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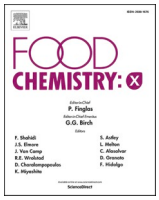

Tuber flours improve intestinal health and modulate gut microbiota composition

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ABSTRACT

The different health effects between starch and whole flour from tubers are rarely studied. Here, we investigated the effects of cassava flour (CF), cassava starch (CS), potato flour (PF), and potato starch (PS) on gut health and gut microbiota of normal rats. Feed analysis showed that CF and PF diet provided significantly more slowly digestible and resistant starch, less rapidly digestible starch. Compared with rats fed with PS and CS diets, rats fed with PF and CF diets gained less body weight and have tighter intestinal barrier. Butyric acid contents were increased by tuber flours. CF and PF selectively promoted the relative abundance of *Akkermansia* and *Eubacterium ruminantium* in cecal and colonic content. In conclusion, tuber flour has intestinal protection, body weight control, and gut microbiota improving ability compared with starch. The different composition of starch might be the basis for these effects.

1. Introduction

Whole foods are highly recommended by nutritionists as their health effects are gradually revealed in the past decades (Wright, Wilson, Smith, Duncan, & McHugh, 2017; Xi & Liu, 2016). Whole grain has been reported to have protective effects against obesity, cardiovascular diseases, type 2 diabetes, and cancers (Mourouti et al., 2016; Ye, Chacko, Chou, Kugizaki, & Liu, 2012). The mechanisms underlying these health-promoting activities are often related to the high content of dietary fibre compared with refined grain. Growing evidence showed that gut microbiota was essential for the beneficial effects of these food components (Costabile et al., 2008; Han et al., 2018; Zhao et al., 2018). Gut microbiota ferments dietary fibres into short chain fatty acids (SCFAs), which can be used by colonic cells as energy resources and influence host metabolism (Den Besten, Van Eunen, Groen, Venema, Reijngoud, & Bakker, 2013; Natarajan et al., 2016).

However, whether tuber flours have similar health effects compared with starch are not so sure. Tubers such as cassava, potato, and yam are staple foods in some area and they are usually processed to starch for better preservation. Apart from the different content in fibres and phytochemicals between tuber flour and starch, the composition of starch is vital to their health effects as well. Based on the different digestibility, starch can be divided into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) (Englyst, Kingman, & Cummings, 1992) and their potential health influences have been studied (Toraya-Avilés, Segura-Campos, Chel-Guerrero, & Betancur-Ancona, 2017). In the process of starch production, the composition of RDS, SDS, and RS might change as well (Abioye et al., 2017). They would also influence the growth of gut microbiota and the production of SCFAs. Thus, the different health influences between tuber flour and starch need to be verified.

The composition of gut microbiota affected many physiological processes and developments of diseases such as obesity, type 2 diabetes, inflammatory bowel disease, and gut barrier integrity (Sommer & Bäckhed, 2013). The metabolite of gut microbiota such as SCFAs and lipopolysaccharide (LPS) can pass the gut barrier into host plasma or

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directly stimulate intestinal epithelial cells to influence the host health (Natarajan et al., 2016; Smith et al., 2013). While some toxins produced by harmful bacteria would also be found in plasma when the intestinal permeability increased (Mass, Kubera, & Leunis, 2008). Thus, the integrity of the gut barrier is a key factor that reflects the health status of the intestine.

In this work, we tested cassava starch (CS), cassava flour (CF), potato starch (PS), and potato flour (PF) to verify the hypothesis that tuber flour have improvement effects on intestinal health and gut microbiota composition compared with their starch, and SCFAs play an important role in it. The *in vitro* models could not reflect the exact health status of gut microbiota, gut permeability and blood profiles. The connection between these profiles was not available in *in vitro* models. Thus, the use of animal model was necessary and was commonly applied by previous studies. The changes of gut permeability and blood glucose and lipid profiles by different diets were monitored as well as the gut microbiota composition.

2. Materials and methods

2.1. Materials and chemical reagents

PS and PF were provided by Neimenggu Fuguang Food Company limited. CS and CF was provided by the Chinese Academy of Tropical Agricultural Sciences. Acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, and isovaleric acid were purchased from Aladdin (Shanghai, China). The primary antibody to GAPDH (K200057M) was purchased from Beijing Solarbio Science & Technology Co., Ltd (Beijing, China). The primary antibodies to ZO-1 (sc-33725), Occludin (sc-133256), Claudin-1 (sc-81796), and Claudin-4 (sc-376643) were purchased from Santa Cruz Biotechnology (Dallas, TX, USA). Secondary antibodies conjugated with HRP of anti-rat (BL002A) and anti-mouse (BL001A) were purchased from Biosharp (Hefei, Anhui, China). Deionized water was obtained with a Milli-Q water purification system (Millipore, Billerica, MA, USA). Reagents that were necessary if not mentioned were purchased from China National Pharmaceutical Group Co., Ltd (Shanghai, China).

2.2. Animals and diets

Male Sprague Dawley (SD) rats (7 weeks of age) were purchased from Shanghai Jihui Laboratory Animal Care Co., Ltd (Shanghai, China). After 7 days of acclimatization, 24 rats were randomly divided into four groups (n = 6 per group) as follows: PS, PF, CS, and CF diet group. The groups were named after the diet animals consumed in the experiment. For example, Rats in PS diet group were fed with PS diet feed listed in Table S1. The feed formula of four diets was based on the animal feed formula of American Society for Nutrition (AIN-93 M) provided by Trophic Animal Feed High-tech Co., Ltd (Jiangsu, China) and the exact composition are listed in Table S1. Diets were stored at 4℃ and rats were kept under specific-pathogen-free (SPF) condition with different diets and water ad libitum for 7 weeks with 12 h day and 12 h night light cycle. After the diet intervention, rats were anesthetised by pentobarbital and sacrificed by cardiac puncture and decapitation. All animal experiments were approved by the Animal Ethical and Welfare Committee of Zhejiang Chinese Medical University (Approval No. IACUC-20181008-11) and followed the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

2.3. Nutritional composition analysis

Dry matter, crude protein, crude fat, crude ash and crude fibre were analysed using Chinese national standards GB/T 6435-2014, GB/T 5009.5-2016, GB/T 6433-2006, GB/T 6438-2007 and GB/T 6434-2006, respectively. Carbohydrate was calculated by the content of dry matter minus crude protein, crude fat, and crude ash (Ifon & Bassir, 1980). RDS, SDS, and RS were determined by the Englyst method (Englyst et al., 1992).

