The impact of diets varying in carbohydrates resistant to endogenous enzymes and lignin on populations of *Ascaris suum* and *Oesophagostomum dentatum* in pigs

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SUMMARY

The impact of diets varying in type and level of carbohydrates resistant to endogenous enzymes and lignin on the establishment and location of *Ascaris suum* and *Oesophagostomum dentatum* was investigated experimentally. Fifty worm-free pigs, from a specific pathogen-free farm were used. The animals were assigned randomly to 5 diets and infected with 600 infective *A. suum* eggs and 6000 infective larvae of *O. dentatum* per pig. The diets consisted of a traditional ground barley plus protein feed (diet A), commercial full-constituent pelleted feed (diet B), barley flour plus protein (diet C), barley flour, inulin (Raftiline® ST, ORAFTI, Tienen, Belgium), sugar beet fibre plus protein (diet D), and barley flour, wheat bran, and protein (diet E). The faecal egg excretion was followed and the pigs were slaughtered at 8 weeks p.i. and samples taken from the small and large intestine. Intestinal contents were analysed for worm burdens, worm location and female worm fecundity along with the concentration of insoluble (chromic oxide) and soluble (polyethylene glycol-4000) markers, lignin, non-starch polysaccharides (NSP) and organic acids. In all diet groups *A. suum* worm burdens were low and comparable, whereas the *O. dentatum* worm burdens were significantly higher in pigs fed the diets with high levels of NSP and lignin (diets A and E) than in pigs fed diets B, C, and D. The present study suggests that a diet rich in lignin and insoluble NSP's provides favourable conditions for the establishment of *O. dentatum* in the large intestine of pigs while it is unlikely that the concentration of short-chain fatty acids and pH plays any major role.

Key words: pigs, *Ascaris suum*, *Oesophagostomum dentatum*, dietary fibre, oligosaccharides, nutrition.

INTRODUCTION

Experimental studies have shown repeatedly that pigs inoculated with *Ascaris suum* exhibit variable establishment of intestinal worm burdens (Anderson *et al*. 1973; Nilsson, 1982). This has usually been attributed to different infectivity of the larval batches used, variations in individual host immune reactions, or both. A pilot study (Petkevičius *et al*. 1985; Bjørn, Roepstorff & Nansen, 1996), however, indicated that diets which differed mainly in the content of carbohydrates, which were not digested by endogenous enzymes (raffinose oligosaccharides and non-starch polysaccharides (NSP)) in the small intestines, gave significant differences in the establishment, gut location, and fecundity of *Oesophagostomum dentatum* in the large intestine of pigs. These results were in accordance with data which have shown that growth and maturation of gastrointestinal parasites can be dependent on the type and the amount of carbohydrates (Von Brand, 1979; Nesheim, 1984; Crompton, 1991). It has been found previously that the carbohydrate composition and the level of lignin plays an important role for the physico-chemical environment in the gut lumen and for the microbial fermentation in the large intestine of pigs (Bach Knudsen *et al*. 1991, 1993; Johansen *et al*. 1996). These changes may have implications for the health of the epithelial cells lining the large intestine as these cells obtain most of their energy from short-chain fatty acids (SCFA), in particular butyrate produced by microbial fermentation in the

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large intestine (Sakata, 1995). Moreover, a diet rich in carbohydrates, mainly undigested in the small intestines, stimulates peristalsis and increases faecal bulk (Graham, Hesselman & Åman, 1986; Bach Knudsen & Hansen, 1991). Experiments with pigs, however, have shown repeatedly that a diet rich in NSP and lignin are beneficial to many intestinal parasites, particularly those which have predominantly anaerobic metabolism, such as *Oesophagostomum* spp. (Herbert *et al*., 1969).

The aim of the present investigation was to substantiate a previous study on the effect of the type of level of carbohydrates entering the large intestine on the establishment and location of *A. suum* and *O. dentatum* in the gut (Petkevičius *et al*. 1995; Bjørn *et al*. 1996). Pigs were fed the 2 conventional diets used in the previous study along with 3 experimentally formulated diets. The 2 conventional diets consisted of ground barley supplemented with a protein mixture and a pelleted full constituent feed mixture. The 3 experimental diets were formulated to provide different levels and types of carbohydrates to the large intestine. The experimental diets were based on barley flour supplement with a protein mixture, and 2 diets where parts of the barley flour were replaced with either inulin (Raftiline® ST, ORAFTI, Tienen, Belgium) plus sugar beet fibre or wheat bran, respectively. Inulin plus sugar beet fibre were chosen as they were expected to be rapidly and almost completely fermented by the microflora in the large intestine (Graham *et al*. 1986), whereas wheat bran was expected to be more resistant to fermentation in the large intestine (Bach Knudsen & Hansen, 1991).

MATERIALS AND METHODS

Experimental design

Fifty male Landrace/Yorkshire/Duroc cross-breed pigs were purchased from a commercial specific pathogen-free farm. The animals were approximately 12-weeks-old on arrival and their average weight was 37 kg (S.D. 5·1 kg) at the start of the experiment. The pigs were divided randomly into 5 groups each of 10 animals. The pens were disinfected thoroughly prior to the introduction of the pigs and at regular intervals during the study period. The animal's excreta was removed daily and pigs were kept on the clean floor without bedding. The experimental pigs had free access to water via drinking nipples.

Prior to inoculation, faecal examinations of all pigs were performed and helminth eggs were not found. The pigs were kept for 3 weeks in the pens prior to the parasite inoculations, to allow them to adapt to the environment and to the different diets.

After the adaptation period, all pigs in each group were inoculated by stomach tube with a single dose of 6000 infective larvae of *O. dentatum* EH-strain (Roepstorff, Bjørn & Nansen, 1987) and 600 infective *A. suum* eggs of a strain isolated in 1993 from an organic pig farm.

The 5 groups were fed 2 conventional diets and 3 experimental diets. The 2 conventional diets consisted of either whole grain ground barley plus protein concentrate (3:1) (diet A) or a commercial full-constituent pelleted feed mixture (diet B). The 3 experimental diets were composed of a low dietary fibre (DF) diet based on barley flour supplemented with a protein concentrate (3:1) (diet C) and 2 high fibre diets consisting of barley flour, inulin (Raftiline® ST, ORAFTI, Tienen, Belgium) and dried sugar beet fibre (80·1 %:7 %:12·9 %) plus protein concentrate (3:1) (diet D) and barley flour and wheat bran (64 %:36 %), plus protein concentrate (3:1) (diet E). To the diets were added 2 g/kg of chromic oxide and 8 g/kg of polyethylene glycol 4000 (PEG) as insoluble and soluble markers, respectively. The chemical composition of the experimental diets are shown in Table 1. The diets were fed twice daily (with 12 h intervals). The amount of feed offered was according to the Danish standard ration for growing pigs, initially 1·9 kg/pig/day at 37 kg, increasing to 2·6 kg/pig/day over the 8 week period.

