The influence of the period of adaptation on the digestibility of diets containing different types of indigestible polysaccharides in rats

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In balanced experiments with rats the influence of the period of adaptation on nutrient digestibility in diets containing cellulose (CEL), guar gum (GG), pectin (PEC) or retrograded high amylose maize starch (RS) was studied. Inclusion level was 80 g/kg diet DM except for the retrograded high-amylose maize starch, where the level was 316 g/kg diet DM. A diet containing normal maize starch only acted as a control diet (FF). The apparent digestibilities of DM, NSP, starch and protein were determined after adaptation periods of 4 d and 2, 4, 6 and 8 weeks. The digestibility of nutrients was affected by diet ($P < 0.001$) as well as by the period of adaptation ($P < 0.001$). The digestibilities of DM, NSP and starch increased asymptotically during the course of the experiment. The asymptotic progress over time was most pronounced for the GG, PEC and RS diets. The estimated periods of adaptation required for stable DM digestibility were approximately 1 week for the GG, PEC and RS diets and < 4 d for the FF and CEL diets. The digestibility of NSP in the GG and PEC diets was also stable after approximately 1 week, while it was stable from < 4 d for the CEL diet. However, PEC increased the faecal content of uronic acids for at least 2 weeks. A stable starch digestibility required 1 month in the case of RS and 3–10 d for the other diets. The high faecal content of glucose for the RS diet decreased during all 8 weeks but was still high at the close of the experiment. The apparent protein digestibility changed over time in a parabolic rather than an asymptotic fashion. The GG, PEC and RS diets increased the amount of N excreted by the faecal route.

Adaptation to diet: Polysaccharide digestibility: Resistant starch: N excretion

It is well documented that the digestibility of a diet is affected by certain types of dietary fibre (Hansen *et al.* 1992; Levrat *et al.* 1993; Longland *et al.* 1993) and by α-amylase-resistant starches (Goodlad & Mathers, 1992). However, little attention has been given to the influence of the period of adaptation (time from introduction of a new diet) when measuring the digestibility of diets high in fibre. The digestibility of foods and feeds is often measured by use of a rat model (e.g. Björck *et al.* 1986; Nyman *et al.* 1990; Brunsgaard *et al.* 1994), but the adaptation period practised varies considerably between workers; some use only 4 d (e.g. Eggum, 1973; Nyman *et al.* 1990) while others use as long as 6 weeks (Lajvardi *et al.* 1993).

In the case of polysaccharides not digested by endogenous enzymes secreted in the small intestine, hereafter referred to as enzyme-indigestible polysaccharides (EIDPS), the period of adaptation may be particularly critical. In the gastrointestinal tract the EIDPS have a diversity of effects evolving over days and even weeks. The hypertrophic effect of certain EIDPS on the gut tissue is well documented (Johnson & Gee, 1986; Ikegami *et al.* 1990),

as is the stimulatory effect of EIDPS on the activity of the microflora in the hind gut (Bach Knudsen *et al.* 1993; Jensen & Jørgensen, 1994). The fermentability and viscosity of the EIDPS seem to be important determinants of the physiological effects. Evidence has been presented that the tissue hypertrophy is interactively affected by type of EIDPS and adaptation period (Brunsgaard *et al.* 1995). Likewise, adaptation of the microbial activity appears to be influenced by time as well as by EIDPS fermentability (Tulung *et al.* 1987; Levrat *et al.* 1991). It thus seems likely that the apparent digestibility of nutrients is affected not only by the presence of, and the type of, EIDPS in the diet, but also by the period of adaptation. The measured nutritional value of foods and feeds could then depend on the time allowed for diet adaptation. The adaptation period required for determining the nutritional value could well be longer in the case of foods and feeds with a high EIDPS content than of diets with a low EIDPS content. A systematic investigation of the importance of period of adaptation for the degree of digestibility is apparently lacking.

The objectives of the present study were (1) to determine whether the period of adaptation to a new diet has any impact on the digestibility of protein, DM and polysaccharides and (2) to determine whether the type of polysaccharide has any influence on the progress of digestibility adaptation.

MATERIALS AND METHODS

Diets

Five diets differing only in the composition of polysaccharides (644 g/kg diet DM) were used. The control diet (diet FF) contained autoclaved maize starch as a source of digestible starch. In the experimental diets, part of the maize starch was replaced by pectin (PEC; 80 g), cellulose (CEL; 80 g), guar gum (GG; 80 g) or a retrograded high-amylose maize starch (RS; 316 g containing 80 g resistant starch). The pectin was obtained from Copenhagen Pectin A/S, Lille Skensved, Denmark, the guar gum from Ferraton A/S, Haslev, Denmark, and the cellulose from A. G. Frisenette Aps, Ebeltoft, Denmark. The retrograded high-amylose maize starch was produced by Cerestar, Vilvoorde, Belgium, for the European FLAIR Concerted Action on Resistant Starch (EURESTA). The product was a high-amylose maize starch (Hylon 7), which had been extruded (170° with high incorporation of water), then stored at 4° for 48 h, dried and finally milled. The full compositions of the diets are indicated in Table 1.

Before initiation of the experiment the rats were kept on a commercial rat chow containing 176 g protein and 177 g NSP/kg DM (25 g as soluble NSP). Apparent digestibility of the rat chow was 0·82 for DM and 0·80 for protein.

Animals

Rat experiments were performed using juvenile male Wistar rats with an initial weight of 76·9 (SE 0·31) g.

