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## A fucoidan from sea cucumber *Pearsonothuria graeffei* with well-repeated structure alleviates gut microbiota dysbiosis and metabolic syndromes in HFD-fed mice†

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A high-fat diet (HFD) has been a major contributor to increasing morbidity caused by metabolic syndromes. Functional foods from natural sources are potential choices for addressing metabolic diseases because they provide many health benefits with a low level of adverse side effects. In our former reports, fucoidan from *Pearsonothuria graeffei* (fuc-Pg), a type of sulfated polysaccharides with a repeating structure, was shown to be a potential functional food ingredient. In this work, we investigated the effects of fuc-Pg on gut microbiota dysbiosis and metabolic syndromes caused by HFD. Our results indicated that fuc-Pg could reduce weight gains, alleviate hyperlipidemia, and protect the liver from steatosis in HFD-fed mice. Meanwhile, fuc-Pg decreases serum inflammatory cytokines and reduces macrophages infiltrating into adipose tissue. The gut microbiota dysbiosis caused by HFD was alleviated by administration of fuc-Pg, mainly working in the colon. Fuc-Pg increased abundances of Bacteroidetes and Actinobacteria while decreased Firmicutes and Proteobacteria. Our results indicated that fuc-Pg could be a functional food for gut microbiota dysbiosis and metabolic syndromes.

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### 1. Introduction

In recent years, the importance of gut microbiota has been emphasized by researchers. Many metabolic disorders are supposed to be related with quantitative and qualitative changes in gut microbiota composition and function, such as inflammatory bowel diseases, obesity, non-alcoholic fatty liver disease (NAFLD), diabetes, and cardiovascular disease.<sup>1,2</sup> Taxonomic composition of the gut microbiota varies greatly among individuals, due to both intrinsic and extrinsic factors (physical activity, medication, cultural habits, diet, etc.).<sup>3</sup> Diet has been one of most important factors that affect gut microbiota.<sup>4</sup> Many researchers report that a high-fat diet (HFD) can disturb the balance of the gut microbiota.<sup>5,6</sup> A gut microbiota impairment is known as dysbiosis. Bacteroidetes, Firmicutes, Proteobacteria and Actinobacteria are the main phyla in the gut microbiota and 90% of gut microbiota belong to

Bacteroidetes and Firmicutes.<sup>7</sup> HFD consumption generally leads to a decrease in Bacteroidetes and an increase in Firmicutes, alterations that have been associated with obesity and subsequent development of chronic diseases.<sup>8</sup> A higher ratio of Firmicutes to Bacteroidetes (F/B) has been associated with an improved capacity for energy harvest and storage, enhanced gut permeability and inflammation.<sup>9,10</sup> In recent years, gut microbiota has even been considered as a therapeutic target for metabolic disorders.<sup>11</sup>

Dietary polysaccharides that escape digestion in the small intestine and reach the large bowel where they are available for fermentation appear especially important for health.<sup>12</sup> Short chain fatty acids (SCFAs) are the products of polysaccharides fermented by bacteria, which may be the primary means by which benefit occurs.<sup>13</sup> In recent years, several researchers have reported that polysaccharides from food sources prevent gut microbiota dysbiosis and metabolic syndromes caused by diets.<sup>14–16</sup> Polysaccharides extracted from fuzhuan brick tea could increase microbiota phylogenetic diversity and reduce the relative amounts of Erysipelotrichaceae, Coriobacteriaceae, and Streptococcaceae, which are related to metabolic disorders.<sup>15</sup> Ganoderma lucidum polysaccharides could improve insulin sensitivity by alleviating inflammation and regulating gut microbiota composition.<sup>14</sup> Dietary fucoidans from algae improved metabolic syndromes in association with an

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increased abundance of benign microbes, such as *Akkermansia* and *Alloprevotella*.<sup>16</sup>

In our recent study, the structure of fucoidan from sea cucumber *Pearsonothuria graeffei* (fuc-Pg) was identified by a combination of nuclear magnetic resonance and mass spectrometry.<sup>17</sup> Fuc-Pg has been proved to be a novel sulfated fucoidan with a tetrasaccharide repeating unit. The molar ratio of fucose to sulfate is 1 : 0.8 in fuc-Pg. This fucoidan contains 4-*O*-mono- and 2,4-di-*O*-sulfated groups, which are important for its functional activities. Fuc-Pg exerts powerful effects in terms of reducing obesity and improving lipid profile, due to its 4-*O*-sulfation and random coil conformation.<sup>18,19</sup> In the present work, we investigated the effects of fuc-Pg on gut microbiota dysbiosis and metabolic syndromes caused by HFD. Meanwhile, the effect of fuc-Pg on the spatial structure of gut microbiota is also included in our study.

## 2. Materials and methods

### 2.1 Materials

Sea cucumber *Pearsonothuria graeffei* were purchased from a local market in Qingdao, Shandong, China. Q Sepharose Fast Flow anion-exchange resin was purchased from GE Healthcare, USA. Papain and cysteine were purchased from Fluka (Seelze, Germany).