2.4. Serum biochemical assay

After the intake of 7 weeks of different diets, rats' blood samples were collected via cardiac puncture. Blood samples were then centrifuged at 4000 r/min for 10 min and serum was collected and stored at −80℃ until further analysis. The concentration of fasting blood glucose (FBG), serum total triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) were measured by the automatic biochemical analyser (Laola, Nanjing, Jiangsu, China).

2.5. Paraffin section and H&E stain

Freshly isolated small intestine was fixed in 4% paraformaldehyde overnight, then dehydrated and embedded in paraffin. Tissues were then sectioned and stained with haematoxylin and eosin (H&E). ImageJ 1.52a (National Institutes of Health, Bethesda, MD, USA) was used for villus height and crypt depth measurement.

2.6. Western blot analysis

Western blot analysis was performed according to previous study (Li et al., 2018). Briefly, 20 μg protein samples were separated by SDS-PAGE and transferred to PVDF membranes. Membranes were blocked in 5% milk-TBST and incubated overnight at 4℃ in the primary antibody. Antibodies including GAPDH, ZO-1, Occludin, Claudin-1, and Claudin-4 were used. Peroxidase-conjugated anti-mouse IgG or anti-rat IgG was used as the secondary antibody. The protein bands were visualized using ChemiScope series (Clinx Science Instruments, Shanghai, China). Grey value of protein bands were quantified using ImageJ 1.52a (National Institutes of Health, Bethesda, MD, USA). The original images were shown in Fig. S1.

2.7. SCFAs measurements

Ileal (IL), cecal (CC), and colonic (LI) contents were collected after rats sacrifice and stored at −80℃. SCFAs were measured by gas chromatography according to the reported method with some modification (Kim, Lee, Go, Ahn, Kim, & Hong, 2018). Briefly, frozen sample (100 mg) was dissolved in 1 mL deionized water and 20 μL 0.6 N HCl. The mixture was then vortexed and centrifuged at 10,000 r/min for 5 min at 4 °C. The supernatant was collected and pass through a 0.22 μm filter for further analysis.

Agilent 6890 N GC system equipped with a flame ionization detector (FID) and HP-INNOWax column (30 m × 0.25 mm, 0.25 μm) was used. The parameters of GC were as follows: The carrier gas was nitrogen with a flow rate of 1 mL/min; 0.5 μL of samples were injected into the gas system, the split ratio was 10:1; The inlet temperature and FID detector temperature was 270 °C; Initial column temperature, 100 °C, then programmed to 180 °C at 10 °C/min and held for 1 min, then programmed to 230 °C at 50 °C/min. Acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, and isovaleric acid were used as standards.

2.8. Gut microbiota analysis

Microbial DNA extraction, detection and data analysis were based on a previous study with some modification (Zhang, Chen, Zhang, & Lin, 2016). Briefly, total genomic DNA from 100 mg of ileal, cecal, and colonic contents was extracted using the E.Z.N.A. Stool DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's protocols. 1% agarose gel electrophoresis was used for genomic DNA detection.

The V4-V5 region of 16S rDNA was amplified by PCR (LongGene A300, Hangzhou, Zhejiang, China) (first keep at 95 °C for 2 min, followed by 25 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, and the final extension at 72 °C for 5 min) from microbial genomic DNA using primers with eight-base sequences unique to each sample (barcode), the sequences are 515F 5′-barcode-GTGCCAGCMGCCGCGG-3′ and 907R 5′-CCGTCAATTCMTTTRAGTTT-3′. PCR reactions were performed in triplicate. 0.4 μL of FastPfu Polymerase, 0.8 μL of each primer (5 μmol/L), 2 μL of 2.5 mmol/L dNTPs, 4 μL of 5 × FastPfu Buffer, and 10 ng of template DNA were mixed to 20 μL. PCR products were then detected by 2% agarose gel electrophoresis, using AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) to recover the PCR product. Then using QuantiFluor -ST (Promega, USA) for the purification. Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 300) on an Illumina MiSeq at Mingke Biotechnology (Hangzhou) Co., Ltd. (Hangzhou, Zhejiang, China) with MiSeq Reagent Kit v3 (600-cycle).

Raw fastq files were demultiplexed, quality-filtered using QIIME (version 1.17) with the following criteria: (i) The 250 bp reads were truncated at any site receiving an average quality score < 20 over a 10 bp sliding window, discarding the truncated reads that were shorter than 50 bp. (ii) exact barcode matching, 2 nucleotide mismatch in primer matching, reads containing ambiguous characters were removed. (iii) only sequences that overlap longer than 10 bp were assembled according to their overlap sequence. Reads which could not be assembled were discarded (Zhang et al., 2016).

Operational taxonomic units (OTUs) were clustered with 97% similarity cut-off using UPARSE (version 7.1 http://drive5.com/uparse/) and chimeric sequences were identified and removed using UCHIME. The phylogenetic affiliation of each 16S rRNA gene sequence was analyzed by RDP Classifier (http://rdp.cme.msu.edu/) against the silva (SSU115)16S rRNA database using a confidence threshold of 70% (Zhang et al., 2016). The functional potential of gut microbiota was predicted using CowPI (Wilkinson et al., 2018), which is a dedicated version of Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) for rumen microbiome.

