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Evaluation of fermentability of acid-treated maize husk by rat caecal bacteria *in vivo* and *in vitro*

BY HIROSHI HARA[1], YUTAKA SAITO[1], HIROSHI NAKASHIMA[2] AND SHUHACHI KIRIYAMA[1]

[1] *Department of Bioscience and Chemistry, Faculty of Agriculture, Hokkaido University, Sapporo 060, Japan*

[2] *Development Department Pharmaceutical Section, Fujinomiya Factory, Terumo Corporation, Fujinomiya-city, Shizuoka 418, Japan*

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Fermentable energy in insoluble dietary fibre (DF) sources was evaluated by *in vivo* and *in vitro* methods using rats. Test diets contained 50 and 100 g maize husk or organic-acid-treated maize husk/kg diet. Soluble fractions were removed from both the DF sources by washing. The acid treatment increased digestibility by a microbial hemicellulase from 12·7% to 32·6%. The fermentability of DF was evaluated by measurement of the production rate of short-chain fatty acids (SCFA) in a short-term *in vitro* incubation of the caecal contents of rats fed on test diets for 22 d. The production rates of the major SCFA, acetic, propionic and butyric acids, were increased by feeding both DF sources, and these production rates in the acid-treated DF group were significantly higher than those in the untreated DF group. The production rate of a minor SCFA, isovaleric acid, was decreased by feeding both diets. The production rate of total SCFA in rats given the acid-treated maize husk was 32·6% higher than that in rats given the untreated maize husk. The fermentable energy in DF was estimated *in vivo* by subtracting the faecal excretion of DF energy from ingested DF energy. The fermentable energy in DF was increased by the acid treatment (32·5% in maize husk and 63·4% in acid-treated maize husk), which agreed with the SCFA production rate predicted in the caecum. These results indicate that a short-term incubation of caecal contents is a useful method for evaluation of the fermentability of DF sources, and that acid treatment can increase the fermentability of an insoluble DF source.

Short-chain fatty acids: Dietary fibre: Caecum: Rat

Measurement of the fermentability of ingested dietary fibre (DF) is important for evaluation of the energy value of a diet and the physiological effects of the DF sources in the colon. The end products of fermentation, mainly short-chain fatty acids (SCFA), are known to influence several functions of the colon, for example ionic transport (Argenzio *et al.* 1975), colonic motility (Yajima, 1985) and mucosal-cell proliferation (Sakata, 1987), and may be related to the incidence of colon cancer.

The aim of the present study was to evaluate the production rate of SCFA in the rat caecum and to evaluate the fermentable energy in the ingested DF sources by measuring faecal energy excretion derived from DF. The caecum is a major site of SCFA production in rats (Rémésy & Demigné, 1976).

The production rate of SCFA in the caecum was measured by a short-term *in vitro* incubation of the caecal contents of rats immediately after death. This may reflect the *in vivo* SCFA production rate of caecal contents. This method was described by Carroll & Hungate (1954) using the contents of the bovine rumen. This simple method has been used with rabbit (Hoover & Heitmann, 1972) and porcupine caecal contents (Johnson & McBee, 1967).

The DF sources used in our study were maize husk, which is a readily available insoluble fibre source, and maize husk treated with a heated organic acid in order to increase its fermentability. This may be a new source of highly fermentable insoluble DF.

MATERIALS AND METHODS

Animals and diets

Expt 1. In vitro *SCFA production rates by caecal contents.* Male Sprague–Dawley rats (Japan SLC, Hamamatsu, Japan), weighing 125–150 g, were divided into three groups of six rats by randomized block design. The rats were fed on a semi-purified powdered diet containing 250 g casein/kg (fibre-free stock diet, Table 1) for 12 d under a meal-feeding regimen of 6 h (10·00–16·00 hours) every day in order to coordinate the postprandial condition. DF sources (100 g/kg diet) were added to the fibre-free stock diet to determine the effects on SCFA production rates by the caecal contents. The rats were given a fibre-free diet or diets containing the DF sources for 22 d by meal feeding. On the last day aortic blood was withdrawn under pentobarbital anaesthesia (Nembutal, sodium pentobarbital, 50 mg/kg body weight; Abbott Co., North Chicago, IL, USA) 5 h after feeding the test diet, and immediately the caecum was removed together with its contents. The liver was also removed.

