Journal of Functional Foods 55 (2019) 193–210

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

# Effect of the sulfation pattern of sea cucumber-derived fucoidan oligosaccharides on modulating metabolic syndromes and gut microbiota dysbiosis caused by HFD in mice

Shan Li[a], Junhui Li[a], Guizhu Mao[a], Lufeng Yan[a], Yaqin Hu[a], Xingqian Ye[a], Ding Tian[a], Robert J. Linhardt[b], Shiguo Chen[a,*]

[a] Zhejiang Key Laboratory for Agro-Food Processing, Department of Food Science and Nutrition, Fuli Institute of Food Science, Zhejiang University, Hangzhou 310058, China
[b] Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY 12180, USA

ARTICLE INFO

*Keywords:*
Fucoidan oligosaccharides
Metabolic syndromes
Gut microbiota dysbiosis
Structure and function relationship

ABSTRACT

Fucoidans from sea cucumbers are potential functional food ingredients with a well-defined repeating structure. However, there have been fewer function studies of fucoidan oligosaccharides. In the present study, we first compare the functional effects of fucoidan oligosaccharides from *Pearsonothuria graeffei* (Dfuc-*Pg*) and *Isostichopus Badionotus* (Dfuc-*Ib*) in a high fat diet (HFD)-fed mouse model. Sulfation pattern was the only structural difference in the two fucoidan oligosaccharides. Both Dfuc-*Pg* and Dfuc-*Ib* inhibited hyperlipidemia, obesity, and inflammation caused by HFD. Notably, Dfuc-*Pg* could inhibit macrophages infiltrating into adipose tissue and had better anti-inflammatory activity than Dfuc-*Ib*. Meanwhile, both fucoidan oligosaccharides could reverse gut microbiota dysbiosis, particularly colonic microbiota dysbiosis by decreasing abundance of Firmicutes while increasing abundance of Bacteroidetes. However, Dfuc-*Ib* dominated with 2-*O*-sulfo groups increased abundance of Proteobacteria. Dfuc-*Pg* dominated with 4-*O*-sulfo groups maintained a better balanced gut microbiota profile. Our results shed a new insight into structure-function relationship of sulfated oligosaccharides.

## 1. Introduction

Over the past decade, molecular interactions linking the gut microbiota with host energy metabolism, lipid accumulation, and immunity have been identified (Boulangé, Neves, Chilloux, Nicholson, & Dumas, 2016). The microbiota-driven immune responses in metabolic tissues and the host nutrient-sensing mechanisms of gut microbial metabolites, such as lipopolysaccharide (LPS) and short-chain fatty acids (SCFAs), are clearly associated with obesity, non-alcoholic fatty liver disease (NAFLD), diabetes, and related metabolic syndromes (Arora & Backhed, 2016; Boulangé et al., 2016; Leung, Rivera, Furness, & Angus, 2016).

Extensive researches on the gut microbiota has shown that diet modulates the composition and function of a protective community of microbes in humans and other mammals (De Filippo et al., 2010; Sonnenburg & Bäckhed, 2016). In contrast, a diet with excessive nutrition such as fat and sucrose will cause gut microbiota dysbiosis (Li, Watanabe, & Kimura, 2017). For example, a diet rich in saturated fatty acids shifts the composition of the microbiota in the mice (Caesar, Tremaroli, Kovatchevadatchary, Cani, & Bäckhed, 2015). European children having a typical Western diet show a significantly higher ratio of Firmicutes to Bacteroidetes (F/B) than those rural African children having a diet low in fat and animal protein and high in fiber (De Filippo et al., 2010). Results of some intervention and observational studies confirm previous findings suggesting that a decrease of Bacteroidetes and an increase of Firmicutes are correlated with obesity (Wolters et al., 2018). A higher ratio of Firmicutes to Bacteroidetes positively correlates to body mass index (BMI) in an adult Ukrainian population

*Abbreviations:* HFD, high-fat diet; Dfuc-*Pg*, degraded fucoidan oligosaccharide from *Pearsonothuria graeffei*; Dfuc-*Ib*, degraded fucoidan oligosaccharide from *Isostichopus badionotus*; HFD, high-fat diet; TC, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment-insulin resistance index; TNF-α, tumour necrosis factor; LPS, lipopolysaccharide; IOD, integrated optical density; F/B, the ratio of Firmicutes to Bacteroidetes

* Corresponding author at: College of Biosystem Engineering and Food Science, Zhejiang University, Hangzhou 310058, China.
*E-mail address:* chenshiguo210@163.com (S. Chen).

https://doi.org/10.1016/j.jff.2019.02.001
Received 20 September 2018; Received in revised form 2 February 2019; Accepted 2 February 2019

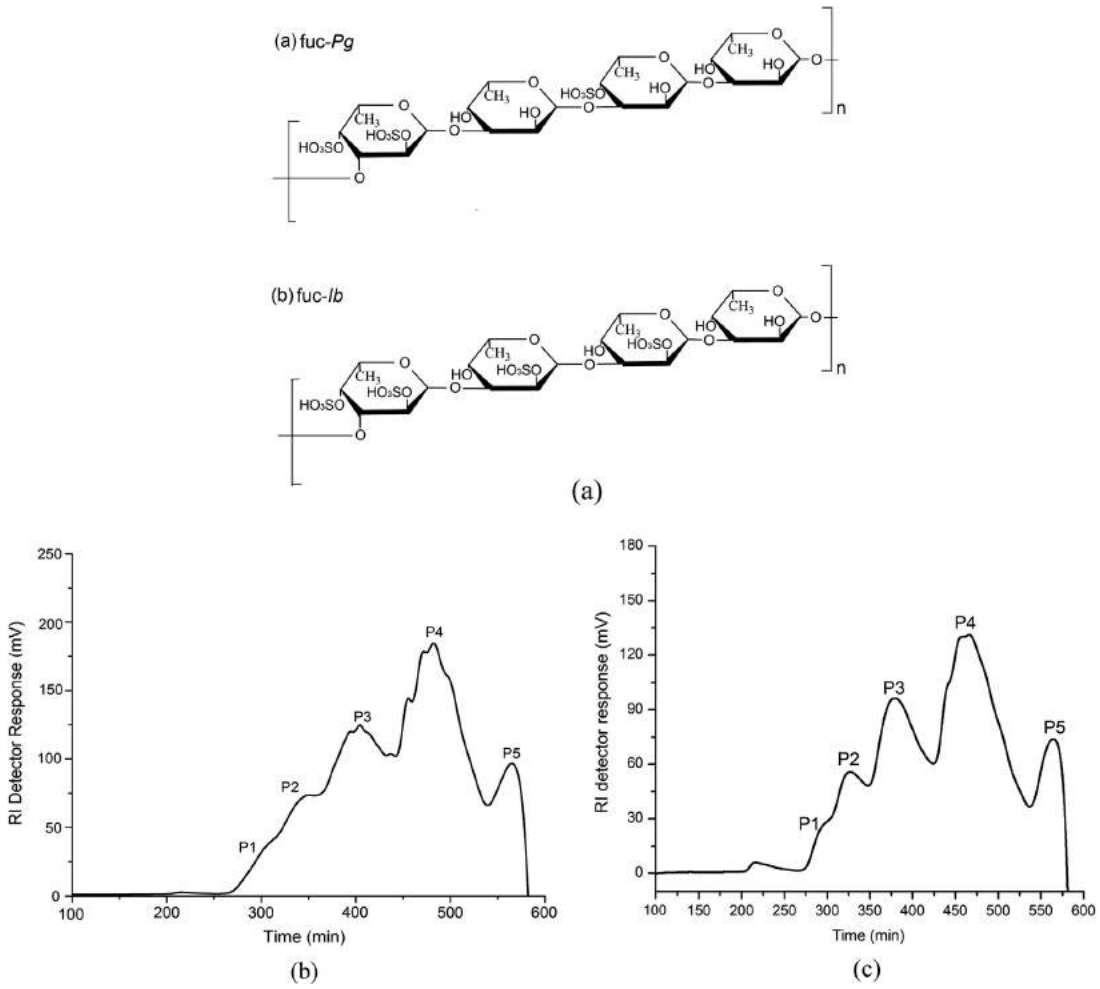



**Fig. 1.** The structural information of Dfuc-*Pg* and Dfuc-*Ib*. Chemical structures of the well-repeated tetrasaccharide units of fuc-*Pg* and fuc-*Ib* (Fig. 1a); Fractionation of Dfuc-*Pg* by gel filtration chromatography on a Superdex 30 column (1.6 × 100 cm) (Fig. 1b); Fractionation of Dfuc-*Ib* by gel filtration chromatography on a Superdex 30 column (1.6 × 100 cm) (Fig. 1c); The $^{1}$H NMR spectrum of fuc-*Pg* and Dfuc-*Pg* (Fig. 1d); The $^{1}$H NMR spectrum of fuc-*Ib* and Dfuc-*Ib* (Fig. 1e).

(Koliada et al., 2017). This phenomenon suggests that dietary target of gut microbiota could be novel therapeutic strategy for the prevention of metabolic syndromes.

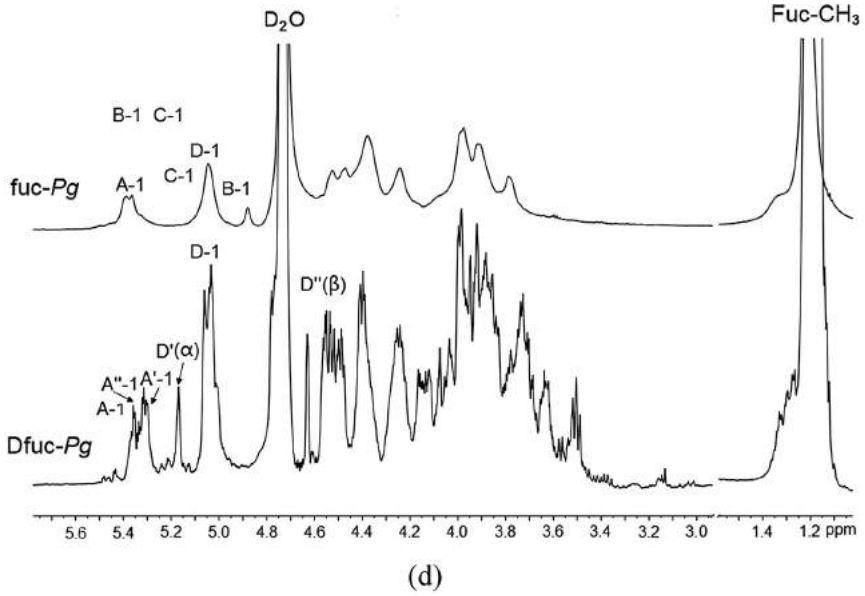
Certain polysaccharides are considered dietary fibres, which are beneficial for gut health (Hartley, May, Loveman, Colquitt, & Rees, 2016). Dietary fiber is defined as carbohydrate polymers with ten or more monomeric units obtained from natural or synthetic resources, that cannot hydrolyze by endogenous enzymes in small intestine of human beings but have shown physiological effects benefiting health (Cummings, Mann, Nishida, & Vorster, 2009). Recent, marine polysaccharides, and oligosaccharides by controlled degradation, can be fermented by gut microbiota of mice to impact intestinal ecology (Shang et al., 2018). Alginate, a marine plant polysaccharide, and its low molecular weight derivatives have prebiotic potential and increase the abundance of beneficial bacteria in the mice (Ramnani et al., 2012). Fucoidans are marine type of polysaccharides with a backbone of L-fucose units with rich sulfate ester groups (Ale & Meyer, 2013). Marine algae and invertebrates are two main resources for fucoidans. Unlike fucoidan obtained from algae, fucoidans extracted from marine invertebrates have simple, linear structures, comprised of repeating oligosaccharide units. Fucoidan derived from algae could alleviated dietary-induced metabolic syndromes by enriching microbes such as *Akkermansia muciniphila*, *Alloprevotella*, *Blautia* and *Bacteroides* in the HFD-fed mice (Shang et al., 2017). However, there are limited studies in the function of fucoidan oligosaccharide derivatives.

In our previous studies, fucoidan oligosaccharides were prepared from *Pearsonothuria graeffei* (Dfuc-*Pg*) and *Isostichopus badionotus* (Dfuc-*Ib*) by mild acid hydrolysis (Chen et al., 2012; Hu et al., 2015). Fucoidans extracted from sea cucumbers have simple, linear structures, composed of repeating units of oligosaccharides. They differ in their sulfation pattern (i.e., 2-O-, 4-O-and 2, 4-O-sulfo group substitution) among different species. Both Dfuc-*Pg* and Dfuc-*Ib* have similar straight-chain structures and composed of fucose. Their structural differences are decided by their sulfation pattern. The fucoidan tetrasaccharides of Dfuc-*Pg* are dominated with 4-*O*-sulfo groups, while the tetrasaccharides of Dfuc-*Ib* dominated with 2-*O*-sulfo groups. In the present work, we chose the two oligosaccharides to explore the effect of fucoidan oligosaccharides on metabolic syndromes and gut microbiota dysbiosis caused by HFD. They structure-function relationship were also investigated in this study.

