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Evaluation of large-intestinal parameters associated with dietary treatments designed to reduce the occurrence of swine dysentery

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Diets containing soluble NSP (sNSP) and resistant starch (RS) increase hindgut fermentation in pigs, which in turn increases the incidence of swine dysentery (SD) after infection with the intestinal spirochaete *Brachyspira hyodysenteriae*. In the present study pigs were fed diets based on either wheat or sorghum, fed either raw or treated by extrusion, and/or with the addition of dietary enzymes to reduce RS and/or sNSP content. The aim was to determine the effects of these treatments on pig performance, large intestinal fermentation and expression of SD. Weaned pigs (*n* 132) were fed experimental diets for 4 weeks, when half the pigs in each treatment group were euthanased and samples collected to assess the influence of the diet on hindgut fermentation. The remaining pigs then were infected with *B. hyodysenteriae*, and monitored for development of SD. In general, compared with pigs fed raw wheat, fermentation in all parts of the large intestine was reduced either by feeding raw sorghum-based diets, or by feeding diets that were extruded. The addition of enzymes that degrade RS or sNSP reduced fermentation only in the distal parts of the large intestine. The incidence of SD was lower in pigs fed sorghum-based diets, and some of the extruded diets, but none of the dietary treatments offered full protection against SD. Multiple regression analysis of the results from all three experiments showed that colonisation by spirochaetes was highly related to dietary sNSP concentrations, whilst development of SD was similarly influenced by RS content of the diet.

Resistant starch: Soluble non-starch polysaccharide: Dietary enzymes: Swine dysentery: *Brachyspira hyodysenteriae*

Swine dysentery (SD) is a colitis of pigs resulting from infection with the anaerobic spirochaetal bacterium *Brachyspira* (*Serpulina*) *hyodysenteriae*. Recently, attempts have been made to control SD by manipulation of dietary ingredients and the use of specific dietary additives. Such studies established that diets which promote hindgut fermentation lead to a higher incidence of SD amongst groups of pigs, and predicted that the disease could be prevented by reducing the amount of fermentable fibre that reached the large intestine (LI) (Siba *et al.* 1996). Retrospectively, by analysing the diets that caused high expression of the disease, Pluske *et al.* (1996) found that the presence of two rapidly fermentable dietary ingredients commonly found in cereal grains, resistant starch (RS) and soluble NSP (sNSP), predisposed pigs to SD. Similarly, feeding a diet based on cooked white rice and animal protein, which contained very low levels of sNSP and RS, reduced hindgut fermentation in pigs, and offered complete protection against SD after experimental infection (Pluske *et al.* 1996; Siba *et al.* 1996). Unfortunately, this diet was impractical for use on a large scale in pig production because of the difficulties in processing the rice and its high cost compared with other cereal grains.

Cereal grains used in pig production differ in their RS and sNSP contents. Wheat and sorghum are commonly used to feed pigs in Australia, but both are rich in RS, and raw wheat also contains high levels of sNSP (Kopinski *et al.* 1995; Pluske *et al.* 1996). A reduction of RS content

Abbreviations: ESor, extruded sorghum; EW, extruded wheat; LI, large intestine; RS, resistant starch; RSor, raw sorghum; RW, raw wheat; SD, swine dysentery; sNSP, soluble NSP; VFA, volatile fatty acids.

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in cereal grain can be achieved by various forms of feed processing that cause starch gelatinisation. Starch in the grain can be altered by physical processes including grinding, milling, moist-heat treatment, flaking and extrusion (Bengala Freire *et al.* 1991; Carter & Leibholz, 1991; Gill *et al.* 1996; Pluske *et al.* 1996). Studies intended to reduce LI fermentation and hence SD after experimental infection have used cereal grains that were steam-flaked (Siba *et al.* 1996), or hammer-milled, extruded or steam-flaked (Pluske *et al.* 1996). Extrusion was found to be the most effective method to achieve this with wheat- and sorghum-based diets. These studies also showed that diets that were low in RS still resulted in a high incidence of SD in experimentally-infected pigs, an effect which was then thought to be due to residual sNSP. The anti-nutritive effect of sNSP can be reduced by the dietary inclusion of specific sNSP-degrading enzymes which partially depolymerise sNSP, resulting in lower digesta viscosity (Bedford & Classen, 1992; Choct *et al.* 1995, 1996). Improved growth, weight gain, protein and starch digestibility, and better feed conversion have been achieved in pigs by supplementing wheat- or barley-based diets with sNSP-degrading enzymes, as a result of a better nutrient availability in the small intestine (Graham *et al.* 1989; Inborr *et al.* 1991*a*,*b*). Various treatments have been applied to sorghum-based diets to improve their nutritive value for animals. Heat treatments such as extrusion and steam-flaking increased starch digestibility (Buchanan-Smith *et al.* 1968; Mills *et al.* 1994; Kemm & Brand, 1996), while reduction of the sorghum particle size by fine grinding resulted in an increase of the apparent ileal digestibility of DM, starch, gross energy and protein (Owsley *et al.* 1981). The addition of enzymes to sorghum-based diets to enhance performance in finishing pigs has been limited to dietary supplementation of cellulases (Kim *et al.* 1994).

The overall aim of the present study was to identify a cost-effective, commercially applicable pig diet which would provide protection against SD. Three experiments were undertaken using diets based on wheat or sorghum, investigating the effects of grain extrusion and addition of an RS-degrading enzyme (both to reduce RS), and/or addition of an sNSP-degrading enzyme (to reduce the viscous effect of sNSP). The influences of these diets on pig performance, fermentation in the large intestine, and on the expression of SD after experimental infection were recorded. Results of the influences of these diets on the microflora have been published elsewhere (Durmic *et al.* 2000).

Experimental methods

General

The present research was reviewed and approved by the Murdoch University Animal Ethics Committee in accordance with the NH&MRC/CSIRO/AAC Code of Practice for the Care and Use of Animals for Experimental Purposes. Each experiment involved (per treatment) twelve Large White × Landrace weaner pigs, of mixed gender, purchased from a specific pathogen-free farm (Wandalup Farms, Mandurah, Australia), weaned at 21 d, and housed in individual or group pens at Medina Research Station, Medina, Australia. Pigs were fed the experimental diets *ad libitum* for 4–5 weeks, and were monitored for daily weight gain, and daily feed intake. Feed conversion ratio was calculated as average daily feed intake divided by average daily gain. When the pigs reached the required weight range (20–30 kg), their final body weight was recorded, and they were transferred to Murdoch University. One half of the pigs on each diet were killed, and samples collected to assess hindgut fermentation. In these pigs, empty body weight was calculated as final body weight minus LI weight. The remaining pigs were housed in an isolation house, infected with *B. hyodysenteriae* and monitored for development of SD over 4 weeks.