Faecal samples were collected from the rectum every week for worm egg counts and for evaluation of faecal consistency. Samples of the feed mixtures were collected for chemical analysis, the last 2 weeks before slaughter. The pigs were weighed every week throughout the experiment. All pigs were slaughtered at 8 weeks post-inoculation (p.i.).

Parasitological techniques

Faecal egg counts were monitored using a modified McMaster technique with a lower level of detection of 20 eggs/g (epg). At the end of the experiment, after an overnight fast, all animals were killed by CO_2 suffocation, followed by exsanguination. The entire small and large intestines were removed immediately and separated from the mesenteries. The small intestine was divided into 4 sections of approximately equal length (designated 1, 2, 3, 4 from the anterior end). The contents of each section were collected by passing luke-warm water twice through the intact section, and then washing over a sieve of mesh size 212 μm. The large intestine was divided into the caecum (Ce) and into 4 (Co1-0–20 %, Co2-21–40 %, Co3-41–60 %, and Co4-5-61–100 %) segments of colon and rectum. From the contents of each section representative subsamples of 20 % were taken. Samples for detailed chemical analysis were taken from 6 randomly selected pigs in each diet group. These samples were taken from the distal small intestine ($\sim$ 4 m), the caecum and the various parts of the colon and are referred to as ileum (I), caecum (Ce) and colon 1–5 (Co1–5).

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Oesophagostomum dentatum was collected from digesta and washings of the caecum and colon using a modified agar-gel method described by Slotved *et al*. (1996). All the agar-gel samples were incubated in physiological saline for 24 h at 37 °C. In order to estimate how quickly worms migrate from the agar, 20 % of the gels were transferred to new containers after 1 h of incubation. This allowed worms that migrated in the first h and in the following 23 h to be counted separately. After harvesting, worms were transferred to screw-capped plastic tubes, and fixed and stored in iodine solution (6·25 % iodine + 31·25 % potassium iodide + 62·5 % distilled water). The samples were decolorized with 3 % thiosulfate solution and the worms subsequently counted. The developmental stages of *O. dentatum* were determined using the criteria of Goodey (1926). The sex of adults of both species was recorded. Specimens of *A. suum* were weighed and their length measured using a ruler. White spots on the surface of the livers were counted at the time of slaughter.

Analyses of feed and intestinal contents

All analyses were performed in duplicate. Analyses of lactic acid (LA) and short-chain fatty acids (SCFA) were performed on wet materials by the gas chromatographic method of Richardson *et al*. (1989) and PEG by the turbidimetric method of Hyden (1955); pH was also performed on wet materials, whereas the other analyses were carried out on freeze-dried materials. The dry matter (DM) content of feed was determined by drying at 105 °C to constant weight and of digesta materials by freeze-drying.

Protein (N × 6·25) was determined by the Kjeldahl method using a Kjell-Foss 16200 autoanalyser, ash in accordance to the Association of Official Analytical Chemists (1975), chromic oxide (Cr_2O_3) measured by the method of Schürch, Loyd & Crampton (1950), whereas fat (hydrochloric acid-fat) was extracted with diethyl ether after acid-hydrolysis (Stoldt, 1952). Low molecular weight (LMW) sugars were quantified by high performance liquid chromatography according to the method of Bach Knudsen & Li (1991), inulin as described by Bach Knudsen & Hessov (1995), and starch analysed by an enzymatic method described by Bach Knudsen *et al*. (1994). Total, soluble and insoluble non-starch polysaccharides (NSP) and their constituent sugars in plant materials and diets and total NSP in digesta were determined as alditol acetates for neutral sugars and by the colormetrical method for uronic acids using a modification of the Uppsala (Theander & Åman, 1979; Theander & Westerlund, 1986) and Englyst (Englyst, Wiggins & Cummings, 1982) procedures as described by (Bach Knudsen *et al*. 1994). Cellulose was calculated as:

$$\text{cellulose} = \text{NSP}_{\text{glucose (12 mol l}^{-1})} - \text{NSP}_{\text{glucose (1 mol l}^{-1})},$$

non-cellulose polysaccharides (NCP) were calculated as:

$$\text{NCP} = (\text{arabinose} + \text{xylose} + \text{mannose} + \text{galactose} + \text{glucose} + \text{uronic acids})$$

and soluble NSP (S-NSP) as:

$$\text{S-NSP} = \text{Total-NSP} - \text{I-NSP},$$

where I-NSP are insoluble NSP.

Klason lignin was measured gravimetrically as the residue resistant to 12 mol l^{-1} H_2SO_4 (Theander & Åman, 1979; Theander & Westerlund, 1986).

Statistical analysis

In all analyses data from different pigs are assumed to be independent. All analyses are performed with analysis of variance techniques assuming either univariate or multivariate normality.

Faecal egg counts (epg) were recorded weekly until slaughter at 8 weeks after inoculation. Due to non-response data epg are given only from weeks 3 to 8 for *O. dentatum* and from weeks 7 and 8 for *A. suum*. The diet effects were analysed by a multivariate analysis of variance assuming that the transformed counts $\log_{10}(\text{epg}+10)$ for each pig are multivariate normal with an unconstrained covariance common to all pigs and with mean

$$\text{E}(\log_{10}(\text{epg}+10)_{ijk}) = \alpha_i + \beta_j + \gamma_{ij},$$

where α_k is the diet main effect, β_j is the main effect of week, and γ_{ij} represents the diet week interaction, index k represents the pig.

Concerning the data on worm burden in each intestinal section, only 6 of the 50 pigs had *A. suum* worms; hence only limited information is available and no statistical analysis was performed. *O. dentatum* worms were observed in sections Ce and Co1 to Co5, but data from sections Co4 and Co5 were combined. The diets were contrasted for each section and analysed by means of a one-way analysis of variance.

As an indication of the mean position of the *O. dentatum* worms along the large intestine, the average location of worms for each pig was calculated as

$$\text{'Location'} = \frac{\sum_{s\in\{\text{Ce, Co1, Co2, Co3, Co4–5}\}} \text{I(s)} \times swc(s)}{\sum_{s\in\{\text{Ce, Co1, Co2, Co3, Co4–5}\}} swc(s)}$$

where I(Ce) = 1, …, I(Co4–5) = 5 and $swc(s)$ is the worm count in section s. The effect of the diet on the average location was investigated by one-way analysis of variance.

