British Journal of Nutrition (2004), **92**, 95–104
DOI: 10.1079/BJN20041173

Inter-relationship of microbial activity, digestion and gut health in the rabbit: effect of substituting fibre by starch in diets having a high proportion of rapidly fermentable polysaccharides

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(Received 20 August 2003 – Revised 13 January 2004 – Accepted 22 March 2004)

Caecal microbial activity, digestion and gut health were analysed in the young rabbit, in response to fibre substitution by starch, in diets with high proportions of rapidly fermentable polysaccharides (pectins + hemicelluloses:acid-detergent fibre (ADF) ratio of 1·7). A range of five diets corresponding to a 60 % linear reduction of the ADF level (230 to 92 g ADF/kg) without changes in the fibre quality, and to a corresponding linear increase in dietary starch, was given *ad libitum* to young rabbits from 18 d until 70 d of age. A one half reduction of the ADF level resulted in a sharp increase in energy digestibility (+25 units), associated with a lower feed intake (−35 %) and to an increase of the mean retention time (+6 h) in the whole digestive tract. Despite large variations in the fibre intake (20 to 59 g ADF/d), the fibre digestive efficiency remained similar among the five diets. Starch ileal concentrations were low after 4 weeks of age (< 5 %), and variations with age were significant when the dietary starch level was over 19 %. A 65 % lower biomass production was measured when the ADF level progressed from 230 to 165 g/kg, and no precise relationship was found with fermentative activity. Reducing the fibre intake led to a linear decrease of caecal volatile fatty acids concentrations, and to higher pH and NH_3 levels. An increased occurrence of mortality by diarrhoea was registered with the lowest fibre intake. It can be concluded that a sufficient supply of fibre, with high proportions in rapidly fermentable polysaccharides, stimulates the maturation of microbial activity and reduces the occurrence of diarrhoea.

Rabbits: Dietary fibre: Gut health: Caecal microbial activity

Dietary fibre exhibits numerous effects along the digestive tract; on intake, rate of passage, digestive efficiency, and microbial activity (Bach Knudsen, 2001; Wenk, 2001). Previous studies on fibre digestion in the single-stomached animal have generally only addressed some of these effects on digestion (Gidenne *et al.* 1998; De Blas *et al.* 1999). Moreover, fibre can also affect digestive health. This was clearly demonstrated in the young rabbit, where a fibre deficiency affects digestive processes (Bellier & Gidenne, 1996) and leads to digestive problems and increased morbidity and mortality (Bennegadi *et al.* 2001). Digestive disorders seem to originate in a poor or unbalanced caecal microbial activity of the symbiotic flora (Buddington & Weiher, 1999). However, a more comprehensive view of fibre's nutritional role is difficult to attain, particularly in order to understand more clearly how fibre acts in the relationship between digestive parameters and the susceptibility to digestive problems, as outlined by Montagne *et al.* (2003).

The generic term 'fibre' encompasses several components having their own effect on digestion and health. Briefly, for animal feeds the procedure of Van Soest *et al.* (1991) allows a routine fractionation of some fibre fractions using several analytical criteria; for example, the acid-detergent fibre (ADF) criterion recovers the cellulose and lignins fractions. The impact of these particular fractions on the prevention of digestive problems in the growing rabbit has been studied (Perez *et al.* 1994, 1996), but with dietary models containing low proportions of rapidly fermentable polysaccharides (RFP). These polysaccharides have been shown to play a specific role in rabbit digestion (Gidenne & Bellier, 2000; Gidenne & Perez, 2000), microbial activity (Garcia *et al.* 2002) and health (Perez *et al.* 2000). Moreover, few studies have addressed the effect of fibre without changes in its quality on digestive parameters and microbial activity.

Therefore, the present study aimed to quantify changes in digestive processes and microbial activity (fermentation

Abbreviations: ADF, acid-detergent fibre; DAPA, diaminopimelic acid; MRT, mean retention time; NDF, neutral-detergent fibre; RFP, rapidly fermentable polysaccharides; VFA, volatile fatty acids.

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and biomass) and their relationship with digestive health, with a dietary model having a high proportion of RFP, where starch replaced fibre and produced a 60 % linear decrease in ADF supply, without changes in the proportions of the major fibre fractions.

Material and methods

Diets, experimental design and animal housing

The dietary model consisted of five diets arranged to obtain a similar decrease in every fibre fraction (Table 1) substituted by starch. We chose the ADF criterion to express more easily the fibre supply, as it corresponds to a standardised procedure. The five diets, named by their ADF concentration, covered a range from 90 to 230 g ADF/kg; the ADF:starch ratio progressing from 9 to 0·2. Compared with more classical feeds (De Blas *et al.* 1999), the experimental diets contained a high proportion of RFP (i.e. hemicelluloses + pectins), reaching 70 % of the water-insoluble cell wall, and accordingly, the proportion of RFP relative to other fibre fractions was high and similar among the five diets (for example, RFP:ADF = 1·4). Water and feeds (pellets of 3·5 mm) were given *ad libitum*. No antibiotics were included in the feed or in the water.

The study was organised in three trials, for assessing: first, the microbial activity and starch digestion at the ileum; second, the digestive efficiency and rate of passage; third, the growth and health status. All rabbits were housed in a closed and ventilated room (18 ± 2°C), with 12 h light (7.00–19.00 hours), and handled according to the principles for the care of animals in experimentation, in agreement with French national legislation. Animals had free access to water and feeds, and they were not prevented from eating their caecotrophes.