### 2.2 Isolation and purification of fuc-Pg

Crude sea cucumber fucoidan was prepared based on a previously described method with some modifications.<sup>20,21</sup> Briefly, the dry sea cucumber body wall (*ca.* 100 g) was minced and homogenized. The homogenate was digested with papain at 60 °C for 10 h in a solution containing 5 mM EDTA and 5 mM cysteine, and then subjected to centrifugation (2000g, for 15 min at 4 °C). Polysaccharides in the clear supernatant fractions were precipitated with 160 mL of 10% cetylpyridinium chloride solution. After incubation at room temperature for 24 h, the mixture was centrifuged (2000g for 15 min). The precipitated sulphated polysaccharide was dissolved with 1000 mL of 3 M NaCl : Ethanol (100 : 15, v/v) solution and then 600 mL of 95% ethanol were added to precipitate fCS-Pg (chondroitin sulfate from *Pearsonothuria graeffei*). After centrifugation (2000g, 15 min) and removal of the precipitate, another 900 mL of ethanol was added to the supernatant for a final concentration of 60%. The precipitate formed was collected by centrifugation (2000g, 15 min) and dissolved in water before dialysis against water for 24 h. The retained solution was lyophilized and crude fucoidan was obtained.

The crude fucoidan 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<sup>-1</sup>. Carbohydrate fractions were collected every 6 min with a test tube. Polysaccharide content was determined by the improved phenol-sulfuric acid method at 490 nm.<sup>22</sup> The purified polysaccharide was collected, dialyzed, and lyophilized. Then, the

purity of fucoidan was analyzed by high performance liquid chromatography (HPLC) with a Waters Ultrahydrogel 2000 column (2000 Å, 7.8 × 300 mm). As shown in the ESI Fig. 1,† the purified fucoidan was only a narrow peak on the gel filtration chromatography.

### 2.3 Animals and experimental design

Forty eight-week old 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) with a 12 h light cycle. They were fed with a commercial chow for a week to acclimatize to animal facilities, then weighed and randomly divided into four groups of 10 mice. One group was fed regular chow (the control group); one group receiving HFD served as the negative control; two groups receiving HFD were given fuc-Pg in doses of 20 and 80 mg kg<sup>-1</sup> and were named as fuc-Pg-L and fuc-Pg-H respectively. The HFD in this study contained 60% kcal% fat and its composition was shown in the ESI Table 1.† A certain amount of fucoidan according to the dosages of 20 and 80 mg kg<sup>-1</sup> was dissolved in 0.2 mL distilled water and administered orally by metallic gavage needle. The mice had free access to food and water during the experimental period. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Zhejiang University and approved by the Animal Ethics Committee of Zhejiang University with approval number ZJU20181160.

### 2.4 Biochemical analysis of serum indices

At the end of the experimental period (6 weeks), the mice were fasted for 24 h, weighed and anesthetized. Their livers and fat tissues were quickly removed and weighed. Blood samples were collected from the eyeballs for following analysis: levels of serum lipids including total cholesterol (TC), triglyceride (TG) and low density lipoprotein cholesterol (LDL-C) levels were measured enzymatically by assay kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China) according to the manufacturer's instructions. Fasting blood-glucose level was also measured enzymatically by assay kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China). HOMA-IR (model assessment of insulin resistance) was calculated by fasting blood glucose (mmol L<sup>-1</sup>) × fasting insulin (mIU L<sup>-1</sup>)/22.5. The TNF-α, LPS, and insulin contents were measured using commercial ELISA kits (R&D Systems, USA) according to the manufacturer's instructions.

### 2.5 Liver and epididymal fat histology

After the blood samples were gathered, the liver and epididymal fat tissue samples were collected. Tissues were fixed with neutral formalin solution for 48 h, dehydrated through ascending grades of alcohol, cleared in benzene and embedded in low melting point paraffin wax. Sections, 3 μm thick, were cut and stained with hematoxylin and eosin (HE staining) for light microscopic examinations.

## 2.6 Immunohistochemistry

The paraffin sections of visceral adipose from mice were subjected to deparaffination, antigen retrieval, endogenous peroxidase activity blocking. Thereafter, slides were incubated with CD68 primary antibody (Santacruz Biotechnology Inc., USA) and horseradish peroxidase (HRP)-conjugated secondary antibody. After 3,3-diaminobenzidine (DAB) immunostaining, harris hematoxylin counterstaining, dehydration and coverslipping, 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 Real-time quantitative PCR

