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Pigs experimentally infected with *Serpulina hyodysenteriae* can be protected from developing swine dysentery by feeding them a highly digestible diet

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SUMMARY

Weaner pigs ($n = 72$) were fed 1 of 4 diets. These were based on either cooked rice and animal protein, cooked rice and lupin, wheat and lupin, or wheat and animal protein. Twenty-six of the pigs were slaughtered after 1 month. Those fed the highly digestible cooked rice and animal protein diet had drier colonic contents and faeces, lighter large intestines, and the contents of their large intestines had increased pH values and decreased total VFA concentrations. The other 46 pigs were orally challenged with broth cultures of *Serpulina hyodysenteriae*, and were monitored for faecal excretion of the spirochaetes, and for the development of swine dysentery (SD). None of 18 pigs fed the cooked rice and animal protein diet developed colonic changes or disease, whereas most pigs on the other diets developed mucohaemorrhagic colitis and dysentery. The reduced fermentation that occurred in the large intestines of pigs fed cooked rice and animal protein was associated with a subsequent failure of colonization by *S. hyodysenteriae*, and resultant protection against SD.

INTRODUCTION

Swine dysentery (SD) occurs in weaner and grower pigs throughout the world [1], and is one of the most economically important endemic diseases of pigs. The condition is a severe mucohaemorrhagic colitis resulting from infection with the anaerobic spirochaetal bacterium, *Serpulina* (*Treponema*) *hyodysenteriae* [2–4]. The bacteria colonize and damage both the colon and caecum [5].

Diets containing cereal grain and protein supplements are used widely for pig production. Many of these grains are rich in dietary fibre, which initially was defined as those components in a plant cell wall such as cellulose, hemicellulose, pectins and lignins, that are resistant to all digestive enzymes [6]. These components are classified as non-starch polysaccharides (NSP), and are the major substrates for microbial fermentation in the porcine large intestine [7]. The extent of the microbial breakdown is influenced greatly by the nature of the carbohydrate, which is highly dependent upon the type of diet consumed. The large intestine provides an excellent habitat for the establishment of a large and diverse variety of bacterial species, about 10^{10} cells per gut content, with strict anaerobes being the most common organisms present [8, 9]. These bacteria are fastidious and require specific environmental conditions, including correct pH, temperature, redox potential, osmolality, anaerobiosis, endogenous secretions, enzyme activities and dry matter content of ingesta to enable them to be actively involved in the breakdown of the NSP or other substrates in the large intestine. Such environmental conditions can be manipulated to either increase or decrease bacterial activity; for example, studies have shown that the population of the microflora in the large intestine increases greatly after pigs are fed a diet containing high levels of fibre [9, 10]. The major end products of fermentation are short-chain or volatile fatty acids (VFAs: acetic, propionic and butyric acid) [11, 12], and consequently

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the concentration of these increases as increased amounts of fermentable fibre reach the large intestine [13]. The principle dietary function of VFAs is to serve as energy sources for the pig [14].

Certain diets have been shown to create inhibitory conditions against bacterial pathogens in the large intestine of rabbits and pigs [15–17]. For example, it was reported that SD did not occur in pigs on an infected farm after a highly fibrous (cellulose/hemicellulose) diet, based on maize silage, was fed [16]. The new diet resulted in changes in fermentation in the proximal colon, and it was speculated that its protective effect was due to its low base content, which interacted with VFAs produced to create an unfavourable environment for *S. hyodysenteriae*.

Serological and clinical studies in Australia have shown that even though *S. hyodysenteriae* is present in certain herds, SD does not necessarily develop [18, 19]. The reasons for this are unclear, but in view of the above considerations this study was conducted to investigate the role of diet in determining the susceptibility of pigs to SD. This paper reports our findings on the effect of diets based on two feed grains, rice (*Oryza sativa*) and wheat (*Triticum aestivum*), supplemented with animal protein or lupin (*Lupinus angustifolius*).