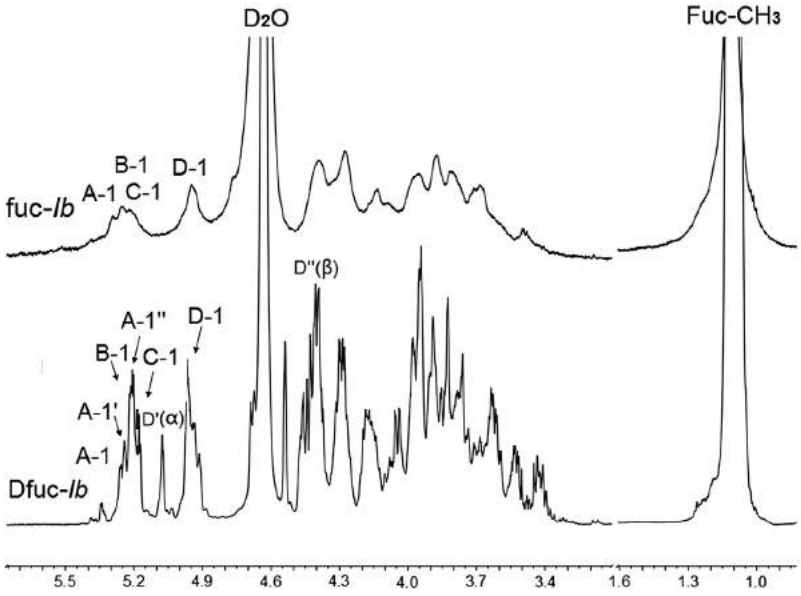
## 2. Materials and methods

### 2.1. Materials

Sea cucumbers *Isostichopus badionotus* and *Pearsonothuria graeffei*




(d)




(e)

**Fig. 1.** (*continued*)

were purchased from a local market in Qingdao, Shandong, China. Waters Ultrahydrogel 2000 and 250 columns were acquired from Waters (Milford, USA) and Q Sepharose Fast Flow anion-exchange resin from GE Healthcare, USA. Papain and cysteine were purchased from Fluka (Seelze, Germany).

### 2.2. Preparation of the Dfuc-Pg and Dfuc-Ib by mild acid hydrolysis

Crude sea cucumber polysaccharides were prepared based on a previously described method with some modifications (Chen et al., 2012; Hu et al., 2015). The crude polysaccharide solution was fractionated by anion-exchange chromatography on a Q-Sepharose Fast Flow column (4.6 × 20 cm) with elution by a linear gradient of 0–3 M NaCl in 1000 min at a flow rate of 2 mL/min. Carbohydrate fractions were collected every 6 min. Polysaccharide content was determined by the improved phenol-sulfuric acid method at 490 nm (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The purified polysaccharide was collected, dialyzed, and lyophilized. Then, the purity of fucoidan was analysed by high performance liquid chromatography (HPLC) with a Waters Ultrahydrogel 2000 column.

For partial depolymerization of the polysaccharide fuc-*Pg* and fuc-*Ib*, mild acid hydrolysis was carried out with 0.05 M $H_2SO_4$ at 65 °C for 5 h. The hydrolytic products were analysed by HPLC with a Waters Ultrahydrogel 250 column for determination of the molecular weight distribution. The oligosaccharide mixture was fractionated by gel filtration chromatography on a Superdex 30 column (1.6 × 100 cm) eluted with 0.3 M $NH_4HCO_3$ at a flow rate of 0.3 mL/min, and monitored by refractive index. The oligosaccharide fractions having a

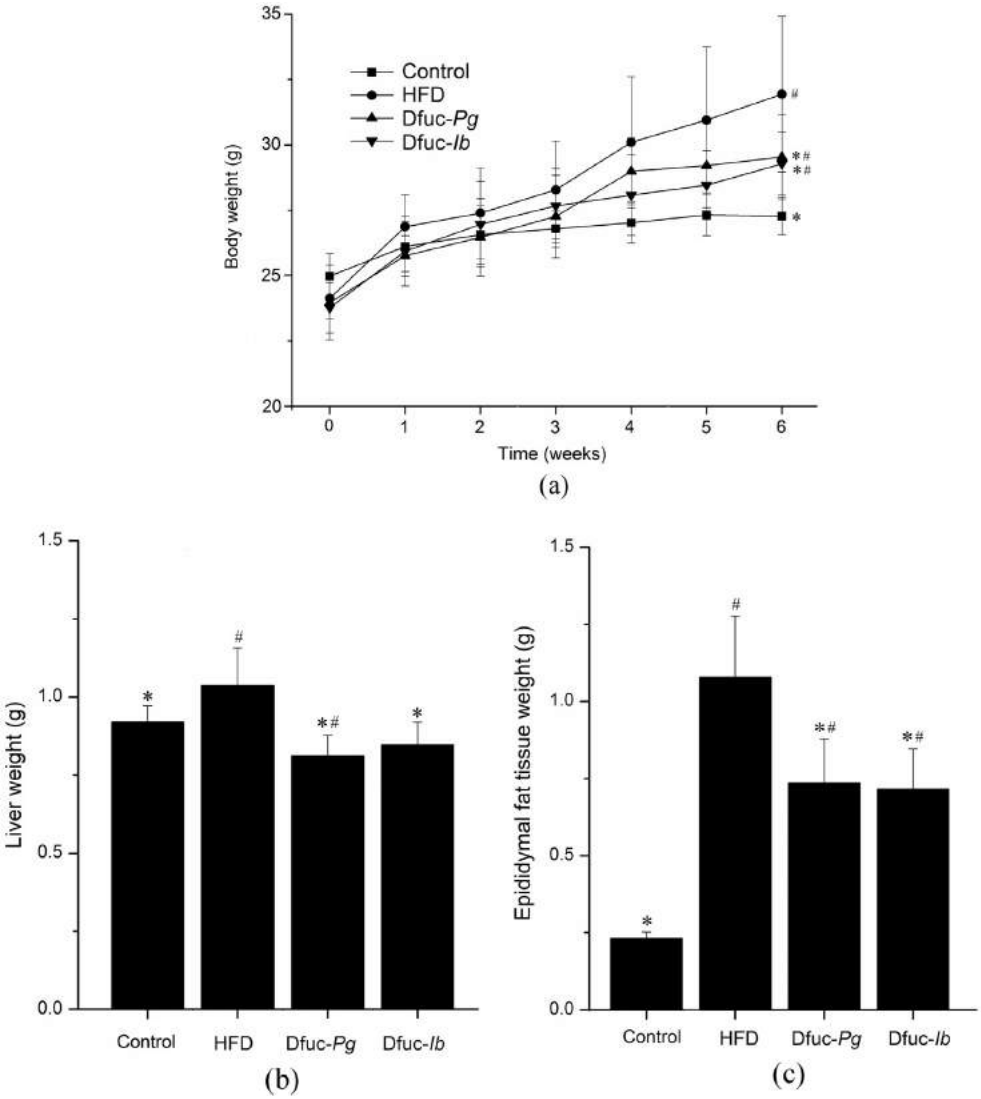



**Fig. 2.** Fucoidan oligosaccharides reduced body weight, liver weight and fat tissue weight in the HFD-fed mice. Body weight (a), liver weight (b) and epididymal fat weight (c). All data are expressed as mean ± SD (n = 10). Differences were assessed by ANOVA. $^{*}P < 0.05$: compared with the HFD group. $^{\#}P < 0.05$: compared to the control group.

degree of polymerization of 4–12 were selected and lyophilized. The detailed structural information was confirmed by $^{1}$NMR.

Polysaccharides and Oligosaccharides were co-evaporated with D2O (99.9%) twice by lyophilisation respectively and consequently dissolved in 500 µl D2O (99.9%) containing 0.1 µl acetone. The NMR spectra were recorded by Bruker AVANCE III 600 (Bruker, German). Experiments were conducted at room temperature. The number of scans (ns) in each experiment was dependent on the sample concentrations.

### 2.3. Animals and experimental design

Forty C57BL/6J mice, male, weighting from 24 ± 2 g, were purchased from the Shanghai Slaccas Laboratory Animal Company (Certificate Number SCXK (hu) 2007–0005, Shanghai, China). The animals were housed in stainless steel cages at room temperature (25 ± 2 °C) and 12 h light cycle. The animals were fed with a commercial chow for a week to acclimatize to animal facilities. The animals were then weighed and randomly divided into four groups of ten mice. One group were fed regular chow (the control group) and three groups were fed on a HFD with 60% kcal fat. The composition of 60% HFD was shown in the Supplementary Table 1, while regular chow shown in the Supplementary Table 2. One group received HFD served as a negative control (the HFD group) and two groups received HFD and were given Dfuc-*Pg* (the Dfuc-*Pg* group) and Dfuc-*Ib* (the Dfuc-*Pg* group) in dose of 80 mg/kg/day, respectively. Fucoidan oligosaccharides were dissolved in distilled water and administered orally by metallic gavage needle according to weight of every mouse. The mice were allowed free access to food and water during the experimental period. Food consumption and weight gain were measured once each week. All animal maintenance and use procedures were in accordance with the guidelines of the Laboratory Animal Centre at Zhejiang University.

### 2.4. Biochemical analysis of serum

After 6 weeks, the mice were withheld food for 12 h, weighed, and then blood samples were collected from the orbital sinus under anaesthetized conditions. The liver and adipose tissue were collected from each animal and weighed and frozen at −80 °C. Serum TC, TG, HDL-C,

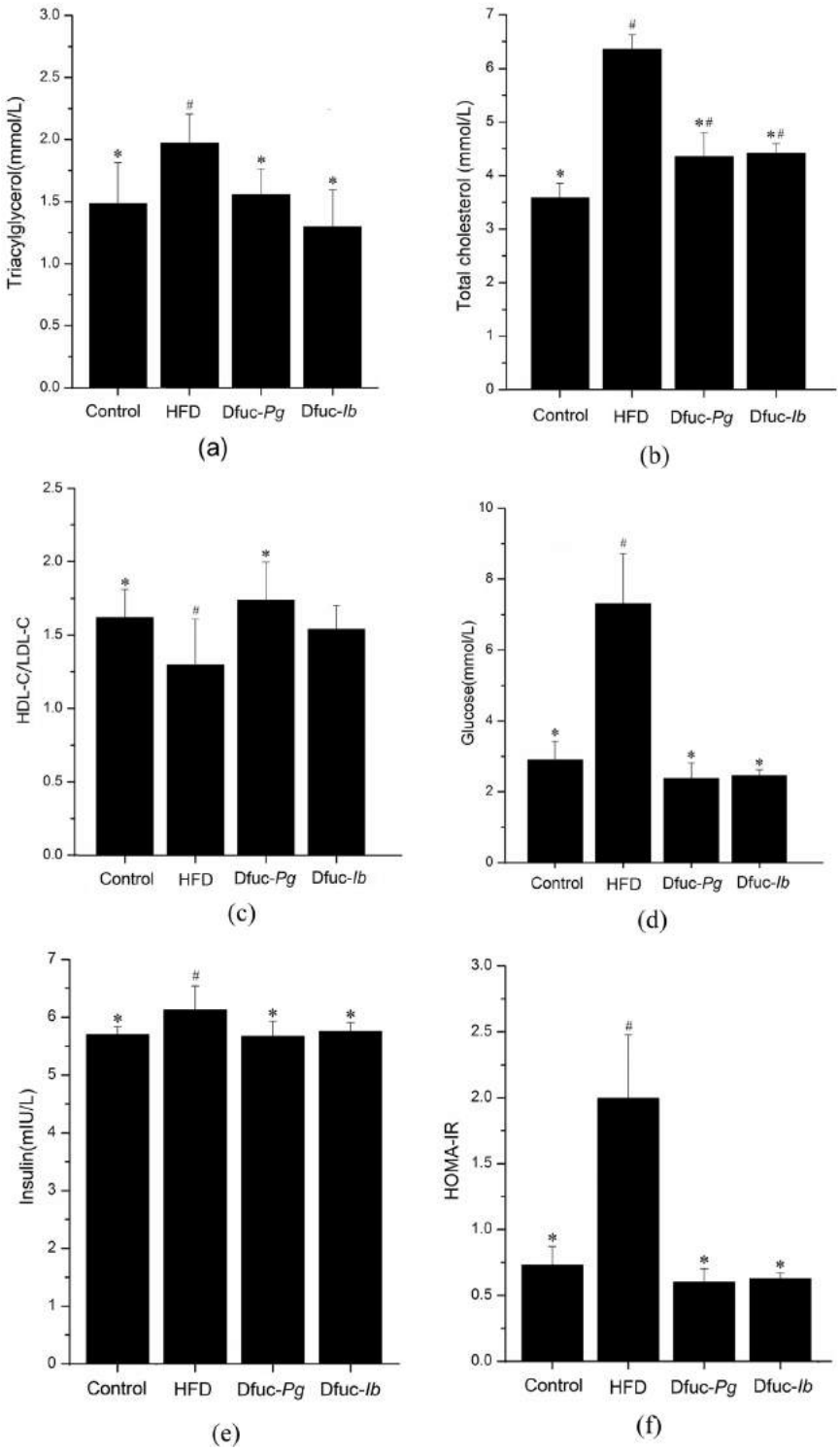



**Fig. 3.** Fucoidan oligosaccharides attenuated high lipid levels and improved glucose-insulin homeostasis in the serum of HFD-fed mice. triglyceride (a); the ratio of high density lipoprotein cholesterol and low density lipoprotein cholesterol (HDL/LDL) (b); Total cholesterol (c); fasting blood glucose (d); fasting insulin (e); HOMA-IR (f), calculated by fasting blood glucose (mmol $L^{-1}$) × fasting insulin (mIU/L)/22.5. All data are expressed as mean ± SD (n = 10). Differences were assessed by ANOVA. $^{*}P < 0.05$: compared with the HFD group. $^{\#}P < 0.05$: compared to the control group.

