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Effect of Dietary Fiber on Microbial Activity and Microbial Gas Production in Various Regions of the Gastrointestinal Tract of Pigs

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The microbial activity, composition of the gas phase, and gas production rates in the gastrointestinal tract of pigs fed either a low- or a high-fiber diet were investigated. Dense populations of culturable anaerobic bacteria, high ATP concentrations, and high adenylate energy charges were found for the last third of the small intestine, indicating that substantial microbial activity takes place in that portion of the gut. The highest microbial activity (highest bacterium counts, highest ATP concentration, high adenylate energy charge, and low pH) was found in the cecum and proximal colon. Greater microbial activity was found in the stomach and all segments of the hindgut in the pigs fed the high-fiber diet than in the pigs fed the low-fiber diet. Considerable amounts of O_2 were found in the stomach (around 5%), while the content of O_2 in gas samples taken from all other parts of the gastrointestinal tract was <1%. The highest concentrations and highest production rates for H_2 were found in the last third of the small intestine. No methane could be detected in the stomach or the small intestine. The rate of production and concentration of methane in the cecum and the proximal colon were low, followed by a steady increase in the successive segments of the hindgut. A very good correlation between in vivo and in vitro measurements of methane production was found. The amount of CH_4 produced by pigs fed the low-fiber diet was 1.4 liters/day per animal. Substantially larger amounts of CH_4 were produced by pigs fed the high-fiber diet (12.5 liters/day). Also, the daily rate of CO_2 production in the gut was higher for the pigs fed the high-fiber diet than for the pigs fed the low-fiber diet (212 versus 46 liters/day). Although the highest microbial activity was found in the cecum and proximal colon and although hydrogen production is an obligate part of anaerobic fermentation in the hindgut, only small net amounts of hydrogen were produced in these segments. Furthermore, only small amounts of methane were produced in the cecum and proximal colon. This strongly indicates that hydrogen sinks other than methane production are involved in hydrogen removal in the cecum and proximal colon of pigs.

The gastrointestinal tract of pigs contains a large and diverse microbial population, with cell population densities in excess of 10^{10} cells per g of gut content in the large intestine, the vast majority of which are strictly anaerobic bacteria.

In recent years, the microbial fermentation of nonstarch polysaccharides or dietary fiber in the gastrointestinal tract of monogastric animals has been a subject of considerable interest. The extent of microbial breakdown of nonstarch polysaccharides is influenced by the nature of the carbohydrate polymers present and the degree of lignification (2). The main products of nonstarch polysaccharide fermentation are short-chain fatty acids, lactate, ammonia, and various gases (29). The short-chain fatty acids produced are physiologically important especially in the large intestine, in which butyrate in particular is required to maintain the health of the epithelial cells lining the gut (37).

Three gases, H_2, CH_4, and CO_2, are produced in appreciable volumes by the intestinal microbiota (27, 29). It has been indirectly shown by using respirometry that methanogenesis occurs in the lower gastrointestinal tract of pigs, but in contrast to the situation for ruminants, methanogenesis accounts for minimal loss of digestible energy in pigs (5). A number of investigators have used breath and flatus H_2 measurements to quantitate fermentation in the large intestine (27, 40). However, there exists very little information about the relative rates of gas production in different regions of the gastrointestinal tracts of humans and monogastric animals.

The present study was undertaken to provide more information about the microbial activity, the composition of the gas phase, and the relative rates of gas production in various regions of the gastrointestinal tract of pigs.

MATERIALS AND METHODS

Animals and diets. The experiment was carried out with eight 7-month-old pigs (two litters of four castrated males). Two pigs from each litter were fed a low-fiber diet based on barley and wheat starch as the carbohydrate sources, and two were fed a high-fiber diet based on barley supplemented with pea fiber and pectin as the carbohydrate source. The compositions of the diets are shown in Table 1.

The animals were housed individually and were fed restrictively (3% of body weight per day) three times a day at 0700, 1500, and 2200. The drinking water (2.5 to 3.0 liters day^{-1}) was thoroughly mixed with the feed. After at least 2 months on the experimental diets, the pigs were sacrificed individually by intravenous injection of an overdose of 20% sodium pentobarbital 4 h after the morning feed. Within 5 min after slaughter, the gastrointestinal tract was removed and separated by ligatures into the 12 sections shown in Fig. 1. Samples (0.5 ml) were rapidly removed from gas bubbles in each segment with

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TABLE 1. Compositions of diets

Component	% of diet	
	Low fiber	High fiber
Barley	25.06	52.74
Wheat starch	58.02	
Casein	3.25	1.32
Fish meal	7.58	3.07
Soybean oil	3.00	3.00
Pea fiber		35.00
Pectin		2.50
Calcium carbonate		0.27
Dicalcium phosphate	0.97	1.58
Monocalcium phosphate	1.59	
Sodium chloride	0.33	0.32
Vitamin-mineral mixture[a]	0.20	0.20

[a] Supplied per kilogram of food: 4,000 IU of vitamin A, 1,000 IU of vitamin D_3, 50 mg of vitamin E (*dl*-α-tocoferylacetate), 2 mg of vitamin K_3, 4 mg of vitamin B_2, 10 mg of D-pantothenic acid, 0.02 mg of vitamin B_{12}, 250 mg of $FeSO_4 \cdot 7H_2O$, 100 mg of ZnO, 36 mg of Mn_3O_4, 80 mg of $CuSO_4 \cdot 5H_2O$, 260 μg of potassium iodide, and 660 μg of Na_2SeO_3.