Diet analysis

DM, crude protein, fat, crude fibre, ash and gross energy in the diets were analysed as described in the Association of Official Analytical Chemists (1988) methods. Estimations of the total starch in the diet or digesta were made by using a Megazyme Total Starch Assay Kit (Megazyme, Warriewood, Australia). Samples of diet (0·5 g) were washed with ethanol to remove simple sugars and then treated with dimethylsulfoxide followed by a 5 min incubation in boiling water to gelatinise RS. Incubation with thermostable α-amylase (5 min at 100°C) followed by amyloglucosidase digestion (30 min at 50°C) converted starch to glucose. Released glucose was measured spectrophotometrically by the glucose oxidase method with readings taken at 420 nm. Measurement of RS in the diet was by the method of Muir & O'Dea (1993). NSP and their sugars were measured by GLC after enzymic–chemical hydrolysis of NSP (Englyst *et al.* 1982, 1992; Englyst, 1989; Choct *et al.* 1995).

Post-mortem examination of non-infected animals

Food was removed from the pigs 1 h before they were killed. Pigs were stunned using a captive bolt gun, and exsanguinated. The abdominal cavity was opened, the LI tied off and removed from the body, and the caecum excised from the colon. Sections of LI were weighed full and after emptying the digesta. Digesta samples were collected from the caecum, proximal colon and distal colon. Measurements of pH, LI weight, volatile fatty acids (VFA) and ATP concentrations were made as described previously (Pluske *et al.* 1996). The total amount of VFA present in the caecum or colon was calculated by multiplying VFA concentrations by the weight of digesta. To estimate DM of digesta, 1 g samples of wet digesta were weighed in tared boats and air-dried in a hot-air oven at 60°C to constant weight. The samples were re-weighed and the DM content calculated. Total starch in digesta samples (0·5 g) was measured as described for total starch in the diet.

Experimental infection and monitoring for swine dysentery

Pigs brought to Murdoch University animal isolation house were allowed to acclimatise for 1 d, and then fasted for 24 h

before experimental infection. For infection, *B. hyodysenteriae* strain 155/23 (serogroup A) was obtained from the collection held by the Reference Centre for Intestinal Spirochaetes at Murdoch University. This was propagated in Kunkle's pre-reduced anaerobic Trypticase soya broth supplemented with 2 % fetal bovine serum and a 1 % ethanolic cholesterol solution (Kunkle *et al.* 1986). Each animal was infected via a stomach tube by administering approximately 100 ml of a broth culture containing 10^8 viable mid-log phase cells/ml. This was repeated on two consecutive days (i.e. each animal was dosed three times). Pigs were monitored daily for clinical signs of SD (depression, lack of appetite and diarrhoea with blood and mucus). Rectal swabs were taken from each pig before exposure and then every 2–3 d for the duration of the experiment. The swabs were cultured for the presence of *B. hyodysenteriae*, as described previously (Jenkinson & Wingar, 1981; Durmic *et al.* 2000). Pigs were euthanased within 24 h of the onset of clinical signs, otherwise 4 weeks after being experimentally inoculated. The caecum and colon were examined grossly to look for evidence of mucohaemorrhagic colitis, consistent with SD (Alexander & Taylor, 1969). Swabs from the caecal and colonic walls were collected from each pig and cultured for *B. hyodysenteriae*.

Statistical analysis

Data were analysed by ANOVA using Statview 4.02 and SuperAnova for Macintosh (ABACUS Concepts, CA, USA). Means were compared using Fisher's protected least significant method, and statistical significance was accepted at $P<0{\cdot}05$. Numbers of pigs developing SD within treatments in each experiment were compared using Fisher's exact test. The relationship between colonisation by *B. hyodysenteriae* (number of challenged pigs detected positive by rectal swabs taken during the infection period), the incidence of SD (number of challenged pigs developing SD), and fermentation parameters in pigs on the eleven dietary treatments over all three experiments was tested using simple linear regression and by stepwise multiple linear regression.

Animals and diets used in experiment 1

A total of forty-eight weaned pigs, weighing 6·50 (SEM 0·27) kg were allocated to four treatments on the basis of live weight. Pigs in each group were fed a wheat-based diet (Table 1). Wheat was fed either raw (RW diet), extruded (EW diet), raw with enzyme (RW–enzyme), or extruded with enzyme (EW–enzyme). Wheat for extrusion was ground to pass a 1·5 mm screen, and then extruded (Hunts Pet Foods, Naval Base, Western Australia, Australia) in a twin-screw extruder having a 1·7 m barrel (80 to 90 bar pressure). Wheat was passed through three heat zones (90°C, 120°C and 160°C) and exited through the die at 130°C, with a total residence time of 35–40 s. Both raw wheat and extruded wheat were mixed with other ingredients, except enzyme, and then hammer-milled. A

Table 1. Composition of the experimental diets in experiments 1, 2 and 3 (percentage of air-dry diet)

	Basal diets				
		Experiment 2 (Extruded wheat-based)		Experiment 3 (Sorghum-based)	
Ingredient	Experiment 1 (Wheat-based)	Weaner	Grower	Weaner	Grower
Wheat, 12 % CP (raw or extruded)	78·0	77·9	85·3	–	–
Sorghum, 10 % CP (raw or extruded)	–	–	–	69·3	80·6
Meat and bone meal, 50 % CP	5·8	2·8	4·3	7·4	6·8
Fish meal, 65 % CP	6·6	9·6	7·2	13·0	8·3
Blood meal, 85 % CP	2·0	1·8	1·7	3·0	3·0
Soyabean meal, 45 % CP solvent	6·0	–	–	–	–
Rapeseed oil	1·0	2·3	1·0	1·0	1·0
L-Lysine-HCl	0·23	0·24	0·24	0·06	0·11
DL-Methionine	0·03	–	0·01	–	–
L-Threonine	0·09	0·10	0·08	0·02	–
Vitamin and mineral premix	0·15	0·15	0·15	0·15	0·15
Choline chloride 50 %	0·04	0·04	0·04	0·04	0·04
Skimmed milk powder	–	5·0	–	6·2	–
Analysed nutrient contents					
Water	9·9	–	9·5	8·8	8·8
CP	20·6	–	20·0	19·4	19·2
Fat	4·7	–	2·8	5·4	5·6
Crude fibre	1·9	–	2·6	1·6	1·6
Ash	4·4	–	4·8	5·5	5·4
N-free extract	58·5	–	60·3	59·4	59·5
Calculated nutrient and energy contents					
DE (MJ/kg)	14·58	15·0	14·59	14·6	14·5
Available lysine (g/MJ DE)	0·75	0·75	0·65	0·85	0·65
Ca	0·95	0·80	0·80	1·40	1·08
P	0·52	0·48	0·45	0·77	0·59

CP, crude protein; DE, digestible energy.

commercially available dietary enzyme preparation (Porzyme® 8300; Finnfeeds International, Marlborough, UK), containing a guaranteed minimum activity of 5000 U/g endo-1,4-β-xylanase (*EC* 3·2·1·8; sNSP-degrading) and 500 U/g subtilisin (*EC* 3·4·21·62; protease) was then mixed into diets at the manufacturer's recommended inclusion rate of 1 kg/tonne. Weaned pigs were fed the experimental diets until they reached 25·62 (SEM 0·69) kg (uninfected pigs) or 21·62 (SEM 0·34) kg (pigs for infection).

