



# LUND UNIVERSITY

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

Henningsson, Åsa; Nyman, Margareta; Björck, Inger

*Published in:*  
British Journal of Nutrition

*DOI:*  
[10.1079/BJN2003782](https://doi.org/10.1079/BJN2003782)

2003

[Link to publication](#)

*Citation for published version (APA):*  
Henningsson, Å., Nyman, M., & Björck, I. (2003). Influences of dietary adaption and source of resistant starch on short-chain fatty acids in the hindgut of rats. *British Journal of Nutrition*, 89(3), 319-327.  
<https://doi.org/10.1079/BJN2003782>

*Total number of authors:*  
3

### General rights

Unless other specific re-use rights are stated the following general rights apply:  
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

### Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117  
221 00 Lund  
+46 46-222 00 00

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

Åsa M. Henningsson\*, E. Margareta, G. L. Nyman and Inger M. E. Björck

*Applied Nutrition and Food Chemistry, Center for Chemistry and Chemical Engineering,  
Lund University, P.O. Box 124, SE-221 00 Lund, Sweden*

(Received 10 January 2002 – Revised 23 September 2002 – Accepted 17 October 2002)

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  $\alpha$ -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,

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; Monsma & 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 (Annisson & 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  $\alpha$ -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 amylose 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; Brunsgaard *et al.* 1995), whereas retrograded HAS required a longer intervention period (1 month; Brunsgaard *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 and 6.0 months adaptation, respectively (Le Blay *et al.* 1999). In another study, however, no change in faecal pattern of SCFA was found in rats fed RPS or retrograded potato starch over a 5-month period (Kleessen *et al.* 1997).

The aim of the present investigation was to study the pattern of SCFA formed during fermentation of two types of RS using a rat experimental model. The pattern of SCFA was measured at different sites along the hindgut, and the possible influence of the adaptation time was investigated. Ungelatinised crystalline starches rich in RS 2, RPS and HASg were chosen as substrates.

## Materials and methods

### *Diets and animals*

RPS (Lyckeby Stärkelsen AB, Kristianstad, Sweden) or HAS (Hi-maize™; Penford Australia, Lane Cove, New South Wales, Australia) was included into diets at a level

of 100 g indigestible carbohydrates/kg DM. The composition of the test diets is listed in Table 1. The DM content of the diets was adjusted with wheat starch, a starch source that has been shown to be completely digested and absorbed in the small bowel of rats, and thus does not contribute to hindgut fermentation (Björck *et al.* 1987).

Male Wistar rats (B&K Universal, Stockholm, Sweden) with an initial weight of 72 (SD 7) g were randomly divided into groups of seven. Rats used for the first 13 d of the study were housed individually in metabolism cages (Berggren *et al.* 1993). After 7 d of adaptation to the diet, a 5 d balance experiment followed when faeces were collected daily for determination of fermentation of RS and faecal dry weight. Faeces were kept at  $-20^{\circ}\text{C}$  and then freeze-dried and milled before analysis of starch. The experimental diets were fed to the rats for another 24 h, and during this time fresh faeces were collected on dry ice for determination of the faecal excretion of SCFA ('13 d' in Tables 2, 4–5). Rats used for studying SCFA following 28 and 42 d of ingestion were kept singly in cages with wire-mesh floors. Faeces were collected the last 5 h of the experiment, frozen at  $-80^{\circ}\text{C}$  and saved for analysis of the concentration of SCFA. To facilitate determination of fermentability, the feed intake was restricted to 12 g DM/d during the first 13 d. The feed during the longer adaptation was given to the rats *ad libitum* and found to be between 15 and 17 g DM/d (Table 2). At the end of the various adaptation periods, the animals were killed using  $\text{CO}_2$ . The caecum and colon were removed immediately and the colon divided into a proximal and a distal part and then kept frozen ( $-80^{\circ}\text{C}$ ) until analysed for SCFA. The protocol of the animal experiment