1.0-ml plastic syringes fitted with 25-gauge needles, and the composition of the gas was measured by gas chromatography. To obtain a representative sample of gas, each syringe was flushed 8 to 10 times with intestinal gas before the final sample was taken. Then the needle of the syringe was inserted into a rubber stopper until the gas was analyzed. All analyses were carried out within 30 min after collection. No changes in the composition of the gas within the syringes could be detected for up to 2 h of storage. All gas samples were analyzed in duplicate. Then the contents of each gastrointestinal segment were quantitatively collected for determination of content weight, dry matter, pH, adenine nucleotide, total anaerobic bacteria, and gas production capacity.

In vivo methane production. Within the last week prior to slaughter, the CH_4 excretion over a 24-h period was measured by means of a respiration unit working according to the open-air-circulation principle (31). The CH_4 concentration in the outgoing air was measured continuously by means of an infrared gas analyzer (URAS 3 G; Hartman and Braun, Frankfurt, Federal Republic of Germany) as described by Christensen and Thorbek (5).

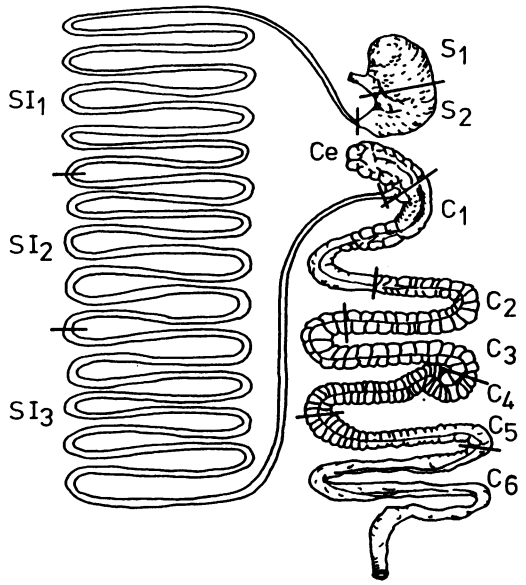

FIG. 1. Sampling sites in the gastrointestinal tract. SI, small intestine; S, stomach; Ce, cecum; C, colon.

In vitro gas production. Samples of gut content were taken to the laboratory for processing within 10 min of collection. A sample (5.0 g) from each segment was weighed into 20 ml of anaerobic buffer (13) in 125-ml serum bottles under a constant flow of O_2-free CO_2. The serum bottles were sealed with butyl rubber stoppers. Immediately before the incubations were initiated, the gas phase of each serum bottle was changed to O_2-free N_2 by three successive cycles of evacuation and refilling with N_2, using a manifold fitted to a vacuum pump and a cylinder of N_2. Serum bottles were connected to the manifold by 18-gauge needles. The N_2 was scrubbed free from O_2 by being passed over hot (110°C) BASF catalyst R3-11. The bottles were removed from the manifold under a slight overpressure of N_2. The bottles were then placed in a shaking water bath at 38°C, and the pressure was adjusted to atmospheric pressure with a 25-gauge needle. Gas samples (250 μl) were removed from the headspace of the serum bottles at zero time and after 1, 2, 4, 6, and 24 h with a gas-tight syringe, and CO_2, CH_4, N_2, and H_2 were measured on a Perkin-Elmer 900 gas chromatographic unit equipped with a Carlo Erba HWD 430 hot-wire thermal conductivity detector and a column of Porapak Q (80/100 mesh; 200 cm long by 1/8 in. [ca. 3.2 mm] inside diameter) operated at 50°C with argon as the carrier gas. The amounts of CO_2, CH_4, and H_2 were calculated from the area of each peak relative to the area of the N_2 peak in order to compensate for the pressure increase in the bottles during incubation.

Total counts of anaerobic bacteria. Intestinal contents from each segment (10 g) were rapidly transferred under a flow of CO_2 gas into flasks containing 90 ml of anaerobic dilution solution (13). This suspension was transferred to a CO_2-flushed plastic bag and homogenized in a stomacher laboratory blender (Seward Medical, London, United Kingdom) under CO_2 for 2 min. Then 10-fold dilutions were made in anaerobic dilution solution by the technique of Miller and Wolin (32). Duplicate samples (0.1 ml) were removed and inoculated onto rumen fluid-glucose-cellobiose-agar plates (13) in an anaerobic cabinet (10:10:80 CO_2-H_2-N_2 atmosphere). The plates were then incubated in the anaerobic cabinet at 38°C for 7 days and the counts of total anaerobic bacteria were determined.

