British Journal of Nutrition (2002), **88**, 489–497
DOI: 10.1079/BJN2002699

Large bowel fermentation of maize or sorghum–acorn diets fed as a different source of carbohydrates to Landrace and Iberian pigs

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(Received 26 October 2001 – Revised 24 May 2002 – Accepted 2 July 2002)

Twenty-four finishing pigs (twelve Iberian and twelve Landrace) were used in a growing and slaughtering experiment. Animals were fed two diets differing in their ingredients, maize (diet C) or sorghum–acorn (diet A). At an average weight of 107·0 kg pigs were slaughtered and hindgut digesta sampled to study the effect of breed and diet on large bowel fermentation. Flows of digesta to the hindgut compartment were estimated based on an indigestible flow marker (Cr_2O_3) and were higher in Iberian than in Landrace pigs ($P<0·001$), and higher in animals fed diet A than diet C ($P=0·07$). The higher flows in Iberian pigs were mainly associated with a higher voluntary feed intake (3·50 *v.* 2·70 kg/d, $P<0·01$) and lower ileal digestibility of NSP (−12·8 *v.* 47·8, $P<0·01$). Differences between diets were mainly associated with a lower ileal digestibility of starch from diet A (89·2 *v.* 96·9 %, $P=0·06$), although no differences in the resistant starch content were observed *in vitro*. Fermentation of different carbohydrates through the large bowel showed that NSP-glucose had lower digestibility in Iberian than in Landrace pigs (62·5 *v.* 94·2 %, $P<0·001$), but no differences were observed in starch, or other NSP-fibre fractions (arabinose, xylose and galactose). The type and amount of carbohydrates reaching the large bowel were related to the diet but also to breed, and promoted differences in the fermentative activity associated with different volatile fatty acid patterns and changes in microbial enzymic activity.

Carbohydrates: Large bowel fermentation: Landrace pigs: Iberian pigs

The components of the diet that are poorly digested in the small intestine of swine provide a substrate for microbial growth that allows the animal to utilise part of the energy of these substrates, the bacterial fermentation products. The amount of energy so absorbed in the large bowel of pigs is not negligible and may contribute up to 25 % of the maintenance energy requirements (Yen *et al.* 1991). However, energy derived from fermentation endproducts is utilised with lower efficiency than energy absorbed in the small intestine (Noblet *et al.* 1994), and may determine to some extent the energy values of digestible components. Among energy sources, carbohydrates are the most abundant form of energy in plant materials and, as such, are the most widely available source of energy for feeding single-stomached animals. Carbohydrates, which include low-molecular-weight sugars, starch and NSP, provide a heterogeneous substrate (Bach Knudsen, 1997). In most mixed diets, starch constitutes the main source of carbohydrates in the small intestine while NSP, commonly referred to as dietary fibre, are mostly known to escape digestion in the small intestine and are fermented to different extents by the caecal and colonic bacteria (Jensen, 2001).

However, some starchy foods may also increase hindgut fermentation (Anderson *et al.* 1981). In this regard, Englyst *et al.* (1982) identified a starch fraction in cornflakes, which was resistant to α-amylase (*EC* 3.2.1.1.), now termed resistant starch (RS). In contrast to structural resistance of NSP to mammalian enzymes, resistance of starch occurs for a number of chemical, technological and physiological reasons (Gallant *et al.* 1992; Annison & Topping, 1994). RS occurs for a number of reasons, which have been used for RS classification into three main types (Englyst *et al.* 1992): RS1 includes that trapped within whole plant cells and food matrices; RS2 comprises those granules from certain plants that are gelatinised

Abbreviations: Ad, adenine; Gn, guanine; PB, purine bases; RS, resistant starch; TT, transit time; VFA, volatile fatty acids.

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poorly and hydrolysed slowly by α-amylases; RS3 comprises retrograded starches. Thus, foregut starch digestion has been shown to be conditioned by physiological variables including chewing and individual variation in transit (Champ, 1992; Englyst *et al.* 1992). In this context, the swine digestive system may have an influence on the nutritive value of each energy source and especially the starch fraction.

The present study aims to evaluate the effect of breed on the fractional digestion of carbohydrates in the foregut and hindgut compartments. Two genetically distinct strains of pigs, Landrace, a breed improved for lean growth rate, and the indigenous Iberian pigs, traditionally fattened in field conditions, have been previously shown to differ in their ability to digest carbohydrate (Morales *et al.* 2001) and offer an opportunity to study animal factors conditioning digestion of starch and NSP in the large intestine. In order to promote variation of carbohydrates characteristics, animals were fed *ad libitum* two diets differing in their carbohydrate ingredients (maize *v.* sorghum and acorn). We studied the microbial fermentation by measuring different parameters of the microbial activity, such as the purine bases (PB) content and the enzymic carbohydrase activity, and the simultaneous carbohydrate disappearance and distribution of volatile fatty acids (VFA) and pH along the large intestine.

A preliminary account of part of the present study has been published (Morales *et al.* 2001; Pérez *et al.* 2001).