Expt 2. In vivo *fermentable energy in maize-husk preparations.* Male Sprague–Dawley rats (Japan SLC), weighing 135–155 g, were divided into three groups of six rats which were fed on the fibre-free stock diet for 7 d. DF sources (50 g/kg diet) were added to the fibre-free stock diet to make the test diets. The rats were fed on a fibre-free diet or diets containing the DF sources for 21 d, and their faeces were collected from the 8th to the 10th day (period 1) and from the 19th to the 21st day (period 2) after feeding the test diets, in order to measure gross energy excretion in faeces. The faeces were collected daily, and were frozen immediately.

Throughout the experiments the rats were housed individually in wire-bottomed cages, and kept in a temperature controlled room at $23 \pm 2°$ maintaining a 12 h light-dark cycle.

Fibre sources were prepared from a commercially available maize husk (Oji-Cornstarch Co. Ltd, Ichihara, Japan). The maize husk was heated in a citric acid solution, pH 3·0, at 120° for 30 min and washed (acid-treated maize husk). The untreated maize husk was heated in water (120° for 30 min) and washed to remove residual starch. These preparations were ground to a particle size of 20 μm or less, and dried.

The chemical composition of the DF sources is shown in Table 1. Both preparations contained about 800 g or more total DF/kg, and 90% or more of the total DF was insoluble DF.

Analyses

The protein, lipid and ash contents of the DF preparations were analysed by the methods of the Association of Official Analytical Chemists (1990). Total and insoluble DF contents of the preparations were measured by the enzymic-gravimetric method of Prosky *et al.* (1985). The insoluble DF was estimated from the weight of the insoluble fraction of the enzymically-digested solution in Prosky's procedure, which was separated by filtration. Acid and neutral detergent fibres were measured by the methods of Van Soest (1963) and Van Soest & Wine (1967) respectively.

Microbial hemicellulase (containing pectinase activity originating from *Aspergillus niger* (Type M; Tanabe Seiyaku, Osaka, Japan), 100 g of enzyme source/kg of substrate) digestibilities of the fibre preparations *in vitro* were 12·7% in untreated maize husk and 32·6% in acid-treated maize husk, which were estimated by reducing the dry weight of water-insoluble substances after hemicellulase digestion for 20 h at 37° (pH 4·5).

The caeca with contents were weighed, the contents were taken out into 10 ml autoclaved

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Table 1. *Composition of test diets (g/kg diet) and dietary fibre sources (g/kg preparation)*

	Stock diet	
Casein*	250	
Maize oil†	50	
Mineral mixture‡	40	
Vitamin mixture§	10	
Granulated vitamin E‖	1·0	
Choline bitartrate	4·0	
Maize husks	100 (Expt 1) or 50 (Expt 2)	
Sucrose	to make 1 kg	
Composition of maize husks	Untreated	Acid-treated¶
Protein**	88	116
Lipid††	41	52
Ash	7·7	8·2
Dietary fibre		
Total	822	790
Insoluble	788	760
Neutral detergent	784	635
Acid detergent	205	331

* Casein (ALACID; New Zealand Dairy Board, Wellington, New Zealand).
† Retinyl palmitate (7·66 μmol/kg diet) and ergocalciferol (0·0504 μmol/kg diet) were added to maize oil.
‡ The mineral mixture was prepared according to the AIN-76 Workshop held in 1989 (Reeves, 1989). It provided (mg/kg diet): Ca 4491, P 2997, K 3746, Mg 375, Fe 100, I 0·32, Mn 10·0, Zn 34·7, Cu 6·00, Na 4279, Cl 6542, Se 1·05, Mo 1·00, Cr 0·50, B 0·50, V 0·25, Sn 2·00, As 1·00, Si 20·0, Ni 1·00, F 2·72, Co 0·20.
§ The vitamin mixture was prepared in accordance with the AIN-76 mixture (American Institute of Nutrition, 1977) except that menadione and L-ascorbic acid were added to make 5·81 μmol/kg (American Institute of Nutrition, 1980) and 284 μmol/kg diet (Harper, 1959) respectively.
‖ Vitamin E granules (Juvela; Eisai Co., Tokyo, Japan) supplied 423 μmol all-*rac*-α-tocopheryl acetate/kg diet.
¶ For details of treatments, see p. 720.
** Protein content was measured by the Kjeldahl method (N × 6·25).
†† Lipid content was measured as fat acidity following the procedure of the Association of Official Analytical Chemists (1990).