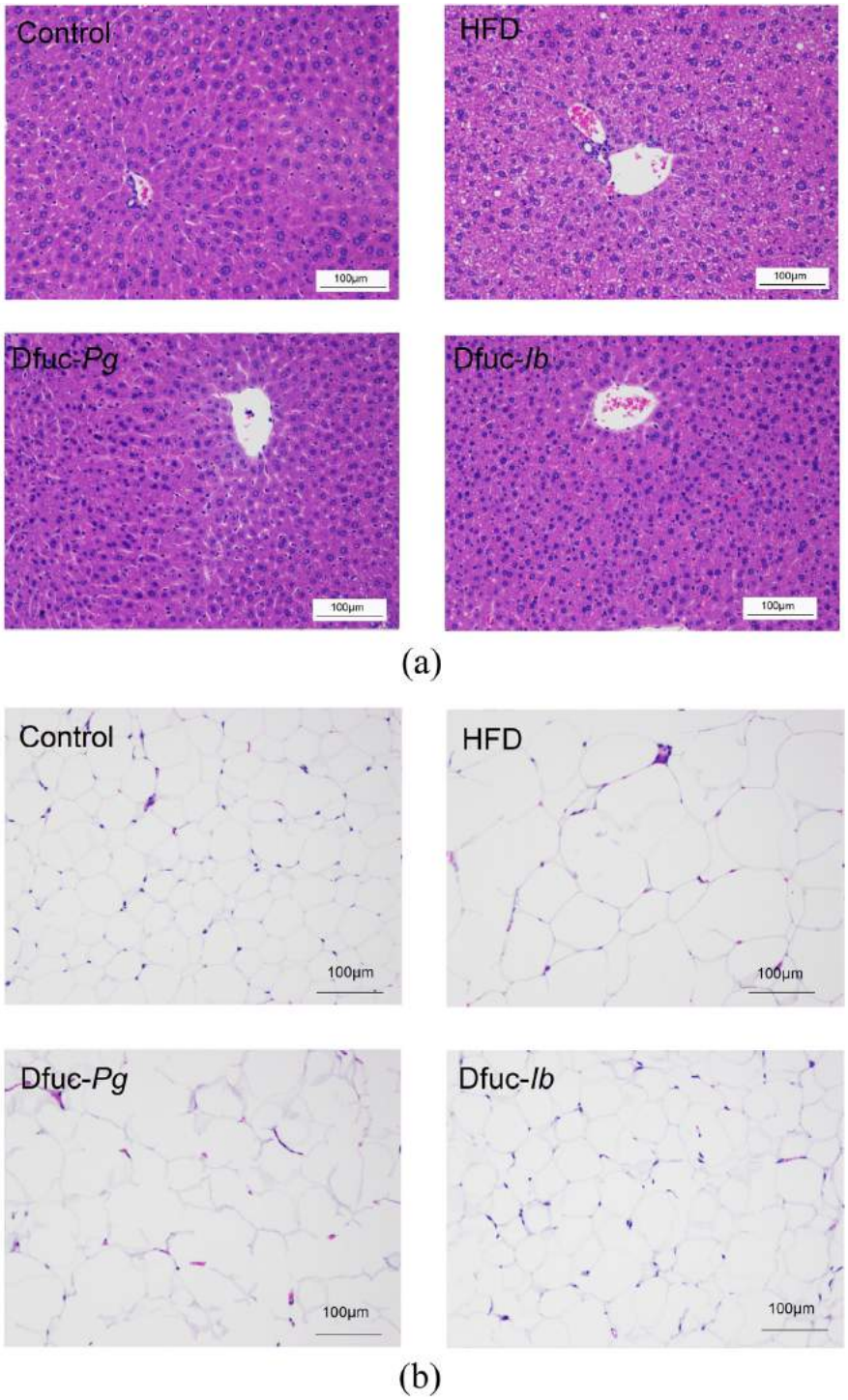



**Fig. 4.** Fucoidan oligosaccharides attenuated hepatic steatosis and adipocyte hypertrophy in HFD-induced obese mice. Histological assessment of liver in HFD-induced obesity mice (HE stain, 200× magnification) (a); histological assessment of adipose tissue in HFD-induced obesity mice (HE stain, 200× magnification) (b).

LDL-C, and glucose levels were measured enzymatically by commercial kit. TNF-α, LPS, and insulin contents were measured using commercial ELISA kits (R&D Systems, USA).

### 2.5. Liver and epididymal fat histology

After the blood samples were gathered, the liver and epididymal fat tissue samples were collected. Part of these tissues were cut and fixed with neutral formalin solution for 48 h, dehydrated through ascending grades of alcohol, cleared in benzene and embedded in low melting

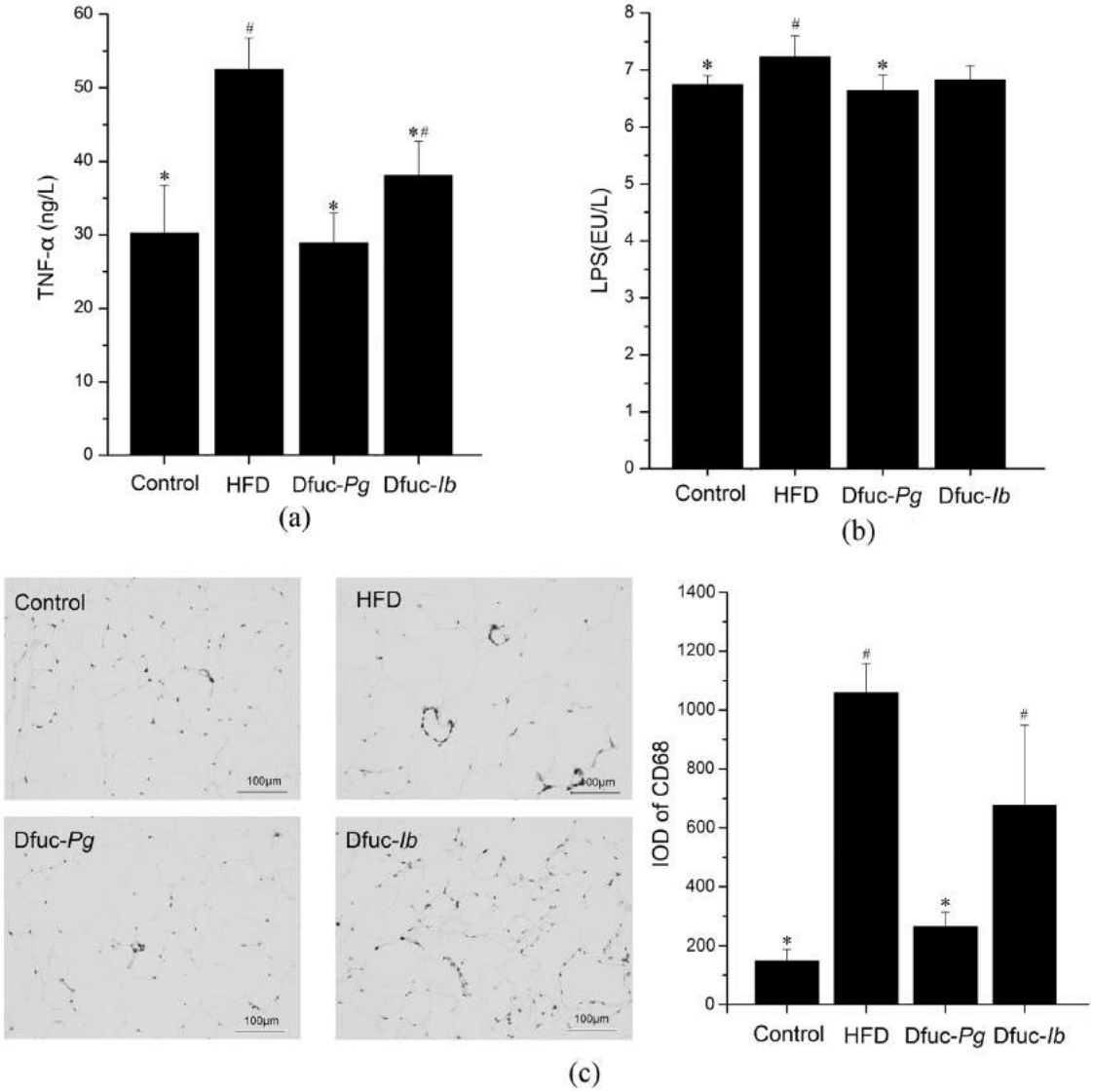



**Fig. 5.** Fucoidan oligosaccharides had different effects on suppressing the chronic inflammation caused by HFD. Effect of Dfuc-*Pg* and Dfuc-*Ib* administration on serum TNF-α (a), LPS (b) and immunohistochemical staining for CD68 (c) in the fat tissue. The stained areas were viewed using an optical microscope at 200×. All data are expressed as mean ± SD (n = 10). Differences were assessed by ANOVA. $^{*}P < 0.05$: compared with the HFD group. $^{\#}P < 0.05$: compared to the control group.

point paraffin wax. Sections, 3 μm thick, were cut and stained with hematoxylin and eosin (HE staining) for light microscopic examinations.

### 2.6. Immunohistochemistry

Part of adipose tissue from mice were immersed in 4% paraformaldehyde for 48 h and then dehydrated through a serial alcohol gradient, and embedded in paraffin wax blocks. Incubate sections in 3 changes of xylene, 15 min each. Dehydrate in 2 changes of pure ethanol for 5 min, followed by dehydrate in gradient ethanol of 85% and 75% ethanol, respectively, 5 min each. Wash in distilled water and cut into slides. Immerse the slides in sodium citrate antigen retrieval solution (pH 6.0) and maintain at a sub-boiling temperature for 8 min, standing for 8 min and then followed by another sub-boiling temperature for 7 min. Wash three times with PBS (pH 7.4) in a Rocker device. Immerse in 3% $H_2O_2$ and incubate at room temperature for 15 min, kept in dark place. Then wash again three times with PBS (pH 7.4) in a Rocker device. Eliminate obvious liquid, mark the objective tissue with liquid blocker pen. Cover objective tissues with 3% BSA at room temperature for 30 min. Slides were incubated with CD68 primary antibody (Santacruz Biotechnology Inc., USA) overnight at 4 °C. Wash slides three times with PBS (pH 7.4) in a Rocker device. Cover objective tissue with secondary antibody (appropriately respond to primary antibody in species) labelled with HRP, incubate at room temperature for 50 min. Dry sections slightly, add fresh prepared DAB chromogenic reagent to marked tissue. Manage reaction time by observing in microscopy until nucleus shows brown-yellow. Then stop developing reaction by wash in running tap water. Counterstain in nucleus with Hematoxylin staining solution for 3 min and wash in tap water. Treat with the differentiate solution for a few seconds, wash in running tap water. Back to blue by bluing solution, wash in running tap water. Dehydrate successively in

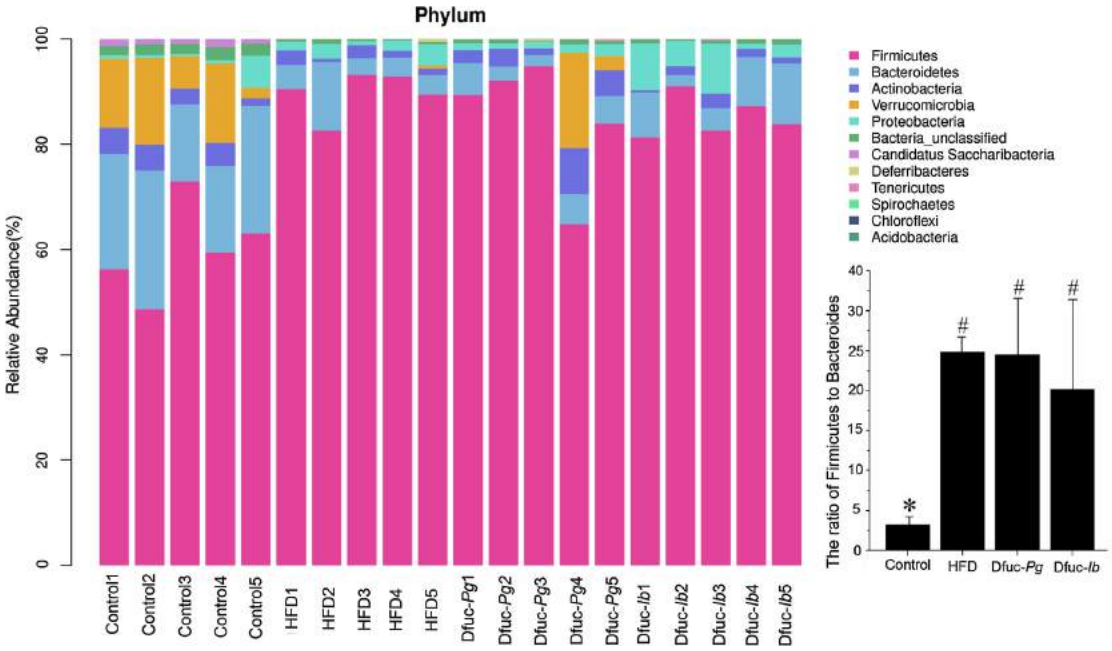



**Fig. 6.** Composition of cecal microbiota in control, HFD, Dfuc-*Pg*, and Dfuc-*Ib* groups at phylum level (a); Box chart of relative abundance of colonic microbiota at the phylum level (b). Composition of cecal microbiota in control, HFD, Dfuc-*Pg*, and Dfuc-*Ib* groups at genus level (c); Box chart of relative abundance of colonic microbiota at the gens level (d); Taxonomic cladogram of LEfSe analysis of cecal microbiota of control, HFD, Dfuc-*Pg*, and Dfuc-*Ib* groups. The small figure inserted in the Fig. 6a was the ratio of Firmicutes to Bacteroidetes, which was expressed as mean ± SD (n = 5). Differences were assessed by ANOVA. $^{*}P < 0.05$: compared with the HFD group. $^{\#}P < 0.05$: compared to the control group.

gradient ethanol of 75%, 85%, and 2 changes of pure ethanol, respectively. Clear in xylene for 5 min and mount with resin mounting medium. The sections were observed in DS-Ri1-U3 Nikon digital imaging system and the positive integral optical density (IOD) of CD68 in the immunohistochemical pictures was analysed by Image pro-plus 6.0 software.