The effect of the diet on the proportion of female *O. dentatum* worms in each pig was investigated by analysis of variance on the arcsine transformed proportions.

For pigs with a positive female adult worm burden at week 8, fecundity of *O. dentatum* or *A. suum* female worms was estimated by dividing the week 8

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faecal egg count by the adult female worm burden. The diets were contrasted by means of a one-way analysis of variance on log-transformed fecundity data.

Mean transit time (MTT) of liquid and solids in the caecum and colon was calculated as:

$$\text{MTT(h)} = \frac{\text{Mass of marker in segment (mg)}}{\text{Daily intake of marker (mg)}} \times 24 \text{ h}.$$

The content of polysaccharide residues was calculated as anhydrosugars and all apparent digestibilities were calculated relative to Cr_2O_3 content:

$$\text{Digestibility of X(s)} = \left[1 - \frac{Cr_2O_3 \times X(s)}{Cr_2O_3(s) \times X(d)}\right] \times 100,$$

where X(d) and X(s) are the concentrations of specific nutrients in the diet *d* and in digesta materials (s) from segments of the GI tract (s∈{I, Ce, Co1, Co2, Co3, Co4, Co5}). When calculating starch digestibility it was assumed that free glucose in digesta materials was derived from starch.

Data on concentrations and digestibilities of nutrients were analysed by means of multivariate analysis of variance, since observations from the same pig must be assumed to be correlated. The mean response is assumed to be a linear function of the effects of diet and section: $EY_{ijk} = \alpha_i + \beta_j + \gamma_{ij}$, where α_i is the diet main effect, β_j is the section main effect and γ_{ij} is the diet section interaction, index k represents the pig. The covariance model is a first-order auto-regressive model, hence $\text{Var}(Y_{ijk}) = \sigma^2$ and $\text{Cov}(Y_{ijk}, Y_{ij,k}) = \sigma^2\rho^{|j-j|}$, this model gave a nice compromise between simplicity and appropriateness of fit. The interpretation is in accordance with the sections forming a sequence of compartments only interacting with the closest neighbour. Model checking by residual analysis was done in all analyses.

Ethical considerations

The experiment was approved by the Danish Animal Ethical Committee (animal experiment permission 1994-101-115). Meetings were held with the agricultural and laboratory technicians to explain the purpose of the experiment and what was required from the persons handling the pigs.

RESULTS

Composition of diets

The composition of the diets is shown in Table 1. Protein varied from 161 g/kg DM (diet D) to 190 g/kg DM (diet B), fat from 37 g/kg DM (diet D) to 61 g/kg DM (diet B), total carbohydrates (LMW-sugars, starch, inulin and NSP) from 628 g/kg DM (diet B) to 730 g/kg DM (diet D) and Klason lignin from 9 g/kg DM (diet C and D) to 25 g/kg DM (diet A). The ratio between total carbohydrates and carbohydrates resistant to endogenous enzymes (sum of raffinose oligosaccharides, inulin and NSP) was 0·21 for diet C, ~ 0·27 for diet A and B and 0·31–0·33 for diet D and E with NSP being the predominant source. Diet D contained the highest level (154 g/kg DM) of carbohydrates (raffinose oligosaccharides, inulin and S-NCP) that is presumed to be readily available for the microflora in the large intestine.

Animal health and performance

There were no clinical signs of parasitosis in any of the pigs during the experiment. The faecal consistency was softer and more 'fatty' in pigs when fed diets B and D than when fed diets A and E, and thicker from the pigs on diet C. The dry matter content of faecal materials was significantly lower ($P < 0·001$) for the pigs on diet D (14·3 %) compared to that on diets A, B, C and E (22·3–24·1 % dry matter).

The weight of the pigs allocated to diets A–E was at the start of the experiment: diet A 37·4 kg (S.D. 5·1); diet B 39·9 kg (S.D. 6·1); diet C 34·6 kg (S.D. 3·0); diet D 37·2 kg (S.D. 3·3) and diet E 36·2 kg (S.D. 5·7). At slaughter the weight of the pigs on the 5 diets were 72·2 kg (S.D. 12·7), 79·3 kg (S.D. 12·1), 72·9 kg (S.D. 6·8), 76·3 kg (S.D. 6·2) and 68·7 kg (S.D. 9·8). The differences between groups were not significant.

Ascaris suum eggs were first detected 6 weeks after inoculation (Fig. 1), and subsequently there was a gradual and parallel rise in mean egg counts of all experimental groups. Time after inoculation affected *A. suum* egg counts ($P < 0·01$), but the diet and time × diet interaction were not significant.

Oesophagostomum dentatum eggs were first detected in individual pigs 3 weeks after inoculation (Fig. 2), and were present in the faeces of all pigs from the fourth week and onwards. A significant difference ($P < 0·001$) was obtained between group A and the other dietary groups in relation to time post-inoculation, the diet and time × diet.

Worm burdens

Details on the intensity of infection and location of *A. suum* recovered at week 8 p.i. are given in Table 2. No significant difference ($P > 0·05$) was found in the prevalence of *A. suum* between the experimental groups. A few pigs in each group contained worms all of each were adult. Female worms were more numerous than males: 73 % in groups A and C, 67 % in group B of the total worm burden. In both sexes, there was no difference between groups in worm length or worm weight.

At the time of slaughter, the mean number of superficial white spots per liver (Table 2) was low in all groups, ranging from 1·9–4·6.

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Table 1. Chemical composition (g/kg DM) of the experimental diets

Diet*	A	B	C	D	E
Ash	47	60	48	44	52
Protein (N × 6·25)	163	190	179	161	180
Fat	46	61	43	37	48
LMW-sugars	34	47	38	29	37
Starch	485	425	532	472	442
Inulin	N.D.	N.D.	N.D.	67	N.D.
Cellulose	37	34	26	24	35
NCP	133 (50)†	122 (40)	107 (51)	138 (77)	160 (48)
Rhamnose	1 (0)	2 (0)	1 (0)	2 (0)	3 (0)
Fucose	1 (0)	1 (1)	1 (0)	3 (1)	2 (1)
Arabinose	25 (6)	26 (6)	19 (6)	34 (16)	37 (6)
Xylose	45 (5)	41 (6)	23 (5)	19 (3)	56 (6)
Mannose	5 (2)	5 (1)	6 (2)	5 (1)	5 (1)
Galactose	10 (5)	14 (8)	12 (6)	13 (8)	12 (5)
Glucose	38 (27)	22 (12)	34 (26)	37 (26)	37 (22)
Uronic acids	10 (5)	13 (6)	13 (6)	29 (22)	13 (7)
Total NSP	170	156	133	162	195
Klason lignin	25	21	9	9	24
Dietary fibre	195	177	142	174	217

* LMW-sugar, low molecular weight sugars; NSP, non-starch polysaccharides; NCP, non-cellulosic polysaccharides.
† Values in parentheses are soluble NCP.
N.D., Not determined.

