Influences of dietary adaptation and source of resistant starch on short-chain fatty acids in the hindgut of rats

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The effect of adaptation time on the concentration and pattern of short-chain fatty acids (SCFA) formed in the hindgut of rats given resistant starch (RS) in the form of raw potato starch (RPS) or high-amylose maize starch (HAS) was evaluated. Each starchy material was tested in diets containing 100 g indigestible carbohydrates/kg DM, and fed for 13, 28 and 42 d. At the end of each period, the content of SCFA was determined in caecum, distal colon and faeces. The caecal concentration of total and individual SCFA increased for both diets with increasing adaptation time. The concentration of butyric acid was higher in the group fed RPS than in that fed HAS at all adaptation times. The caecal proportion of butyric acid was low both in rats fed RPS and HAS (6 and 4 %, respectively) following 13 d of adaptation. However, after 28 d of adaptation, the proportion of butyric acid had increased to 19 % in rats given RPS. A longer adaptation period (42 d) did not increase the proportion of butyric acid further. With HAS, there was also a significant (P<0.01) increase in the proportion of butyric acid with longer adaptation time. However, the increase was much slower and the proportion of butyric acid reached 6 and 8 % after 28 and 42 d respectively. It is concluded that the pattern of SCFA formed from RS in rats is dependent on adaptation time. It cannot be excluded that the different patterns of SCFA reported in the literature for RS may be due to the time of adaptation.

Resistant starch: Fermentation: Short-chain fatty acids: Butyric acid: Rats

Short-chain fatty acids (SCFA; mainly acetic, propionic and butyric acid) are formed during microbial fermentation of carbohydrates in the colon. There is increasing evidence that SCFA, especially butyric acid, play an essential role in the maintenance of the colonic mucosa. Butyric acid is the main energy substrate for the colonocytes (Roediger, 1982) and has been suggested to play a role in the prevention and treatment of diseases of the colonic mucosa, such as distal ulcerative colitis (Cummings, 1997) and cancer (Scheppach et al. 1995). A diminished oxidation of butyrate in the colonocytes has been suggested to contribute to the genesis of ulcerative colitis (Roediger, 1980) and enemas with butyric acid have been associated with reduced symptoms in patients with ulcerative colitis (Scheppach et al. 1992). Although butyric acid serves as the primary energy source for the normal colonic epithelium and stimulates growth of colonic mucosa, the growth of colon tumour cell lines has been reported to be obstructed by butyrate (Whitehead et al. 1986). Butyrate also appears to induce cell differentiation (Barnard & Warwick, 1993) and to stimulate apoptosis (Hague et al. 1995) in tumour cell lines.

Starches have been shown to produce high proportions of butyric acid by in vitro fermentation in human faecal inocula (Englyst et al. 1987; Weaver et al. 1992; Bradburn et al. 1993; Casterline et al. 1997). Starch that reaches the colon has also been shown to increase the faecal concentration of butyric acid in human subjects. Thus, administration of an α-amylase inhibitor, acarbose, resulted in a specific increase in faecal concentrations of butyric acid in normal subjects (Scheppach et al. 1988; Weaver et al. 1997). Further, when adding the resistant starch (RS) source high-amylose maize starch (HAS) to the diet, the faecal concentration (Phillips et al. 1995; Noakes et al. 1996) or daily excretion (van Munster et al. 1994) of butyric acid increased. However, of these studies, only the study by Noakes et al. (1996) showed an increase in the faecal proportion of butyric acid. In another study, different sources of RS, such as raw starch from potatoes and bananas and retrograded starch from wheat and maize, were given to normal subjects (Cummings et al. 1996). Of these substrates, only raw potato starch (RPS) gave an increased faecal proportion of butyric acid. However,

**Abbreviations:** HAS, high-amylose maize starch; RPS, raw potato starch; RS, resistant starch; SCFA, short-chain fatty acids.

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studies in human subjects are scarce. In addition, experimental conditions, such as the intake of indigestible carbohydrates, are difficult to control. Moreover, acarbose, frequently used to increase starch delivery to the colon and thus enable studies of SCFA formation from starch, may affect microbial enzymes involved in fermentation, as judged from experiments in rats (A Berggren, I Björck and M Nyman, unpublished results).

