Gastrointestinal implications in pigs of wheat and oat fractions

2. Microbial activity in the gastrointestinal tract

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The present work was undertaken to study the microbial activity in various segments of the gastrointestinal (GI) tract of pigs as influenced by the source and level of wheat and oat dietary fibre (DF). Eight experimental diets were prepared from wheat and oat fractions and studied in a series of two experiments using wheat flour as the DF-depleted control. The diets in Expt 1 were based on wheat flour and three iso-DF enriched diets comprising fractions rich in wheat aleurone, pericarp/testa or bran. In Expt 2, oat bran was added to wheat flour to achieve the same DF intake level as in Expt 1. This series included further diets based on rolled oats and rolled oats plus oat bran. The eight diets were given to thirty-two ileal-cannulated pigs, with sixteen pigs in each experiment. After a total period of 34 d (Expt 1) and 42 d (Expt 2), the pigs were slaughtered 4 h post-feeding and samples taken for adenine nucleotides (adenosine 5′-triphosphate (ATP); adenylate energy charge (AEC)), organic acids (lactic acid (LA); short chain fatty acids (SCFA)) and pH at twelve sites of the GI tract. The microbial activity as measured by the ATP concentration was low in the stomach and the cranial two-thirds of the small intestine, but tended to increase in the distal third. In the caecum a sharp rise in microbial activity was observed; the highest level was found for the diet providing most fermentable substrates. In all the diets but the rolled oats + oat bran diets, microbial activity showed a descending pattern as the digesta moved through the colon. In the large intestine source and level of residues had a marked influence on microbial activity. LA was the chief organic acid in the stomach and small intestine (10–40 mmol/l) while LA relative to SCFA was a minor component in the caecum and colon (10–20 mmol/l). The contribution of SCFA to total organic acids was reciprocal to LA, i.e. low in the stomach and small intestine (< 20 mmol/l) and high in the caecum and colon. In the large intestine the concentration of SCFA decreased from 100–140 mmol/l in the caecum and proximal colon to 40–80 mmol/l in the distal colon. The acetic:propionic acid ratio increased from the caecum to the distal colon. With the diets based on oat alone (rolled oats; rolled oats + oat bran) the increase was less significant. DF addition and oats in particular increased the butyric acid molar ratio, from 0·06–0·08 for the wheat flour diet to 0·10–0·12 for the diet based on rolled oats + oat bran. For the same two diets the proportion of isobutyric and isovaleric acids increased more rapidly with the wheat-flour diet compared with the rolled oats + oat bran diet.

Microbial activity: Adenylate energy charge: Dietary fibre: Pig

Microbial fermentation occurs to a varying degree in the gastrointestinal (GI) tract of most mammals including pigs and man (Argenzio & Southworth, 1975; Clemens *et al.* 1975; Cummings, 1982; Cummings & Englyst, 1987). Transit of fluid and particulate markers through the stomach and small intestine of pigs is generally rapid with little or no accumulation at any point (Clemens *et al.* 1975). These conditions are unfavourable for the establishment of prolific microbial growth and most studies show a relatively low microbial activity in these GI segments (Fuller *et al.* 1960; Savage, 1977). In contrast, in the large intestine, materials may be retained for prolonged periods of time (20–38 h) (Hill, 1969; Keys & DeBarthe, 1974), which allows prolific microbial growth (Decuypere & Van der

Heyde, 1972). Concurrently extensive microbial degradation of endogenous and exogenous materials is seen in these GI segments (Mason & Just, 1976; Graham *et al.* 1986; Bach Knudsen & Hansen, 1991). The principal energy substrate for microbial fermentation is carbohydrate, with the end-products: lactic acid (LA), short-chain fatty acids (SCFA; acetic, propionic and butyric acids) and various gases (hydrogen, carbon dioxide, methane). LA is the chief organic acid present in digesta from the stomach and small intestine (Argenzio & Southworth, 1974; Clemens *et al.* 1975), while SCFA predominate in the large intestine (Argenzio & Southworth, 1974; Clemens *et al.*, 1975).

The renewed interest in the role of dietary fibre (DF) in health and disease has focused attention to the GI tract and metabolic implications of various DF sources (Cummings, 1982; Cummings & Branch, 1982). SCFA are the main anions in the large intestine of pigs (Argenzio & Whipp, 1979) and of man (Cummings *et al.* 1979, 1987). The SCFA produced are rapidly absorbed from the gut lumen of simple-stomached animals (McNeil *et al.* 1978; Argenzio & Whipp, 1979), stimulate sodium and water absorption (Argenzio & Whipp, 1979) and play an important role in the energy supply (Bach Knudsen & Hansen, 1991). The SCFA absorbed might have different effects in the body. Butyrate is believed to have important implications for the metabolism, structure and function of epithelial cells lining the large intestine (Ryan *et al.* 1979; Cummings & Branch, 1982; Sakata & Yajima, 1984) where it is the preferred fuel over glucose (Roediger, 1980), while propionate may modify hepatic metabolism (Chen *et al.* 1984).

The adenosine nucleotides are energy couplers between catabolic and anabolic processes in all living cells, and measurements of ATP (adenosine 5′-triphosphate), ADP (adenosine 5′-diphosphate) and AMP (adenosine 5′-monophosphate) have been used as indicators of the amount of energy available for metabolic processes (Chapman *et al.* 1971). Although rumen studies by Forsberg & Lam (1977) have caused doubt about the use of the ATP concentration as an indicator of microbial activity in rumen fluid, studies at this Institute with rats and pigs (Bach Knudsen *et al.* 1982*a*, *b*; 1984; Jensen, 1988) suggest that ATP might be a useful indicator for microbial activity in the GI tract of simple-stomached animals. Studies by Swedes *et al.* (1975) have shown that the rate of protein synthesis and the potential for cellular biosynthesis are more closely correlated with changes in adenylate energy charge (AEC) than with fluctuations in absolute concentrations of intracellular adenine nucleotides. AEC is defined as $(ATP+0{\cdot}5\ ADP)/(ATP+ADP+AMP)$ and was originally proposed to describe the regulation of enzyme reactions which synthesize or utilize ATP (Atkinson & Walton, 1967).

