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Effects of dietary fibers and their mixtures on short chain fatty acids and microbiota in mice guts

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Dietary fiber (DF) can be broken down into short-chain fatty acids (SCFAs) such as acetic, propionic and *n*-butyric acid by gut microbiota to obtain energy. Therefore, dietary fibers have effects on the balance of gut microbiota and the production of SCFAs. In the four-week feeding, mice were fed with four dietary fibers, including pectin, resistant starch (RS), fructo-oligosaccharide (FOS) and cellulose. The results showed that the mice body-weight gain was the smallest (7.0 ± 2.3 g) when the mixture of RS–FOS–cellulose was ingested, followed by the mixture of RS–cellulose (7.2 ± 3.5 g) and FOS–cellulose (8.3 ± 2.5 g). Ingestion of the mixture of pectin–FOS–cellulose, RS–FOS and RS–FOS–cellulose can respectively increase the diversity of the gut microbiota with 12, 11 and 11 terminal restriction fragments (TRFs) detected (digested by *Hha* I). The maximum amount of total SCFAs were produced by the mixture of FOS–cellulose (5.504 ± 0.029 μmol mL^{-1}), followed by pectin–FOS–cellulose (3.893 ± 0.024 μmol mL^{-1}) and pectin–RS–FOS–cellulose (3.309 ± 0.047 μmol mL^{-1}). In conclusion, the addition of DFs (pectin, RS, FOS and cellulose), in single or mixture pattern, can exert different effects. An amount of 10.7% of single DF in the diet cannot be conducive to the balance of gut microbiota after ingestion for a long time, however, it can help with body weight loss like the mixtures of DFs in this study; FOS is a very important component in the mixture of DFs for both the balance of the gut microbiota and the production of SCFAs.

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Introduction

Dietary fiber (DF) consists of the remnants of plant cells resistant to hydrolysis (digestion) by the alimentary enzymes of man, whose components are hemicelluloses, celluloses, lignins, oligosaccharides, pectins, gums and waxes. Most of them are indigestible, as these components cannot be degraded by the enzymes present in the large intestine, but they are exposed to bacterial enzymatic activities that can partially degrade them.[1] The mammal intestinal tract, especially the large intestine, harbors magnanimous bacteria that reach 10^{11-12} cells per gram of contents. The gut microbiota provides a multitude of functions for the host, including nutrient processing (*e.g.*, complex sugar breakdown), regulation of host fat storage and protection against pathogens. Moreover, certain gastrointestinal diseases, such as inflammatory bowel disease or necrotizing enterocolitis, have been linked to gut microbiota.[2–4] The gut bacteria mainly obtain energy by breaking down dietary fibers, and the extent of this degradation depends on the type of bacterial flora.[5]

The most important end products of dietary fibers fermented in the gut are the short-chain fatty acids (SCFAs) such as acetic acid, propionic acid, and *n*-butyric acid,[6] which may directly influence the health of the gut. As the various SCFAs have very different metabolic fates (*e.g.*, acetic acid is absorbed and reaches the liver and muscles where it is used as an energy source, whereas butyric acid is a preferred energy source of colonocytes and induces cellular differentiation in the colon cell lines), the extent of fermentation and the pattern of SCFA is likely to be of crucial importance in determining the physiological effects of a particular carbohydrate.[7]

Therefore, the balance of gut microbiota and the production of SCFAs should be two of the reasons of positive effects of dietary fibers on health. The effect of DFs on microbiota and SCFA production have been investigated in previous studies, and most of them were based on DFs added in single or mixture pattern with the method of *in vitro* fermentation.[6,8–12] The comparative effects of different DFs (pectin, resistant starch, fructo-oligosaccharide and cellulose) and their mixtures are seldom studied through *in vivo* fermentation.

In this research, four types of representative DFs, including pectin, resistant starch (RS, type II), fructo-oligosaccharide (FOS) and cellulose, were added to the normal feed of mice in single or mixture pattern in order to compare the effects of different dietary fibers ingested on the gut microbiota and SCFA production in mice.