2.9. Statistical analysis

Data were expressed as mean ± standard deviation. The data were analysed with SPSS 20.0 software. Significant difference ($P < 0.05$) between groups were evaluated by one-way ANOVA. Tukey's post hoc test was used for multiple comparisons. All results were confirmed from three independent experiments.

3. Results

3.1. Feed analysis, body weight gain, energy intake, energy efficiency, and blood biochemical profiles

We analysed the nutritional composition of the experimental diets. Results were listed in Table S2. There was no significant difference between four groups in crude fibre. The content of RDS, SDS, and RS were significantly different in diets of flour and starch group. CF and PF diet have higher SDS and RS content and less RDS content compared with CS and PS diet, respectively. Tuber flours also contained more crude ash, which represents the minerals. In addition, the crude fat in potato diets were lower than that of cassava diets and potato flour diet had more crude protein than other diets.

After 7 weeks intake of different diets, the body weight gain of flour groups were significantly lower than that of corresponding starch group (Table 1). The final body weight of rats showed no significant differences between groups. The feed intake was not significantly different between groups either. The feed efficiency was the ratio of body weight gain (g) to feed intake (g). It represented the efficiency of feed converted to rat's body weight. CF and PF diet had a lower feed efficiency compared with their starch diet. No significant difference in FBG, TG, TC, HDL-C, and LDL-C was observed between rats fed with different diets (Table S3).

Table 1
Body weight gain, energy intake, and energy efficiency of different groups of rats[a].

	initial body weight (g)	final body weight (g)	body weight gain (g)	feed intake (g)	feed efficiency
Cassava starch group	329±11	548±18	224±9 a	1176.5±45.4	0.190
Cassava flour group	331±16	497±20	180±11 b	1237.0±143.1	0.146
Potato starch group	324±15	514±2	188±1 a	1156.8±70.1	0.162
Potato flour group	326±14	468±3	154±5 c	1116.9±105.7	0.138

[1] Values in the same column do not share the same lowercase letter are significantly different ($P < 0.05$)

3.2. Small intestinal health status

The representative H&E stain of the small intestine was shown in Fig. 1A–D. These figures showed that the small intestine structural integrity of rats fed with tuber flours were better than starch groups. We also measured the villus height/crypt depth ratio, which was an indicator of intestinal digestive and absorptive capacity as well as the gut health status. CF group had the highest (Fig. 1E) villus height/crypt depth ratio, which was significantly higher than PF and PS group ($P < 0.05$), CS and PS group was lower than CF and PF group, respectively, but the differences were not statistically significant ($P > 0.05$).

The relative abundance of four important tight-junction-related proteins were evaluated by western blot and GAPDH was used as the internal standard. The representative bands and grey intensity analysis results were shown in Fig. 1F, G. Rats fed with tuber flours showed higher Occludin contents in the small intestine. Similar trends were observed in the expression of ZO-1, Claudin-1, and Claudin-4. Moreover, the expression of ZO-1 between potato and cassava groups were not statistically different.

3.3. Gut microbiota difference

The gut microbiota composition of ileal, cecal, and colonic contents were investigated by V4-V5 regions sequencing of the 16S rRNA gene. Alpha diversity of ileal, cecal, and colonic microbiota were analysed and results were shown in Fig. 2. Tuber flour diets fed rats showed higher observed OTUs, Chao1 and Shannon index and lower Simpson index than that of their starch diets fed rats in ileal, cecal, and colonic microbiota. Rats fed with cassava diets had higher observed OTUs, Chao1 index, Shannon index and lower Simpson index than potato diets especially for the colonic microbiota. In most diet groups, the Chao1 and Shannon index increase as the intestinal digesta flows (ileal microbiota < cecal microbiota < colonic microbiota) and the Simpson index decreased as the intestinal digesta flows. Beta-diversity analysis with hierarchical clustering (Fig. 3A–C) and Bary-Curtis distance based PCoA (Fig. 3D–F) showed that cecal and colonic microbiota resulted from different diets clustered separately from each other. While in ileum, it was not clearly clustered.

At phylum level, Firmicutes and Bacteroidetes were the most abundant phylum in cecal, and colonic microbiota (Fig. 4B, C). However, in ileum, the relative abundance of Actinobacteria and Proteobacteria