Considerably more results on the formation and pattern of SCFA from fermentation of RS are available in animals. Studies in rats (Mallett et al. 1988; Gee et al. 1991; Berggren et al. 1995; Monsmà & Marlett, 1995) and pigs (Topping et al. 1993; Brown et al. 1997; Bird et al. 2000) have shown important variations in fermentation profiles with different types of RS as substrates. One explanation could be that the production of butyric acid may vary between different sources of RS (Anisson & Topping, 1994). RS are generally classified into three types (Englyst et al. 1992): starch trapped in the cell wall of plants and thereby physically inaccessible to α-amylase (RS 1); starch stored in granules in the native crystalline form that can be made accessible to enzymes by gelatinisation (RS 2); starch that has been retrograded after cooling of gelatinised starch (RS 3). Chemically modified starches have been described as RS 4 (Brown, 1996). The distribution between amylase and amylopectin in the starch molecule may be of importance for the profile of SCFA formed. Wang et al. (1999) demonstrated that different bacterial strains are involved in the degradation of these two molecules.

Another factor that might influence fermentation characteristics is the adaptation time. With respect to dietary fibre, e.g. wheat bran, pectin, cellulose and guar gum, even a short adaptation time (between 5 and 7 d) appears to yield stable fermentation in rats as judged from determination of extent of fermentation (Nyman & Asp, 1985; Bruunsgaard et al. 1995), whereas retrograded HAS required a longer intervention period (1 month; Bruunsgaard et al. 1995). Concerning the effect of adaptation time on the profile of SCFA from RS, differing results have been obtained in rats. In rats fed RPS, the proportion of butyric acid in the caecum increased with time, from 13 to 28 % following 0·5 in rats. In rats fed RPS, the proportion of butyric acid in the caecum increased with time, from 13 to 28 % following 0·5

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### Table 1. Composition of test diets (g/kg)

<table>
<thead>
<tr>
<th>Ingredient*</th>
<th>RPS</th>
<th>HAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPS</td>
<td>157</td>
<td>188</td>
</tr>
<tr>
<td>Wheat starch</td>
<td>515</td>
<td>504</td>
</tr>
<tr>
<td>Casein</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Maize oil</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Mineral mixture†</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td>Vitamin mixture†</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

RPS, raw potato starch; HAS, high-amylose maize starch.

* RPS (Lyckeby Stärkelsen AB, Kristianstad, Sweden); HAS (Hi-maize™; Penford Australia, Lane Cove, New South Wales, Australia), wheat starch (Ceresst, Krefeld, Germany), casein (Sigma Chemical Company, St Louis, MO, USA), sucrose (Danisco Sugar, Malmö, Sweden), maize oil (Maizola; Best-foods Nordic A/S, Copenhagen, Denmark), mineral mixture (Apoteket, Malmö, Sweden), vitamin mixture (Apoteket, Malmö, Sweden), choline chloride (Aldrich-Chemie, Steinheim, Germany), DL-methionine (Sigma Chemical Company).

† Containing (g/kg): CuSO₄·5H₂O 0·37, ZnSO₄·7H₂O 1·40, KH₂PO₄ 332·10, NaH₂PO₄·2H₂O 171·80, CaCO₃ 324·40, KI 0·068, MgSO₄ 57·20, FeSO₄·7H₂O 1·25, MnSO₄·H₂O 3·40, CoCl₂·6H₂O 0·020, NaCl 101·70.

‡ Containing (g/kg): menadione 0·62, thiamin hydrochloride 0·62, riboflavin 0·62, pyridoxine hydrochloride 0·62, calcium pantothenate 6·25, nicotinic acid 6·25, folic acid 0·25, inositol 0·25, p-amino-benzoic acid 0·25, biotin 0·05, cyanocobalamin 0·00375, retinyl palmitate 0·187, calciferol 0·00615, α-tocopherolacetate 25·00, maize starch 941·25.
Resistant starch and short-chain fatty acids

Table 2. Feed intake, body-weight gain and caecal wet content in rats fed either raw potato starch (RPS) or high-amylose maize starch (HAS) (Mean values with their standard errors for seven rats per group)†

<table>
<thead>
<tr>
<th></th>
<th>13 d</th>
<th></th>
<th>28 d</th>
<th></th>
<th>42 d</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RPS</td>
<td>HAS</td>
<td>RPS</td>
<td>HAS</td>
<td>RPS</td>
<td>HAS</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>Feed intake (g/d)</td>
<td>12.0</td>
<td>0.0</td>
<td>11.9</td>
<td>0.1</td>
<td>15.7</td>
<td>0.4</td>
</tr>
<tr>
<td>Body-weight gain (g/d)</td>
<td>3.5</td>
<td>0.1</td>
<td>3.7</td>
<td>0.1</td>
<td>5.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Caecal wet content (g)</td>
<td>2.9***</td>
<td>0.2</td>
<td>1.7</td>
<td>0.1</td>
<td>6.1*</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Mean values were significantly different from those in the HAS group (one-way ANOVA): *P<0.05, ***P<0.001.
† For details of diets and procedures, see Table 1 and p. 320.

was approved by the Ethics Committee for Animal Studies at Lund University.