Experiment 1: microbial activity, bacterial biomass production, and starch digestion in the small intestine

From 18 d until weaning (at 28 d of age), the diets ADF23, ADF17 and ADF9 were given *ad libitum* to three groups of ten litters (equalised at birth, to nine kits) caged separately from their mothers who received a commercial diet. The doe was introduced to the litter cage for milking (for 5 min, at 8.00 hours). After weaning, the growing rabbits were caged in individual wire net cages, and were fed the same diet that was given before weaning. At weaning (28 d), and at 42, 56 and 70 d of age, one kit/litter was killed for ileal and caecal digesta sampling, by sudden cervical dislocation (American Veterinary Medical Association, 1986) at the end of the caecotrophy period (13.00 hours). A segment of terminal ileum (200 mm before the ileo-caecal junction) was sampled for further analyses of starch, while the whole caecal content was sampled for microbial activity analyses, i.e. biomass production and fermentation pattern.

The pH of the caecal digesta was taken immediately after laparotomy, with a glass electrode pH meter (pH

Table 1. Ingredients and chemical composition of experimental diets

Diet...	ADF23	ADF20	ADF17	ADF14	ADF9
Ingredients (g/kg diet)					
Dehydrated beet pulp	490	400	310	220	130
Dehydrated lucerne	150	122	95	68	40
Wheat straw	150	122	95	68	40
Soya-bean meal	165	169	172	175	180
Wheat	22	162	300	438	576
Salt	4·0	4·5	5·0	6·5	6·0
DL-Methionine	3·0	2·5	2·0	1·5	1·0
Dicalcium phosphate	11·0	10·0	9·0	7·0	6·0
Calcium carbonate	–	3·0	7·0	11·0	16·0
Minerals and vitamins mixture	5·0	5·0	5·0	5·0	5·0
Chemical composition (g/kg as fed)					
DM	911	915	904	908	903
Crude protein (N × 6·25)	158	165	162	153	165
Starch	25	98	196	277	374
WICW*	475	414	356	294	207
NDF	423	369	311	272	194
ADF	230	198	165	142	92
ADL	40	37	24	23	18
ADF:starch ratio	9·2	2·0	0·8	0·5	0·2
Uronic acids	58	47	40	31	17
Pectins†	137	115	92	72	50
RFP‡	327	286	238	202	152
Hardness of pellets§	13·7	11·4	9·4	8·0	6·4

ADF, acid-detergent fibre; WICW, water-insoluble cell walls; NDF, neutral-detergent fibre; ADL, acid-detergent lignins; RFP, rapidly fermentable polysaccharides.

Minerals and vitamins mixture provided the following: Robenidine®, 66 mg/kg; vitamin A, 540 mg retinol/kg; vitamin D_3, 5 mg/kg; vitamin B_1, 300 mg/kg; vitamin E, 6000 mg/kg. The mixture also provided oligo elements: Cu^{2+}, 4000 mg/kg; Fe^{2+}, 14 000 mg/kg; Zn^{2+}, 20 000 mg/kg; Mn^{2+}, 7000 mg/kg.

* According to Brillouet *et al.* (1988).

† Sum of uronic acids + associated neutral sugars (water-insoluble fractions).

‡ Sum of pectins + hemicelluloses (NDF-ADF).

§ Hardness expressed in kg; force to break a pellet (KHAL index, mean of fifteen measurements).

95; WTW, Weilheim, Germany). Portions of the caecal digesta samples (1 g fresh matter) were placed in tubes containing H_3PO_4 or H_2SO_4 (2 %, v/v) storage solution (2 and 3 ml/tube), respectively for further analyses of SCFA and NH_3, and stored at − 18°C.

The caecal biomass production was measured individually, using diaminopimelic acid (DAPA) as an internal marker of the bacterial protein (Broderick & Merchen, 1992). The biomass production was only measured at 56 d of age, and on four rabbits of groups ADF23, ADF17 and ADF9 used for the digestibility measurements (experiment 2), because the procedure was costly and time-consuming. In rabbits, the bacterial biomass is either excreted in hard or soft faeces. Assuming that no DAPA was hydrolysed or absorbed between the caecum and the rectum, the daily production of bacterial biomass was calculated by dividing the daily excretion of DAPA by the mean DAPA concentration in the caecal bacteria. Thus, quantitative excretion of DAPA was measured individually in hard faeces during a 4 d period (50 and 54 d of age), and soft faeces excretion was evaluated in rabbits wearing a collar to prevent caecotrophy (at 48 and 55 d of age). At 56 d of age, four rabbits/group were slaughtered to sample the caecal digesta, and DAPA was determined on caecal bacteria extracted by differential centrifugation (Legay-Carmier & Bauchart, 1989).