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

Ingredient*	RPS	HAS
RPS	157	
HAS		168
Wheat starch	515	504
Casein	120	120
Sucrose	100	100
Maize oil	50	50
Mineral mixture†	48	48
Vitamin mixture‡	8	8
Choline chloride	2	2
DL-Methionine	1	1

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 (Cerestar, Krefeld, Germany), casein (Sigma Chemical Company, St Louis, MO, USA), sucrose (Danisco Sugar, Malmö, Sweden), maize oil (Mazola; Bestfoods 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):  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  0.37,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  1.40,  $\text{KH}_2\text{PO}_4$  332.10,  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  171.80,  $\text{CaCO}_3$  324.40, KI 0.068,  $\text{MgSO}_4$  57.20,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  7.70,  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$  3.40,  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  0.020, NaCl 101.70.

‡ Containing (g/kg): menadione 0.62, thiamin hydrochloride 2.50, riboflavin 2.50, pyridoxine hydrochloride 1.25, calcium pantothenate 6.25, nicotinic acid 6.25, folic acid 0.25, inositol 12.50, *p*-aminobenzoic acid 1.25, biotin 0.05, cyanocobalamin 0.00375, retinyl palmitate 0.187, calciferol 0.00613,  $\alpha$ -tocopherol acetate 25.00, maize starch 941.25.

**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)†

	13 d				28 d				42 d			
	RPS		HAS		RPS		HAS		RPS		HAS	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Feed intake (g/d)	12.0	0.0	11.9	0.1	15.7	0.4	15.0	0.3	16.9	0.4	16.8	0.7
Body-weight gain (g/d)	3.5	0.1	3.7	0.1	5.5	0.2	5.3	0.2	5.1	0.2	5.2	0.3
Caecal wet content (g)	2.9***	0.2	1.7	0.1	6.1*	0.6	4.7	0.3	7.1	0.5	6.5	0.5

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 ethanol and analysed as liberated glucose after solubilisation in KOH and enzymatic treatment with a thermostable  $\alpha$ -amylase (Termamyl 300L DX; Novo Nordisk A/S, Copenhagen, Denmark) and amyloglucosidase (Roche Diagnostics, Mannheim, Germany) according to Björck & Siljeström (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 & Siljeström, 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†

(Mean values with their standard errors for seven rats per group)

	RPS		HAS	
	Mean	SEM	Mean	SEM
Faecal dry weight (g/d)	1.0***	0.1	0.5	0.0
Fermentability of RS (%)	54.5***	6.4	97.4	0.7

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*-butyldimethylsilyl)-*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†

	(Mean values with their standard errors for seven rats per group)												Statistical significance of effect: <i>P</i>			
	13 d			28 d			42 d			Diet	Time	Diet × Time				
	RPS	HAS		RPS	HAS		RPS	HAS					Mean	SEM	Mean	SEM
Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	
Caecum																
Acetic	72.2	3.3	69.5	6.3	94.2	15.7	7.3	109.7	7.3	134.7	14.1	106.0	8.2	0.529	<0.0001	0.107
Propionic	12.6	1.4	12.6	1.2	15.2	2.0	1.8	20.5	1.8	20.6	2.4	23.1	2.2	0.099	<0.0001	0.376
Butyric	5.2***	0.2	3.5	0.3	27.2**	5.0	0.7	7.9	0.7	27.6*	6.5	11.3	1.4	<0.0001	<0.0001	0.032
Total	102.8	4.1	95.1	7.3	143.7	19.7	8.5	153.5	8.5	193.9	16.8	159.7	11.6	0.304	<0.0001	0.224
Distal colon																
Acetic	49.2	3.1	57.3	5.4	53.6	9.4	5.7	64.8	5.7	67.6	10.7	73.7	3.6	0.173	0.049	0.933
Propionic	6.8	1	7.4	1.2	8.0	1.3	1.1	7.7	1.1	8.9	1.7	11.5	1.2	0.359	0.043	0.522
Butyric	3.7	0.5	3.9	0.5	12.0*	2.2	0.7	5.8	0.7	14.4	3.5	8.4	1.2	0.009	0.001	0.137
Total	69.8	3.5	80.3	6.5	79.01	0.6	6.8	86.4	6.8	98.4	11.8	103.9	4.7	0.236	0.007	0.951
Faeces																
Acetic	48.9	2.6	57.3	5.2	46.9*	7.2	3.7	67.4	3.7	57.4*	6.6	81.0	6.6	–‡	–‡	–‡
Propionic	7.1	0.8	5.5	0.8	10.4	3.2	0.9	6.1	0.9	6.0	0.5	8.1	1.2	–‡	–‡	–‡
Butyric	4.0	0.7	3.2	0.5	10.8*	1.6	0.7	6.3	0.7	10.2	2.6	8.4	2.0	–‡	–‡	–‡
Total	67.9	3	74.1	7.8	73.3	5.3	4.6	87.3	4.6	84.8	6.0	106.0	9.7	–‡	–‡	–‡