MATERIALS AND METHODS

Animals

One-month-old Large White pigs ($n = 72$) were purchased from a commercial specific-pathogen-free herd known to be free of SD. The animals were divided into groups on a random basis, and were housed in these groups in an isolation house in adjacent pens with raised mesh floors. Pens were divided from each other by removeable open-mesh wire walls that permitted close contact between the animals.

Diets

Three experimental and 1 commercial diet, each made to the same weaner specifications, were fed to the pigs. Their compositions by dry weight were 77% cooked rice and 18% animal protein (RA diet), 64% cooked rice, 15% dehulled lupin and 13% animal protein (RL diet), 62% whole wheat, 15% dehulled lupin, 11·5% animal protein and 3% peas (WL diet), and 75% wheat and 17% animal protein (WA diet). Other components of all diets were soya bean meal (3%), soy oil, salt, soya, choline chloride, lysine, pig starter PMX and DF-750. Long grain white rice (Doongara) was purchased from the Australian Ricegrowers Co-operative Ltd. Water was added at a ratio of 2 to 1 (v/v), and this was cooked for 20 min at 121 °C. in an autoclave. The other ingredients were purchased separately from Milne Feeds Pty Ltd, Western Australia, and were mixed. Each diet was formulated to contain 14·7 MJ/Kg digestible energy, 20% crude protein and 1·27% lysine (expressed on a dry matter basis). The WL diet was an Australian commercial weaner diet in unpelleted form, not containing antimicrobial compounds, and was purchased directly from Milne Feeds Pty Ltd. All the diets were mixed with water and fed *ad libitum*.

Experimental design

After 1 month of feeding, 8 pigs on the RA diet, 5 on the RL diet, 7 on the WL diet and 6 on the WA diet were slaughtered to investigate the effect of these diets on the large intestine of uninfected pigs. The remaining 46 pigs were used in infectivity trials to determine their susceptibility to experimentally-induced SD.

Experimental induction of swine dysentery

The infection phase of the study involved 3 separate animal trials, in which the pigs ($n = 46$) were fed 1 of the 4 diets for 4 weeks before and up to 8 weeks after being challenged with broth cultures of *S. hyodysenteriae*. In trial 1, 4 pigs were fed the RA diet, and 4 received the commercial WL diet. In trial 2, 8 pigs were fed each of the same 2 diets. In this experiment 2 healthy pigs that were originally on the RA diet subsequently were transferred to the commercial WL diet, 4 weeks after the challenge with cultures of *S. hyodysenteriae*. At the time of this transfer all surviving pigs again were challenged on one occasion with *S. hyodysenteriae*. In trial 3, 4 pigs received the commercial WL diet, and the other 3 diets each were fed to 6 pigs.

Growth rates

The pigs were weighed weekly. Growth rate was calculated as average increase in weight per day over the experimental period. This was shorter (1 month)

for uninfected pigs than for those subsequently challenged.

Bacterial strains

Serpulina hyodysenteriae strain Western Australia 15 (serogroup A) was used to infect pigs in the first and third infectivity trials, and strain Western Australia 1 (serogroup B) was used in the second infectivity trial. Both strains originally were isolated from outbreaks of SD in piggeries in Western Australia. Broth cultures of spirochaetes stored in 2·0 ml vials at −70 °C were thawed and grown at 37 °C in the prereduced anaerobic broth medium of Kunkle and colleagues [20]. Spirochaetes in mid-log phase were used to inoculate pigs. Pigs were challenged orally with 10^{10} viable cells of *S. hyodysenteriae* in 100 ml of broth culture, daily for 3 days. On the first day feed was removed 18 h before inoculation, and was returned 4 h after inoculation: on the 2 remaining days the feed was removed in the mornings 2 h prior to inoculation, and returned 4 h after inoculation. Once clinical signs became apparent in a group of pigs, the feed was temporarily removed and the animals in the different experimental groups were allowed to mix for 2 h each day, so as to maximize the opportunity for transmission of infection.

