Ginseng soluble dietary fiber can regulate the intestinal flora structure, promote colon health, affect appetite and glucolipid metabolism in rats

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ABSTRACT

In this study, the effects of ginseng water-soluble dietary fiber (ginseng-SDF) on metabolism, appetite and colon health in rats were investigated. The results showed that ginseng-SDF could improve the glucolipid metabolism, especially in the triglyceride levels. Ginseng-SDF also increased satiety and delayed gastric emptying by regulating the appetite hormone levels as ghrelin, glucagon-like peptide-1, peptide YY, and cholecystokinin. In addition, ginseng-SDF improved intestinal structures and enhanced fecal short-chain fatty acids concentrations (especially acetic acid and butyric acid). More importantly, ginseng-SDF affected the abundance of Firmicutes and Bacteroides, and significantly promoted the proliferation of probiotics and cellulolytic bacteria such as *Lactobacillus*, *Bifidobacterium* and *Ruminococcus_bromii*. Among them, *Bifidobacterium pseudolongum*, *Lactobacillus helveticus* and *R. bromii* were correlated with blood glucose and blood lipid levels. These results suggested that ginseng-SDF could alter the intestinal flora structure, promote colon health, and ultimately have a positive impact on glucolipid metabolism and energy homeostasis.

1. Introduction

The rapid development of modern civilization has brought about great changes in our diet and lifestyle. The incidences of obesity and metabolic syndrome with common features of glucolipid metabolism disorders, have risen sharply and become the number one public health problem of modern society (Barber, Kabisch, Pfeier & Weickert, 2020). The intestinal flora represent the largest and most complex "organ" in the human body, and its powerful functions have been extensively studied and revealed in recent years. The intestinal flora can coevolve with the host, providing an important and stable environment for the intestinal system to digest complex nutrients, produce small molecule metabolites, and regulate the immune system (Zhao et al., 2021; Nie, Chen, Hu, Fan & Nie, 2019). Diet is thought to be one of the most important factors in determining the composition of the intestinal flora. Dietary fiber can regulate the contents of Firmicutes and Bacteroidetes—the two main phyla of intestinal microbes in the human body—and increase the abundance of probiotics such as *Lactobacillus*, *Bifidobacterium* and cellulolytic bacteria (*Roseburia*, *Ruminococcus*, etc.) (Coker, Moyne, Rodionov & Zengler, 2021; Canfora, Meex, Venema & Blaak, 2019).

Dietary fibers (DFs) are defined as indigestible carbohydrates inherent in plants (or microorganisms), including naturally isolated or synthetic cellulose that has physiological benefits to human health (Wang et al., 2021; Barber et al., 2020). The intestinal flora constitute an important medium for exhibiting the beneficial roles of dietary fiber. After being fermented by intestinal flora, DFs can reduce the intestinal lumen pH by producing small molecule metabolites represented by short-chain fatty acids (SCFAs), restrict the proliferation of harmful bacteria by competitive exclusion effects, and reduce lipopolysaccharide and harmful metabolism compounds (indole and hydrogen sulfide, etc.) to build healthy ecosystems (Zhao et al., 2018). Importantly, SCFAs can bind to G-protein coupled receptors (GPCRs) to induce the expression of hormones glucagon-like peptide 1 (GLP-1) and peptide YY (PYY) secreted by intestinal enteroendocrine L cells. The release of these two hormones has been shown to be critical for glucolipid metabolism and appetite regulation (Nie, Chen, Hu, Fan & Nie, 2019).

Although various commercial DFs have been used in food, people have been pursuing healthier and more effective dietary fiber prebiotic products. DFs from Chinese herbal medicine have an innate advantage

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in this regard. Modern studies have confirmed that many well-known traditional Chinese medicines, such as *Dendrobium officinale*, *Plantagenum officinale*, *Cyanthus sinensis*, and *Panax ginseng*, are rich in dietary fiber and have significant effects on lowering levels of glucose and lipids, reducing inflammation and regulating the intestinal flora (Hua et al., 2020b; Hua et al., 2019; Nie, Chen, Hu, Fan & Nie, 2019). Our previous study found that ginseng residue is extremely rich in dietary fiber, of which the content of water-soluble dietary fiber (ginseng-SDF) is approximately 10%, the molecular weight is 3000–200 Da, and the main monosaccharide component is glucose (58.03%). Ginseng-SDF has good enzyme inhibitor activity against α-amylase and α-glucosidase *in vitro*, which suggests that it may have certain health effects *in vivo* (Hua et al., 2020b). In addition, we also found that probiotics such as *Lactobacillus* could proliferate in MRS medium with ginseng-SDF as the only carbon source, which also suggested that ginseng-SDF might have prebiotic properties. However, there are few reports on the metabolic regulation and intestinal health effects of ginseng-SDF. This study aims to investigate the short-term intervention effects of ginseng-SDF on glucolipid metabolism, appetite levels and the intestinal environment in rats, reveal its possible health effects, and lay a foundation for its further application in functional foods.

2. Materials and methods

2.1. Ethical statementaaa

The animal experiment was conducted in accordance with the Regulations of the People's Republic of China on the Control of Laboratory Animals and approved by the Laboratory Animal Management and Ethics Committee of the Institute of Special Animal and Plant Sciences, Chinese Academy of Agricultural Sciences (NO. ISAPSAEC-2020-021).

2.2. Materials

The boiled ginseng residue (10-fold volume of water, 2 h/time × twice) was dried at 60 °C and grinded. Ginseng-SDF was extracted from the residue and dried by freeze-drying according to the method of Hua et al. (2020b) Ginseng-SDF contains 51.36% total sugars, 10.02% protein, and no fat. High performance liquid chromatography showed that it do not contain ginsenoside (Hua et al., 2020b).

2.3. Animal experiment design

Twenty-four SPF male SD rats (6-week old, 150.0 ± 5.0 g) with a qualification number of SYXK (Ji) 2018-0001 were purchased from Liaoning Changsheng Biotechnology Co., Ltd. (Liaoning, China). Feed and bedding materials were purchased from the same company. The experimental drinking water was treated by high temperature and high pressure sterilization. Rats were raised in an SPF-animal house (20–22 °C temperature and 50–55% humidity) to ensure the light condition for 12 h day/night cycle and drink and feed freely. Adaptive feeding for 7 days, then rats were divided into 4 groups randomly (n = 6/group), including the control group (physiological saline, Normal), the low-dose group (200 mg/kg·bw, SDFL200), the medium-dose group (400 mg/kg·bw, SDFM400), the high-dose group (800 mg/kg·bw, SDFH800). 2 mL of physiological saline or various-doss of ginseng-SDF were gavaged to corresponding groups for 15 days. During the intervention, the general health status of the rats were observed.

2.4. Sample collection and metabolic index determination

Rats were fasted for 12 h (drinking water unlimited) before the sacrifice, then fasting blood glucose was measured by tail venous blood (Roche glucometer, ACCU-CHEK Performa, Germany). Rats were anesthetized by pentobarbital sodium (0.5–1.0 mg/kg·bw). Blood was collected by heart puncture. Serum was collected by centrifuged at 4 °C,

3000 rpm for 10 min. Serum total cholesterol (TC), total triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) were tested according to the instructions of Kits (Nanjing Jian-cheng Bioengineering Research Institute, Jiangsu, China). Fasting insulin, appetite factors as GLP-1, PYY, cholecystinin (CCK) and ghrelin levels were tested by the ELISA Kits (Shanghai Enzyme-linked Biotechnology Co., Ltd., China). Colonic tissue were dissected, and fixed by 10% neutral formalin to make the paraffin section, observed the histological changes. Feces were collected under sterile conditions and stored at −80 °C.

