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Flaxseed gum reduces body weight by regulating gut microbiota

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ABSTRACT

The function of flaxseed gum (FG) on blood glucose control makes it possible for body weight loss. This experiment was to investigate the anti-obesity effect of FG and the alteration of gut microbiota. Diets with high, middle and low doses of FG were applied to feed obese rats for 5 weeks. The body weights, serum biochemical indices, body fats, short-chain fatty acid (SCFA) contents and metagenomic information of gut microbiota were analyzed. The results showed the FG diet reduced body weights, body fats and total triacylglycerols, and reshaped rat's cecal microbial compositions. The anti-obesity effect of FG could be achieved by appetite suppression by reducing the relative abundance of Firmicutes and/or the Firmicutes/Bacteroidetes ratio and regulating some specific bacteria. The genus *Clostridium* might be the key one for the degradation of FG and production of SCFAs. SCFAs may not be involved in this weight-loss effect.

1. Introduction

Flax (*Linum usitatissiumum* L.) is a blue flowering annual herb. Flax produces small flat seeds, also named as flaxseed. Human have been consuming flaxseed since ancient times. Flaxseed is a valuable and important edible oil source (Cunnane, & Thompson, 1995). It is cultivated in more than 50 countries including Canada, India, China, United States, and Ethiopia, etc. Flax has been cultivated for fiber as well as for medicinal purposes and as nutritional product (Tolkachev, & Zhuchenko, 2000). Recently, new interest in flaxseed is due to its health benefits. Its functional components include lignans (secoiso-laricir-esinol diglucoside (SDG) being the predominant form), α-linolenic acid, and soluble flaxseed gum (FG, also named as Flax mucilage) (Hall Iii, Tulbek, & Xu, 2006).

FG accounts for 2–10% (w/w) in flaxseed. It is extracted mainly from the layer of flaxseed hull with water. FG can be further separated into neutral (NFG) and acidic (AFG) fraction using ion exchange chromatography (IEC). The neutral fraction constitutes L-arabinose, D-xylose, D-galactose and arabinoxylan; acidic fractioncontains L-rhamnose, L-fucose, L-galactose and D-galactouronic acid (Wanasundara & Shahidi, 1994).

FG *in vitro* exhibited a high bile acid binding capacity and generated high amount of acetate and propionate, which indicates that FG may lower serum cholesterol (Fodje, Chang, & Leterme, 2009; Denis, Barbara, & Dominique, 2007; Theuwissen, & Mensink, 2008). Alzuetaetal (2003) have also reported that flaxseed gum could selectively stimulate the growth of *Lactobacilli in vivo*. These reports indicate that FG is a potential prebiotics. However, there is no further report on FG inducing body weight loss via regulating gut microbiota. This study aimed to investigate the anti-obesity effect of FG *in vivo* and the alteration of gut microbiota.

2. Materials and methods

2.1. Animal, diets and sample preparation

The animal experiments were approved by the Institutional Animal Care and Use Committee of Jinan University. Totally 54 male Sprague Dawley rats (4 weeks of age), were bought from Guangdong Medical Laboratory Animal Center (GDMLAC). Forty-eight of the 54 rats were used to build obese model. In brief, after a 10-days adaption with a standard diet (Li, et al., 2015), rats were used to build obese models by feeding them a high-fat diet for 5 weeks. The 50% of the obese model rats with higher body weights were further randomly divided into four groups (6 rats for each) for the following experiment. The four groups individually caged for another 5-weeks trial with (1) a high-flaxseed gum diet (containing 30% flaxseed gum) (Group FG_H), (2) a medium-flaxseed gum diet (containing 20% flaxseed gum) (Group FG_M), (3) a low-flaxseed gum diet (containing 10% flaxseed gum) (Group FG_L) and the standard diet (Group Con). Six of the 54 rats were fed the standard

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Table 1
Diet recipe (%).