Total RNA was isolated using a total tissue TRIzol® Plus RNA Purification Kit (Invitrogen, USA). Equal amounts of total RNA were used to synthesize cDNA with the Quant II fast RT kit (Tools, Taiwan). Quantitative real-time reverse-transcription PCR (qRT-PCR) was performed in triplicate using SYBR Green, 384-well plates and the CFX384 Touch Real-Time PCR System (Bio-Rad, USA). Each well was loaded with a total of 20 µl containing 1 µl of cDNA, 1 µl of target primers, and 8 µl of SDW (sterilized deionized water) and 10 µl of Power SYBR® Green Master Mix. Hot-start PCR was performed for 40 cycles, with each cycle consisting of denaturation for 15 s at 94 °C, annealing for 30 s at 60 °C and elongation for 30 s at 72 °C. The primers of CD68, forward: 5' TGTCTGATCTTGCTAGGACCG 3', Reverse: 5' GAGAGTAACGGCCTTTTGTGA 3'. The housekeeping gene 18S RNA was used as normalization. The primers of 18S RNA, forward: 5' CGGACACGGACAGGATTGACA 3', Reverse: 5' CCAGACAAATCGCTCCACCACTA 3'. PCR products were quantitated using the software iCycler iQ5 (Bio-Rad, USA). The mRNA relative expression levels were expressed as the ratio of signal intensity for the target genes to that of the housekeeping gene.<sup>23</sup>

## 2.8 16S rRNA gene analysis

After the blood samples were gathered, the cecum and colonic contents were collected. All contents were frozen in liquid nitrogen immediately and then were stored in the -80 degree centigrade. Five samples of every group were selected randomly for 16S rRNA analysis. DNA was extracted from the cecal and colon solid 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. Nuclear-free water was used for blank. The total DNA was eluted in 50 µL of elution buffer and stored at -80 °C until measurement in the PCR by LC-Bio Technology Co., Ltd. The 16S rRNA gene (V3 and V4 region) from cecum and colon microbiota was amplified using the universal primers as follows: 319F (5'-ACTCCTACGGGAGCAG-3') and 806R (5'-GGACTACCAGGGTATCTAAT-3').<sup>24</sup> The PCR reactions were carried out in an ABI GeneAmp PCR System 9700 (Applied Biosystems, Foster City, USA). The 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 by Shanghai Majorbio Bio-pharm Biotechnology Co., Ltd (Shanghai, China).

Samples were sequenced on an Illumina MiSeq platform according to the manufacturer's recommendations, provided by LC-Bio. 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. Quality filtering on the raw tags were performed under specific filtering conditions to obtain the high-quality clean tags according to the FastQC (V 0.10.1). Chimeric sequences were filtered using Verseach software (v2.3.4). Sequences with  $\geq 97\%$  similarity were assigned to the same operational taxonomic units (OTUs) by Verseach (v2.3.4). 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 to study phylogenetic relationship of different OTUs. Information on OTUs' abundance was normalized using a standard of sequence number corresponding to the sample with the least sequences. Alpha diversity is applied in analyzing the complexity of species diversity for a sample through 4 indices, including Chao1, Shannon, Simpson and Observed species.<sup>25</sup> All indices in our samples were calculated with QIIME (Version 1.8.0). Beta diversity analysis was used to evaluate differences of samples in species complexity. Beta diversity was calculated by principal coordinates analysis (PCoA) and cluster analysis by QIIME software (Version 1.8.0). 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. The Kruskal-Wallis rank sum test was applied to detect features that were significantly different between assigned taxa and the LDA was used to quantify the effect size of each feature. A significance alpha value of less than 0.05 and an effect size threshold of 2 were used for this analysis.

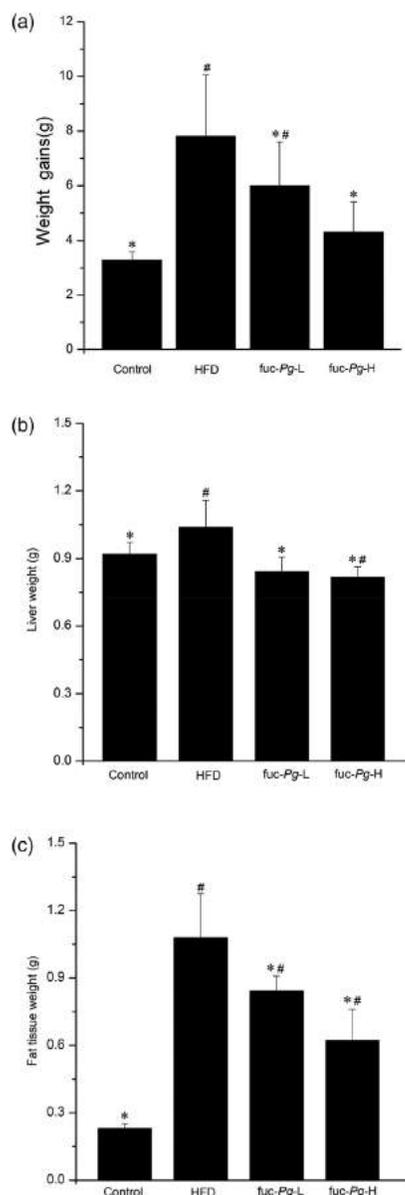
## 2.9 Statistical analysis

Data are expressed as means  $\pm$  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 variance, and then the means were compared by using Tukey's test. Confidence levels for statistical significance were set at  $P < 0.05$ .