2.5. Determination of fecal moisture content and pH

Fecal weight was measured accurately. Oven drying to constant weight, the mass difference of fecal sample was determined and the water content percentage was calculated. 0.3 g fecal sample and 3 mL H_2O were added in a centrifuge tube, and the tube was placed on the XW-80A Vortex oscillator (TissuePrep TP24, Tianjin, China) and shaked thoroughly (Huxi Co., Ltd, Shanghai, China). After centrifuged at room temperature (3000 rpm) for 10 min, the supernatant was taken and pH value was measured using a pH meter (FE28-Standard, METTLER TOLEDO, Shanghai, China).

2.6. Histological observation

The fixed colon tissue were used to make paraffin sections by the following steps as, dehydrated with ethanol, transparent with xylene, impregnated with wax, embedding, sectioning and thermal fixation (37 °C for 12 h-24 h). The nucleus was dyed with hematoxylin for 5–10 min, after repeated cleaning, the cytoplasm was redyeing with eosin. Photographs were taken under light microscope (IX53, Olympus Corporation, Japan) after sealed with neutral gum. The villi and crypt lengths were measured by Olympus CellSens standard software.

2.7. Content analysis of SCFAs

1.0 mL 4 °C precooled PBS solution (0.1 mol/L, pH 7.4) was added to 0.2 g fece, then fully shaken and grinded with 5 steel balls in the high-throughput Tissue Lyser (TissuePrep TP24, Tianjin, China) at low temperature (20 Hz/s, 30 s × 3 times). The supernatant was evaporated and added with 1.0 mL 50% methanol aqueous solution for resolution. After filtering by 0.22 μm filtermembrane, supernatant was stored at 4 °C and detected as soon as possible.

SCFAs were detected by gas chromatography. To be specific, a series of standard substances with concentration gradient (Total volume 1 mL, 0.8 mL, 0.6 mL, 0.4 mL, 0.2 mL mixed with 0.2 mL internal standard 2-ethylbutyric acid) were prepared, including acetic acid (57.65 mmoL/L reserve fluid), propionic acid (53.63 mmoL/L reserve fluid), butyric acid (17.45 mmoL/L reserve fluid), isobutyric acid (3.29 mmoL/L reserve fluid), valeric acid (4.61 mmoL/L reserve fluid) and isovaleric acid (3.67 mmoL/L reserve fluid). 1.0 mL of fece supernatant and standards were blended with 0.2 mL internal standard 2-ethylbutyric acid (in 25% metapopic acid, final concentration 2.88 mmoL/L). Centrifuged at 4 °C (13,000 rpm) for 5 min. The supernatant was filtered by a 0.22 μm filtermembrane and tested on an Aglient 7890A gas chromatograph system. Headspace sampler conditions: thermostatic furnace temperature 70id, final concentration 2.88 mmoL/L). Centrifuged at t 4 °C and detected as soon , constant temperature time and pressure time of the sample bottle were 160.0 kPa, 10 min, and 1 min, respectively. GC cycle time was 22 min. The chromatographic column was set at 30 m × 0.32 mm × 0.32 μm DB-FFAP with high-purity nitrogen carrier gas (1.5 mL/min), and the injection inlet temperature was 220 °C. Flame ionization detector with the temperature of 250 °C. Heating conditions: the initial temperature was 60 °C, 10 °C/min, rised to 180 °C.

2.8. Microbiome analysis based on the 16S rRNA sequencing

About 0.2 g fecal sample was stored in dry ice and sent to Shanghai Personal Biotechnology Co., Ltd. for 16S rRNA detection of intestinal microbial. Microbial genomic DNA was extracted from fecal samples using the QIAamp DNA Fecal Mini Kit (QIAGEN, No. 51504) according to the manufacturer's instructions. The quality of all DNA samples were determined by agarose gel electrophoresis. The 16S rRNA V3-V4 region was selected to design the polymerase chain reaction amplification primers (Forward primer: ACTCCTACGGGAGGCAGCA; Reverse primer: GGACTACHVGGGTWTCTAAT). Illumina NovaSeq platform was used for paired-end sequencing. The obtained sequences were denoised, merged and non-chimeric by DADA 2 method of QIIME 2 software, and then high-quality reads were divided into operational taxonomic units (OTUs) with 97% similarity. The abundance of OTU of each sample reached species level. The OTUs were compared and annotated in the Greengenes database (Release 13.8, http://greengenes.secondgenome. com/). For taxonomic composition analysis, which was performed on the feature table after the removal of Singleton, visualize the composition distribution of each sample at the six classification levels of phylum, class, order, family, genus, and species. Use R language and VennDiagram package to draw the Venn diagrams of common/unique OTUs between different groups (Venn; https://en.wikipedia.org/wiki/Venn _diagram). Use R language and PheatMap package to calculate the clustering results of each sample and each taxon, and the results of species composition heat map was presented in the form of interactive graph.

2.9. Statistical analysis

Values are presented as mean ± SD. Statistical software SPSS 19.0 was used for one-way analysis of variance (ANOVA), and LSD postmortem test was used to compare the differences between ginseng-SDF intervention groups and normal group. *$P < 0.05$ and **$P < 0.01$ were considered significant or extremely significant.

3. Results

3.1. Effects of ginseng-SDF on blood glucose and blood lipid levels

As shown in Table 1, compared with the normal group, the ginseng-SDF intervention groups had reduced fasting blood glucose, fasting insulin and insulin resistance index (HOMA-IR) to varying degrees, and the effect observed in the SDFH800 group was significant ($P < 0.01$). In terms of blood lipid, ginseng-SDF intervention groups had no significant effects on TC but significant reductions in TG and the arteriosclerosis index (AI) ($P < 0.01$). Notably, the levels of HDL-C and LDL-C in the SDFH800 group were significantly increased ($P < 0.05$) compared to those in the normal group. In general, ginseng-SDF intervention can not only promote the growth of rats, but also have positive effects on their blood glucose and blood lipid levels, especially on TG levels.

3.2. Effect of ginseng-SDF on appetite hormone levels

At present, it is generally believed that the nutritional effect of DFs in the intestinal tract has an important effect on the body's satiety. Therefore, we measured the levels of several major appetite hormones in serum and colon tissue (Fig. 1). The results showed that the levels of appetite hormones in serum and colon tissues were generally increased compared to the normal group, except that the serum PYY concentration in the SDFL200 group was decreased ($P < 0.01$). Serum ghrelin concentration in the SDFM400 and SDFH800 groups was significantly increased ($P < 0.01$), serum GLP-1 and PYY concentrations in the SDFH800 group were significantly increased ($P < 0.05$ or $P < 0.01$), and colon PYY and CCK concentrations in the SDFL200 and SDFH800 groups were significantly increased ($P < 0.05$ or $P < 0.01$). It can be seen from the above results that ginseng-SDF has effects on satiety hormones and can increase both anorexia hormone and ghrelin levels. This suggests that ginseng-SDF may have both the satiety characteristic of dietary fiber and the "nourishing" characteristic of ginseng for strengthening the body and promoting digestion, and the final growth level of rats should be the result of a combination of multiple factors, such as hormone regulation and metabolism regulation. It should be pointed out that in regard to the anatomy, we observed that the stomachs of rats in the ginseng-SDF intervention groups kept more feed residues (data not shown), which further confirms the inference that ginseng-SDF could delay gastric emptying and enhance the sense of satiety.