Animals and diets used in experiment 2

A total of thirty-six pigs weighing 6·33 (SEM 0·21) kg were allocated to three treatments on the basis of live weight. Diets were based on extruded wheat (Table 1). Enzyme 1 ('experimental') was included in the EW–enzyme 1 diet, and enzyme 2 (Porzyme® tp 100, a commercially available multi-enzyme mix) was added to the EW–enzyme 2 diet. Both enzymes were provided by Finnfeeds International Ltd. Enzyme 1 had a guaranteed minimum activity of 1500 U/g xylanase and 2000 U/g α-amylase (*EC* 3·2·1·1; RS-degrading). Enzyme 2 had a guaranteed minimal activity of 150 U/g β-glucanase (*EC* 3·2·1·8; sNSP-degrading), 4000 U/g xylanase and 1000 U/g α-amylase activity. Both enzyme mixes were included in the diets at the manufacturer's recommended level of 1 kg/tonne. Pigs received weaner diets until they reached approximately 15 kg, when they were given diets formulated for growers. One pig in the EW–enzyme 2 group was excluded from the experiment because of poor performance that was not related to the experiment. The other pigs remained healthy before autopsy or experimental infection. Pigs were grown to a weight of 33·43 (SEM 0·93) kg (uninfected pigs), or 26·84 (SEM 0·67) kg (pigs for infection).

Animals and diets used in experiment 3

A total of forty-eight pigs weighing 6·09 (SEM 0·17) kg were allocated to four groups on the basis of live weight. Diets were based on sorghum (Table 1). Sorghum (MR 31; Pacific Seed, Queensland, Australia) was milled to pass a 1·2 mm screen and then included in the diet either raw or extruded. Extrusion of sorghum was carried out as described in experiment 1 for wheat. Enzyme 3 ('experimental') with a guaranteed minimum activity of 2500 U/g α-amylase, 800 U/g xylanase and 6000 U/g protease (Finnfeeds International Ltd.) was included at 1 kg/tonne. Pigs were fed a weaner formulation until they reached approximately 15 kg, when they were transferred to a diet formulated for growers. They remained on the grower diet until they reached 24·13 (SEM 0·86) kg (uninfected pigs) or 23·91 (SEM 0·40) (pigs for infection). One pig fed the raw sorghum-based diet (RSor) was removed from the trial because of poor performance not related to the experiment. All other pigs remained healthy during the first part of the trial.

Results

Experiment 1

Results of dietary analyses are shown in Table 2. Extrusion of wheat resulted in a small reduction in total starch, and a substantial reduction in RS, but no other major changes in composition. Results of animal performance are shown in Table 3. The only significant effect observed ($P<0·05$) was a greater average daily gain in pigs fed RW with enzyme added. Results of analysis of large intestinal fermentation parameters are shown in Table 4. The pH values of the digesta in all samples were below 7·0, but became less acidic towards the distal parts of the LI, with both forms of treatment significantly ($P<0·01$) increasing pH values in the distal colon. The relative weights of full sections of the LI were similar with all the dietary treatments. The pigs consuming the EW diets in general had drier digesta than those fed the raw diets, with a significant effect being found in the proximal colon. The starch concentration in the LI was significantly reduced by extrusion, but not by enzyme addition. The VFA concentration and VFA pool in the colon were significantly reduced by extrusion, whilst enzyme addition only had a significant effect on the VFA pool in the colon. Concentrations of ATP generally declined towards the distal parts of the LI, except in the group fed RW–enzyme where higher levels were found in the proximal colon than in the caecum. Extrusion caused a significant reduction in ATP levels in both the caecum and proximal colon.

Four pigs each on the RW and the EW–enzyme diet, three on the RW–enzyme diet and two on the EW diet developed clinical signs of SD following experimental challenge. An additional two pigs on each of the EW–enzyme and RW–enzyme diets, and three on the EW diet excreted spirochaetes in their faeces after challenge,

Table 2. Starch and non-starch polysaccharide contents in the diets analysed (mg/g diet)*

	Experiment 1		Experiment 2	Experiment 3			
Diet...	RW	EW	EW (Grower)†	RSor (Weaner)	ESor (Weaner)	RSor (Grower)	ESor (Grower)
Total starch	54·6	50·1	50·1	69·0	70·8	66·3	69·3
Resistant starch	13·4	0·6	0·3	9·9	6·6	13·5	6·1
Soluble NSP	1·0	1·0	1·0	0·2	0·2	0·2	0·2
Insoluble NSP	6·0	6·3	6·3	3·6	3·7	3·6	3·7
Free sugars	1·5	1·6	1·6	0·7	0·8	0·7	0·8

RW, raw wheat; EW, extruded wheat; RSor, raw sorghum; ESor, extruded sorghum.
* For details of diets and procedures, see Table 1 and pp. 161–162.
† Analysis was not undertaken on the weaner diet used in the initial stages of experiment 2.