Monitoring for disease

Pigs were checked twice daily for signs of SD, including depression, lack of appetite and diarrhoea. Faeces were collected, every second day post-inoculation, and cultured for spirochaetes.

Necroscopy examination of pigs

The pigs were killed and subjected to post mortem examination either after 4 weeks (non-infected pigs), or for the infected pigs within 24 h of blood being observed in their faeces, or between 1–2 months post-infection if they had not developed signs of SD. Contents of the large intestine were collected for bacterial culture from the caecum, proximal colon, distal colon and rectum.

Isolation of spirochaetes

The contents from the large intestinal sites collected at post mortem, and the faeces that were collected every second day post-infection, were cultured on Trypticase Soy agar (BBL Microbiology Systems, Cockeysville, MD, USA) supplemented with 5% defibrinated ovine blood, spectinomycin 400 μg/ml, colistin 25 μg/ml, and vancomycin 25 μg/ml [21]. The plates were placed in anaerobic jars (BBL) under an atmosphere of 94% N_2 and 6% CO_2 at 37 °C for 5 days, and any strongly haemolytic spirochaetes sub-cultured, grown in Kunkle's broth medium [20], tested for indole production by addition of Kovac's reagent after extraction with zylene, and subjected to slide agglutination to determine serotype [22].

Weight of the large intestine and its contents

The large intestines were tied off at the ileo-caecal junction and at the rectum, and removed. The caecum was tied off and excised from the colon, and the two portions of the large intestine were weighed with their contents intact. Approximately 10 ml of the intestinal contents were collected from the caecum, the first loop of the colon, the apex of the spiral of the colon, and the rectum. The remainder of the intestinal contents was then removed, and the caecum and colon were reweighed empty.

Calculation of dry matter content

Approximately 2·0 g of each fresh faecal sample was put into a tared dish and heated in a hot air oven (Watson Victor Ltd, Australia) at 105 °C for 72 h. Each sample was reweighed and the dry matter content calculated.

Measurement of pH

The pH values of the large intestinal contents of pigs were determined within 5 min of death using a portable pH meter (Orion Research Inc., Boston, USA). Distilled water was added to viscous samples (some proximal colon and most rectal samples) to aid measurement. Calibration of the pH electrode was checked every 5 samples.

Estimation of Volatile Fatty Acid (VFA) concentrations

A modification of the method of Pethick and colleagues [23] was used to estimate the VFA concentration in the intestinal contents, using gas liquid chromatography (GLC). Gut contents were diluted 1:1 w/v with 10 N phosphoric acid and

centrifuged at 4 °C for 10 min at 3000 rpm in a bench-top centrifuge (MSE, Sussex, UK). The supernatant was collected into a capped tube and maintained at 0–5 °C before 0·5 μl was used for analysis within 24 h. Chromatography was performed using a Varian 3700 chromatograph (Varian, Palo Alto, CA, USA) and an FFAP 15 m × 0·53 mm × 1·2 u film capillary column (Cat. no. 19684, Alltech Assoc. Inc., Sydney, Australia). Glass wool (5 cm) was packed in a guard column to prevent blockages in the column.

The VFA concentrations of proximal and distal colon were averaged to estimate the total VFA concentration in the colonic tract, based on the weight of the contents.

Data analysis

Analysis of the data was by one-way ANOVA using Statview 4.02 (PICA Software, USA) for the Macintosh. Means were compared using the Fisher's Protected Least Significant method.

RESULTS

Dry matter content of the large intestinal contents of uninfected pigs

There was a clear pattern for an increasing dry matter of contents as the digesta passed from the caecum to the rectum (Table 1). The increase in dry matter was most pronounced for pigs fed the RA diet. The faeces of the pigs fed the RA diet were dry, hard and black, whilst those of the pigs fed the other diets were moist, soft and yellowish-brown.