Experimental methods

The experiment was performed at the Experimental Unit of the Universitat Autònoma de Barcelona and received prior approval from the Animal Protocol Review of this institution.

Animals and diets

Twenty-four castrated male finishing pigs (twelve Landrace, twelve Iberian; mean body weight 88·4 (SD 6·4) kg) were housed in eight pens (three animals/pen) in an environmentally controlled building. Replicates were randomly divided into two groups offered *ad libitum* a maize- (diet C) or a sorghum–acorn- (diet A) based diet in a 2 × 2 factorial and randomised complete block design. Ingredient and analysed nutrient contents of diets are presented in Table 1. Diet A was composed of sorghum (275 g/kg) and decorticated acorns (125 g/kg), the fruit of the *Quercus* genus, which replaced partially the maize content of diet C (754 g/kg). Despite the differences in the ingredients, both diets contained similar amounts of NSP, starch and RS, as measured *in vitro*.

We reduced the shell proportion of ground acorns (up to 12 %) by rough grinding. Diets were equalised for shell and fatty acids content by incorporating isolated shells and olive oil in the maize-based diets. Cr_2O_3 was included (1·5 g/kg) as an indigestible marker.

Experimental protocol

Pen feed intake and individual body weight were recorded every 2 weeks. Pigs were slaughtered at an average weight of 107·0 kg without previous fastening in a commercial slaughterhouse after CO_2 stunning. The whole gut was immediately excised and caecum, colon (proximal, medium and distal) and rectum were ligated, removed and weighed. The intestinal contents were collected and homogenised quickly and samples preserved (on average within 20 min after slaughtering). Ileal digesta were sampled from a 300 mm length segment, approximately 100 mm anterior to the ileal–caecal junction. Caecal digesta samples were frozen in liquid N_2 and stored at −70°C until their analysis for microbial enzymic activities. Caecal, colonic and rectal digesta samples were also acidified with H_3PO_4 (approximately 4 g fresh weight/ml 5 % (w/w) H_3PO_4, 1 % (w/w) mercuric chloride and 50-mM 3-methyl valerate as internal standard) and stored at −20°C until their analysis for VFA concentration. The rest of the ileal, caecal, medium colonic and rectal digesta were freeze-dried and milled for their analysis for Cr, carbohydrates and PB.

Analytical procedures

Chemical analyses of the diets and digesta were conducted according to the Association of Official Analytical Chemists (1984) for DM, ash, crude protein and fat, and according to Goering & Van Soest (1970) for lignin.

Table 1. Composition and analysed nutrient content of the maize-based diet (diet C) and the sorghum–acorn-based diet (diet A)

	Diet C†	Diet A†
Ingredients (g/kg)		
Maize	754	376
Sorghum	–	275
Semi-decorticated acorn	–	125
Soyabean meal	197	195
Acorn shell	15	–
Soyabean oil	–	7
Olive oil	12	–
Vitamin and mineral premix*	22	22
Nutrient analysis (g/kg DM)		
Crude protein	161·9	171·9
Crude fat	52·4	53·3
NSP		
Glucose	65·2	65·5
Galactose	13·0	13·4
Xylose	19·4	16·6
Arabinose	16·6	15·3
Starch	561·0	513·4
Resistant starch	107·0	106·0
Lignin	14·4	18·2
Gross energy (MJ/kg)	16·62	16·48

* Provided the following/(kg diet): $CaCO_3$, 7·1 g; $CaHPO_4$, 7·9 g; NaCl, 2·3 g; vitamin A, 2100 mg; vitamin D_3, 43 mg; vitamin E, 10 mg; vitamin K_3, 1 mg; vitamin B_1, 1 mg; vitamin B_2, 4 mg; vitamin B_6, 2 mg; vitamin B_{12}, 20 μg; biotin, 10 μg; niacin, 18 mg; Ca-d-pantothenic acid, 10 mg; choline, 175 mg; Fe, 80 mg; Zn, 110 mg; Cu, 90 mg; Mn, 50 mg; Co, 0·1 mg; I, 1 mg; Se, 0·2 mg.

† Ethoxiquin® (150 mg) and Luctamold® (500 mg)/kg feed were added to both diets.

Gross energy was determined by adiabatic calorimetry. Total starch and NSP fractions of feed and digesta samples were measured by the method of Theander (1991). Briefly, total starch was determined as glucose liberated after an enzymic incubation with thermostable α-amylase (Sigma ref. A-4551) for 1 h at 100°C, and amyloglucosidase (Sigma ref. A-3514; St Louis, MO, USA) for 4 h at 60°C. NSP were precipitated with 80 % (v/v) ethanol (1 h at 4°C), and hydrolysed with sulfuric acid, using myoinositol as internal standard. RS was measured by the method of Berry (1986) modified by Champ (1992) as the part of starch not hydrolysed by incubation with α-amylase for 16 h at 37°C. Hydrolysis products were extracted in 80 % (v/v) ethanol and discarded. RS was then made soluble with 4-M KOH and hydrolysed with amyloglucosidase for 90 min at 65°C. Monosaccharides released from total and resistant starch, and NSP were analysed as alditol acetates by GLC. Cr_2O_3 concentration in feed and digesta was determined by atomic absorption spectrophotometry following the method of Williams *et al.* (1962).