Analysis of starch

An in vitro model (Åkerberg et al. 1998) was used for determination of RS in the test materials. Six human subjects performed simulated mastication using glass beads for 15 s and then rinsed their mouths with 5 ml water, and thereafter the saliva was pooled. Pooled saliva (5 ml) was transferred to a beaker containing the test product and water. The pH was adjusted to 1.5 and pepsin (Merck, Darmstadt, Germany) was added. Thereafter, the samples were incubated at 37°C for 30 min. The pH was adjusted to 5.0 after addition of pancreatin (Sigma Chemical Company, St Louis, MO, USA) and amyloglucosidase (Boehringer Mannheim, Mannheim, Germany). The suspension was incubated for 16 h at 40°C. Undigested starch was precipitated with ethan-ol and analysed as liberated glucose after solubilisation in KOH and enzymatic treatment with a thermostable a-amylase (Termamyl 300L DX; Novo Nordisk A/S, Copenhagen, Denmark) and amyloglucosidase (Boehringer Mannheim, Mannheim, Germany) according to Björck & Siljestrom (1992). Pooled saliva was used instead of an initial chewing of the sample since the product was not a realistic food item, but a dry flour. The analysis was performed six times per sample. Total starch in faeces was analysed as described earlier (Björck & Siljestrom, 1992) and when corrected for the small amounts of free glucose it was regarded as RS.

Table 3. Faecal dry weight and fermentability of resistant starch (RS) in rats fed either raw potato starch (RPS) or high-amylose maize starch (HAS) following 12 d of ingestion†

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RPS</td>
<td>HAS</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>Faecal dry weight (g/d)</td>
<td>1.0***</td>
<td>0.1</td>
</tr>
<tr>
<td>Fermentability of RS (%)</td>
<td>54.5***</td>
<td>6.4</td>
</tr>
</tbody>
</table>

Mean values were significantly different from those in the HAS group (one-way ANOVA): ***P<0.001.
† For details of diets and procedures, see Table 1 and p. 320.

Analysis of NSP

NSP in the test materials were isolated using the enzymatic method of Asp et al. (1983). The composition of the isolated fibre residue was analysed by GLC on a DB-225 column (J&W Scientific, Folsom, CA, USA) for the neutral sugars as their alditol acetates and spectrophotometrically for the uronic acids (Theander et al. 1995). Non-starch glucose was calculated as the difference between the total glucose content measured by GLC and the total amount of starch in the isolated fibre residue. Triplicate samples were used.

Determination of short-chain fatty acids

The amount of SCFA (acetic, propionic, isobutyric, butyric, isovaleric, valeric, caproic and heptanoic acid) and succinic acid in caecum and colon contents were analysed by a GLC method (Richardson et al. 1989). The intestine content was homogenised (Polytron®, Kinematica, Luzern, Switzerland) with 2-ethylbutyric acid (internal standard). HCl was added to protonise the SCFA, which were then extracted with diethyl ether and silylated with N-(tert-butyldimethylsi-lyl)-N-methyltrifluoroacetamide (Sigma Chemical Company). The samples were allowed to stand for 48 h to complete derivatisation. Samples were analysed using GLC (HP 6890; Hewlett-Packard, Wilmington, DE, USA) equipped with an HP-5 column (Hewlett-Packard), and integrated by Chem Station software (Hewlett-Packard).

Calculations and statistical evaluation

The faecal excretion of RS (%) was calculated as the amount of starch found in faeces divided with the ingested amount of RS and multiplied by 100. The caecal pool of SCFA was calculated by multiplication of the concentration of SCFA in the caecum (mmol/kg) by the total weight of the caecal contents (kg). The faecal excretion of SCFA was calculated by multiplication of the concentration of SCFA in the faeces (mmol/kg) by the weight of faeces (kg) excreted during the last 24 h of the experiment (‘13 d’ in Tables 2, 4–5).