Measurement of ATP in digesta. The concentration of adenine nucleotides in digesta was determined by the luciferin-luciferase method (30). Digesta were extracted with PCA-EDTA (5.0 g of digesta per 10.0 ml of 2 M cold perchloric acid containing 10 mM EDTA), carefully mixed on a vibratory mixer, and immediately frozen at −80°C. At this stage, samples could be stored for several months without any loss of activity. After thawing, mixing, and centrifugation (5,500 × *g*, 30 min, 4°C), 2 ml of the supernatant was neutralized with KOH (0.5 M) to pH 7.4 to 7.6 after addition of 0.4 ml of Tris buffer (0.2 M; pH 7.4). The precipitated $KClO_4$ was removed by centrifugation (5,500 × *g*, 10 min, 4°C), and adenine nucleotides were measured. ATP was measured directly by use of an ATP monitoring kit from LKB-Wallace (38) with a 1251 C luminometer (LKB-Wallace). ADP and AMP were measured after enzymatic conversion into ATP as described by Kimmich et al. (23) except that neutralization was to the cresol purple endpoint at pH 7.8.

The adenylate energy charge (AEC) is defined as follows: AEC = (ATP + 0.5ADP)/(ATP + ADP + AMP) (4).

Dry matter and pH determinations. The dry matter content

TABLE 2. Amounts of digesta and dry matter contents of digesta in various segments of the digestive tracts of pigs fed the low- and high-fiber diets

Segment[a]	g (wet wt) of digesta (mean ± SD)		% Dry matter (mean ± SD)	
	Low-fiber diet	High-fiber diet	Low-fiber diet	High-fiber diet
S_1	1,440 ± 960	1,600 ± 660	25.2 ± 3.9	19.1 ± 1.2
S_2	610 ± 260	1,490 ± 410	26.2 ± 4.2	18.6 ± 1.0
SI_1	30 ± 40	80 ± 60	20.4 ± 4.4	13.7 ± 2.9
SI_2	140 ± 65	790 ± 205	19.8 ± 3.0	12.9 ± 1.0
SI_3	220 ± 160	1,075 ± 200	18.3 ± 1.8	9.6 ± 0.7
Cecum	205 ± 25	1,080 ± 500	12.7 ± 2.5	8.2 ± 0.8
C_1	255 ± 70	1,245 ± 255	18.5 ± 1.7	10.6 ± 1.1
C_2	200 ± 35	775 ± 410	22.1 ± 3.2	12.4 ± 1.3
C_3	185 ± 65	675 ± 135	22.9 ± 3.2	13.6 ± 0.8
C_4	180 ± 65	750 ± 325	24.7 ± 3.3	15.0 ± 0.8
C_5	80 ± 10	610 ± 245	28.1 ± 1.7	16.7 ± 1.6
C_6	95 ± 35	515 ± 210	29.7 ± 1.2	19.8 ± 2.5
Total (kg)	3.6 ± 1.1	10.7 ± 2.0		

[a] As indicated in Fig. 1.

of digesta was determined after freeze-drying. The pH in digesta was measured immediately after slaughter with a combined glass-reference electrode (GK 2401C; Radiometer, Copenhagen, Denmark).

Statistical procedure. The results were statistically treated by analysis of variance (37a), with adjustments for all identified sources of variation. Means for significant treatment differences were compared by the least significant difference test.

RESULTS

Amounts and water content of digesta in various segments of the digestive tract. The amounts of digesta (wet weights) in various segments of the gastrointestinal tracts of the pigs fed the low- and the high-fiber diets are shown in Table 2. It is evident that the amount of digesta in all segments of the gastrointestinal tract is larger in the pigs receiving the high-fiber diet than in the pigs receiving the low-fiber diet (10.7 versus 3.6 kg for the entire gut). Furthermore, in the pigs receiving the low-fiber diet, 57% of the content found in the entire gastrointestinal tract was found in the stomach, while only 11, 6, and 27% were found in the small intestine, the cecum, and the colon, respectively. The same measurements for the pigs fed the high-fiber diet were as follows: stomach, 29%; small intestine, 18%; cecum, 10%; and colon, 43%.

The dry matter contents of digesta in the various segments of the gastrointestinal tract are also shown in Table 2. The relative water content was significantly higher ($P < 0.01$) in all segments of the gut in the pigs receiving the high-fiber diet.

Total counts of culturable bacteria. The density of total culturable bacteria was higher in the caudal half of the stomach (Fig. 2) than in the cranial half of the stomach, and there was a further decline in the first third of the small intestine. A substantial population of culturable bacteria was found in the