Experiment 2: digestive efficiency and rate of passage measurements

According to the 'European' reference method (Perez *et al.* 1995), faecal apparent digestibility was measured individually between 42 and 46 d of age, on five further groups of nine rabbits, housed in individual metabolism cages and fed the five diets from weaning (28 d). The rate of passage of liquids and fibre particles was measured between 49 and 56 d of age, on three groups of seven rabbits (ADF23, ADF17 and ADF9), by following the faecal excretion of a dose of ^{51}Cr-EDTA and a dose of ^{141}Ce-labelled fibre particles (Gidenne, 1994). The rate of passage between mouth and rectum was obtained by giving orally the two markers simultaneously (at 9.00 hours) using a modified syringe of 1 ml. Then, the faecal excretion was fractionated in thirty-six samples during 96 h by means of an automatic faecal sampler (API, Castanet, France) adapted for use in rabbit metabolism cages. After drying, faeces were directly analysed for their marker content in a gamma spectrometer (model 5530; Packard Instruments, Downersgrove, IL, USA). The digesta mean retention time (MRT) was algebraically calculated by numerical integration of the marker quantity excreted in faeces: $MRT = \Sigma Mi \times ti/\Sigma\ Mi$, where ti was the time that had elapsed between marker administration and the ith defecation and Mi was the quantity of marker excreted. MRT includes the transit time, which was the time that had elapsed between marker administration and the first marker appearance in the faeces. Transit time reflects the retention time of digesta without a delay in the mixing compartments. Thus, it represents the rate of passage in the tubular segment of the tract, i.e. mainly in the small intestine and also in the distal colon (Gidenne, 1994).

Experiment 3: feed intake, growth and digestive health status

Intake and growth performances were examined weekly on five groups of forty rabbits, fed *ad libitum* the five experimental diets, from weaning (at 28 d of age) to slaughter at 79 d, and housed in individual cages. Health status measurement included a daily inspection of the mortality by diarrhoea. Gut health was also approached through an individual inspection (three times per week) of all clinical signs of digestive problems (transitory diarrhoea, presence of mucus in excreta, abdomen dilatation, abnormal intake behaviour, etc). Morbidity rate was obtained from animals having an abnormally low intake or weight (under 3 SD below the mean of the group) or clinical signs of digestive problems. Health risk index was the sum of morbid and dead animals.

Chemical analyses

DM was determined in feeds, faeces and caecal contents by heating for 24 h at 103°C. Measurements of fibre fractions (neutral-detergent fibre (NDF), ADF, acid-detergent lignin) in feeds were made according to the sequential procedure of Van Soest *et al.* (1991). Measurements were made using an amylolytic pre-treatment with a thermostable amylase (Association Française de Normalisation, 1997; European Group on Rabbit Nutrition, 2001). In feeds, the water-insoluble cell wall content was determined according to Brillouet *et al.* (1988), and water-insoluble uronic acids were determined according to Blumenkrantz & Asboe-Hansen (1973). Pectins were calculated as the sum of uronic acids and associated neutral sugars (from the tables of Bach-Knudsen, 1997). RFP were calculated as the sum of pectins and hemicelluloses (NDF − ADF). Starch was measured in the diet and ileal digesta, by determination of the glucose resulting from enzymic hydrolysis with the hexokinase (*EC* 2.7.1.1)–glucose 6-phosphate dehydrogenase (NAD) (*EC* 1.1.149) system (Boehringer, Mannheim, Germany). NH_3 concentration was measured by the technique of Weatherburn (1967) with an auto-analyser (Technicon, Domont, France) and was determined spectrophotometrically at 660 nm according to Verdow *et al.* (1977). Volatile fatty acids (VFA) were analysed by GLC (CP9000; Chrompack, Middelburg, The Netherlands) on a semi-capillary column (Bellier, 1994).

DAPA was determined with an automatic amino acid analyser (Beckman 6003; Beckman-Coulter, Gagny, France). Samples were lyophilised and hydrolysed for 24 h in HCl (6 M). Amino acids were separated by chromatography through an ion exchanger column and measured by spectrophotometry (570 nm) after reaction with ninhydrin. DAPA concentration was estimated both in hard and soft faeces and on pure bacteria isolated from the caecum by differential centrifugation.

Statistical analyses

Data for growth, intake, digestive efficiency and rate of passage were subjected to a monofactorial ANOVA (effect of the diet), using the general linear model

Table 2. Caecal characteristics and fermentative activity of 4- and 6-week-old rabbits fed diets with varying fibre:starch ratio (experiment 1)*
(Mean values and residual standard errors)

	Age 28 d					Age 42 d				
	ADF23 (*n* 10)	ADF17 (*n* 10)	ADF9 (*n* 10)	RSE	*P*	ADF23 (*n* 10)	ADF17 (*n* 10)	ADF9 (*n* 10)	RSE	*P*
Caecal content (g/kg live weight)	36·1	40·9	45·9	3·2	0·15	104·7^{a}	67·2^{b}	73·0^{b}	6·3	0·001
pH	6·50	6·18	6·37	0·12	NS	5·74	5·97	6·09	0·21	NS
NH_3 (mmol/l)	18·8^{a}	13·4^{b}	14·6^{b}	1·3	0·025	10·0^{a}	11·5^{a}	15·8^{b}	1·0	0·003
Total VFA (mmol/l)	39·8^{a}	56·6^{b}	52·7^{ab}	4·1	0·036	95·4^{a}	70·5^{ab}	64·5^{b}	6·8	0·012
VFA (mmol/mol)										
Acetate	84·6	89·2	88·2	1·6	NS	87·5	82·1	84·0	1·8	NS
Propionate	7·8	3·8	3·9	0·9	0·009	4·0	4·0	3·2	0·4	NS
Butyrate	6·6	6·2	6·6	0·9	NS	8·1	13·5	15·6	1·8	0·13
Propionate:butyrate	1·21^{a}	0·70^{ab}	0·63^{b}	0·14	0·029	0·63	0·31	0·35	0·11	0·14
VFA pool (mM)	0·46^{a}	0·98^{b}	0·79^{ab}	0·11	0·021	7·0^{a}	4·0^{b}	3·9^{b}	0·66	0·007