phosphate buffer solution (pH 8·0) in a vial without exposure to air, and suspended. The vial was sealed with a silicone septum filled with N_2, and incubated at 37°. Samples of the suspension of the caecal contents (1 ml) were collected by a long injection needle (18G × 90 mm; Terumo Corp., Tokyo, Japan) through the silicone septum, both before and 4 h after an incubation. The incubation was started within 5 min after death. A linear increase of the SCFA concentration in the incubation fluid during 4 h was confirmed in rats fed on a fibre-free or maize-husk diet in a previous experiment in which the incubation fluid was sampled through the needle 1, 2 and 4 h after the incubation. The 4 h incubation period was adopted in order to measure minor SCFA production rates correctly. The suspension of caecal contents was deproteinized by perchloric acid (final concentration 50 g/l) cooled in ice, and the supernatant was added to a KOH solution to precipitate perchloric acid and to form potassium salts of the SCFA. Individual SCFA were measured by GLC (Shimadzu GC-14A with a glass column (1600 mm × 3 mm) packed with 80–100 mesh chromosorb W-AW DMCS with H_3PO_4 (100 ml/l) as a liquid phase; Shimadzu Corporation, Kyoto, Japan) after adding H_3PO_4.

The faeces were freeze-dried, weighed and milled. Gross energy in DF preparations and powdered faeces was measured by bomb calorimetry (Model CA-3; Shimadzu Corporation).

Protein content of the liver was measured by the modified Lowry's method (Lowry *et al.*

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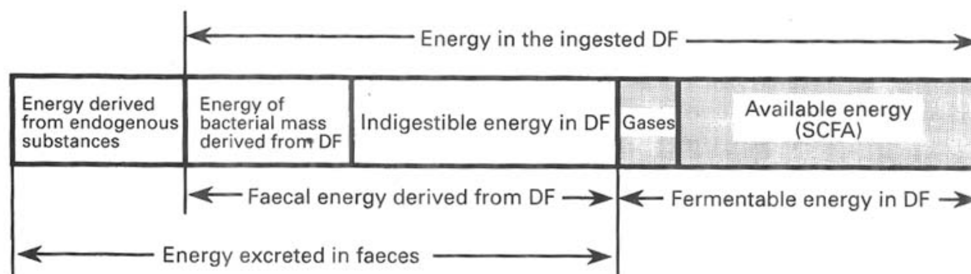

Fig. 1. Illustration of the destination of ingested dietary fibre (DF) and the origins of energy in faeces.

1951; Sugawara, 1975). Glycogen content was estimated photometrically with anthrone (Koehler, 1952) after alkaline solubilization of the liver tissue. Blood glucose concentration was measured by an enzymic method (Glucose B-test Wako; Wako Pure Chemical Industry, Tokyo, Japan), and cholesterol and triacylglycerol concentrations in the liver were estimated using enzymic procedures (T-CHO and TG-EN respectively; Kainos Laboratories Inc., Tokyo, Japan) after Folch's extraction (Folch *et al.* 1957) of saponified liver and hexane extraction of liver respectively.

Calculation and statistics

The feed efficiency ratio was calculated as body weight gain (g) per g of the stock diet in the test diets fed to rats.

The production rates of SCFA by the caecal contents were expressed per whole caecum, and the initial SCFA concentration in the caecum was expressed as amount per g wet caecal contents and per whole caecum. The production rate was calculated from the increase in the concentration of each SCFA in the suspension fluid of the caecal contents over the 4 h incubation period. The initial amount and concentration of SCFA in the caecum were evaluated from the concentrations of SCFA in the suspension before an incubation.

Fermentable energy in DF sources (%) in the test diets was calculated as:

$$\frac{\text{energy in DF consumed (kJ/3 d)} - \text{faecal energy derived from DF (kJ/3 d)}}{\text{energy in DF consumed (kJ/3 d)}} \times 100 \quad (1).$$

Faecal energy derived from DF was estimated by subtracting the endogenous energy in faeces from the faecal energy of each rat in the DF groups. The endogenous energy in faeces was calculated as:

$$\frac{\text{average faecal energy in fibre-free group} \times \text{wt of non-fibre components of diet consumed by rat of fibre group}}{\text{average food intake in fibre-free group}} \quad (2).$$

The endogenous energy in faeces was considered to be proportional to the intake of the fibre-free component of the diet in each of the test diets. The fermentable energy is illustrated in Fig. 1.