Materials and methods

Source of dietary fiber

Four commercial dietary fibers [pectin (98%), cellulose (99%), resistant starch (RS, 98%) (type II) (Qiyun, Guangzhou, China);

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Table 1 Dietary fibers added in normal diet (%) in different groups

Group	Pectin	RS	FOS	Cellulose	Others	Group	Pectin	RS	FOS	Cellulose	Others
A	6	—	—	—	94	BD	—	3	—	3	94
B	—	6	—	—	94	CD	—	—	3	3	94
C	—	—	6	—	94	ABC	2	2	2	—	94
D	—	—	—	6	94	ABD	2	2	—	2	94
AB	3	3	—	—	94	ACD	2	—	2	2	94
AC	3		3	—	94	BCD	—	2	2	2	94
AD	3	—	—	3	94	ABCD	1.5	1.5	1.5	1.5	94
BC	—	3	3	—	94	Control	—	—	—	—	100

fructo-oligosaccharide (FOS, 98%) (Liangzi Gaoke, Jiangmen, China)] were used in this study.

Feeding with DFs

Additional DFs (6%) were blended into the normal diet (Table 2) in different proportions as presented in Table 1. The tested groups were divided into three categories: single DF (group A, B, C and D), mixture of two DFs (group AB, AC, AD, BC, BD and CD) and mixture of multiple DFs (group ABC, ABD, ACD, BCD and ABCD).

Animals and diets

160 experimental male Kunming mice (20 days old), with initial weights of 21.1 ± 4.0 g, were provided by the Animal Center of Southern Medical University, China. They were divided into 16 groups as labeled in Table 1, with 10 mice in each group. After one adaptive week, the tested mice were fed with diet containing DFs while the controlled mice were fed with normal diet. The experimental period was 4 weeks. During this period the environment was maintained at a temperature of 23 ± 2 °C and a relative humidity of 50% to 60%, with a light/dark cycle of 12 h. Padding was renewed three times a week, and the mice had free access to food and water during the experiment. All animal experiments were performed in compliance with the relevant laws and institutional guidelines. The animal experiments were approved by the Research Animal Administration Center at Jinan University (Guangzhou, China).

Collection of the whole gut contents, extraction of total bacterial genome DNA and SCFA

The mice were cervically dislocated to death and then dissected after being weighed. Since the volume of the gut content is very small for mice, the entire of the gut content was collected and immediately placed into a 10 mL EP tube, for storage at −20 °C for further use.

Table 2 Composition of normal diet (%)

Corn flour	Wheat bran	Yeast powder	Starch	Soybean powder	Bone powder	Oil	Fish powder	Salt	Vitamin mix and mineral mix
45	10	2	20	13	1	2.5	5	1	0.5

The protocol of stool DNA out kits (Tiandz Co., China) were performed on the colon homogenate, in preparation to extract and purify the total DNA of each sample. The total DNA samples were characterized with agarose gel electrophoresis for integrity and size. The DNA was adjusted to 40 ng μL^{-1} and stored at −20 °C before use as a template for PCR. 2 μL of the DNA sample was added to 48 μL PCR mixtures for each PCR reaction.

SCFA extraction was performed as followed: 200 mg of the gut contents of each group was weighed; the gut contents were then suspended in 1.6 mL of sterile distilled water followed by the addition of 0.2 mL of hexanoic acid; 0.4 mL of 50% aqueous H_2SO_4 and 2 mL of diethyl ether were the added; the sample was the mixed with an orbital shaker for 45 min; this mixed sample was then centrifuged at 3000 rpm for 5 min at room temperature; anhydrous $CaCl_2$ was then added to remove residual water; the extracts were transferred into a new tube and stored at −20 °C until use.[13]