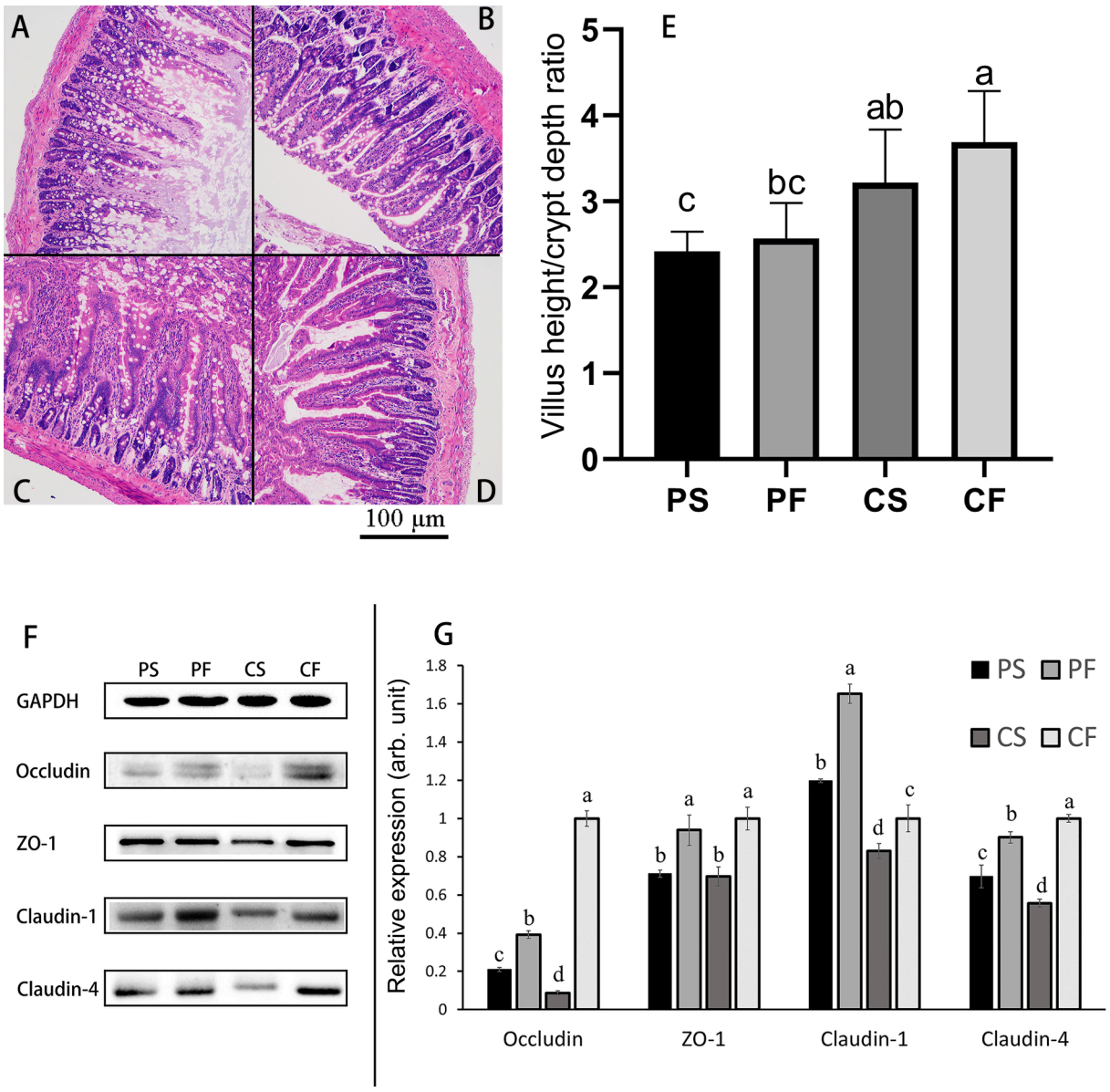

Fig. 1. Paraffin section image analysis and western blot analysis of tight-junction proteins. Representative H&E stained paraffin section image of small intestine from rats fed with potato starch (PS) (A), potato flour (PF) (B), cassava starch (CS) (C), and cassava flour (CF) (D) after 7 weeks. The villus height/crypt depth ratio of small intestine from rats fed with PS, PF, CS, and CF (E). Representative bands of GAPDH, Occludin, ZO-1, Claudin-1, and Claudin-4 from rats fed with different diets (F). Relative abundance of Occludin, ZO-1, Claudin-1, and Claudin-4 from rats fed with different diets (G). Values are presented as the mean ± SD. Groups that do not share the same lowercase letter are significantly different ($P < 0.05$).

were sometimes higher than Bacteroidetes (Fig. 4A). No significant difference was found between groups (Fig. 4D–F). At genus level, *Bacteroidetes S24-7 group_norank* was the most abundant in cecal and colonic microbiota (Fig. S2). Cecal *Allobaculum*, *Eubacterium ruminantium* and *Akkermansia* were selectively promoted by tuber flour. Colonic *Faecalibaculum*, *Lachnospiraceae_uncultured*, *Eubacterium ruminantium* and *Akkermansia* were also promoted by tuber flour. In addition, the relative abundance of cecal and colonic *Bacteroidetes S24-7 group_norank* was lower in rats fed with flour diets than that of starch diets. The relative abundance of *Akkermansia* in tuber flour groups was higher than that in starch groups both in cecal and colonic microbiota. The PICRUSt analysis was applied to predict the functional difference at KEGG level (Fig. S3). The flour diet in cassava and potato showed same trends in amino acid metabolism, replication and repair, and energy metabolism compared with starch diet for ileal microbiota. The flour diet fed rats showed lower amino acid metabolism and higher replication and repair activity for their colonic microbiota. However, it was reversed in cecum. Cecal and colonic microbiota showed no difference in energy metabolism. As for carbohydrate metabolism activity, the microbe in ileal, cecum, and colon showed similar trends that PF was lower than PS, while CF was higher than CS.

3.4. SCFAs in ileal, cecal, and colonic contents

The total SCFAs concentrations were the summation of acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, and isovaleric acid. In ileal contents, CF group achieved the highest total SCFAs concentration, which was significantly higher than that of CS group (Fig. 5A), while there was no significant difference between PS and PF group. As for cecal and colonic total SCFAs concentration, there was no statistical differences between four groups (Fig. 5B, C). However, the butyric acid concentration were significantly higher in tuber flour than