The proportion of butyric acid was calculated as the proportion of butyric acid of the three major SCFA (i.e. acetic, propionic and butyric acid) and this was calculated for each rat before statistical evaluation.
Table 4. Concentration (mmol/kg) of short-chain fatty acids in caecum, distal colon and faeces of rats fed a diet containing raw potato starch (RPS) or high-amylose maize starch (HAS) for 13, 28 or 42 d†

<table>
<thead>
<tr>
<th></th>
<th>13 d</th>
<th>28 d</th>
<th>42 d</th>
<th>Statistical significance of effect: P</th>
<th>Diet</th>
<th>Time</th>
<th>Diet × Time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>RPS</td>
<td>HAS</td>
<td>RPS</td>
<td>HAS</td>
<td>RPS</td>
<td>HAS</td>
<td>RPS</td>
</tr>
<tr>
<td>Caecum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic</td>
<td>72·2</td>
<td>3·3</td>
<td>69·5</td>
<td>6·3</td>
<td>94·2</td>
<td>15·7</td>
<td>109·7</td>
</tr>
<tr>
<td>Propionic</td>
<td>12·6</td>
<td>1·4</td>
<td>12·6</td>
<td>1·2</td>
<td>15·2</td>
<td>2·0</td>
<td>20·5</td>
</tr>
<tr>
<td>Butyric</td>
<td>5·2***</td>
<td>0·2</td>
<td>3·5</td>
<td>0·3</td>
<td>7·9</td>
<td>0·7</td>
<td>27·6*</td>
</tr>
<tr>
<td>Total</td>
<td>102·8</td>
<td>4·1</td>
<td>95·1</td>
<td>7·3</td>
<td>143·7</td>
<td>19·7</td>
<td>153·5</td>
</tr>
<tr>
<td>Distal colon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic</td>
<td>49·2</td>
<td>3·1</td>
<td>57·3</td>
<td>5·4</td>
<td>53·6</td>
<td>9·4</td>
<td>64·8</td>
</tr>
<tr>
<td>Propionic</td>
<td>6·8</td>
<td>1·1</td>
<td>7·4</td>
<td>1·2</td>
<td>8·0</td>
<td>1·3</td>
<td>7·7</td>
</tr>
<tr>
<td>Butyric</td>
<td>3·7</td>
<td>0·6</td>
<td>3·9</td>
<td>0·5</td>
<td>12·0</td>
<td>2·2</td>
<td>5·8</td>
</tr>
<tr>
<td>Total</td>
<td>69·8</td>
<td>4·1</td>
<td>80·3</td>
<td>6·5</td>
<td>153·5</td>
<td>19·7</td>
<td>193·9</td>
</tr>
<tr>
<td>Faeces</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic</td>
<td>48·9</td>
<td>3·1</td>
<td>57·3</td>
<td>5·4</td>
<td>46·9**</td>
<td>7·2</td>
<td>67·4</td>
</tr>
<tr>
<td>Propionic</td>
<td>7·1</td>
<td>0·8</td>
<td>5·5</td>
<td>0·8</td>
<td>10·4</td>
<td>2·2</td>
<td>6·1</td>
</tr>
<tr>
<td>Butyric</td>
<td>4·0</td>
<td>0·7</td>
<td>3·2</td>
<td>0·5</td>
<td>10·8*</td>
<td>2·2</td>
<td>6·3</td>
</tr>
<tr>
<td>Total</td>
<td>71·9</td>
<td>4·6</td>
<td>74·1</td>
<td>6·5</td>
<td>73·3</td>
<td>16·8</td>
<td>84·8</td>
</tr>
</tbody>
</table>

Mean values were significantly different from those in the HAS group (one-way ANOVA): *, P < 0·05; **, P < 0·01; ***P < 0·001.

†For details of diets and procedures, see Table 1 and p. 320.

‡Two-way ANOVA could not be performed, as some rats did not defecate during the sampling time.
All statistical analyses were performed with the Mini-
Tab statistical software package (version 13.0; Minitab Inc., State College, PA, USA). In Tables 2 and 3, mean values were analysed by one-way ANOVA using the General Linear Model procedure according to Minitab. In Tables 4 and 5, mean values were analysed by two-way ANOVA to assess the effects of diet, adaptation time and interactions between the diet and time on the concentration of SCFA in caecum and distal colon. The analysis was not performed on faecal data because some values were missing. When significant differences were found, individual means were analysed by one-way ANOVA to assess the effects of diet at each adaptation time. The level of significance was $P < 0.05$.