Dry matter content of the large intestinal contents of infected pigs

In contrast to the uninfected pigs, in infected pigs the dry matter content of the large intestinal contents was relatively constant as the digesta moved from the caecum to the rectum, except for pigs fed the RA diet. The latter animals showed a pattern of dry matter change down the tract that was similar to their uninfected counterparts (Table 1).

Growth rate of uninfected pigs

Weaner pigs on all 4 diets grew at an acceptable rate, but those on the WL diet grew the fastest, and those on the RL diet grew significantly ($P \leqslant 0{\cdot}05$) slower (Table 1).

Growth rate of infected pigs

There was no significant difference in growth rate amongst the infected pigs on the 4 diets (Table 1).

Weight of the large intestine and its contents in uninfected pigs

The caecae of the uninfected animals that were fed the RA diet were significantly lighter than those of the pigs on the other 3 diets (Table 2), and caecal contents were lighter for both rice-based diets. Pigs that consumed diets based on wheat grains had heavier colons than pigs fed rice-based diets, but the colonic contents were significantly heavier when wheat and/or lupin grains were fed compared with the RA diet.

Weight of the large intestine and its contents in infected pigs

Infected pigs that consumed the RA diet had significantly lighter caecae than the pigs fed the other diets (Table 2). The caecal contents were significantly heavier in pigs fed the WL diet. The colons of pigs fed the RA and WL diets were significantly lighter than those of pigs fed the other 2 diets. Pigs fed the 2 rice-based diets had the least colonic contents.

pH values of large intestinal contents in uninfected pigs

The pH values of the contents of the caecum and proximal colon of uninfected pigs fed the RA diet were significantly higher (mean pHs at both sites 6·3) than those of pigs fed the other three diets (pH ranges at the two sites 5·4–5·7 and 5·7–5·9 respectively) (Table 3). The pH values of the contents increased in the distal colon in all groups, and were higher in the pigs that were fed both rice-based diets than in pigs fed the wheat-based diets.

pH values of large intestinal contents in infected pigs

In infected animals the pH value of the caecal contents for pigs fed the diets that were supplemented with animal protein were significantly higher (mean pH 6·45) than for pigs fed the two diets supplemented

Table 1. *Dry matter content of the large intestinal contents, and the growth rate of normal and infected pigs fed the different diets*

	Diet*			
	RA	RL	WL	WA
Uninfected pigs				
No. of pigs	8	5	7	6
Dry matter content (%)				
Caecum	9·2 ± 1·7[a]	14·2 ± 1·0[b]	11·7 ± 0·7[a]	13·0 ± 0·2[b]
Proximal colon	20·1 ± 2·0[a]	15·8 ± 0·5[b]	16·8 ± 1·6[b]	15·6 ± 0·3[b]
Distal colon	25·0 ± 1·1[a]	16·2 ± 0·6[b]	19·2 ± 1·7[b]	16·8 ± 0·1[b]
Faeces	36·1 ± 3·8[a]	18·6 ± 0·2[b]	26·0 ± 1·3[c]	18·8 ± 0·3[b]
Growth rate† (g/day)	310 ± 20·0[a]	237 ± 20·2[b]	319 ± 19·0[a]	273 ± 13·4[a,b]
Infected pigs				
No. of pigs	16	6	16	6
Dry matter content (%)				
Caecum	8·8 ± 0·7[a]	14·0 ± 1·3[b]	13·5 ± 0·6[b]	14·8 ± 0·8[b]
Proximal colon	19·1 ± 1·2[a]	14·8 ± 1·6[a,b]	14·3 ± 1·6[b]	13·0 ± 3·0[b]
Distal colon	26·0 ± 0·7[a]	16·5 ± 2·0[b]	14·7 ± 1·6[b]	13·1 ± 3·9[b]
Faeces	45·0 ± 0·7[a]	13·3 ± 1·3[b]	14·6 ± 1·6[b]	15·6 ± 3·5[b]
Growth rate (g/day)	533 ± 36·8	551 ± 52·9	564 ± 32·2	529 ± 37·4

* Diets: RA, cooked rice and animal protein; RL, cooked rice and lupin; WL, wheat and lupin (commercial diet); WA, wheat and animal protein.