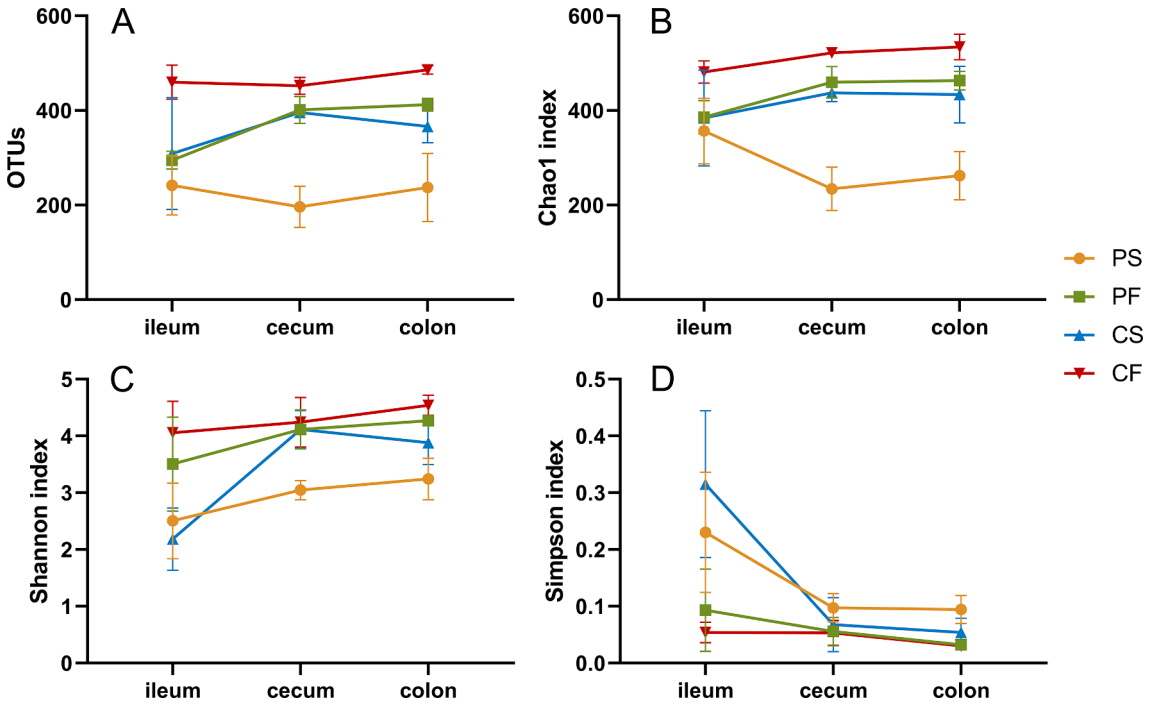

Fig. 2. Alpha diversity indexes of gut microbiota. OTUs (A), Chao1 index (B), Shannon index (C), and Simpson index (D) of ileal, cecal, and colonic contents from rat fed with potato starch (PS), potato flour (PF), cassava starch (CS), and cassava flour (CF).

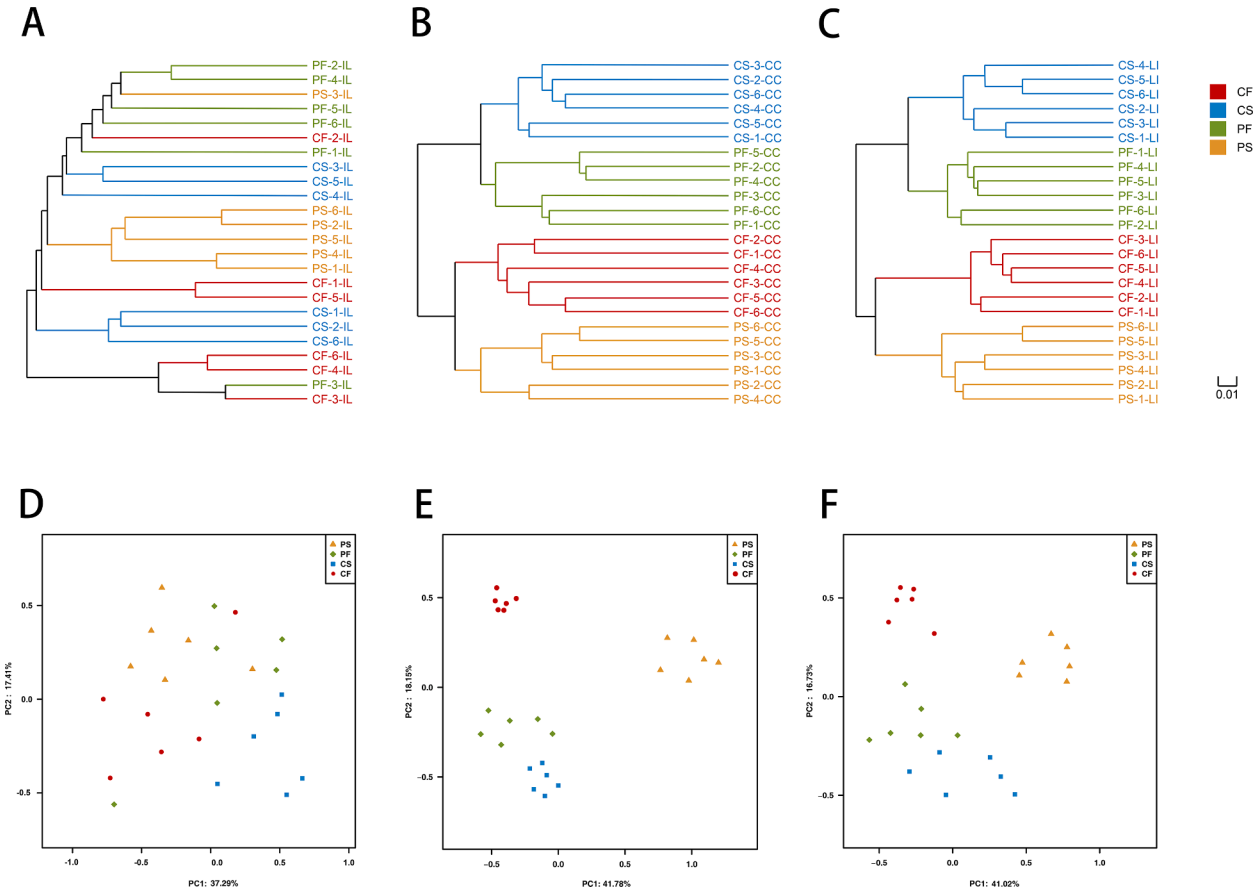

Fig. 3. Beta diversity analysis of gut microbiota. Hierarchical clustering of ileal (A), cecal (B), and colonic (C) contents. Plots of Bary-Curtis distance based PCoA of ileal (D), cecal (E), and colonic (F) contents from rat fed with potato starch (PS), potato flour (PF), cassava starch (CS), and cassava flour (CF).