Table 3. Average daily intake (ADI), average daily gain (ADG), feed conversion ratio (FCR) and final body weights (BW) in all pigs, and empty body weights (EBW) in non-infected pigs in the three experiments*

(Mean values and standard errors of the difference)

	Experiment 1							Experiment 2						Experiment 3						
	Diet					Significance		Diet				Significance		Diet					Significance	
	RW	RW−Enz	EW	EW−Enz	SED	Ext	Enz	EW	EW−Enz 1	EW−Enz 2	SED	Enz 1	Enz 2	RSor	RSor−Enz	ESor	ESor−Enz	SED	Ext	Enz
No. of pigs	12	12	12	12				12	12	11				11	12	12	12			
ADI (g/d)	618	638	596	642	58·8	NS	NS	608[a]	611[a]	510[b]	46·8	NS	†††	403	402	389	389	43·7	NS	NS
ADG (g/d)	417[a]	487[b]	399[a]	438[a]	22·0	NS	†	406	389	367	32·1	NS	NS	195	189	181	168	26·6	NS	NS
FCR (g/g)	1·5	1·3	1·6	1·5	0·2	NS	NS	1·5	1·6	1·4	0·2	NS	NS	2·1	2·2	2·2	2·4	0·2	NS	NS
BW (kg)	23·9	24·1	23·6	22·9	0·6	NS	NS	36·5[a]	32·6[b]	30·7[b]	0·9	†	††	25·3[a]	25·4[a]	23·4[b]	22·1[c]	1·3	††	NS
No. of pigs	6	6	6	6				6	6	5				5	6	6	6			
EBW (% BW)	94·7	96·0	95·9	95·9	0·5	NS	NS	96·5	96·7	96·9	0·7	NS	NS	96·9	96·4	96·6	96·5	1·1	NS	NS

RW, raw wheat; RW−Enz, raw wheat−enzyme; EW, extruded wheat; EW−Enz, extruded wheat−enzyme; Ext, extrusion; Enz, enzyme; EW−Enz 1, extruded wheat−enzyme 1; EW−Enz 2, extruded wheat−enzyme 2; RSor, raw sorghum; RSor−Enz, raw sorghum−enzyme; ESor, extruded sorghum; ESor−Enz, extruded sorghum−enzyme.

[a,b,c]Mean values within a row for an experiment with unlike superscript letters were significantly different ($P<0·05$).

*For details of diets and procedures, see Tables 1 and 2 and pp. 161–162.

†$P<0·05$, ††$P<0·01$, †††$P<0·001$.

Table 4. Large-intestinal fermentation parameters in non-infected pigs in experiment 1*
(Mean values and standard errors of difference)

	Diet					Significance	
	RW	RW−Enz	EW	EW−Enz	SED	Ext	Enz
No. of autopsied pigs	6	6	6	6			
pH of digesta							
Caecum	5·75	5·62	5·58	5·44	0·11	NS	NS
Proximal colon	5·75	5·74	6·11	5·96	0·18	NS	NS
Distal colon	6·10[a]	6·57[b]	6·61[b]	6·80[b]	0·16	††	†
Full LI weights (% BW)							
Caecum	0·88	0·83	0·96	0·84	0·12	NS	NS
Colon	4·38	3·12	3·09	3·30	0·41	NS	NS
Whole LI	5·26	3·95	4·05	4·14	0·53	NS	NS
DM (mg/g of digesta)							
Caecum	243	196	252	214	33·4	NS	NS
Proximal colon	178[a]	182[a,c]	246[b]	222[b,c]	16·4	†††	NS
Distal colon	245	243	274	274	18·0	NS	NS
Starch (mg/g of digesta)							
Caecum	13·72[a]	6·32[b]	2·58[b]	3·11[b]	2·38	††	NS
Proximal colon	10·19[a]	6·24[b]	0·64[c]	2·04[c]	1·31	†††	NS
Distal colon	7·19[a]	2·14[b]	0·17[b]	0·00[b]	1·42	††	NS
VFA concentration (μmol/g of digesta)							
Caecum	244	287	235	242	29·3	NS	NS
Colon	208[a]	261[a]	197[b]	219[a,b]	32·5	†	NS
LI	220[a]	270[b]	210[a]	227[a]	30·9	NS	†
VFA pool (mmol/digesta)							
Caecum	43	38	47	32	8·01	NS	NS
Colon	165[a]	72[b]	85[b]	67[b]	21·12	†	†
LI	208[a]	110[b]	132[b]	99[b]	22·57	NS	††
ATP (nmol/g of digesta)							
Caecum	0·52[a]	0·28[a,b]	0·16[b]	0·73[a]	0·011	††	NS
Proximal colon	0·30[a]	0·42[a]	0·09[b]	0·44[a]	0·083	†	NS
Distal colon	0·17	0·17	0·14	0·23	0·062	NS	NS

RW, raw wheat; RW−Enz, raw wheat−enzyme; EW, extruded wheat; EW−Enz, extruded wheat−enzyme; Ext, extrusion; Enz, enzyme; LI, large intestine; BW, final body weight; VFA, volatile fatty acids.
[a,b,c]Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* For details of diets and procedures, see Tables 1 and 2 and pp. 161–162.
†$P<0·05$, ††$P<0·01$, †††$P<0·001$.

but did not develop disease. These treatment differences in disease occurrence were not significant. All pigs with clinical signs had characteristic pathological changes in the LI, and *B. hyodysenteriae* was subsequently isolated from them at post mortem.

Experiment 2

Extrusion of wheat in experiment 2 reduced RS levels even more than in experiment 1 (Table 2). The inclusion of enzyme 2 to the diet significantly ($P<0·001$) depressed daily intake, and pigs fed both enzymes had significantly ($P<0·01$) lower final BW (Table 3). Addition of enzyme 1 resulted in a significant increase in pH values in the caecum (Table 5). Pigs receiving enzyme 2 had significantly lighter full colons than the other pigs (Table 5). Pigs fed the diet mixed with enzyme 1 had significantly drier digesta in the proximal colon (Table 5). Starch concentrations rapidly declined from the proximal to the distal parts of the LI, with the concentration in the caecum and proximal colon being significantly lower ($P<0·01$) when pigs were fed diets that contained either of the enzymes (Table 5). The VFA concentration and pool did not differ significantly between the treatment groups (Table 5). Both enzymes reduced concentrations of ATP in the LI. With enzyme 1, significant effects were observed in the proximal and distal colon, while enzyme 2 only had this effect in the proximal colon (Table 5).

Five pigs in the EW group and three in each of the EW−enzyme 1 and EW−enzyme 2 groups developed clinical signs of SD. The sixth pig in the EW group and two additional pigs in the EW−enzyme 2 group shed spirochaetes in their faeces, but did not develop disease. These treatment differences were not significant for either disease occurrence or colonisation. All pigs with clinical signs had pathological changes consistent with SD at post mortem, and *B. hyodysenteriae* was recovered from the lesions.