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.

**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†

	(Mean values with their standard errors for seven rats per group)												Statistical significance of effect: <i>P</i>				
	13 d			28 d			42 d			Diet	Time	Diet × Time					
	RPS	Mean	SEM	HAS	Mean	SEM	RPS	Mean	SEM				HAS	Mean	SEM		
Caecal pool (μmol)																	
Acetic	18.0	119.8	13.2	572.1	93.5	514.0	48.1	952.0*	106.0	678.9	54.9	0.013	<0.0001	0.222			
Propionic	5.3	21.5	2.2	89.6	11.6	97.3	12.6	146.3	18.7	144.6	11.0	0.762	<0.0001	0.625			
Butyric	1.2	6.0	0.5	171.5**	38.3	37.5	4.7	201.6*	51.5	71.1	7.3	<0.0001	<0.0001	0.037			
Total	24.1	163.6	15.5	874.6	128	721.6	65.1	1380.0*	143.0	1013.0	59.7	0.004	<0.0001	0.337			
Faecal excretion (μmol/d)																	
Acetic	10.2	59.4	8	—†	—†	—†	—†	—†	—†	—†	—†	—†	—†	—†			
Propionic	2.4	5.7	0.9	—†	—†	—†	—†	—†	—†	—†	—†	—†	—†	—†			
Butyric	1.8	3.4	0.7	—†	—†	—†	—†	—†	—†	—†	—†	—†	—†	—†			
Total	14.8	76.7	10	—†	—†	—†	—†	—†	—†	—†	—†	—†	—†	—†			

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.

All statistical analyses were performed with the Minitab<sup>®</sup> 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<sup>®</sup>. 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 was, however, 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 of adaptation, rats given RPS had a higher caecal pool of SCFA and faecal excretion of SCFA than those fed HAS ( $P < 0.01$ ), due to a higher content of all the three main SCFA. The caecal pools of SCFA increased linearly with adaptation time for both substrates (RPS  $R^2$  0.71,  $P < 0.001$ ; HAS  $R^2$  0.86,  $P < 0.001$ ). After 28 d, there was a higher caecal butyric acid pool ( $P = 0.0046$ ) with RPS than HAS, but no difference regarding other SCFA. Following 42 d of adaptation, RPS still gave a higher caecal pool of butyric acid than RPS ( $P = 0.0275$ ). In addition, the pool of total SCFA and acetic acid was also higher in rats given RPS than in those given HAS at this time ( $P < 0.05$ ).

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).