PCR and enzyme digestion

Terminal restriction fragment (TRF) patterns were obtained by extraction of the sample DNA, PCR with a fluorescent labeled primer to amplify the 16S rRNA genes, and digestion with a restriction endonuclease (*Hha* I) to generate phylotype specific fragments.[14] The primer sets for total bacteria were labeled with FAM, 7f (5′-FAM-AGAGTTTGATYMTGGCTCAG-3′) and 1510r (5′-ACGGYTACCTTGTTACGACTT-3′).[15] Amplification was performed using an Eppendorf Mastercycler™ thermal cycler with the following program: an initial 5 min denaturation at 94 °C followed by 35 cycles of 45 s denaturation at 94 °C, 30 s annealing at 52 °C, and 30 s extension at 72 °C. After the final cycle, 2 min elongating at 72 °C completed the PCR. The following reaction cocktail was used throughout: 20 ng template DNA, determined with BioPhotometerplus (Nano-100, Eppendorf, German); 0.025 U μL^{-1} Taq DNA polymerase (Takara); 10× reaction buffer (Mg^{2+} plus) as supplied by manufacturer; 0.25 mM of each primer (synthesized by Shanghai Sangong, China); and 0.2 mM of each of the dNTPs (Takara, Japan). The reactions were carried out at a final volume of 50 μL.

PCR products were further purified by the New Probe PCR purification kit (New Probe, China) using the manufacturer's protocol to remove unincorporated nucleotides and primers. The PCR cleanup products were quantified by determining the absorption of the samples at 260 nm. Enzyme digestion was performed on each PCR product using *Hha* I (Takara). Each 40 μL digestion used 75 ng DNA, 1 U enzyme and 4 μL

10× buffer (0.4 μL 20 μg BSA mL^{-1} were added to *Hha* I digestions). Samples were digested for 4 h at 37 °C and inactivated for 20 min at 65 °C. The sizes of the fluorescently labeled fragments were sent to Invitrogen (Carlsbad, USA) for TRF profile determination.

Data analysis of TRFLP

Terminal restriction fragment length polymorphism (TRFLP) analysis was used to provide a quantitative measure of the community composition without providing precise taxonomic classifications.[16] Relative peak areas of each TRF were determined by dividing the area of the peak of interest by the total area of peaks within the following threshold values: a lower threshold of 50 bp and an upper threshold of 600 bp. A threshold for relative abundance was applied at 0.5%, and only TRFs with higher relative abundances were included in the remaining analyses.

Replicate profiles from total bacterial DNA extractions and PCR reactions for each sample were compared to identify the subset of reproducible fragment sizes. The average area of each reproducible peak was calculated. Terminal fragments smaller than 50 bp were excluded from further analysis. Fragments differing by less than 1 bp length were clustered. The standardized binning criteria used to identify the subset of reproducible peaks were as previously described.[17,18] Ribotype richness (S) equals the total number of distinct fragments in a profile.

SCFA detection

An external standard method was used for SCFA detection following the method described previously.[13] Standard curves were accomplished using different concentrations of acetic, propionic, butyric and lactic acid. A hydrogen flame detector was used and the conditions for gas chromatography was as follows: carrier gas was N_2; split ratio was 10 : 1; flow rate was 2.0 mL min^{-1}; chromatographic column was DB-FFAP; temperature programming was 120 °C for 5 min, temperature rose to 250 °C with 15 °C min^{-1} and kept for 1 min; burner temperature was set at 280 °C and sample volume was 2 μL. Data of SCFA was analyzed using the software of SPSS Statistics version 17.0.

Results

Weight gain and feed consumption

During the four weeks of feeding, the mice fed the diet containing additional dietary fiber (6%) (Table 1) consumed more feed than the control group without significant difference ($P > 0.05$). The weight gain of mice in all tested groups was significantly less than that of the control mice ($P < 0.01$ for group BD, CD and BCD, $P < 0.05$ for the other tested groups) (Table 3).