# 3 Results

## 3.1 Fuc-Pg alleviated obesity caused by HFD

After six weeks of feeding, the HFD group gained more body weight (Fig. 1a), liver weight (Fig. 1b), and fat tissues weight (Fig. 1c) compared with the control group ( $P < 0.05$ ). When supplemented with fuc-Pg, lower body weight, liver weight,



**Fig. 1** The effects of fucoidan on weight gains (a), liver weight (b) and fat tissue weight (c) in the HFD-fed mice. All data are expressed as mean  $\pm$  SD ( $n = 8$ ). \* $P < 0.05$ : compared with HFD group. # $P < 0.05$ : compared to normal control group.

and fat tissues weight were observed in the fuc-Pg groups ( $P < 0.05$ , compared with HFD group). Meanwhile, fuc-Pg showed a dose-dependent effect on reducing obesity. The fuc-Pg-H group had a lower weight gain than the fuc-Pg-L group.

### 3.2 Fuc-Pg inhibited hyperlipidemia and hyperglycaemia in HFD-fed mice

As shown in the Table 1, HFD-fed mice had higher serum TC, LDL-C, and TG levels than the control group ( $P < 0.05$ ), indicat-

ing hyperlipidemia. Fuc-Pg intervention significantly decreased serum TC, LDL-C, and TG levels in the HFD-fed mice ( $P < 0.05$ , compared with the HFD group). Remarkably, the serum glucose level in the HFD group was much higher than the control group ( $7.30 \pm 1.42$  vs.  $2.90 \pm 0.53$ ,  $P < 0.05$ ). Although HFD significantly increased the serum insulin level ( $P < 0.05$ ), there was no great disparity between the HFD and control groups in insulin levels. The HOMA-IR of the HFD group was much higher than that of control group. Our results indicated that fuc-Pg had powerful effects on inhibiting hyperglycaemia caused by HFD. The glucose and insulin level in the fuc-Pg-H group was even lower than control group.

### 3.3 Fuc-Pg attenuated liver steatosis in HFD-fed mice

In this section, the histomorphology of the liver was examined by H&E staining. In the control group, liver cells were neatly distributed with abundant cytoplasm. The HFD caused damages in liver cells (Fig. 2) with enlargement of liver cell volume, the structural disorder of hepatic lobules, and visible cavitation. Compared with the HFD group, a relatively normal structure of hepatic lobules, significantly improved fatty degeneration of liver cells was noted in fuc-Pg-L and fuc-Pg-H groups. Therefore, fuc-Pg reduced the accumulation of lipid droplets in the hepatocytes of hyperlipidemic mice and improved liver function.

### 3.4 Fuc-Pg reduced low-grade inflammation in HFD-fed mice

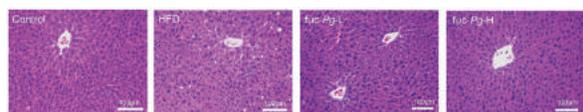
In this section, the inflammation caused by HFD was investigated, including serum TNF- $\alpha$  and lipopolysaccharide (LPS) levels. TNF- $\alpha$  is a multifunctional cytokine that mediates key roles in acute and chronic inflammation. Inhibiting TNF- $\alpha$  activities in inflammatory bowel disease has been remarkably successful.<sup>26</sup> The higher TNF- $\alpha$  level in the HFD group was reversed by a high-dosage of fuc-Pg (Fig. 3a). LPS was reported to initiate obesity and insulin resistance caused by HFD.<sup>27</sup> Fig. 3b indicated that a 60% HFD increased the serum LPS level slightly (although  $P < 0.05$  compared with the control group). Our results were different from former report that a 4-week high-fat diet chronically increased plasma LPS concentration two to three times.<sup>27</sup>

More inflammatory factors would be produced under the stimulation of HFD. Meanwhile, macrophages were induced into M1 by the higher inflammatory factors and then produced more inflammatory factors. One of outcomes caused by this phenomenon is macrophages infiltrating into adipose tissue, aggravating obesity caused by HFD.<sup>28</sup> Thus, we measured the CD68 by RT-PCR and immunohistochemical staining in the fat tissues. CD68 is a surface marker of macrophages.<sup>29</sup> The expression of CD68 in fat tissue was significantly increased by HFD intervention ( $P < 0.05$  compared with the control group). Administration of fuc-Pg could decrease the expression of CD68 in HFD-fed mice ( $P < 0.05$  compared with the HFD group). A higher dosage of fuc-Pg even had similar expression levels in the control group ( $P > 0.05$ ). The immunohistochemical staining of CD68 in the fat tissue also supports the results of RT-PCR (Fig. 3d). These results indicated that fuc-Pg could effectively reduce macrophages infiltrating into adipose