In addition, we also measured the levels of serum inflammatory factors (Supplementary Fig. S1)**rresponding author . Compared with the normal group, the levels of TNF-α ($P < 0.05$), IL-1β ($P < 0.01$) and IL-2 ($P < 0.05$) were significantly decreased in the SDFL200 group. The levels of IL-6 ($P < 0.05$) and IL-8 ($P < 0.01$) were significantly increased in the SDFM400 group, and the levels of IL-8 ($P < 0.05$) and IL-10 ($P < 0.01$) were significantly increased in the SDFH800 group. The low dose of ginseng-SDF (200 mg/kg) had a certain inhibitory effect on proinflammatory cytokines, while the high dose of ginseng-SDF (800 mg/kg) mainly showed a promoting effect on anti-inflammatory cytokines, such as IL-10.

3.3. Effects of ginseng-SDF on colon health

3.3.1. Effects of ginseng-SDF on fecal quality, SCFA levels and colon morphosis

Since DFs are mainly fermented in the colon, we focused on the effects of ginseng-SDF on colon health, including fecal quality, colon morphology and fecal intestinal flora structure. As shown in Table 2, compared with the normal group, the ginseng-SDF intervention groups had no significant effects on the pH of feces ($P > 0.05$), and the fecal water content in the SDFM400 group was significantly increased ($P < 0.01$). In terms of colon morphology, ginseng-SDF intervention made the intestinal villi more orderly and compact, the number of villi increased slightly, and the villi height and crypt depth changed significantly (Fig. 2 and Table 2). The V/C (villi height/crypt depth) ratio of these two groups was significantly increased ($P < 0.01$) compared with the normal group, suggesting that the digestive and absorptive capacities of the colon in rats were enhanced.
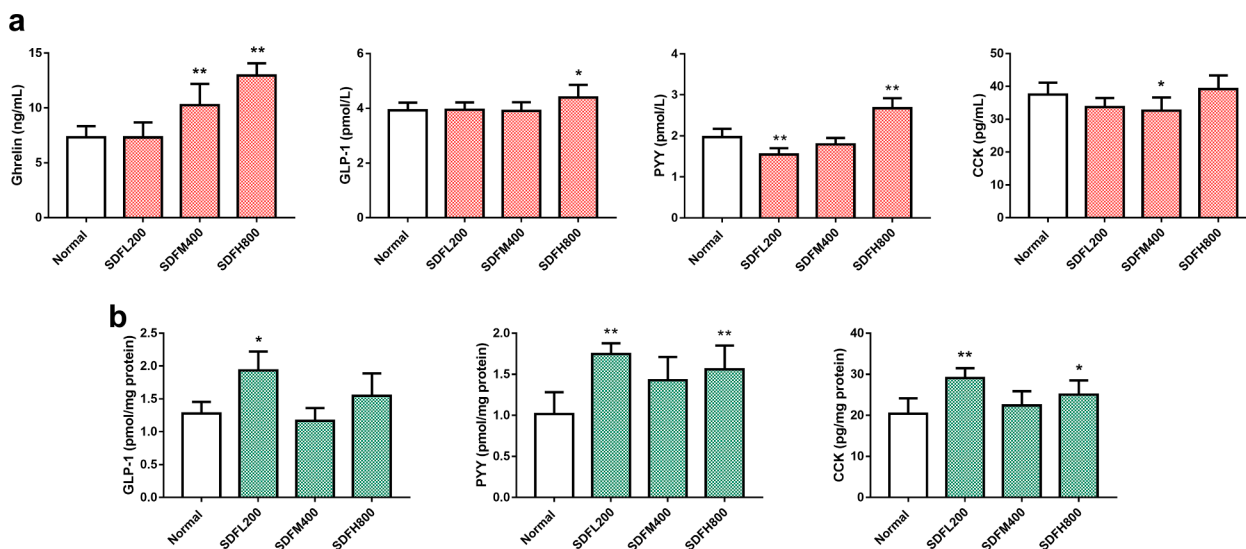
Table 1

Effect of ginseng-SDF on blood glucose and blood lipid levels in rats (mean ± SD).

Groups	Glucose(mmol/L)	Insulin(mU/L)	HOMA-IR	TC(mmol/L)	TG(mmol/L)	HDL-C(mmol/L)	LDL-C(mmol/L)	AI
Normal	6.15 ± 0.44	3.64 ± 0.38	0.99 ± 0.24	2.52 ± 0.43	0.97 ± 0.14	0.88 ± 0.17	0.35 ± 0.06	1.86 ± 0.28
SDFL200	6.01 ± 0.47	3.61 ± 0.49	0.96 ± 0.15	2.30 ± 0.39	0.72 ± 0.11**	0.95 ± 0.08	0.40 ± 0.12	1.42 ± 0.31**
SDFM400	5.89 ± 0.48	3.49 ± 0.36	0.91 ± 0.13	2.15 ± 0.15	0.64 ± 0.08**	0.90 ± 0.07	0.38 ± 0.10	1.39 ± 0.12**
SDFH800	5.39 ± 0.35**	3.30 ± 0.28	0.79 ± 0.07**	2.59 ± 0.31	0.68 ± 0.07**	1.11 ± 0.14*	0.44 ± 0.09*	1.33 ± 0.22**

HOMA-IR, insulin resistance index was calculated as [Fasting blood glucose (mmol/L) × Fasting insulin (mU/L)] / 22.5. TC, total cholesterol; TG, total triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. AI (Arteriosclerosis index) = (TC-HDL-C)/HDL-C. *$P < 0.05$ and ** $P < 0.01$ means significantly different from normal group.

Fig. 1. Effects of ginseng-SDF on the serum (a) and colon (b) appetite hormones levels in rats. * ($P < 0.05$), ** ($P < 0.01$) means the significant results compared with the normal group.

Table 2
Effects of ginseng-SDF on fecal quality and colon morphosis in rats (mean ± SD).

Groups	Feces moisture content (%)	pH	Colon tissue Villus height (μm)	Crypt depth (μm)	V/C
Normal	34.87 ± 3.22	6.15 ± 0.25	215.22 ± 14.48	109.43 ± 10.11	1.97 ± 0.33
SDFL200	38.80 ± 3.34	6.02 ± 0.30	238.65 ± 12.04*	86.26 ± 9.08*	2.77 ± 0.28**
SDFM400	42.40 ± 3.11**	6.09 ± 0.29	213.35 ± 10.02	98.71 ± 8.61	2.16 ± 0.22
SDFH800	37.99 ± 4.19	6.04 ± 0.29	244.64 ± 17.28*	65.47 ± 11.24**	3.74 ± 0.40**

V/C: villi height/crypt depth; *$P < 0.05$ and ** $P < 0.01$ means significantly different from normal group.

In addition, we measured the fecal SCFA levels (Table 3). The results showed that the effect of ginseng-SDF on fecal SCFAs was dose-related. The content and percentage of butyrate in the SDFL200 group were significantly increased ($P < 0.05$), while the content of acetic acid was decreased ($P < 0.05$). The contents of propionic acid and isovaleric acid in the SDFM400 group were significantly increased ($P < 0.01$), while the contents of acetic acid and valeric acid were further decreased ($P < 0.01$). The contents of total SCFAs, acetic acid and butyric acid in the SDFH800 group were significantly increased ($P < 0.05$), but the contents of propionic acid ($P < 0.05$) and valeric acid ($P < 0.01$) were significantly decreased compared with those in the normal group. As the most important small molecule metabolites of the intestine, acetic acid, propionic acid and butyric acid increased to varying degrees, suggesting that ginseng-SDF can play a wider role in health by regulating the

metabolism of the intestinal flora. It is important to point out that the increase in SCFAs was closely related to the selective stimulation of SCFA-producing bacteria by ginseng-SDF.