In the preceding paper (Bach Knudsen & Hansen, 1991) we reported the effect of wheat and oat DF on digestion and bulking properties in pigs. The aim of the present part of the investigation was to study the effect of the various DF sources on microbial activity and products formed in various segments of the GI tract of pigs.

EXPERIMENTAL

Experimental Diets

The experimental diets comprised the eight diets characterized with regard to dietary composition and bulking properties in the preceding paper (Bach Knudsen & Hansen, 1991). The diets were based on refined wheat flour (WF) (low DF, control), wheat flour + aleurone (WFA), wheat flour + pericarp/testa (WFPT), wheat flour + wheat bran (WFWB), wheat flour + oat bran (WFOB), rolled oats (RO) and rolled oats + oat bran (ROOB). The diets were tested in a series of two experiments using diet WF as the low DF control (diets WF1 and WF2, Table 1). The low DF control provided on the day of

slaughtering 62 g DF/d; diet WFA, WFPT, WFWB and WFOB 95–116 g DF/d; diet RO 168 g DF/d; and diet ROOB 194 g DF/d.

Animals and feeding

A total of thirty-two ileal-cannulated pigs, sixteen pigs (four herds of four littermates) in each series of experiments, were used. The sixteen pigs were divided into four groups (one group/diet) with one littermate from each herd in each group. After finishing the balance experiment, the pigs were fed for an additional 6 d in Expt 1 or 14 d in Expt 2. On day 34 (Expt 1) and day 42 (Expt 2) the pigs were given the morning ration and killed 4 h post-feeding. Immediately after slaughtering, the GI tract was removed and separated by ligatures into twelve sections (Clemens *et al.* 1975). These comprised the cranial and caudal halves of the stomach (S_1, S_2), three equal segments of the small intestine (SI_1, SI_2, SI_3), the caecum (Ce) and six segments of the colon (C_1, C_2, C_3, C_4, C_5, C_6). The latter consisted of the proximal, two ascending, two descending and the distal segments. With the exception of the stomach there was hardly any exchange between the contents of the different segments of the gut before ligaturing. The total contents of each GI segment were carefully collected for determination of dry matter, pH, adenine nucleotides, LA and SCFA. The samples for determination of adenine nucleotides were collected in cold perchloric acid/ethylenediamine tetra-acetic acid (PCA/EDTA), mixed and stored at −80°, while the other samples were frozen immediately and stored at −20° until analysed. For samples drawn for ATP determinations it was particularly important to avoid contamination with blood and mucosa.

Analytical methods

Analyses of SCFA and LA were performed on undried samples whereas the other analyses were carried out on freeze-dried material. Dry matter contents of GI samples were determined by freeze drying. The pH in digesta was measured using a glass electrode (Type G2040/C; Radiometer A/S, Copenhagen, Denmark) using a mercury–mercury chloride electrode as reference (Type K9040; Radiometer A/S). Total LA (D and L) was determined by means of specific enzymes in a coupled enzymic reaction with nicotinamide-adenine dinucleotide (NAD^+) and SCFA by gas–liquid chromatography as described by Bach Knudsen & Hansen (1991).

The concentration of adenine nucleotides in digesta contents was estimated by the luciferin–luciferase (*EC* 1.13.12.7) method (McElroy, 1947; Wolstrup & Jensen, 1976). This assay is based on the quantitative measurement of light produced by an enzyme reaction between ATP and luciferin catalysed by firefly luciferase. The light intensity is directly proportional to the concentration of ATP and is calculated as the light intensity relative to that of an ATP standard. The adenine nucleotides in digesta (1·5 g) were extracted with PCA/EDTA (2 M-$HClO_4$/10 mM-EDTA; 3 ml; 0°) and carefully mixed using a whirlmixer. After centrifugation (5500 *g*, 30 min, 0°), 2·0 ml of the supernatant fraction was neutralized with potassium hydroxide (0·5 M) to pH 7·0–7·5. In order to obtain a highly buffered sample, 0·4 ml Tris-buffer (0·2 M, pH 7·4) was added before neutralization. After recentrifugation (5500 *g*, 10 min, 4°) the amount of adenine nucleotides was measured. ATP was determined by use of an ATP monitoring kit (LKB-Wallace AB, Sweden), with a luminometer (1251 Luminometer; LKB-Wallace AB) (Thore, 1979). ATP in the samples was determined directly, while ADP and AMP were measured after conversion to ATP. ADP was converted to ATP by phosphoenolpyruvate (PEP) (Boehringer Mannheim GmbH, Mannheim, Germany) and pyruvate kinase (*EC* 2.7.1.40; PK; Boehringer Mannheim GmbH), and AMP by PEP, PK myokinase (*EC* 2.7.4.3;

Boehringer Mannheim GmbH). The adenine nucleotides in the digesta samples are expressed as AEC calculated as:

$$\text{AEC} = \frac{(\text{ATP} + 1/2\text{ADP})}{(\text{ATP} + \text{ADP} + \text{AMP})} \quad (1)$$

Statistical analysis

The analytical data from the various sampling points of the GI tract were tested separately for each of the two experiments for homogeneity according to Barlett's test (Snedecor & Cochran, 1973). The homogeneity test showed that for AEC, SCFA molar ratio and pH the values for all GI segments could be pooled, whereas for ATP, SCFA and LA the data set was homogeneous only within the stomach and small intestine ($(S_1, S_2, SI_1, SI_2, SI_3)$ or within the large intestine (Ce, C_1, C_2, C_3, C_4, C_5, C_6) respectively. After doing the homogeneity test, the analytical values within the previous stated groups were subjected to statistical analysis with a two-way analysis of variance (ANOVA) model (Snedecor & Cochran, 1973):

$$X_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk} \quad (2)$$

where X_{ij} is the dependent variable (i.e. ATP values etc.), μ is the overall mean, α_i is the effect of diet, β_j is the effect of GI segment, and ϵ_{ijk} is a normally distributed random variable.