PB (adenine (Ad) and guanine (Gn)) in digesta samples (40 mg) were determined by HPLC (Makkar & Becker, 1999), after their acid hydrolysis with 2 ml 2-M-perchloric acid at 100°C for 1 h, including 0·5 ml 1-mM allopurinol as internal standard. The microbial enzymes were extracted from the caecal contents by hydrolysis with lysozyme (Silva *et al.* 1987), harvested from the supernatant fraction after centrifugation at 23 000 ***g*** for 15 min, and kept frozen (− 20°C) until their analysis, in less than a month. Polysaccharidase activity of the enzyme extract was measured according to the method of Nelson-Somogyi (Ashwell, 1957) against carboxymethylcellulose (Sigma ref. C-8758; carboxymethylcellulase activity), xylan from oat spelts (Sigma ref. X-0627; xylanase activity), soluble starch from potato (PANREAC 121096; Barcelona, Spain; amylase activity) and waxy starch from maize (Sigma ref. S-9679; amylopectinase activity). The activity of the enzymic extract was expressed as nmols of neutral sugars released/ml extract per min and referred either to the dry weight of digesta (total enzymic activity) or the PB content (bacterial enzymic activity). VFA concentration in deproteinised digesta was determined by GLC, following the method proposed by Jouany (1982).

Calculations and statistical analysis

Apparent digestibility along the intestine (ileum, caecum, intermediate colon and rectum) and daily flow of neutral sugars and PB in gastrointestinal segments were calculated by the marker (Cr) ratio method. In particular, the daily flow of a nutrient through the ileum, caecum or colon was calculated as the ratio between concentrations of nutrient and Cr in digesta, multiplied by Cr intake/d. Caecal and colonic transit time (TT) were calculated as the mass of Cr present in the organ divided by Cr intake (Goodlad & Mathers, 1987). Data were subjected to ANOVA, and a Tukey follow-up test was used for comparisons of means using the general linear model available in Statistical Analysis Systems statistical software package version 6.11 (SAS Institute, Cary, NC, USA) for a factorial arrangement of treatments. A two-tailed P value of $<0{\cdot}05$ was considered significant.

Results

Food intake and carbohydrates flow to the large bowel

Mean voluntary intake of Iberian pigs was significantly higher ($P=0{\cdot}004$) than that of Landrace (3497 *v.* 2699 g DM/d; Table 2), but no differences were observed between experimental diets. Iberian pigs also showed higher flows of carbohydrates to the large intestine, with a tendency for glucose (122·1 *v.* 63·2 g/d; $P=0{\cdot}10$), and significantly for galactose (54·0 *v.* 26·5 g/d; $P=0{\cdot}003$) and arabinose + xylose (198·2 *v.* 81·6 g/d; $P=0{\cdot}001$) contained in the NSP, although not significantly for starch (139·9 *v.* 90·4 g/d; $P=0{\cdot}45$). Between diets, diet A tended to promote a higher amount of starch entering the large intestine than diet C (174·3 *v.* 56·0 g/d; $P=0{\cdot}08$).

Hindgut digesta contents (i.e. caecum + colon) were

Table 2. Voluntary feed intake, daily flows to the hindgut of starch and NSP fractions, digesta content and transit time in caecum and colon of Landrace and Iberian finishing pigs fed a maize-based diet (diet C) or a sorghum–acorn-based diet (diet A)†

	Landrace		Iberian			Probability		
	Diet C	Diet A	Diet C	Diet A	SE	*P* Breed	*P* Diet	*P* Breed×diet
Feed intake (g/d)	2626	2771	3461	3532	199·0	**	NS	NS
Daily flows to the hindgut (g)								
Starch	31·3	149·5	80·7	199·1	66·71	NS	0·08	NS
NSP								
Glucose	45·8	80·7	97·1	147·2	35·49	0·10	NS	NS
Arabinose + xylose	73·1	90·2	209·8	186·5	25·57	***	NS	NS
Galactose	24·4	28·6	45·5	62·5	8·30	**	NS	NS
Digesta content (g)								
Caecum	257	412	213	231	61·7	*	NS	NS
Colon	1882	2382	1442	1251	220·7	**	NS	NS
Transit time (h)								
Caecum	1·29	1·59	0·81	0·66	0·260	*	NS	NS
Colon	21·89	16·87	11·24	4·76	1·678	***	**	NS

NS, $P>0{\cdot}10$; *$P<0{\cdot}05$, **$P<0{\cdot}01$, ***$P<0{\cdot}001$.
† For details of diets and procedures, see Table 1 and p. 490.

significantly higher ($P<0·01$) in Landrace (2139 and 2794 g) than in Iberian pigs (1655 and 1482 g) fed diet C and diet A, respectively (Pérez *et al.* 2001). No significant differences were observed between diets. TT in the large bowel was significantly higher in Landrace than Iberian pigs ($P<0·001$) and with diet C than diet A ($P=0·018$), averaging 23·2 and 18.5 h in Landrace and 12.1 and 5.4 h in Iberian pigs fed diet C and A, respectively.