### 2.7. 16S rDNA analysis

DNA was extracted from the cecal and colon contents of mice by using the E.Z.N.A. ®Stool DNA Kit (D4015, Omega, Inc., USA) according to manufacturer's instructions. The reagent, which was designed to uncover DNA from trace amounts of sample, has been shown to be effective for the preparation of DNA of most bacteria (Chen, Zhang, Cheng, Zheng, & Zhang, 2018). Nuclease-free water was used for the blank. The total DNA was eluted in 50 μl of elution buffer and stored at −80 °C until PCR amplification (LC-Bio Technology Co., Ltd, Hangzhou, China). The 16S rDNA gene (V3 and V4 region) from the colon microbiota was amplified using the universal primers as follows (Evans et al., 2014):

319F, 5′-ACTCCTACGGGAGCAG-3′
806R, 5′-GGACTACCAGGGTATCTAAT-3′

PCR reactions were carried out in an ABI GeneAmp PCR System 9700 (Applied Biosystems, Foster City, USA). Amplicons were purified using the AxyPrep DNA gel extraction kit (Axygen Bioscience, Union City, USA) and quantified by a Promega Quantifluor® ST fluorometer (Promega, Madison, USA) before being sequenced on an Illumina Miseq PE250 platform (Majorbio Bio-pharm Biotechnology Co., Ltd, Shanghai, China).

Samples were sequenced on an Illumina MiSeq platform according to the manufacturer's recommendations (LC-Bio Technology Co., Ltd, Hangzhou, China). Paired-end reads were assigned to samples based on their unique barcode and truncated by cutting off the barcode and primer sequence. Paired-end reads were merged using FLASH (Tanja & Salzberg, 2011). Quality filtering on the raw tags was performed under specific filtering conditions to obtain the high-quality clean tags according to the FastQC v0.10.1 (Moolhuijzen, Manners, Wilcox, Bellgard, & Gardiner, 2013). Chimeric sequences were filtered using Verseach software v2.3.4 (Rognes, Flouri, Nichols, Quince, & Mahé, 2016). Sequences with ≥97% similarity were assigned to the same operational taxonomic units (OTUs). Representative sequences were chosen for each OTU, and taxonomic data were then assigned to each representative sequence using the RDP (Ribosomal Database Project classifier). The differences of the dominant species in different groups, multiple sequence alignment were conducted using the PyNAST software (Caporaso et al., 2010) to study phylogenetic relationship of different OTUs. OTU abundance information was normalized using a standard of sequence number corresponding to the sample with the least sequences. Alpha diversity is applied in analysing complexity of species diversity for a sample through 4 indices, including Chao1, Shannon, Simpson and Observed species. All this indices in our samples were calculated with QIIME v1.8.0 (Caporaso et al., 2010). Beta diversity including UniFrac-based principal coordinates analysis (PCoA) and Nonmetric multidimensional scaling (NMDS) analysis were calculated by QIIME software v1.8.0 to evaluate differences of samples in species complexity. Linear discriminant analysis (LDA) effect size (LEfSe) analysis was performed to identify the dimensional gut bacteria and characterize the microbial differences between different treatment groups. A significance alpha value of less than 0.05 and an effect size threshold of 2 were used for this analysis.

### 2.8. Statistical analysis

Data are expressed as means ± SD. Comparisons across groups were performed with SPSS 22.0 (SPSS, Inc., Chicago, IL, USA) using one-way analysis of variance (ANOVA) after testing for homogeneity of

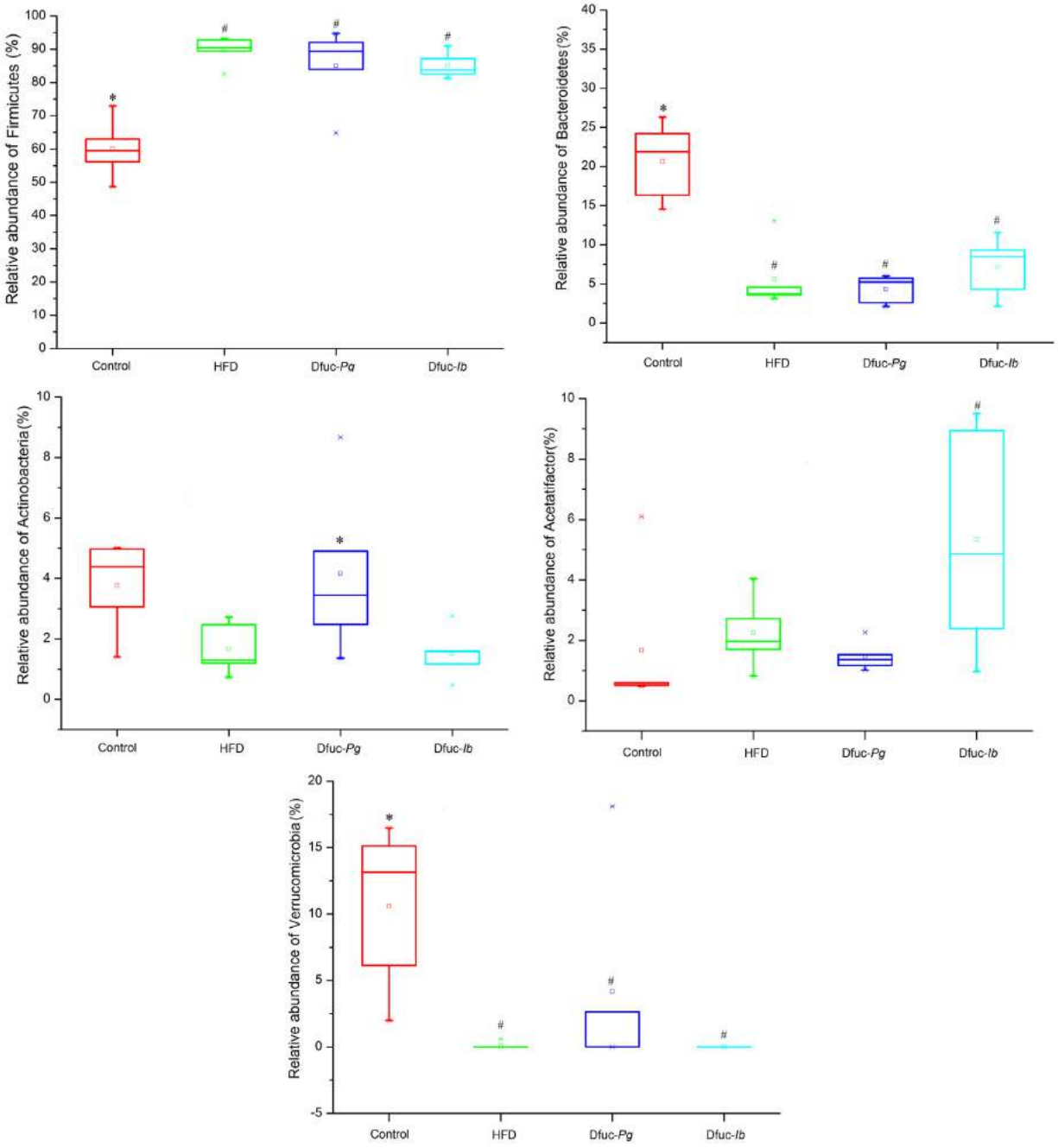



**Fig. 6.** (*continued*)

variance test, and then the means were compared by Tukey's test. Confidence levels for statistical significance were set at $P < 0.05$.

## 3. Results

### 3.1. Structural elucidation of fucoidan oligosaccharides

As shown in the Fig. 1a, we have extracted two types of fucoidans from sea cucumbers, named as fuc-*Pg* (fucoidan from *Pearsonothuria graeffei*) and fuc-*Ib* (fucoidan from *Isostichopus badionotus*) respectively (Chen et al., 2012; Hu et al., 2015). Fuc-*Pg* is dominated by 4-*O*-sulfo groups, while fuc-*Ib* has more 2-*O*-sulfo groups. The molecular size of polysaccharides and the large molecular size of fucoidan have limited their structure elucidation and functional applications. In former researches, we have developed a mild acid hydrolysis method to prepare fucoidan oligosaccharides from sea cucumber with little desulfation (Chen et al., 2012; Hu et al., 2015). During the process of acid hydrolysis, oligosaccharides with regular sequences were obtained. As shown in the Fig. 1b and c, five peaks were found after acid hydrolysis. The major fractions P2, P3 and P4 were proven to contain sulphated dodeca-, octa- and tetrasaccharides by our former studies (Chen et al., 2012; Hu et al., 2015). We collected fucoidan oligosaccharides from P2 to P4. The molecular weight of Dfuc-*Pg* were manly between 760 Da and 2490 Da, while Dfuc-*Ib* were 760 Da to 2906 Da. We further

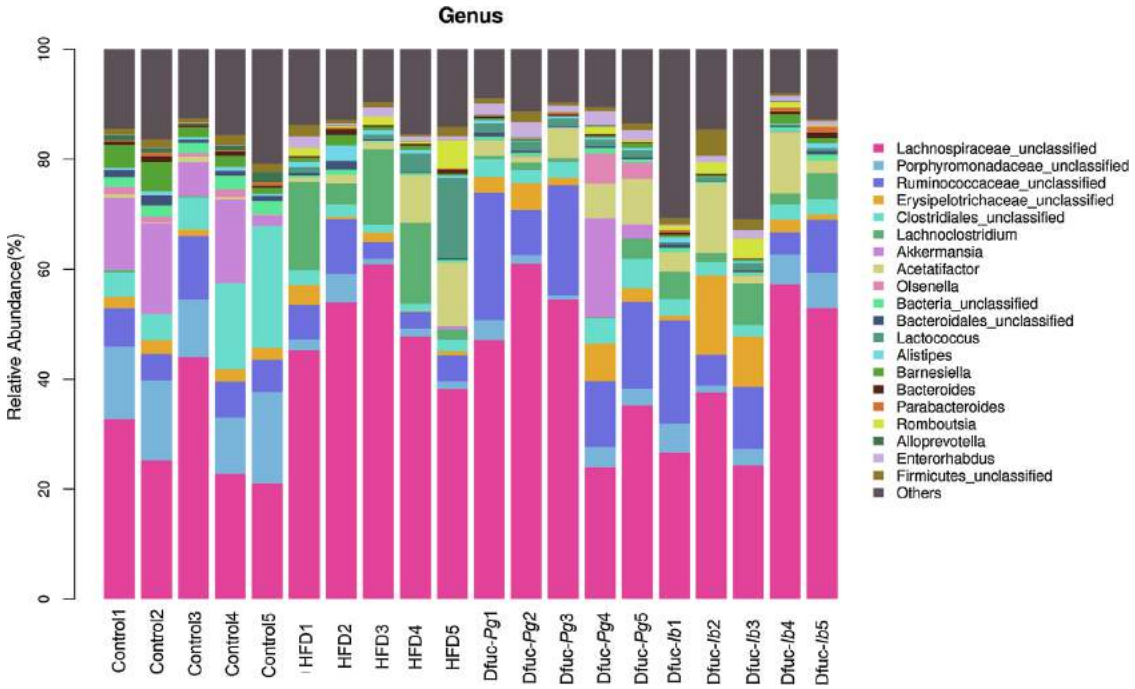



**Fig. 6.** (*continued*)

analyzed the structural information by $^1$H NMR (Fig. 1d and e). According to our results, fucoidans and their oligosaccharides had similar $^1$H NMR spectra, which indicated that the acid preferentially cleaved the glycosidic bond without altering of the structural features. Meanwhile, The signals of anomeric proton A-1, A-1′, A″ indicated that the two fucoidan oligosaccharides contained tetrasaccharides, octasaccharide, and dodecasaccharide.

### 3.2. Fucoidan oligosaccharides reduced obesity in the HFD-fed mice.

C57BL/6J mice were fed with HFD for 6 weeks to set up obesity. During the feeding trial, mice in the HFD group showed higher growth rate body weight than mice in the chow, Dfuc-*Pg*, and Dfuc-*Ib* groups (Fig. 2a). Importantly, mice fed on HFD supplemented with two fucoidan oligosaccharides gained significantly less weight than mice in the HFD group at the ending of trial ($P < 0.05$). Meanwhile, liver weight and epididymal fat weight in the HFD group were also higher than the control group ($P < 0.05$). Both Dfuc-*Pg* and Dfuc-*Ib* could significantly decrease liver and fat tissue weight in the HFD-fed mice ($P < 0.05$, compared with the control group, Fig. 2b and c). Especially, the live weight in the Dfuc-*Pg* group was even lower than that of the control group ($P < 0.05$). Both fucoidan oligosaccharides only alleviated the abnormal body weight and fat tissue weight in the HFD-fed mice.

### 3.3. Fucoidan oligosaccharides attenuated high lipid levels and improved glucose-insulin homeostasis in the serum of HFD-fed mice

Abnormal metabolism of glucose and lipids is the hallmark of metabolic syndrome, which is defined by high levels of two or more factors, including elevated triglycerides, high blood pressure, and increased fasting blood glucose (Sonnenburg & Bäckhed, 2016). In this section, we studied the effects of fucoidan oligosaccharides on the serum lipid profile, fasting blood glucose, insulin, and HOMA-IR in HFD-fed mice.