RESULTS

Digestion of nutrients in the large intestine

The total amount of energy (kJ/d) absorbed from the large intestine varied from 1606 kJ for diet WF1 to 4440 kJ for diet ROOB (Table 2). The bulk of the absorbed energy was derived from digestion of carbohydrates of which NSP (non-starch polysaccharides) was the most important (40·1–106·3 g/d). Starch in Expt 1 contributed only a small amount (3·5–7·7 g/d), while the amount in Expt 2 was much higher (17·5–42·7 g/d). Of the other dietary constituents, low-molecular-weight (LMW) sugars and fructans amounted to 2·0–21·9 g/d, fat to −3·7–9·5 g/d and nitrogen to 1·27–5·31 g/d.

Adenine nucleotides

The ATP concentration (μg/g digesta) was low (1–3 μg/g) in the stomach and proximal small intestine (Fig. 1) . In Expt 1 there was a slight increase in ATP concentration to 5–7 μg/g and further to 7–13 μg/g in the mid- and distal small intestine, while in Expt 2 ATP concentration in all segments of the upper GI tract was below 5 μg/g. The ATP concentration in both experiments increased sharply in the caecum to a level of 26–35 μg/g in Expt 1 and to 30–45 μg/g in Expt 2; the highest level found was for diet ROOB ($P < 0{\cdot}05$). More marked differences for the eight diets were found in the colon, where the general order of ATP content was: diets WF1 < WFPT < WF2 < WFA < WFWB < WFOB < RO < ROOB. When the amount of energy was limiting (diet WF1) there was a rapid decrease in ATP concentration; the concentration in the distal colon was in the same order as in the stomach and proximal small intestine (2–3 μg/g). Addition of DF to achieve iso-DF levels (diets WFA, WFPT, WFWB, WFOB) stimulated microbial activity, with wheat and oat bran and wheat aleurone having a significantly greater effect than pericarp/testa. With the three former DF sources, ATP peaked (33–37 μg/g) in the

Table 1. *Composition of experimental diets (g/kg dry matter)*

	Expt 1				Expt 2			
Diet*...	WF1	WFA	WFPT	WFWB	WF2	WFOB	RO	ROOB
Wheat flour	794	675	744	740	794	705	—	—
Wheat aleurone	—	174	—	—	—	—	—	—
Wheat pericarp/testa	—	—	72	—	—	—	—	—
Wheat bran	—	—	—	82	—	—	—	—
Rolled oats	—	—	—	—	—	—	892	794
Oat bran	—	—	—	—	—	154	—	151
Casein	122	79	105	100	122	66	70	21
Soya-bean oil	46	39	43	42	46	41	—	—
Vitamin/mineral mixture	34	29	32	32	34	30	34	30
Chronic oxide (marker)	4	4	4	4	4	4	4	4

WF1, WF2, wheat flour; WFA, wheat flour + aleurone; WFPT, wheat flour + pericarp/testa; WFWB, wheat flour + wheat bran; WFOB, wheat flour + oat bran; RO, rolled oats; ROOB, rolled oats + oat bran.

* For chemical composition see Bach Knudsen & Hansen (1991).

Table 2. *Calculated† digestion of nutrients (g/d) and absorption of energy (kJ/d) in the large intestine of pigs fed on wheat- and oat-based diets*

	Expt 1				Expt 2			
Diet*...	WF1	WFA	WFPT	WFWB	WF2	WFOB	RO	ROOB
Energy								
Total (kJ)	1606	1858	1286	2302	2405	2328	4214	4440
CHO (kJ)	1134	1427	1152	1760	1607	1708	2781	3059
Nutrients								
Nitrogen (g)	2·19	1·90	1·27	2·50	3·35	2·93	5·31	4·63
Fat (g)	0·6	−0·2	−3·7	0·8	3·3	0·5	9·5	8·8
LMW-sugars + fructans (g)	8·0	13·2	11·6	10·1	21·9	16·8	2·7	2·0
Starch (g)	3·5	4·9	3·5	7·7	26·7	17·5	40·5	42·7
NSP (g)	40·1	58·9	46·3	60·0	44·6	55·2	101·5	106·3

LMW-sugars, low-molecular weight sugars (sum of fructose and sucrose); CHO, carbohydrates; NSP, non-starch polysaccharides; WF1, WF2, wheat flour; WFA, wheat flour + aleurone; WFPT, wheat flour + pericarp/testa; WFWB, wheat flour + wheat bran; WFOB, wheat flour + oat bran; RO, rolled oats; ROOB, rolled oats + oat bran.

* For details of diets, see Table 1.

† Values based on feed intake on the day of slaughtering and digestibility coefficient obtained in the balance period (Bach Knudsen & Hansen, 1991).

proximal colon, while addition of pericarp/testa only resulted in an ATP concentration which was slightly higher than that for diet WF1. The highest ATP concentration (32–46 μg/g) in all segments of the colon was achieved with diet ROOB, where the microbial activity was maintained at a constantly high level in contrast to the other diets where ATP concentration decreased. The correlation between the average ATP concentration of the large intestine and the amount of digested carbohydrates was highly significant (r 0·77, $P < 0·001$; Fig. 2).