**Results**

RPS contained a higher amount of RS (640 g/kg DM) than HAS (591 g/kg DM). Only HAS contained a measurable amount of NSP (14 g/kg DM).

The rats tolerated both diets well and there was no difference in feed intake or body weight gain between the two diets during any of the intervention periods (Table 2). However, ingestion of the RPS diet gave a higher caecum wet weight than the HAS diet following 13 and 28 d of adaptation ($P < 0.05$). Further, the faecal dry weight measured during the balance experiment was twice as high for rats fed RPS than for those fed HAS ($P < 0.001$, Table 3). The two starches were fermented to various extents. RS in HAS was almost completely fermented during the balance experiment, and only about 3.0% of the ingested amount appeared in faeces. RS in RPS, on the other hand, was considerably more resistant and 45.5% appeared in faeces ($P < 0.001$).

The caecal concentration of SCFA increased with adaptation time in rats fed both substrates (Table 4). Further, the concentration of SCFA was higher in caecum than in distal colon or faeces, and this difference increased with prolongation of the adaptation time. The two substrates gave similar caecal concentrations of total SCFA, as well as of acetic and propionic acid after all adaptation periods. Butyric acid formation, however, was dependent on the diet, and the caecal concentration was significantly higher ($P < 0.05$) in rats fed RPS than HAS during the entire experiment. In the distal colon and faeces, the concentration of total SCFA also generally increased with time.

The total faecal excretion of SCFA after 13 d and the caecal pool of SCFA following 13, 28 and 42 d are shown in Table 5. After 13 d, rats given RPS had a higher caecal pool of SCFA and faecal excretion of SCFA than those given HAS ($P < 0.01$). The caecal pool of SCFA increased linearly with adaptation time for both substrates ($R^2 = 0.71, P < 0.001$ for RPS and $R^2 = 0.66, P < 0.001$ for HAS). The total faecal SCFA excretions were not measured after 28 and 42 d.

**Table 5.** Total caecal pool and daily faecal excretion of short-chain fatty acids (SCFA) in rats fed a diet containing raw potato starch (RPS) or high-amylose maize starch (HAS) for 13, 28 or 42 d†

<table>
<thead>
<tr>
<th></th>
<th>RPS</th>
<th>HAS</th>
<th>RPS</th>
<th>HAS</th>
<th>RPS</th>
<th>HAS</th>
<th>Statistical significance of effect: $P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SEM)</td>
<td>Mean (SEM)</td>
<td>Mean (SEM)</td>
<td>Mean (SEM)</td>
<td>Mean (SEM)</td>
<td>Mean (SEM)</td>
<td>Diet x Time</td>
</tr>
<tr>
<td>Caecal pool (µmol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic</td>
<td>207.3** (18.0)</td>
<td>119.8 13.2</td>
<td>572.1 93.5</td>
<td>514.0 48.1</td>
<td>952.0 106.0</td>
<td>678.9 54.9</td>
<td>0.013</td>
</tr>
<tr>
<td>Proprionic</td>
<td>34.6** (5.3)</td>
<td>21.5 2.2</td>
<td>89.6 11.6</td>
<td>97.3 12.6</td>
<td>146.3 18.7</td>
<td>144.6 11.0</td>
<td>0.762</td>
</tr>
<tr>
<td>Butyric</td>
<td>15.5** (2.0)</td>
<td>0.5 0.9</td>
<td>38.3 4.7</td>
<td>70.3 4.7</td>
<td>201.6 51.6</td>
<td>71.0 7.3</td>
<td>0.062</td>
</tr>
<tr>
<td>Total</td>
<td>295.9*** (24.1)</td>
<td>163.6 15.5</td>
<td>387.4 128</td>
<td>721.6 65.1</td>
<td>1380.0 143.0</td>
<td>1013.0 59.7</td>
<td>0.004</td>
</tr>
<tr>
<td>Faecal excretion (µmol/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic</td>
<td>99.0* (10.2)</td>
<td>59.4 8</td>
<td>-†</td>
<td>-†</td>
<td>-†</td>
<td>-†</td>
<td>-†</td>
</tr>
<tr>
<td>Proprionic</td>
<td>14.6* (2.4)</td>
<td>5.7 0.9</td>
<td>-†</td>
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<td>-†</td>
<td>7.3</td>
<td>-†</td>
</tr>
<tr>
<td>Butyric</td>
<td>8.4* (1.8)</td>
<td>3.4 0.7</td>
<td>-†</td>
<td>-†</td>
<td>-†</td>
<td>-†</td>
<td>-†</td>
</tr>
<tr>
<td>Total</td>
<td>137.4** (14.8)</td>
<td>76.7 10</td>
<td>-†</td>
<td>-†</td>
<td>-†</td>
<td>-†</td>
<td>-†</td>
</tr>
</tbody>
</table>