Neutral sugars fermentation

Fig. 1 shows the fractional digestibility (%) through the large bowel compartment of starch and the main NSP constituents: glucose; galactose; xylose + arabinose. Significant differences were observed in the fractional digestibility of individual carbohydrates as affected by the breed or diet. Specifically, starch of diet A was less digested in the small intestine than that of diet C (89·2 *v.* 96·9 %; $P=0·06$); no differences were observed between breeds. Differences between diets were progressively reduced along the large intestine compartment, and reached a complete digestion by the rectum with both diets. The small intestine digestibility of NSP-glucose was not significantly different between treatments. Average values were 67 % in Landrace and 42 % in Iberian pigs; between diets, mean values were 65 and 44 % in animals fed diet C and A, respectively. Large intestine fermentation increased NSP-glucose digestion, but significant differences between Landrace and Iberian pigs were still evident in the whole tract digestibility (94·66 *v.* 60·76 %; $P<0·001$). Fractional digestibility in the ileum of NSP-galactose and -arabinose + xylose was significantly higher in Landrace than Iberian pigs, which unexpectedly presented negative values. Large intestine fermentation in the caecum compensated most differences. However, whole-tract digestibility of arabinose + xylose was still higher ($P<0·001$) in Landrace (39·77) than Iberian (7·09) pigs.

Purine bases and carbohydrase activities in large bowel digesta

PB concentration in digesta is presented in Table 3. Compared with the middle colonic digesta, caecal digesta contained higher concentrations of PB (30–41 *v.* 7–11 μmol/g DM) and Gn:Ad (1·4–1·5 *v.* 1·1–1·2). Significant differences in PB concentration were observed between breeds, being higher in the caecum in Landrace than Iberian (38·7 *v.* 32·1 μmol/g DM; $P=0·047$), and higher in the middle colon in Iberian than Landrace pigs (10·6 *v.* 8·1 μmol/g DM, $P=0·017$). Differences in colonic digesta were significantly increased when PB were expressed on a daily flow basis, being higher in Iberian than Landrace (7·8 *v.* 5·2 mmol/d; $P=0·001$) and in diet A than diet C (8·4 *v.* 4·6 mmol/d; $P=0·016$). Gn:Ad in the middle colon digesta were also significantly higher for Iberian than for Landrace pigs (1·18 *v.* 1·11; $P=0·05$).