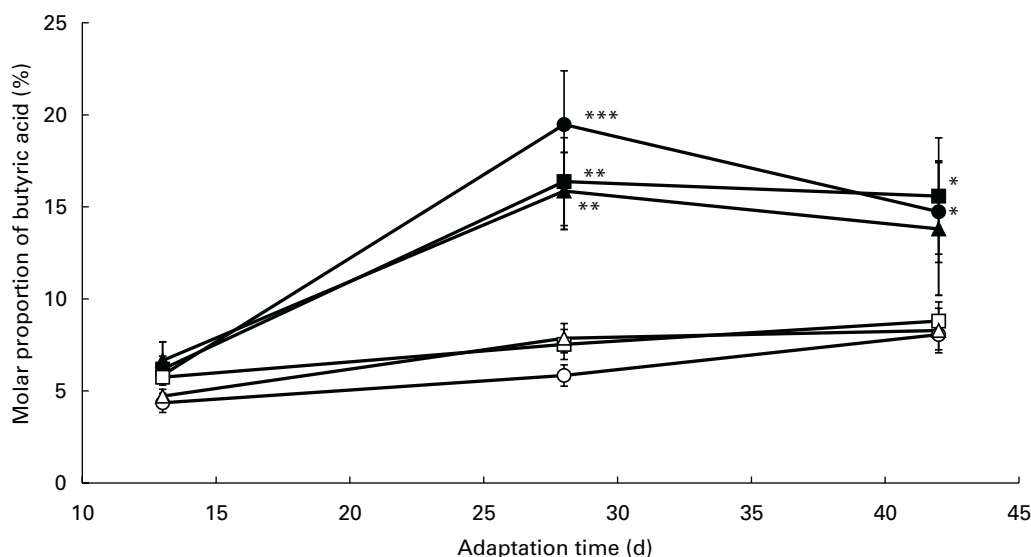
### Discussion

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 have indicated 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 (Åkerberg *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): \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$ .

following 28 and 42 d of adaptation, a phenomenon that not could be seen with HAS or with RPS after 13 d of adaptation. Bloating is caused by production of H<sub>2</sub> and CO<sub>2</sub> during fermentation by some specific bacterial strains. Interestingly, studies in human subjects have shown that RPS may produce higher amounts of breath H<sub>2</sub> 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 explanation 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 amylopectin and amylose in *in vitro* studies (Wang *et al.* 1999). The two starches differ regarding the granule structure and amylose:amylopectin ratio. Potato starch in its native form exists as relatively 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 polyhedral 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 prolonged. Thus, the discrepancy in literature regarding butyric 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 tumorigenesis (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 aberrant 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 adaptation 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 patterns of SCFA formed from different RS substrates. Potential 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.

### Acknowledgements

This work was made possible by financial support from the Swedish Foundation for Strategic Research through the LiFT Programme.

### References

- Åkerberg AK, Liljeberg HG, Granfeldt YE, Drews AW & Björck IM (1998) An *in vitro* method, based on chewing, to predict resistant starch content in foods allows parallel determination of potentially available starch and dietary fiber. *Journal of Nutrition* **128**, 651–660.
- Annon G & Topping DL (1994) Nutritional role of resistant starch: chemical structure vs physiological function. *Annual Reviews of Nutrition* **14**, 297–320.
- Asp N-G, Johansson CG, Hallmer H & Siljeström M (1983) Rapid enzymatic assay of insoluble and soluble dietary fiber. *Journal of Agricultural and Food Chemistry* **31**, 476–482.
- Barnard JA & Warwick G (1993) Butyrate rapidly induces growth inhibition and differentiation in HT-29 cells. *Cell Growth and Differentiation* **4**, 495–501.
- Barry JL, Hoebler C, Macfarlane GT, Macfarlane S, Mathers JC, Reed KA, Mortensen PB, Nordgaard I, Rowland IR & Rumney CJ (1995) Estimation of the fermentability of dietary fibre *in vitro*: a European interlaboratory study. *British Journal of Nutrition* **74**, 303–322.
- Berggren AM, Björck IM, Nyman EM & Eggum BO (1993) Short-chain fatty acid content and pH in caecum of rats given various sources of carbohydrates. *Journal of the Science of Food and Agriculture* **63**, 397–406.
- Berggren AM, Björck IM, Nyman EM & Eggum BO (1995) Short-chain fatty acid content and pH in caecum of rats fed various sources of starch. *Journal of the Science of Food and Agriculture* **68**, 241–248.
- Bird AR, Hayakawa T, Marsono Y, Gooden JM, Record IR, Correll RL & Topping DL (2000) Coarse brown rice increases fecal and large bowel short-chain fatty acids and starch but lowers calcium in the large bowel of pigs. *Journal of Nutrition* **130**, 1780–1787.
- Björck I, Nyman M, Pedersen B, Siljeström M, Asp N-G & Eggum BO (1987) Formation of enzyme resistant starch