AEC in the caecum was 0·60–0·85 in Expt 1 and 0·55–0·75 in Expt 2. Appreciably lower values were found in the three investigated segments of the colon (C_1, C_4, C_6) (Fig. 3). This was particularly the case when giving the control diet (diets WF1 and WF2), providing

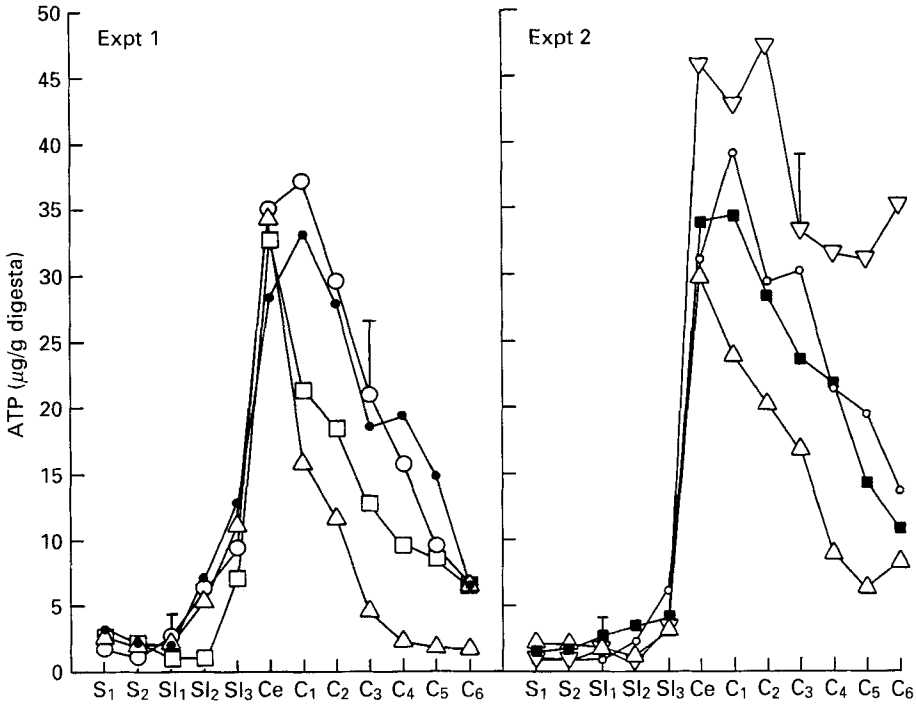

Fig. 1. Expts 1 and 2. ATP concentration of the gastrointestinal contents of pigs. The four diets in Expt 1 were: △—△ wheat flour, ○—○ wheat flour + aleurone, □--□ wheat flour + pericarp/testa, ●—● wheat flour + wheat bran and in Expt 2: △—△ wheat flour, ■—■ wheat flour + oat bran, ○—○ rolled oats and ▽—▽ rolled oats + oat bran. The various segments of the gastrointestinal tract were: S_1 and S_2, cranial and caudal halves of stomach; SI_1, SI_2 and SI_3, three equal segments of small intestine; Ce, caecum; C_1, proximal colon; C_2 and C_3, ascending colon; C_4 and C_5, descending colon; and C_6, distal colon. Values are means with their standard errors represented by vertical bars. For details of diets see Table 1.

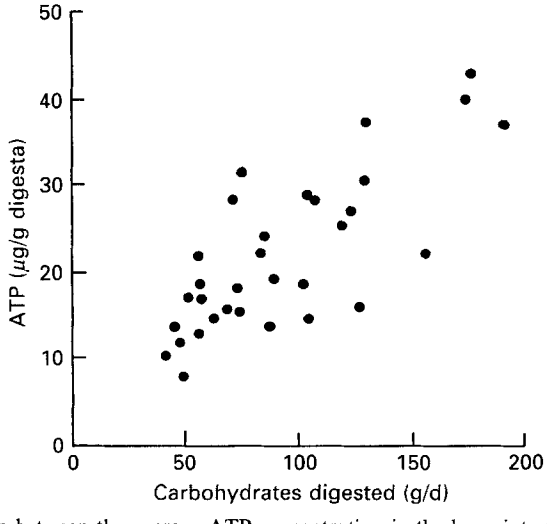

Fig. 2. Correlation between the average ATP concentration in the large intestine and the amount of carbohydrate digested.

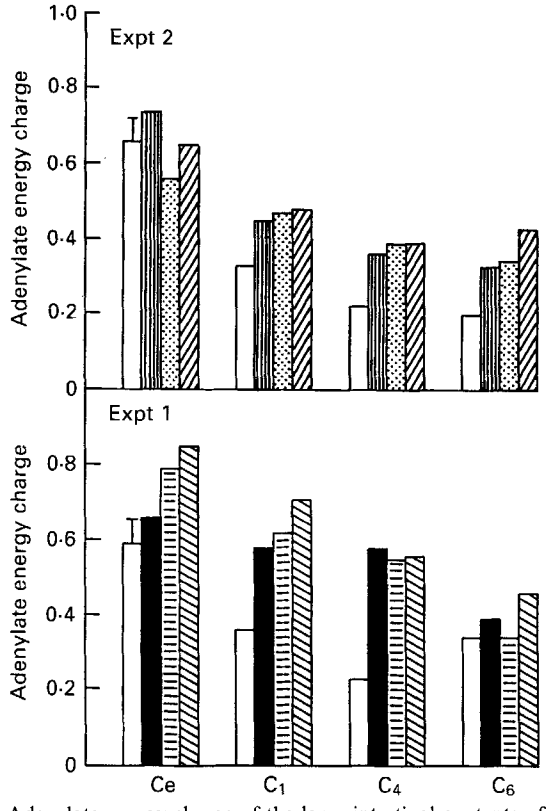

Fig. 3. Expts 1 and 2. Adenylate energy charge of the large intestinal contents of pigs. The four diets in Expt 1 were: □ wheat flour, ■ wheat flour + aleurone, ▤ wheat flour + pericarp/testa, ▧ wheat flour + wheat bran and in Expt 2: □ wheat flour, ▥ wheat flour + oat bran, ▨ rolled oats and ▨ rolled oats + oat bran. The various segments of the gastrointestinal tract were: Ce, caecum; C_1, proximal colon; C_2 and C_3, ascending colon; C_4 and C_5, descending colon; and C_6, distal colon. Values are means with their standard errors represented by vertical bars. For details of diets see Table 1.

values of 0·35–0·37 in the proximal, 0·20–0·25 in the mid- and 0·20–0·34 in the distal colon. When DF was added to the diets, the decreases in AEC were less significant. For the DF-enriched diets in Expt 1, AEC was 0·58–0·71 in the proximal colon, 0·55–0·58 in the mid-colon and 0·34–0·46 in the distal colon. The comparable values obtained in Expt 2 were: proximal colon 0·44–0·48; mid-colon 0·36–0·40 and distal colon 0·32–0·38.