Experiment 3

Extrusion of sorghum caused a reduction in the levels of RS in both weaner and grower diets (Table 2). Pigs fed RSor had a significantly ($P<0·01$) greater final BW than pigs fed the extruded sorghum (ESor) diets (Table 3), but all pigs fed sorghum-based diets had a reduced growth performance compared with wheat-fed pigs in experiments 1 and 2. The pH values of the digesta increased from the caecum to the distal colon (Table 6), and addition of

Table 5. Large-intestinal fermentation parameters in non-infected pigs in experiment 2*
(Mean values and standard errors of the difference)

	Diet				Significance	
	EW	EW–Enz 1	EW–Enz 2	SED	Enz 1	Enz 2
No. of autopsied pigs	6	6	5			
pH						
Caecum	5·90[a]	6·33[b]	6·11[a,b]	0·10	††	NS
Proximal colon	6·17	6·45	6·35	0·08	NS	NS
Distal colon	6·97	6·92	6·69	0·22	NS	NS
Full LI weights (% BW)						
Caecum	0·85	0·69	0·79	0·47	NS	NS
Colon	2·83[a]	2·67[a,b]	2·20[b]	0·26	NS	†
Whole LI	3·47	3·27	3·09	0·31	NS	NS
DM (mg/g of digesta)						
Caecum	184	169	191	29·5	NS	NS
Proximal colon	220[a]	262[b]	241[a,b]	18·3	†	NS
Distal colon	266	279	276	26·5	NS	NS
Starch (mg/g of digesta)						
Caecum	2·81[a]	0·64[b]	0·80[b]	0·6	††	††
Proximal colon	1·36[a]	0·50[b]	0·33[b]	0·18	††	††
Distal colon	0·36	0·06	0·06	0·17	NS	NS
VFA concentration (μmol/g of digesta)						
Caecum	91	87	110	16·8	NS	NS
Colon	78	97	90	31·2	NS	NS
LI	82	94	97	35·4	NS	NS
VFA pool (mmol/digesta)						
Caecum	19	13	17	4·5	NS	NS
Colon	94	107	77	18·9	NS	NS
LI	113	120	94	19·4	NS	NS
ATP (nmol/g of digesta)						
Caecum	1·06	0·53	0·36	0·031	NS	NS
Proximal colon	2·22[a]	0·46[b]	0·75[b]	0·102	††	††
Distal colon	0·90[a]	0·24[b]	1·07[a]	0·030	††	NS

EW, extruded wheat; EW–Enz 1, extruded wheat–enzyme 1; EW–Enz 2, extruded wheat–enzyme 2; LI, large intestine; BW, final body weight; VFA, volatile fatty acids.
[a,b,c]Mean values within a row with superscript letters were significantly different ($P<0·05$).
* For details of diets and procedures, see Tables 1 and 2 and pp. 161–162.
†$P<0·05$, ††$P<0·01$, †††$P<0·001$.

enzyme significantly increased pH values in the distal colon. All pigs had similar intestinal DM contents, and the digesta became drier as it moved along the tract (Table 6). Dietary extrusion significantly decreased the VFA pool but not the VFA concentration in the colon, and it significantly decreased the ATP concentrations in the proximal colon.

One pig each in the RSor and RSor–enzyme groups, three in the ESor and four in the ESor–enzyme group developed clinical signs of SD. Three additional pigs in group RSor, two in group RSor–enzyme and one in group ESor shed spirochaetes in their faeces. The treatment differences in rates of disease and colonisation failed to reach significance. The diseased pigs had pathological changes consistent with SD at post mortem, and *B. hyodysenteriae* was isolated from their LI.

Linear regression and multilinear models

Simple linear regression analyses were conducted between dietary characteristics (eleven dietary treatments), body weight and fermentation parameters in uninfected pigs (*n* 64), and the percentage of experimentally infected pigs colonised with *B. hyodysenteriae* (i.e. shedding *B. hyodysenteriae* in their faeces), and the percentage of pigs developing SD (Table 7). Significant effects on colonisation included dietary RS and sNSP content, empty body weight, caecal weight, caecal pH values, pH values in the distal colon, ATP concentration in the proximal and distal colon, VFA concentration in the caecum and total bacterial numbers in the proximal colon. Significant influences on disease occurrence included dietary RS and sNSP content, empty caecal weight, pH in the distal colon, ATP concentrations throughout the LI, and total bacterial numbers in the proximal colon.

Two multiple regression models then were developed.

Model 1 (colonisation by B. hyodysenteriae*).* Y (% of experimentally challenged pigs that were colonised) = 196·9 (intercept: SEM 34·07; $P<0·0001$) + 20·8 (sNSP in diet, %: SEM 4·19; $P<0·001$) − 22·0 (pH of caecum: SEM 5·48; $P<0·0002$) − 0·12 (VFA colon pool, mmol: SEM 0·035; $P<0·001$). $R^2 = 0·43$ ($P<0·0001$).

Model 2 (development of swine dysentery). Y (% of experimentally challenged pigs that developed SD) = 32·1 (intercept: SEM 8·8; $P<0·0006$) − 1·6 (RS in diet, %: SEM 0·40; $P<0·0002$) + 7·9 (colon full, % final BW: SEM 3·05; $P<0·0119$) + 4·0 (ATP proximal colon, nmol/g digesta: SEM 1·31; $P<0·0036$). $R^2 = 0·34$ ($P<0·0001$).

Table 6. Large-intestinal fermentation parameters in non-infected pigs in experiment 3*
(Mean values and standard errors of the difference)

	Diet					Significance	
	RSor	Rsor−Enz	ESor	Esor−Enz	SED	Ext	Enz
No. of autopsied pigs	5	6	6	6			
pH							
Caecum	5·91	5·99	5·88	6·06	0·11	NS	NS
Proximal colon	5·94	6·02	5·90	6·19	0·09	NS	NS
Distal colon	6·14[a]	6·33[a,b]	6·21[a]	6·70[b]	0·23	NS	†
Full LI weights (% BW)							
Caecum	0·49	0·67	0·63	0·63	0·63	NS	NS
Colon	2·58	2·87	2·78	2·84	0·08	NS	NS
Whole LI	3·07	3·55	3·41	3·47	0·22	NS	NS
DM (mg/g of digesta)							
Caecum	226	220	220	220	31·5	NS	NS
Proximal colon	213	220	229	264	22·4	NS	NS
Distal colon	277	252	271	290	34·5	NS	NS
Starch (mg/g of digesta)							
Caecum	3·00	3·73	1·73	2·98	1·42	NS	NS
Proximal colon	2·69	3·61	1·92	2·58	1·39	NS	NS
Distal colon	1·67	1·21	2·29	2·86	0·81	NS	NS
VFA concentration (μmol/g of digesta)							
Caecum	146	115	127	112	13·5	NS	NS
Colon	154	138	144	125	24·5	NS	NS
LI	151	130	138	121	29·2	NS	NS
VFA pool (mmol/digesta)							
Caecum	14	15	13	12	4·0	NS	NS
Colon	56[a]	49[a,b]	42[b]	39[b]	15·8	††	NS
LI	71[a]	64[a,b]	55[a,b]	50[b]	22·4	†	NS
ATP (nmol/g of digesta)							
Caecum	0·08	0·08	0·04	0·10	0·012	NS	NS
Proximal colon	0·21[a]	0·21[a]	0·08[b]	0·06[b]	0·031	††	NS
Distal colon	0·14	0·07	0·09	0·09	0·042	NS	NS

RSor, raw sorghum; RSor−Enz, raw sorghum−enzyme; ESor, extruded sorghum; ESor−Enz, extruded sorghum−enzyme; Ext, extrusion; Enz, enzyme; LI, large intestine; BW, final body weight; VFA, volatile fatty acids.
[a,b,c]Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* For details of diets and procedures, see Tables 1 and 2 and pp. 161–162.
†$P<0·05$, ††$P<0·01$, ††$P<0·001$.