3.3.2. Effects of ginseng-SDF on the fecal intestinal flora structure

The health effects of DFs in the body are largely achieved by regulating the structure and function of the intestinal flora. As shown in Fig. 3a, the ginseng-SDF intervention groups had no significant effects on the Chao1 and Simpson indexes of fecal intestinal flora α-diversity compared with the normal group ($P > 0.05$). As seen from the results of the PCoA analysis of β-diversity (Fig. 3b), although there were no significant differences between the groups, the distribution range of the intestinal flora in the ginseng-SDF intervention groups began to adjust and was relatively concentrated with increasing dose. At the phylum level (Fig. 3c, 3e), the relative abundances of Firmicutes in the SDFL200 and SDFM400 groups were significantly increased ($P < 0.05$), while the relative abundances of Bacteroidetes were significantly decreased ($P < 0.05$), and the ratios of F/B (Firmicutes/Bacteroidetes) were significantly increased ($P < 0.01$) compared with the normal group. An increase in the abundance of Firmicutes is thought to improve the ability of the host to obtain energy from the diet, to absorb calories and to gain body weight more efficiently (Nikbakht, et al., 2018). In this study, weight gain in rats was accompanied by an increase in Firmicutes abundance. However, further analysis found that there was mainly a significant rise in the abundance of probiotics such as *Lactobacillus* and cellulolytic bacteria such as *Ruminococcaceae* from Firmicutes, and no general inflammation or intestinal pathological phenomena were found in rats. This suggests that unlike known commercial DFs, short-term intervention with ginseng-SDF actually promoted the growth of healthy rats instead of losing weight.

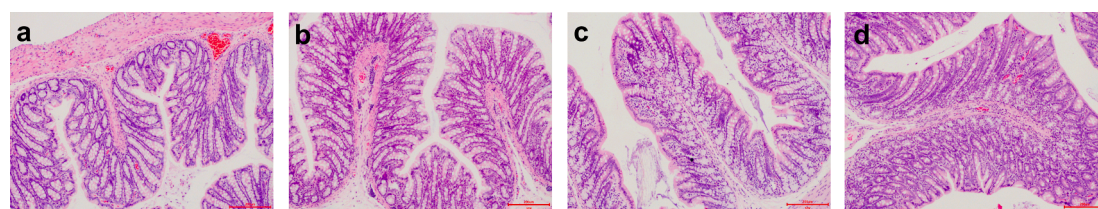At the genus level (Fig. 3d, 3e), *Lactobacillus*, *Bifidobacterium*,

Fig. 2. Effects of ginseng-SDF on the colon morphology in rats. a-d shown the colon tissues H&E staining images of the Normal (a), SDFL200 (b), SDFM400 (c) and SDFH800 (d) groups in turn.

Table 3
Effects of ginseng-SDF on the fecal SCFA levels in rats (mean ± SD).

index	SCFAs	Normal	SDFL200	SDFM400	SDFH800
Content (mmol/L)	Acetic acid	1.75 ± 0.19	1.57 ± 0.16	1.17 ± 0.13**	2.05 ± 0.08*
	Propionic acid	0.64 ± 0.08	0.76 ± 0.10	1.14 ± 0.07**	0.61 ± 0.08
	Butyric acid	0.59 ± 0.06	0.81 ± 0.04*	0.50 ± 0.14	0.79 ± 0.13*
	Valeric acid	0.07 ± 0.02	0.06 ± 0.02	0.05 ± 0.02	0.05 ± 0.01
	Isobutyric acid	0.06 ± 0.02	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.01
	Isovaleric acid	0.06 ± 0.02	0.06 ± 0.01	0.07 ± 0.02	0.07 ± 0.02
	Total SCFAs	3.18 ± 0.20	3.42 ± 0.32	2.99 ± 0.31	3.66 ± 0.14*
Percentage (%)	Acetic acid	55.19 ± 1.17	45.81 ± 2.02*	39.06 ± 1.05**	56.16 ± 2.17
	Propionic acid	20.25 ± 1.98	22.32 ± 2.53	38.17 ± 1.18**	16.71 ± 1.06*
	Butyric acid	18.57 ± 0.99	23.68 ± 1.24*	16.74 ± 1.03	21.58 ± 1.31
	Valeric acid	2.10 ± 0.61	1.85 ± 0.60	1.67 ± 0.67*	1.37 ± 0.27**
	Isobutyric acid	1.99 ± 0.66	1.85 ± 0.32	1.90 ± 0.32	2.02 ± 0.27
	Isovaleric acid	1.89 ± 0.60	1.85 ± 0.29	2.46 ± 0.70**	2.01 ± 0.57

*$P < 0.05$ and ** $P < 0.01$ means significantly different from normal group.

Turicibacter and *Ruminococcaceae_Ruminococcus* had the most remarkable changes in abundance. The relative abundance of *Lactobacillus* increased in the SDFL200 and SDFM400 groups ($P < 0.05$ or $P < 0.01$). On the other hand, in the SDFH800 group, the relative abundance of *Bifidobacterium* and *Ruminococcaceae_Ruminococcus* significantly increased ($P < 0.01$), and the relative abundance of *Turicibacter* significantly decreased ($P < 0.01$). These results suggested the prebiotic potential of ginseng-SDF, which significantly promotes the proliferation of *Lactobacillus* and *Bifidobacterium* and has a significant influence on the abundances of *Ruminococcaceae_Ruminococcus* and *Turicibacter*, which bacteria are easily induced by a DF diet (Crost et al., 2018; David et al., 2014). The changes in these bacterial communities may be one of the important pathways by which ginseng-SDF regulates body weight and metabolism.

3.3.3. Effects of ginseng-SDF on the characteristics in intestinal flora and metabolic pathways

Cluster analysis of 16S rRNA sequences in each group (97% similarity level) was performed using the DADA2 method on the Qiime2 (2019.4) platform, and 5311 operational taxonomic units (OTUs) were obtained in the normal group, 2922 OTUs were obtained in the SDFL200 group, 3140 OTUs were obtained in the SDFM400 group, and 4097 OTUs were obtained in the SDFH800 group (Fig. 4a). Compared with the normal group, the numbers of OTUs in the ginseng-SDF intervention groups decreased by 22.86–44.98%, especially in the SDFL200 and SDFM400 groups. Moreover, the proportion of unique OTUs in each group increased with the dose, and the numbers of unique OTUs in the SDFL200, SDFM400 and SDFH800 groups adjusted from 78.95% (Normal group) to 61.74%, 64.39% and 72.71%, respectively. LEfSe (Linear discriminant analysis Effect Size) analysis showed the evolutionary relationship of different flora among groups (Fig. 4b). The representative bacteria of the normal group came from Firmicutes, while the representative bacteria of the SDFL200 and SDFM400 groups expanded to *Proteobacteria*, *Bacteroidetes*, etc. Among them, *Lactobacillus hamsteri*, *Burkholderia*, *Faecalibacterium prausnitzii* and other bacteria may play a role in host health in several ways, including regulating immunity and metabolism and preventing obesity and inflammation (Barber et al., 2020; Canfora, Meex, Venema & Blaak, 2019).