Organic acids

LA was the major organic acid in the stomach and small intestine (Fig. 4). In the stomach LA levels were 10–20 mmol/l, in the small intestine 20–35 mmol/l, while the concentration was 5–20 mmol/l throughout the caecum and colon.

The variation in SCFA concentration at the various sampling points of the GI tract was generally inverse to that of LA; the concentration of SCFA was low in the stomach (5–10 mmol/l) and the first two-thirds of the small intestine (SI_1, SI_2), while a significant increase to 12–23 mmol/l was measured in the lower portion of the small intestine. In the caecum a sharp rise in SCFA concentration to 100–140 mmol/l was seen. The SCFA concentration in colonic segments was lower, varying from 80–130 mmol/l in the proximal colon to 20–65 mmol/l in the distal colon. A similar descending pattern of SCFA

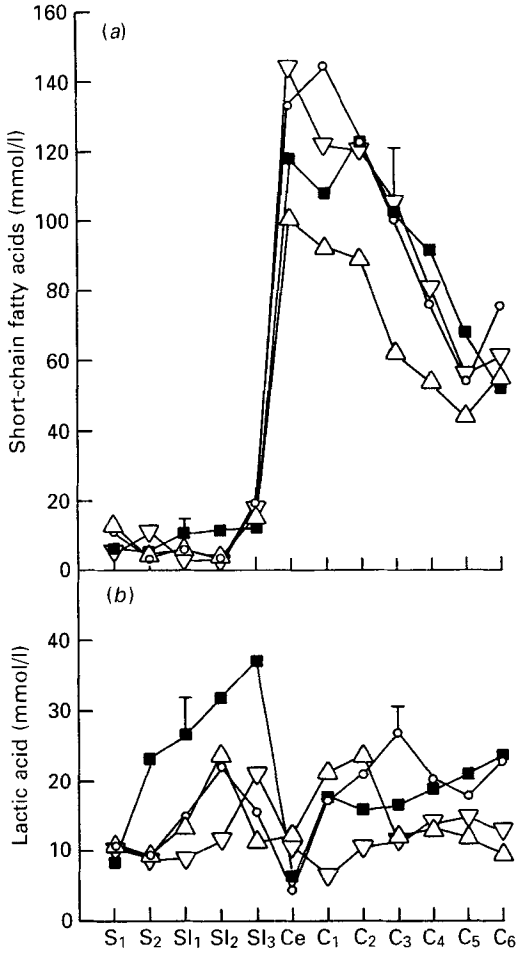

Fig. 4. Expt 2. (*a*) Short-chain fatty acids and (*b*) lactic acid (mmol/l) in the gastrointestinal content of pigs fed on the △—△ wheat flour, ■—■ wheat flour + oat bran, ○—○ rolled oats and ▽—▽ rolled oats + oat bran diets. The various segments of the gastrointestinal tract were: S_1 and S_2, cranial and caudal halves of stomach; SI_1, SI_2 and SI_3, three equal segments of small intestine; Ce, caecum; C_1, proximal colon; C_2 and C_3, ascending colon; C_4 and C_5, descending colon; and C_6, distal colon. Values are means with their standard errors represented by vertical bars. For details of diets see Table 1.

concentration throughout the colon was identified for all diets. For diet WFWB in Expt 1 and diets WFOB, RO and ROOB in Expt 2, however, there was a tendency to a higher SCFA concentration at all sampling points of the colon.

The relative contributions to SCFA of acetic and propionic acids at the various sites of the GI tract were generally inversely related (Figs. 5 and 6). Acetic acid accounted for 0·70–0·95 of SCFA in the stomach and small intestine, 0·45–0·55 in the caecum, 0·50–0·55 in the proximal colon and 0·60–0·70 in the distal colon. In Expt 2, however, when feeding the diets based on oats alone the increase in acetic acid in the caecum and colon was less significant, with values varying from 0·50–0·55 in the caecum to 0·55–0·60 in the distal colon. Propionic acid accounted for 0·05–0·15 in the stomach and small intestine, 0·35–0·40 in the caecum, 0·30–0·35 in the proximal colon and 0·18–0·25 in the distal colon. The contribution of butyric acid to SCFA was much lower (0·02–0·12). In the large intestine, however, DF addition and oats in particular tended to increase the proportion of butyric