Mean values were significantly different from those in the HAS group (one-way ANOVA): *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

† For details of diets and procedures, see Table 1 and p. 320.
‡ Faecal SCFA excretions were not measured after 28 and 42 d.
The molar proportion of butyric acid in the caecum was low with both RPS (6%) and HAS (4%) after 13 d of adaptation (Fig 1). In rats given RPS, the proportion of butyric acid increased significantly to 19% after 28 d of adaptation ($P=0.0017$). A longer adaptation (42 d) did not affect this proportion further. The pattern of SCFA formed in rats fed HAS was also affected by adaptation time, but the increase in the proportion of butyric acid was slower and reached 8% after 42 d ($P=0.0023$). Similar trends were found also for the proportion of butyric acid in distal colon and faeces (Fig. 1).

**Discussion**

Two sources of native RS were used to study the potential effect of adaptation time on the concentration and pattern of SCFA in the hindgut of rats. The rat model used has been shown to correlate well with human experiments with respect to total fermentation of dietary fibre (Nyman et al. 1986). Concerning the formation of SCFA, in vitro incubations with human and rat faeces have been found to give similar profiles from both dietary fibre (Lupton & Villalba, 1988; Barry et al. 1995) and starch (Wyatt & Horn, 1988) and the rat therefore seems to be useful for comparisons of patterns of SCFA formed from different carbohydrate substrates. However, previous studies on rats may indicate that the pattern of SCFA formed from RS may vary due to the length of the feeding time (Le Blay et al. 1999). This is important from a methodological point of view, to enable valid comparisons of potential differences in the formation of SCFA from various carbohydrate substrates.

The fermentation of the RS in HAS was very high and of the same magnitude as in previous studies in rats (Schulz et al. 1993; De Schrijver et al. 1999). RPS was more resistant to fermentation, which has been demonstrated previously (Berggren et al. 1995). Accordingly, the faecal dry weight was higher for rats fed RPS than those given HAS. In spite of higher fermentation of HAS, the rats fed this substrate generally had lower caecal pools of SCFA and lower faecal excretions of SCFA than those fed RPS. Similarly, others have reported lower caecal pools of SCFA in combination with lower faecal weights in rats fed HAS, than rats fed RPS (de Dekere et al. 1995; Ferguson et al. 2000). In these studies, the analysed RS in the substrates were of the same magnitude as in the present investigation (618–650 and 650–670 g/kg for HAS and RPS respectively) and RS was added to yield a level of about 140 (de Dekere et al. 1995) or about 222 (Ferguson et al. 2000) g/kg in the diet. These results suggest that RS may differ in fermentation characteristics and SCFA production depending on origin. The in vitro model for RS determination, used in the present study, has been demonstrated to yield RS contents in agreement with literature results obtained in the ileostomy model for several food products (Äkerberg et al. 1998). However, it cannot be excluded that the differences in SCFA formation from fermentation of HAS and RPS respectively may have emanated from erroneous estimation of the true amount of starch delivered to the rat hindgut.

After a short adaptation (13 d), both starch sources gave similar patterns of SCFA, with low caecal and faecal proportions of butyric acid. The proportion of butyric acid increased with prolongation of the adaptation time with both substrates. However, the increase in the proportion of butyric acid was faster in rats given RPS than in those given HAS and after 28 and 42 d of adaptation, the butyric acid formation was shown to be promoted by RPS. This result is in agreement with previous studies by Le Blay et al. (1999), where the caecal proportion of butyric acid increased with adaptation time in rats fed RPS. It is noteworthy that RPS also caused bloating in the colon.