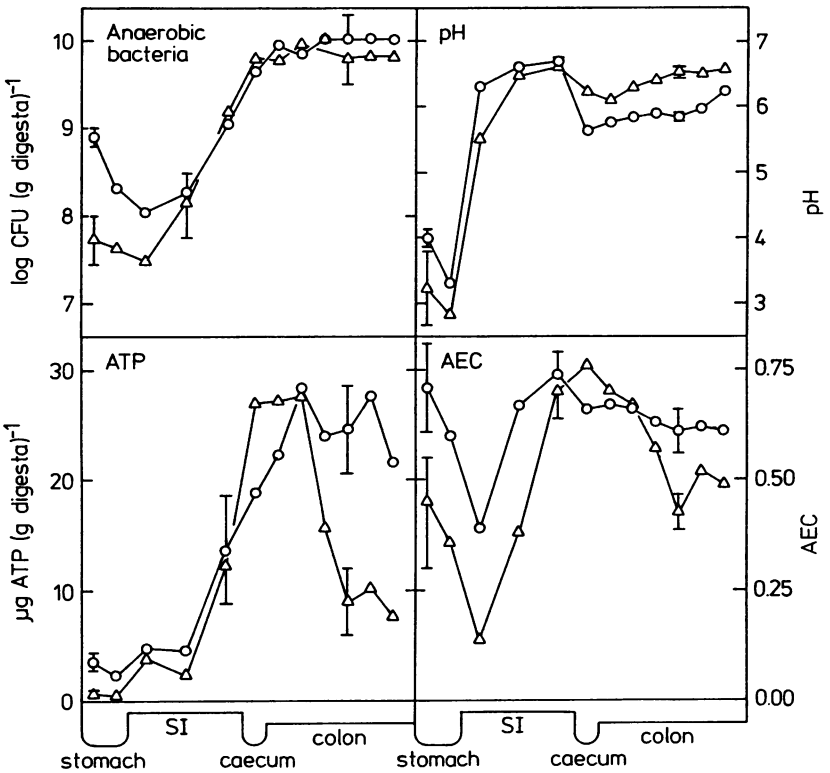

FIG. 2. Total viable counts of anaerobic bacteria ($\log_{10}$ CFU per gram of digesta), pH, ATP concentration (micrograms per gram of gut content), and AEC values in various regions of the gastrointestinal tracts of the pigs fed the low-fiber (△) and the high-fiber (○) diets. Values are means with standard errors represented by vertical bars at representative sampling sites from the stomach (S_1 in Fig. 1), small intestine (SI_3), and hindgut (C_4).

TABLE 3. Microbial activity and total amounts of various gases produced per day in the individual pigs

Pig	Diet type (fiber)	Wt of pig (kg)	mg of ATP	Gas produced (liters/day)			
				In vivo, CH_4	In vitro[a]		
					CH_4	H_2	CO_2
1	Low	131	26.4	1.2	0.8	2.9	49
3	Low	127	30.3	1.6	1.1	1.5	39
5	Low	112	26.9	0.9	1.0	1.3	41
6	Low	132	30.3	1.9	0.9	8.6	54
2	High	131	127.2	9.5	11.1	11.3	249
4	High	127	167.4	16.1	12.7	2.1	157
7	High	116	178.7	16.2	19.7	1.4	196
8	High	144	146.9	8.1	5.7	6.1	245

[a] Calculated from the in vitro production rates and the amounts of digesta in the various regions of the gastrointestinal tract.

last third of the small intestine (8.3×10^9 to 8.8×10^9 bacteria per g of digesta). The highest numbers of culturable bacteria were found in the colon (0.9×10^{10} to 1.6×10^{10} bacteria per g of digesta). Digesta from the stomachs of the pigs receiving the high-fiber diet contained significantly larger ($P < 0.05$) amounts of culturable bacteria than did digesta from the stomachs of the pigs receiving the low-fiber diet (7.8×10^8 and 2.4×10^8 versus 0.9×10^8 and 0.7×10^8, respectively). The amount of bacteria per gram of digesta tended to be somewhat larger in the cecum and the proximal part of the colon in the pigs receiving the low-fiber diet than in the pigs receiving the high-fiber diet, while the opposite was the case in the distal part of the colon. However, none of these differences were statistically significant ($P > 0.10$).

pH of digesta. The pH profiles for various regions of the gastrointestinal tract for the pigs in each group are shown in Fig. 2. The pH values for all sites in the cecum and colon in the pigs receiving the high-fiber diet were significantly ($P < 0.01$) lower than the values for the same sites in the pigs receiving the low-fiber diet.

Adenine nucleotides. The concentration of ATP and the AEC are useful for estimating the microbial activity in the digestive tract of monogastric animals (2, 14). As shown in Fig. 2, the concentrations of ATP in both groups of animals were low in the stomach and the proximal segments of the small intestine, while they increased in the ileal part of the small intestine. The highest ATP concentrations were found in the cecum and the proximal segments of the large intestine. In the pigs receiving the high-fiber diet high ATP concentrations were found throughout the large intestine, while in the pigs receiving the low-fiber diet a steady decrease in ATP concentrations was observed in the distal segments of the large intestine. The concentrations of ATP in the stomachs of the pigs receiving the high-fiber diet were significantly higher than those found in the pigs on the low-fiber diet ($P < 0.05$).

The total amounts of ATP in the entire gastrointestinal tract (means ± standard deviations) were 28 ± 2 mg for the pigs fed the low-fiber diet and 155 ± 23 mg for the pigs fed the high-fiber diet (Table 3).

For the pigs fed the high-fiber diet, the AEC was high (0.71) in the caudal half of the stomach and decreased somewhat in the cranial half (0.60). The lowest AEC values (0.14) were found in the proximal part of the small intestine, while they increased to 0.74 in the ileal part of the small intestine and then decreased slowly in the cecum and the large intestine to 0.61. For the pigs fed the low-fiber diet, the AEC values were lower in the stomach and the small intestine than in the pigs fed the high-fiber diet. AEC values in the proximal part of the large intestine were similar with the two types of diet, but the AEC values in the distal part of the large intestine were lower for the pigs fed the low-fiber diet than for the pigs fed the high-fiber diet.