The experiments comprised a total of 150 rats fed on one of the five diets for up to 8 weeks. The experimental design was a 5×5 factorial design repeated in six blocks. Each block contained five rats per diet at the start. Adaptation periods investigated were 4 d, and 2, 4, 6 and 8 weeks. At the end of each adaptation period one rat from each diet was killed for other purposes. Before killing the rats, urine and faeces were collected during the final 5 d. These samples were used for N analysis. Faecal collections from the remaining rats were done simultaneously so as to obtain sufficient faecal material for the NSP and starch analyses. The six blocks were separated by 2–4 weeks, due to limited animal facilities.

The rats were given equal amounts of feed (g DM) per day at 10% below estimated *ad lib.* feed intake. The meal size was increased gradually from 9 to 17 g DM/d as the animals

Table 1. *Dietary ingredients and chemical composition of the experimental diets*

Diet...	FF	PEC	CEL	GG	RS
Ingredients (g/kg DM):					
Casein*	140	140	140	140	140
Sucrose	80	80	80	80	80
Soyabean oil	80	80	80	80	80
Mineral mixture†	40	40	40	40	40
Vitamin mixture†	16	16	16	16	16
Polysaccharides					
Maize starch	644	564	564	564	328
Pectin	—	80	—	—	—
Cellulose	—	—	80	—	—
Guar gum	—	—	—	80	—
Retrograded amylose‡	—	—	—	—	316
Chemical composition:					
Protein (g/kg DM)	129·5	133·5	136·7	135·6	134·3
Constituent sugars of NSP (g/kg DM)					
Rhamnose	0·8	1·3	1·2	1·0	1·0
Fucose	tr	tr	tr	tr	tr
Arabinose	tr	2·1	tr	1·7	tr
Xylose	tr	tr	2·8	0·3	tr
Mannose	1·0	1·2	1·7	43·1	1·1
Galactose	1·1	4·7	1·1	26·7	1·1
Glucose	9·3	11·3	85·5	10·9	9·4
Uronic acids	1·2	53·5	1·5	1·7	0·7
Starch (g/kg DM)					
Total	678·5	589·0	592·5	581·5	667·5
Rapidly digested	601·5	529·0	531·0	495·5	466·0
Slowly digested	35·0	24·5	27·0	45·0	97·0
Resistant	37·5	37·0	34·5	42·5	104·5

FF, fibre-free control diet; PEC, pectin; CEL, cellulose; GG, guar gum; RS, resistant starch (retrograded high-amylose maize starch); tr, trace.

* Casein with 10 g methionine/kg.

† For details see Eggum (1973).

‡ Corresponding to addition of 80 g resistant starch.

grew. Deviations from intended feed intakes were determined from feed residues. Feed residues and body weights were recorded at the beginning and end of the collection periods.

All animals were housed individually in wire-bottomed cages with free access to water. Temperature and relative humidity were maintained at 23° and 60% respectively. Lighting was controlled by alternating 12 h periods of light and dark.

Analytical methods

DM of faecal collections was determined after freeze drying. The faecal samples were milled before chemical analysis.

N in faeces and urine was analysed in duplicate for each animal collection using a modified Kjeldahl method (KjelFoss 16200 Autoanalyser; Foss Electric A/S, Hillerød, Denmark).

NSP and starch were analysed in pooled samples of faeces from each combination of diet and adaptation period. Pooling was done within blocks and with amounts corresponding proportionally to the amount of faeces excreted (DM). Total NSP and their constituent neutral sugars in faeces were determined as alditol acetates by GLC using a modification of the Uppsala and the Englyst procedures (Bach Knudsen *et al.* 1993). Uronic acids were

determined by a colorimetric method (Scott, 1979). Faecal samples were analysed with and without pre-treatment with dimethyl sulphoxide (DMSO) to dissolve starch. Starch in faeces was determined by difference in the faecal NSP glucose content with and without preceding DMSO dissolution.

Starch in the diets was characterized according to Englyst *et al.* (1992), NSP were determined as alditol acetates by GLC after complete removal of starch by DMSO and enzymes (Bach Knudsen *et al.* 1993) and uronic acids were measured according to Scott (1979).

Calculations and statistical analyses

Digestibilities of DM, NSP, starch and protein (N × 6·25) were calculated using equation 1:

$$\text{apparent digestibility} = \frac{\text{intake (g)} - \text{excreted (g)}}{\text{intake (g)}} \quad (1)$$

using the chemically determined composition of the feed and faeces.

N excretion pattern was expressed as the amounts (mg) of N excreted in the urine and faeces over each of the 5 d collection periods.

Statistics were performed using the GLM procedure of SAS (SAS Institute Inc., 1988). The results from the balanced experiments appeared to be normal and variance to be homogeneous. This was confirmed with a Levene's test of homogeneity of variance (Snedecor & Cochran, 1980). The results were then examined by three-way ANOVA. Main factor components in the model were the diets, the adaptation periods and the blocks, while the interactive component was adaptation period by diet. Least significant differences (LSD) between means of diets and periods were determined according to this model, using the mean square error as the standard error estimate. However, this model does not quantitatively express the influence of time on the nutrient digestibility, thus further analyses were performed with models employing time as a covariate in an asymptomatic model (Snedecor & Cochran, 1980). Digestibilities of DM, starch, NSP and protein were analysed using the following non-linear asymptotic model:

$$Y = A_i \times \exp(\beta_i / t), \quad (2)$$

where A_i is the asymptotic value of the ith diet ($i = 1, 2, 3, 4, 5$) and β_i is the rate of change over time t given the ith diet.