![Fig. 1. Proportion of butyric acid (%) in caecum (●), distal colon (■) and faeces (▲) of rats given raw potato starch and in caecum (○), distal colon (□) and faeces (△) of rats given high-amylose maize starch (HAS), following different adaptation times. For details of diets and procedures, see Table 1 and p. 320. Values are means for seven rats per group with standard errors shown by vertical bars. Mean values were significantly different from those in the HAS group at the same adaptation time (one-way ANOVA): *$P<0.05$, **$P<0.01$, ***$P<0.001$.](image-url)
following 28 and 42 d of adaptation, a phenomenon that
not could be seen with HAS or with RPS after 13 d of adap-
tation. Bloating is caused by production of H₂ and CO₂
during fermentation by some specific bacterial strains.
Interestingly, studies in human subjects have shown that
RPS may produce higher amounts of breath H₂ than
HAS (Olesen et al. 1992). Various bacterial species are
known to use different fermentation pathways (Moore &
Holdeman, 1974; Holdeman et al. 1977), and an explana-
tion of the differences in the pattern of SCFA formed
and gas production between starches could be that different
micro-organisms are involved during the fermentation.
Different bacterial strains have also been shown to be
involved in the degradation of amylose and amylopectin
in in vitro studies (Wang et al. 1990). The two starches
differ regarding the granule structure and amylose:amylo-
pectin ratio. Potato starch in its native form exists as rela-
tively large spherical or ellipsoid granules (Gallant et al.
1992) and has a low amylose:amylopectin ratio (0·25).
Instead, HAS granules are small and can be both polyhed-
radic and irregular with a higher amylose:amylopectin
ratio (4·00). Possibly these differences in physico-chemical
properties affect the type of micro-organisms involved in
fermentation and in the production of SCFA.
With HAS, the increase in the proportion of butyric acid
with adaptation time was less significant. However, it
cannot be excluded that the butyric acid formation with
the two substrates would be similar if adaptation were pro-
longed. Thus, the discrepancy in literature regarding buty-
ic acid formation from RS in rat models (Mallett et al.
1988; Gee et al. 1991; Berggren et al. 1995; Monsma &
Marlett, 1995) might be explained partly by the fact that
various starches, when fed as individual substrates, are
affected differently by the length of the adaptation time.
It may be hypothesised that the length of the intervention
period may influence the pattern of SCFA formed from
RS also in studies on human subjects. Another factor that
has been reported to affect the pattern of SCFA formed
from RS is the level of RS in the diet (Mathers et al. 1997).
As butyric acid has been shown to inhibit growth of
colon cancer cells in vitro (Whitehead et al. 1996) and
stimulate apoptosis (Hague et al. 1995), RS (by its ability
to promote butyric acid production) has been suggested to
protect against colon cancer (Hylla et al. 1998). Further,
epidemiological studies have shown a strong correlation
between a high intake of dietary starch and a low incidence
of colo-rectal cancer, whereas no significant relationship
has been found between NSP and colon cancer (Cassidy
et al. 1994). However, in studies investigating the effect
of RS and cancer prevention using rodent models, varying
results have been obtained. Thus, when intestinal cancer
was induced by azoxymethane in rats, both HAS (Caderni
et al. 1994) and RPS (Thorup et al. 1995) were observed
to be protective. In contrast with these results, RPS
enhanced tumourogenesis (Young et al. 1996), whereas
no effect of this starch was noted in the investigation by
Sakamoto et al. (1996). Of the reports mentioned earlier,
only the study by Sakamoto et al. (1996) specified the
SCFA produced from RS. In that study, the butyric acid
concentration in the distal part of the colon did not differ
from the basal group. Recently, Perrin et al. (2001) found
that only dietary fibres promoting a high and stable butyric
acid production in the rat hindgut decreased the rate of aber-
rant crypt foci in rats. It thus appears as if evaluation of
butyric acid formation from various starches is important
in relation to colon diseases. In this context, it is interesting
to note that RPS generally gave higher concentrations of
butyric acid.
In conclusion, the present study shows that the proportion
of butyric acid formed in rats fed RS, in the form of RPS and
HAS, increases with increasing length of adaptation time.
However, the increase in the proportion of butyric acid
with RPS was faster than with HAS. The impact of adap-
tation time may explain the different patterns of SCFA for
RS reported in the literature. More studies are needed in
order to establish steady-state conditions with respect to pat-
terns of SCFA formed from different RS substrates. Poten-
tial differences in butyric acid production between different
RS sources are important to evaluate, as butyric acid has
been suggested to protect against diseases of the colon
mucosa, such as distal ulcerative colitis and cancer.

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