Data were analysed by one-way analysis of variance (ANOVA; $P < 0{\cdot}05$), and significant differences between diet groups were determined by the least significant difference ($P < 0{\cdot}05$).

RESULTS

Fig. 2 shows that the caecal concentrations of propionic and butyric acids in the acid-treated maize husk group were higher than those in the untreated maize husk group. The

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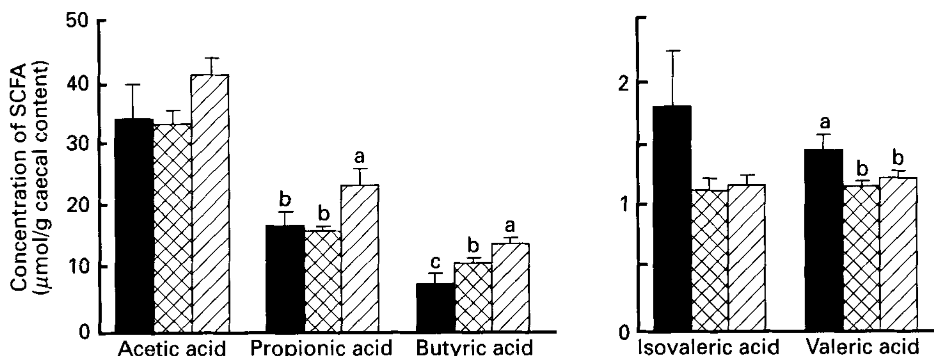

Fig. 2. Initial concentrations (μmol/g wet caecal contents) of short-chain fatty acids (SCFA) in the caeca of rats fed on test diets containing no fibre (■), untreated maize husk (100 g/kg; ▧) or acid-treated maize husk (100 g/kg; ▨) for 22 d. All values are means with their standard errors for six rats. P values estimated by one-way ANOVA were 0·168, 0·018, < 0·001, 0·126 and 0·028 for acetic, propionic, butyric, isovaleric and valeric acids respectively. For each SCFA, diet values with unlike superscript letters are significantly different (LSD): $P < 0.05$.

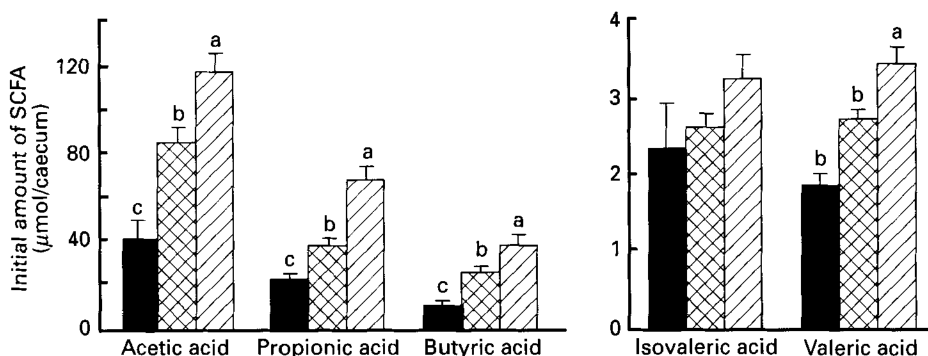

Fig. 3. Short-chain fatty acid (SCFA) content in the caeca of rats fed on test diets containing no fibre (■), untreated maize husk (100 g/kg; ▧) or acid-treated maize husk (100 g/kg; ▨) for 22 d. All values are means with their standard errors for six rats. P values estimated by one-way ANOVA were < 0·001, < 0·001, < 0·001, 0·148 and < 0·001 for acetic, propionic, butyric, isovaleric and valeric acids respectively. For each SCFA, diet values with unlike superscript letters are significantly different (LSD): $P < 0.05$.

initial amounts of the major SCFA, acetic, propionic, and butyric acids, shown in Fig. 3, were significantly increased by feeding both DF sources, and the amounts of these SCFA in the rats fed on the acid-treated maize husk were significantly higher than those of the rats fed on the untreated maize husk. The initial molar ratio of acetic, propionic, and butyric acids in the caecum was about 4:2:1 in each diet group. The amounts of two minor SCFA, isovaleric and valeric acids, in the caecum were found to be very low in all groups.