Ingredients	High-fat diet (D12492)	Standard diet (AIN-93 M)	Flaxseed gum diet		
			Low	Medium	High
Flaxseed gum	0.00	0.00	10.00	20.00	30.00
Corn starch	0.00	46.57	36.57	26.57	16.57
Dextrin	16.35	15.50	15.50	15.50	15.50
Casein	26.17	14.00	14.00	14.00	14.00
Sucrose	9.00	10.00	10.00	10.00	10.00
Cellulose	6.54	5.00	5.00	5.00	5.00
Soybean oil	3.27	4.00	4.00	4.00	4.00
Lard	32.06	0.00	0.00	0.00	0.00
Mineral mix AIN-93	4.58	3.50	3.50	3.50	3.50
Vitamin mix AIN-93	1.31	1.00	1.00	1.00	1.00
L-cystine	0.39	0.18	0.18	0.18	0.18
Cholinebitartrate	0.33	0.25	0.25	0.25	0.25

diet during the whole experimental period (Group Blank). The flaxseed gum used here was purchased from Shandong Zhongkai Ltd. Co., China. The standard diet and high-fat diet served in this experiment were AIN-93 M (Reeves, Nielsen, & Fahey, 1993) and D12492 (Gajda, Pellizzon, Ricci, & Ulman, 2007), respectively; three diets of flaxseed gum were made by replacing corn starch in the standard diet with equivalent flaxseed gum. All recipes of diet were listed in Table 1. Cecal content and serum samples from each group were immediately collected and stored in liquid nitrogen after the rats were sacrificed, and then transferred into a -80 °C refrigerator. Abdominal and epididymal fat were individually weighed after animals were dissected.

2.2. MiSeq sequencing of the V3 region of 16S rRNA genes

Bacterial genomic DNA in rats' cecal contents was extracted by using TIANamp Stool DNA kit (Tiangen, Beijing, China) according to manufacturer's instructions. The primers, P1 and P2 (ACTCCTACGGG AGGCAGCAG and GGACTACHVGGGTWTCTAAT) corresponding to positions 338F to 806R in bacterial 16SrRNA gene, were used to amplify the V3-V4 region of each sample by PCR. PCR reactions were performed in triplicate 20 μL mixture solution containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, and 10 ng of template DNA. PCR reactions were run in a thermocycler PCR system (ABI GeneAmp® 9700, USA) using the following programme: 3 min of denaturation at 95 °C followed by 27 cycles of 30 sec at 94 °C, 30 sec at 55 °C and 45 sec at 72 °C, with a final extension at 72 °C for 10 min. Amplicons were extracted from 2% agarose gels and purified with the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) according to the manufacturer's instructions and quantified using QuantiFluor™ -ST (Promega, USA). Purified amplicons were pooled in equimolar amounts and paired-endsequenced (2 × 250) on an IlluminaMiSeq platform according to the standard protocols.

2.3. Bioinformatics of sequencing data

Raw fastq files were demultiplexed, quality-filtered using QIIME (version 1.9.1) with the following criteria: (i) The 300 bp reads were truncated at any site receiving an average quality score < 20 over a 50 bp sliding window, discarding the truncated reads that were shorter than 50 bp. (ii) Exact barcode matching, 2 nucleotide mismatch in primer matching, reads containing ambiguous characters were removed. (iii) Only sequences that overlap longer than 10 bp were assembled according to their overlap sequence. Reads which could not be assembled were discarded.

Operational Taxonomic Units (OTUs) were clustered with 97% similarity cutoff using Usearch (version 7.1, http://drive5.com/uparse/) and chimeric sequences were identified and removed using UCHIME. The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier (http://rdp.cme.msu.edu/) against the silva (SSU123) 16S rRNA database using confidence threshold of 70% (Amato et al., 2013).

Hierarchical clustering (Hcluster) analysis was performed according to the data matrix of unweighted pair group method with arithmetic mean (UPGMA), and a tree-like structure was built to express and compare the similarity and difference between communities. The distance matrix was calculated by the Bray-Curtis method (Jiang et al., 2013):

$$D_{Bray-Curtis} = 1-2\frac{\sum \min(S_{A,i}, S_{B,i})}{\sum S_{A,i} + \sum S_{B,i}}$$

Among them, $S_{A,i}$ means the amount of sequences in No. i OTU of Sample A; $S_{B,i}$ means the amount of sequences in No. i OTU of Sample B.

2.4. Determination of SCFAs

The concentrations of SCFAs in cecal contents were measured with the method described by Campbell, Fahey, and Wolf (1997).

2.5. Serum biochemical analysis

Blood samples were collected from the tail vein after overnight fasting and centrifuged at 12,000 rpm for 30 min to pellet blood cells, and the serum was stored at −80 °C until further analysis. The analysis of serum total cholesterol (TC), total triacylglycerols (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) was performed via UV–vis spectrophotometer using Konelab 20XTi (Thermo Fisher Scientific, USA).