† Growth rate of uninfected pigs only over the period 4 weeks after weaning.

For each row, figures with different superscripts differ at 5% level of significance.

Table 2. *Mean weight of the large intestine and its contents expressed as a percentage of body weight in uninfected and infected pigs*

	Diet*			
	RA	RL	WL	WA
Uninfected pigs				
Caecum	0·14 ± 0·01[a]	0·23 ± 0·01[b]	0·21 ± 0·02[b]	0·23 ± 0·1[b]
Caecal contents	0·43 ± 0·08[a]	0·55 ± 0·6[a]	1·03 ± 0·08[b]	2·99 ± 0·03[b]
Colon	1·00 ± 0·04[a]	1·03 ± 0·04[a]	1·84 ± 0·03[b]	1·29 ± 0·01[c]
Colonic contents	1·55 ± 0·06[a]	2·92 ± 0·11[b]	2·99 ± 0·35[b]	3·43 ± 0·3[b]
Infected Pigs				
Caecum	0·18 ± 0·01[a]	0·25 ± 0·02[b]	0·23 ± 0·02[b]	0·32 ± 0·02[c]
Caecal contents	0·37 ± 0·04[a]	0·41 ± 0·10[a]	2·18 ± 0·34[b]	0·83 ± 0·27[a]
Colon	0·92 ± 0·05[a]	1·53 ± 0·22[b]	0·99 ± 0·12[a]	2·24 ± 0·31[c]
Colonic contents	0·83 ± 0·08[a]	0·99 ± 0·26[a,b]	1·46 ± 0·11[b]	1·52 ± 0·30[b]

* Diets: RA, cooked rice and animal protein; RL, cooked rice and lupin; WL, wheat and lupin (commercial diet); WA, wheat and animal protein.

For each row, figures with different superscripts differ at 5% level of significance.

with lupin as the protein source (pH range 5·8–6·0) (Table 3). Similar trends were observed in the proximal colon, with pH values in the pigs fed the diets containing animal protein being higher (pH range 6·5–6·7) than those fed the lupin-containing diets (pH range 6·0–6·3). The same trend was seen in the distal colon, but again with the mean pH values being higher than at the more proximal sites.

Table 3. *pH values of the large intestinal contents of uninfected and infected pigs*

	Diet*			
	RA	RL	WL	WA
Uninfected pigs				
pH				
Caecum	$6{\cdot}33 \pm 0{\cdot}16^{a}$	$5{\cdot}76 \pm 0{\cdot}21^{b}$	$5{\cdot}41 \pm 0{\cdot}05^{b}$	$5{\cdot}60 \pm 0{\cdot}09^{b}$
Proximal colon	$6{\cdot}33 \pm 0{\cdot}13^{a}$	$5{\cdot}90 \pm 0{\cdot}08^{b}$	$5{\cdot}80 \pm 0{\cdot}18^{b}$	$5{\cdot}73 \pm 0{\cdot}09^{b}$
Distal colon	$6{\cdot}57 \pm 0{\cdot}10^{a}$	$6{\cdot}62 \pm 0{\cdot}19^{a}$	$6{\cdot}01 \pm 0{\cdot}14^{b}$	$6{\cdot}10 \pm 0{\cdot}12^{b}$
Infected pigs				
pH				
Caecum	$6{\cdot}45 \pm 0{\cdot}16^{a}$	$5{\cdot}81 \pm 0{\cdot}15^{b}$	$6{\cdot}04 \pm 0{\cdot}14^{b}$	$6{\cdot}46 \pm 0{\cdot}03^{a}$
Proximal colon	$6{\cdot}54 \pm 0{\cdot}07^{a}$	$6{\cdot}08 \pm 0{\cdot}15^{b}$	$6{\cdot}34 \pm 0{\cdot}16^{a,b}$	$6{\cdot}73 \pm 0{\cdot}6^{a}$
Distal colon	$6{\cdot}96 \pm 0{\cdot}02^{a}$	$6{\cdot}58 \pm 0{\cdot}11^{a,b}$	$6{\cdot}53 \pm 0{\cdot}17^{b}$	$6{\cdot}81 \pm 0{\cdot}11^{a,b}$

* Diets: RA, cooked rice and animal protein; RL, cooked rice and lupin; WL, wheat and lupin (commercial diet); WA, wheat and animal protein.