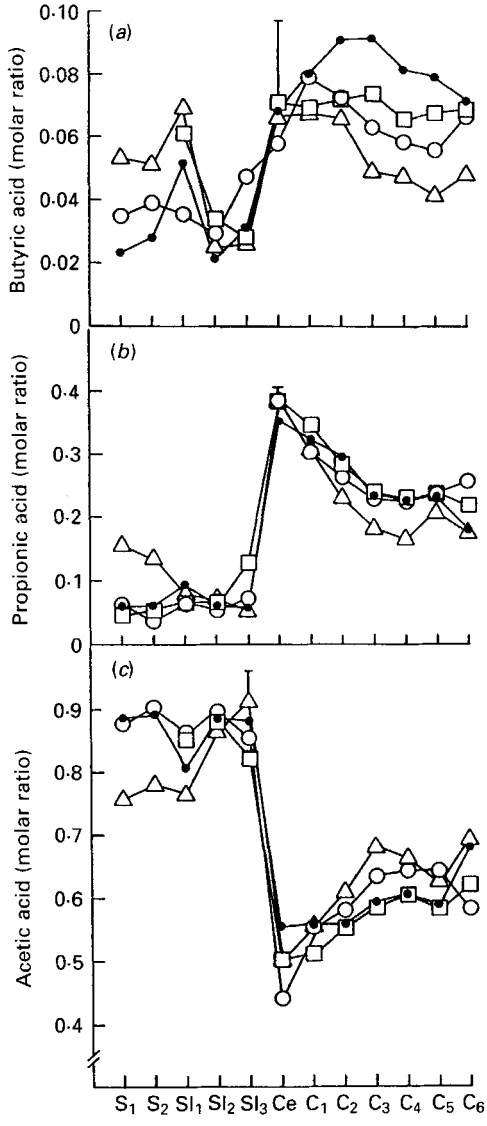

Fig. 5. Expt 1. Molar ratios of the short-chain fatty acids: (*a*) butyric, (*b*) propionic and (*c*) acetic acids in the gastrointestinal contents of pigs fed on the following diets: wheat flour △—△, wheat flour+aleurone ○—○, wheat flour+pericarp/testa □—□ and wheat flour+wheat bran ●—●. The various segments of the gastrointestinal tract were: S_1 and S_2, cranial and caudal halves of stomach; SI_1, SI_2 and SI_3, three equal segments of small intestine; Ce, caecum; C_1, proximal colon; C_2 and C_3, ascending colon; C_4 and C_5, descending colon; and C_6, distal colon. Values are means with their standard errors represented by vertical bars. For details of diets see Table 1.

acid. For the branched-chain fatty acids, isobutyric acid and isovaleric acid, there was a more rapid increase in the large intestine from 0·01 and 0·015 in the caecum to 0·027 and 0·05 in the distal colon when feeding diet WF2 compared with diet ROOB (caecum 0·005 and 0·003, distal colon 0·03 and 0·045 respectively; Fig. 7).

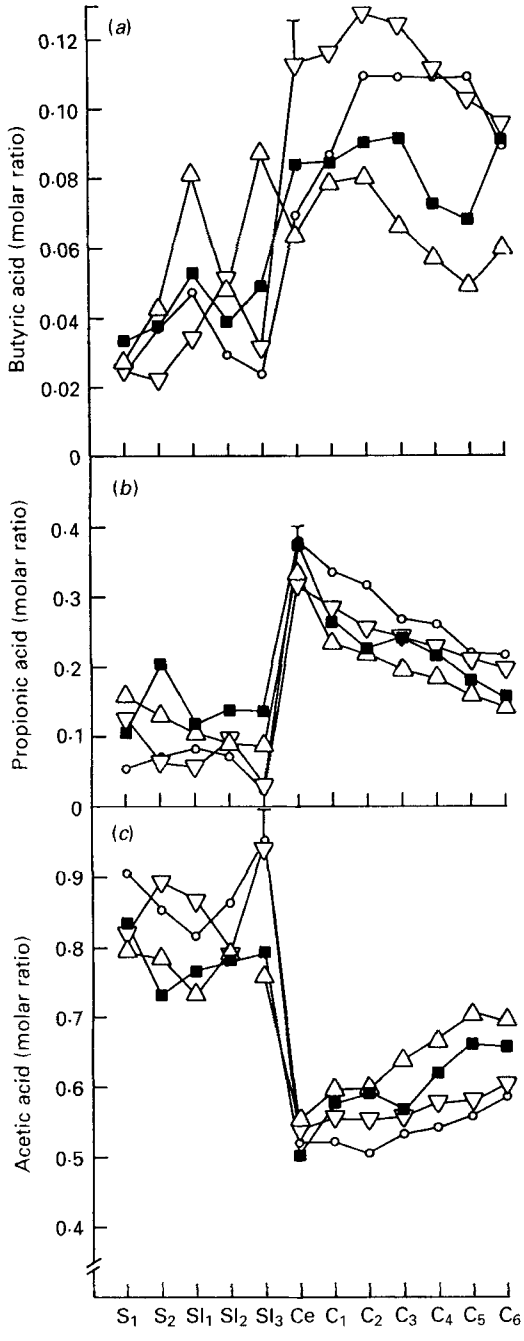

Fig. 6. Expt 2. Molar ratios of the short-chain fatty acids: (*a*) butyric, (*b*) propionic and (*c*) acetic acids in the gastrointestinal contents of pigs fed on the following diets: wheat flour △—△, wheat flour + oat bran ■—■, rolled oats ○–○ and rolled oats + oat bran ▽—▽. The various segments of the gastrointestinal tract were: S_1 and S_2, cranial and caudal halves of stomach; SI_1, SI_2 and SI_3, three equal segments of small intestine; Ce, caecum; C_1, proximal colon; C_2 and C_3, ascending colon; C_4 and C_5, descending colon; and C_6, distal colon. Values are means with their standard errors represented by vertical bars. For details of diets see Table 1.

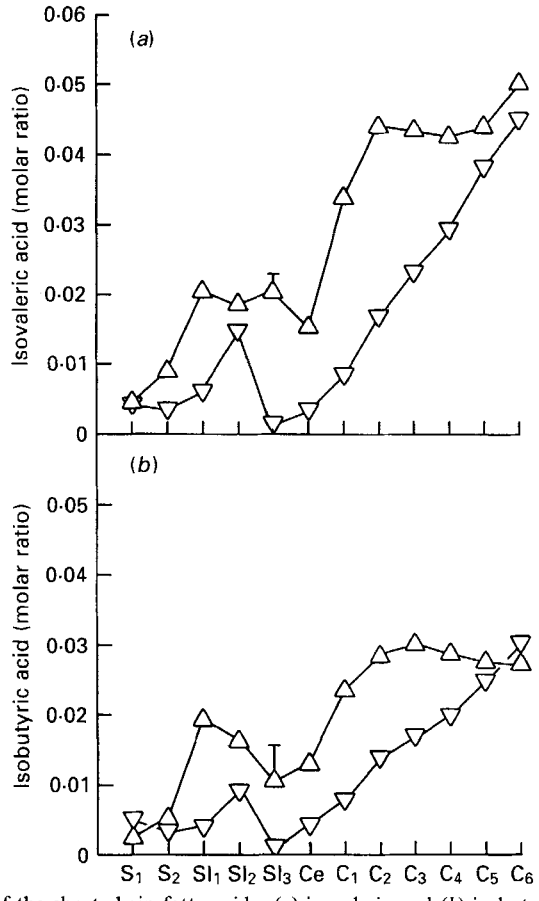

Fig. 7. Molar ratios of the short chain fatty acids: (*a*) isovaleric and (*b*) isobutyric acids in the gastrointestinal contents of pigs fed on the following diets: wheat flour △—△, rolled oats + oat bran ▽—▽. The various segments of the gastrointestinal tract were: S_1 and S_2, cranial and caudal halves of the stomach; SI_1, SI_2 and SI_3, three equal segments of small intestine; Ce, caecum; C_1, proximal colon; C_2 and C_3, ascending colon; C_4 and C_5, descending colon; and C_6, distal colon. Values are means with their standard errors represented by vertical bars. For details of diets see Table 1.