The model was modified according to the results of the three-way ANOVA, i.e. if an interaction effect could not be detected, the covariate parameter estimate (β) was not allowed to vary with the diet.

The rate parameter estimates were tested against the null hypothesis (H_o: $\beta_i = 0$, i.e. no effect of time on the response) using a t test. Contrasts were used to compare parameter estimates of each diet.

Calculations were made to determine the adaptation period required for stable digestibility of nutrients. The digestibility was regarded as stable when the difference between the predicted value and the asymptotic value did not exceed the LSD ($P < 0{\cdot}05$). Calculations were made according to the following equation:

$$T_i = \frac{\beta_1}{\ln(1 - \text{LSD}/A_i)}, \quad (3)$$

where T_i is the adaptation period required by the ith diet, β_i is the estimated rate parameter and A_i the estimated asymptotic value for ith diet.

Table 2. *Body weight, feed efficiency and N utilization of rats fed on diets containing different indigestible polysaccharides*

(Mean values with their standard errors for thirty rats)

	Adaptation period										*P* value*		
	4 d		2 weeks		4 weeks		6 weeks		8 weeks				
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Diet	Period	Diet × period
n	30		30		30		30		30				
Body weight (g)	106·4	0·90	146·1	1·42	229·6	1·43	292·6	3·06	323·9	2·85	$P = 0·003$	$P < 0·001$	$P = 0·035$
Gain (g/5 d)	16·6	0·80	19·4	0·92	28·7	0·70	15·3	1·20	12·1	1·09	$P = 0·132$	$P < 0·001$	$P = 0·154$
Feed intake (g/5 d)	43·7	0·67	55·7	0·75	78·6	0·55	77·6	0·90	75·3	0·83	$P = 0·062$	$P < 0·001$	$P = 0·540$
Relative feed intake (g/d per kg BW)	82	1·3	76	1·00	69	0·7	53	0·6	47	0·5	$P = 0·022$	$P < 0·001$	$P = 0·326$
N intake (mg/5 d)	933·3	16·39	1196·0	16·62	1686·1	14·31	1663·4	17·84	1616·0	19·85	$P = 0·007$	$P < 0·001$	$P = 0·620$
N retained (mg/5 d)	613.9	13·89	757·5	16·23	919·9	20·79	732·6	19·78	515·7	19·94	$P = 0·773$	$P < 0·001$	$P = 0·964$
N excreted (mg/5 d)													
In urine	202·3	9·17	289·5	8·95	560·9	19·16	707·2	22·49	864·5	20·99	$P < 0·001$	$P < 0·001$	$P = 0·237$
In faeces	117·1	6·03	149·1	6·92	205·3	8·76	223·5	9·20	235·8	7·23	$P < 0·001$	$P < 0·001$	$P = 0·615$
Total	319·4	8·11	438·5	9·39	766·2	17·66	930·8	22·79	1100·3	18·56	$P < 0·009$	$P < 0·001$	$P = 0·239$

* Determined by three-way analysis of variance. Block effects not shown.

RESULTS

Body weight and feed intake

Body weights (BW) of rats at the end of the five collection periods are presented in Table 2. Feed intake (g DM/5 d), BW gains (g/5 d), relative feed intake (g DM/d per g BW × 100) and N intake, N excretion and N retention are presented as means with their standard errors for each of the five collection periods.

BW increased progressively over the 8 weeks of feeding ($P < 0{\cdot}001$). Rats receiving the CEL diet or PEC diet had slightly, but significantly, lower BW than rats receiving the FF diet or RS diet. As determined by least square means these rats had a 4% lower BW than the rats fed on the FF diet.

BW gain and feed intake were unaffected by the diet, although there was a tendency ($P = 0{\cdot}062$) for an influence of diet on feed intake. As determined by least square means (not shown), the GG group had a 4% lower feed intake than the FF and CEL groups. However, there was no diet effect on the relative feed intake. Relative feed intake declined progressively from 85 g/kg BW at 4 d of adaptation to 54 g/kg BW at 8 weeks of adaptation.

N intake was primarily determined by the adaptation period as intended, although there was a significant effect of diet. Only the rats fed on CEL had a significantly different N intake and excretion as determined by least square means (see Table 6). The slightly higher N intake and N excretion in the CEL group was due to a combination of a slightly higher N content in the diet *per se* as determined by the chemical analysis (Table 1) and to the CEL group having the lowest feed residues of all groups. The other four groups were not significantly different with respect to N intake and total N excretion (Table 2).

N retention was unaffected by diet. N retention increased during the initial 4 weeks and decreased thereafter to the close of the experiment.

DM, NSP and starch digestibilities

Digestibilities of DM, NSP and starch are depicted as means of each combination of diet and period in Figs 1, 2 and 3 respectively. Results of the statistical analysis are given in Table 3 and the corresponding fitted curves are plotted with the means in Figs 1, 2 and 3.

The digestibilities of DM, NSP and starch were significantly affected by the period of adaptation ($P < 0{\cdot}001$). However, the influence of time on the digestibility of DM and starch was very much related to the diet.

The progress of DM, NSP and starch digestibilities over time was well described by an asymptotic model, the relative model variation (R^2) being in the range of 0·86 to 0·95.