The changes in the production rates of major SCFA in the caecum caused by feeding the DF sources, shown in Fig. 4, tended to be similar to those in the initial amounts of the SCFA. The difference in the production rates between the three SCFA was smaller than the difference in the initial content between the SCFA. The production rate of isovaleric acid was significantly reduced by feeding both the DF sources. The sum of the production rates of all SCFA is shown in Table 2 (values converted from mol to mg). The rate in the acid-treated maize husk group was twofold higher than that in the fibre-free group.

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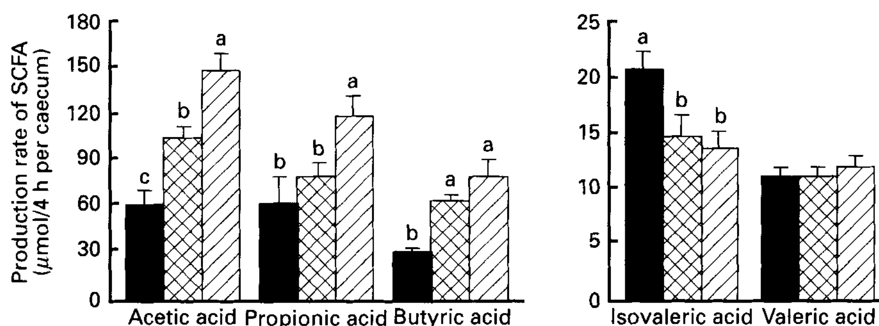

Fig. 4. Production rates of short-chain fatty acids (SCFA) by the caecal contents of rats fed on test diets containing no fibre (■), untreated maize husk (100 g/kg; ⊠) or acid-treated maize husk (100 g/kg; ▨) for 22 d. The values were evaluated from the increments of SCFA concentrations in the suspension of the caecal contents after *in vitro* incubation for 4 h at 37° (pH 8·0). All values are means with their standard errors for six rats. *P* values estimated by one-way ANOVA were 0·001, 0·023, $< 0·001$, 0·026 and 0·660, for acetic, propionic, butyric, isovaleric and valeric acids respectively. For each SCFA, diet values with unlike superscript letters are significantly different (LSD): $P < 0·05$.

Table 2. *Sum of the production rates of short-chain fatty acids (SCFA; mg/4 h per caecum) by the caecal contents of rats fed on fibre-free or maize-husk diets (100 g/kg diet) for 22 d**

(Mean values with their standard errors for six rats)

	SCFA production	
Diet	Mean	SE
Fibre-free	15·49[c]	1·800
Maize husk		
Untreated	21·33[b]	1·133
Acid-treated	28·30[a]	1·825
ANOVA	$P = 0·002$	

[a,b,c] Mean values with unlike superscript letters are significantly different (LSD): $P < 0·05$.
* For details of diets, fibre-treatments and procedures, see Table 1 and pp. 720–722.

Body weight gains and food intakes, shown in Table 3, were not influenced by feeding either of the fibres, but feed efficiency ratio tended to be higher in the acid-treated maize husk group than in the fibre-free group ($P = 0·0875$ in F-test).

Liver weights in the DF groups were lower than those in the fibre-free group, as shown in Table 4. Wet weights of the caecal contents, however, were increased after feeding the DF-containing test diets (Table 4). The results of liver analyses (Table 5) show that the glycogen content of both the DF groups was lower than that of the fibre-free group. Liver protein, triacylglycerol and cholesterol contents were not influenced by feeding DF.

Mean values of blood glucose concentration 5 h after feeding were 10·8, 10·6, and 11·3 mmol/l in the fibre-free, untreated, and acid-treated fibre groups respectively (no significant difference).

Fig. 5 shows faecal energy excretion for 3 d during feeding of the test diets. In period 1, energy excretion was increased significantly by feeding the diets containing DF sources, but there was no difference between the DF groups. In period 2, energy excretion by the rats fed on the acid-treated maize husk diet was significantly lower than that by the rats fed on

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Table 3. *Body weight gain (g/21 d), food intake (g/21 d) and feed efficiency ratio (FER)* in rats fed on fibre-free or maize-husk diets (100 g/kg diet) for 21 d†*

(Mean values with their standard errors for six rats)

Diet	Body wt gain		Food intake		FER	
	Mean	SE	Mean	SE	Mean	SE
Fibre-free	84·8	4·12	262[b]	5·5	0·323[b]	0·0123
Maize husk						
Untreated	84·8	5·01	276[ab]	13·9	0·340[ab]	0·0052
Acid-treated	102·8	8·36	307[a]	16·1	0·370[a]	0·0179

[a,b] Mean values with unlike superscript letters are significantly different (LSD): $P < 0.05$.