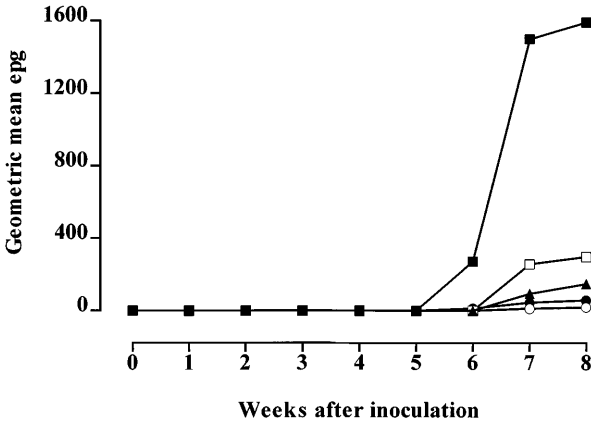

Fig. 1. Geometric mean *Ascaris suum* egg counts of pigs fed ground barley plus protein (group A, ■), commercial pelleted feed (group B, ▲), barley flour plus protein (group C, □), barley flour plus Raftiline plus sugar beet fibre plus protein (group D, ○) and barley flour plus wheat bran plus protein (group E, ●).

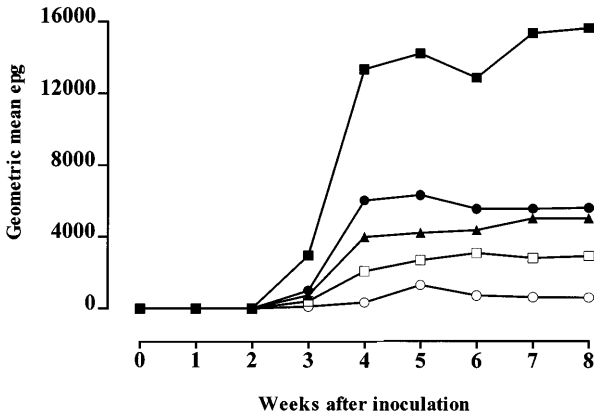

Fig. 2. Geometric mean *Oesophagostomum dentatum* egg counts of pigs fed ground barley plus protein (group A, ■), commercial pelleted feed (group B, ▲), barley flour plus protein (group C, □), barley flour plus Raftiline plus sugar beet fibre plus protein (group D, ○), and barley flour plus wheat bran plus protein (group E, ●).

It was observed that 98·8% of the *O. dentatum* worms that migrated out of the agar-gel did so during the first hour of incubation.

The *O. dentatum* worm burdens are shown in Fig. 3. The total number of worms recovered was significantly higher ($P < 0·001$) in groups A and E, than in groups B, C, and D. The total caecal worm burden was not significantly affected by the diet. In the anterior part of the large intestine (Co1), those pigs given the traditional ground barley plus protein diet (A) had the highest ($P < 0·001$) worm burdens compared with the other groups. Among the other groups, the highest worm burdens were observed in the barley flour plus wheat bran diet pigs (E), but results were not statistically significant ($P > 0·05$). In Co2, pigs from the group D showed lower worm counts than in the group E ($P < 0·05$), but there were no statistically significant differences between the other diet groups. Variable numbers of *O. dentatum* were observed in Co3 and Co4, but the differences between diet groups were not significant.

The mean (S.D.) location of *O. dentatum* in the sections of the large intestine was analysed in Fig. 3. The values for diets A–E were: 2·4 (0·4), 2·9 (0·3), 3·2 (0·4), 2·8 (0·3) and 3·0 (0·3), respectively. The differences observed in worm location were statistically significant between the pigs fed diet A and diets C and E ($P < 0·05$), whereas there was no

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Table 2. Number of *Ascaris suum* in pigs with positive worm counts (all other pigs in each group had no *A. suum* worms), and mean and standard deviation (S.D.) number of white spots in the livers of all pigs

Diet	Total no. of pigs	No. with positive *A. suum* worm counts	Worm burdens of pigs with positive counts (section of small intestine)						No. of white spots (S.D.)
			Pig	0–25 %	26–50 %	51–75 %	76–100 %	Total	
A	10	2	1	21	15	1	0	37	2·4 (1)
			2	0	0	1	0	1	
B	10	1	1	1	3	20	3	27	3·8 (3)
C	10	2	1	0	5	0	0	5	2 (2)
			2	7	47	8	0	62	
D	10	1	1	1	0	0	0	1	4·6 (5)
E	10	0	0	0	0	0	0	0	1·9 (2)

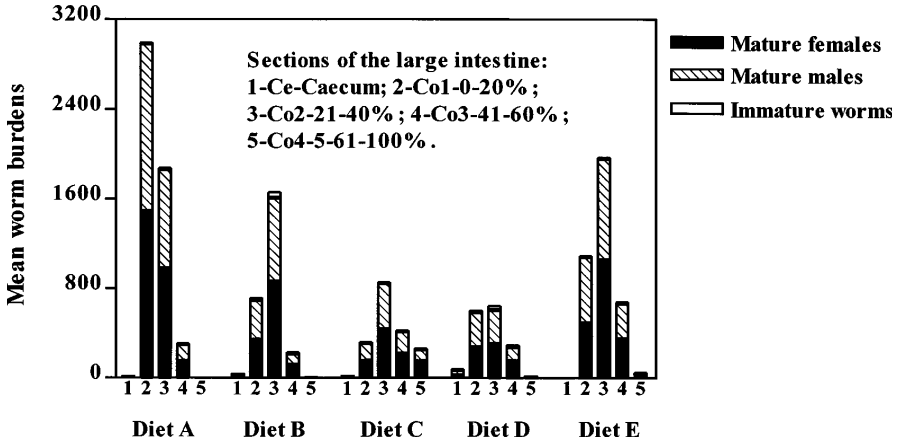

Fig. 3. The mean location of *Oesophagostomum dentatum* worm burdens of pigs fed with 5 experimental diets.

significant difference between the location of worms of the pigs fed either diets B and D relative to diet A or diets C and E.