For DM digestibility (DDM) the rate parameters for the GG, PEC and RS diets were found to be significantly different from the rate parameters for the FF and CEL diets. The rate parameters for the FF and CEL diets did not diverge significantly from 0, thus indicating that within the adaptation period investigated there was no effect of time on the DDM. The rate parameters for the GG, PEC and RS diets indicated that the asymptotic value was approached within the period investigated. The asymptotic values for the GG, PEC and RS diets were close to 0·95. The asymptotic value of DDM was the highest for the FF diet (0·96) and the lowest for the CEL diet (0·88). These differences were significant ($P < 0{\cdot}001$).

The digestibility of NSP was significantly influenced by diet and by period of adaptation ($P < 0{\cdot}001$), while there was no significant interaction between diet and time ($P = 0{\cdot}059$). The asymptotic model estimated the asymptotic value for the CEL diet to be 0·19 which was significantly lower than that for the other two NSP-containing diets. The asymptotic

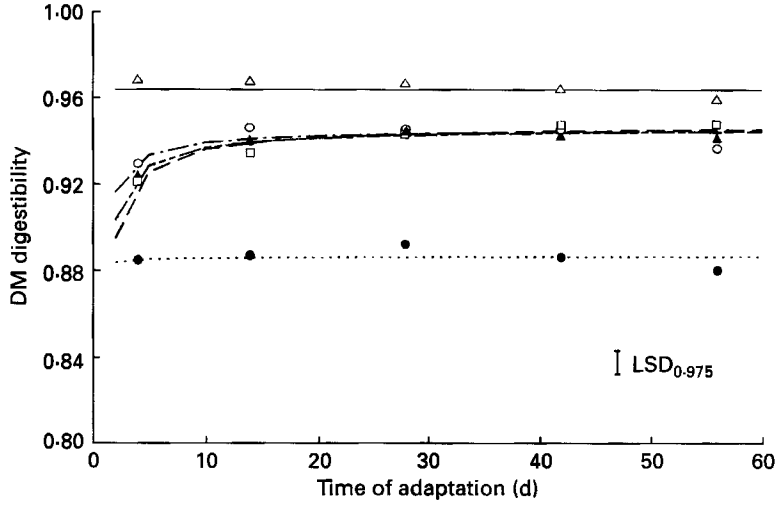

Fig. 1. Effects of adaptation period on the dry-matter digestibility of a fibre-free control diet (FF, △) and diets containing cellulose (CEL, ●), guar gum (GG, ○), pectin (PEC, ▲) and retrograded high-amylose maize starch (RS, □). Values are means for six rats, and the pooled SEM is 0·0035. Fitted curves are depicted for diets FF (——), CEL (····), GG (–·–), PEC (– – –) and RS (—). For details of diets and procedures, see Table 1 and pp. 834–837.

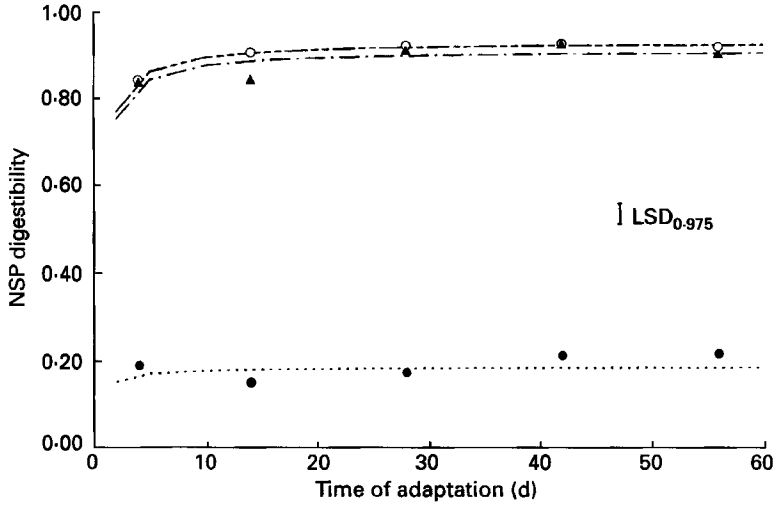

Fig. 2. Effects of adaptation period on the digestibility of the non-starch polysaccharide component of diets containing cellulose (CEL, ●), guar gum (GG, ○), and pectin (PEC, ▲). Values are means of six rats, and the pooled SEM is 0·0159. Fitted curves are depicted for CEL (····), GG (–·–) and PEC (– – –). For details of diets and procedures, see Table 1 and pp. 834–837.

values of NSP digestibility were 0·95 and 0·93 for the PEC and GG diets respectively and did not differ between the two diets.