VFA, volatile fatty acids; NS, not significant ($P > 0·15$).
[a,b] Mean values, within a row, within an age, with unlike superscript letters were significantly different ($P < 0·05$).
* For details of diets and procedures, see Table 1 and pp. 96–97.

procedure (SAS OnlineDoc®, release 8.01 for SunOs; SAS Institute Inc., Cary, NC, USA). Means were compared using the Scheffé test. In addition, the effect of fibre level was analysed as a quantitative variable, and treatments of sums of squares were partitioned into linear effects. The statistical analysis of VFA concentrations was performed according to a bifactorial model; age, diet and interaction. Significant interactions were detected between diet and age effects, at 4 and 6 weeks of age for almost all caecal parameters. Therefore, between 4 and 6 weeks of age, analysis was performed intra-age (Table 2), while between 6 and 10 weeks of age results are presented as mean effects for diet and age (Table 3). Some data from unhealthy animals or animals having a reversed VFA pattern (propionate:butyrate ratio over 2) were not included in the statistical analysis. Categorical data (mortality, morbidity, health risk index) were treated using the Catmod procedure of SAS.

Results

Digestive efficiency, rate of passage and starch digestion in the small intestine (experiment 2)

Voluntary feed intake during the digestibility measurements decreased linearly with the reduction of the dietary fibre level (Table 4), except for diet ADF17 that seemed underconsumed. Whole-tract digestive efficiency of organic matter and energy were greatly and linearly improved (+25 units) from the ADF23 to the ADF9 diet. In parallel, protein digestion was improved by 20 units and reached 90 % for the diets with the lowest fibre:starch ratio.

Digestion of starch before the caecum was investigated through the analysis of residual starch at the terminal ileum. Whatever the age, the concentration of starch in the ileum did not vary for the ADF23 diet (Fig. 1), as the dietary starch was already low (< 30 g/kg DM). The variance analysis indicated that there was no effect of age. However,

Table 3. Caecal characteristics and fermentative activity of rabbits aged between 6 and 10 weeks fed diets with varying fibre:starch ratio (experiment 1)*
(Mean values and residual standard errors)

	Diet			Age (d)				*P*		
	ADF23 (*n* 30)	ADF17 (*n* 30)	ADF9 (*n* 30)	42 (*n* 30)	56 (*n* 30)	70 (*n* 30)	RSE	Diet	Age	Diet × age
Caecal content (g/kg live weight)	89·7^{a}	67·7^{b}	62·5^{b}	82·3^{a}	86·1^{a}	57·0^{b}	2·4	< 0·001	< 0·001	0·09
pH	5·62^{a}	5·79^{ab}	5·85^{b}	5·69	5·86	5·72	0·05	0·048	0·073	NS
NH_3 (mmol/l)	9·5^{a}	11·3^{b}	13·2^{c}	12·5^{a}	11·6^{ab}	10·7^{b}	0·4	< 0·001	0·016	0·07
Total VFA (mmol/l)	85·7^{a}	73·9^{ab}	64·8^{b}	76·6	70·7	74·1	3·2	< 0·001	NS	NS
VFA (mmol/mol)										
Acetate	87·6^{a}	83·4^{b}	76·2^{c}	84·6^{a}	84·1^{a}	79·4^{b}	0·8	< 0·001	< 0·001	< 0·001
Propionate	3·9	4·0	3·6	3·7^{ab}	3·4^{a}	4·3^{b}	0·2	NS	< 0·01	NS
Butyrate	8·1^{a}	12·1^{b}	19·3^{c}	11·1^{a}	12·1^{ab}	15·6^{b}	0·8	< 0·001	< 0·01	< 0·001
Propionate:butyrate	0·23^{a}	0·37^{a}	0·60^{b}	0·44	0·43	0·33	0·05	< 0·001	NS	NS
VFA pool (mM)	9·1^{a}	6·9^{b}	5·2^{c}	5·0^{a}	7·6^{b}	7·7^{b}	0·4	< 0·001	< 0·001	NS

VFA, volatile fatty acids; NS, not significant ($P > 0·15$).
[a,b,c] Mean values within a row, within an effect, with unlike superscript letters were significantly different ($P < 0·05$)
* For details of diets and procedures, see Table 1 and pp. 96–97.

Table 4. Whole-tract digestive efficiency of 6-week-old rabbits fed diets with varying fibre:starch ratio (experiment 2)† (Mean values and residual standard errors)

	Diet						
	ADF23 (*n* 9)	ADF20 (*n* 9)	ADF17 (*n* 9)	ADF14 (*n* 9)	ADF9 (*n* 9)	RSE	Diet effect (*P*)
Intake (g DM/d per kg live weight)	85·5^{a}	64·0^{b}	50·1^{c}	56·7^{bc}	54·4^{bc}	2·9	<0·001
Digestibility coefficient (%)							
Organic matter	60·8^{a}	68·2^{a}	77·4^{b}	77·8^{b}	85·3^{c}	1·4	<0·001*
Crude protein	68·2^{b}	74·4^{b}	84·2^{a}	83·7^{a}	89·9^{a}	1·4	<0·001*
Energy	59·5^{d}	67·0^{c}	76·4^{b}	77·0^{b}	84·4^{a}	1·5	<0·001*
NDF	41·4	44·9	51·7	45·3	49·4	3·2	NS
ADF	28·7	32·2	39·9	32·4	33·1	4·2	NS
Hemicelluloses	56·7	59·7	65·4	59·3	64·0	2·6	0·13
Nutritive value							
Digestible energy (MJ/kg; air-dry basis)	9·42	10·79	12·03	12·24	13·70		
Digestible protein (g/kg; air-dry basis)	108	123	136	128	138		

NDF, neutral-detergent fibre; NS, not significant ($P>0·15$).
^{a,b,c,d} Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* Significant linear effect of the dietary fibre level (either ADF or water-insoluble cell walls) ($P<0·05$).
† For details of diets and procedures see Table 1 and pp. 96–97.

there was a significant effect of diet, with a four times higher ileal starch level with the ADF9 diet compared with the ADF23 diet (49 *v.* 11 g/kg DM). In contrast, when we compared only the ADF9 diet with the ADF17 diet, we found a significantly higher level of starch for rabbits at weaning (53 g/kg DM) compared with older animals (31 g/kg DM).

