon than in the cecum. Furthermore, the proportion of propionic acid produced by cecal bacteria was generally higher than that with bacteria from the mid colon irrespective of the dietary treatment.

**SCFA production related to the degradation of d-tagatose.**
D-tagatose was not degraded when incubated with bacteria isolated from the stomach and distal SI, independent of the dietary treatment of the pigs (values ranged from −1.5 to 0.1 g/(h ⋅ kg)). Figure 3 illustrates the SCFA production by bacteria harvested from the cecum and colon of pigs fed the control or tagatose diet plotted against their d-tagatose degradation. Generally, there was a very low degradation of d-tagatose with bacteria harvested from the pigs in the control group. Thus the degradation of d-tagatose was significantly higher for the bacteria isolated from the mid colon of pigs fed d-tagatose [15.3 ± 1.2 g/(h ⋅ kg), n = 6] compared with the degradation seen with bacteria from the control pigs [1.2 ± 1.2 g/(h ⋅ kg), n = 5, P = 0.0001]. There was no significant difference in the capacity of the microbiota from the cecum to degrade d-tagatose between the control diet [2.7 ± 1.0 g/(h ⋅ kg), n = 6] and the tagatose diet [4.4 ± 1.1 g/(h ⋅ kg), n = 6, P = 0.36]. The mean SCFA production per gram of degraded tagatose was 4.8 ± 1.0 mmol/g for the cecal bacteria and 4.0 ± 1.8 mmol/g for the colonic microbiota of tagatose-adapted pigs. Based on this production and the molar composition, we estimate that when the d-tagatose was fermented by cecal and colonic bacteria adapted to the ingestion of d-tagatose, ~51% of the energy of d-tagatose was recovered as SCFA. Because of the very low level of d-tagatose degradation with bacteria from the control group, it was not possible to estimate an energy value for the unadapted microbiota.

**Gas production during in vitro fermentation of isolated bacteria with and without addition of d-tagatose.** Major increases in hydrogen production were seen during the in vitro incubation of d-tagatose with bacteria isolated from the cecum and colon of tagatose-fed pigs compared with the pigs fed the control diet (Table 2). Significant effects of d-tagatose on methane production were not seen until mid colon where the production in the tagatose group was more than five times the control diet (0.33 ± 0.09 mmol/h) was not significantly different (P = 0.18) from that obtained with the tagatose diet (0.52 ± 0.09 mmol/h). The total SCFA production in the cecum was 3.0 ± 2.7 and 10.7 ± 2.7 mmol/h for the control and tagatose group, respectively (P = 0.06), whereas the productions in the mid colon were 5.1 ± 1.4 and 5.9 ± 1.4 mmol/h, respectively (P = 0.7).

**Rate of production and composition of SCFA during in vitro fermentation with slurries of bacteria with and without addition of d-tagatose.** The presented d-tagatose caused no increase in SCFA or lactic acid production in bacterial slurries from the stomach and distal SI, indicating that the bacteria from these segments of the gastrointestinal tract were not able to degrade d-tagatose in vitro. The rate of SCFA production from d-tagatose obtained with bacteria from the cecum (Table 1) was generally higher for the tagatose group than from the control group, which was significant for formic, propionic, butyric, valeric and caproic acid but not for acetic acid (P = 0.42). The bacteria from the mid colon from the control group had a slower SCFA production than the bacteria obtained from the tagatose-fed pigs. The production of formic, acetic, propionic, butyric, valeric and caproic acids was higher in the latter group than in the former.

**DISCUSSION**
The population of bacteria able to degrade d-tagatose comprised ~10% of the total population of anaerobic bacteria before the pigs started eating the experimental diets. The inclusion of d-tagatose in the diet resulted in ten times as many d-tagatose-degrading bacteria compared with the diet without d-tagatose. The increase in density of the population of total anaerobic...
bacteria per gram of fresh feces and the increase in dry matter content of feces at the beginning of the experiment could be explained by a reduction in the dietary fiber content of the experimental diets (5 g/100 g of dry matter) compared with traditional pig feed (18–20 g/100 g of dry matter).