Starch digestibility was significantly related to the adaptation period and to the type of diet ($P < 0·001$). The rate parameter for starch digestibility was significantly lower for the RS diet. Fig. 3 depicts the gradual approach to an asymptotic value, which in the case of the RS diet progressed throughout the adaptation period investigated. The GG diet had a slightly lower adaptation rate than the FF diet ($P = 0·024$). Starch digestibility of the GG

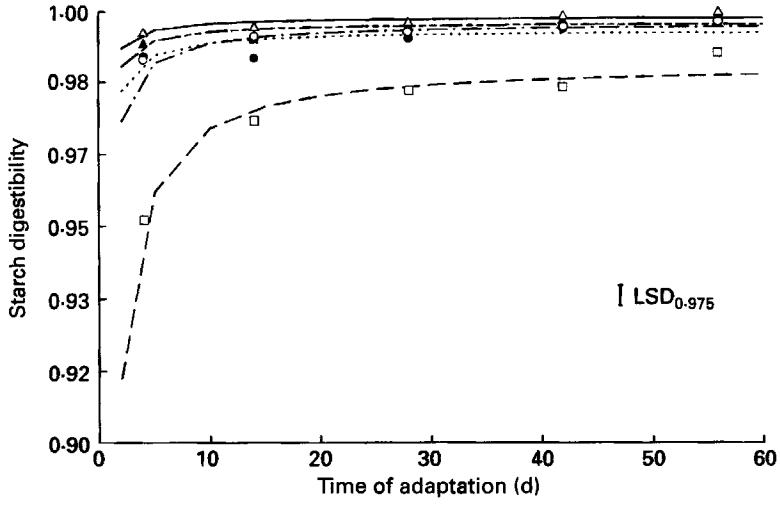

Fig. 3. Effects of adaptation period on the digestibility of starch in a fibre-free control diet (FF, △) and diets containing cellulose (CEL, ●), guar gum (GG, ○), pectin (PEC, ▲) and retrograded high-amylose maize starch (RS, □). Values are means for six rats, and the pooled SEM is 0·0015. Fitted curves are depicted for diets FF (——), CEL (····), GG (–·–), PEC (– – –) and RS (—). For details of diets and procedures, see Table 1 and pp. 834–837.

Table 3. *Effect of diet and the period of adaptation on the apparent digestibility of dry matter, non-starch polysaccharides, starch and protein in growing rats*

	Diet	Apparent digestibility			
		DM	NSP	Starch	Protein
Statistics†					
Diet	—	$P < 0·001$	$P < 0·001$	$P < 0·001$	$P < 0·001$
Period	—	$P < 0·001$	$P < 0·001$	$P < 0·001$	$P < 0·001$
Diet × Period	—	$P < 0·007$	$P < 0·059$	$P < 0·001$	$P < 0·503$
Estimates‡					
Asymptote (A_i)	FF	$0·963^{a}$		$0·999^{a}$	$0·897^{a}$
	CEL	$0·886^{c}$	$0·186^{b}$	$0·995^{a}$	$0·892^{a}$
	GG	$0·945^{b}$	$0·930^{a}$	$0·997^{a}$	$0·838^{c}$
	PEC	$0·945^{b}$	$0·911^{a}$	$0·997^{a}$	$0·845^{c}$
	RS	$0·946^{b}$		$0·988^{b}$	$0·856^{b}$
Rate (b_i)	FF	$0·000^{a}$	—	$-0·014^{*a}$	—
	CEL	$-0·005^{*ab}$	—	$-0·029^{ab}$	—
	GG	$-0·062^{bc}$	—	$-0·046^{b}$	—
	PEC	$-0·090^{c}$	—	$-0·021^{ab}$	—
	RS	$-0·112^{c}$	—	$-0·153^{c}$	—
Rate (b)	—	—	−0·381	—	0·045
R^2‡	—	0·89	0·95	0·85	0·63

FF, fibre-free control diet; CEL, cellulose; GG, guar gum; PEC, pectin; RS, resistant starch (retrograded high-amylose maize starch).

^{a,b,c} For each parameter, values within a column with unlike superscript letters were significantly different, $P < 0·05$.

* Value was not significantly different from zero.

† Determined by three-way ANOVA.

‡ Estimates and relative model variation corresponding to the model: $Y = A_i \times \exp(\beta_i/t)$.

Table 4. *Calculated adaptation period (d) required for stable* digestibility of dry matter, non-starch polysaccharides and starch in experimental diets containing different indigestible polysaccharides*

Diet	DM	NSP	Starch
FF	0	—	3
CEL	0	1	6
GG	5	7	10
PEC	8	7	4
RS	9	—	31

FF, fibre-free control diet; CEL, cellulose; GG, guar gum; PEC, pectin; RS, resistant starch (retrograded high-amylose maize starch).

* Predicted value not significantly different from the asymptotic value ($P < 0.05$).

diet at day 4 was significantly lower than observed in the following adaptation periods. The rate parameter for the FF diet was not significantly different from 0, indicating that starch digestibility for this diet was unaffected by time. The asymptotic values for all diets were close to 1·00, although the RS appeared to have a slightly lower level.

The calculated adaptation period (T_i) required for stable digestibility is presented in Table 4. For DM digestibility the calculated period was 0 d for the FF and CEL diets and 5 d, 8 d and 9 d respectively for the GG, PEC and RS diets. For NSP digestibility the periods were 1 d for the CEL diet and 7 d for the GG and PEC diets. Calculated periods for starch digestibility were 3 d, 6 d, 10 d, 4 d and 31 d respectively for the FF, CEL, GG, PEC and RS diets.

Protein digestibility

Apparent digestibility of protein (ADP) is depicted as means of diets and adaptation periods in Fig. 4 and statistics are presented in Table 3.

The ADP was significantly affected by the type of diet ($P < 0.001$) and by the period of adaptation ($P < 0.001$). No significant interaction between the diet and the adaptation period could be detected ($P = 0.503$).

The progress of ADP over time was poorly described by an asymptotic model, the relative model variation (R^2) being 0·63. The progress over the time investigated was more like a parabola (R^2 0·73, results not shown), the ADP initially increasing and subsequently decreasing (Fig. 4). However, the mean ADP at 4 d, 2 weeks, 4 weeks and 6 weeks were not significantly different for any of the five diets as determined by LSD comparison of means. Only the mean value at 8 weeks was significantly different from the means at previous times. An exception was the PEC diet.