The MRT of the solid phase was increased by 70 % between the ADF23 and ADF9 diets, and by only 30 % for the liquid phase (Table 5). When the feed intake was introduced as a covariate in the statistical model, the effect of the diet remained significant ($P=0·05$) only for the solid phase. Transit in tubular segments (small intestine and distal colon), as represented by the minimal transit time, was similar for the solid and liquid phases (mean 4·2 h). No significant effect of dietary treatment was found for this parameter.

Caecal microbial activity: bacterial biomass production and fermentation profile (experiment 1)

The DAPA concentration decreased from bacterial sample to soft and then hard faeces, and was not affected by the diet (Table 6). Therefore, as hard and soft faeces excretion decreased sharply from the ADF23 to the ADF9 diet, the total DAPA excretion was three times lower with the ADF9 compared with the ADF23 diet. The total bacterial biomass production was thus three-fold higher for rabbits fed the diets with the highest fibre:starch ratio, compared with the two other groups (17·9 *v.* 5·7 g DM/d). This bacterial biomass production accounted for 15·7 and 7·5 % of the feed intake, respectively, for the ADF23 and 'ADF17 + ADF9' rabbits. The recycling of bacteria through caecotrophy accounted for 2·7–7·0 g of the DM. However, DAPA excretion in soft faeces was variable (residual CV = 40 %), because of high variability in the DAPA concentrations. As the crude protein content of bacteria was 481 g/kg DM, the biomass recycled through caecotrophes accounted for 4·1 and 2·7 g crude protein/d for the ADF23 and the 'ADF17 + ADF9' groups. Therefore the recycling of bacterial protein through caecotrophy corresponded to 24·4, 13·7, and 8·8 % of the crude protein intake respectively for the ADF23, ADF17 and ADF9 groups.

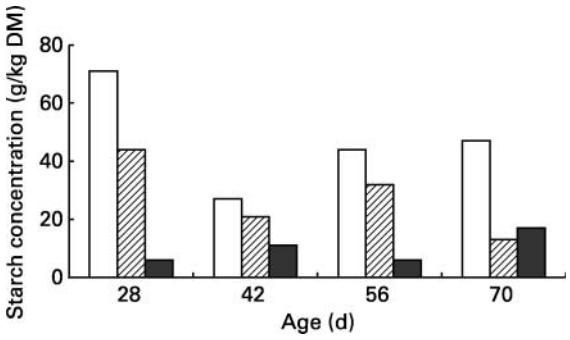

Fig. 1. Starch concentration at the terminal ileum of growing rabbits fed diets with varying fibre:starch ratio (experiment 1). The results of variance analysis with all diets were: effect of age, $P=0·12$; effect of diet, $P<0·001$; age × diet, $P=0·26$; root mean square error, 2·6. (□), ADF9 diet; (///), ADF17 diet; (■), ADF23 diet. For details of diets and procedures, see Table 1 and pp. 96–97.

Significant age × diet interactions were detected, for the caecal characteristics and fermentation pattern, particularly between 28 and 42 d of age, linked to a lower VFA caecal concentration for the ADF23 group (Table 2) compared with the two other groups. The lower feed intake during the first week of the experiment of the rabbits fed the ADF23 diet could explain this effect (Table 7). Conversely at 4 weeks of age, the propionate and NH_3 levels were higher for the rabbits fed the ADF23 diet, while the caecal VFA pool was reduced by one half. Comparatively at 42 d of age, a compensatory effect was observed in the ADF23 group; the VFA level and the caecal pool were significantly higher in the ADF23 compared with the ADF9 rabbits (+20 and + 40 %, respectively). Between 42 and 70 d of age, the VFA level was 25 % lower in the ADF9 group compared with the ADF23 group, while the NH_3 and caecal pH were higher (Table 3). The caecal content was also increased in the rabbits fed the ADF23 diet, compared with the two other groups of rabbits. The pattern of fermentation was also subject to interactions between

Table 5. Rate of passage in 6-week-old rabbits fed diets with varying fibre:starch ratio (experiment 2)*
(Mean values and residual standard errors)

	Diet				
	ADF23 (*n* 6)	ADF17 (*n* 6)	ADF9 (*n* 7)	RSE	Diet effect (*P*)
Live weight (g)	1450	1526	1386	29	NS
Food intake† (g/d per kg live weight)	89·5^{b}	87·5^{ab}	75·8^{a}	1·2	0·012
Mean retention time‡ (h)					
Solid phase (^{141}Ce)	9·7^{b}	11·2^{b}	16·2^{a}	0·4	<0·01
Liquid phase (^{51}Cr)	17·8^{b}	21·2^{ab}	23·7^{a}	0·6	0·032
Minimal transit time§ (h)					
Solid phase (^{141}Ce)	3·8	4·2	4·4	0·4	NS
Liquid phase (^{51}Cr)	4·0	4·5	4·7	0·3	NS

NS, not significant ($P>0·15$).
^{a,b} Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* For details of diets and procedures, see Table 1 and pp. 96–97.
† Mean feed intake during rate of passage measurements (4 d).
‡ Mean retention time, between mouth and rectum ($\Sigma Mi \times ti/\Sigma Mi$; see p. 97).
§ Time of first appearance of marker in faeces.