2.6. Statistical analysis

Results are expressed as mean values and standard deviations. The statistical analysis was performed with SPSS 20.0 software (SPSS Inc., Chicago, Ill., USA). The analysis was conducted by two-tailed *t*-test or one way ANOVA followed by Tukey test. Statistical significance was set at a $P < 0.05$ and highly significance was $P < 0.01$.

3. Results

3.1. Body weight and body weight gains

The body weight gain (BWG) of Group Blank was significantly higher at week 2 ($P < 0.05$) and remained similar since then ($P > 0.05$). Different from Group Blank, the BWGs of Group FG_H, FG_M and FG_L had lower BWG since week 3 ($P < 0.05$). Specifically, Group FG_H and FG_M showed a negative growth of BWG at week 3 while Group FG_L started to loss weight at week 4. The BWG of Group FG_M and FG_L remained negative ($P > 0.05$) while a significant increment of BWG was observed in Group FG_H at week 5 ($P < 0.05$). As for Group Con, its BWG kept increasing from the beginning to the end (Table 2).

3.2. Serum biochemical indices and weights of body fat

The value of total cholesterol (TC) in Group FG_H was significantly lower when compared with that in Con ($P < 0.05$). No significant difference of TC value was found in Group FG_M, FG_L and Blank compared with Group Con (Table 3).

The values of total triacylglycerols (TG) in Group FG_L, FG_M and FG_H were significantly lower than that in Con ($P < 0.05$ or $P < 0.01$), while Group Blank had similar value of TG to that in Group Con ($P > 0.05$) (Table 3).

However, except for the value of low-density lipoprotein cholesterol (LDL-C) in FG_L and Blank, which was significantly higher than that in

Table 2
Body weight gain during the experimental period (g).

	BWG				
	Week 1	Week 2	Week 3	Week 4	Week 5
Group Blank	16.27 ± 09.36^{Bb}	56.15 ± 41.31^{Aa}	21.77 ± 07.72^{Ba}	27.22 ± 08.01^{ABa}	26.72 ± 06.69^{ABa}
Group FG_H	48.73 ± 18.72^{Aa}	41.13 ± 30.23^{Aa}	−17.08 ± 11.81^{Bc}	−30.40 ± 11.92^{Bc}	35.28 ± 14.70^{Aa}
Group FG_M	40.92 ± 10.66^{Aa}	35.18 ± 06.84^{Aa}	−0.53 ± 09.09^{Bb}	−15.88 ± 07.99^{BCb}	−24.05 ± 15.60^{Cb}
Group FG_L	42.95 ± 07.31^{Aa}	37.07 ± 16.09^{Aa}	19.07 ± 05.24^{Ba}	−12.38 ± 03.84^{Cb}	−12.18 ± 03.75^{Cb}
Group Con	41.07 ± 08.17^{Ba}	59.45 ± 11.77^{Aa}	27.25 ± 06.13^{BCa}	25.75 ± 06.07^{Ca}	17.68 ± 09.37^{Ca}

BWG: Body weight gain per week. ^{abc}: Different letters in the same column indicated significant differences were found ($P < 0.05$). ^{ABC}: Different letters in the same line indicated significant differences were found ($P < 0.05$). Group Blank: Normal animals consuming a standard diet throughout; Group FG_H: the group consuming a diet supplemented with 30% flaxseed gum after obese model built; Group FG_M: the group consuming a diet supplemented with 20% flaxseed gum after obese model built; Group FG_L: the group consuming a diet supplemented with 10% flaxseed gum after obese model built; Group Con: the group consuming a standard diet after obese model built.

Group Con ($P < 0.01$ and $P < 0.05$, respectively), LDL-C value in other groups and the values of high-density lipoprotein cholesterol (HDL-C) in Group FG_H, FG_M, FG_L and Blank were similar to that in Con (Table 3).

Additionally, Group FG_H and Blank had highly significantly lower level of the abdominal fat ratio index (AFW/BW, AF index) while Group FG_M had significantly lower level of AF index ($P < 0.05$) when compared with those in Group Con (Table 3).