As determined by the asymptotic model the GG and PEC diets had significantly lower ADP, the ADP being 5–6% lower than that for the FF diet. The estimated ADP of the RS diet was slightly higher than for the GG and PEC diets, but significantly lower (−4%) than for the FF and CEL diets.

Faecal content of diet-characteristic enzyme-indigestible polysaccharide constituent sugars

The faecal contents (mg/g DM faeces) of the diet-characteristic constituent sugars, glucose, mannose + galactose and uronic acids, are presented in Table 5 as means of diets and periods of adaptation. There was a significant effect of diet and of adaptation period

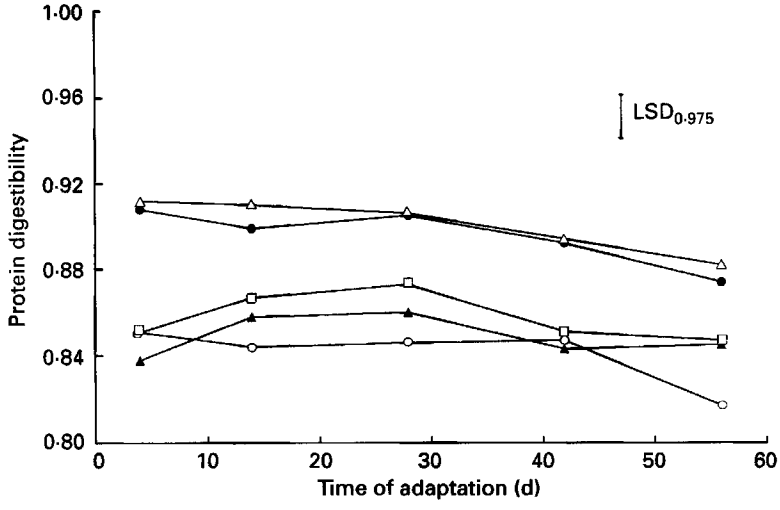

Fig. 4. Effects of adaptation period on the digestibility of protein in a fibre-free control diet (△) and diets containing cellulose (●), guar gum (○), pectin (▲) and retrograded high-amylose maize starch (□). Values are means for six rats, and the pooled SEM is 0·0070. For details of diets and procedures, see Table 1 and pp. 834–837.

($P < 0·001$) on the faecal content. There was also an interaction between diet and adaptation period ($P < 0·001$) for the sugars.

The faecal content of glucose for the CEL diet was 701 mg/g DM faeces at the day 4–9 collection. The faecal glucose content for the CEL diet remained high throughout the 8 weeks investigated, although there was a slightly, but significantly, lower content during the final 2 weeks compared with the initial 2 weeks. The faecal content of glucose for the RS diet declined progressively from 497 mg/g DM faeces at the day 4–9 collection to 148 mg/g faeces at the day 56–61 collection. The faecal glucose content of the RS diet remained significantly higher than the FF, GG and PEC diets throughout the experiment.

The faecal content of mannose + galactose of the GG diet decreased significantly ($P < 0·001$) from 50·2 mg/g DM faeces at days 4–9 to 26·4 mg/g DM faeces at days 14–19, whereafter it remained constant. The faecal content of uronic acids for the PEC diet decreased significantly ($P < 0·01$) from 87·1 mg/g DM faeces at days 4–9 to 61·2 mg/g DM faeces at days 14–19 and further to 19·8 mg/g DM faeces at days 28–33. The content of uronic acids remained constant thereafter.

Faecal samples of the other diets had the same content of mannose + galactose and uronic acids throughout the experiment.

Route of N excretion

Intake of N (mg/5 d) and excretion of N (mg/5 d) in urine and in faeces are presented in Table 6 as means of diets and adaptation periods. There was a significant effect of diet ($P < 0·001$) and of period ($P < 0·001$) on the amount of N excreted in urine and in faeces. No interactive effect was detected for the urine ($P = 0·237$) or the faeces ($P = 0·615$).

The N excretion by the urinary route as well as by the faecal route increased over the time investigated. However, the increase in urinary N excretion was much larger than in the faeces.

The GG and PEC diets had the highest faecal N and lowest urinary N excretion, while the reverse was observed for the CEL and FF diets. The RS diet excretion pattern was closer to the PEC than the FF diet.

Table 5. *Faecal content of major constituent monosaccharides (mg/g DM faeces) in rats fed on diets containing different enzyme-indigestible polysaccharides for up to 8 weeks**

(Values are means of six samples)

Sugar constituent...	Glucose					Mannose + galactose					Uronic acids				
Diet...	FF	CEL	GG	PEC	RS	FF	CEL	GG	PEC	RS	FF	CEL	GG	PEC	RS
Day															
4–9	142·4	701·3	195·6	106·1	496·8	23·0	14·2	50·2	19·3	20·9	4·3	6·5	4·2	87·1	5·7
14–19	115·2	713·5	104·5	96·6	361·7	20·6	13·2	26·4	22·4	24·8	5·7	7·9	4·5	61·2	5·9
28–33	91·1	682·1	82·0	67·3	302·5	21·0	12·9	25·2	23·7	22·9	4·4	7·3	4·8	19·8	5·2
42–47	57·7	644·8	80·3	53·4	239·5	22·0	12·6	27·3	21·3	24·7	5·1	7·1	5·1	12·7	5·3
56–61	71·5	634·1	91·3	92·1	147·9	21·4	13·4	29·4	22·5	25·1	4·1	7·7	6·3	9·5	3·8
Pooled SEM			14·8					1·6					2·7		
Statistics†															
P-value:															
Diet			$P < 0·001$					$P < 0·001$					$P < 0·001$		
Period			$P < 0·001$					$P = 0·006$					$P = 0·001$		
Diet × Period			$P < 0·001$					$P < 0·001$					$P < 0·001$		

FF, fibre-free control diet; CEL, cellulose; GG, guar gum; PEC, pectin; RS, resistant starch (retrograded high-amylose maize starch).