High concentrations of TG and/or TC and low ratio of HDL/LDL are considered as characteristic features of dyslipidemia (Liu, Zheng, Zhang, Yao, & Gao, 2014). In our study, serum TG (Fig. 3a) and TG (Fig. 3b) levels increased while the ratio of HDL-C/LDL-C (Fig. 3c) decreased in the HFD group ($P < 0.05$ compared with the control group). It was demonstrated that both fucoidan oligosaccharides had the ability to decrease TC and TG levels while increased the ratio of HDL-C/LDL-C in the serum of HFD-fed mice ($P < 0.05$ compared with the HFD group). The levels of TG in the Dfuc-*Pg* and Dfuc-*Ib* groups were recovered close to the control group ($P > 0.05$ compared with the control group). Dfuc-*Pg* showed better effect on improving the ratio of HDL-C/LDL-C ($P > 0.05$ compared with the control group) than Dfuc-*Ib* ($P < 0.05$ compared with the control group). The data showed that both fucoidan reversed the hyperlipidemia caused by HFD in mice.

Fasting blood glucose and insulin were measured in this study to explore the effects of fucoidan oligosaccharides on glucose-insulin homeostasis. Fasting blood glucose was increased twice in the HFD group compared with the normal control group ($P < 0.05$) and decreased by both fucoidan oligosaccharides to a normal level ($P > 0.05$ compared with the control group, $P < 0.05$ compared with the HFD group) (Fig. 3d). Insulin level was slightly increased in the HFD group and normalized by fucoidan oligosaccharides (Fig. 3e). HOMA-IR (homeostasis model assessment-insulin resistance index) analysis confirmed that both Dfuc-*Pg* and Dfuc-*Ib* could improve glucose-insulin homeostasis (Fig. 3f). In the present study, the glucose level of HFD group was greatly increased twice than that of the control group. However, the insulin level was slightly increased by HFD. HFD feeding for six weeks may be not long enough to cause severe hyperinsulinemia. According to early reports, serum glucose of mice feeding on HFD elevated linearly throughout the study period. In contrast, serum insulin increased progressively by time (Winzell & Ahrén, 2004). Serum insulin could markedly increase by HFD at nine weeks (Wei, Lane, Lane, Padanilam, & Sansom, 2004). In the present study, fucoidan oligosaccharides improved the glucose-insulin homeostasis in the HFD-fed mice.

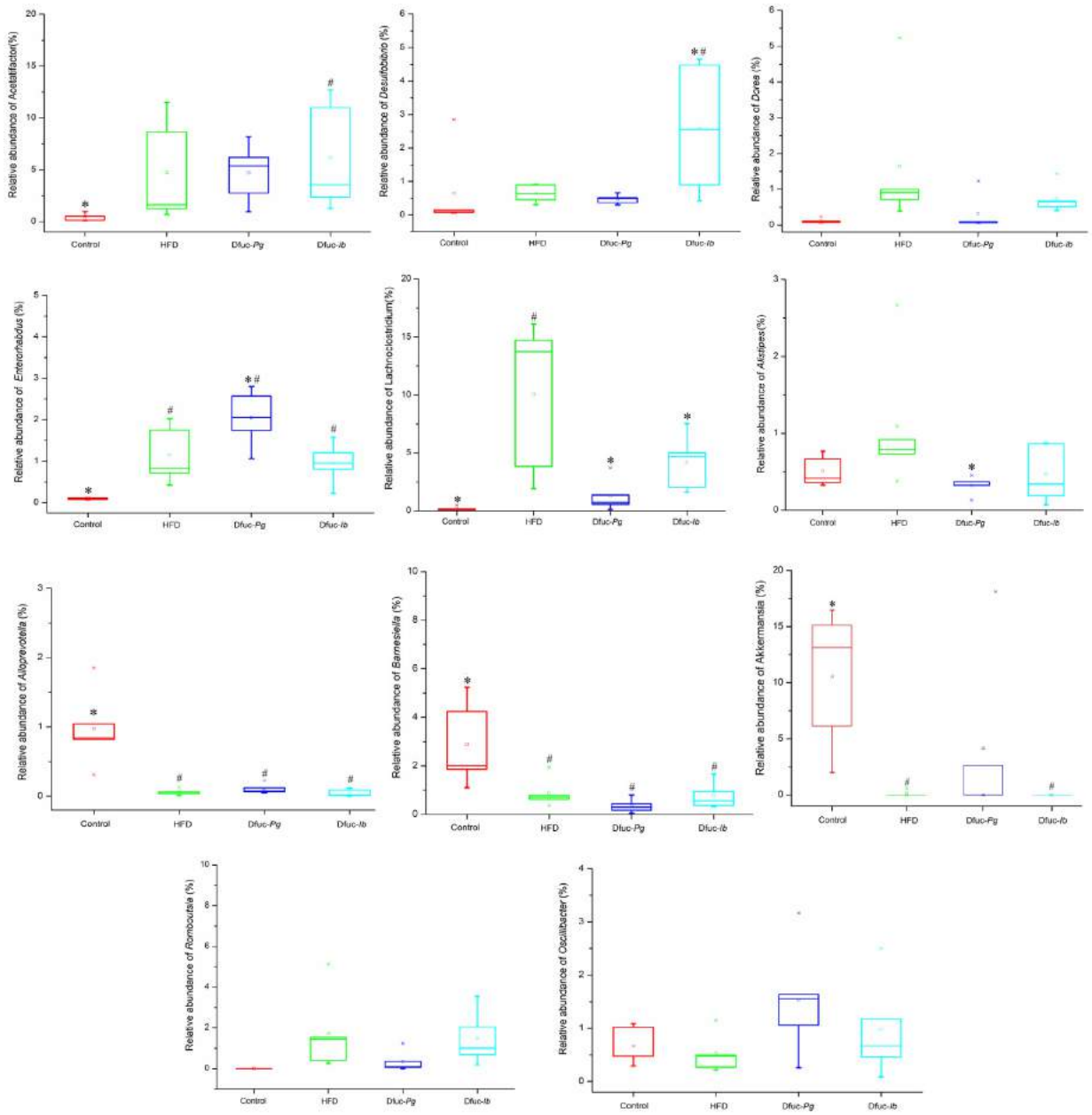



**Fig. 6.** (*continued*)

### 3.4. Fucoidan oligosaccharides attenuated liver steatosis and adipocyte hypertrophy in HFD-induced obese mice.

Histopathological examination of liver tissues showed a normal intact structure of hepatic lobules, round shape of liver cells, and central location of nuclei (Fig. 4a). In the HFD group, the structural disorder of hepatic lobules, fatty degeneration of liver cells within hepatic lobules, mixed fatty degeneration characterized by macrovesicular steatosis, enlargement of liver cell volume, and visible cavitation within the cytoplasm were noted. Compared to the HFD group, the relatively normal structure of hepatic lobules significantly improved fatty degeneration of liver cells in hepatic lobules and less visible necrosis of hepatocytes were noted both in the Dfuc-*Pg* and Dfuc-*Ib* group. Both fucoidan oligosaccharides could protect liver from damages induced by HFD.

In addition, Fig. 4b showed that the adipocyte sizes of the HFD group were much bigger than that of the control group. While, oral administration of both Dfuc-*Pg* and Dfuc-*Ib* could reduce lipid accumulation in adipose tissues, thus preventing adipocyte hypertrophy in HFD-induced obese mice.

### 3.5. Fucoidan oligosaccharides alleviated HFD-induced low-grade inflammation

Chronic inflammation is often a complication accompanying metabolic diseases such as obesity and type 2 diabetes (Gregor & Hotamisligil, 2011). As shown in Fig. 5a and b, there were significant increases in TNF-α and LPS levels in blood of HFD-fed mice compared with those of control mice ($P < 0.05$). Both Dfuc-*Pg* and Dfuc-*Ib* had effects on lowering serum TNF-α and LPS levels ($P < 0.05$, compared

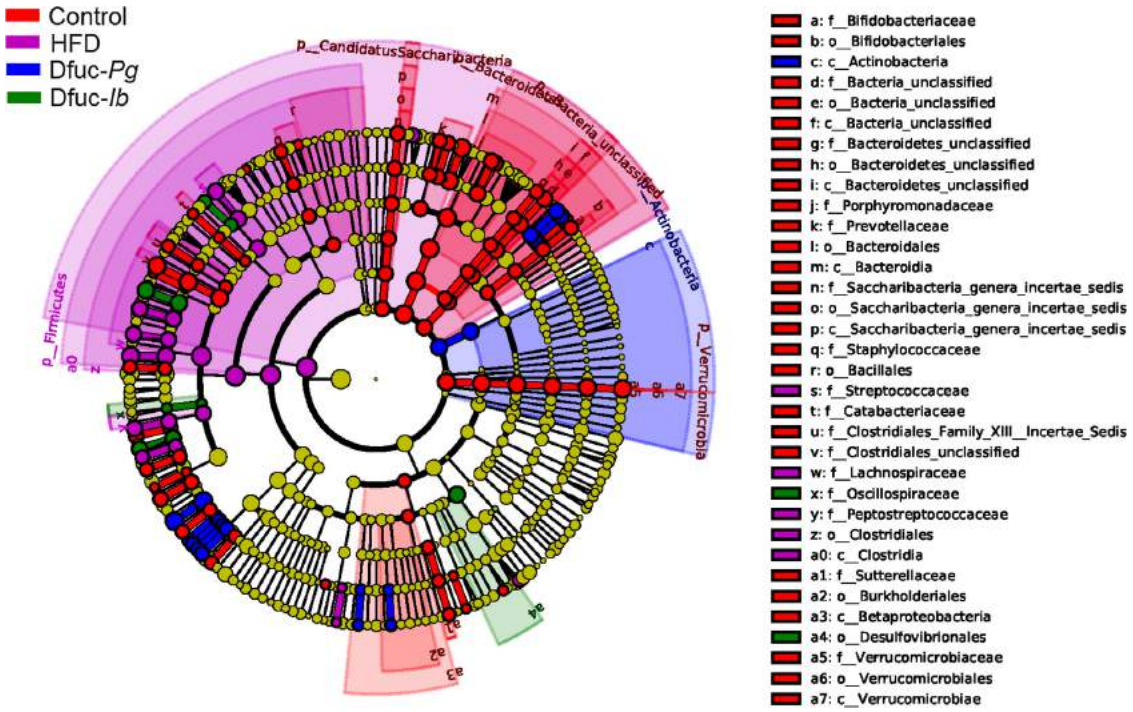



**Fig. 6.** (*continued*)

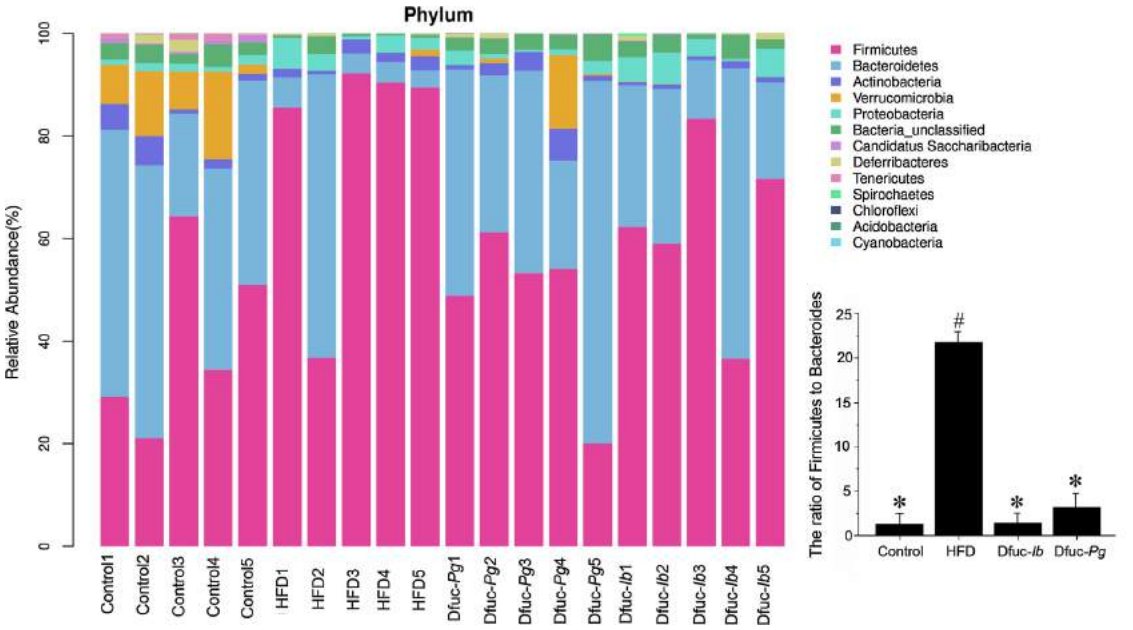



**Fig. 7.** Composition of colonic microbiota in control, HFD, Dfuc-*Pg*, and Dfuc-*Ib* groups at phylum level (a); Box chart of relative abundance of colonic microbiota at the phylum level(b); Composition of colonic microbiota in control, HFD, Dfuc-*Pg*, and Dfuc-*Ib* groups at genus level (c); Box chart of relative abundance of colonic microbiota at genus level(d); Taxonomic cladogram of LEfSe comparison of colonic microbiota of control, HFD, Dfuc-*Pg*, and Dfuc-*Ib* groups (e). The small figure inserted in the (a) was the ratio of Firmicutes to Bacteroidetes, which was expressed as mean ± SD (n = 5). Differences were assessed by ANOVA. $^{*}P < 0.05$: compared with the HFD group. $^{\#}P < 0.05$: compared to the control group.

with the HFD group). Dfuc-*Pg* exhibited better anti-inflammatory activity than Dfuc-*Ib*. The TNF-α and LPS levels in the Dfuc-*Pg* group levels ($P > 0.05$ compared with the control group) were lower than these of the Dfuc-*Ib* group ($P < 0.05$, compared with the control group).