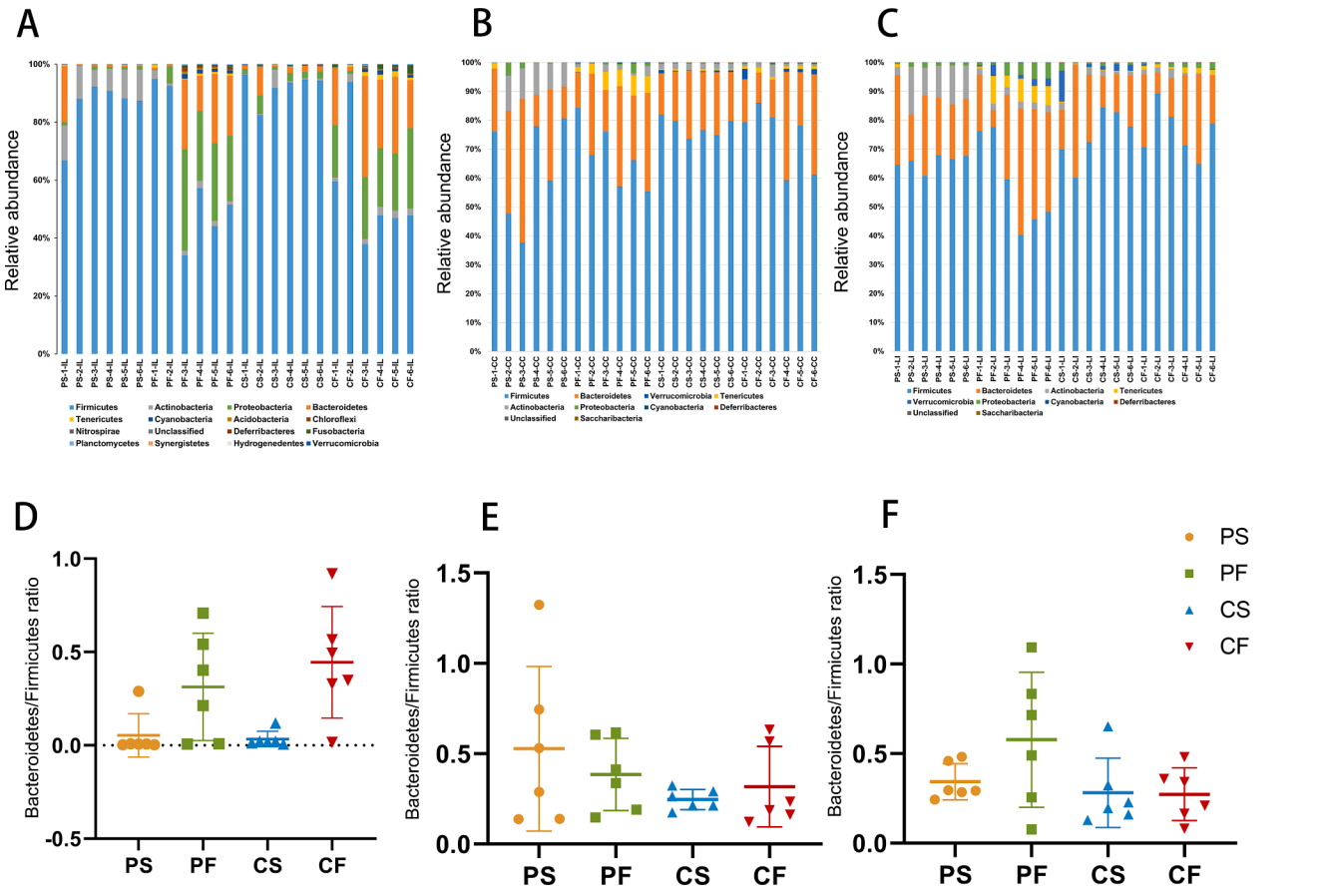

Fig. 4. Relative abundance of gut microbiota at phylum level. Bar graph of different diet on the relative abundance of microbiota in ileum (IL) (A), cecum (CC) (B), and colon (LI) (C) and Bacteroidetes/Firmicutes ratio in ileum (D), cecum (E) and colon (F) from rat fed with potato starch (PS), potato flour (PF), cassava starch (CS), and cassava flour (CF).

that of starch groups in ileal, cecal and colonic digesta.

4. Discussion

Whole food is gradually popular due to their potential health benefits for human and grain is the most studied whole food as their partial losses during food processing. The nutritional differences between tuber flour and processed starch were rarely studied. The content of dietary fibre, phytochemicals, minerals, proteins, and fats between starch and corresponding flour might be different. RS is also considered as a type of dietary fibre and the various health effects of starch with different digestibility is gradually revealing. RS and other dietary fibre are utilised by gut microbiota and the content and type of dietary fibre have great impacts on the composition and amount of human gut microbiota. The changed gut microbiota will further affect the health of the host started from their habitat, the intestine. Thus, it is possible that flour has a better promoting effect on intestinal health compared with starch.