- during autoclaving of wheat starch: studies in vitro and in vivo. *Journal of Cereal Science* **6**, 159–172.
- Björck IME & Siljeström MA (1992) In-vivo and in-vitro digestibility of starch in autoclaved pea and potato products. *Journal of the Science of Food and Agriculture* **58**, 541–553.
- Bradburn DM, Mathers JC, Gunn A, Burn J, Chapman PD & Johnston ID (1993) Colonic fermentation of complex carbohydrates in patients with familial adenomatous polyposis. *Gut* **34**, 630–636.
- Brown I (1996) Complex carbohydrates and resistant starch. *Nutrition Reviews* **54**, S115–S119.
- Brown I, Warhurst M, Arcot J, Playne M, Illman RJ & Topping DL (1997) Fecal numbers of bifidobacteria are higher in pigs fed *Bifidobacterium longum* with a high amylose cornstarch than with a low amylose cornstarch. *Journal of Nutrition* **127**, 1822–1827.
- Brunsgaard G, Bach Knudsen KE & Eggum BO (1995) The influence of the period of adaptation on the digestibility of diets containing different types of indigestible polysaccharides in rats. *British Journal of Nutrition* **74**, 833–848.
- Caderni G, Luceri C, Spagnesi MT, Giannini A, Biggeri A & Dolara P (1994) Dietary carbohydrates modify azoxy-methane-induced intestinal carcinogenesis in rats. *Journal of Nutrition* **124**, 517–523.
- Cassidy A, Bingham SA & Cummings JH (1994) Starch intake and colorectal cancer risk: an international comparison. *British Journal of Cancer* **69**, 937–942.
- Casterline LJ, Oles CJ & Ku Y (1997) In vitro fermentation of various food fiber fractions. *Journal of Agricultural and Food Chemistry* **45**, 2463–2467.
- Cummings JH (1997) Short-chain fatty acid enemas in the treatment of distal ulcerative colitis. *European Journal of Gastroenterology and Hepatology* **9**, 149–153.
- Cummings JH, Beatty ER, Kingman SM, Bingham SA & Englyst HN (1996) Digestion and physiological properties of resistant starch in the human large bowel. *British Journal of Nutrition* **75**, 733–747.
- de Deckere EA, Kloots WJ & van Amelsvoort JM (1995) Both raw and retrograded starch decrease serum triacylglycerol concentration and fat accretion in the rat. *British Journal of Nutrition* **73**, 287–298.
- De Schrijver R, Vanhof K & Vande Ginste J (1999) Nutrient utilization in rats and pigs fed enzyme resistant starch. *Nutrition Research* **19**, 1349–1361.
- Englyst HN, Hay S & Macfarlane GT (1987) Polysaccharide breakdown by mixed populations of human faecal bacteria. *FEMS Microbiology Ecology* **45**, 163–171.
- Englyst HN, Kingman SM & Cummings JH (1992) Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition* **46**, Suppl. 2, S33–S50.
- Ferguson LR, Tasman-Jones C, Englyst H & Harris PJ (2000) Comparative effects of three resistant starch preparations on transit time and short-chain fatty acid production in rats. *Nutrition and Cancer* **36**, 230–237.
- Gallant DJ, Bouchet B, Buleon A & Perez S (1992) Physical characteristics of starch granules and susceptibility to enzymatic degradation. *European Journal of Clinical Nutrition* **46**, Suppl. 2, S3–S16.
- Gee JM, Faulks RM & Johnson IT (1991) Physiological effects of retrograded, alpha-amylase-resistant cornstarch in rats. *Journal of Nutrition* **121**, 44–49.
- Hague A, Elder DJ, Hicks DJ & Paraskeva C (1995) Apoptosis in colorectal tumour cells: induction by the short chain fatty acids butyrate, propionate and acetate and by the bile salt deoxycholate. *International Journal of Cancer* **60**, 400–406.
- Holdeman LV, Cato EP & Moore WEC (1977) *Anaerobic Laboratory Manual*, 4th ed., Blackburg, VA: Virginia Polytechnic Institute and State University.