There was no significant difference in the average numbers of female and male *O. dentatum* in all the experimental groups. Examination of the sexual maturity of *O. dentatum* showed that at 8 weeks p.i. 99 % of the worm population had reached maturity.

Worm fecundity

At week 8 there were 8, 2, 2, 2 and 3 pigs with positive *A. suum* faecal egg counts from the groups offered diets A–E, respectively. Worms were recovered from 2, 1, 2, 1, and 0 of these animals and *A. suum* fecundity was not significantly affected by the diet. Back-transformed mean *O. dentatum* fecundity (epg per adult female worm (standard deviation)) was 6·0 (3·4), 3·2 (2·2), 1·8 (1·5), 0·8 (0·8), and 2·2 (1·6) in the 5 experimental groups, respectively. The diet had a significant effect on the fecundity of group A compared to the other groups (B–E).

Worm burden, faecal egg counts and dietary constituents

The total worm number within the individual segments of the large intestine was, in general, poorly correlated to the concentration of dietary residues. Of the different segments, the highest correlations were found for total NSP in Co1 where the correlation was 0·33 ($P < 0·072$). Significantly higher correlations were identified between undigested fibre residues (NSP plus lignin) measured in Co5 and the total worm burden ($r = 0·675$; $P < 0·0001$) and faecal egg counts ($r = 0·596$; $P < 0·0005$).

Accumulation of markers in the gastrointestinal tract

The accumulation of chromic oxide and PEG at the ileum and in the various segments of large intestine is shown in Figs 4 and 5. The concentration of chromic oxide in ileal digesta was ~ 4 g/kg DM for diet A and B. With diet A, there was hardly any increase in the concentration in the caecum, whereas

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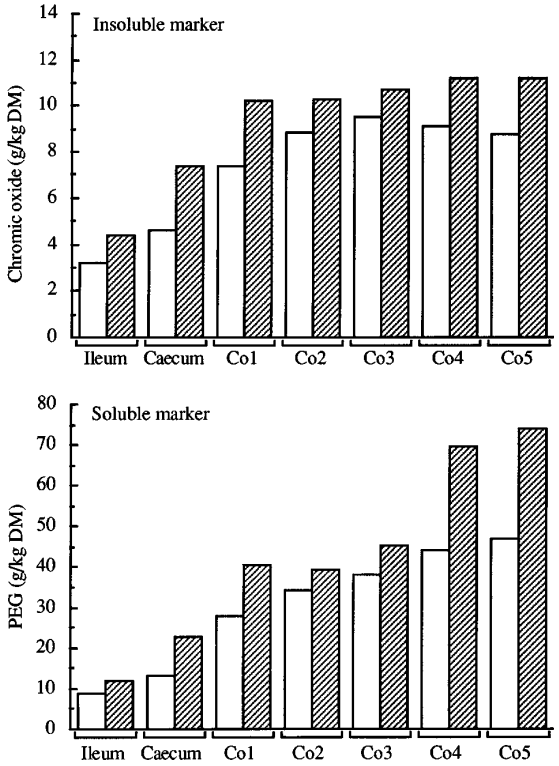

Fig. 4. Concentration (g/kg DM) of insoluble and soluble markers in ileum, caecum and colon 1–5 when feeding diets A (□) and B (▨). *P* values for insoluble marker are: Diet = 0·0247, Segment = 0·0001, Diet × Segment = 0·355, S.D. = 1·84 and $\rho = 0{\cdot}71$ and for soluble marker: Diet = 0·16, Segment = 0·0001, Diet × Segment = 0·81, S.D. = 5·3 and $\rho = 0{\cdot}53$.

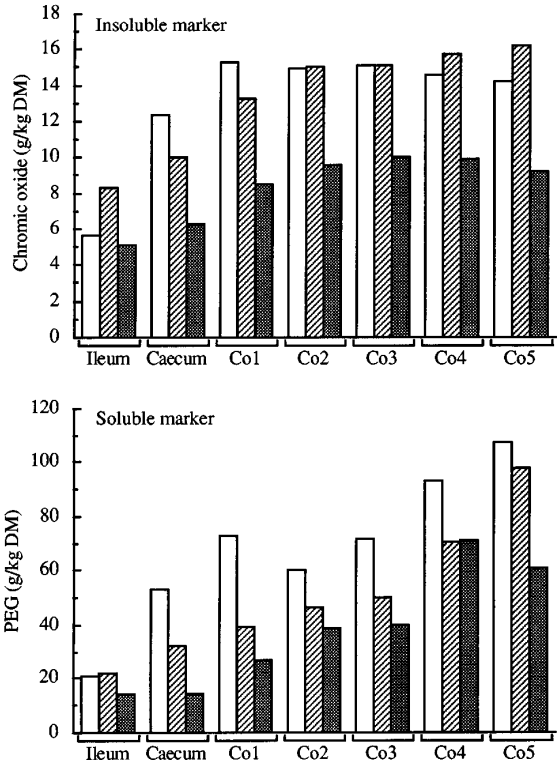

Fig. 5. Concentration (g/kg DM) of insoluble and soluble markers in ileum, caecum and colon 1–5 when feeding diets C (□), D (▨) and E (■). *P* values for insoluble marker are: Diet = 0·0001, Segment = 0·0001, Diet × Segment = 0·001, S.D. = 1·70 and $\rho = 0{\cdot}74$ and for soluble marker: Diet = 0·015, Segment = 0·0001, Diet × Segment = 0·21, S.D. = 5·8 and $\rho = 0{\cdot}34$.

it increased to 7 g/kg DM in Co1 and further to 9–10 g/kg DM in the remaining parts of the colon. Higher concentrations of chromic oxide were found with diet B in the caecum (7·5 g/kg DM) and in all segments of the colon (10·5–11·5 g/kg DM).

The concentration of chromic oxide in ileal digesta from diets C and E was ~ 5 g/kg DM compared to 8·3 g/kg DM for diet D. With diet C, there was a rapid increase to 12·3 g/kg DM in the caecum and further to 13·5–15·0 g/kg DM in all segments of colon. With diet D and E, the increase in chromic oxide concentration was slower; the plateau level of 15–16 g/kg DM and 9·2–10·0 g/kg DM for the 2 diets, respectively, was not reached until Co2.