TRFLP analysis of the total gut bacteria

Total gut microbiota varied significantly when the mice ingested the different dietary fiber(s) that were added to their normal feed (Fig. 1 and Table 4). Compared with the area of the total peaks in the control group (47013 ± 8823), the amounts of total gut bacteria increased the most in groups D and AC (79298 ± 13001 and 79460 ± 23125, $P < 0.01$), followed by groups ACD and BD (69511 ± 18105 and 63790 ± 16678, $P < 0.05$); while the amounts of total gut bacteria decreased the most in groups A and B (1395 ± 293 and 2001 ± 575, $P < 0.01$), followed by groups C, AD and ABCD (14749 ± 8368, 18927 ± 1268 and 23111 ± 5989, $P < 0.01$), as well as group CD (27156 ± 11588, $P < 0.05$); the other groups showed no significant changes ($P > 0.05$).

Table 3 Initial and final weights (g), weight gain (g) and diet consumption (g) of Kunming mice fed with experimental diets for 28 days

Experimental groups	Initial weight (g)	Final weight (g)	Weight gain (g)	Diet consumption (g)
A	21.1 ± 1.8	30.6 ± 3.7	10.3 ± 2.8[a]	164.3 ± 12.9
B	20.7 ± 2.3	30.8 ± 5.2	9.5 ± 3.1[a]	168.1 ± 16.6
C	21.8 ± 2.9	31.9 ± 7.4	10.1 ± 2.5[a]	170.2 ± 11.5
D	22.1 ± 1.5	31.0 ± 6.9	9.6 ± 1.8[a]	161.5 ± 14.3
AB	20.3 ± 3.4	27.6 ± 1.4	9.3 ± 3.7[a]	172.2 ± 13.3
AC	19.2 ± 2.0	30.4 ± 6.8	10.8 ± 2.9[a]	166.7 ± 15.2
AD	20.5 ± 1.6	29.6 ± 8.1	9.4 ± 2.1[a]	168.6 ± 10.7
BC	19.9 ± 2.8	29.5 ± 7.5	9.4 ± 4.0[a]	169.7 ± 13.2
BD	20.0 ± 2.1	28.3 ± 7.1	7.2 ± 3.5[b]	171.1 ± 9.5
CD	21.6 ± 1.7	29.7 ± 5.6	8.3 ± 2.5[b]	163.5 ± 15.7
ABC	20.8 ± 3.8	29.3 ± 2.5	9.5 ± 2.9[a]	160.2 ± 15.5
ABD	21.6 ± 1.7	30.4 ± 3.9	9.1 ± 2.0[a]	160.3 ± 14.8
ACD	22.5 ± 2.0	32.1 ± 4.7	10.6 ± 1.8[a]	173.8 ± 11.6
BCD	20.7 ± 2.6	27.0 ± 2.2	7.0 ± 2.3[b]	165.7 ± 14.1
ABCD	21.2 ± 3.3	31.4 ± 3.5	9.3 ± 3.6[a]	159.7 ± 9.5
Control	20.5 ± 2.0	33.0 ± 4.2	13.4 ± 3.2	165.5 ± 11.2

[a] $P < 0.05$, compared to the control group. [b] $P < 0.01$, compared to the control group.

The tested groups had distinctly diverse TRFs. The 69 bp fragment was the most dominant of the whole bacterial community fragments, especially for groups A (100%) and B (100%). Additionally, the 100 bp fragment shared dominance with a relative abundance of 21.4% in group C. The fragments varied in all the other groups, as 56, 60, 87 and 100 bp existed in multi-groups (≥7 groups), while the other 15 fragments occurred in fewer groups (<7 groups) (Table 4).

Compared to the control group, all of the tested groups showed an increased relative abundance of 69 bp. The amount of TRFs decreased in the single DF groups, except group D (10 TRFs). The most TRFs (12) were found in group ACD, followed by groups BC and BCD (11). There were 11 groups with less TRFs (<10), 3 groups (groups ACD, BC and BCD) with more TRFs (>10) than the control group (10) and one group (group D) with the same number. Groups A, B, C and ABD presented much fewer TRFs (1, 1, 2 and 5, respectively) (Table 4).