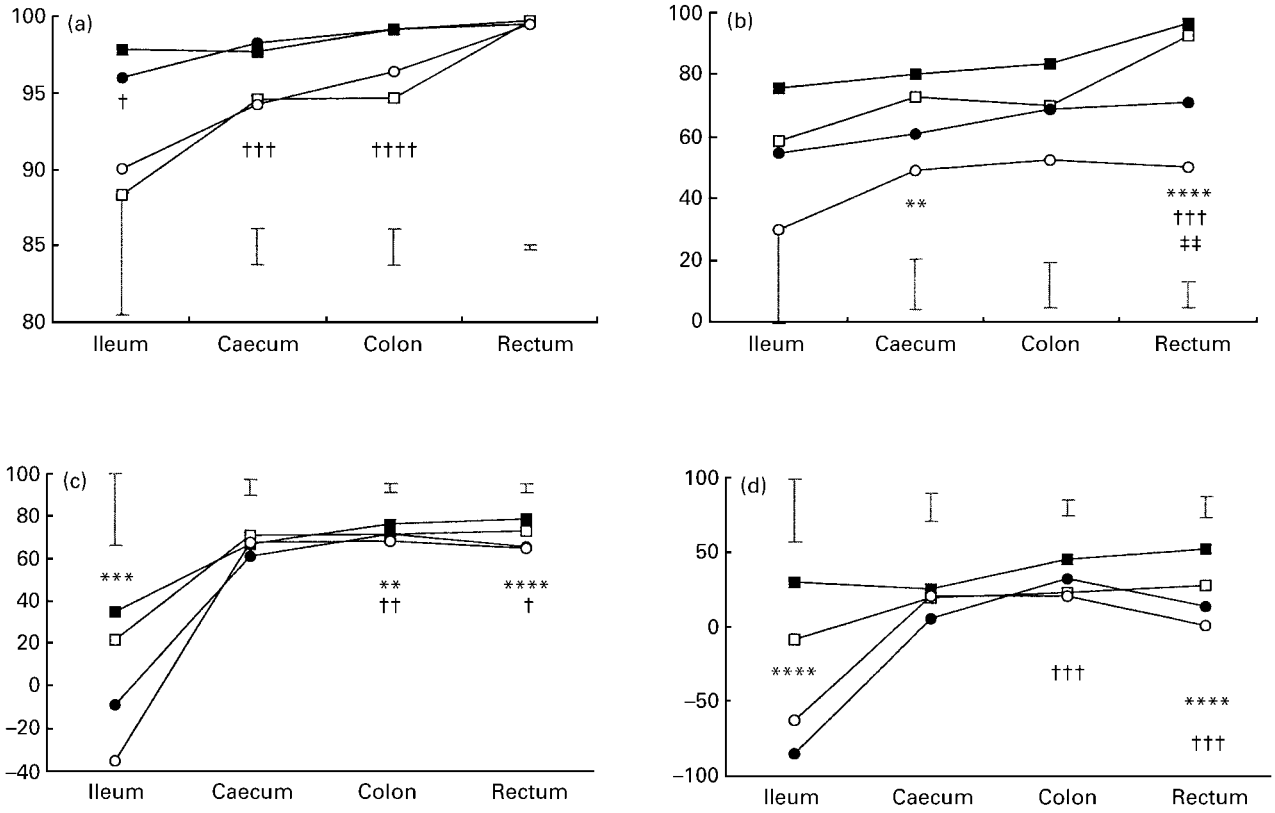

Fig. 1. Fractional digestibility of starchy glucose (a), NSP-glucose (b), NSP-galactose (c) and NSP-arabinose + xylose (d) in ileal, caecal, middle colonic and and rectal digesta of finishing pigs fed a maize-based diet (■, Landrace; ●, Iberian) or a sorghum–acorn-based diet (□, Landrace; ○, Iberian). Standard errors are represented by vertical bars. $^{**}P<0·05$, $^{***}P<0·01$, $^{****}P<0·001$ (effect of breed); †$P<0·10$, ††$P<0·05$, †††$P<0·01$, ††††$P<0·001$ (effect of diet); ‡‡$P<0·05$ (effect of breed × diet).

Table 3. Concentration and daily flow of purine bases, and guanine:adenine in caecal and middle colonic digesta of Landrace and Iberian pigs fed a maize-based diet (diet C) or a sorghum–acorn-based diet (diet A)†

	Landrace		Iberian			Probability		
	Diet C	Diet A	Diet C	Diet A	SE	*P* Breed	*P* Diet	*P* Breed×diet
Purine bases (μmol/g DM)								
Caecum	40·7	36·7	33·8	30·3	3·35	*	NS	NS
Colon	7·5	8·6	10·5	10·7	1·13	*	NS	NS
Daily flow of purine bases (mmol/d)								
Caecum	26·7	32·6	29·1	30·5	3·38	NS	NS	NS
Colon	3·21	7·22	5·99	9·64	1·10	**	*	NS
Guanine:adenine								
Caecum	1·44	1·46	1·49	1·47	0·040	NS	NS	NS
Colon	1·10	1·12	1·18	1·18	0·039	*	NS	NS

NS, $P>0·10$; *$P<0·05$, **$P<0·01$.
† For details of diets and procedures, see Table 1 and p. 490.

Mean values of total enzymic activities (nmol/g DM and min; Table 4) averaged 300 for carboxymethylcellulase, 838 for xylanase, 717 for amylase and 439 for amylopectinase. Iberian pigs showed higher values than Landrace pigs for carboxymethylcellulase, xylanase and amylopectinase ($P<0·05$). There was no dietary effect, except for a higher amylopectinase activity in Iberian pigs given diet A (interaction of breed × diet; $P=0·045$). Bacterial enzymic activities, as expressed on a PB ratio, better describe the specific activity of caecal microflora against carbohydrates. Significant differences were observed between breeds, showing the bacterial population in Iberian pigs to have a higher enzymic activity than in Landrace pigs. A higher amylopectinase activity was also observed ($P<0·05$) for the microbial population of animals fed diet A.

Large bowel pH and volatile fatty acid concentrations

Average pH values (Fig. 2) in ileal, caecal, middle colon and rectal digesta were 6·1, 5·6, 5·8 and 6·2, respectively, and no differences were observed between treatments. VFA concentrations (Fig. 2) ranged from 95–200 μmol/g digesta, reached the highest concentrations in the proximal (183·2) and medium (175·5) colonic digesta, and decreased progressively as digesta approached the rectum (111·9 μmol/g). Between treatments, concentrations of VFA tended to be lower in proximal colonic digesta of Iberian than in Landrace pigs (172·2 *v.* 194·2 μmol/g; $P=0·06$). No significant differences were observed in the rest of the large intestine.

Individual VFA profiles in the proximal and distal colon digesta are presented in Fig. 3. In the proximal colon, Iberian pigs showed higher propionate (31·0 *v.* 24·4; $P<0·001$) and lower acetate (56·1 *v.* 60·1; $P=0·040$) percentages than Landrace. No significant differences were observed between diets. A defined pattern throughout the large bowel for acetate, propionate and butyrate was not observed. However, the percentage of propionate in Iberian pigs decreased through the colon as the percentage of acetate increased. Branched-chain VFA showed increasing values from proximal (1·0 %) to distal colon (3·6 %), where they were lower in Iberian than Landrace pigs ($P<0·05$) and with diet A than diet C ($P<0·05$).