Table 6. Caecal bacterial biomass production in growing rabbits (8 weeks old) fed diets with varying fibre:starch ratio (experiment 1)*
(Mean values and residual standard errors)

Diet...	ADF23 (*n* 4)	ADF17 (*n* 4)	ADF9 (*n* 4)	RSE	Diet effect (*P*)
Faecal excretion† (g DM/d)					
Hard faeces	40·9^{a}	16·25^{b}	11·06^{b}	2·2	<0·01
Soft faeces	20·9^{a}	6·9^{b}	6·8^{b}	2·5	<0·01
DAPA concentration‡ (mg/g DM)					
Bacteria in the caecum	2·26	2·41	2·85	0·49	NS
Hard faeces	0·61	0·41	0·54	0·08	NS
Soft faeces	0·73	0·80	0·92	0·11	NS
DAPA excretion‡ (mg/d)					
Hard faeces	24·1^{a}	6·5^{b}	6·1^{b}	8·6	<0·01
Soft faeces	14·8^{a}	5·6^{b}	6·3^{b}	17·2	0·010
Production of biomass‡ (g DM/d)					
Hard faeces	11·0^{a}	3·4^{b}	2·4^{b}	1·0	<0·01
Soft faeces	7·0^{a}	3·0^{b}	2·5^{b}	1·1	0·038
Total	17·9^{a}	6·3^{b}	5·0^{b}	1·9	<0·01

DAPA, diaminopimelic acid; NS, not significant ($P>0·15$).
^{a,b} Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* For details of diets and procedures, see Table 1 and pp. 96–97.
† Measured on nine rabbits per diet, during the 4 d faecal collection period (50–54 d of age).
‡ Measured on four rabbits per diet, selected from the nine rabbits used for faecal collection.

the effect of age and that of diet; acetate proportions were higher at 6 weeks of age in the ADF9 group and then decreased with age (with a reverse situation for butyrate), while for the ADF17 and ADF23 groups acetate and butyrate proportions remained similar with age. This suggests that fermentation patterns were incompletely established in the ADF9 rabbits, compared with the other groups. Indeed, between 6 and 10 weeks of age, the effect of age appeared weak in terms of VFA and NH_3 concentrations and pH, suggesting that fermentative activity was already installed at 42 d of age.

Intake regulation, growth and gut health (experiment 3)

Mean feed intake from 28 to 79 d of age decreased linearly by 23 % when the fibre:starch ratio was reduced from 19 to 0·6. Accordingly, between diets ADF23 and ADF9, the decrease in fibre intake was greater (from 59 to 20 g ADF/d) than the increase in starch intake (+32 g; from 3 to 35 g/d). However, during the first week of the experiment, diets ADF23 and ADF20 tended to be less consumed, with respect to their fibre:starch ratio, compared with the other diets having a lower fibre content (Table 7). An increase in pellet hardness was measured with the inclusion of beet pulp from ADF9 to ADF23 (Table 1). This may have caused the lower consumption, and the lower growth registered from 28 to 37 d of age for the ADF23 compared with the ADF20 group (−30 %). After the first week post-weaning, this effect decreased but remained significant for the periods 28–51 d and 28–79 d of age. We also measured a linear negative effect on post-weaning growth ($P<0·05$) of decreasing the fibre level, from ADF20 to ADF9. With the decrease of the fibre level, the feed conversion index logically decreased linearly, as the digestible energy content of the diets increased (Table 4).

Table 7. Intake and growth of growing rabbits fed diets with varying concentrations fibre:starch ratio (experiment 3)†
(Mean values and residual standard errors)

	Diet						
	ADF23 (*n* 40)	ADF20 (*n* 40)	ADF17 (*n* 40)	ADF14 (*n* 40)	ADF9 (*n* 40)	RSE	Diet effect (*P*)
Period 28–37 d of age							
Intake (g/d)	56·9^{ab}	59·2^{ab}	63·9^{a}	56·6^{ab}	54·8^{b}	1·94	0·01
Weight gain (g/d)	26·8^{c}	39·1^{a}	38·7^{a}	36·2^{ab}	34·4^{b}	1·03	<0·01
Period 28–51 d of age							
Intake (g/d)	91·8^{a}	89·2^{a}	81·6^{b}	74·8^{bc}	70·5^{c}	1·72	<0·01*
Weight gain (g/d)	37·1^{b}	41·5^{a}	39·2^{ab}	38·4^{ab}	37·9^{ab}	0·85	<0·001
Feed conversion	2·49^{a}	2·17^{b}	2·10^{bc}	1·96^{cd}	1·88^{d}	0·039	<0·01*
Period 28–79 d of age							
Intake (g/d)	123·7^{a}	118·3^{ab}	109·9^{bc}	104·8^{c}	94·7^{c}	2·13	<0·01*
Weight gain (g/d)	35·6^{b}	38·4^{a}	38·5^{a}	37·8^{ab}	36·4^{ab}	0·68	<0·01
Feed conversion	3·46^{a}	3·10^{b}	2·85^{c}	2·76^{c}	2·60^{d}	0·036	<0·01*

^{a,b,c,d} Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* Significant linear effect of the dietary fibre level (water-insoluble cell walls) ($P<0·05$).
† For details of diets and procedures, see Table 1 and pp. 96–97.