SCFAs in the mice guts

Acetic acid. The concentration of acetic acid was highest in group A (1.579 ± 0.007 μmol mL^{-1}), among the single DF category, while it was highest in group CD (1.780 ± 0.010 μmol mL^{-1}) among the two-DF mixture category, and in group ABCD (1.675 ± 0.012 μmol mL^{-1}) among the multiple DF mixture category. These three values were distinctly different from the other data in the respective categories ($P < 0.05$), besides, they were less than the value (1.937 ± 0.004 μmol mL^{-1}) of the control group ($P < 0.01$).

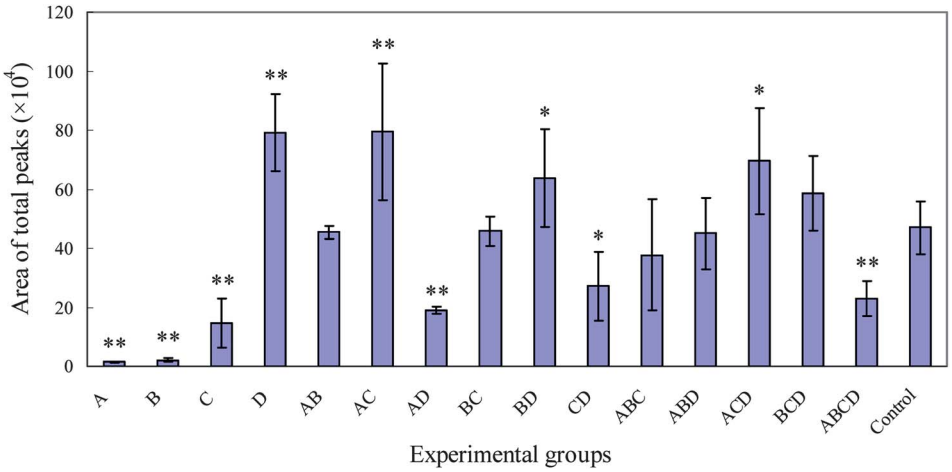

Fig. 1 Areas of the total peaks of mice gut microbiota for each group (digested by *Hha* I). * $P < 0.05$, compared to the control group; ** $P < 0.01$, compared to the control group.

Table 4 Relative abundance (%) of terminal restriction fragments (*Hha* I) in the mice guts after ingesting additional dietary fiber(s)

TRFs (bp)	52	56	58	60	63	67	69	71	74	87	95	100
A	—[a]	—	—	—	—	—	100.0	—	—	—	—	—
B	—	—	—	—	—	—	100.0	—	—	—	—	—
C	—	—	—	—	—	—	78.6	—	—	—	—	21.4
D	—	—	—	0.9	0.7	1.1	77.4	—	—	7.4	—	2.3
AB	—	3.3	—	3.5	—	—	77.2	0.7	—	11.5	—	3.8
AC	—	0.6	—	0.6	0.8	—	73.8	—	—	6.9	1.7	14.7
AD	1.5	—	—	—	—	—	77.1	—	—	13.7	2.4	3.0
BC	—	0.8	—	—	—	—	71.9	0.9	—	7.8	1.7	7.8
BD	—	0.9	—	—	—	2.8	73.8	—	—	7.3	—	1.3
CD	—	1.2	0.8	1.1	—	—	78.3	—	—	13.8	1.2	1.6
ABC	5.3	1.4		0.7	1.0	—	71.5	—	—	14.7	—	5.4
ABD	—	—	—	—	—	—	84.2	—	—	8.7	3.3	1.6
ACD	0.9	0.6	—	0.6	—	—	78.0	0.5	—	9.8	—	1.4
BCD	—	1.1	—	1.8	0.8	—	75.3	—	—	7.5	2.4	2.8
ABCD	—	1.6	—	1.2	—	—	63.6	2.1	—	13.7	3.2	—
Control	—	2.7	—	3.3	0.9	—	61.7	—	0.8	14.3	—	2.6