As for the epididymal fat ratio index (EFW/BW, EF index), only Group FG_H showed a highly significantly lower level when compared with that in Group Con ($P < 0.01$) (Table 3)

3.3. Contents of short-chain fatty acids (SCFAs) in cecal contents

No significant difference of acetate and propionate content was found in Group FG_H, FG_M and FG_L compared with those in Group Con ($P > 0.05$). Both Group FG_H and FG_L had less butyrate than Group Con with high significance ($P < 0.05$). The content of total SCFAs in Group FG_M was significantly higher ($P < 0.05$) while that in Group FG_H was lower than in Group Con with very significant difference ($P < 0.01$) (Table 4).

3.4. Overall information and distribution of cecal microbiota

The operational taxonomic unit (OTU) number in Group FG_H and Con was similar. But they were significantly lower than that in Group FG_M and FG_L. No significant difference of OTU number was found between Group FG_M and FG_L (Table 5). The value of Chao1 index was found significantly higher in Group FG_L and FG_M when compared with that in Group FG_H ($P < 0.05$), while this value in Group Con was similar to that in FG_M and FG_H ($P > 0.05$). There was no significant difference of the ACE index value between Group FG_L and FG_M, and also between Group FG_H and Con ($P > 0.05$). But the ACE indices in the former two groups were significantly higher than the latter two ($P < 0.05$). Both values of Shannon and Simpson index in Group FG_M, FG_L and Con were similar and they all significantly higher than Group FG_H ($P < 0.05$) (Table 5).

Different group had various gut microbial distribution in a four-quadrant of Nonmetric Multidimensional Scaling plot (NMDS plot) (Fig. 1). There was a clear boundary that can be found between the sample dots of Group FG_M and Group FG_L together with Con. Additionally, the most sample dots of Group FG_L and Con shared a close location on the right side of the NMDS plot. In the meanwhile, the most sample dots of Group FG_M and Group FG_H similarly located on the left of the NMDS plot.

The number of shared OTU among four groups showed that all groups shared 382 OTUs, and Group FG_H, FG_M and FG_L shared 417 OTUs. Besides, 398 OTUs were shared in Group FG_L and Con while 234 OTUs were shared in Group FG_M and FG_H (Fig. 2).

3.5. Compositions of cecal microbiota

The relative abundance (RA) of each group at both phylum and genus level was displayed and RA < 0.1% was classified into the others at genus level (Fig. 3).

At phylum level, the significantly highest RA of Firmicutes was detected in Group Con ($P < 0.05$), and the RA of Firmicutes increased with dose dependence on FG content in feed. A similar tendency of TM7 was also found. Differently, the RA of Proteobacteria and Cyanobacteria in Group Con were the lowest among all of four groups ($P < 0.05$), but they became higher as increasing FG content. Besides, Group FG_M presented the significant highest RA value of Elusimicrobia ($P < 0.05$)

Table 3
Serum biochemical indices (mM) and body fat weight indices (%) in each group.

	TC[1]	TG[1]	HDL-C[1]	LDL-C[1]	AFW/BW[2]	EFW/BW[2]
Group Blank	1.33 ± 0.25	0.38 ± 0.07	0.38 ± 0.07	0.17 ± 0.04*	1.72 ± 0.27**	1.89 ± 0.39
Group FG_H	0.88 ± 0.24*	0.55 ± 0.24**	0.25 ± 0.10	0.10 ± 0.06	0.77 ± 0.51**	1.21 ± 0.46**
Group FG_M	1.05 ± 0.44	0.60 ± 0.28**	0.29 ± 0.11	0.10 ± 0.07	1.93 ± 0.63*	2.05 ± 0.46
Group FG_L	1.39 ± 0.13	0.66 ± 0.30*	0.28 ± 0.06	0.19 ± 0.05**	2.63 ± 0.59	2.63 ± 0.33
Group Con	1.29 ± 0.28	1.15 ± 0.31	0.31 ± 0.08	0.11 ± 0.04	2.78 ± 0.38	2.31 ± 0.39

TC: Total cholesterol; TG: Total triacylglycerols; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; AFW: Abdominal fat weight; EFW: Epididymal fat weight; BW: Body weight.
Group Blank: Normal animals consuming a standard diet throughout; Group FG_H: the group consuming a diet supplemented with 30% flaxseed gum after obese model built; Group FG_M: the group consuming a diet supplemented with 20% flaxseed gum after obese model built; Group FG_L: the group consuming a diet supplemented with 10% flaxseed gum after obese model built; Group Con: the group consuming a standard diet after obese model built.
[1] With the unit of mM.
[2] With the unit of %.
* $P < 0.05$ when compared with Group Con.
** $P < 0.01$ when compared with Group Con.