The HFD-induced macrophages infiltration in adipose tissue is another phenomenon indicating inflammation(Bai & Sun, 2015). The macrophages infiltration was marked by CD68, a surface marker of macrophages (Yan et al., 2016). Thus, we performed immunohistochemical staining using antibody against macrophages

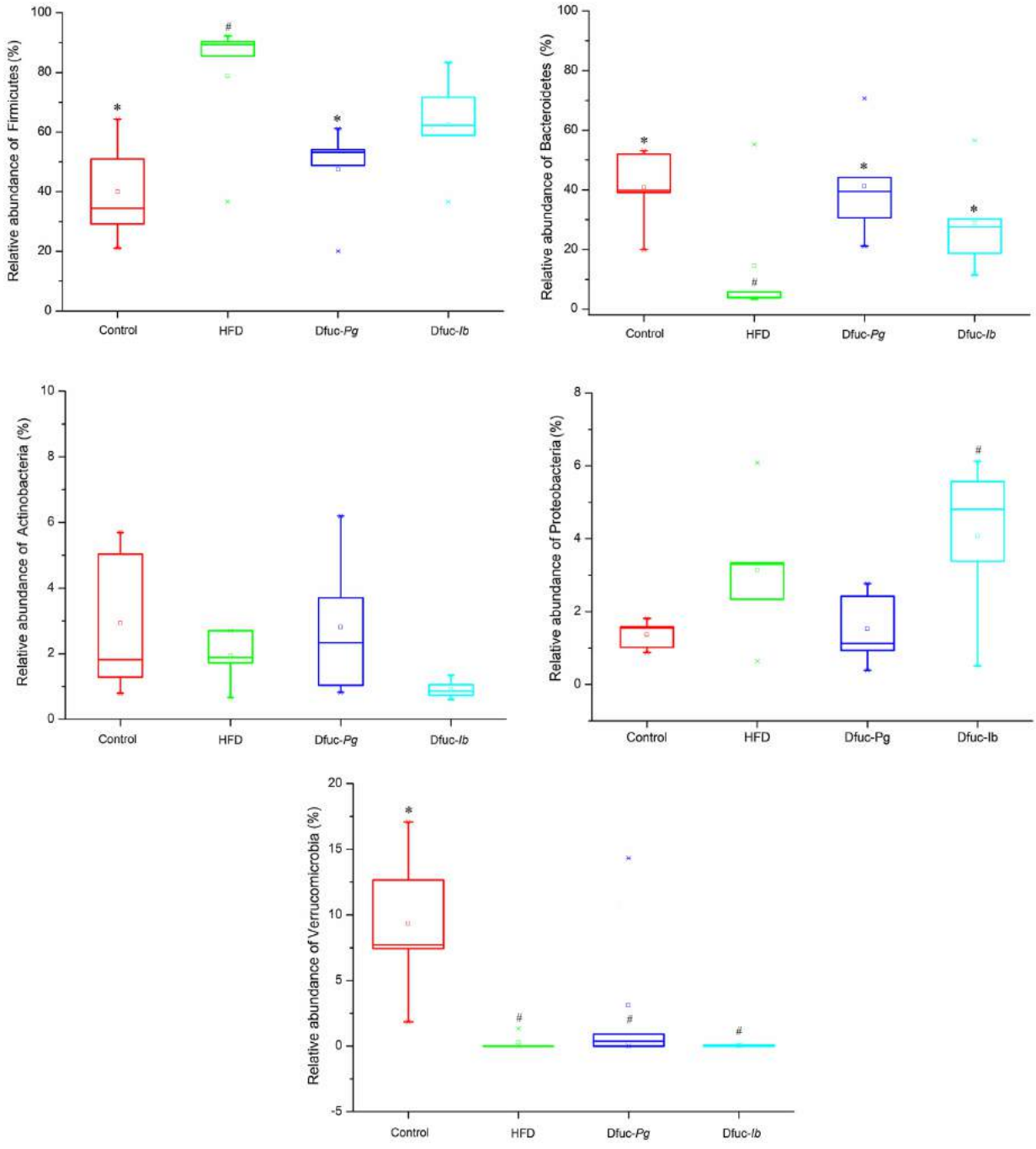



**Fig. 7.** (*continued*)

(CD68) in the fat tissue. CD68-positive staining area in the fat tissue was far more extensive in the HFD-fed mice than in normal control mice (Fig. 5c). Dfuc-*Pg* administration markedly reduced CD68-positive staining area in the fat tissue ($P < 0.05$, compared with the HFD group). However, Dfuc-*Ib* had only a moderate effect on reducing macrophage infiltration into adipose tissue in the HFD-fed mice ($P > 0.05$ compared with the HFD group).

### *3.6. Fucoidan oligosaccharides modulated cecal microbiota in HFD-fed mice*

We examined the effects of fucoidan oligosaccharides on gut microbiota composition by performing analysis of bacterial 16S rRNA (V3–V4 region) in cecal contents. The bacterial diversity and richness of cecal microbiota were shown in the Supplementary Table 3. A reduced observation of species and the Chao1 index of the HFD group indicated that bacterial richness was decreased by HFD ($P < 0.05$, compared to the control group. Treatment with Dfuc-*Pg* and Dfuc-*Ib* exerted almost no influence on these. In addition, the lower Shannon index and

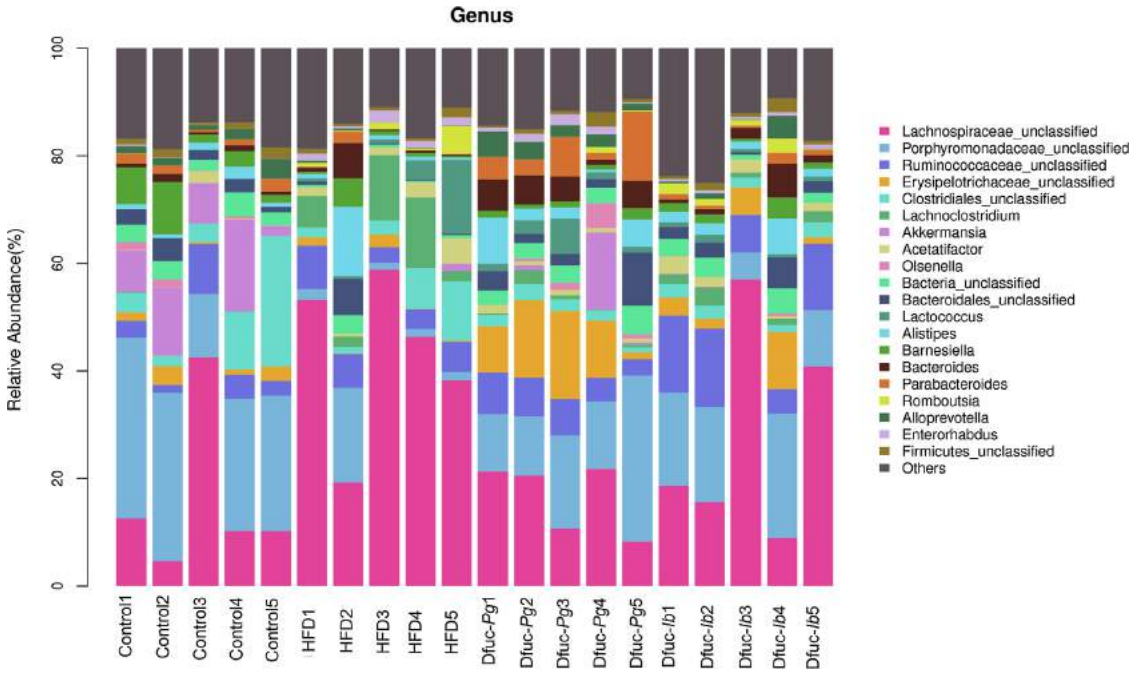



**Fig. 7.** (*continued*)

Simpson index of the HFD group indicated that bacterial diversity was decreased by HFD ($P < 0.05$), compared to the control group. Thus, Dfuc-*Pg* and Dfuc-*Ib* had no effect on cecal bacterial diversity in the HFD-fed mice. UniFrac-based principal coordinates analysis (PCoA) and Nonmetric multidimensional scaling (NMDS) analysis were performed to better understand the composition of gut microbiota in the four groups. The results revealed a distinct clustering of microbiota composition between the control and HFD groups (Supplementary Fig. 2a and b). Meanwhile, supplementation with fucoidan oligosaccharides had the similar cecal microbiota composition with the HFD group. More specific analysis of the cecal microbiota composition was performed at phylum and genus levels.

Firmicutes and Bacteroidetes are predominant phyla in the gut microbiota of mice (Shang et al., 2016). In addition, less abundant bacterial phyla, such as Actinobacteria, Proteobacteria, and Verrucomicrobia (Murphy et al., 2010; Shang et al., 2017), are also present. As shown in the Fig. 6a and b, HFD changed notably in cecal bacterial composition compared with that of the control group, increasing the abundance of Firmicutes ($P < 0.05$) and Proteobacteria ($P > 0.05$), and decreasing the abundance of Bacteroidetes ($P < 0.05$), Actinobacteria ($P > 0.05$), and Verrucomicrobia ($P < 0.05$). Thus, the ratio of Firmicutes to Bacteroidetes was increased by HFD ($P < 0.05$, compared with the control group). Dfuc-*Pg* had no significant effect on the abundance of Firmicutes, Bacteroidetes, and Verrucomicrobia in the cecal microbiota of HFD-fed mice ($P > 0.05$ compared with the HFD group). The abundance of Actinobacteria was significantly increased ($P < 0.05$, compared with the HFD group) while Proteobacteria slightly decreased. Dfuc-*Ib* had no effect on the abundance of Firmicutes, Bacteroidetes, Actinobacteria, and Verrucomicrobia in the HFD-fed mice ($P > 0.05$ compared with the HFD group). However, the abundance of Proteobacteria in the Dfuc-*Ib* was much higher than that of the control group, even more than the HFD group ($P < 0.05$, compared with both control and HFD group).

We further analysed the characterization of the cecal microbiota based on the genus level (Fig. 6c and d). Compared to the control group, the HFD group had higher abundance of *Lachnoclostridium*, *Acetatifactor*, *Alistipes*, *Enterorhabdus*, and lower abundance of *Alloprevotella*, *Barnesiella* and *Akkermansia* than the control group. Among these genus, HFD significantly affected the abundance of *Lachnoclostridium*, *Enterorhabdus*, *Alloprevotella*, and *Barnesiella* ($P < 0.05$, compared with the control group). Oral administration of Dfuc-*Pg* significantly reversed the ratio of *Lachnoclostridium* and *Alistipes* ($P < 0.05$, compared with the HFD group) in HFD-fed mice. Dfuc-*Ib* significantly decreased the abundance of *Alistipes* ($P < 0.05$, compared with the HFD group). However, the abundance of *Desulfovibrio* in the Dfuc-*Ib* was much higher than that of the other three groups ($P < 0.05$), which belongs to Proteobacteria.

LEfSe analyses were used here to identify the specific composition of cecal microbiota in each group (Fig. 6e and Supplementary Fig. 2c). The specific bacterium in the control group was characterized by Bacteroidetes, Bacteroidia, Porphyromonadaceae, Prevotellaceae, Verrucomicrobia, Verrucomicrobiaceae, Akkermansia, *Barnesiella*, *Olsenella*, and *Alloprevotella*. The specific bacterium in the HFD group was characterized by a higher amount of Firmicutes, Lachnospiraceae, Clostridia, Clostridiales, *Lachnoclostridium*, Streptococcaceae, *Lactococcus*, *Romboutsia*, *Dorea*, Desulfovibrionaceae, *Ruminococcus*, and *Acetatifactor*. In the Dfuc-*Pg* group, there was higher abundance of *Allobaculum*, *Olsenella*, and Actinobacteria compared with other three groups. As for Dfuc-*Ib*, it was found higher abundance of Desulfovibrionales, *Acetatifactor*, and *Oscillibacter* compared with other three groups. The LEfSe analysis showed that Dfuc-*Pg* had more powerful effects on reversing cecal microbiota dysbiosis than Dfuc-*Ib*.