**Table 1** The effects of fuc-Pg on the serum biochemical parameters in HFD-fed mice

Groups	TC (mmol L <sup>-1</sup> )	LDL-C (mmol L <sup>-1</sup> )	TG (mmol L <sup>-1</sup> )	Glucose (mmol L <sup>-1</sup> )	Insulin (mIU L <sup>-1</sup> )	HOMA-IR
Control	3.58 ± 0.28*	0.93 ± 0.12*	1.48 ± 0.33*	2.90 ± 0.53*	5.69 ± 0.14*	0.73 ± 0.14*
HFD	6.36 ± 0.27 <sup>#</sup>	1.17 ± 0.20 <sup>#</sup>	1.97 ± 0.23 <sup>#</sup>	7.30 ± 1.42 <sup>#</sup>	6.12 ± 0.42 <sup>#</sup>	1.99 ± 0.48 <sup>#</sup>
Fuc-Pg-L	5.85 ± 0.67 <sup>#</sup>	1.12 ± 0.18	1.75 ± 0.46	3.65 ± 0.56*	5.60 ± 0.21*	0.90 ± 0.15*
Fuc-Pg-H	4.71 ± 0.43*	1.09 ± 0.14	1.56 ± 0.28	2.32 ± 0.35*	5.45 ± 0.17*	0.56 ± 0.08*

\* $P < 0.05$ : Compared with HFD group. <sup>#</sup> $P < 0.05$ : Compared to normal control group. Data are presented as mean ± SD,  $n = 8$ . The control group was supplied with common commercial chow and all the other groups were supplied with HFD.

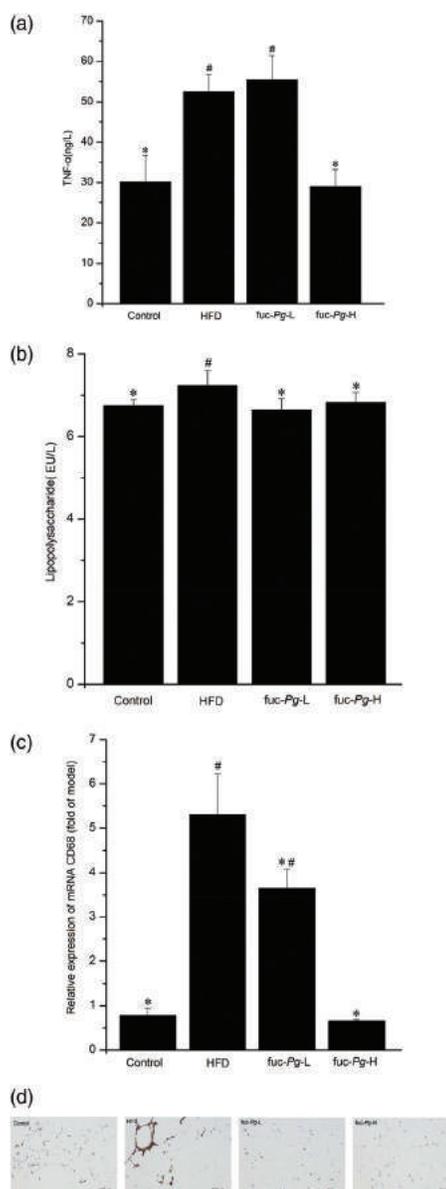
**Fig. 2** The effects of fuc-Pg on the liver histology in HFD-induced obese mice by HE stain (200× magnification).

tissue. In addition, Fig. 3d also shows that the adipocyte sizes in the control and fuc-Pg groups are similar to one another. However, the adipocytes in the HFD group were much bigger than the other three groups. These results indicated that fuc-Pg could reduce lipid accumulation in adipose tissues, thus reducing body weight gains.

### 3.5 Fuc-Pg modulated gut microbiota in HFD-fed mice

In this section, we investigated how fuc-Pg modulated the profiles of both cecal and colonic microbiota in the HFD-fed mice. Since a high dosage, 80 mg kg<sup>-1</sup>, had more profound effect in improving lipid profile, this dose was selected to investigate the effects of fuc-Pg on gut microbiota. Both in the cecum (ESI Table 2 and Fig. 2a†) and the colon (ESI Table 3 and Fig. 2b†), the reduced Chao1 index, Simpson index, and Shannon index of the HFD group indicate that alpha diversity is decreased by HFD ( $P < 0.05$ , compared to the control group). Treatment with fuc-Pg had no effect on bacterial alpha diversity in the HFD-fed mice. Nonmetric multidimensional scaling (NMDS) analysis and weighted uniFrac-based principal coordinates analysis (PCoA) were performed to better understand the similarity of gut microbiota between samples and groups. The results revealed a distinct clustering of microbiota composition between the control and HFD groups. Treatment with fuc-Pg showed different effects on the profiles of cecal and colonic microbiota. As shown in the Fig. 4a and b, the control group showed different clustering of cecal microbiota with the fuc-Pg group. However, a similar profile of colonic microbiota was found between the control and fuc-Pg group (Fig. 4c and d). The results indicated that fuc-Pg alleviated gut microbiota dysbiosis mainly in the colon. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster of cecal and colonic microbiota also demonstrated this result (ESI Fig. 3†).