PEG accumulated to a higher degree than chromic oxide in all segments of the large intestine. This was particularly the case in Co4 and Co5 where there was a strong increase in the PEG concentration.

Accumulation of lignin and NSP residues

The concentration of lignin and NSP components are shown in Tables 3 and 4. Lignin was present at a level of 48–59 g/kg DM in ileal digesta from the pigs fed diet A and B, 93–99 g/kg DM in caecal digesta while it increased to 133–144 g/kg DM in colon with diet A and to 162–175 g/kg DM with diet B. The concentration of NSP in ileal digesta was 384–414 g/kg DM; 85–104 g/kg DM was present as cellulose and 299–302 g/kg DM as NCP. The concentration of NSP in the caecum and colon was higher at all sites with diet B than with diet A. This was primarily due to a higher concentration of NCP whereas the concentration of cellulose was almost the same with the 2 diets. The most significant difference between the NCP residues of the 2 diets was seen for xylose and glucose; the former being consistently higher for diet A while the opposite was the case with $NCP_{glucose}$.

The level of lignin in ileal digesta was ~ 40 g/kg DM with diets C–E (Table 4). In the caecum, the level was 64 g/kg DM (diet D), 75 g/kg DM (diet C) and 86 g/kg DM (diet E) and in Co1 122 g/kg DM (diet C), 140 g/kg DM (diet D) and 158 g/kg DM (diet E). The increase in concentration in the remaining colon was marginal. NSP accounted for 299 g/kg DM (diet C) and up to 383 g/kg DM (diet E) of the ileal digesta. In the caecum, the concentration of NSP increased to 311–520 g/kg DM while there was a gradual decrease in the concentration, in particular for diets D and E, toward the distal part of the colon. The level of cellulose in

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Table 3. The concentration (g/kg DM) of lignin, total non-starch polysaccharides (NSP), cellulose and non-cellulosic polysaccharides (NCP) and the digestibility of starch, non-starch polysaccharides (NSP) and organic matter (OM) in the gastrointestinal tract of pigs fed the traditional ground barley plus protein supplemented feed (A) and the commercial full-constituent pellets feed (B)

	Content (g/kg DM)								Digestibility					
	Lignin		NSP		Cellulose		NCP		Starch*		NSP		OM	
Diet	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Ileum	59	48	414	384	104	85	302	299	0·89	0·96	−0·21	0·08	0·53	0·64
Caecum	93	99	498	509	149	143	340	366	0·92	0·98	−0·05	0·23	0·66	0·79
Colon1	133	170	499	505	136	138	360	367	1·00	1·00	0·37	0·47	0·80	0·85
Colon2	142	167	460	470	129	129	331	341	1·00	1·00	0·49	0·53	0·83	0·86
Colon3	141	162	433	469	123	132	309	337	1·00	1·00	0·57	0·57	0·85	0·88
Colon4	144	173	439	482	128	138	311	344	1·00	1·00	0·53	0·58	0·84	0·88
Colon5	135	175	423	453	124	129	293	324	1·00	1·00	0·54	0·61	0·84	0·88
S.D.	27		63		17		52		0·01	0·003	0·22		0·07	
ρ	0·62		0·52		0·25		0·66		0·39		0·65		0·62	
P values for:														
Diet	0·1167		0·60		0·98		0·48		0·0005		0·18		0·029	
Segment	0·0001		0·0001		0·0001		0·002		0·0137		0·0001		0·0001	
D × S	0·19		0·80		0·62		0·8		0·38		0·57		0·33	

* Starch was only present at trace levels in the colon and the analysis is consequently based on ileum and caecum.
S.D., Standard deviation.

Table 4. The concentration (g/kg DM) of lignin, total non-starch polysaccharides (NSP), cellulose and non-cellulosic polysaccharides (NCP) and the digestibility of starch, non-starch polysaccharides (NSP) and organic matter (OM) in the gastrointestinal tract of pigs fed barley flour plus protein (C), barley flour, inulin, sugar beet fibre and protein (D) and barley flour, wheat bran, and protein (E)

	Content (g/kg DM)												Digestibility								
	Lignin			NSP			Cellulose			NCP			Starch*			NSP			OM		
Diet	C	D	E	C	D	E	C	D	E	C	D	E	C	D	E	C	D	E	C	D	E
Ileum	39	40	42	299	326	383	71	78	83	223	248	300	0·96	0·99	0·98	0·04	0·33	0·11	0·65	0·69	0·57
Caecum	75	64	86	311	370	520	100	119	138	212	250	382	0·99	0·99	0·98	0·50	0·44	0·14	0·83	0·79	0·69
Colon1	122	140	158	266	332	493	85	100	128	188	232	365	1·00	1·00	1·00	0·64	0·62	0·37	0·86	0·84	0·76
Colon2	136	144	160	259	323	435	81	96	111	179	228	325	1·00	1·00	1·00	0·64	0·67	0·48	0·86	0·86	0·79
Colon3	143	146	157	295	313	430	92	92	111	207	221	320	1·00	1·00	1·00	0·64	0·68	0·51	0·87	0·86	0·80
Colon4	141	159	169	297	301	417	92	88	107	208	213	310	1·00	1·00	1·00	0·62	0·70	0·53	0·86	0·87	0·80
Colon5	136	154	157	304	292	434	94	87	114	210	206	321	1·00	1·00	1·00	0·60	0·73	0·47	0·85	0·87	0·78
S.D.	31			53			21			41						0·14			0·06		
ρ	0·69			0·66			0·70			0·68						0·40			0·43		
P values for:																					
Diet	0·37			0·0001			0·036			0·0001						0·0007			0·0001		
Segment	0·0001			0·0001			0·0001			0·0003						0·0001			0·0001		
D × S	0·57			0·12			0·48			0·087						0·0002			0·84		

* No model was fitted since observations were very close to 1·00.
S.D., Standard deviation.