pH

Digesta pH in the cranial and caudal halves of the stomach was quite similar (Fig. 8). pH increased along the small intestine, reaching values of 6·6–7·2 near the ileo-caecal junction. The pH then dropped to 5·7–6·8 in the caecum. In the colon pH increased particularly for the DF-depleted wheat flour diet which in the mid-colon (C_3–C_5) had a pH of 7·5. This can be compared with 6·7–6·8 ($P < 0·05$) with diets WFA and WFWB and 6·0–6·5 with diets WFOB, RO and ROOB. Diet WFPT was in between these two extremes. In the distal colon, however, pH was about 6·5–6·8 for all diets.

DISCUSSION

The microbial activity measured as the ATP concentration of digesta confirms that the microbial activity at the various sites of the pig GI tract varies. Under normal physiological conditions the population of micro-organisms is low in the stomach and the cranial half of the small intestine (Fuller *et al.* 1960), while it increases rapidly to 10^7–10^9 culturable bacteria per gram digesta, mostly lactobacilli and streptococci, in the distal part of the

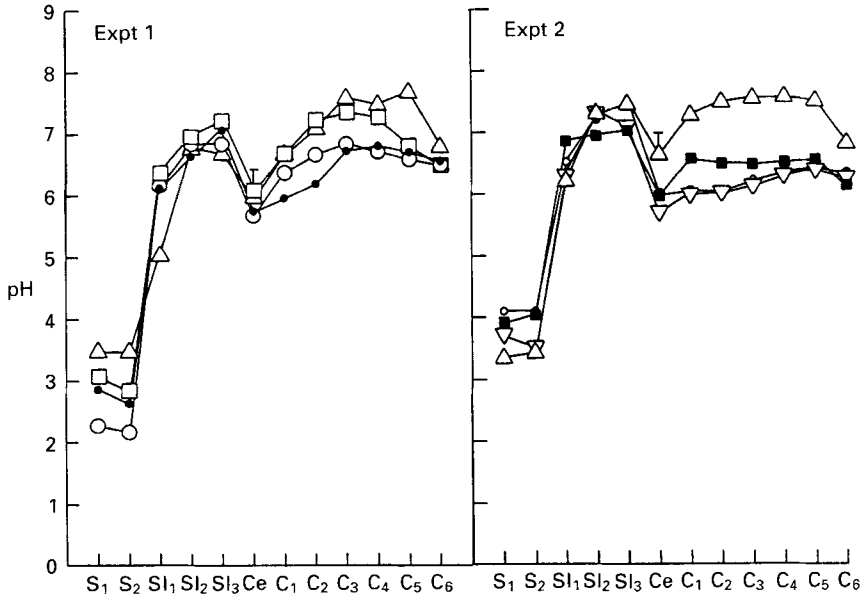

Fig. 8. Expts 1 and 2. pH in gastrointestinal contents of pigs. The four diets in Expt 1 were: △—△ wheat flour, ○—○ wheat flour + aleurone, □—□ wheat flour + pericarp/testa, ●—● wheat flour + wheat bran and in Expt 2: △—△ wheat flour, ■—■ wheat flour + oat bran, ○—○ rolled oats and ▽—▽ rolled oats + oat bran. The various segments of the gastrointestinal tract were: S_1 and S_2, cranial and caudal halves of the stomach; SI_1, SI_2 and SI_3, three equal segments of small intestine; Ce, caecum; C_1, proximal colon; C_2 and C_3, ascending colon; C_4 and C_5, descending colon; and C_6, distal colon. Values are means with their standard errors represented by vertical bars. For details of diets see Table 1.

small intestine (Savage, 1977). The large intestine of pigs (and man) contains a vast number of different micro-organisms (400–500 species in man; Borriello, 1986) and reaches concentrations as high as 10^{11}/g digesta (Decuypere & Van der Heyde, 1972). Studies at this Institute with pigs confirmed these results, giving values for microbial density in the lower third of the small intestine of 10^8–10^9, in the caecum of 10^9–10^{10}, and in the mid- and distal colon of 10^{10}–10^{11} (Jensen, 1988).

Although the density of micro-organisms in the caecum and colon is believed to be quite constant, the decrease in ATP concentration and AEC values from the caecum and proximal colon to the distal colon suggests a decrease in metabolic activity once the energy becomes limiting during the movement through the large intestine. Studies with pure rumen bacteria (Forsberg & Lam, 1977; Nuzback *et al.* 1983) and with mixed populations from pig's caecum (B. B. Jensen, unpublished) in batch cultures have shown that the ATP pool size is higher during early exponential growth, but decreases rapidly during the stationary phase. The substrates in caecum and proximal colon with all diets are the readily fermentable carbohydrates, such as starch and sugar residues, mucus and soluble NSP which are known to favour propionic-producing micro-organisms (Leng, 1969; Wolin, 1975). The consequence is a rapid proliferation, a decrease in pH and an increase in propionic acid levels. As digesta moves aborally it is depleted of readily fermentable energy sources. The substrate available in the distal colon comprises, therefore, the least degradable cell wall materials of the ligno-cellulose type (Bach Knudsen & Hansen, 1991). These conditions are known to favour acetic acid-producing micro-organisms (Leng, 1969). At the stage of energy limitation the microflora certainly become more proteolytic. However, as judged from the descending ATP concentration in the colon and the relative

proportion of branched-chain fatty acids of SCFA, derived from fermentative breakdown of branched-chain amino acids (Macfarlane *et al.* 1986), the state of energy limitation occurs at various stages along the colon depending on dietary composition. With the diets providing the least fermentable substrate (the control diets) there was a much more rapid decrease in ATP concentration and a concurrent increase in branched-chain fatty acids compared with the diet providing the most fermentable substrates (diet ROOB).