HFD caused higher abundances of Firmicutes and Proteobacteria, and lower abundances of Actinobacteria, Bacteroidetes, and Verrucomicrobia compared with the control group both in the cecum (Fig. 5a) and the colon (Fig. 5b).

**Fig. 3** The effects of fucoidan on suppressing the chronic inflammation caused by HFD. Effect of fuc-Pg on serum TNF- $\alpha$  (a), LPS (b), relative expression of CD68 mRNA (c) and immunohistochemical staining for CD68 in the fat tissue (d). \* $P < 0.05$ : compared with HFD group. <sup>#</sup>  $P < 0.05$ : compared to normal control group.



Further analysis was performed at genus level (as shown in the Fig. 5c and d). The HFD group had high abundance of *Lachnospirillum*, *Acetatifactor*, *Lactococcus*, *Romboutsia*, *Enterorhabdus*, and *Dorea*, and low abundance of *Akkermansia*, *Olsenella*, *Barnesiella*, and *Alloprevotella* in the cecum. Fuc-Pg decreased the abundance of *Acetatifactor* and *Lactococcus* while increased the abundance of *Olsenella* and *Alloprevotella* in the HFD-fed mice. As for the profile of cecal microbiota, the HFD group had high abundance of *Lachnospirillum*, *Acetatifactor*, *Lactococcus*, *Alistipes*, *Romboutsia*, *Enterorhabdus*, *Desulfovibrio*, *Olsenella* and *Dorea*, and low abundance of *Akkermansia*, *Barnesiella*, *Alloprevotella*, and *Parabacteroides* compared to the control group in the colon. Fuc-Pg had lower *Acetatifactor*, *Lachnospirillum*, *Desulfovibrio*, *Dorea* and higher *Parabacteroides*, *Alloprevotella*, *Olsenella* in the colon of HFD-fed mice.

LEfSe analysis was performed to explore the differences in microbiota profile between the control, HFD, and fuc-Pg groups. Taxa with an LDA score threshold >3 were included in the LEfSe, and we ignored the counting of very low abundance bacterium. Fig. 6a and ESI Fig. 4a† showed the cecal microbiota profile in the control, HFD, and fuc-Pg group. The results showed that the control group were dominated by Parasutterella, Bacillales, Bifidobacteriales, Bifidobacteriaceae, Bifidobacterium, *Prevotella*, *Alloprevotella*, Prevotellaceae, *Barnesiella*, Actinobacteria, Verrucomicrobia, Verrucomicrobiales, Verrucomicrobiae, Verrucomicrobiaceae, *Akkermansia*, Porphyromonadaceae, Bacteroidetes, Bacteroidia, and Bacteroidales. In the HFD group, the cecal microbiota were dominated by Firmicutes, Lachnospiraceae, Clostridia, Clostridiales, *Lachnospirillum*, *Acetatifactor*, Streptococcaceae, *Lactococcus*, *Romboutsia*, *Dorea*, *Enterorhabdus*, Oscillibacter, and Oscillospiraceae. The fuc-Pg group had higher abundance in *Olsenella*, Actinobacteria, and *Oscillibacter*. Then, the colonic microbiota was analysed by LEfSe analysis (Fig. 6b and ESI Fig. 4b†). Similar to the cecal microbiota profile, the dominant bacteria in the control group were characterized by a higher amount of Verrucomicrobia, Verrucomicrobiaceae, *Akkermansia*, Porphyromonadaceae, Bacteroidales, Bacteroidia, Bacteroidetes while the HFD had higher amount of Firmicutes, Lachnospiraceae, Clostridia, Clostridiales, *Lachnospirillum*, Streptococcaceae, *Lactococcus*, Desulfovibrionales, Desulfovibrionaceae, *Desulfovibrio*, *Romboutsia*, *Dorea*, and *Acetatifactor* compared with the HFD group. However, the profile of colonic microbiota was different from that of the cecum in the fuc-Pg group. The dominant colonic bacteria in the fuc-Pg group was characterized by higher amounts of Bacteroidetes, Bacteroidales, Bacteroidia, *Olsenella*, *Prevotellaceae*, and *Alloprevotella*.