TRFs (bp)	105	120	147	177	188	190	198	200	253	402	600
A	—	—	—	—	—	—	—	—	—	—	—
B	—	—	—	—	—	—	—	—	—	—	—
C	—	—	—	—	—	—	—	—	—	—	—
D	—	—	—	0.9		0.7	—	6.9	1.8	—	—
AB	—	—	—	—	—	—	—	—	—	—	—
AC	—	—	—	—	0.9	—	—	—	—	—	—
AD	—	—	2.3	—	—	—	—	—	—	—	—
BC	0.9	—	1.2	3.7	—	1.1	—	2.2	—	—	—
BD	—	—	—	—	—	1.1	2.2	5.4	—	—	5.2
CD	—	—	—	—	—	—	—	—	—	—	2.0
ABC	—	—	—	—	—	—	—	—	—	—	—
ABD	—	—	—	—	—	—	2.2	—	—	—	—
ACD	—	—	0.9	1.3	—	0.7	—	2.9	1.6	—	—
BCD	—	4.3	—	—	—	—	0.9	1.5	0.6	—	—
ABCD	—	—	—	—	—	1.7	10.1	—	—	—	—
Control	—	—	—	—	—	—	—	8.5	3.7	1.5	—

[a] The relative abundance of TRF was lower than 0.5% or none.

Propionic acid. The concentration of propionic acid in group A was 0.595 ± 0.010 μmol mL^{-1}, which was significantly higher than that of the other groups in the single DF category ($P < 0.05$). The concentration of propionic acid (0.684 ± 0.003 μmol mL^{-1}) was highest in group BC, in the two-DF mixture category, which was significantly different from the other results ($P < 0.05$). For the multiple DF mixture category, the concentration (0.635 ± 0.014 μmol mL^{-1}) in group ABCD was the highest with significant difference ($P < 0.05$). The control group showed a value of 0.606 ± 0.008 μmol mL^{-1}. There was also no significant difference ($P > 0.05$) between the control group (0.606 ± 0.008 μmol mL^{-1}) and the highest one (group A, 0.595 ± 0.010 μmol mL^{-1}) in the single DF category.

Butyric acid. Group A showed high production of butyric acid (0.241 ± 0.010 μmol mL^{-1}). For the two-DF mixture category, no significant effect was observed in groups BD (0.359 ± 0.008 μmol mL^{-1}) and CD (0.368 ± 0.009 μmol mL^{-1}) ($P > 0.05$). For the multiple DF mixture category, the concentration in group ACD (0.351 ± 0.007 μmol mL^{-1}) was the highest amongst the groups ($P < 0.05$), however, this value was insignificantly different from that of groups BD and CD ($P > 0.05$). The value of the control group was 0.307 ± 0.006 μmol mL^{-1}, which was significantly lower than the groups mentioned above ($P < 0.05$), except for group A.

Lactic acid. As for the concentration of lactic acid, concentrations of 1.530 ± 0.008 μmol mL^{-1} for group B , 2.702 ± 0.007 μmol mL^{-1} for group CD, and 1.516 ± 0.004 μmol mL^{-1} for group ACD, were the highest values within their relative category. Additionally, the values of groups B and ACD showed no significant difference ($P > 0.05$). The value of the control group (0.152 ± 0.011 μmol mL^{-1}) was significantly lower than the highest values of lactic acid mentioned above ($P < 0.01$).