### 3.7. Fucoidan oligosaccharides modulated colonic microbiota in HFD-fed mice

It has been reported that the composition of gut microbiota have different space distribution in the mice (Xiaofei & Xuewu, 2015). In this part, colonic microbiota composition of four group was investigated. Similar with the effects of HFD on the cecal microbiota, HFD decreased diversity and richness of cecal microbiota while both fucoidan oligosaccharides had no effect on these indices (Supplementary Table 4). For colonic contents, PCoA and NMDS revealed a distinct clustering of colonic microbiota composition for four groups (Supplementary Fig. 2a

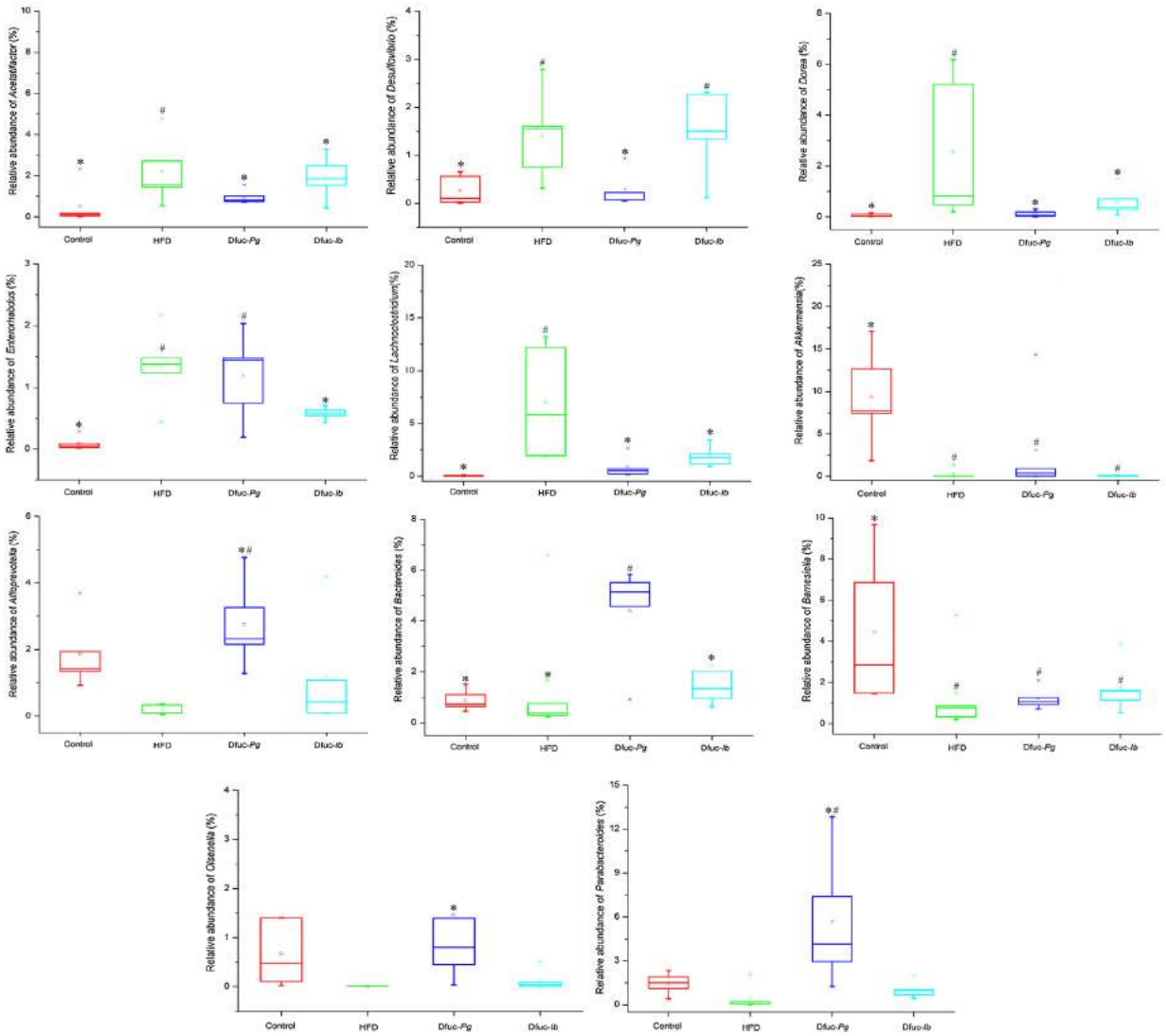



**Fig. 7.** (*continued*)

and b). Like the cecal bacterial composition, HFD changed the colonic bacterial composition compared to the effect of normal chow in the control group. However, fucoidan oligosaccharides had more powerful effects on the composition of colonic microbiota.

HFD increased the abundance of Firmicutes ($P < 0.05$) and Proteobacteria ($P > 0.05$), and decreased the abundance of Bacteroidetes ($P < 0.05$), Actinobacteria ($P > 0.05$), and Verrucomicrobia ($P < 0.05$)) in the colon, compared to those of the control group (Fig. 7a and b). The ratio of Firmicutes to Bacteroidetes was increased by HFD ($P < 0.05$, compared with the control group). Remarkably, Dfuc-*Pg* decreased the abundance of Firmicutes to normal levels in the colonic microbiota of HFD-fed mice while the abundance of Bacteroidetes was increased to normal levels ($P < 0.05$ compared with the HFD group; $P > 0.05$ compared with the control group). The ratio of Firmicutes to Bacteroidetes in HFD-fed was reversed by Dfuc-*Pg* ($P < 0.05$, compared with the HFD group). Dfuc-*Pg* slightly increased the abundance of Actinobacteria while decreased the abundance of Proteobacteria. Dfuc-*Ib* decreased the abundance of Firmicutes and Bacteroidetes ($P < 0.05$ compared with the HFD group), while no effects on the abundance of Actinobacteria and Verrucomicrobia in the HFD-fed mice ($P > 0.05$, compared with the HFD group). The abundance of Proteobacteria in the Dfuc-*Ib* group was also much higher than that of the HFD group ($P < 0.05$ compared with the HFD group).

At genus level in the colon (Fig. 7c and d), the HFD group significantly increased the abundance of *Lachnoclostridium*, *Acetatifactor*, *Enterorhabdus*, *Desulfovibrio* and *Dorea*, and decreased the abundance of *Akkermansia*, *Barnesiella*, *and Olsenella* compared to the control group ($P < 0.05$). Oral administration of Dfuc-*Pg* reversed the ratio of *Lachnoclostridium*, *Acetatifactor*, *Desulfovibrio*, *Dorea*, *Alloprevotella*, and *Olsenella* in HFD-fed mice ($P < 0.05$ compared with the HFD group). The abundance of *Parabacteroides*, *Alloprevotella* and *Bacteroides* was significantly increased by Dfuc-*Pg*, even much higher than the control group ($P < 0.05$ compared with both the control and HFD groups). In the Dfuc-*Ib* group, the abundance of *Lachnoclostridium*, *Acetatifactor*, *Dorea*, *Enterorhabdus*, were restored in the colon of HFD-fed mice ($P < 0.05$ compared with the HFD group). However, the abundance of *Desulfovibrio* was significantly increased by Dfuc-*Ib* ($P < 0.05$ compared with both the control group and HFD group).

The results of LEfSe analysis of four groups were shown in the Fig. 7e and Supplementary Fig. 3c. The specific bacterium in the control group, Bacteroidetes, Bacteroidia, Porphyromonadaceae, *Prevotella*, *Bifidobacterium*, Verrucomicrobia, Verrucomicrobiaceae, *Akkermansia*,

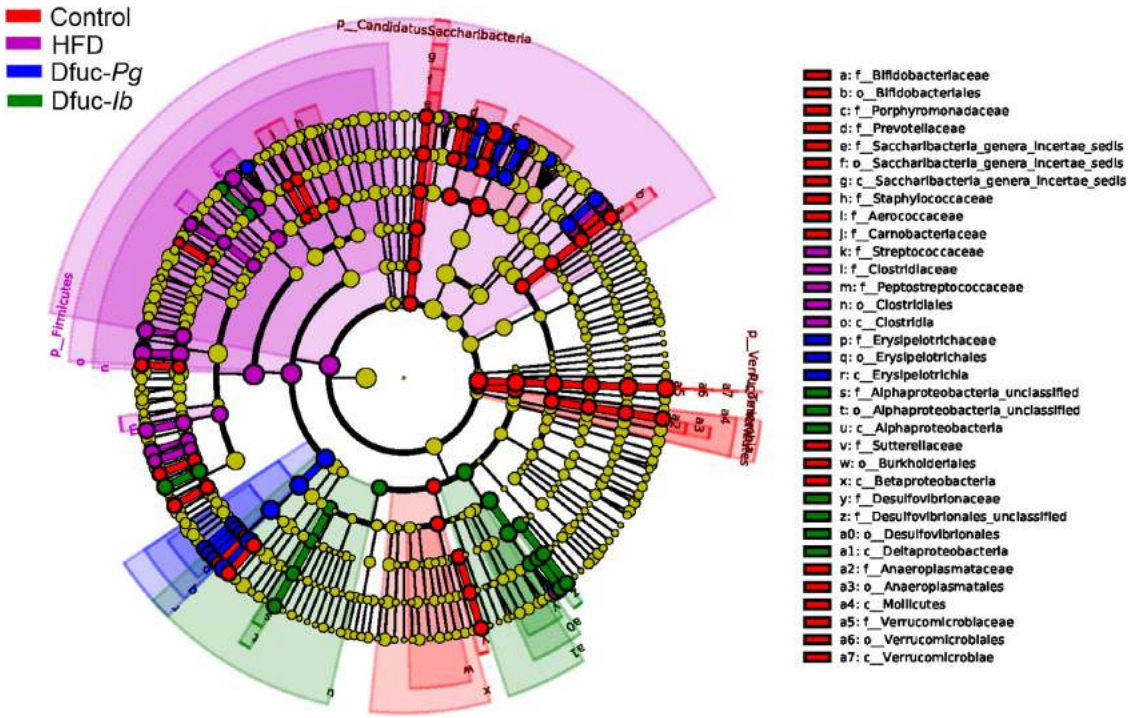



**Fig. 7.** (*continued*)

and Eryipelotrichaceae. The specific bacterium in the HFD group was characterized by a higher amount of Firmicutes, Lachnospiraceae, *Clostridia*, Clostridiales, *Lachnoclostridium*, Streptococcaceae, *Lactococcus*, Desulfovibrionales, Desulfovibrionaceae, *Desulfovibrio*, *Romboutsia*, and *Dorea*. In the Dfuc-*Pg* group, there were higher abundance of Eryipelotrichaceae, *Allobaculum*, *Ruminococcus*, *Olsenella*, *Alloprevotella*, and *Parabacteroides* compared with the other three groups. As for Dfuc-*Ib*, it was found that the abundance of Erysipelotrichaceae, Desulfovibrionales, *Acetatifactor*, *Streptococcus*, and *Oscillibacter* were higher than these of other three groups.

## 4. Discussion

The anti-hyperlipidemia activity of fuc-*Pg* and fuc-*Ib* has been studied in our former work (Li et al., 2017). The fuc-*Pg* dominated with 4-*O*-sulfo fucose branches exert excellent hypolipidemic activity in Sprague Dawley rats fed on HFD, whereas fuc-*Ib* showed only a modest effect. In recent years, fucoidan has been found to improve metabolic syndromes by affecting gut microbiota (Shang et al., 2017). In addition, there might be a structure-dependent relationship between fucoidan and modulation of the gut microbiota (Shang et al., 2016). However, there are limited studies to reveal the effects of fucoidan oligosaccharides on metabolic syndromes and gut microbiota dysbiosis.

In the present study, fucoidan oligosaccharides were found alleviating metabolic syndromes and gut microbiota dysbiosis caused by HFD in mice. Our results showed that the obesity in the HFD-fed mice was alleviated by both fucoidan oligosaccharides. The lower liver weight, fat tissue weight, and smaller adipocyte sizes in the Dfuc-*Pg* and Dfuc-*Ib* groups confirmed the anti-obesity effects of fucoidan oligosaccharides, which may be associated with the altered gut microbiota. The F/B in the colonic contents of HFD-fed mice was decreased sharply to a normal level by both of Dfuc-*Pg* and Dfuc-*Ib*. Higher F/B was found to be associated with obesity in HFD-fed mice (Yan et al., 2016). Fucoidan oligosaccharides decreased the relative abundance of *Lachnoclostridium*, *Acetatifactor* and *Dorea* in the colon of HFD-fed mice. The three genera belong to Firmicutes, which have been reported to be associated with obesity and its metabolic complications. The genus *Lachnoclostridium* within the family Lachnospiraceae is found increasing abundance in obese mice (Zhao et al., 2017) while *Acetatifactor* is a novel bacterium isolated from the intestine of an obese mouse (Pfeiffer et al., 2012). Reduced cardiovascular disease risk in human is associated with decreased *Dorea* (Wang et al., 2016). Meanwhile, our results showed that Dfuc-*Pg* (Fig. 7d) improved the abundance of *Alloprevotella*, *Barnesiella*, *Parabacteroides*, and *Bacteroides*, which belong to Bacteroidetes. Dfuc-*Ib* increased the relative abundance of *Bacteroides* in the HFD-fed mice. *Alloprevotella* is negatively correlated with the serum TG, TC and LDL-C levels but positively correlated with the serum HDL-C levels (Guo et al., 2018). *Bacteroides* are members of the human gut microbiota that confer myriad benefits on their hosts and contribute to carbohydrate utilization (Schwalm & Groisman, 2017). The increased relative abundance of *Bacteroides* in both Dfuc-*Pg* and Dfuc-*Ib* groups indicated that fucoidan oligosaccharides also can be utilized in the colon of mice by *Bacteroides*.

The abundance of Actinobacteria was increased by Dfuc-*Pg* both in the cecal and colon of HFD-fed mice. However, Dfuc-*Ib* had no effects on relative abundance of Actinobacteria. A further analysis found that *Olsenella* genus was responsible for the increased Actinobacteria phylum. *Olsenella* is also part of the normal bacterial flora in the human gut (Zhang et al., 2014). In a former report, fructo-oligosaccharides (FOS) can stimulate the growth of *Olsenella*, which provided a new target for the interactions between FOS and gut bacteria (Mao et al., 2015). The results in the present study showed fucoidan oligosaccharide dominated with 4-*O*-sulfation increased the abundance of *Olsenella*.

Our results indicated that Dfuc-*Pg* administration could effectively improve chronic inflammation in HFD-induced obese mice by reducing serum TNF-α, LPS, and macrophages infiltration into adipose tissue while Dfuc-*Ib* had a weaker effect. The presence of a 4-*O*-sulfo group substitution pattern of fucoidan showed more powerful effects on anti-inflammatory activity. Notably, Dfuc-*Ib* was found to increase the abundance of *Desulfovibrio* (belonging to Desulfovibrionaceae and Proteobacteria), both in the cecum and colon of HFD-fed mice.

*Proteobacteria* expansion is a microbial signature of colonic epithelial dysfunction (Litvak, Byndloss, Tsolis, & Bäumler, 2017). The family Desulfovibrionaceae is a potentially important group of LPS producers (Xiao et al., 2014). An increment of *Desulfovibrio* is associated with inflammatory bowel diseases (Berry & Reinisch, 2013). However, the side effects of Dfuc-*Ib* can be neutralized by its other beneficial effects on gut microbiota.