For each row, figures with different superscripts differ at 5% level of significance.

Table 4. *Estimated total VFA concentration (mmol) in the large intestine of uninfected and infected pigs*

	Diet*			
	RA	RL	WL	WA
Uninfected pigs				
Caecum	9686 ± 2398^{a}	7543 ± 735^{a}	28546 ± 3919^{b}	10024 ± 1469^{a}
Colon	12699 ± 2756^{a}	$27229 \pm 4381^{a,b}$	39535 ± 7865^{b}	32969 ± 3256^{b}
Infected pigs				
Caecum	26795 ± 4295^{a}	11664 ± 3810^{a}	62022 ± 7737^{b}	15508 ± 4752^{a}
Colon	45737 ± 5269^{a}	41202 ± 11590^{a}	132469 ± 20444^{b}	72450 ± 24872^{b}

* Diets: RA, cooked rice and animal protein; RL, cooked rice and lupin; WL, wheat and lupin (commercial diet); WA, wheat and animal protein.

For each row, figures with different superscripts differ at 5% level of significance.

Production of VFAs in the large intestine of uninfected pigs

Both rice-based diets resulted in relatively low VFA production in the caecum and colon of uninfected pigs (Table 4). This was particularly marked for the colonic contents of pigs on the RA diet. The inclusion of wheat or lupin grain increased total VFAs in the colon.

Acetate was the major acid produced at all three large intestinal sites, on all diets, followed by propionate and then butyrate (Table 5). At all sites the RA diet resulted in a relative increase in acetate, and a decrease in propionate and butyrate compared with the other diets.

Production of VFAs in the large intestine of infected pigs

Total VFA was very high in the caecum and colon of the infected pigs fed the WL diet (Table 4). Pigs fed both rice-based diets had the lowest VFA concentrations.

In infected pigs acetate again was the principle VFA produced at all 3 sites, followed by propionate and butyrate respectively (Table 5). The molar proportion of acetate was greatest in pigs fed the RA diet, and propionate and butyrate production was decreased. The molar proportion of VFAs in the distal colon was almost the same in pigs fed all 4 diets.

Incidence of swine dysentery

Pooled results of the occurrence of disease in infected pigs on the 4 diets are shown in Table 6. In the first trial, all 4 pigs fed the commercial WL diet developed diarrhoea, passed specks of blood and mucus in the faeces, and showed depression and a lack of appetite:

Table 5. *Estimated VFA molar proportions* (%) *in the large intestine of uninfected and infected pigs*

	Diet*			
	RA	RL	WL	WA
Uninfected pigs				
Caecum	66:22:7†	49:35:9	49:28:13	58:29:9
Proximal colon	66:22:7	41:42:10	58:28:9	47:32:14
Distal colon	66:20:8	58:28:9	58:28:9	57:29:9
Infected pigs				
Caecum	66:23:5	57:25:11	61:24:11	52:30:12
Proximal colon	63:23:6	56:27:9	61:26:8	56:24:12
Distal colon	65:22:5	65:23:10	63:23:8	65:22:8

* Diets: RA, cooked rice and animal protein; RL, cooked rice and lupin; WL, wheat and lupin (commercial diet); WA, wheat and animal protein.

† Ratio (acetic:propionic:butyric).