Composition of gas from various segments of the gastrointestinal tract. Gas samples were taken from the 12 sites along the gut (Fig. 1), and their compositions were analyzed. The gas compositions in the various segments show similar trends for the two groups of pigs (Fig. 3). In both groups, low levels of H_2 were detected in gas from the stomach, followed by a steady increase along the small intestine reaching a maximum (21 to 28%) in the last third of the small intestine. Gas from the cecum and the first segment from the large intestine also contained substantial amounts of H_2, while the amounts in gas from the other segments of the large intestine were small. The amount of H_2 in gas from the cecum was larger for the pigs receiving the high-fiber diet than for the pigs receiving the low-fiber diet ($P < 0.05$). No methane could be detected in gas from the stomach or small intestine from any of the pigs. Small amounts of CH_4 were found in gas from the cecum, followed by a steady increase in the following segments, reaching concentrations as high as 29 to 37% in the rectum.

The percentage of CO_2 in the stomachs of the pigs receiving the high-fiber diet was much higher than that in the pigs receiving the low-fiber diet (43 to 47 versus 16 to 18%; $P < 0.05$). For both group of pigs, the percentage of CO_2 in the small intestine was around 30%. It increased in the cecum and the first segment of the large intestine to a level of 60 to 70%, followed by a steady decrease in the following segments. This decrease was, however, more pronounced in the pigs on the low-fiber diet. The residual gas was composed predominantly of N_2 (results not shown).

The analytical procedure used in the present investigation was not able to separate small amounts of O_2 from N_2, and quantitative results for O_2 are not presented here. However, we did find considerable amounts of O_2 in gas from the stomach (around 5%), while the concentrations of O_2 in gas samples taken from all other parts of the gastrointestinal tract were less than 1%.

In vivo methane production. In vivo methane production measured for the individual pigs is given in Table 3. The average daily methane production was 1.4 liters of CH_4 pig^{-1} day^{-1} (range, 0.9 to 1.9 liters) for the pigs fed the low-fiber diet and 12.5 liters of CH_4 pig^{-1} day^{-1} (range, 8.1 to 16.2 liters) for the pigs fed the high-fiber diet.

In vitro gas production. In vitro gas production was determined by using 20% slurries of gut content from the various segments of the gastrointestinal tract as described in Materials and Methods. Typical time courses of CO_2, H_2, and CH_4 production are shown in Fig. 4.

Apart from the slurries of stomach content in which an initial burst in CO_2 production was observed, the CO_2 production was linear for the first 4 h. The CO_2 production rate was calculated from the slope of the lines between 1 and 4 h. Large quantities of carbon dioxide were produced by contents from all segments of the gastrointestinal tract.

With the contents from the stomach, the cecum, and the large intestine, constant H_2 production was detected during the first 2 to 4 h. After this time, no further net production of H_2 could be detected with contents from the cecum and large intestine. With contents from the middle and last thirds of the small intestine, a continuous increase in H_2 production was detected. The H_2 production rate was calculated from the slope of the lines between 0 and 2 h. The highest H_2 production rates were detected with content from the small intestine.

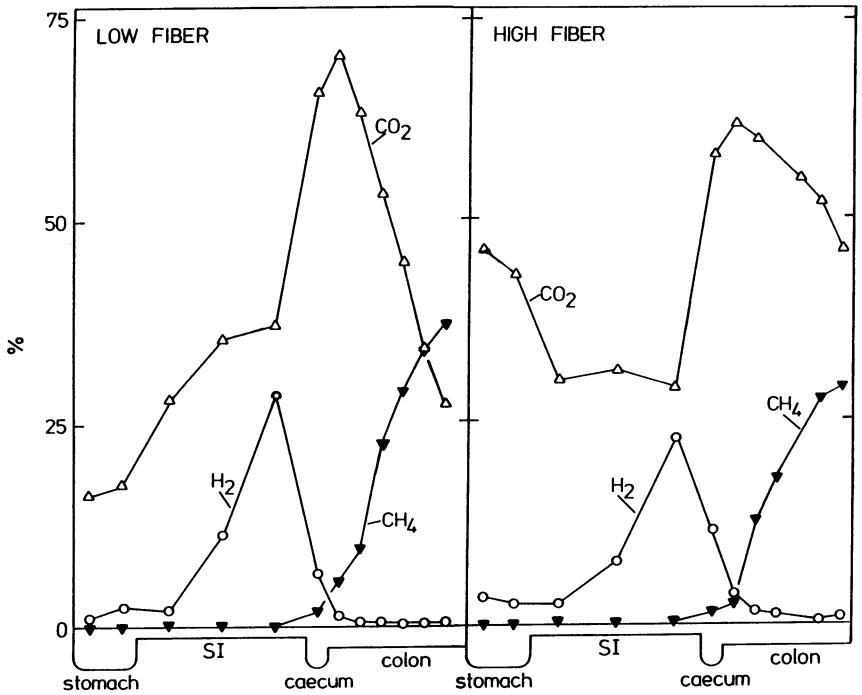

FIG. 3. Compositions of gases from various regions of the gastrointestinal tracts of the pigs fed the low- and high-fiber diets.