* Feed efficiency ratio was evaluated by the following equation. Feed efficiency ratio = body weight gain/(food intake × 0·90).

† For details of diets and procedures, see Table 1 and pp. 720–722.

Table 4. *Weights of liver and caecum (g/100 g body wt) in rats fed on fibre-free or maize-husk diets (100 g/kg diet) for 22 d**

(Mean values with their standard errors for six rats)

Diet	Liver		Caecal wall		Caecal content	
	Mean	SE	Mean	SE	Mean	SE
Fibre-free	4·80[a]	0·161	0·34	0·032	0·54[b]	0·088
Maize husk						
Untreated	4·21[b]	0·050	0·37	0·015	1·12[a]	0·055
Acid-treated	4·19[b]	0·139	0·38	0·032	1·22[a]	0·062
ANOVA	$P = 0.013$		NS		$P < 0.001$	

NS, not significant.

[a,b] Mean values with unlike superscript letters are significantly different (LSD): $P < 0.05$.

* For details of diets and procedures, see Table 1 and pp. 720–722.

Table 5. *Liver protein (g/liver), triacylglycerol (g/liver), cholesterol (mg/liver) and glycogen (g/liver) content in rats fed on fibre-free or maize-husk diets (100 g/kg) for 22 d**

(Mean values with their standard errors for six rats)

Diet	Protein		Triacylglycerol		Cholesterol		Glycogen	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Fibre-free	1·61	0·062	0·217	0·0292	24·6	3·04	0·475[a]	0·0548
Maize husk								
Untreated	1·56	0·080	0·197	0·0502	21·1	1·84	0·238[b]	0·0495
Acid-treated	1·63	0·105	0·256	0·0325	22·4	1·65	0·216[b]	0·0267
ANOVA	NS		NS		NS		$P = 0.011$	

NS, not significant.

[a,b] Mean values with unlike superscript letters are significantly different (LSD): $P < 0.05$.

* For details of diets and procedures, see Table 1 and pp. 720–722.

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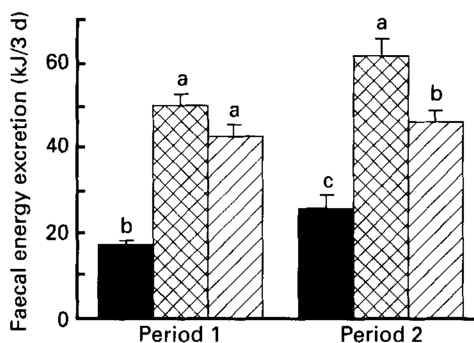

Fig. 5. Energy excretion in faeces over 3 d in rats fed on test diets containing no fibre (■), untreated maize husk (50 g/kg; ⊠) or acid-treated maize husk (50 g/kg; ▨). Period 1 is from the 8th day to the 10th day, and period 2 is from the 19th day to the 21st day. All values are means with their standard errors for six rats. P values estimated by ANOVA were $< 0{\cdot}001$ in period 1 and $< 0{\cdot}001$ in period 2. For each period, diet values with unlike superscript letters are significantly different (LSD): $P < 0{\cdot}05$.

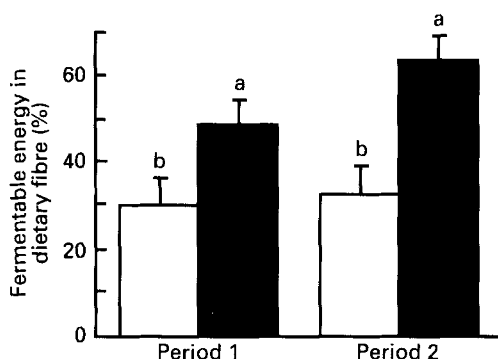

Fig. 6. *In vivo* fermentabilities of dietary fibre from untreated maize husk (□) and acid-treated maize husk (■) estimated from energy intakes and excretion into faeces of fibre preparations. Period 1 is from the 8th day to the 10th day, and period 2 is from the 19th day to the 21st day. All values are means with their standard errors for six rats. P values estimated by ANOVA were 0·129 in period 1 and 0·022 in period 2. For each period, diet values with unlike superscript letters are significantly different (LSD): $P < 0{\cdot}05$.

the untreated maize-husk diet. Also in period 2, fermentable energy in the acid-treated maize husk shown in Fig. 6 was twofold higher than that of the untreated maize husk.