Taken together, these data suggest that both fucoidan oligosaccharides had effects on alleviating obesity and its metabolic complications, such as hyperlipidemia, hyperglycemia, and inflammation in the HFD-fed mice. Further studies indicated that the functional effects of fucoidan oligosaccharides may be associated with their effects on altering gut microbiota. In the present study, both cecal and colonic microbiota in mice had been investigated by 16 s DNA. The results indicated that fucoidan oligosaccharides mainly altered the colonic microbiota. However, former studies have indicated that fucoidans could be absorbed by intestinal tract in rats but the absorption efficiency is very low (Nagamine, Nakazato, Tomioka, Iha, & Nakajima, 2014). In addition, the fucoidan with lower molecular weight has much better absorption and bioavailability than the high-molecular-weight fucoidan (Zhao et al., 2016). In our study, we did not perform the absorption of fucoidan oligosaccharides. Considering the low molecular of fucoidan oligosaccharides in used in the present work, further study should be confirmed whether fucoidan oligosaccharides can be absorbed by small intestine and their effects on inner metabolism.

## 5. Conclusions

The present study demonstrated that fucoidan oligosaccharides can alleviate metabolic syndromes such as hyperlipidemia, obesity, and inflammation in HFD-fed mice. Meanwhile, fucoidan oligosaccharides modulate both cecal microbiota and colonic microbiota in the HFD-fed mice, and their main acting location was colon. Both of the two oligosaccharides can reverse the ratio of *Firmicutes* and *Bacteroidetes* in the colonic microbiota, suggest their potential therapeutic application. In addition, the sulfation pattern represents a structural factor that modulates gut microbiota. The combined results of this study suggest that Dfuc-*Pg,* dominated by 4-*O*-sulfo groups, is a better agent for reversing metabolic syndromes and gut microbiota dysbiosis in the HFD-fed mice. Dfuc-*Ib,* with more 2-*O*-sulfo groups, alleviates metabolic syndromes and gut microbiota dysbiosis. However, Dfuc-*Ib* increased bacterium associated with inflammation. The security of Dfuc-*Ib* as functional food needs further investigated.

## Conflict of interest

The authors have declared no conflicts of interest for the article: Effect of the sulfation pattern of sea cucumber-derived fucoidan oligosaccharides on modulating metabolic syndromes and gut microbiota dysbiosis caused by HFD in mice.

## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2019.02.001.

## References


Ale, M. T., & Meyer, A. S. (2013). Fucoidans from brown seaweeds: An update on structures, extraction techniques and use of enzymes as tools for structural elucidation. *RSC Advances, 3*(22), 8131–8141.

Arora, T., & Backhed, F. (2016). The gut microbiota and metabolic disease: Current understanding and future perspectives. *Journal of Internal Medicine, 280*(4), 339–349.

Bai, Y., & Sun, Q. (2015). Macrophage recruitment in obese adipose tissue. *Obesity Reviews, 16*(2), 127–136.

Berry, D., & Reinisch, W. (2013). Intestinal microbiota: A source of novel biomarkers in inflammatory bowel diseases? *Best Practice & Research Clinical Gastroenterology, 27*(1), 47–58.

Boulangé, C. L., Neves, A. L., Chilloux, J., Nicholson, J. K., & Dumas, M.-E. (2016). Impact of the gut microbiota on inflammation, obesity, and metabolic disease. *Genome Medicine, 8*(1), 42.

Caesar, R., Tremaroli, V., Kovatchevadatchary, P., Cani, P. D., & Bäckhed, F. (2015). Crosstalk between gut microbiota and dietary lipids aggravates WAT inflammation through TLR signaling. *Cell Metabolism, 22*(4), 658–668.

Caporaso, J. G., Bittinger, K., Bushman, F. D., Desantis, T. Z., Andersen, G. L., & Knight, R. (2010b). PyNAST. *Bioinformatics*.

Caporaso, J., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F., Costello, E., ... Knight, R. (2010a). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods, 7*(5), 335–336.

Chen, S. G., Hu, Y. Q., Ye, X. Q., Li, G. Y., Yu, G. L., Xue, C. H., & Chai, W. G. (2012). Sequence determination and anticoagulant and antithrombotic activities of a novel sulfated fucan isolated from the sea cucumber *Isostichopus badionotus*. *Biochimica Et Biophysica Acta-General Subjects, 1820*(7), 989–1000.

Chen, Y., Zhang, X., Cheng, L., Zheng, X., & Zhang, Z. (2018). The evaluation of the quality of Feng Huang Oolong teas and their modulatory effect on intestinal microbiota of high-fat diet-induced obesity mice model. *International Journal of Food Sciences & Nutrition, 69*(7), 842–856.

Cummings, J. H., Mann, J. I., Nishida, C., & Vorster, H. H. (2009). Dietary fibre: An agreed definition. *Lancet, 373*(9661), 365–366.

De Filippo, C., Cavalieri, D., Di Paola, M., Ramazzotti, M., Poullet, J. B., Massart, S., ... Lionetti, P. (2010). Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proceedings of the National Academy of Sciences of the United States of America, 107*(33), 14691–14696.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry, 28*(3), 350–356.

Evans, C. C., LePard, K. J., Kwak, J. W., Stancukas, M. C., Laskowski, S., Dougherty, J., ... Ciancio, M. J. (2014). Exercise prevents weight gain and alters the gut microbiota in a mouse model of high fat diet-induced obesity. *Plos One, 9*(3).

Gregor, M. F., & Hotamisligil, G. S. (2011). Inflammatory mechanisms in obesity. *Annual Review of Immunology, 29*(1), 415–445.

Guo, W., Pan, Y., Li, L., Li, T., Liu, B., & Lv, X. (2018). Ethanol extract of Ganoderma lucidum ameliorates lipid metabolic disorders and modulates the gut microbiota composition in high-fat diet fed rats. *Food Funct, 9*(6), 3419–3431.

Hartley, L., May, M. D., Loveman, E., Colquitt, J. L., & Rees, K. (2016). Dietary fibre for the primary prevention of cardiovascular disease. *Cochrane Database of Systematic Reviews, 1*.

Hu, Y. Q., Li, S., Li, J. H., Ye, X. Q., Ding, T., Liu, D. H., ... Chen, S. G. (2015). Identification of a highly sulfated fucoidan from sea cucumber *Pearsonothuria graeffei* with well-repeated tetrasaccharides units. *Carbohydrate Polymers, 134*, 808–816.

Koliada, A., Syzenko, G., Moseiko, V., Budovska, L., Puchkov, K., Perederiy, V., ... Tkach, S. (2017). Association between body mass index and Firmicutes/Bacteroidetes ratio in an adult Ukrainian population. *BMC Microbiology, 17*(1), 120.

Leung, C., Rivera, L., Furness, J. B., & Angus, P. W. (2016). The role of the gut microbiota in NAFLD. *Nature Reviews Gastroenterology & Hepatology, 13*(7), 412–425.

Li, S., Li, J., Zhi, Z., Wei, C., Wang, W., Ding, T., ... Chen, S. (2017). Macromolecular properties and hypolipidemic effects of four sulfated polysaccharides from sea cucumbers. *Carbohydrate Polymers, 173*, 330–337.

Li, X., Watanabe, K., & Kimura, I. (2017). Gut microbiota dysbiosis drives and implies novel therapeutic strategies for diabetes mellitus and related metabolic diseases. *Frontiers in Immunology,* 8.

Litvak, Y., Byndloss, M. X., Tsolis, R. M., & Bäumler, A. J. (2017). Dysbiotic Proteobacteria expansion: A microbial signature of epithelial dysfunction. *Current Opinion in Microbiology, 39*, 1–6.

Liu, W., Zheng, Y., Zhang, Z., Yao, W., & Gao, X. (2014). Hypoglycemic, hypolipidemic and antioxidant effects of Sarcandra glabra polysaccharide in type 2 diabetic mice. *Food & Function, 5*(11), 2850–2860.

Mao, B., Li, D., Zhao, J., Liu, X., Gu, Z., Chen, Y. Q., ... Chen, W. (2015). Metagenomic insights into the effects of Fructo-oligosaccharides (FOS) on the composition of fecal microbiota in mice. *Journal of Agricultural & Food Chemistry, 63*(3), 856–863.

Moolhuijzen, P. M., Manners, J. M., Wilcox, S. A., Bellgard, M. I., & Gardiner, D. M. (2013). Genome sequences of six wheat-infecting fusarium species isolates. *Genome Announc, 1*(5).

Murphy, E. F., Cotter, P. D., Healy, S., Marques, T. M., O'Sullivan, O., Fouhy, F., ... Stanton, C. (2010). Composition and energy harvesting capacity of the gut microbiota: Relationship to diet, obesity and time in mouse models. *Gut, 59*(12), 1635–1642.

Nagamine, T., Nakazato, K., Tomioka, S., Iha, M., & Nakajima, K. (2014). Intestinal absorption of fucoidan extracted from the brown seaweed Cladosiphon okamuranus. *Marine Drugs, 13*(1), 48–64.

Pfeiffer, N., Desmarchelier, C., Blaut, M., Daniel, H., Haller, D., & Clavel, T. (2012). Acetatifactor muris gen. nov., sp. nov., a novel bacterium isolated from the intestine of an obese mouse. *Archives of Microbiology, 194*(11), 901–907.

Ramnani, P., Chitarrari, R., Tuohy, K., Grant, J., Hotchkiss, S., Philp, K., ... Rowland, I. (2012). In vitro fermentation and prebiotic potential of novel low molecular weight polysaccharides derived from agar and alginate seaweeds. *Anaerobe, 18*(1), 1–6.

Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahé, F. (2016). VSEARCH: A versatile open source tool for metagenomics. *Peerj, 4*(10), e2584.

Schwalm, N., & Groisman, E. (2017). Navigating the gut buffet: Control of polysaccharide utilization in Bacteroides spp. *Trends in Microbiology, 25*(12), 1005–1015.

Shang, Q. S., Jiang, H., Cai, C., Hao, J. J., Li, G. Y., & Yu, G. L. (2018). Gut microbiota fermentation of marine polysaccharides and its effects on intestinal ecology: An overview. *Carbohydrate Polymers, 179*, 173–185.

Shang, Q., Shan, X., Cai, C., Hao, J., Li, G., & Yu, G. (2016). Dietary fucoidan modulates

the gut microbiota in mice by increasing the abundance of Lactobacillus and Ruminococcaceae. *Food & Function, 7*(7), 3224–3232.
Shang, Q., Song, G., Zhang, M., Shi, J., Xu, C., Hao, J., ... Yu, G. (2017). Dietary fucoidan improves metabolic syndrome in association with increased Akkermansia population in the gut microbiota of high-fat diet-fed mice. *Journal of Functional Foods, 28*, 138–146.
Sonnenburg, J. L., & Bäckhed, F. (2016). Diet–microbiota interactions as moderators of human metabolism. *Nature, 535*, 56–64.
Tanja, M., & Salzberg, S. L. (2011). FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics, 27*(21), 2957–2963.
Wang, Y., Ames, N. P., Tun, H. M., Tosh, S. M., Jones, P. J., & Khafipour, E. (2016). High molecular weight barley β-glucan alters gut microbiota toward reduced cardiovascular disease risk. *Frontiers in Microbiology, 7*, 129.
Wei, P., Lane, P. H., Lane, J. T., Padanilam, B. J., & Sansom, S. C. (2004). Glomerular structural and functional changes in a high-fat diet mouse model of early-stage Type 2 diabetes. *Diabetologia, 47*(9), 1541–1549.
Winzell, M., & Ahrén, B. (2004). The high-fat diet-fed mouse: A model for studying mechanisms and treatment of impaired glucose tolerance and type 2 diabetes. *Diabetes, 53*(Suppl 3), S215–S219.
Wolters, M., Ahrens, J., Romaní-Pérez, M., Watkins, C., Sanz, Y., Benítez-Páez, A., ... Günther, K. (2018). Dietary fat, the gut microbiota, and metabolic health – A systematic review conducted within the MyNewGut project. *Clinical Nutrition*.
Xiao, S. M., Fei, N., Pang, X. Y., Shen, J., Wang, L. H., Zhang, B. R., ... Zhao, L. P. (2014). A gut microbiota-targeted dietary intervention for amelioration of chronic inflammation underlying metabolic syndrome. *Fems Microbiology Ecology, 87*(2), 357–367.
Xiaofei, X., & Xuewu, Z. (2015). Lentinula edodes-derived polysaccharide alters the spatial structure of gut microbiota in mice. *PLOS One, 10*(1), e0115037.
Yan, C., Zou, S., Hui, X., Li, M., Zan, T., Min, X., & Xu, X. (2016). Hypoglycemic activity of the Baker's yeast β-glucan in obese/type 2 diabetic mice and the underlying mechanism. *Molecular Nutrition & Food Research, 60*(12), 2678–2690.
Zhang, J., Guo, Z., Lim, A., Zheng, Y., Koh, E., Ho, D., ... Zhang, H. (2014). Mongolians core gut microbiota and its correlation with seasonal dietary changes. *Scientific Reports, 4*, 5001.
Zhao, X., Guo, F., Hu, J., Zhang, L., Xue, C., Zhang, Z., & Li, B. (2016). Antithrombotic activity of oral administered low molecular weight fucoidan from *Laminaria Japonica*. *Thrombosis Research, 144*, 46–52.
Zhao, L., Zhang, Q., Ma, W., Tian, F., Shen, H., & Zhou, M. (2017). A combination of quercetin and resveratrol reduces obesity in high-fat diet-fed rats by modulation of gut microbiota. *Food & Function, 8*, 4644–4656.