After an initial lag phase of 1 h, the production of CH_4 was linear during the next 5 h. No methane production could be detected in material from the stomach or the small intestine. Methane production could be detected in gut content from the cecum, followed by a steady increase in the production rate in the successive segments of the large intestine.

The mean carbon dioxide, hydrogen, and methane production rates for all four pigs fed each of the two diets are shown in Fig. 5. By multiplying the gas production rate (milliliters of gas per gram of gut content per day) by the total amount of digesta in each segment and adding these values together, the total amount of gas produced per pig per day could be estimated. These estimates are given for the individual pigs in Table 3. The average daily methane production was estimated to be 0.9 (range, 0.8 to 1.1) liters of CH_4 pig^{-1} day^{-1} for the pigs fed the low-fiber diet and 12.3 (range, 5.7 to 19.7) liters of CH_4 pig^{-1} day^{-1} for the pigs fed the high-fiber diet.

The average daily CO_2 production was estimated to be 212 (range, 157 to 249) liters of CO_2 pig^{-1} day^{-1} for the pigs fed the high-fiber diet and 46 (range, 39 to 54) liters of CO_2 pig^{-1} day^{-1} for the pigs fed the low-fiber diet (Table 3).

Substantial production of H_2 was detected only with samples from the small intestine. However, large variations between pigs in hydrogen production were detected (Fig. 5). The average daily H_2 production was estimated to be 5.2 (range, 1.4 to 11.3) liters of H_2 pig^{-1} day^{-1} for the pigs fed the high-fiber diet and 3.6 (range, 1.3 to 8.6) liters of H_2 pig^{-1} day^{-1} for the pigs receiving the low-fiber diet (Table 3).

DISCUSSION

Gut content and microbial activity. In agreement with the results presented here, previous authors have found that increasing levels of crude fiber in pig diets increase the amount of digesta in the gastrointestinal tract (20) and cause a decrease in the dry matter content of digesta (1, 28). On the other hand, our finding that a high level of fiber intake seems to increase the pH of gastric digesta is in contrast to the findings of other investigators. Drochner and Coenen (9) found a slightly reduced pH of gastric digesta 3 to 5 h after feeding in pigs fed 6% crude fiber, while Johansen (17) found no effect of increasing fiber content on the pH of the gastric digesta.

The high ATP concentrations and the high AEC values found in stomach digesta from the pigs fed the high-fiber diet indicate that substantial microbial activity takes place in the stomachs of these pigs. This is possibly a consequence of the higher pH of the digesta due to the buffering capacity of the fiber or because microenvironments of high pH exist within the highly unhomogeneous stomach contents. That substantial microbial metabolism takes place in the stomach of pigs has been suggested by several authors (6, 8) and is demonstrated by the high concentration of fermentation products such as lactic acid and other organic acids found in gastric contents (2).

The dense population of culturable anaerobic bacteria, the high ATP concentration, and the high AEC values found in the last third of the small intestine indicate that not only is that part of the gastrointestinal tract heavily colonized by microorganisms, but also the supply of nutrients is plentiful and the microbes are in a metabolically very active state. It is generally accepted that the AEC is important in the control of key catabolic and anabolic pathways (21), and studies have demonstrated that certain ranges of AEC values correlate with different physiological conditions. The AEC has a maximum value of 1.0, when all the adenylate is in the form of ATP, and a minimum value of 0.0, when all the adenylate is in the form of AMP. Values between 0.7 and 0.9 are typical in microorganisms which are actively growing and reproducing under optimal conditions. Values in the range of 0.5 to 0.7 have been measured in organisms which are under suboptimal conditions.

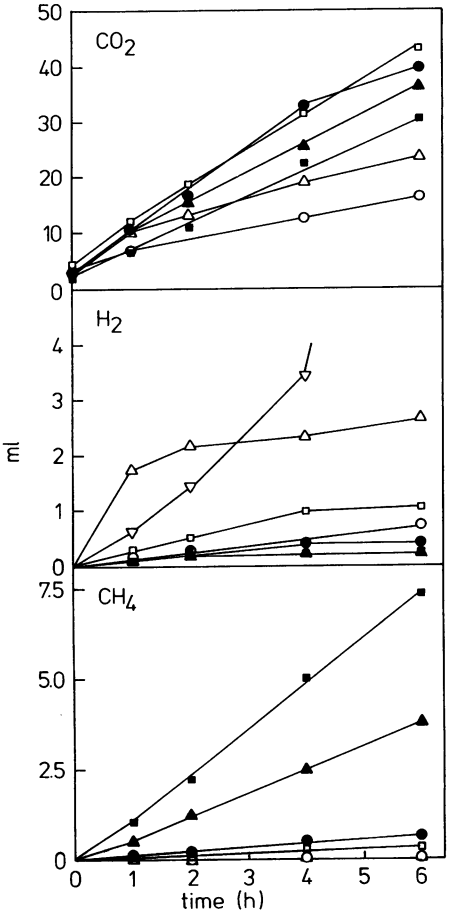

FIG. 4. Time course of carbon dioxide, hydrogen, and methane production by gut contents from various regions of the gastrointestinal tract of a pig fed the high-fiber diet. Slurries (20% [wt/vol]) were incubated anaerobically in serum bottles; no exogenous substrate was added. Sites: ○, S_1; ▽, SI_1; △, SI_3; □, cecum; ●, C_1; ▲, C_4; ■, C_6.