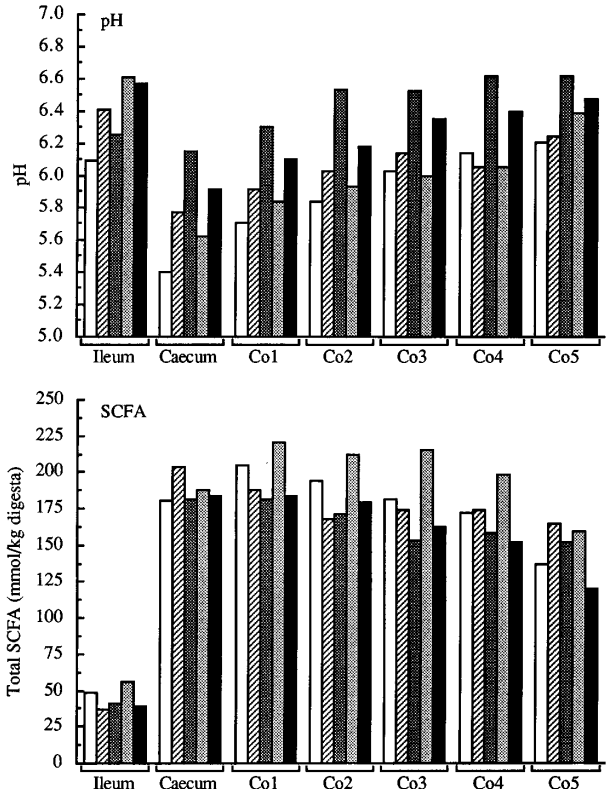

Fig. 6. pH and the concentration (mmol/kg digesta) of short chain fatty acids (SCFA) in ileum, caecum and colon 1–5 when feeding diets A (□), B (▨), C (■), D (⬚) and E (■). *P* values for pH are: Diet = 0·0005, Segment = 0·0001, Diet × Segment = 0·0010, S.D. = 0·26 and $\rho = 0{\cdot}64$ and for total short-chain fatty acids: Diet = 0·0110, Segment = 0·0001, Diet × Segment = 0·51, S.D. = 31·9 and $\rho = 0{\cdot}47$.

ileal digesta was the same for the 3 diets. In the caecum and colon, however, the concentration increased from diet C to diet E. This tendency was even more pronounced with NCP where there clearly was an increase in the concentration at all sampling points from 179–223 g/kg DM for diet C to 300–382 g/kg DM for diet E. Of the NCP residues the most significant difference was found for arabinose, xylose and glucose.

Digestibility of polysaccharides and organic matter

The digestibility of starch and NSP at the ileum was 0·89 and −0·21, respectively, with diet A and 0·96 and 0·08, respectively, with diet B (Table 3). In the caecum, the digestibility increased for diet A to 0·92 for starch and to −0·05 for NSP and for diet B to 0·98 (starch) and 0·23 (NSP). Starch was completely degraded in proximal colon, whereas with NSP there was a gradual rise through the colon for diet A to 0·54–0·61 for both diets. The digestibility of organic matter followed the trend for starch and NSP; from 0·53 and 0·64, respectively, at the ileum to 0·84 and 0·88 for the 2 diets in mid and distal colon.

The digestibility of starch at the ileum varied from 0·96 to 0·99 for diets C–E, in the caecum it was 0·98–0·99; the remaining starch being degraded in the proximal colon. The digestibility of NSP at the ileum was 0·04 for diet C and 0·11 for diet E, whereas it was significantly higher (0·33) for diet D. The digestibility of diet C increased rapidly to 0·50 in the caecum reaching a plateau level of 0·60–0·64 in the proximal colon. With diets D and E, the increase was slower, the digestibility being 0·44 (diet D) and 0·14 (diet E) in the caecum, and 0·62 and 0·37, respectively, in the proximal colon. For both diets, there was a gradual rise in the NSP digestibility to reach 0·70–0·73 (diet D) and 0·47–0·51 (diet E) in the distal colon. The digestibility of organic matter was 0·57 for diet E at the ileum and 0·65–0·69 for diets C and D. From the ileum and onwards, the digestibility increased by ∼ 0·20 units for diet C, ∼ 0·18 units for diet D and ∼ 0·21 units for diet E. The digestibility of the inulin (diet D) was 0·85 at the ileum increasing to 0·99 in the caecum.

Organic acids and pH

The concentration of SCFA in ileal digesta was 38–56 mmol/kg digesta (Fig. 6) and of LA in the range from 16 to 51 mmol/kg digesta. LA disappeared almost completely in the large intestine, whereas there was a sharp rise to 175–200 mmol SCFA/kg digesta in the caecum, decreasing gradually to 120–160 mmol/kg digesta in Co5. In ileal materials, acetate accounted for 85–90 % of SCFA. The molar proportion changed significantly from the ileum to the large intestine in which the following molar proportions were found; acetate 46–54 %; propionate 27–34 %; butyrate 8–17 % and branched-chain fatty acids (BCFA), 1–5 %. Whereas the molar proportion of acetate was reasonably constant in all segments of the large intestine, there was a trend toward a lower molar proportion of proprionate in the distal colon compared to the caecum and proximal colon. The opposite was the case with BCFA; the molar proportion of this acid increased from 1 to 2·3 % in the caecum and from 3·4 to 4·7 % in the distal colon. The most significant dietary effect was identified for diet A where acetate was lower and butyrate higher than for the other diets and for diet C and D where, respectively, the highest and lowest molar proportions of BCFA were seen in most segments of the large intestine as compared to the other 3 diets.

The pH was 6·1–6·6 in ileal digesta (Fig. 6). In the caecum the pH decreased to 5·4 for diet A, 5·7 for diet B, 6·1 for diet C, 5·6 for diet D, and 5·9 for diet E. For all diets, there was a gradual increase in pH to reach a level of 6·2–6·6 in Co5. The pH in digesta was at all sampling points higher with diet C (6·2–6·7) and diet E (5·9–6·5) when compared to the rest of the diets.

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Table 5. Mean transit time (h) in caecum and colon

	Diet					
	A	B	C	D	E	S.D.
Insoluble marker						
Caecum	1·6	1·6	1·5	2·0	1·4	0·86
Colon	12·1‡	10·9‡	11·3‡	15·9‡	9·2‡	2·92
Total large intestine	13·7†,‡	12·5†,‡	12·8†,‡	17·7†	10·6‡	3·11
Soluble marker						
Caecum	0·9	1·1	1·3	1·5	0·7	0·69
Colon	11·9‡	10·7‡	11·4†,‡	13·7†	9·3‡	3·04
Total large intestine	12·0	11·7	12·7	15·2	10·0	3·14

†,‡ Values in the same row with different superscripts are significantly different ($P < 0·05$).
S.D., Standard deviation.

Mean transit time

The MTT in the caecum was estimated to be 1·4–2·0 h for the insoluble phase marker and 0·7–1·5 h for the liquid phase marker (Table 5). The MTT in the colon was significantly higher with values for insoluble phase in the range 9·2–15·9 h and for the liquid phase 9·3–13·7 h. For both markers, MTT was significantly higher for diet D than for the other diets, in particular diet E.