Discussion

The experiments conducted here were intended to identify cereal grains and practical dietary processing techniques or treatments that would reduce the incidence of SD in pigs. None of the treatments used was able to provide complete disease protection, and, in each experiment, differences in numbers of pigs in each treatment both becoming colonised by *B. hyodysenteriae* (as assessed by faecal excretion) and developing disease failed to reach significance. In part, this lack of statistical significance may have resulted from there being too few animals included in each treatment group. To help overcome this lack of numbers in the treatment groups, and to try to establish whether there was a link between diet and spirochaete colonisation and disease development, regression analysis was conducted using data collected over all three experiments. This analysis, which involved data from sixty-four uninfected pigs analysed against results for the sixty-six experimentally infected pigs, showed that low levels of dietary RS and sNSP, and the resultant intestinal indices related to low levels of fermentation in the LI, were associated with reduced colonisation by *B. hyodysenteriae* and with less frequent expression of SD. The subsequent multiple regression model for colonisation was particularly heavily driven by levels of dietary sNSP, whilst that for disease was more influenced by dietary RS. Other direct indices of hindgut fermentation also contributed to the models. Although the details of these simple models are not necessarily completely robust, they are generally consistent with our previous findings relating to the influences of diet on SD (Pluske *et al.* 1996, 1998; Siba *et al.* 1996; Hampson *et al.* 2001).

Diets in the first two experiments were based on wheat, because it is a common ingredient used in pig diets in Australia. Extrusion was undertaken with the aim of improving starch digestibility, and enzymes were added to increase starch digestion and/or degrade sNSP. In the first experiment, pigs eating EW unexpectedly had a lower intake and gain than those fed RW, whilst addition of sNSP-degrading enzymes improved daily gain, but as a result of higher food intake rather than improved nutrient conversion. Extrusion did substantially reduce the amount of dietary RS present, and consequently hindgut fermentation was suppressed, as indicated by higher pH values, less VFA and lower ATP than in pigs fed RW. This relative suppression also correlated well with a previously reported reduced numbers of RS-degrading bacteria in these pigs (Durmic *et al.* 2000). The effects of sNSP-degrading enzymes on fermentation tended to be less marked than those

Table 7. Correlation between intestinal parameters, colonisation by *Brachyspira hyodysenteriae**, and development of swine dysentery (SD)†

	Colonisation		SD incidence	
Parameter	R^2	*P* value	R^2	*P* value
Diet				
Resistant starch in diet (%)	0·132	0·003	0·119	0·005
Soluble NSP in diet (%)	0·264	<0·001	0·202	<0·001
Body weight				
BW (kg)	<0·060	>0·05	<0·060	>0·05
EBW (% BW)	0·269	<0·001	<0·060	>0·05
Large intestine weight (% BW)				
Caecum full	0·097	0·012	<0·060	>0·05
Caecum empty	0·091	0·015	0·090	0·016
Caecum digesta	0·074	0·029	<0·060	>0·05
Colon full	<0·060	>0·05	<0·060	>0·05
Colon empty	<0·060	>0·05	<0·060	>0·05
Colon digesta	<0·060	>0·05	<0·060	>0·05
pH				
Caecum	0·133	0·003	<0·060	>0·05
Proximal colon	<0·060	>0·05	<0·060	>0·05
Distal colon	0·076	0·029	0·117	0·006
ATP concentration (nmol/g of digesta)				
Caecum	<0·060	>0·05	0·132	0·003
Proximal colon	0·157	0·001	0·129	0·003
Distal colon	0·135	0·003	0·083	0·022
VFA pool (mmol)				
Caecum	0·095	0·013	<0·060	>0·05
Colon	<0·060	>0·05	<0·060	>0·05
Total bacterial numbers (log_{10} CFU/g digesta)				
Proximal colon	0·202	<0·001	0·243	<0·001

BW, final body weight; EBW, empty body weight; VFA, volatile fatty acids; CFU, colony-forming units.

* Pigs were recorded as colonised if they shed *B. hyodysenteriae* in their faeces in the period following experimental infection. Pigs were recorded as being diseased if they developed clinical signs of SD. Not all colonised pigs developed SD. The correlations were based on sixty-four uninfected pigs, eleven dietary treatments (for resistant starch and soluble NSP results), and sixty-six experimentally-infected pigs in the eleven groups, a proportion of which became colonised (*n* 46) or which developed disease (*n* 33).

†For details of procedures, see p. 165.

associated with extrusion. In the pigs fed the RW−enzyme diet, starch concentrations, and consequently some other indicators of fermentation, were reduced when enzyme was present in the diet. In these animals, however, there was a disparity between assessments of fermentation based on pH and ATP concentrations, and those based upon VFA concentrations and gut weights. Such differences have been reported previously, with a suggestion that presence or absence of different dietary ingredients may affect different fermentation parameters (Pluske *et al.* 1998; Govers *et al.* 1999). For example, NSP are known to drag substrate down the tract, and shift fermentation to more distal parts of the colon (Philips *et al.* 1995; Pluske *et al.* 1998; Govers *et al.* 1999). In the pigs receiving enzyme, fermentation occurred more in the proximal than in the distal parts of the LI, probably as a result of enzymic depolymerisation of viscous sNSP. These smaller polymers may increase the rate of passage of the ileal digesta, and rapidly carry solubilised starch and the hydrolysed sNSP to the LI where they undergo fermentation. As a consequence, addition of enzyme failed to substantially reduce fermentation in EW-based diets, and did not extend protection against SD beyond that achieved with extrusion alone. It is still possible that improved protection might occur with an EW-based diet if a starch-degrading enzyme and an sNSP-degrading enzyme were both added, so as to remove any starch that remains after extrusion.