* For details of diets and procedures, see Table 1 and pp. 834–837.

† Determined by three-way ANOVA.

Table 6. *Nitrogen intake and excretion (urine and faeces; mg/5 d) of rats receiving diets containing different enzyme-indigestible polysaccharides for up to 8 weeks**

(Values are means for six animals)

	N intake					N in urine					N in faeces				
Diet...	FF	CEL	GG	PEC	RS	FF	CEL	GG	PEC	RS	FF	CEL	GG	PEC	RS
Day															
4–9	903	974	903	922	965	237	240	140	202	193	79	90	133	147	136
14–19	1158	1203	1231	1206	1181	313	323	259	284	268	104	121	192	171	157
28–33	1643	1732	1667	1707	1681	567	627	477	556	578	154	164	256	239	213
42–47	1655	1757	1681	1597	1627	689	856	678	661	653	176	190	257	250	244
55–61	1615	1705	1567	1586	1606	899	961	772	799	892	191	214	283	243	247
Pooled SEM			32·9					30·1					10·1		

FF, fibre-free control diet; CEL, cellulose; GG, guar gum; PEC, pectin; RS, resistant starch (retrograded high-amylose maize starch).

* For details of diets and procedures, see Table 1 and pp. 834–837.

DISCUSSION

The sources of EIDPS chosen for the present experiment included purified fibre sources and starch which have been extensively used for research purposes. They were selected for their diversity in viscosity and fermentability. The choice of purified EIDPS had the advantage of negligible levels of N or anti-nutritional components that could otherwise interfere with the digestibility of the diet components examined.

In the investigation we used growing rats, with the disadvantage that the effect of adaptation period is confounded by a possible effect of BW changes as well as a possible effect of changes in feed intake. It cannot be ruled out that the BW of the rats influences the fermentation capacity of the hind gut (Van Soest *et al.* 1982). In a previous experiment the effect of increasing amounts of feed intake on subsequent digestibility was found to be only weakly, but negatively, related to feed intake (Larsen *et al.* 1991). In rat experiments researchers are often confronted with the interaction of growth on the measurement in question. The use of a control group (FF diet) and equal feed intakes for all rats in the present investigation, however, makes it possible to compare measurements over time, at least from the time when the pre-experimental diet is no longer part of the gut contents. It cannot be ruled out that remnants of the previous diet were present in the gut at the initiation of the first collection. The investigation does, however, indicate that the period of adaptation influences the apparent digestibility of nutrients. The extent of this time effect appears to depend on the composition of the dietary polysaccharides. This is in contrast to a previous investigation by Thorbek *et al.* (1982) who concluded that time did not influence the measurement of digestibility. Differences in the composition of the diets investigated are likely to cause at least part of this discrepancy. Thorbek *et al.* (1982) used diets more closely resembling a rat chow, whereas the diets in the present investigation were quite different from the rat chow fed before the experiment. In the present investigation the mean values of DM, starch and protein digestibility of each diet did not vary more than 0·05 over the time investigated. Differences will probably be even smaller and difficult to detect when considering a change from one diet to another, not very different, diet.

The change in nutrient digestibility over time was, in the case of DM, NSP and starch, well described by an asymptotic model. The asymptotic progress of digestibility was most pronounced for the diets containing GG, PEC and RS, while the digestibility of the FF and CEL diets appeared to change very little during the course of the experiment. In case of the GG and PEC diets the digestibility of DM, NSP and starch continued to increase for 2–3 weeks, while the digestibility of starch in the RS diet increased during the entire experimental period. This progress over time may, at least partly, be caused by changes in the fermentation capacity in the caecum and colon of the rats. The extent of fibre fermentation has been shown to be a function of time combined with the fermentability of the fibre (Tulung *et al.* 1987). Several workers have suggested that the composition of the microflora changes towards micro-organisms that are efficient in fermenting the EIDPS provided (Tulung *et al.* 1987; Edwards, 1993). Since the extent of fermentation is positively correlated with the retention time in the hind gut (Van Soest *et al.* 1982), an increasing retention time during the course of the experiment could contribute to the increasing digestibility of DM, NSP and starch. Retention time is positively related to the BW, at least when comparing species (Van Soest *et al.* 1982).

In a previous investigation (Brunsgaard *et al.* 1995) we found that the size of the caecum and colon in rats increased with BW and even more so when fermentable EIDPS was added to the diet. The increase in the size of the caecum and colon takes place within 1–2 weeks from introducing a diet containing highly fermentable EIDPS, while in the case of diets with less-fermentable EIDPS the increase in the size of the colon and caecum takes place over a longer period of time (Brunsgaard *et al.* 1995; G. Brunsgaard, unpublished results).