In order to prove the potential gut health benefits of tuber flours, we chose cassava and potato flour as the experimental materials. The corresponding starch was also synthesised by repeated washing and precipitation of flour. To maintain the general health status of rats, the flours and starches were administrated through designed feeds. The corn starch in AIN-93M was replaced by cassava flour, cassava starch, potato flour, and potato starch and CF, CS, PF, and PS diet were obtained. Then we analysed the nutritional composition of the four diets. Due to the addition of casein and soybean oil, the protein and fat were not significantly different between flours and starch (Table S2) except that PF diet have more protein than PS diet ($P < 0.05$). Crude ash showed that flours did have more mineral than starch. This was due to the low addition amount of mineral (Table S2). Different from what we expected, there was no significant difference between four diets in crude fibre. All four diet contained only about 0.1% crude fibre, it was relatively low, compared with previous reports (Akinfala, Amusan, & Adeyemi, 2019). This might be the reason why no significant difference was found. Considering that RS was also recognized as dietary fibre, we analysed the content of RDS, SDS, and RS. Flour diets had more SDS and RS, less RDS (Table S2). The RDS, SDS, and RS content determined *in vitro* were reliable indicators of glycaemic index (Zhang & Hamaker, 2009). They might affect body weight, type 2 diabetes and many other glycolipid metabolism-related diseases (Keenan et al., 2015; Lehmann & Robin, 2007). The higher RS and SDS in CF and PF diet indicated that tuber flour might be beneficial to gut health compared with starch.

Rat model was applied to study the potential health effects of four diets on human. Eight-week-old SD rats ate the four diets for 7 weeks. Although the final body weight showed no significant difference between groups, the results of body weight gain and feed efficiency indicated that long term administration of tuber flour might be beneficial for those people who wanted to control their body weight. Similar results were found in rats fed with whole grain diet and refined grain diet (Han et al., 2018). And lower feed efficiency of flour diets supported the speculation (Table 1). The blood lipids concentration were consistent with final body weight results. No significant difference was found between groups. The FBG was a key indicator of insulin resistance and diabetes. It also showed no significant difference between four groups. All these results indicated that short-term administration of flour and starch diets had limited difference on body weight, blood glucose level and related metabolic status of normal people. However, the results of RDS, SDS, and RS composition as well as the body weight gain

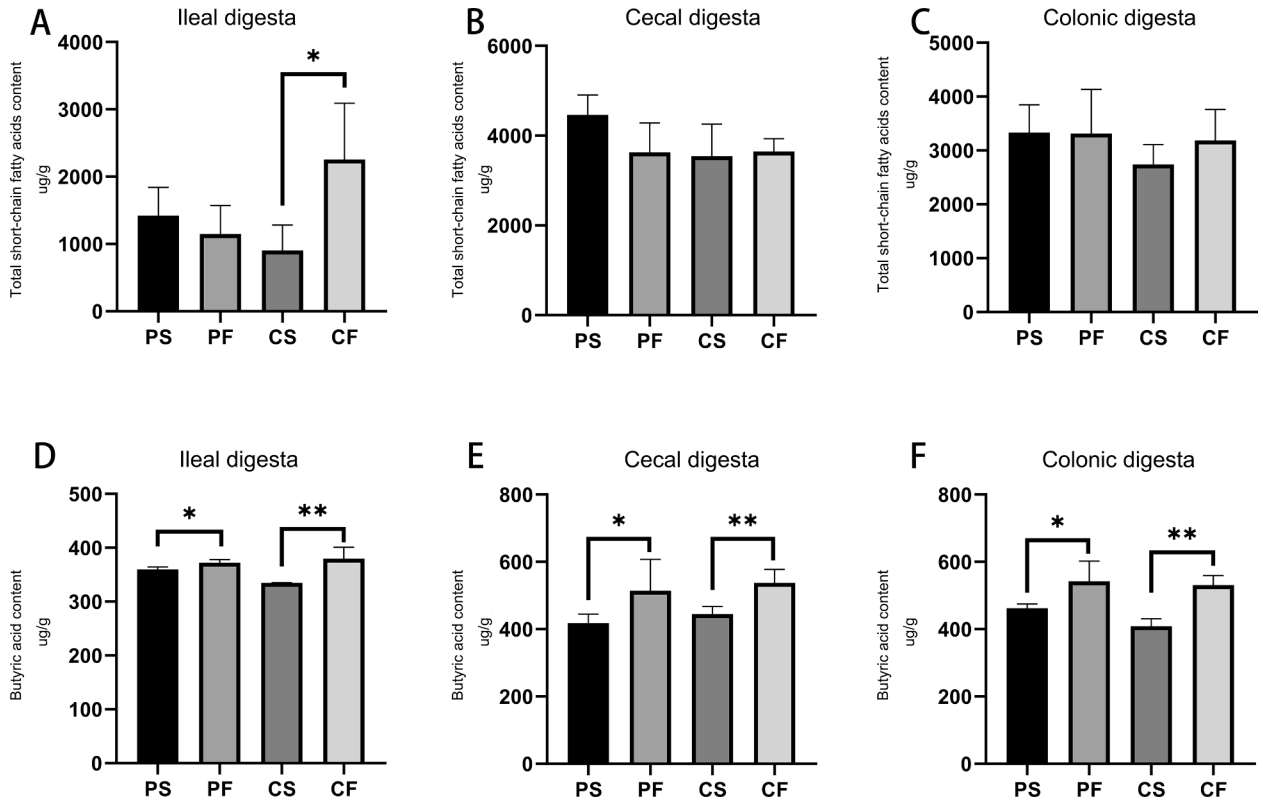

Fig. 5. SCFAs concentration in intestine. Total SCFAs concentrations of ileal (A), cecal (B), and colonic (C) digesta from rats fed with different diets. Butyric acid content of ileal (D), cecal (E), and colonic (F) digesta from rat fed with potato starch (PS), potato flour (PF), cassava starch (CS), and cassava flour (CF).

encouraged us to study the potential benefits of flour diets on gut health.

Thus, we investigated the intestinal absorptive function and gut permeability of the rats. The intact villus structure was crucial for human digestive and absorptive function. The result of higher villus height/crypt depth ratio indicated that tuber flour might have a better gut protective activities on human gut function and cassava was better than potato (Fig. 1E). The relative abundance of tight-junction-related protein was used to illustrate the gut permeability. The results indicated that tuber flour had a protective effect on gut mucosa epithelium tight junction (Fig. 1F, G). Reduced intestinal permeability would lead to a reduced risk of related diseases such as inflammatory bowel disease (Michielan & D'Incà, 2015) and non-alcoholic fatty liver disease (Leung, Rivera, Furness, & Angus, 2016). Tuber flour showed a health protective ability compared with starch.