Table 4. Enzymatic acitivities (nmol glucose or xylose released/ml per min per g DM (total activity) or /μmol purine bases per min (bacterial activity)) of the caecal contents against carboxymethylcellulose, xylan, amylose and amylopectin of Landrace and Iberian pigs fed on a maize-based diet (diet C) or a sorghum–acorn-based (diet A)†

	Landrace		Iberian			Probability		
	Diet C	Diet A	Diet C	Diet A	SE	*P* Breed	*P* Diet	*P* Breed×diet
Total activity								
Carboxymethylcellulose	290	170	395	344	64·4	*	NS	NS
Xylanase	703	588	895	1164	154·8	*	NS	NS
Amylase	635	583	741	909	137·3	NS	NS	NS
Amylopectinase	193[b]	247[b]	265[b]	1052[a]	197·0	*	*	*
Bacterial activity								
Carboxymethylcellulose	6·9	5·1	12·5	12·7	2·02	**	NS	NS
Xylanase	18·4	16·2	27·1	43·6	7·41	**	NS	NS
Amylase	15·6	16·6	25·7	28·4	5·49	*	NS	NS
Amylopectinase	4·6	6·5	7·2	35·0	8·15	*	*	0·06

[a,b]Group mean values with unlike superscript letters within a row were significantly different ($P<0·05$).
NS, $P>0·10$; *$P<0·05$, **$P<0·01$.
† For details of diets and procedures, see Table 1 and p. 490.

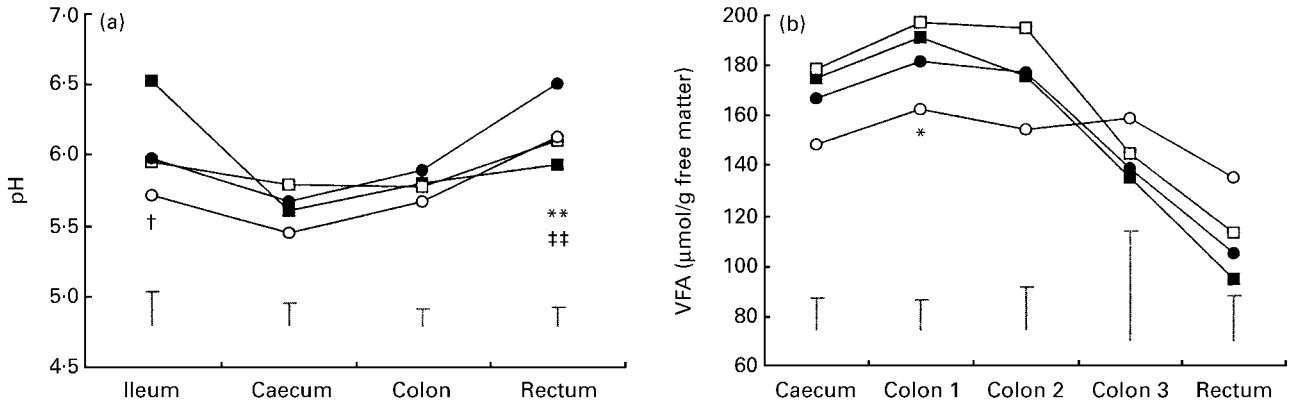

Fig. 2. pH (a) and volatile fatty acid (VFA) concentration (b) in ileal, caecal, colonic and rectal digesta of finishing pigs fed a maize-based diet (■, Landrace; ●, Iberian) or a sorghum–acorn-based diet (□, Landrace; ○, Iberian). Standard errors are represented by vertical bars. $^{*}P<0{\cdot}10$, $^{**}P<0{\cdot}05$ (effect of breed); †$P<0{\cdot}10$ (effect of diet); ‡‡$P<0{\cdot}05$ (effect of breed × diet).

Discussion

The extent of microbial fermentation in the large bowel of growing pigs may depend on dietary factors or the breed. In particular, dietary parameters other than the NSP content may reduce digestion in the small intestine, for example the content of RS (Champ *et al.* 1998), protein–starch interactions (Rooney & Pflugfelder, 1986) or anti-nutritional factors (Lizardo *et al.* 1995). Moreover, comparison between breeds has also indicated differences in the feed intake and whole tract digestibility (Morales *et al.* 2001). In particular, Iberian pigs, an indigenous breed from the South-west Iberian Peninsula and traditionally fattened in field conditions, were compared in the present study with Landrace pigs to examine animal factors that might affect fore- and hindgut carbohydrate digestion.