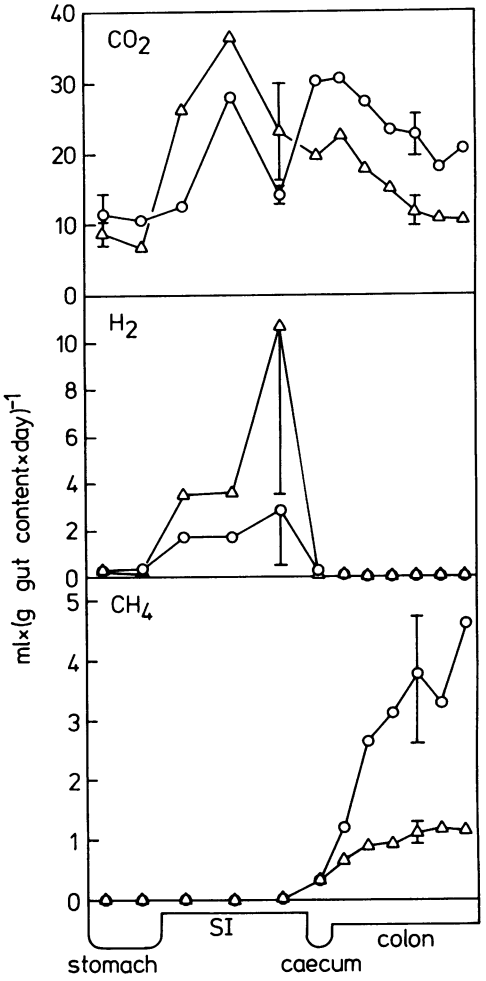

FIG. 5. Mean carbon dioxide, hydrogen, and methane production in various regions of the gastrointestinal tracts of the pigs fed the low-fiber (△) and the high-fiber (○) diets. Values are means with standard errors represented by vertical bars at representative sampling sites from the stomach (S_1), small intestine (SI_3), and hindgut (C_4).

Values below 0.5 have been associated with irreversible loss of viability under deleterious conditions.

The density of microorganisms was quite constant throughout the cecum and large intestine both in the pigs receiving the high-fiber diet and in the pigs receiving the low-fiber diet. However, the concentration of ATP and the AEC values fall progressively in the regions toward the distal colon. By contrast, the pH was lowest in the cecum and proximal colon and was higher in the distal colon. These changes in pH, ATP concentrations, and AEC values clearly indicate that although the density of culturable bacteria is almost constant throughout the hindgut, the maximal microbial fermentation occurs in the cecum and proximal colon, in which substrate availability is greatest. It has previously been shown that carbohydrates are the principal energy substrate for microbial fermentation in the large intestine of pigs (2, 15). The higher ATP concentrations and AEC levels in combination with the lower pH values found in all segments of the hindgut of the pigs fed the high-fiber diet compared with those fed the low-fiber diet confirm that fermentable carbohydrates are important energy substrates for large intestinal microbial fermentation and show that it is possible to increase microbial fermentation in the hindgut of pigs by increasing the amount of fiber in the diet.

Gas composition and gas production. In contrast to the enormous numbers of analyses of gastrointestinal digesta which have been performed, there have been surprisingly few analyses of gas samples obtained from various gut regions of monogastric animals and humans. As in the present investigation with pigs, the five gases N_2, O_2, CO_2, H_2, and CH_4 also appear to constitute more than 99% of intestinal gas in humans and dogs (26). Normally, negligible amounts of H_2 and CH_4 are present in the stomachs of humans and dogs, and N_2 (80%) and CO_2 (3 to 17%) are the dominating gases. However, with gastric stasis H_2 can reach concentrations as high as 28% (10, 22, 35). CO_2 (5 to 15%) and N_2 (80%) are also the dominating gases in the small intestines of humans and dogs, but in contrast to the findings for pigs, H_2 is normally not detected (26). Also in contrast to the situation for pigs, only small amounts of methane are found in the hindgut of humans (some humans produce no methane and others produce only small amounts) (39). On the other hand, H_2 is always found in high concentrations (10 to 20%) in gas samples from the hindgut of humans. As we have found with pigs, the ingestion of dietary

fiber by humans is also associated with an increase in the concentration of CH_4 and CO_2 in the hindgut gas (26).

The higher concentration of CO_2 found in gas from the stomachs of the pigs fed the high-fiber diet correlates very well with the high level of microbial activity (ATP concentrations and AEC values) found in the stomachs of these pigs.