- Hylla S, Gostner A, Dusel G, Anger H, Bartram HP, Christl SU, Kasper H & Scheppach W (1998) Effects of resistant starch on the colon in healthy volunteers: possible implications for cancer prevention. *American Journal of Clinical Nutrition* **67**, 136–142.
- Kleessen B, Stoof G, Proll J, Schmiedl D, Noack J & Blaut M (1997) Feeding resistant starch affects fecal and cecal microflora and short-chain fatty acids in rats. *Journal of Animal Science* **75**, 2453–2462.
- Le Blay G, Michel C, Blottière HM & Cherbut C (1999) Enhancement of butyrate production in the rat caecocolonic tract by long-term ingestion of resistant potato starch. *British Journal of Nutrition* **82**, 419–426.
- Lupton JR & Villalba N (1988) Fermentation of fiber to short-chain fatty acids: a comparative study with humans, baboons, pigs and rats. *FASEB Journal* **2**, A1201.
- Mallett AK, Bearn CA, Young PJ, Rowland IR & Berry C (1988) Influence of starches of low digestibility on the rat caecal microflora. *British Journal of Nutrition* **60**, 597–604.
- Mathers JC, Smith H & Carter S (1997) Dose-response effects of raw potato starch on small-intestinal escape, large-bowel fermentation and gut transit time in the rat. *British Journal of Nutrition* **78**, 1015–1029.
- Monsma DJ & Marlett JA (1995) Rat cecal inocula produce different patterns of short-chain fatty acids than fecal inocula in in vitro fermentations. *Journal of Nutrition* **125**, 2463–2470.
- Moore WE & Holdeman LV (1974) Human fecal flora: the normal flora of 20 Japanese-Hawaiians. *Applied Microbiology* **27**, 961–979.
- Noakes M, Clifton PM, Nestel PJ, Le Leu R & McIntosh G (1996) Effect of high-amylose starch and oat bran on metabolic variables and bowel function in subjects with hypertriglyceridemia. *American Journal of Clinical Nutrition* **64**, 944–951.
- Nyman M & Asp NG (1985) Dietary fibre fermentation in the rat intestinal tract: effect of adaptation period, protein and fibre levels, and particle size. *British Journal of Nutrition* **54**, 635–643.
- Nyman M, Asp NG, Cummings J & Wiggins H (1986) Fermentation of dietary fibre in the intestinal tract: comparison between man and rat. *British Journal of Nutrition* **55**, 487–496.
- Olesen M, Rumessen JJ & Gudmand-Hoyer E (1992) The hydrogen breath test in resistant starch research. *European Journal of Clinical Nutrition* **46**, Suppl. 2, S133–S134.
- Perrin P, Pierre F, Patry M, Berreur M, Pradal G, Bornet F, Meflah K & Menanteau J (2001) Only fibres promoting a stable butyrate producing colonic ecosystem decrease the rate of aberrant crypt foci in rats. *Gut* **48**, 53–61.
- Phillips J, Muir JG, Birkett A, Lu ZX, Jones GP, O'Dea K & Young GP (1995) Effect of resistant starch on fecal bulk and fermentation-dependent events in humans. *American Journal of Clinical Nutrition* **62**, 121–130.
- Richardson AJ, Calder AG, Stewart CS & Smith A (1989) Simultaneous determination of volatile and non-volatile acidic fermentation products of anaerobes by capillary gas chromatography. *Letters in Applied Microbiology* **9**, 5–8.
- Roediger WE (1980) The colonic epithelium in ulcerative colitis: an energy-deficiency disease? *Lancet* **2**, 712–715.
- Roediger WE (1982) Utilization of nutrients by isolated epithelial cells of the rat colon. *Gastroenterology* **83**, 424–429.
- Sakamoto J, Nakaji S, Sugawara K, Iwane S & Munakata A (1996) Comparison of resistant starch with cellulose diet on 1,2-dimethylhydrazine-induced colonic carcinogenesis in rats. *Gastroenterology* **110**, 116–120.