Table 6. *Pooled results from three trials showing incidence of disease in pigs fed different diets and challenged with* S. hyodysenteriae

	Diet*			
	RA	RL	WL	WA
No. of pigs challenged	16	6	16	6
No. of pigs that shed *S. hyodysenteriae* in their faeces	3	6	13	5
Mean duration (days) of faecal shedding in these pigs	4·6	5·4	8·5	5·6
No. of pigs that developed swine dysentery	0	5	10	3
Incidence of disease (%)	0	83·3	62·5	60

* Diets: RA, cooked rice and animal protein; RL, cooked rice and lupin; WL, wheat and lupin; WA, wheat and animal protein.

they were slaughtered between 9 and 15 days post-inoculation. Each pig showed gross and microscopic evidence of a severe mucohaemorrhagic colitis. The 4 pigs that were fed the experimental RA diet remained healthy throughout the experiment, and no abnormalities were detected in their large intestines on post-mortem examination.

In the second trial, 5 of the 8 pigs fed the WL diet developed SD over the period 8–28 days post-inoculation, while all 8 pigs fed the RA diet remained healthy. Of the 2 pigs on the RA diet that were transferred to the WL diet, 1 died suddenly 2 days later with signs of clostridial enterotoxaemia, whilst 6 days later the other pig developed SD. These pigs are not included in Tables 1–5. None of the 3 surviving pigs on the WL diet nor the 6 that were still on the RA diet subsequently developed SD.

In trial three, 5 of the 6 pigs that were fed the RL diet became diseased within 6 days after inoculation. Three of the 6 pigs that were fed the WA diet became diseased 11 days after inoculation, and 1 of the 4 pigs fed the WL diet developed SD on day 42. The 6 animals fed the RA diet remained healthy throughout the experiment.

Isolation and identification of *S. hyodysenteriae*

Spirochaetes identified as *S. hyodysenteriae*, and belonging to the same serogroup as used to inoculate the pigs, were recovered from the faeces and/or intestinal contents of most animals fed the WL, WA and RL diets, but they were isolated from only 3 of the 16 experimentally-challenged pigs that were fed the RA diet (Table 6): 1 of these 3 pigs shedded spirochaetes over a period of 4 days in the second trial, and the other 2 pigs shed them over a 4–5 day period at different times in the third trial. Spirochaetes also were isolated from a colonic scrapping taken at necropsy from one of the latter pigs, 13 days after spirochaetes were last isolated from its faeces. Faecal shedding by pigs on the other three diets ranged from 4–15 days, with the group means varying from 5·4–8·5 days (Table 6).

Pathological changes in the large intestine of infected pigs

No gross nor microscopic abnormalities were seen in the large intestines of healthy pigs. The large intestine of all pigs identified on clinical grounds as having SD showed variable congestion and haemorrhage, with accumulations of mucus and fibrin-containing pseudo-

membranes in regions throughout the colon. Lesions in the caecum were usually confined to mild congestion.

DISCUSSION

The primary source of carbon for fermentation in the large intestine is dietary fibre which consists mainly of non-starch polysaccharides, both soluble and insoluble (NSP) [24]. An additional source of readily fermentable carbon is (i) resistant starch; that is starch which escapes digestion in the small intestine [24] and (ii) oligosaccharides of the raffinose series. The diet consisting of cooked rice as the energy source and animal protein as the protein supplement was extensively digested and absorbed in the small intestine of the normal pigs. Cooked white rice contains *c.* 85% total starch (most of which is readily digested in the small intestine) and 2% NSP, of which 60% is soluble [25, 26]. Consequently little substrate would have entered the large intestine and this led to reduced microbial fermentation, as indicated by the higher pH values and low total VFA concentration of the large intestinal contents. Greater fermentation occurred with the diets that contained either wheat or lupin. Dehulled lupin grain is a high protein legume which has virtually no starch [27] but contains high levels of fermentable substrates, including 29% NSP, of which 16% are soluble and 8% are oligosaccharides [28]. The NSPs and oligosaccharides are fermented in the large intestine to VFAs [7, 14, 29, 30], with a resultant decrease in pH values [11]. Whole wheat contains about 65% total starch and 12% NSP, of which 20% is soluble [27]. An unknown proportion of the starch in raw wheat would be 'resistant starch' that is not digested in the small intestine [31]. The soluble NSPs and oligosaccharides derived from the lupin and wheat, and the resistant starch from the wheat proved to be excellent substrates for microbial fermentation, as indicated by increased VFA concentrations and lower pH values in the large intestinal contents of pigs receiving these ingredients.