Changes of gut microbiota have a large impact on gut health including the villus height/crypt depth ratio and intestinal permeability (Tremaroli & Bäckhed, 2012). Thus, we further analysed the gut microbiota difference between different diets to understand the mechanism of the health promoting effects of tuber flours. The observed OTUs is the detected species number and Chao1 index is the estimated exact species number, they are both positively correlated to the microbiota richness and diversity (Kim et al., 2017). Simpson index is the possibility that random pick of two OTUs, they are the same, it indicates the diversity of microbiota. The Simpson index is negatively correlated to microbiota richness and diversity (Kim et al., 2017). The Shannon index is positively correlated to the microbiota evenness (Kim et al., 2017). The results of OTUs, Chao1, Simpson, and Shannon index indicated that tuber flours increased the gut microbial richness and diversity compared with starch. In addition, cassava diets were better than potato diets in improving intestinal microbiota richness and diversity. As the intestinal digest flows, the microbiota diversity, richness, and evenness increased. Previous studies found similar results (Li et al., 2020). The β-diversity showed that diet reshaped the gut microbiota in cecum and colon, but not in ileum (Fig. 3). The low abundance of microbes in ileum might be the reason why it was not clustered with diet. This indicated that gut health difference mentioned above might be from the different gut microbiota.

In order to determine if the gut microbiota was associated with the gut absorptive function and permeability as well as the exact species that mediated the health effects of flour diet, we further investigated the relative abundance of specific phylum and genus. Bacteroidetes/Firmicutes ratio is an important microbial marker for host obesity (Ley, Turnbaugh, Klein, & Gordon, 2006). Bacteroidetes/Firmicutes ratio is usually lower in obese individuals than in lean ones. The no significant difference result of the ratio was consistent with the results of final body weight (Table 1). Specific genus of cecal and colonic microbe were upregulated or downregulated by tuber flour diet compared with starch diet. *Akkermansia* is a group of Gram-negative bacteria that is essential for human mucus layer integrity (Anhê et al., 2015). The relative higher abundance of *Akkermansia* in tuber flour groups was consistent with their higher gut integrity (Fig. 1F, G). Besides, *Akkermansia* is beneficial for body weight control (Everard et al., 2013), anti-inflammation (Anhê et al., 2015), type 2 diabetes (Qin et al., 2012) and cancer (Weir et al., 2013) in human. This indicated that long-term administration of tuber flour might have other benefits for normal people compared with starch. *Eubacterium ruminantium* is an important fibrolytic bacterial specie (Koike & Kobayashi, 2009) and SCFAs producing bacterium (Luo et al., 2019), it was promoted by flour diets. *Bacteroidetes S24-7 group_norank* is also a group of SCFAs producing bacteria in human gut microbiota, its relative abundance was significantly lower in rats fed with tuber flour. The SCFAs are key metabolites that human gut microbiota produced and affect host health such as gut permeability (Suzuki, Yoshida, & Hara, 2008). The changes in SCFAs content needed to be verified. Moreover, we applied PICRUSt analysis to predict the functions of gut microbiota. Results showed some difference between flour and starch diets in carbohydrate metabolism, amino acid metabolism, replication and repair,

and energy metabolism. This provided evidences that the different content of RDS, SDS, and RS affected the functional properties of potential health benefits for human of flour diets.

We analysed the SCFAs concentration in ileal, cecal and colonic digesta. The total SCFAs between groups showed no significant difference in cecal and colonic digesta. Only CF group had a higher total SCFAs than CS group in ileal digesta. This did not indicate that SCFAs was not the mediator of the health benefits of reshaped gut microbiota. Because different types of SCFAs showed different biological activities in human (Den Besten et al., 2013). The butyric acid exhibits various therapeutic effects in colon cancer, stomach and intestine diseases, inflammation, allergy, mitochondrial dysfunction, and neurological disease (Jiang, Fu, Yang, Xu, Wang, & Yang, 2018). It is the most reported SCFA that improves the tight-junction of intestine. The butyric acid can be used as energy resource by intestinal epithelial cells, thus improves the human gut health (Van Immerseel et al., 2010). Tuber flour diet increased the concentration of butyric acid in gut digesta and showed gut barrier protective effects compared with starch diet. The higher content of SDS and RS provided more material for butyric acid producing bacteria (Table S2).

5. Conclusion

In summary, we found that SD rats fed with tuber flour diet gained less body weight and exhibited better gut health status than those fed with corresponding starch diet. This indicates long-term administration of tuber flours would have a health promoting effects on body weight, gut permeability and related metabolic diseases. These health benefits may be related to their higher SDS and RS content rather than the fibre. The higher SDS and RS content in tuber flour diets increased the richness and diversity of butyric acid-producing gut microbiota as well as the relative abundance of *Akkermansia*, and *Eubacterium ruminantium*. Butyric acid concentration was then improved, but total SCFAs concentration was not significantly changed. Higher butyric acid content helped to improve the gut integrity. Microbiota transplantation may be applied to further confirm that microbiota participated the health benefits of tuber flour diets. The long-term effects of tuber flour on body weight and glucose-lipid metabolism and many other health benefits are need to be verified.

CRediT authorship contribution statement

Tao Xu: Conceptualization, Investigation, Methodology, Formal analysis, Writing – original draft. **Weisu Huang:** Resources, Writing – review & editing. **Jiajia Liang:** Investigation, Methodology, Formal analysis. **Yongheng Zhong:** Investigation, Resources, Writing – review & editing. **Qi Chen:** Investigation, Writing – review & editing. **Fan Jie:** Investigation. **Baiyi Lu:** Conceptualization, Validation, Funding acquisition, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2021.100145.

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