- Scheppach W, Bartram HP & Richter F (1995) Role of short-chain fatty acids in the prevention of colorectal cancer. *European Journal of Cancer* **31A**, 1077–1080.
- Scheppach W, Fabian C, Sachs M & Kasper H (1988) The effect of starch malabsorption on fecal short-chain fatty acid excretion in man. *Scandinavian Journal of Gastroenterology* **23**, 755–759.
- Scheppach W, Sommer H, Kirchner T, Paganelli GM, Bartram P, Christl S, Richter F, Dusel G & Kasper H (1992) Effect of butyrate enemas on the colonic mucosa in distal ulcerative colitis. *Gastroenterology* **103**, 51–56.
- Schulz AG, Van Amelsvoort JM & Beynen AC (1993) Dietary native resistant starch but not retrograded resistant starch raises magnesium and calcium absorption in rats. *Journal of Nutrition* **123**, 1724–1731.
- Theander O, Åman P, Westerlund E, Andersson R & Pettersson D (1995) Total dietary fiber determined as neutral sugar residues, uronic acid residues, and Klason lignin (the Uppsala method): collaborative study. *Journal of AOAC International* **78**, 1030–1044.
- Thorup I, Meyer O & Kristiansen E (1995) Effect of potato starch, cornstarch and sucrose on aberrant crypt foci in rats exposed to azoxymethane. *Anticancer Research* **15**, 2101–2105.
- Topping DL, Illman RJ, Clarke JM, Trimble RP, Jackson KA & Marsono Y (1993) Dietary fat and fiber alter large bowel and portal venous volatile fatty acids and plasma cholesterol but not biliary steroids in pigs. *Journal of Nutrition* **123**, 133–143.
- van Munster IP, Tangerman A & Nagengast FM (1994) Effect of resistant starch on colonic fermentation, bile acid metabolism, and mucosal proliferation. *Digestive Diseases and Sciences* **39**, 834–842.
- Wang X, Conway PL, Brown IL & Evans AJ (1999) In vitro utilization of amylopectin and high-amylose maize (Amylomaize) starch granules by human colonic bacteria. *Journal of Applied Environmental Microbiology* **65**, 4848–4854.
- Weaver GA, Krause JA, Miller TL & Wolin MJ (1992) Cornstarch fermentation by the colonic microbial community yields more butyrate than does cabbage fiber fermentation; cornstarch fermentation rates correlate negatively with methanogenesis. *American Journal of Clinical Nutrition* **55**, 70–77.
- Weaver GA, Tangel CT, Krause JA, Parfitt MM, Jenkins PL, Rader JM, Lewis BA, Miller TL & Wolin MJ (1997) Acarbose enhances human colonic butyrate production. *Journal of Nutrition* **127**, 717–723.
- Whitehead RH, Young GP & Bhathal PS (1986) Effects of short chain fatty acids on a new human colon carcinoma cell line (LIM1215). *Gut* **27**, 1457–1463.
- Wyatt GM & Horn N (1988) Fermentation of resistant food starches by human and rat intestinal bacteria. *Journal of the Science of Food and Agriculture* **44**, 281–288.
- Young GP, McIntyre A, Albert V, Folino M, Muir JG & Gibson PR (1996) Wheat bran suppresses potato starch-potentiated colorectal tumorigenesis at the aberrant crypt stage in a rat model. *Gastroenterology* **110**, 508–514.