Digestibility measurement procedures

Large amounts of digesta were collected from the caecum, colon and rectum after slaughtering, and fractional digestibility results obtained were consistent with other published results (Glitsø *et al.* 1998; Canibe & Bach Knudsen, 2001). However, smaller intestine digesta samples were collected from the terminal ileum, which resulted in strange results, with negative values observed for galactose and arabinose–xylose fractions for Iberian pigs. It appears that the samples obtained from the ileum of slaughtered pigs were not representative of the total flow, probably due to phase separations between specific components of the digesta and the indigestible markers (Danfaer & Fernandez, 1999). This phenomenon could be aggravated by gut contractions at slaughter, and were probably related to the high variability of the slaughter method (Prawirodigdo *et al.* 1998). Over the last 30 years, several studies have examined the digestion of NSP anterior to the caecum in pigs fitted with a cannula in the terminal ileum or by slaughter. However, none of these indicates the overall superiority of any one procedure (Donkoh *et al.* 1994). In particular, the negative digestibility of some NSP fractions has also been observed after cannulation (Jørgensen *et al.* 1996; Glitsø *et al.* 1998; Canibe & Bach Knudsen, 2001). Cannulation has also received extensive criticism

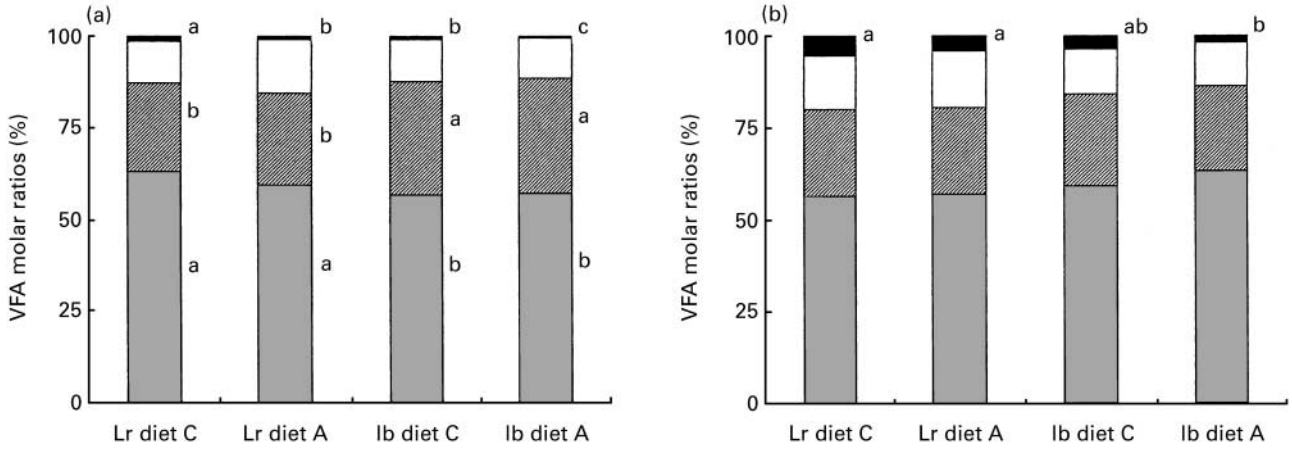

Fig. 3. Volatile fatty acids (VFA) molar ratios of acetate (■), propionate (▨), butyrate (□) and branched-chain VFA (▩) in proximal (a) and distal (b) colonic digesta of Landrace (Lr) and Iberian (Ib) pigs fed a maize-based diet (diet C) or a sorghum–acorn-based diet (diet A). ^{a,b,c}Group means with unlike letters for a fatty acid fraction were significantly different ($P<0{\cdot}05$).

revealing social concern about invasive procedures and the influence of surgery on the physiology of the animals (Canibe & Bach Knudsen, 2001).

Foregut digestion

Our results indicate that a considerable proportion of the NSP-glucose (54·7 *v.* 77·7 %) and most starch (93·1 *v.* 99·7 %) disappeared in the upper intestine. Moreover, significant differences were observed between breeds, NSP digestion being higher in Landrace pigs. The disappearance of NSP in the upper intestine is caused by fermentation to VFA, depending on the composition and structure of the dietary fibre. Most β-glucans, approximately up to 0·4 of pentose sugars, such as xylose and arabinose, but no cellulose are digested before the terminal ileum (Fadel *et al.* 1989; Glitsø *et al.* 1998; Bach Knudsen & Canibe, 2000). Marked differences between breeds observed in the present experiment could be due to longer TT and a higher bacterial density in the small intestine of Landrace pigs.

In both breeds higher amounts of starch from diet A escaped small intestine digestion (Fig. 1). Considering that no differences between diets in the RS content were observed *in vitro*, it appears that foregut digestion of starch involves variables not accounted for by the analytical procedures *in vitro*. The amount of starch escaping foregut digestion *in vivo* may depend on the extent of chewing, the rate of orocaecal transit, or the amount of starch ingested (Chapman *et al.* 1985; Englyst & Cummings, 1990). It has also been suggested that starch resisting hydrolysis *in vitro* could be affected by the particle size after the grinding of feed samples compared with the particle size resulting from chewing *in vivo* (Englyst *et al.* 1992). Nevertheless, it is remarkable that no interactions were observed with the animal breed.