Total acids. The amount of total acids was the summation of acetic, propionic, butyric and lactic acid. From Table 5, 3.304 ± 0.034 μmol mL^{-1} for group B, 5.504 ± 0.029 μmol mL^{-1} for group CD and 3.893 ± 0.024 μmol mL^{-1} for group ACD were the largest values of total acids in the respective categories, all of

Table 5 Various SCFA production in mice intestinal contents (μmol mL^{-1})

Category	Group	Acetic acid	Propionic acid	Butyric acid	Lactic acid	Total acid
Single DF	A	1.579 ± 0.007^a	0.595 ± 0.010^a	0.241 ± 0.010^a	0.181 ± 0.005	2.596 ± 0.032
	B	1.120 ± 0.010	0.470 ± 0.011	0.184 ± 0.005	1.530 ± 0.008	3.304 ± 0.034^a
	C	0.955 ± 0.012	0.561 ± 0.023	0.155 ± 0.006	0.229 ± 0.008	1.899 ± 0.049
	D	1.037 ± 0.013	0.472 ± 0.017	0.215 ± 0.006	0.538 ± 0.008^a	2.263 ± 0.045
Mixture of two DFs	AB	0.733 ± 0.003	0.231 ± 0.012	0.213 ± 0.005	0.337 ± 0.013	1.513 ± 0.033
	AC	0.665 ± 0.019	0.301 ± 0.007	0.174 ± 0.670	0.670 ± 0.005	1.810 ± 0.041
	AD	1.130 ± 0.008	0.513 ± 0.013	0.174 ± 0.011	0.359 ± 0.005	2.177 ± 0.037
	BC	0.175 ± 0.014	0.684 ± 0.003^a	0.278 ± 0.005	0.472 ± 0.005	1.608 ± 0.027
	BD	1.122 ± 0.009	0.384 ± 0.007	0.359 ± 0.008	1.886 ± 0.005	3.750 ± 0.028
	CD	1.780 ± 0.010^a	0.655 ± 0.003	0.368 ± 0.009^a	2.702 ± 0.007^a	5.504 ± 0.029^b
Mixture of multiple DFs	ABC	1.498 ± 0.006	0.407 ± 0.003	0.262 ± 0.012	1.130 ± 0.004	3.297 ± 0.025
	ABD	1.224 ± 0.006	0.371 ± 0.004	0.168 ± 0.006	1.179 ± 0.008	2.941 ± 0.025
	ACD	1.542 ± 0.009	0.484 ± 0.005	0.351 ± 0.007^a	1.516 ± 0.004^a	3.893 ± 0.024^a
	BCD	1.387 ± 0.005	0.509 ± 0.011	0.244 ± 0.011	0.961 ± 0.007	3.102 ± 0.034
	ABCD	1.675 ± 0.012^a	0.635 ± 0.014^a	0.249 ± 0.010	0.750 ± 0.011	3.309 ± 0.047^a
—	Control	1.937 ± 0.004^b	0.606 ± 0.008	0.307 ± 0.006	0.152 ± 0.011^b	3.002 ± 0.029

^{a} $P < 0.05$, compared with other data in their own category. ^{b} $P < 0.01$, compared with other data in the same column.

which were significantly different from the value (3.002 ± 0.029 μmol mL^{-1}) of the control group ($P < 0.01$).

Discussion

The four dietary fibers have all been reported for their effect on animal health, including the loss of body weight, the production of SCFAs and the balance of gut microbiota.[19–22] According to our results, a 6% amount of the four single dietary fibers or their mixtures significantly made the mice gain less body weight in all tested groups compared with the control group, which can be due to the reduced energy they ingested. Since the normal diet already contained 10% wheat bran, which had been determined to contain about 50% of dietary fiber,[23] and soybean powder (13% in normal diet) also contains about 30% of dietary fiber,[24] all the tested mice in this study ingested a total amount of 14.4% dietary fiber ($94\% \times 10\% \times 50\% + 94\% \times 13\% \times 30\% + 6\% = 14.4\%$) in their diet. It has been reported in the literature that 10% or more of dietary fiber in the diet can significantly decrease an animal's body weight gain,[19–21] which agrees with our results. Additionally, there is no significant difference of body weight gain between all the tested groups in our study.