In experiment 2, although RS was apparently reduced by dietary extrusion even more than in experiment 1, water content, starch concentrations in the digesta, the VFA pool in the colon, and ATP concentrations in the LI were higher than in experiment 1, and more animals developed SD. To an extent this different outcome may have reflected minor differences in the composition of the diets in the two experiments. On the other hand, the differences found following extrusion in the two experiments are consistent with the experiences of other workers, who have found that extrusion of carbohydrate sources has variable effects on growth performance in young pigs, and that the degree of gelatinisation is not a major factor in explaining this variation (Hongtrakul *et al.* 1998). Osman *et al.* (1970) have suggested that failure to reach optimal conditions during processing might actually strengthen bonds within the starch granules, making them less susceptible to host enzyme attack. Other reasons for the incomplete digestion of starch might include differences in the physical form of the food, formation of complexes with fat or protein, and the presence of α-amylase inhibitors in the diet, but

could also be due to inadequate chewing, shorter transit time in the gut or higher viscosity of the digesta (Bengala Freire *et al.* 1991; Muir & O'Dea, 1992; Muir *et al.* 1995). Another possible explanation relates to the replacement of soyabean meal with skimmed milk in the second experiment. Although the effect of skimmed milk on hindgut fermentation is not well known, it is rich in the disaccharide lactose, which is usually poorly digested in young weaned pigs (Redel *et al.* 1997). Hence the lactose could be expected to be available for bacterial fermentation in the LI.

The addition of enzymes containing xylanase and α-amylase activity (enzyme 1) or xylanase, β-glucanase and α-amylase activity (enzyme 2) to the wheat-based diet resulted in a reduction in hindgut fermentation, with enzyme 1 being somewhat more efficient than enzyme 2. Enzyme 2 had less α-amylase activity than enzyme 1. Consistent with a reduced fermentation, pigs that received enzymes had a slightly reduced incidence of SD compared with pigs fed the same diet without enzymes (three of six developed disease with both enzyme treatments, compared with five of six not receiving enzyme).

In both experiments using wheat-based diets, the extent of the protection offered by dietary treatments was limited, probably due to the high content of sNSP and RS in wheat. Consequently, in experiment 3, sorghum was evaluated since it has a lower sNSP content than wheat. The level of sNSP in the sorghum diets was confirmed to be low, and similar to that found in a previous highly digestible and protective diet based on cooked rice (Pluske *et al.* 1996). Furthermore, as expected, the RS content of the sorghum was lower in the extruded diets than in the raw diets. Hindgut fermentation was reduced by extrusion (as indicated by a reduced VFA pool and ATP concentrations) or by enzyme addition (as indicated by increased pH values and DM), and this was in good correlation with reduced bacterial numbers found previously (Durmic *et al.* 2000). However, not many of these differences were significant, and hence it is difficult to distinguish the effects of extrusion or enzyme addition from those of the initial fine grinding of the sorghum. Particle size of sorghum has a great effect on nutrient digestibility in pigs (Cabrera *et al.* 1994; Kopinski & Willis, 1996), and this is likely to have contributed to the effects seen. Apparently, as a result of fine-grinding, and in the absence of significant amounts of sNSP, dietary RS was exposed to intestinal amylase, and digested and absorbed before arrival at the LI. Consequently all groups of pigs had a relatively low incidence of SD compared with pigs fed wheat-based diets, with the pigs fed the RSor diets having the lowest incidence. The extruded sorghum diets resulted in a somewhat higher incidence of SD than raw diets, and there was no clear explanation for this difference. Extrusion disrupts the plant cell wall matrix, and can increase sNSP content by solubilising insoluble NSP; however, such differences were not seen in the analysed NSP contents of the diets (Table 2). Furthermore, indices of fermentation were similar with both RSor and ESor diets, and the addition of the enzyme mix to both raw and extruded diets had no additional protective effect against SD.

Conclusions

The results of the present study confirm the substantial role of fermentable fibre, especially RS and sNSP, in the development of SD. They also indicate that with an appropriate selection of grain types and/or with some of the proposed dietary treatments, hindgut fermentation and consequently SD can be reduced. In terms of reducing SD, the choice of a finely-ground grain inherently low in sNSP (sorghum) achieved better results than applying dietary extrusion or addition of starch-degrading enzymes to wheat. Unfortunately the extent of the effect in the present study was not adequate to fully protect pigs from developing SD, and an appropriate combination of grain selection, processing and dietary enzyme inclusion to obtain full protection against SD is yet to be determined.

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References

Alexander TJL & Taylor DJ (1969) The clinical signs, diagnosis and control of swine dysentery. *Veterinary Record* **85**, 59–63.

Association of Official Analytical Chemists (1988) *Official Methods of Analysis*, 14th ed. Washington, DC: AOAC.

Bedford MR & Classen HL (1992) Reduction of intestinal viscosity through manipulation of dietary rye and pentosane concentration is affected through changes in the carbohydrate composition of the intestinal aqueous phase and results in improved growth rate and food conversion efficiency of broiler chicks. *Journal of Nutrition* **122**, 560–569.

Bengala Freire J, Aumaiter A, Peiniau J & Leberton Y (1991) Apparent ileal digestibility of starch and α-galactosides from peas by early weaned pigs: effect of extrusion. In *The 5th Congress on Digestive Physiology in Pigs*, pp. 395–400 [MWA Verstegen, J Huisman and LA den Hartog, editors]. Wageningen, Netherlands: Pudoc Wageningen.

Buchanan-Smith JG, Totsek R & Tillman AD (1968) Effect of methods of processing on digestibility and utilization of grain sorghum by cattle and sheep. *Journal of Animal Science* **27**, 525–530.

Cabrera MR, Hancock JD, Hines RH, Behnke KC & Bramel-Cox PJ (1994) Sorghum genotype and particle size affect milling characteristics, growth performance, nutrient digestibility, and stomach morphology in finishing pigs. *Journal of Animal Science* **72**, Supp. 2, 55.

Carter RR & Leibholz J (1991) Effects of extrusion of wheat on dry matter and starch digestibility in the young pig. In *Manipulating Pig Production III*, p. 86 [PD Cranwell, editor]. Werribee, Victoria, Australia: Australasian Pig Science Association.

Choct M, Hughes RJ, Trimble RP, Angkanaporn K & Annison G (1995) Non-starch polysaccharide-degrading enzymes increase the performance of broiler chickens fed wheat of low apparent metabolizable energy. *Journal of Nutrition* **125**, 485–492.