Hindgut digestion

Hindgut fermentation compensated most differences observed in the amount of carbohydrates escaping small intestine digestion (Fig. 1), except for Iberian pigs, which did not degrade NSP-glucose as efficiently as Landrace pigs. The fraction analysed as NSP-glucose is composed in cereals of cellulose (approximately two-thirds) and non-cellulosic glucose (one-third), mainly from β-glucans (Bach Knudsen, 1997). This indicates an average involvement of both components on the fractional digestibility of NSP-glucose. Theoretically, differences in the effective degradation of NSP could be associated with differences in the microbial activities or the time available for degradation. There is unlikely to be an inherent restriction on microbial degradation in Iberian pigs, due to the high enzymic activities observed in caecum digesta and bacteria (Table 4). On the other hand, the TT of digesta in the large bowel was much lower in Iberian than in Landrace pigs (Table 2). Many studies of plant cell-wall fermentation have established that fermentation is relatively slow (Van Soest *et al.* 1983) and variable among fibre sugars (Salvador *et al.* 1993), which means that variations in the TT might influence the extent of NSP digestion. Stephen *et al.* (1987) tested this hypothesis by modifying the TT of digesta in the large bowel of human volunteers. Reducing TT had little effect on the faecal output of the major pentose sugars, but increased the output of cellulose. Our results on NSP-glucose digestibility, partly influenced by cellulose, are consistent with those of Stephen *et al.* (1987).

Microbial activity and fermentation parameters

Although nucleic acids and PB are among the most commonly used naturally occurring microbial markers for rumen studies (Pérez *et al.* 1997; Makkar & Becker, 1999), to our knowledge this is the first time that PB analysis has been used to quantify microbes in the large intestine. To obtain basic information on hindgut microbial proliferation, we assumed an average composition (324 μmol PB/g microbial crude protein) similar to bacteria isolated from rumen liquid samples (Pérez *et al.* 1997). Average microbial mass content estimated in digesta was 109 mg microbial crude protein/g DM in the caecum, and significantly lower in the middle colon (29 mg microbial crude protein/g DM). Bacteria inhabiting the colon of single-stomached animals obtain their energy mainly from dietary sugars that escaped foregut digestion (Bergman, 1990). However, as fermentable carbohydrates decline along the colon, bacteria switch to the degradation of proteinaceous material and autolysis (Reid & Hillman, 1999). Branched-chain VFA ratios also increased from the proximal to distal colon, being considered characteristic products of the fermentation of certain amino acids. PB in caecal digesta were lower in Iberian than in Landrace pigs, while the opposite occurred in the middle colon (especially on a daily flow basis), which reflects the higher extent of fermentation in the distal compartments of Iberian pigs. Concomitantly with the higher PB concentrations, significantly higher Gn:Ad were observed in caecal than in colonic digesta and in the middle colon of Iberian than in Landrace pigs. Differences in Gn:Ad may reflect changes in the bacterial community composition in the gastrointestinal tract (Apajalahti *et al.* 1998). In the present experiment, the caecal population of Iberian pigs showed higher total and bacterial enzymic activities than Landrace, which could reflect a selection of different species of bacteria by differences in the amount and type of substrates fermented and/or changes in the residence time of digesta (Table 2).

Endproducts of this fermentation have been described previously (Pérez *et al.* 2001). The results showed increases in caecal and proximal colon VFA content and a simultaneous fall in pH. Butyrate molar responses were observed in caecal digesta of Landrace pigs fed diet A (breed × diet; $P=0{\cdot}05$), while Iberian pigs fed diet A showed a tendency for propionate to increase in the proximal colon and acetate in distal colon digesta. Many studies indicate that the nature and the amount of VFA produced are closely related to the type of sugars fermented (Salvador *et al.* 1993; Casterline *et al.* 1997). *In vivo* studies (Mathers *et al.* 1997; Topping *et al.* 1997) have provided evidence that starch fermentation leads to relatively higher molar ratios of butyrate, whereas Salvador

et al. (1993) suggest that xylose is the most suitable of fibre sugars for the production of butyrate *in vitro*. Mathers & Dawson (1991) collated data on caecal TT and molar proportion of butyrate from four separate studies and observed that caecal butyrate increased sharply when caecal TT decreased below about 0·75 d. The present results in Iberian pigs conflict somewhat with those of Mathers & Dawson (1991). It is remarkable that the caecum of Iberian pigs also fermented significantly higher amounts of arabinose, xylose and galactose, which could affect the higher propionate production. Mortensen *et al.* (1988) reported that the degradation of pentose sugars leads to the production of propionic acid. Salvador *et al.* (1993) also cited the involvement of glucose, arabinose and xylose, but suggested that the formation of propionic acid was less predicted by sugar disappearance than other VFA, such as acetate and butyrate.

The present study provides an opportunity to evaluate factors modifying the sites and amounts of carbohydrate fermentation in pigs, based on the comparison of two genetically distinct strains (Serra *et al.* 1998) when feeding on diets differing in their carbohydrate sources. Some fractions of NSP were shown to be fermented in appreciable percentages before reaching the large bowel, and differences between breeds may have been due to differences in TT. The type and amount of carbohydrates reaching the large bowel, related to the diet but also to breed, promoted differences in the microbial proliferation through the large bowel associated with different VFA patterns and changes in microbial activity.

Acknowledgements

This study has been supported by the CICYT-funded Project AGF98-0506.

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