Heat maps at the species level can further reveal the influences of ginseng-SDF intervention on the fecal intestinal flora structure (Fig. 4c). Compared with the normal group, the relative abundances of *L. hamsteri*, *Lactobacillus salivarius* and *Streptococcus alactolyticu* in the SDFL200 group, and the relative abundances of *Bifidobacterium bifidum*, *Lactobacillus vaginalis*, *Clostridium ruminantium* and *Acinetobacter rhizosphaerae* in the SDFM400 group were all significantly increased. In the SDFH800 group, the relative abundances of *Bifidobacterium pseudolongum*, *Bifidobacterium animalis*, *Lactobacillus helveticu*, *Selenomonas lacticifex*, *Ruminococcus bromii*, *Ruminococcus flavefaciens*, *Clostridium clostridioforme* and *Parabacteroides distasonis* were increased significantly. However, the abundances of *Lactobacillus pontis*, [*Ruminococcus*] *gnavus* and *Ruminococcus callidus* in the normal group were low. Random forest analysis can identify the complex nonlinear dependence between variables to enable a more effective and accurate classification of intestinal flora samples from each group (Thompson, Johansen, Dunbar & Munsky, 2019). As shown in Fig. 4d, a variety of probiotics were significantly affected by ginseng-SDF. The relative abundances of *L. salivarius* and *L. hamsteri* were significantly increased in the SDFL200 group and became the dominant strains of this group. The relative abundances of *R. bromii*, *B. pseudolongum* and *L. helveticus* significantly increased and became the dominant strains of the SDFH800 group. These strains are thought to play an important role in supporting human colon-resistant starch fermentation and lowering serum triglyceride and cholesterol levels in mice fed a high-fat diet (Bo et al., 2020; Baxter et al., 2019; Crost et al., 2018). The above results reveal the prebiotic properties of ginseng-SDF, namely, including the specific proliferation effect on *Lactobacillus* and *Bifidobacterium*. In addition, ginseng-SDF also increased the relative abundances of *R. bromii*, *C. clostridioforme* and other cellulolytic bacteria, a result which was closely related to the chemical nature of dietary fiber.

After examining the structural characteristics, we used Picrust2 software to observe the influences of intestinal flora on metabolic pathways. As shown in Fig. 4f, there were no significant abundance differences in the overall metabolic pathways among groups, which may be related to the intervention time and dose of ginseng-SDF. Metabolic pathways in each group were concentrated in the aspects of metabolism and genetic information processing, among which the metabolism-related pathways accounted for the highest proportion of the total. As further shown in Fig. 4e, compared with the normal group, although the difference was not significant, the abundances of lipid metabolism and xenobiotics biodegradation and metabolism in the sinseng-SDF intervention groups increased.

3.3.4. Effects of ginseng-SDF on the correlations between intestinal flora and glucolipid metabolism indexes

To further reveal which intestinal strains play a role in glucolipid metabolism, we analyzed the correlations between the relative abundances of several major probiotics and cellulolytic bacteria and blood glucose and lipid indicators. As shown in Fig. 5, the significantly increased *Lactobacillus* abundance in the SDFL200 and SDFM400 groups was negatively correlated with serum TC, TG and HDL-C levels, and positively correlated with LDL-C, FBG and FINS levels. The significantly increased abundance of *Bifidobacterium* in the SDFH800 group was positively correlated with TC and LDL-C and negatively correlated with TG, HDL-C and FBG levels. This result was similar to the weight and TC effects of mannan-oligosaccharide on the obesity and gut microbiota in mice fed a high-fat diet (Wang et al., 2018). However, even among the probiotics, different strains play different roles. The abundance of *L. helveticus* significantly increased in the SDFH800 group and was positively correlated with the HDL-C and LDL-C levels and negatively correlated with the FBG level. Similarly, the abundance of *R. bromii* was significantly increased in the SDFH800 group, significantly positively correlated with the HDL-C and LDL-C levels and negatively correlated with the FBG level. In addition, the abundance of all strains except *L. salivarius* were negatively correlated with the TG level. Except for
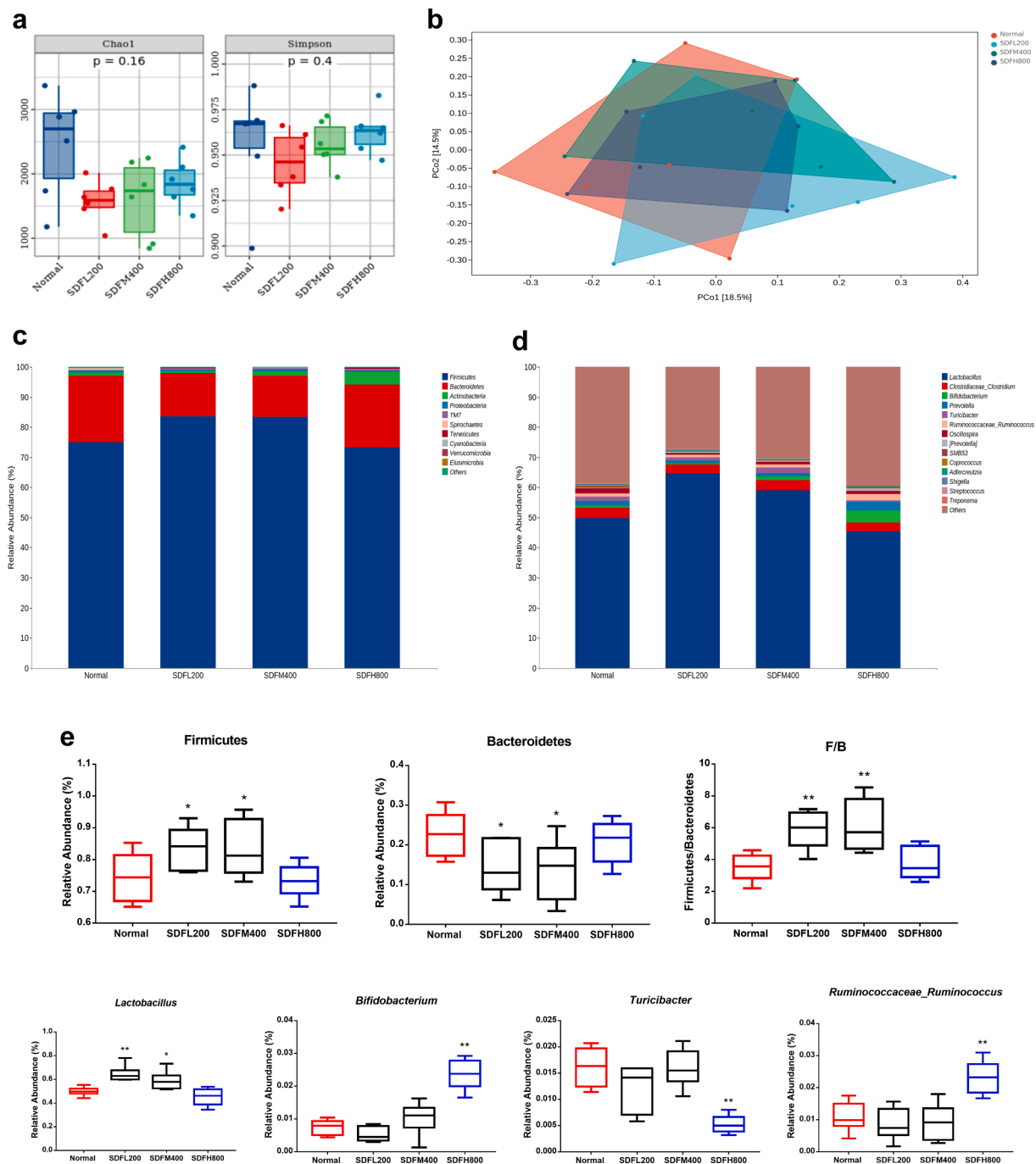
Fig. 3. Effects of ginseng-SDF on the intestinal flora structure in rats. a, Chao1 and Simpson index of α-diversity; b, PCoA analysis of β-diversity; c and d, taxonomic composition of intestinal flora on the phylum and genus levels; e, species with major differences on the phylum and genus levels. * ($P < 0.05$), ** ($P < 0.01$) means the significant results compared with the normal group.