Different dietary fibers also exerted effects on the composition of the gut microbiota, as well as the body weight gain. Previous work has shown differences in gut bacteria after administration of different sources of fiber to pigs.[21,25] In this study, the mixtures of dietary fibers can distinctly increase the diversity of gut microbiota. As shown in Table 4, the mixture of pectin–FOS–cellulose presented the best impact (12 TRFs), followed by the mixtures of RS–FOS and RS–FOS–cellulose. Thus, FOS seems to be the key component in the mixtures of the dietary fibers, however, single FOS did not show a stronger ability in improving the diversity of the gut microbiota compared with cellulose. The four kinds of DF in this study consist of different monomers or monosaccharides, while three of them (pectin, RS, FOS) are soluble. The impact of DFs on gut microbiota can be attributed to their different structures, which can determine their fermentable extent and stimulate the growth of specific bacteria in the gut.[26]

In this study, the groups with pectin, RS and FOS had the minimum amounts of total gut bacteria (Fig. 1) and TRFs (Table 4), indicating that the diversity of the gut microbiota decreased. As the TRFs were prepared with single restriction enzyme *Hha* I, the amount of total gut bacteria might be underestimated.[27] The detected bacteria in group D, with cellulose, were much more than that in group AD, with pectin–cellulose, and group ABCD, with pectin–RS–FOS–cellulose ($P < 0.01$), which might result from the inhibition of pectin, RS and FOS on the growth of other dominant bacteria which are undetected. The changes of total gut bacteria deserved to be focused on in further studies.

In Table 4, 23 TRFs were presented in all tested groups *via Hha* I digestion. Bacteria with a TRF of 69 bp must be the major one for hydrolyzing different dietary fibers, since it dominated in all tested groups, followed with TRFs of 87 and 100 bp (Table 4). These TRFs need to be further cloned and identified. No other report has been focused on these bacteria and their ability to break down different dietary fibers. Although it is hard to present the whole gut microbiota with a single enzyme (*Hha* I), the enzyme used in this study, the result of total gut microbiota and TRFs can reflect the changing trends of gut microbiota, especially the diversity of the total gut microbiota.[27]

The fluctuation of gut microbiota, influenced by the composition of the DFs, will change the SCFAs present in the mice guts.[8] The production of SCFAs in descending order was acetic, propionic and butyric acid in each group (Table 5), in accordance with the previous study;[8] and most of them showed significant differences ($P < 0.05$, not labeled in Table 5 since it is universal in most groups) when compared to each other, except for several data points of butyric acid production, which implied that different DFs and their mixtures have different effects on the production of SCFAs.

According to the amount of SCFAs in each group, the production of SCFAs changed along with the different dietary

fiber(s) added in the diet. The highest content of total acids was produced from the mixture of FOS and cellulose in all tested groups, secondly from the mixture of pectin, FOS and cellulose, and then from the mixture of RS and cellulose. As for the single DFs, pectin can produce more acetic, propionic and butyric acid, but less lactic acid than RS in the gut. All added dietary fibers decreased the content of acetic acid compared with the normal diet (about 5% dietary fibers). However, the mixtures of RS–FOS and FOS–cellulose produced the highest amount of propionate, and the FOS–cellulose mixture still had the highest amount of butyric and lactic acids (Table 5).

Conclusion

The addition of pectin, RS, FOS and cellulose in single or mixture pattern into the normal diet presented different impacts on the mice guts. 14.4% of single DF in the diet cannot be conducive to the balance of gut microbiota after ingestion for a long time, however, it can help with body weight loss like the mixtures of DFs in this study; FOS is a very important component in a mixture of DFs for both the balance of gut microbiota and the production of SCFAs. This is the first report about the effects of DF on gut microbiota changes and SCFAs production when they are added as a mixture.

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