Although the response to D-tagatose in terms of number of D-tagatose–degrading bacteria was rapid, there was a reduction in the pH of the feces on d 1 (equivalent to d 3 after the first exposure to D-tagatose), which was very clear for the pigs fed the tagatose diet. Presumably, at this stage the microbiota in the proximal part of the colon was not adapted to digestion of D-tagatose, and their ability to degrade D-tagatose was limited. Consequently, more D-tagatose would pass on to the more distal parts of the colon before it was completely fermented, which would lead to a larger SCFA production in this area and a lower pH of the excreta. After adaptation (on d 8 and d 15), D-tagatose could be fully fermented in the proximal large intestine, and less substrate would be available for the microbiota in the distal large intestine, leading to a higher pH compared with the period just after introduction of D-tagatose to the diet. These results suggest that a certain period of adaptation is necessary before the gastrointestinal microbiota can digest D-tagatose. This was confirmed in the in vitro fermentation studies with isolated bacteria, where the rate of D-tagatose degradation and SCFA production was larger for the adapted bacteria (from the pigs in the tagatose group) than the unadapted bacteria. The results also indicate that the large intestinal bacteria have the capacity to metabolize D-tagatose at much higher levels than the 10% added to the diet of this experiment. The lack of difference between dietary groups in the SCFA production during in vitro incubation of digesta from the mid colon of pigs after a 20-d adaptation period suggests that at this point all D-tagatose was fermented in the proximal part of the colon and did not reach the mid colon. Indeed, the in vitro incubations showed that the bacteria in the distal colon had a high capacity to digest D-tagatose, although the microbial activity

### TABLE 1

*Rate of production and molar composition of short chain fatty acids (SCFA) produced after 4 h from D-tagatose by bacterial slurries harvested from pigs fed either the control or the tagatose diet*  

<table>
<thead>
<tr>
<th></th>
<th>Cecum</th>
<th>Colon</th>
<th></th>
<th></th>
<th>Rate of production, mmol/(h · kg digesta)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Tagatose</td>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>Tagatose</td>
</tr>
<tr>
<td>Formate</td>
<td>0.24 ± 0.85</td>
<td>3.74 ± 0.85*</td>
<td></td>
<td></td>
<td>0.03 ± 0.59</td>
<td>2.34 ± 0.59*</td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>3.85 ± 1.11</td>
<td>5.25 ± 1.11</td>
<td></td>
<td></td>
<td>4.62 ± 2.09</td>
<td>15.70 ± 2.03***</td>
<td></td>
</tr>
<tr>
<td>Propionate</td>
<td>0.63 ± 0.93</td>
<td>4.49 ± 0.93*</td>
<td></td>
<td></td>
<td>0.01 ± 0.38</td>
<td>2.55 ± 0.38***</td>
<td></td>
</tr>
<tr>
<td>Butyrate</td>
<td>1.25 ± 1.06</td>
<td>5.67 ± 1.06*</td>
<td></td>
<td></td>
<td>1.09 ± 2.47</td>
<td>30.04 ± 2.47***</td>
<td></td>
</tr>
<tr>
<td>Valerate</td>
<td>0.75 ± 0.90</td>
<td>5.67 ± 0.90**</td>
<td></td>
<td></td>
<td>0.50 ± 1.02</td>
<td>9.16 ± 1.02***</td>
<td></td>
</tr>
<tr>
<td>Caproate</td>
<td>0.15 ± 0.07</td>
<td>0.41 ± 0.07*</td>
<td></td>
<td></td>
<td>0.32 ± 0.57</td>
<td>4.58 ± 0.57***</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>6.93 ± 4.35</td>
<td>25.22 ± 4.35*</td>
<td></td>
<td></td>
<td>6.54 ± 4.76</td>
<td>64.29 ± 4.76***</td>
<td></td>
</tr>
</tbody>
</table>

* 1 Values are means ± SEM, n = 8.