those of *L. hamsteri*, *L. salivarius* and *L. vaginalis*, the abundances of the other strains were negatively correlated with FBG levels. These results preliminary support the conclusion that ginseng-SDF may improve glucolipid metabolism by regulating the intestinal flora.

4. Discussion

4.1. Glucolipid metabolism regulation

This study investigated the effects of ginseng-SDF on blood glucolipid metabolism, appetite levels and colon health in rats (especially the fecal intestinal flora) to reveal its possible health effects. The results

showed that ginseng-SDF could further control blood glucolipid metabolism (especially TG), reduce HOMA-IR and AI, increase the serum ghrelin levels, and increase the levels of the colon appetite hormones GLP-1, PYY and CCK. These effects may be related to the positive effects of ginseng-SDF on colon health, including the improvement of colon morphology, the increased in the abundances of probiotics (such as *Lactobacillus* and *Bifidobacterium*) and cellulolytic bacteria (such as *Ruminococcaceae* and *Clostridium*), and the regulation of fecal SCFA levels.

As an low-molecular-weight sugar, ginseng-SDF had good water-holding capacity and viscosity (Hua et al., 2020b), which not only helps resist digestion and absorption by the small intestine but also
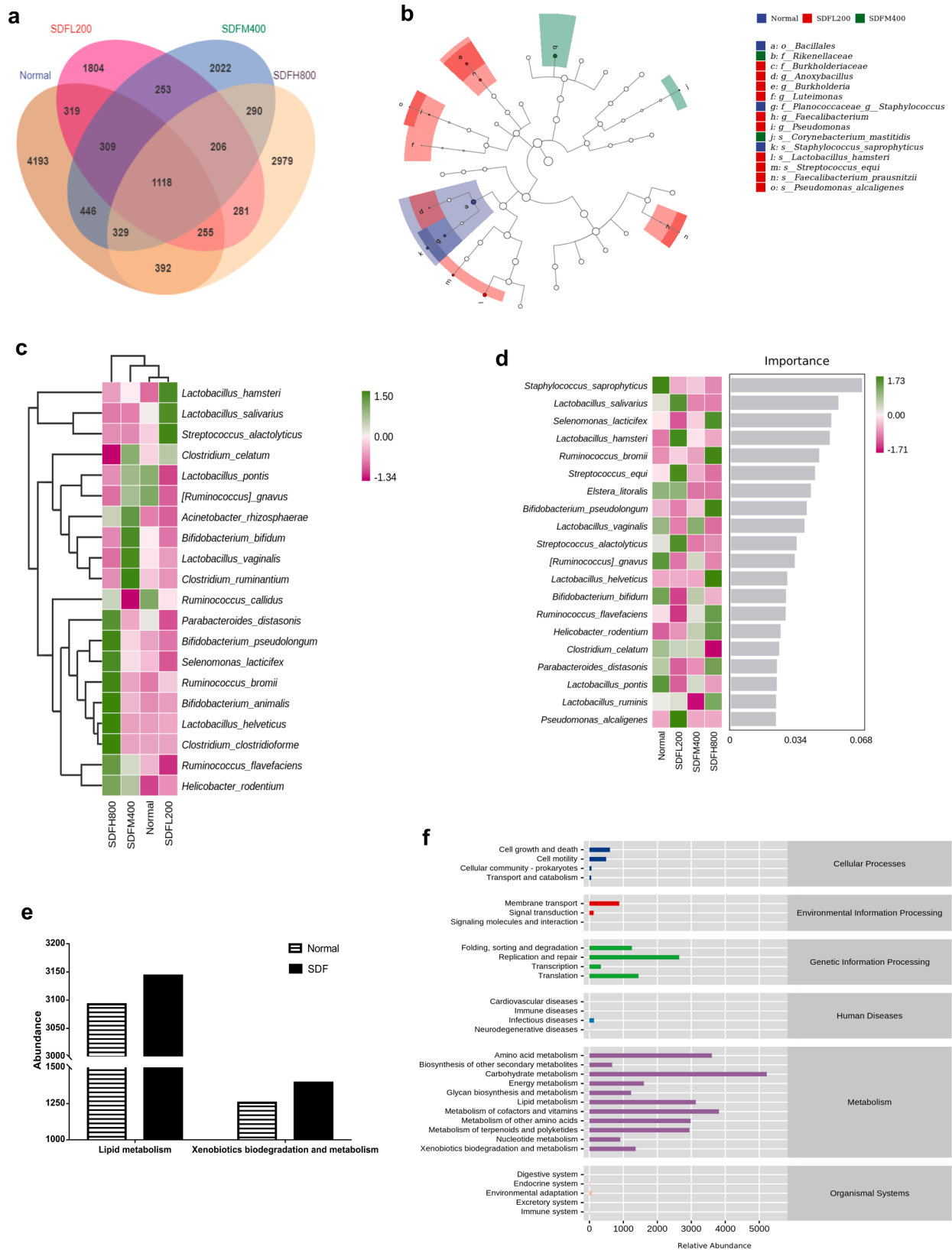
Fig. 4. Intestinal flora characteristics and metabolic pathways levels in rats. a, Venn diagram; b, LEfSe analysis of biomarker among groups (LDA threshold is 2); c, Heat map of species composition; d, Random forest model of species level; e, The up-regulated metabolic pathways affected by ginseng-SDF; f, Metabolic pathways analysis (KEGG database) of the ginseng-SDF intervention groups.
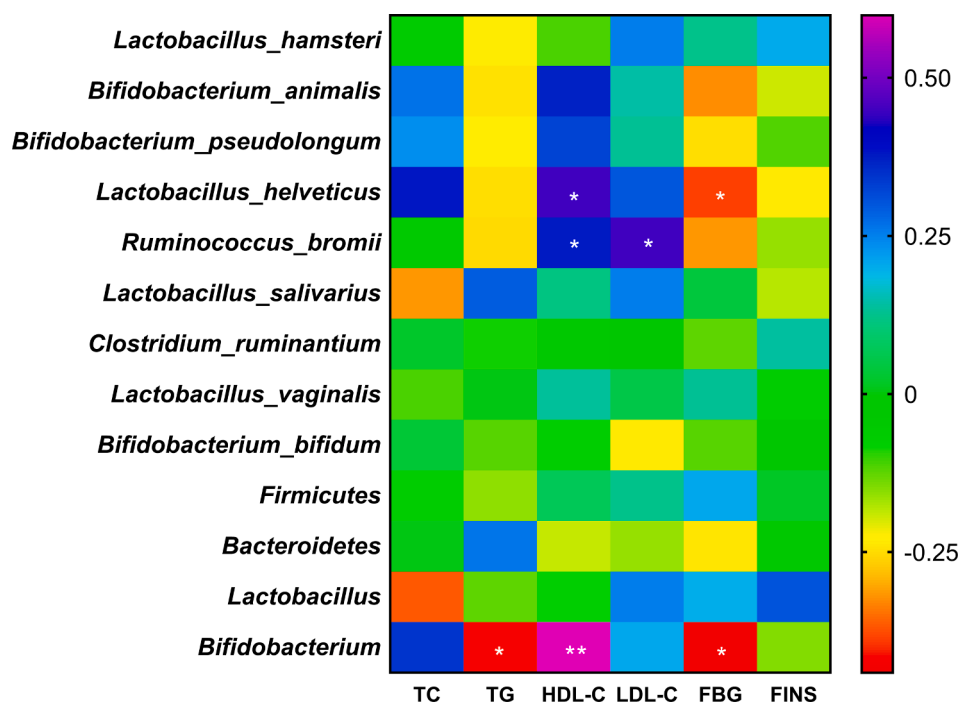
Fig. 5. Effects of ginseng-SDF on the Pearson correlation between the intestinal flora abundance and glucolipid metabolism indexes analyzed by SPSS 19.0 software. * ($P < 0.05$), ** ($P < 0.01$) means the significant results compared with the normal group.