In Expt 2 body weight gains and food intakes of both DF groups were the same as those of the fibre-free group. Mean values for body weight gain and food intake by eighteen rats were 156·7 g/21 d and 394 g/21 d respectively.

DISCUSSION

The present study shows that the fermentable energy in acid-treated maize husk is twofold higher than that in untreated maize husk, and that the SCFA production rate in the caecum of rats fed on the acid-treated maize husk is higher than that of rats fed on the untreated maize husk. The results indicate that the acid treatment enhances the caecal fermentation of maize husk. The organic acid treatment may change the structure of the insoluble DF source. These results also show that *in vitro* caecal SCFA production is correlated with the *in vivo* utilization of energy from the DF sources.

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Production rates of SCFA have been measured *in vivo* by the isotope dilution technique (Yang *et al.* 1970; Parker, 1976) or the nylon bag method (Robertson *et al.* 1987). Production activities have also been measured by *in vitro* incubation systems with colonic digesta and faeces. With the *in vivo* methods it is difficult to measure all the SCFA species in many diet groups. In the *in vitro* method the SCFA production is usually estimated by incubation for many hours after addition of exogenous substances to the incubation medium (McBurney & Thompson, 1987; Costa *et al.* 1989). The exogenous substance may change the SCFA production rate. Van Nevel & Demeyer (1988) showed that lowering pH decreases the production rate of SCFA and changes the proportion of the SCFA produced in the *in vitro* incubation of rumen contents.

In the present report the SCFA production rate in the caecum was measured by a short-term *in vitro* incubation of rat caecal contents without addition of any exogenous substrate. A buffered medium was used in order to prevent lowering the pH of the incubation medium. The use of the buffered medium (pH 8) and dilution of the caecal contents may possibly influence SCFA production. However, the production rates of SCFA shown in Fig. 4 were similar to the SCFA concentrations in the human proximal colon reported by Cummings *et al.* (1987). In this study the average time of sampling the human colonic contents was about 3 h after sudden death (stop of the blood flow), so the human values roughly represent the production rate of SCFA by the colonic contents for 3 h. Therefore the production rate of SCFA evaluated by the *in vitro* method in the present report may reflect the *in vivo* production rate of SCFA.

The concentration and content of SCFA in the colonic digesta have been used as indices of the fermentation of DF in many studies (Stanogias & Pearce, 1985; Mathers & Dawson, 1991; Fleming *et al.* 1992). However, these indices are determined not only by SCFA production rate but also by SCFA absorption rate in the colon. In the present study the changes in the initial amounts of the major SCFA in the caecum between diet groups were correlated with the increases in the SCFA production rates. In contrast, the initial amount of each SCFA did not reflect the production rate of each SCFA. That is, the differences in the production rates between acetic, propionic, and butyric acids were smaller than the differences between the initial amounts of these SCFA in the caecum. These findings suggest that butyric or proprionic acid in the caecum is absorbed faster than acetic acid. The amounts of valeric and isovaleric acids in the caecum were much less than the production rates of these minor SCFA. The result suggests that the minor SCFA are absorbed rapidly from the caecum.

The decrease in production rate of isovaleric acid caused by feeding DF sources was found to be opposite to changes in the production of the major SCFA. The precursor of isovaleric acid is known to be leucine (Zarling & Ruchim, 1987), and the lower production rate of the minor SCFA represents the decrease in the leucine concentration of the caecal content, which may be caused by the enhancement of the luminal bacterial growth by the supplement of DF.

Table 5 shows that the decrease in liver weight in both DF groups was the result of the lower glycogen content. The decrease in glycogen content may correspond to that in the liver weight because glycogen is stored with a large amount of water in the tissue. Glycogen content was measured in the absorptive state (5 h after feeding), which was supported by high concentrations of blood glucose. Insoluble DF is not known to influence gastric emptying rate (Tinker & Schneeman, 1989). Therefore, the lower glycogen content in the DF groups may not be due to a difference in sugar absorption, but may be due to the enhancement of hormone secretion, for example, glucagon. Wolever *et al.* (1991) observed an increase in plasma glucagon following rectal infusion of acetic and propionic acids.

In summary, a short-term *in vitro* incubation of rat caecal contents was a useful method

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for measurement of the production rate of SCFA in DF-fed rats and for evaluation of the fermentability of a DF source. Acid-treated maize husk was evaluated as a new insoluble DF source with high fermentability.

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