### TABLE 2

*Gas production from D-tagatose incubated with bacteria from the cecum and mid colon of pigs fed the tagatose or control diet*  

<table>
<thead>
<tr>
<th></th>
<th>Hydrogen</th>
<th>Methane</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Tagatose</td>
<td>SEM</td>
<td>Control</td>
<td>Tagatose</td>
<td>SEM</td>
</tr>
<tr>
<td>Stomach</td>
<td>0</td>
<td>−1</td>
<td>0.4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Distal SI2</td>
<td>−3</td>
<td>4</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cecum</td>
<td>3</td>
<td>50***</td>
<td>8</td>
<td>13</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>Mid colon</td>
<td>0</td>
<td>120*</td>
<td>37</td>
<td>44</td>
<td>244*</td>
<td>51</td>
</tr>
</tbody>
</table>

* 1 Values are means ± SEM, n = 8.

2 SI, small intestine.

* 0.05, ** 0.01, *** 0.001; significantly different from control.
fermentable carbohydrate sources, which has been found therefore unexpected. Lactulose is one of few other easily for the microbiota in the small intestine of the pigs is chemically modified monosaccharide, is totally unavailable in cannulated and intact pigs. Our finding that D-tagatose, a glucose and mannitol (Canibe and Bach Knudsen 1991, Bach Knudsen and Hansen 1997, Christensen et al. 1999), sorbitol, galacturonic and glucuronic acid (Mortensen et al. 1988), neosugar (Berggren et al. 1993) and fructooligosaccharides (Campbell et al. 1997, Gibson and Wang 1994, Le Blay et al. 1995). Our results support this for pigs but are in contrast to the results of Christensen et al. (1999), who found that adaptation to the diet was less important if the contribution of SCFA from the fecal inulin produced during in vitro incubation was subtracted from the total production.

Based on the in vitro incubations we estimated that ~51% of the energy in D-tagatose was recovered as SCFA after microbial fermentation. It is important to note that this value was obtained from actively metabolizing bacteria, which were not limited in the amounts of available D-tagatose. It is well known that bacteria growing in a medium with excess carbohydrate will accumulate the carbohydrate and store it in the bacterial cells (Dawes and Senior 1961). It is likely that an extended fermentation time, which was not allowed in the present study, was responsible for the incomplete fermentation. We would have expected to recover more energy from D-tagatose if it had been added to the list of components that may beneficially enhance the production of butyric acid in the large intestine.

why? D-tagatose degradation in the distal colon was lower than in the cecum and proximal colon (Lærke and Jensen 1999). However, the possibility remains that a higher microbial activity caused by fermentation of D-tagatose would have been seen in the more distal part of the large intestine if the pigs had been killed later than 3 h after the morning meal. At 3 h after a meal, dietary material present in the large intestine of pigs mostly stems from the preceding meal fed 19 h earlier, where D-tagatose, which was found to be a readily fermentable substrate, would have been fermented.

The rates of SCFA production in digesta from the cecum and mid colon found in this investigation were from three (acetic acid) to seven (propionic acid) times higher than the rates reported by Holtug et al. (1992) for pigs calculated from 6 h in vitro incubations. This difference is probably due to the shorter incubation time used in this investigation. The rates of SCFA production would have been underestimated in this study if they had been based on incubation times >30 min, because the rate of SCFA production decreased after prolonged fermentation.

Strikingly, no D-tagatose degradation and SCFA or lactic acid production took place with bacteria isolated from the stomach and distal small intestine, neither from the adapted nor from the unadapted pigs, suggesting that the microbiota in the upper gastrointestinal tract were not able to adapt to utilization of D-tagatose as substrate for microbial fermentation. Consequently, the SCFA produced in the upper gastrointestinal tract would be expected to stem from other components of the diet, which was supported by the observation that the total amount and composition of SCFA and lactic acid present at slaughter was not significantly different in the two dietary groups (Lærke and Jensen, 1999).

Other easily fermentable carbohydrates such as raffinose-oligosaccharides and mannotel (Canibe and Bach Knudsen 1997, Gdala et al. 1997, Saunders and Wiggins 1981), inulin (Bach Knudsen and Hessov 1995, Graham and Aman 1986) and β-glucans (Bach Knudsen and Hansen 1991, Bach Knudsen et al. 1993, Johansen et al. 1997, Sundberg et al. 1996) have been found to be degraded to variable extents at the terminal ileum in healthy ileostomy patients, ileum cannulated and intact pigs. Our finding that D-tagatose, a chemically modified monosaccharide, is totally unavailable for the microbiota in the small intestine of the pigs is therefore unexpected. Lactulose is one of few other easily fermentable carbohydrate sources, which has been found fully recovered from ileostomies (Saunders and Wiggins 1981).