## 4 Discussion

Fucoidans are a family of sulfated polysaccharides extracted from marine algae and echinoderms that mainly consist of a fucose polysaccharide modified with *O*-sulfo groups. The struc-

tures of fucoidans from marine algae are often complicated by the presence of monosaccharides, having a variety of glycosidic linkages and side branches. Unlike algae fucoidans, fucoidan extracted from marine invertebrates have simple, linear structures, composed of repeating units of oligosaccharides.<sup>30</sup> Fucoidans from sea cucumbers can alleviate metabolic syndromes caused by high-fat and high-sucrose diet, lowering serum glucose, TG, TC, body weight gain, improving adiponectin, and inhibiting insulin resistance.<sup>31</sup> Moreover, they can normalize PKB/GLUT4 insulin signaling cascades in skeletal muscle<sup>32</sup> and PI3K/PKB/GSK-3 $\beta$  signaling in liver tissues.<sup>33</sup> In our recent work, fuc-Pg dominated with 4-*O*-sulfation exerted anti-hyperlipidemia and anti-obesity activities.<sup>18</sup> However, there is little research involving in the effects of fucoidans from sea cucumbers on metabolic complications and gut microbiota dysbiosis caused by HFD.

Our data indicated a HFD containing 60% fat energy could induce obesity, mild chronic inflammation, hyperglycemia, and gut microbiota dysbiosis. As expected, fuc-Pg could significantly suppress the HFD-induced body weight gain, adipose tissue weight, as well as liver weight. Higher serum biochemical parameters, including TC, TG, LDL-C, glucose, insulin, and HOMA-IR may also reflect that HFD caused energy metabolism disorders in C57BL/6J mice. In particular, fuc-Pg in a high dosage reversed these parameters in HFD-fed mice close to the control group. Meanwhile, fuc-Pg protected the liver from steatosis. The low-grade inflammation in HFD-fed mice was also reversed by fuc-Pg. In this work, the gut microbiota dysbiosis caused by HFD were found both in the cecum and the colon. However, according to the results of 16s rRNA gene analysis and LEfSe analysis, fuc-Pg was found to alleviate gut microbiota dysbiosis mainly in the colon.

Our results support the idea that the ratio of *Firmicutes* to *Bacteroidetes* (F/B) is positively correlated to obesity.<sup>34,35</sup> Supplemental fuc-Pg could reverse the ratio of F/B to a normal level in HFD-fed mice from 21.8 to 1.56 in the colon of HFD-fed mice, which was similar to the control group 1.27. However, fuc-Pg had limited effects on that in the cecum. Fuc-Pg increased the abundance of *Bacteroidetes* in the colon of HFD-fed mice such as Bacteroidales, Bacteroidia, Prevotellaceae, *Parabacteroides* and *Alloprevotella*. *Alloprevotella* has been well illustrated to be responsible for the degradation of diverse polysaccharides and fibres, a member of short chain fatty acid (SCFA) producers.<sup>36</sup> *Parabacteroides* can provide a defense against the invasion of exogenous microorganisms in the intestinal microbiota by producing bacteriocins.<sup>37</sup> In contrast, administration of fuc-Pg decreased *Firmicutes* phylum, such as Lachnospiraceae, *Lachnospirillum*, Clostridia, Clostridiales, *Dorea*, and *Acetatifactor* in the colon of HFD-fed mice. Most of these microorganisms are significantly associated with metabolic diseases. *Dorea* is associated with reduced cardiovascular disease risk.<sup>38</sup> The abundance of *Acetatifactor* was decreased by fuc-Pg both in the cecum and the colon. *Acetatifactor* was first isolated from the intestine of obese mouse.<sup>39</sup> Meanwhile, fuc-Pg decreased the abundance of *Lactococcus* in the cecal of HFD-fed mice, which was signifi-