The fact that energy under many conditions may be a limiting factor for microbial growth in the colon is certainly the main reason for the relatively low AEC values obtained in the colon compared with the caecum and rumen (0·68–0·92) (Erfle *et al.* 1979; 1981; Wallace & West, 1982). For the control diets, AEC in the colon was 0·2–0·4, far below 0·5 where viability under in vitro conditions is lost (Chapman *et al.* 1971). However, even when sufficient amounts of energy reached the large intestine to keep ATP concentrations high (diet ROOB), the AEC in the colon was below 0·5. For diet ROOB the relatively low AEC values may be due to its lack of bulky substances (Bach Knudsen & Hansen, 1991) which facilitates a longer retention time in the large intestine and, thus, causes a reduction in survival of bacterial cells. One factor worth discussing here is the sampling procedure. Studies in vitro aiming at a simulation of the conditions during slaughtering; e.g. the exposure of gut contents to air, the time elapsed between slaughtering and sampling (5–20 min) and the temperature fall (37–25°), show no drastic effect on the fraction of the adenylate pool containing anhydride-bound phosphate of high energy for hydrolysis (B. B. Jensen, unpublished results). However, it is critical that care was taken to avoid drastic cooling of the digesta before adenylates were extracted in the perchloric acid solution. On this basis it is concluded that the low AEC values are not due to bad sampling procedures.

The results from the present study confirm that the carbohydrates, in particular NSP, are the principal energy substrates for large intestine microbial fermentation. These findings also support those from the digestibility trial of Bach Knudsen & Hansen (1991) showing that the chemical as well as the structural composition of DF is an important factor in its GI effects along the GI tract. Compared with diet WF1, the microbial activity increased as more substrate passed to the large intestine, e.g. in the form of starch (diet WF2 *v.* WF1), as the availability of DF polysaccharides at iso-DF levels rose (diets WFOB > WFA > WFWB > WFPT), or at increasing levels of DF (diet RO and ROOB *v.* the other six diets).

The importance of fermentation to man and other simple-stomached animals, however, lies in the products formed and their fate in the body. In agreement with other pig studies (Argenzio & Southworth, 1974; Clemens *et al.* 1975), LA is the chief organic acid in the stomach and small intestine while SCFA predominate in the caecum and colon. Comparison of the SCFA results obtained in the present study with those obtained in similar studies with pigs, however, shows some marked differences (Argenzio & Southworth, 1975; Imoto & Namioka, 1978). In the studies of Argenzio & Southworth (1975), and Imoto & Namioka (1978) there was a constant acetate:propionate ratio and total SCFA concentration as the digesta moved through the large intestine. In the present study the acetate:propionate ratio increased for all diets except diets RO and ROOB. The SCFA concentration decreased over the same distance. These differences are most likely due to the relatively low DF levels of the current diets compared with conventional pig diets (Graham *et al.* 1986). Apart from being scarce in fermentable substrates, the low DF level results in a prolonged retention of digesta in the large intestine and, thus, an increased total absorption of nutrients. In ponies which have a slower digesta transit time than pigs, the proportion between the acids is similar to that found in the present study (Argenzio & Southworth, 1975; Argenzio *et al.* 1974).

Assuming that the equations for converting carbohydrates and proteins into SCFA given

for man (Miller & Wolin, 1979; Macfarlane *et al.* 1986) are valid for pigs, the amount of fermented carbohydrates in the large intestine, in theory, leads to a production (mmol/d) of from approximately 500 for diet WF1 to 1500 for diet ROOB and the amount of fermented protein from approximately 20 for diet WFPT to 90 for diet RO. In addition there is an unknown quantity of LA produced in the stomach and small intestine. Apart from providing the host with significant amounts of energy (Bach Knudsen & Hansen, 1991) the SCFA produced might have more specific GI and metabolic effects (Cummings & Englyst, 1987). Butyrate, particularly, is considered to have important implications for metabolism, structure and function of epithelial cells lining the large intestine (Ryan *et al.* 1979; Cummings & Branch, 1982; Sakata & Yajima, 1984) where it is the preferred fuel over glucose (Roediger, 1980). Interestingly enough, DF addition, and oat DF in particular, not only caused an increase in butyric acid production in accordance with the overall SCFA, but the type of DF source also affected the amount of butyric acid relative to the other SCFAs. Hence, while the total production of SCFAs increased from approximately 500 mmol/d for diet WF1 to 1500 mmol/d for diet ROOB; butyric acid production increased from 35 mmol/d to 170 mmol/d for the two diets. Certainly this is one of the most important factors to consider when discussing the mitogenic response in the large intestine of certain DF sources (Lupton *et al.* 1988).

An association between a high DF intake and a low faecal pH was noted by Walker *et al.* (1979). The pH in the caecum and colon decreased as a consequence of fermentation of carbohydrates; the effect, however, was strongly influenced by the source and amount of residues reaching the large intestine. In the diets providing most fermentable substrates (diets RO and ROOB) the pH was maintained in the range of 6·0–6·5 in all segments of the caecum and colon, while pH increased from approximately 6·0 in the caecum to 7·5–8·0 in the lower segments of the colon when feeding the DF-depleted wheat flour diet. The lumen pH may influence the growth and metabolism of colonic epithelial cells (Lupton *et al.* 1988) and modify the degradation of bile acids. Under acid conditions the 7α-dehydroxylation of primary bile acids is inhibited possibly through a direct effect on the enzyme or the poor solubility of these compounds in acid conditions (Cummings & Branch, 1982).

The present and the previous study (Bach Knudsen & Hansen, 1991) demonstrate that the effects of various types of cereal DF on the GI tract were highly correlated with the chemical as well as the structural composition of cell wall materials. It is the belief of the authors that the effects described here in the present model experiment with pigs are valid even for man.

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