Acetate was the predominant VFA produced in the caecum and colon of the pigs fed all 4 diets, but particularly so in those fed the RA diet, where total fermentation was reduced. The relatively low level of propionate with the RA diet was expected, since propionic acid-producing organisms tend to favour readily fermentable substrates such as oligosaccharides and resistant starch [31]. The weight and volume of the large intestine tends to increase when diets high in dietary fibre are consumed [13, 32–4], and this would explain why the pigs fed the RA diet had less bulky and drier colonic contents and faeces, and a lighter large intestine than animals fed the other diets. Overall, the RA diet was highly digestible, and this led to there being restricted large intestinal microbial fermentation, with lesser and drier contents, and a ligher and smaller large intestinal tract.

The total VFA content in the large intestine of the older (infected) pigs increased, presumably as a result of their greater feed intake and their larger size. At the same time the pH values at these sites also increased, and this was not explained. This increase was not simply a result of the development of diarrhoea, as it also was seen in healthy pigs in the RA group. As the pig matures the digestion of starch in the small intestine increases [35], and so less resistant starch would enter the large intestine to be fermented. This then might result in a relative elevation of pH values.

None of the pigs that were fed the RA diet and were inoculated with *S. hyodysenteriae* developed SD. This protection occurred with 2 different virulent strains of *S. hyodysenteriae*. Only 3 of 16 animals showed evidence of transient colonization with spirochaetes, implying that the protective influences of the diet likely resided in its ability to inhibit colonization by these organisms. When 2 pigs fed the RA diet were transferred to the commercial WL diet, 1 died of acute clostridial enterotoxaemia, whilst the other developed SD. This provided further evidence for the protective effect of the highly digestible RA diet on development of SD.

Previous work by Prohaszka and Lukacs [16], suggesting that feeding a diet high in cellulose/hemicellulose (i.e. maize silage) can alter the expression of SD, must be treated with caution since the work did not involve controlled inoculation studies. Despite this, a diet high in cellulose/hemicellulose may result in a fermentation pattern, as assessed by VFA ratio of the digesta, similar to that obtained with the protective RA diet used in this study. Thus a major dietary residue entering the large intestine on the RA diet would have been cellulose/hemicellulose, since the remaining components of the ration (gelatinised starch and animal protein) would have been extensively digested in the small intestine. Both diets would result in a relatively slow rate of fermentation that is typical for cellulose, and would produce a similar pattern of VFAs and so therefore qualitatively a similar microbial flora.

It is known from work in gnotobiotic pigs, and in mice, that different components of the microflora of the large intestine can either enhance [36–8] or reduce [39] colonization by *S. hyodysenteriae*, and subsequently can influence the expression of SD. A similar explanation has been put forward as to why certain chemotherapeutic agents with no effect on *S. hyodysenteriae* can be used to control SD: these agents are thought to inhibit other components of the microflora that normally interact to enhance colonization by *S. hyodysenteriae* [40]. The RA diet has been shown to reduce overall levels of fermentation in the large intestine, hence its protective effect likely operates through some unspecified alteration in the microflora of the large intestine. Whilst this could be a direct effect, it also could be indirect. For example, both diet and microflora can influence production of mucins in the large intestine [41], and mobility of *S. hyodysenteriae* in the mucus is important for its ability to colonize [42]. Another possibility is that significantly drier contents of the colon in pigs on the RA diet acted to inhibit survival of the spirochaetes at these sites. Further work will attempt to define the alterations occurring in the microflora, and in patterns of fermentation in pigs fed the RA diet, and will seek to identify other commercially viable highly digestible diets that offer protection against SD.

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