inhibits the diffusion of glucose and the absorption of triglycerides, improves postprandial blood glucose and insulin responses, increases satiety and reduces energy intake. Ginseng-SDF has the ability to bind taurocholate and glycocholate *in vitro* due to its a lot of hydroxyl groups (Hua, Fan, Lu, Dong & Sun, 2020a). This may inhibit bile reabsorption and accelerate fecal bile acid excretion. In response to this loss, the liver stimulates the release of cholic acid by increasing the LDL-C receptor, causing more LDL-C to be isolated from the blood (Shah et al., 2020; Jesch & Carr, 2017; Threapleton et al., 2013). This may be the reason for the increased serum LDL-C content in the SDFH800 group. In conclusion, the physicochemical properties of ginseng-SDF contribute to its hypoglycemic and lipid-lowering effects in the form of physical effects in the intestinal tract, which directly affect appetite and energy intake (Poutanen et al., 2017).

In addition to the physicochemical properties, SCFAs produced by intestinal flora fermentation of DFs can play an important role in glucolipid metabolism. In this study, ginseng-SDF increased the contents of acetic acid, propionic acid and butyric acid in feces (Table 3). The main metabolic benefit of SCFAs is the activation of intestinal gluconeogenesis (IGN) in enterocytes (Blaak et al., 2020). Acetic acid is the main type of human SCFA (Rivera-Piza & Lee, 2020), and it is a signaling molecule in IGN and lipogenesis metabolism that inhibits basal and β-adrenergic receptor-mediated adipocyte breakdown, thereby contributing to insulin sensitivity (Canfora, Meex, Vlema & Blaak, 2019). Human experiments have shown that oral acetic acid has beneficial effects on body weight, serum triglyceride and postprandial glucose levels (Moffett, Puthillathu, Vengilote, Jaworski & Namboodiri, 2020). Propionic acid is primarily utilized by the liver for IGN and inhibition of cholesterol synthesis, leading to weight loss, glucose tolerance and increased insulin sensitivity (Wang et al., 2019; Wijdeveld, Nieuwdorp & Ijzerman, 2020). Similar to propionic acid, butyric acid can initiate IGN through the gut-brain axis and promote glucose metabolism and weight regulation. Butyric acid can enhance the ability to fight against metabolic disorders through a series of mechanisms, such as activating the expression of AMP-activated protein kinase (AMPK) and glucose transporter 4 (GLUT4) in adipose tissue, reversing the intestinal flora dysregulation caused by a high-fat diet, and stimulating the biosynthesis of lipoxin

(Gao et al., 2019). Importantly, both butyric acid and propionic acid activate IGN in enterocytes through complementary mechanisms that do not involve GPR41/43 (Blaak et al., 2020). This may also be the potential mechanism of ginseng-SDF in glucolipid metabolism.

4.2. Appetite regulation

Appetite regulation is one of the core functions of DFs as a food ingredient, intake of DFs prebiotics has a strong effect on the appetite hormone levels (Barber, Kabisch, Pfeier & Weickert, 2020). DFs are metabolized by intestinal flora to produce SCFAs, which stimulate intestinal L cells to secrete anorexia hormones GLP-1 and PYY responding by GPCRs as GPR41/FFAR3 and GPR43/FFAR2, thereby enhancing satiety, reducing chronic inflammation, controlling energy intake and glucolipid metabolism (Xie, Jones, Rayner & Wu, 2020). GLP-1 can slow gastric emptied in healthy and T2DM patients, inhibit the production of lipid proteins (such as apolipoprotein ApoB-48), affect the synthesis and transport of chylomicron in intestinal epithelial cells, so as to reduce lipid metabolism in mice (Xie, Jones, Rayner & Wu, 2020). Human studies shown that supplementation of fructooligosaccharides can lead to an increase in PYY level, a decrease in ghrelin level, and a decrease in postprandial glucose and insulin levels, accompanied by an increase in satiety and a decrease in subjective hunger (Parnell & Reimer, 2009). Intra CCK at physiological doses (0.6–0.8 pmol/kg/min) and allergic doses (1.8/2.6 pmol/kg/min) inhibited hunger and energy intake in healthy adults (Brennan et al., 2008). The combined response of these hormones is an important signal that affects gastric empting, pancreatic insulin and glucagon secretion, energy intake, and postprandial blood sugar levels (Xie, Jones, Rayner & Wu, 2020). In this study, serum and colon GLP-1, PYY and CCK levels were increased to varying degrees (Fig. 1), suggesting that ginseng-SDF may delay gastric emptied, enhance satiety, control glucolipid metabolism and weight gain by regulating the hormone above.

Notably, ginseng-SDF also resulted in a significant increase in serum ghrelin levels, which may be a factor contributing to the increase in feed intake. Ghrelin is a powerful stimulant, the serum ghrelin level can reflect the degree of satiety (Nunez-Salces et al., 2020). Ghrelin is

involved in the communication between the gut-brain axis composed of gastrointestinal tract, central nervous system and enteric nervous system, thus affecting glucose metabolism and energy homeostasis in the body (Huang et al., 2019). In addition, ghrelin can act as a neuroprotective agent to affect the body health, and has been associated with the regulation of autophagy in Parkinson's disease, Alzheimer's disease and Hinton's disease (Wang et al., 2020). To sum up, ginseng-SDF can not only suppress appetite, but may also promote the health of multiple organs such as nervous system and immune system through hormone regulation. This is also consistent with the traditional Chinese medicine theories such as ginseng can strengthen the body and prolong the life, suggesting the functional diversity of ginseng-SDF.