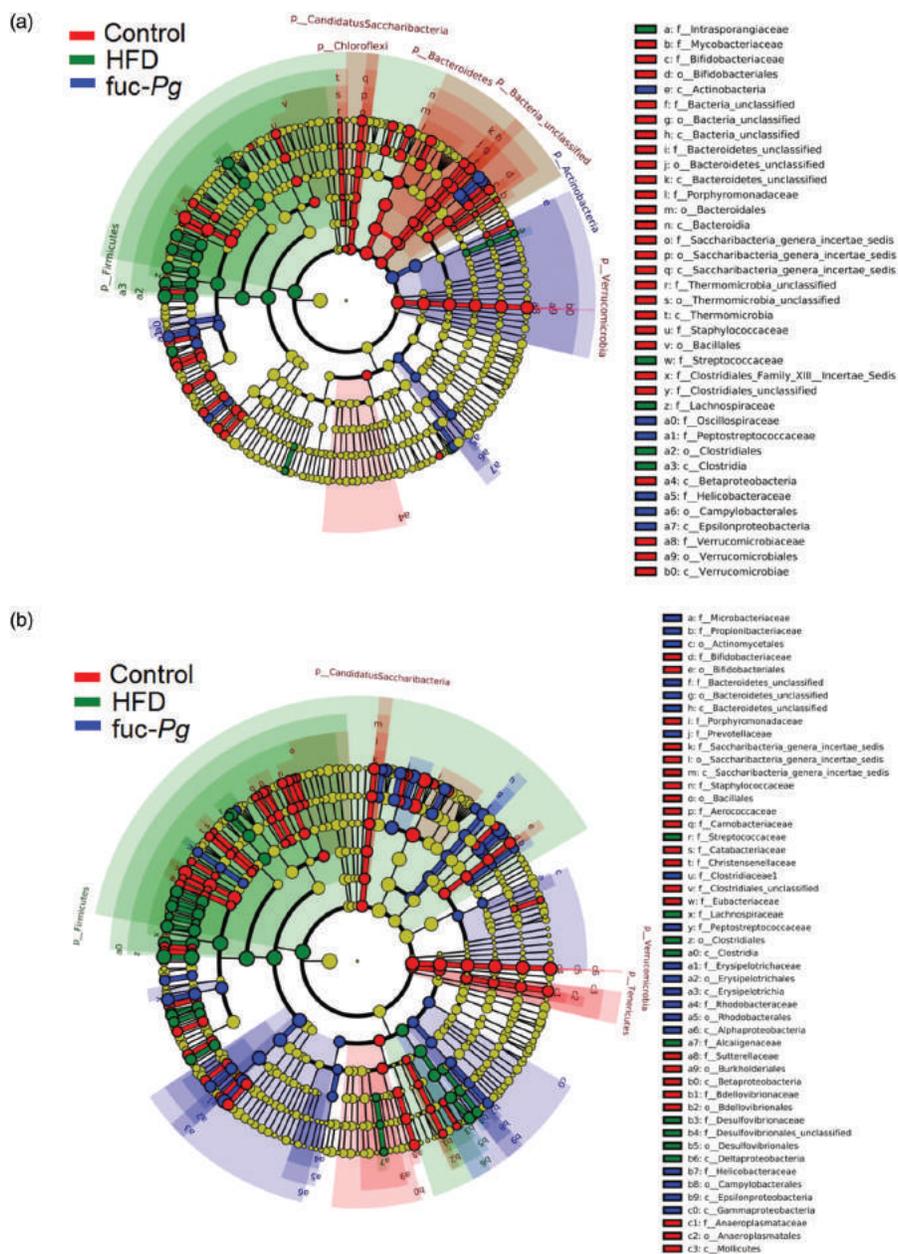


Fig. 6 LefSe comparison of cecal microbiota. Taxonomic cladogram e of cecal microbiota between the control, HFD and fuc-Pg group (a); taxonomic cladogram of cecal microbiota between the fuc-Pg and HFD group (b).

cantly increased in the obesity-prone mice compared with obesity-resistant phenotype mice.<sup>40</sup> In the colon of HFD-fed mice, fuc-Pg decreased the ratio of *Desulfovibrio* genus, belonging to Desulfovibrionaceae, Desulfovibrionales, and Proteobacteria. *Desulfovibrio* is a kind of microbe positively related to inflammation.<sup>41</sup> Remarkably, fuc-Pg increased the abundance of *Actinobacteria* both in the cecum and the colon of HFD-fed mice, even higher than the control group. Our

study showed that fuc-Pg increased abundance of *Actinobacteria* via the high abundance of *Olsenella*. *Olsenella* is also part of the normal bacterial flora in the human gut.<sup>42</sup> However, little is known about the role that *Olsenella* plays in the human health and diseases. Fructo-oligosaccharides (FOS), as a kind of prebiotics can stimulate the growth of *Olsenella*, which provided a new target for the interactions between FOS and gut bacteria.<sup>43</sup>

## 5 Conclusions

Fuc-*Pg* is a kind of fucoidan with well-repeated structure. The 4-*O*-sulphation patterns endow this fucoidan with high biological activities. In the present work, we demonstrated that fuc-*Pg* could reduce obesity, reverse lipid disorder, protect the liver, and suppress inflammation induced by HFD. Meanwhile, administration of fuc-*Pg* caused a more balance gut microbiota in HFD-fed mice. A comparison between cecal and colonic microbiota was performed in our study. The results indicated that the modulation of fuc-*Pg* on microbiota mainly worked in the colon. Fuc-*Pg* increased the abundance of benign microorganisms such as *Parabacteroides*, *Alloprevotella* and *Olsenella*. On the other hand, administration of fuc-*Pg* decreased microorganisms positively associated with obesity, inflammation, and other metabolic diseases, such as *Dorea*, *Desulfovibrio*, and *Acetatifactor* in the colon of HFD-fed mice. Thus, our data demonstrate that fuc-*Pg* could be a potential prebiotic agent that may be used for the treatment of obesity and its complications.

## Abbreviations

HFD	High-fat diet
fuc- <i>Pg</i>	Fucoidan from <i>Pearsonothuria graeffei</i>
TC	Total cholesterol
TG	Triglyceride
HDL-C	High density lipoprotein cholesterol
LDL-C	Low density lipoprotein cholesterol

## Conflicts of interest

There are no conflicts to declare.

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