A recent investigation has reported that high dissolved-O_2 concentrations (50 to 20% of air saturation) are present in digesta contents from all segments of the digestive tract of pigs (12). Although our measuring system was not optimal for measuring small concentrations of O_2, we can conclude that apart from the stomach the concentration of O_2 was <1% in all other segments of the gastrointestinal tract. High levels of O_2 content in colonic gas have occasionally been measured by other investigators (24). However, a potential source of error is the interchange between the gaseous atmosphere of the gut and the surrounding air between slaughter and sample collection or during measurements. In fact, we find it hard to believe that high dissolved-O_2 concentrations can exist in an environment such as the gut, in which a dense population of facultatively anaerobic bacteria and plenty of respiratory substrate are present. Furthermore, it is difficult to explain why the vast majority of the hindgut bacteria are strict anaerobes (including methanogenic bacteria) if aerobiosis is the rule in the hindgut.

In general, a very good correlation between the concentrations of gases in the gas phase from a specific place in the gut and the in vitro rate of production of gases at the same site was found. The highest concentrations and highest production rates for H_2 were found for the last third of the small intestine, while only small concentrations and low rates of production of H_2 were detected in the cecum and colon. In contrast to that, high-level production of H_2 in the small intestine in humans is found only if there is an overgrowth of bacteria. This indicates that although the digestive systems of humans and pigs are similar, a substantially higher level of microbial activity takes place in the small intestine in pigs.

The rate of production of methane as well as the concentration of methane in the gas phase increased throughout the colon. In agreement with our results, Robinson et al. (36) found that no methane production could be detected on incubation of the contents from the small intestine and stomach and that hydrogen gas accumulated to a greater extent in in vitro incubations of pig small intestinal contents than in incubated cecal or colonic samples from pigs.

It is evident from the data shown in Table 3 that there exists very good agreement between the in vivo and the in vitro measurements of CH_4 excretion. In general, the amount of CH_4 excreted by the pigs fed the low-fiber diet in vivo was estimated to be 1.4 liters day^{-1} and in vitro to be 1.0 liters day^{-1}. Substantially larger amounts of CH_4 were produced by the pigs fed the high-fiber diet (12.5 liters day^{-1} in vivo and 12.2 liters day^{-1} in vitro). Also, the daily CO_2 production in the gut was greater in the pigs fed the high-fiber diet than in those fed the low-fiber diet (210 versus 50 liters day^{-1}).

The estimates of CH_4 production for pig gut samples obtained in this study with the pigs fed the high-fiber diet (130 kg live weight) are similar to the excretion of 12 liters day^{-1} reported by Christensen and Thorbek (5) for 120-kg pigs. Christensen and Thorbek (5) found that CH_4 excretion increased with increasing feed intake, probably because a greater amount of undigested material reached the hindgut. This is also in agreement with our results showing that increasing amounts of dietary fiber in the diet increase CH_4 production. Using in vitro incubations, Robinson et al. (36) estimated the CH_4 excretion by 85-kg pigs to be 1.2 liters day^{-1}. This rate is clearly much lower than the CH_4 excretion rate of 5 to 7 liters day^{-1} found for 85-kg pigs by Christensen and Thorbek (5) and also lower than the values found in this study. The lower CH_4 excretion rate found by Robinson et al. (36) could be due to the fact that they were using filtered samples of gut content in their in vitro studies. As they pointed out, filtration of gut content through two layers of cheesecloth to remove large particles may also have resulted in a loss of methanogenic bacteria as well as organisms supplying the methanogens with H_2 and substrate for these H_2-producing bacteria. Therefore, unfiltered gut samples were used in the present investigation.

Hydrogen sinks. As pointed out above, the ATP, AEC, and pH data indicate that the highest level of microbial activity takes place in the cecum and the proximal colon. Since H_2 production is an obligate part of anaerobic fermentation in the hindgut (3, 33), one would expect H_2 production to be high in the cecum and the proximal part of the colon and to steadily decrease in the successive segments of the colon, but that was not the case. However, a number of possible pathways for disposal of H_2 in the gut exist (41). A small proportion may pass through the gut wall into the bloodstream and to the lungs, where it is excreted in breath. Alternatively, H_2 can be metabolized by the large intestinal microflora. Possible hydrogen sinks include saturation of unsaturated fatty acids (18), reduction of nitrate to ammonia (16), reduction of sulfate to sulfide (11), reduction of CO_2 to methane (36), and reduction of CO_2 to acetate (7, 25, 34).

It is well-known that in the rumen almost all microbially produced H_2 is further metabolized to methane by methanogenic bacteria. However, our results show that only small amounts of CH_4 are produced in the cecum and proximal colon of pigs. Robinson et al. (36) also found a 10-fold-lower level of CH_4 production by cecal material than by colonic material. This indicates that hydrogen sinks other than methane production are involved in H_2 disposal in the cecum and proximal colon of pigs. Jørgensen and Just (19) showed that unsaturated fatty acids are saturated distal to the ileal-caecal junction in pigs, and Christensen and Thorbek (5) have shown that inclusion of soybean oil in a basal diet of pigs reduces the amount of CH_4 excreted. Furthermore, the presence of acetogenic bacteria in pigs has been shown (7), and pigs probably contain sulfate-reducing bacteria in the gut, as humans do.

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