4.3. Colon health regulation

Compared with insoluble dietary fiber, soluble dietary fiber has better intestinal fermentability and can produce more SCFAs (Wang et al., 2019). The fermentation characteristics of ginseng-SDF, such as the fermentation rate, the SCFA composition, and the microbial growth factors, are determined by its chemical structure. In general, acetic acid and butyric acid are produced mainly from aldehydes (e.g., glucose, xylose, galactose, and mannose), and propionic acid is produced mainly from ketones (e.g., fructose and arabinose) (Hu, Nie, Li & Xie, 2013). Studies have confirmed that the degradation of resistant starch mainly produces acetic acid and butyric acid, pectin, the degradation of guar gum and arabinoxylan mainly produces acetic acid and propionic acid, while the degradation of inulin and β-glucan produce more propionic acid and butyric acid (Jonathan et al., 2012). The main monosaccharide component of ginseng-SDF is glucose (58%), and ginseng-SDF also contains galactose, mannose, rhamnose, uronic acid and other components. It was speculated that ginseng-SDF has a dextran main chain and a complex side chain structure (Hua et al., 2020b), so it can have an effect on a variety of SCFAs.

In this study, ginseng-SDF did not have a significant impact on the diversity of fecal intestinal flora but had a significant impact on the flora structure (Figs. 3-4). This is similar to the results of Kim et al. (2020), who studied mice fed a low-cellulose diet (LCD) and a high-cellulose diet (HCD) diets for 3 months. According to the report of Kim et al. (2020), although LCD and HCD had no effect on bacterial diversity, they resulted in changes in abundances of *Oscillibacter* and *Akkermansia* organisms. In a 2-week study of healthy adults, a vegetable diet rich in inulin resulted in a decrease in the α-diversity of the intestinal flora, a slight difference in β-diversity, and a significant increase in the abundances of probiotics, such as *Bifidobacterium*. Participants showed a higher sense of fullness and a lower desire to eat sweets and high-fat foods (Hiel et al., 2019). The above results confirmed that although ginseng-SDF has no significant effect on the diversity of the intestinal flora, it could "optimize" the intestinal flora structure of healthy rats, increase the abundances of *Lactobacillus* and *Bifidobacterium*, and show a significant prebiotic effect.

In this study, the abundances of biomarkers such as *L. salivarius*, *L. hamsteri* (SDFL200), *B. pseudolongum*, *L. helveticus*, and *R. bromii* (SDFH800) were significantly increased (Fig. 4c) and were correlated with blood glucose and lipid levels (Fig. 5). This further suggests that ginseng-SDF may affect the metabolic level by regulating the intestinal flora. It has been reported that *L. salivarius* can directly reduce the expression of monosaccharide transporters in intestinal cells, significantly reduce fasting blood glucose levels, and improve glucose tolerance and blood lipid levels in diabetic mice (Hsieh et al., 2020). *L. helveticus* produces butyric acid in adult feces and reduces the cardiometabolic risk of diet-induced obesity mice (Perazza et al., 2020; Le Roy et al., 2015). Interestingly, the health effects of *L. helveticus* seem to differ by sex. Studies have shown that *L. helveticus* can increase the caloric intake and leptin levels of offspring female rats without affecting their body weight. However, the influences of a Western diet on weight gain and calorie intake in offspring male rats were reduced by *L. helveticus* without changing leptin levels, and the effect was more

significant in male rats (Myles et al., 2020).

B. pseudolongum, the abundance of which was significantly increased in the SDFH800 group, is considered to have great potential in controlling obesity and lipid metabolism. It has been reported that *B. pseudolongum* has a strong ability to hydrolysis resistant starch and is also the key bacteria that promotes the increase in *Bifidobacterium* (such as *B. animalis*) (Centanni et al., 2018). *B. pseudolongum* can significantly reduce the weight gain, serum triglyceride level and visceral fat mass of obese mice induced by a high-fat diet, significantly reverse intestinal flora problems such as diversity reduction and F/B ratio imbalance, and increase the relative abundances of *Butyricimonas* and *Bifidobacterium*. In conclusion, *B. pseudolongum* can reduce metabolic syndrome and control obesity by changing the structure of the intestinal flora in the host (Bo et al., 2020). In this study, we also obtained similar results. This suggests that *B. pseudolongum* may be a key target of ginseng-SDF in promoting intestinal health and improving metabolism. *R. bromii* is a cellulolytic bacterium and represents another important strain affected by ginseng-SDF. *R. bromii* is recognized as a key species for resistant starch degradation (Baxter et al., 2019). Amylosomes, complex multienzyme complexes composed of starch hydrolases, were found in the genome of *R. bromii*. This is the root cause of its outstanding ability to resist starch hydrolysis (Mukhopadhya et al., 2018; Ze, Duncan, Louis & Flint, 2012). *R. bromii* has good ability to utilize monosaccharides and oligosaccharides such as galactose and glucose. Ginseng-SDF is a small molecule sugar mainly composed of monosaccharides such as glucose and galactose, which could be the structural reason that it can promote the proliferation of *R. bromii*. Besides, studies have confirmed that diets with higher resistant starch contents can increase the abundance of Firmicutes and decrease the abundance of Bacteroidetes. The mechanism may be that the starch hydrolytic products produced by *R. bromii* of Firmicutes promote the proliferation of butyrate producers, resulting in an increase in the acetic acid and butyric acid concentrations and a decrease in the environmental pH (Vital et al., 2018). This is also consistent with the increased acetic acid and butyric acid levels observed in the SDFH800 group, suggesting that ginseng-SDF may modulate SCFA levels and intestinal health through a similar mechanism. In conclusion, ginseng-SDF has been shown to promote the proliferation of probiotics and cellulolytic bacteria, increase the concentration of fecal SCFAs and enhance intestinal health, and has a considerable application prospects in regulating glucolipid metabolism and improving appetite.

5. Conclusion

This study explored the 15-days intervention effects of ginseng-SDF on the metabolism, appetite and colon health in rats. Our study showed that ginseng-SDF could further improve the glucolipid metabolism (especially TG) levels in healthy rats. In addition, ginseng-SDF is able to increase satiety and energy homeostasis by regulating appetite hormone levels, delaying gastric emptying. The above effects are closely related to its influence on colon health. Ginseng-SDF enhanced intestinal structures such as villi and crypts, and increased the concentrations of fecal SCFAs (especially acetic acid and butyric acid). More importantly, ginseng-SDF strongly affected the abundances of Firmicutes and Bacteroidetes and significantly promoted the proliferation of probiotics and cellulolytic bacteria such as *Lactobacillus*, *Bifidobacterium* and *R. bromii*. Among them, the abundances of *L. helveticus* and *R. bromii* were significantly correlated with blood glucose and blood lipid levels. These results suggest that ginseng-SDF may improve the intestinal environment by promoting the proliferation and metabolism of specific probiotics and ultimately have a positive impact on metabolism and energy homeostasis in rats. This study reveals the health effects of ginseng-SDF and lays a foundation for its application in health foods.

Ethical statement

Animal experiment was conducted in accordance with the Regulations of the People's Republic of China on the Control of Laboratory Animals and approved by the Laboratory Animal Management and Ethics Committee of the Institute of Special Animal and Plant Sciences, Chinese Academy of Agricultural Sciences (NO. ISAPSAEC-2020–021).

CRediT authorship contribution statement

Mei Hua: Investigation, Writing - original draft, Writing - review & editing, Visualization, Funding acquisition. **Meiling Fan:** Conceptualization, Investigation, Resources, Writing - review & editing, Supervision. **Zhiman Li:** Methodology, Formal analysis, Investigation, Visualization. **Jiyue Sha:** Methodology, Formal analysis, Visualization. **Shanshan Li:** Conceptualization, Resources, Writing - review & editing. **Yinshi Sun:** Conceptualization, Resources, Writing - review & editing, Supervision, Project administration.

Declarations of Competing Interest

No conflict of interest regarding the publication.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2021.104534.

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