DISCUSSION

The results of the present study suggest that there is an important interaction between the dietary composition and the parasite burden in the host. We varied the amount and composition of dietary residues potentially available for fermentation in the large intestine by changing type and level of NSP, the level of lignin, and by the inclusion of inulin. These changes undoubtly influenced the physicochemical environment, the transit time, and fermentation pattern in the large intestine which most likely are responsible for the variations among the pigs in the establishment, fecundity, and location of intestinal helminths in the gut. Thus, *O. dentatum* worm burdens were significantly higher in pigs fed the diets with the highest content of NSP and lignin (diets A and E) than in the other dietary groups. A thorough analysis of the data showed that the depression of *O. dentatum* epg of pigs fed the commercial full constituent feed mixture (diet B) and the 2 experimental diets with either the low level of DF (diet C) or the high level of DF in the form of fermentable fibres (diet D) already had started from 3 weeks p.i. and continued throughout the experiment. This suggests that the negative influence of these diets on the *O. dentatum* population started in the pre-patent period before the initiation of egg production. Moreover, the low level of DF (diet C) and the increased fermentation in the large intestine (diet D) led to a more distal location of *O. dentatum* and resulted in a reduced fecundity of the female worms.

The diet may potentially influence the establishment of *O. dentatum* in the large intestine either directly by the presence of the undigested dietary residues or indirectly through the stimulation of microbial growth which, in turn, may enhance the formation of SCFA and lower the luminal pH. A common feature of the diets giving rise to the highest (diet A and E) and lowest (diet C and D), respectively, faecal egg counts and worm burdens are the contrasting digestibilities, with diet A and E having low and diet C and D high digestibilities of NSP residues and organic matter. Diets A and E are characterized by high levels of lignin, cellulose, and insoluble NCP's which are known to be the dietary factors responsible for low faecal digestibility and increased luminal and faecal bulk (Graham *et al.* 1986; Bach Knudsen & Hansen, 1991). In contrast, diets C and D have lower levels of these dietary constituents and relatively more of the fibres are soluble components. Soluble NCPs are known to be readily fermented in the large intestine providing less luminal and faecal bulk (Graham *et al.* 1986; Bach Knudsen & Hansen, 1991). Diet B, with faecal egg counts, worm burdens and dietary characteristics more closely related to diets A and E than to the 2 other diets, fits reasonably well to this general view. These data thus suggest that a diet with a high level of lignin and insoluble NSP's may provide favourable conditions for the establishment of *O. dentatum* in the large intestine of pigs.

The caecum and proximal colon are, as shown in this and previous studies, the principal sites for carbohydrate fermentation in the large intestine of pigs (Ustin & Epifanov, 1991; Bach Knudsen, Jensen & Hansen, 1993; Canibe & Bach Knudsen, 1996). In these segments, the fermentation is consequently active, with a high production of SCFA and an acidic pH. As the digesta moves distally in the large intestine, the residues are depleted for the most readily fermentable nutrients, the concentration of

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SCFA starts to decline, and the pH begins to rise. This is also found in the present study where the plateau level for digestibility of NSP and the concentration of insoluble marker was reached in Co2, with little further increase beyond that point. It is noticeable that with all the diets there was consistently a low level of worms in the caecum, whereas with all but diet A, the highest worm burdens were found in Co2. The reason for this variation in location could be that the environment in the mid-colon is less variable than in the caecum and proximal colon. For a parasite with a predominant anaerobic metabolism, i.e. *O. dentatum* (Bolla, 1987), this may be an advantage as the parasite needs to adapt less to changes in the luminal environment. Moreover, in naturally infected sows (Herbert, Lean & Nickson, 1969), faecal egg counts increased when a diet rich in carbohydrates, mainly bran and potatoes, was given.

Some previous studies have analysed the influence of carbohydrates on parasite population biology. Increased fecundity, worm burden, and promoted growth and sexual development of *Moniliformis dubius* occurred in rats fed on host dietary fructose (Crompton, Singhvi & Keymer, 1982; Keymer *et al.* 1983*b*). The survival and reproduction of *Moniliformis* are dependent on the carbohydrates liberated at different rates from the intestinal tract of the host during digestion and absorption (Nesheim *et al.* 1977, 1978; Parshad, Crompton & Nesheim, 1980). Absence or restriction of availability of dietary carbohydrates resulted in decreased establishment and growth of *Hymenolepis diminuta* (Roberts, 1980; Keymer, Crompton & Singhvi, 1983*a*).

Numerous studies have described the potential and actual consequences of helminthosis on host nutrition (Nesheim, 1984, 1993; Bundy & Golden, 1987; Stephenson, 1987, 1993; Solomons, 1993). A common feature of infection with intestinal nematodes is a reduction in voluntary feed intake, digestibilities of dry and organic matter, decrease in efficiency of feed utilization, significantly higher nitrogen output, and a rise in plasma urea concentration (Armour *et al.* 1987; Parkins & Holmes, 1989; Blackburn *et al.* 1991; Knox, Steel & Leng, 1994). Host food intake is reduced depending on either the infective dose given to the host or the number of established parasites present (Crompton, 1984, 1991).

Different dietary constituents other than carbohydrates have been found to be important to parasites. The larvae of *A. suum* became established more readily in the intestines of pigs which were fed on diets of oats than in pigs which received diets of milk (Kelley, Olsen & Hoerlein, 1959). *Ascaris suum* infection significantly reduced food intake and fat digestion in pigs fed diets low in protein (Forsum, Nesheim & Crompton, 1981). In pigs infected with single infections of *O. dentatum* (Poelvoorde & Berghen, 1978), or mixed infections with *A. suum* and *O. dentatum* (Costa *et al.* 1979), and fed a low protein diet, a reduction of faecal egg counts and numbers of worms was found compared to control animals. In addition to dietary protein both macrominerals and trace elements can influence the host–parasite relationship (Coop & Holmes, 1996). In the current study, the diets contained comparative amounts of protein and minerals, and it is considered unlikely that the different parasite burdens obtained in the experimental groups can be explained by differences in the protein and mineral content of the feed.

In conclusion, the results of this study substantiate previous findings (Petkevičius *et al.* 1995; Bjørn *et al.* 1996) that the level of carbohydrates, not digested by endogenous enzymes, do not influence adult *A. suum* burdens, but modify the environment in the large intestine and thereby significantly affect establishment, fecundity, and place of location in the gut of *O. dentatum* in pigs. The results further suggest that undigested NSP and lignin has a significant impact on these parameters.

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