Mortality occurred for two-thirds of the cases between 28 and 49 d of age. Symptoms were always an acute and short diarrhoea (during 1 to 3 d), and at autopsy no clinical signs of inflammation were registered, but only liquid digesta content in the caecum and in the small intestine. Mortality was significantly higher with the lowest fibre: starch ratio compared with the highest one (Table 8), with the number of dead rabbits in the ADF23 group one-third (*n* 4) that of the ADF9 group (*n* 13). Morbidity was here characterised mainly by transitory falls in feed intake and growth, and by transient diarrhoea symptoms (in six cases). Morbidity occurred for 50 % of the cases, during the 2 weeks after weaning, with diet having no effect. Therefore, the health risk index linearly increased (Maentel–Hentzel test; $P<0·05$) with the reduction of the fibre level.

Discussion

Feed intake regulation and digestion according to dietary fibre level

With our dietary model in the present study, when fibre was replaced by starch in a large range of variation, feed intake was closely (r^2 0·99) and equally related to starch or dietary fibre level. This model differed from those employed in previous studies by its high level of RFP, and in respect of the sharp reduction in the fibre:starch ratio; in the present study feed intake was moderately reduced (−23 %, −0·6 %/unit NDF). In comparison, with diets rich in poorly digested fibres (lignocellulose) previous studies reported a higher decrease of the feed intake; −1·3 %/unit NDF (Blas *et al.* 1994; Gidenne *et al.* 2000). The higher nutritive value of RFP would explain their moderate effect of intake level compared with poorly digested fibres. Consequently, the prediction of feed intake for the growing rabbit should take into account the fibre quality and not only the fibre level. High protein digestion was recorded and expressed the fact that from ADF23 to ADF9 low-digestible proteins from fibre sources (mainly lucerne) were replaced by more digestible protein from wheat or soya beans.

Besides, feed intake variations explained the changes in the retention time of liquids. Thus, the rate of passage of particles also seemed linked to the dietary fibre level, as already observed (Fraga *et al.* 1984; Bellier & Gidenne, 1996). However, our values of transit appeared 50 % shorter than in previous studies with diets similar in ADF levels, but poor in RFP (Bellier, 1994; Bellier & Gidenne, 1996). This suggests that digestible fibres are also important in the regulation of the rate of passage of digesta, in agreement with previous studies in rabbits (Gidenne & Bellier, 2000; Gidenne & Perez, 2000), but contrary to results obtained in man and in pigs with pectins (Hillman *et al.* 1983; Bailoni *et al.* 1999).

Table 8. Mortality by lethal diarrhoea and morbidity§ in rabbits fed diets with varying fibre:starch ratio (experiment 3)†

	Diet					
	ADF23 (*n* 40)	ADF20 (*n* 40)	ADF17 (*n* 40)	ADF14 (*n* 40)	ADF9 (*n* 40)	Diet effect (*P*)
ADF : starch ratio	9·2	2·0	0·8	0·5	0·2	
Mortality (%)	10·0^{a}	10·0^{a}	7·5^{a}	12·5^{a}	32·5^{b}	0·02
Morbidity (%)	20·0	40·0	37·5	42·5	35·0	0·25
Health risk index‡ (%)	30·0^{a}	50·0^{ab}	45·0^{ab}	55·0^{b}	67·5^{b}	0·04*

^{a,b} Percentages within a row with unlike superscript letters were significantly different ($P<0·05$).
* Significant linear effect of the dietary fibre level (ADF or water-insoluble cell walls) (Maentel–Hentzel, test; $P<0·05$).
† For details of diets and procedures, see Table 1 and pp. 96–97.
‡ Morbidity + mortality.
§ Measured between 28 and 70 days of age.

An improvement of digestion when starch is substituted for fibre is a classical trend (Lebas *et al.* 1982; Fraga *et al.* 1984), but has never been quantified for a large range of fibre:starch ratios. Energy digestibility was here highly negatively correlated with fibre levels (−1 unit/unit NDF, −1·8 unit/unit ADF; r^2 0·96). A similar fall in energy digestion with increased fibre level was reported in previous studies (De Blas *et al.* 1986; Bellier & Gidenne, 1996), although the origin and quality of fibre differed from the present study. This confirmed that fibre itself has no specific negative effect on diet digestion, and also that a fibre criterion could be used conveniently to predict energy digestibility in the rabbit.

Starch digestion in the ileum, as measured by the concentration of undigested starch in the terminal ileum, was not affected by age when the starch level remained under 10 %, although it has been demonstrated that starch digestion develops until 42 d of age (Scapinello *et al.* 1999). We observed also, that from a starch level of 10 %, the digestive capacity for starch seemed saturated at 28 d of age, but not after.

Impact of fibre level and high proportions of rapidly fermentable polysaccharides on microbial activity

As microbial activity is directly affected by the flow of nutrients entering the caecum, it was important to estimate the ileal flow of starch compared with fibre. Using previous results of ileal flow (Gidenne *et al.* 2000), ileal starch flow in the present study was lower than 1·5 g/d for the highest starch intake (diet ADF9), while ileal fibre flow was ten times higher. Therefore, in contrast to fibre, the starch flow was not considered to be a major factor in the control of microbial activity, in agreement with the recent results of Gidenne *et al.* (2004*a*) obtained with digestible or resistant starches.