An interesting observation was the high production of valeric acid obtained by in vitro fermentation of digesta from the cecum and colon of the pigs fed D-tagatose and also by incubation of the isolated bacteria with D-tagatose. The results indicate that D-tagatose may particularly stimulate the production of this acid but whether this is due to a shift in metabolism of the normal gut flora or proliferation of specific groups of bacteria is not known. Very few bacteria have been shown to produce valeric acid as a major fermentation product (Holdeman et al. 1977), and it has even been suggested that it is exclusively a product of amino acid fermentation (Rasmussen et al. 1988). However, enhanced amino acid catabolism in the tagatose-fed pigs is not a likely explanation for the difference seen between the two experimental diets in this study because the production of iso- and isovaleric acid, which also are products of protein degradation, were very small and comparable in the two experimental diets.

The bacterial slurries, especially from the colon of the tagatose-adapted pigs, also produced significant amounts of butyric acid, which is believed to be clinically important because of its metabolic role in the health of the colon mucosa (Roediger 1980) and its protective role against colon-rectal cancer (Young and Gibson 1994). Previous reports of carbohydrates that stimulate butyric acid production in the lower gut of monogastrics include starch (Englyst et al. 1987, Mathers and Smith 1993, Silvester et al. 1995, Weaver et al. 1992), oat bran (Bach Knudsen et al. 1993, Christensen et al. 1999), sorbitol, galacturonic and glucuronic acid (Mortensen et al. 1988), neosugar (Berggren et al. 1993) and fructooligosaccharides (Campbell et al. 1997, Gibson and Wang 1994, Le Blay et al. 1999). From this study it appears that D-tagatose can be added to the list of components that may beneficially enhance the production of butyric acid in the large intestine.

It is also noteworthy that the bacteria isolated from the cecum of both dietary groups produced high proportions of propionic acid, whereas the bacteria harvested from the mid colon did not. Previous studies have concluded that the site of collection and adaptation to the diet had a significant influence on the composition of the SCFA produced in vitro and in vivo in rats (Berggren 1996, Monsma and Marlett 1995). Our results support this for pigs but are in contrast to the results of Christensen et al. (1999), who found that adaptation to the diet was less important if the contribution of SCFA from the fecal inulin produced during in vitro incubation was subtracted from the total production.
tics) was 35–54%, whereas starch fermentation yielded ~60%. Mikkelsen and Jensen (1997) reported an energy yield of 59% from fermentation of fructo-oligosaccharides. According to Hungate (1966), the energy content in SCFA is ~60% of that of the substrate during the fermentation of carbohydrates in the rumen, whereas Cummings (1994) has suggested an upper limit of 60% energy yield for carbohydrate fermentation in the colon and a fixed energy yield of 75% was suggested by McNeil (1984).

Some of the energy in D-tagatose was lost in the form of various gasses. Substantial amounts of hydrogen could be produced by the fermentation of D-tagatose with bacteria isolated from the cecum and colon, while large amounts of methane were produced during incubation of D-tagatose with bacteria isolated from the mid colon. In that respect, D-tagatose resembles other easily fermentable carbohydrates such as fructo-oligosaccharides, galacto-oligosaccharides and lactulose, which have also been shown to increase hydrogen production (Crist et al. 1992, Djouzi and Andrieux, 1997). A high gas production in the large intestine can have several negative effects on the host, including flatulence, abdominal pain and diarrhea. Increased flatulence after continuous ingestion of large amounts of D-tagatose (30 g/d) has been reported in man (Buemann et al. 1999).

In conclusion, the proportion of D-tagatose–degrading bacteria in feces was dependent on the presence of D-tagatose in the diet. The rate of D-tagatose fermentation was higher for bacteria adapted to D-tagatose compared with unadapted bacteria. D-tagatose was not utilized as substrate for the microbiota in the colon of pigs adapted to D-tagatose was two to three times higher than from the unadapted pigs. In addition to short chain fatty acids, D-tagatose could also be metabolized by the bacteria of the large intestine to form hydrogen and methane.

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