This corresponds well with the present investigation demonstrating that diets containing easily fermentable EIDPS require a shorter period for adaptation than do less-fermentable EIDPS. Non-fermentable EIDPS such as cellulose seem to be unaffected by the period of adaptation.

The apparent digestibility of retrograded high-amylose maize starch increased over all 8 weeks investigated, thus adaptation in apparent digestibility can extend over a rather long time. A similar observation was made by Faulks *et al.* (1989) on resistant pea starch and to some extent on resistant maize starch. They observed a gradual decline in the faecal content of starch and an increase in apparent digestibility of pea starch throughout the 18 d of the experiment. The resistant maize starch used in their study appeared to be less resistant than the pea starch used in the same study and also less resistant than the retrograded maize starch used in the present study. Some researchers have found an almost complete fermentation of resistant starch (Andrieux *et al.* 1992), while others have found substantially lower values (Bach Knudsen *et al.* 1988; Gee *et al.* 1991). These differences could be attributable in the type of resistant starch as well as to the period allowed for adaptation before the measurement. It is interesting that the acute effects of resistant starch are closer to those of cellulose than to those of pectin (Ranganathan *et al.* 1994), while the long-term effects are closer to those of fermentable fibre (Demigné & Rémésy, 1982; Brunsgaard *et al.* 1995).

Incorporation of the retrograded high-amylose maize starch in the experimental diet (RS) was based on the analytical value, which is supposed to reflect the ileal digestibility of this starch in humans (Englyst *et al.* 1992). The ileal starch digestibility in rats may be different from that in humans. Therefore the amount of starch that was truly resistant in the rat was uncertain and may not have been the intended 80 g/kg ingested feed. NSP digestibility has been reported to be consistently lower in the rat than in man, while there seems to be good agreement in digestibility of energy (Bach Knudsen *et al.* 1994).

The authors believe that the time-dependent increase in the ability to digest resistant starch is principally due to increases in the fermentation capacity in the hind gut of the rat. However, the present investigation does not rule out that an increase in the digestive and absorptive capacity of the small intestine (Ikegami *et al.* 1990) accounts for part of the increase in starch digestibility. Evidence has been presented that the small intestine is capable of increasing the rate of retrograded starch utilization after 2 weeks of adaptation (Faulks *et al.* 1992). Hypertrophy of the small intestinal tissue is observed for retrograded starch (Brunsgaard *et al.* 1995). An increase in the length of the small intestine could increase the amount of starch digested and absorbed during the passage through the small intestine. The significance of the length of the small intestine for subsequent starch digestion and absorption still needs to be investigated. However, the present and other reports (Lajvardi *et al.* 1993) indicate that hypertrophy of the small intestine is insufficient to prevent completely the resistant starch from reaching the hind gut.

The relatively high faecal contents of uronic acids (markers for pectin) and mannose + galactose (markers for guar gum) during the early adaptation period are inconsistent with the general belief that pectin and guar gum are readily fermented by the gut flora (Spiller, 1994). Our investigation indicates that the hind gut of the rat requires at least 2–3 weeks to obtain the capacity needed for total pectin and guar-gum fermentation. Humans and pigs seem to be much better fermenters of pectin (Cummings *et al.* 1979; den Hartog *et al.* 1988). In a comparative study by Bach Knudsen *et al.* (1994), pectin in the form of citrus fibre was inefficiently fermented by rats, but not by man. The adaptation period used in their rat experiment was 17 d. According to the results of the present investigation this was insufficient to obtain full fermentation capacity.

The apparent digestibility of protein in the present study did not follow an asymptotic

pattern within the time investigated. The pattern was more like a parabola with a small increase initially and a larger decrease subsequently. This time-related change in ADP is supposed to be partly, if not totally, related to an increase in metabolic N with increasing BW (Eggum, 1973) and not to an adaptation in digestibility *per se*. The lower ADP of the GG and PEC diets compared with the FF diet is in agreement with the results of other workers (Nyman & Asp, 1982). A greater amount of faecal N causes the ADP depression of these diets. This is most probably mediated by an increase in the microbial fixation of metabolic and endogenous N in the hind gut (Mosenthin *et al.* 1992; Levrat *et al.* 1993). However, it cannot be ruled out that differences in ileal digestibility of dietary protein contributed to the observed differences in ADP. The choice of casein, a highly digestible protein (Eggum, 1973), as the only source of N should ensure minimal amounts of dietary protein escaping endogenous enzyme digestion.

The substantially higher faecal N excretion in the rats receiving the fermentable EIDPS compared with the FF diet is in agreement with other reports (Mosenthin *et al.* 1992; Levrat *et al.* 1993). Despite the high faecal N excretion of the rats given fermentable EIDPS, the predominant route of N excretion was, at all times for all five diets, the urine. This became even more pronounced as the dietary N supply exceeded the N requirement of the rats.

In conclusion, the digestibility of nutrients appears to be influenced by time, especially so when the diet contains fermentable fibre or α-amylase-resistant starch. The calculated adaptation period required for pectin or guar gum was 1 week, while retrograded starch required at least 1 month. However, the faecal contents of diet-characteristic sugars were elevated for 3 weeks in the case of pectin and 2 months in the case of retrograded starch. Changes in N excretion patterns were dominated by changes in metabolic N rather than diet adaptation although an increase in hind gut supply of fermentable polysaccharides significantly increased the amount of N excreted by the faecal route.

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