Choct M, Hughes RJ, Wang J, Bedford MR, Morgan AJ & Annison G (1996) Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch

polysaccharides in chickens. *British Poultry Science* **37**, 606–621.
Durmic Z, Pethick DW, Mullan BP, Schulze H, Accioly JM & Hampson DJ (2000) Extrusion of wheat or sorghum and/or addition of exogenous enzymes to pig diets influences the large intestinal microbiota but does not prevent development of swine dysentery following experimental challenge. *Journal of Applied Microbiology* **89**, 678–686.
Englyst HN (1989) Classification and measurement of plant polysaccharides. *Animal Feed Science and Technology* **23**, 27–42.
Englyst HN, Quigley ME, Hudson GJ & Cummings JH (1992) Determination of dietary fibre as non-starch polysaccharides by gas–liquid chromatography. *Analyst* **117**, 1707–1714.
Englyst HN, Wiggins HS & Cummings JH (1982) Determination of the non-starch polysaccharides in plant foods by gas–liquid chromatography of constituent sugars as alditol acetates. *Analyst* **107**, 307–318.
Gill BP, Garcia S, Hillman KH & Schulze H (1996) Effect of wheat processing and enzyme supplementation of weaner diets on piglet performance and gut health. *Journal of Animal Science* **62**, 635–641.
Govers MJ, Gannon NJ, Dunshea FR, Gibson PR & Muir JG (1999) Wheat bran affects the site of fermentation of resistant starch and luminal indexes related to colon cancer risk: a study in pigs. *Gut* **45**, 840–847.
Graham H, Fadel JG, Newman CW & Newman RK (1989) Effect of pelleting and β-glucanase supplementation on ileal and fecal digestibility of a barley-based diet in the pig. *Journal of Animal Science* **67**, 1293–1298.
Hampson DJ, Pluske JR & Pethick DW (2001) Dietary manipulation of enteric disease. In *Digestive Physiology of Pigs*, pp. 247–261 [JE Lindberg and B Ogle, editors]. Wallingford, England: CABI Publishing.
Hongtrakul K, Goodband RD, Behnke KC, Nelssen JL, Tokach MD, Bergstrom JR, Nessmith WB & Kim IH (1998) The effects of extrusion processing of carbohydrate sources on weanling pig performance. *Journal of Animal Science* **76**, 3034–3042.
Inborr J, Aherns F & Schmotz M (1991*a*) Effect of supplementary enzymes on ileal nutrient digestibility and post-weaning performance of weaner pigs. In *Manipulating Pig Production III*, p. 84 [PD Cranwell, editor]. Werribee, Victoria, Australia: Australasian Pig Science Association.
Inborr J, Bedford MR, Patience JF & Classen HL (1991*b*) The influence of supplementary feed enzymes on nutrients disappearance and digesta characteristics in the GI-tract of early weaned pigs. In *The 5th Congress on Digestive Physiology in Pigs*, pp. 405–410 [MWA Verstegen, J Huisman and LA den Hartog, editors]. Wageningen, Netherlands: Pudoc Wageningen.
Jenkinson SR & Wingar CR (1981) Selective medium for the isolation of *Treponema hyodysenteriae*. *Veterinary Record* **24**, 384–385.
Kemm EH & Brand TS (1996) Grain sorghum as an energy source for growing pigs. *Pig News and Information* **17**, 87N–89N.
Kim IH, Hancock JD, Hines RH & Risley CR (1994) Effects of cellulase and a bacterial feed additive on the nutritional value of sorghum grain for finishing pigs. *Journal of Animal Science* **72**, Suppl 2, 55.
Kopinski JS, Martin P, Blight GW, Pytko A & Van Melzen P (1995) Non starch polysaccharides in Australian cereals. In *Recent Advances in Animal Nutrition in Australia 1995*, p. 179 [JN Rowe and JV Nolan, editors]. University of New England, Armidale, NSW: Department of Animal Science.
Kopinski JS & Willis JS (1996) Recent advances in utilisation of sorghum for growing pigs. In *Third Australian Sorghum Conference*, **93**, 235–250 [MA Foale, RG Henzell and JFP Kneipp, editors]. Melbourne, Tamworth: Australian Institute of Agricultural Science.
Kunkle RA, Harris DL & Kinyon JM (1986) Autoclaved liquid medium for propagation of *Treponema hyodysenteriae*. *Journal of Clinical Microbiology* **24**, 669–671.
Mills CG, Hines RH, Hancock JD & Gugle TL (1994) Extrusion of sorghum grain and soybeans for lactating sows. *Journal of Animal Science* **72**, Suppl. 2, 66.
Muir JG, Birkett A, Brown I, Jones G & O'Dea K (1995) Food processing and maize variety affects amounts of starch escaping digestion in the small intestine. *American Journal of Clinical Nutrition* **61**, 82–89.
Muir JG & O'Dea K (1992) Measurements of resistant starch: factors affecting the amount of starch escaping digestion *in vitro*. *American Journal of Clinical Nutrition* **56**, 123–127.
Muir JG & O'Dea K (1993) Validation of an *in vitro* assay for predicting the amount of starch that escapes digestion in the small intestine of humans. *American Journal of Clinical Nutrition* **57**, 540–546.
Osman HF, Theurer B, Hale WH & Mehen SM (1970) Influence of grain processing on *in vitro* enzymatic starch digestion of barley and sorghum grain. *Journal of Nutrition* **100**, 1133–1140.
Owsley WF, Knabe DA & Tanksley TDJ (1981) Effects of sorghum particle size on digestibility of nutrients at the terminal ileum and over total digestive tract of growing-finishing pigs. *Journal of Animal Science* **52**, 557–566.
Philips J, Muir JG, Birkett A, Lu ZX, Jones GP & O'Dea K (1995) Effect of resistant starch on fecal bulk and fermentation-dependent events in humans. *American Journal of Clinical Nutrition* **62**, 121–130.
Pluske JR, Durmic Z, Pethick DW, Mullan BP & Hampson DJ (1998) Confirmation of the role of rapidly fermentable carbohydrates in the expression of swine dysentery in pigs after experimental infection. *Journal of Nutrition* **128**, 1737–1744.
Pluske JR, Siba PM, Pethick DW, Durmic Z, Mullan BP & Hampson DJ (1996) The incidence of swine dysentery in pigs can be reduced by feeding diets that limit the amount of fermentable substrate entering the large intestine. *Journal of Nutrition* **126**, 2920–2933.
Redel CA, Shulman RJ & Tivey DR (1997) Determinants of lactose digestion in the miniature pig. *Digestive Diseases and Sciences* **42**, 137–144.
Siba PM, Pethick DW & Hampson DJ (1996) Pigs experimentally infected with *Serpulina hyodysenteriae* can be protected from developing swine dysentery by feeding them a highly digestible diet. *Epidemiology and Infection* **116**, 207–216.