Three ways were used to investigate microbial activity; fibre digestion, fermentative activity, and biomass production. Although the fibre supply covered a large range (5 to 13 g ADF/d), the fibre digestive efficiency remained similar, in agreement with Hoover & Heitmann (1972) and Gidenne *et al.* (2000). This suggests that even for a sharp reduction of fibre supply, the efficacy of flora to digest cell-wall polysaccharides was maintained. The sharp increase in the retention time of digesta was probably a favourable factor to maintain this efficiency in fibre digestion. In contrast, biomass production of 7-week-old rabbits was sharply increased only when the dietary fibre level exceeded 16 % of ADF, associated with the rise in hard and soft faeces production, and seemed not related to digesta transit. Contrasting results have been obtained in the literature about the link between the effect of fibre and fermentative activity, but they were obtained with diets poor in RFP. We here found a clear correlation between fibre level and caecal VFA concentration, and this effect was maintained until 10 weeks of age. For levels of fibre under 20 % of ADF, this lower fermentative activity was associated with a lower biomass production, probably originating in an insufficient flow of fermentable nutrients, such as RFP. However, bacterial biomass production seemed not closely related to VFA caecal concentrations that are more a qualitative criterion than a quantitative one.

In agreement with the literature, we found a clear rise in butyrate and a decrease in acetate proportions when the fibre:starch ratio decreased. Apart from the fibre level, the voluntary feed intake level affected the microbial activity, as observed in the 4-week-old rabbits that underconsumed a fibre-rich diet (ADF23) and had a low fermentative activity. This confirmed the results of Gidenne *et al.* (2002), indicating that the caecal microbial activity of the young rabbit is controlled by the fibre level and by the feed intake level.

Resistance to digestive troubles and rapidly fermentable polysaccharides

In the growing rabbit, the favourable role of cell-wall intake on the occurrence of non-specific enteropathy has been evidenced recently (Bennegadi *et al.* 2001). In contrast, the impact of starch intake on gut health is more questionable, since even with high levels of resistant starch (from crude potato, or maize), the health risk index remained quite unaffected (Pinheiro & Gidenne, 2000; Gidenne *et al.* 2004*b*). Consequently, the impact of the dietary fibre:starch ratio is mainly provided by the variations in cell-wall intake, probably in relation to its impact on caecal microbial activity. The linear reduction of growth rate observed with the decrease in fibre supply reflected an alteration of gut health. Moreover, mortality by diarrhoea in the present study was increased, but only for the lowest level of fibre, while in previous studies the incidence of diarrhoea linearly increased with fibre level (Perez *et al.* 1996). This suggests that the threshold for a protective effect of fibre on digestive health would be modified according to the proportions of fibre fractions, and this suggests a possible role of RFP. But the mechanism remains unclear, except that RFP are able to affect the microbial activity, despite the relatively short retention time (about 6–8 h) in the rabbit caecum. Better gut health in the young rabbit in the present study was also associated with a short retention time and high caecal VFA level and biomass production, and lower pH and NH_3 concentration. This agrees with the hypothesis of Prohaszka (1980) about the antibacterial effect of non-dissociated VFA, observed at caecal pH of about 6·0. We further hypothesised that a high caecal turnover rate combined with a high supply of fermentable fibre would improve and stabilise the microbial activity. Indeed, at 6 weeks of age, the rabbits fed the low-fibre diet showed an incompletely matured fermentation pattern with lower butyrate levels, in contrast to those fed high-fibre diets. In the young rabbit, the butyrate level was effectively particularly decreased in the case of diarrhoea (Gidenne, 1997). Moreover, between 4 and 10 weeks of age, the fermentation pattern evolved with a higher butyrate concentration, which is associated with a lower susceptibility to digestive problems. The anti-inflammatory effect of butyrate and its specific role on mucosal integrity have also been highlighted in several studies, in rats (Andoh *et al.* 1999) and in calves (Mentschel *et al.* 2001). In piglets, Correa-Matos *et al.* (2003) found that fermentable fibre

enhances intestinal function and reduces the severity of symptoms of digestive problems. This outlined the interactions between maturation of the digestive system of the host (digestion, mucosa integrity, etc.) and development of the digestive ecosystem, on the susceptibility to digestive problems.

Conclusion and perspectives

An increased occurrence of digestive problems in the young rabbit was associated with a low fibre intake, a longer retention time and a reduced microbial activity as investigated through qualitative and quantitative criteria. Conversely, a high intake of fibre containing high proportions of RFP appears to stimulate the maturation of microbial activity in the young rabbit. Even if starch digestive capacity is saturated at weaning with 10 % of dietary starch, an overshoot of the intestinal capacity to digest the starch does not seem to be a major factor of control of digestive health in the young rabbit.

The present study provides a better understanding of inter-relationships between several digestive parameters (transit and digestion), bacterial biomass production and fermentation activity, and gut health of the young rabbit. The digestive maturation of the host and microbial activity clearly interact with the resistance to digestive problems. In perspective, studies on the inter-relationships between microbial activity, mucosal integrity and digestive health should be carried out to identify resistance factors to diarrhoea in the young.

Acknowledgements

The authors wish to thank Gérard Blanchart for his advice in DAPA analysis (ENSAIA, Nancy), Carole Bannelier and Veronique Tartié (INRA, Station de Recherches Cunicoles, Toulouse) for their technical collaboration in chemical analyses, and Jacques and Jean De Dapper for their assistance in animal care and feeds manufacture.

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