Table 4
The content of cecal short-chain-fatty-acid in each group (μg/g).

	Acetate	Propionate	Butyrate	Total
Group FG_H	123.27 ± 32.37	070.85 ± 19.73	023.78 ± 024.40**	218.72 ± 062.20**
Group FG_M	142.53 ± 24.97	105.55 ± 06.72	384.92 ± 108.08	675.18 ± 075.20*
Group FG_L	121.65 ± 19.80	094.13 ± 08.30	227.21 ± 022.45**	437.10 ± 007.66
Group Con	108.12 ± 47.46	080.65 ± 44.65	309.93 ± 019.61	500.71 ± 100.24

Group FG_H: the group consuming a diet supplemented with 30% flaxseed gum after obese model built; Group FG_M: the group consuming a diet supplemented with 20% flaxseed gum after obese model built; Group FG_L: the group consuming a diet supplemented with 10% flaxseed gum after obese model built; Group Con: the group consuming a standard diet after obese model built.

* $P < 0.05$ when compared with Group Con.

** $P < 0.01$ when compared with Group Con.

while Group FG_H showed the highest RA value of Verrucomicrobia ($P < 0.05$).

At genus level, the RA of *Clostridium*, Unclassified_*Enterobacteriaceae*, Unclassified_*YS2*, Unclassified_*Burkholderiales*and *Veillonella*increased as the content of FG increased. Conversely, the RA of Unclassified_*Clostridiales*, *Lactobacillus*, Unclassified_*[Mogibacteriaceae]*, *Ruminococcus*, *Oscillospira*, Unclassified_*Coriobacteriaceae* and *Turicibacter* reduced with the increasing content of FG in diet. Additionally, the RA of *Roseburia*, *[Ruminococcus]*, Unclassified_*Peptostreptococcaceae* and Unclassified_*F16* were the highest in Group Con ($P < 0.05$) while they were similar in Group FG_H, FG_M and FG_L ($P > 0.05$). Moreover, the lowest RA of Unclassified_*Rikenellaceae* and highest RA of *Sutterella*, *Serratia*, *Akkermansia* and Unclassified_*Burkholderiales* were found in Group FG_H ($P < 0.05$) while the RA of all these bacteria were similar among Group FG_M, FG_L and Con ($P > 0.05$). Lastly, the RA of *Prevotella* and Unclassified_*Elusimicrobiaceae* in Group FG_M were significantly higher than those in any other groups ($P < 0.05$).

4. Discussion

Dietary fibers had been reported to have multiple biological functions including body weight control via microbial modulation (Nicolucci, Hume, Martinez, Mayengbam, Walter, & Reimer, 2017). As a dietary fiber, FG consumption can also lower blood glucose and cholesterol (Thakur, Mitra, Pal, & Rousseau, 2009; Au, Goff, Kisch, Coulson, & Wright, 2013). Hence, it was suspected to have the activity of body weight control. However, our previous study discovered that soybean fiber didn't reduce body weight even though it also regulates gut microbiota (Li et al., 2015). It seems that dietary fibers can alter gut microbiota but not always present the same biological effects.

In the current research, the diets containing 10%, 20% and 30% FG were separately feed obese rats to discover its anti-obese activity and the relationship with gut microbiota. We found FG in feeds lowered body weights different from soybean fiber, and only a weak dose-dependence in total triacylglycerols and body fat. There is no dose-dependence discovered in cecal SCFAs and bacterial community diversity Moreover, the flaxseed gum intervention reshaped the composition of cecal microbiota by reducing Firmicutes and/or the Firmicutes/Bacteroidetes ratio, as well as regulating some specific bacteria, such as the genus *Clostridium*, with dose-dependence.

Soybean fiber could alter the compositions of rats' gut microbiota but without reducing their body weights in our previous study (Li et al., 2015). We further found those altered bacteria were totally different from those in the presentt study. The family Prevotellaceae, Bacteroidaceae, Ruminococcaceae and/or Desulfovibrionaceae, which showed a higher and/or lower RA after consuming soybean fiber in the previous study (Li et al., 2015), remained similar according to the results in this research. It seems that bacteria in these families were less relative to body weight loss. But in this study, we found FG, one of dietary fibers, controlled rats' body weights via regulating different types of microbiota. Obese rats consumed a FG diet presented a significantly lower Firmicutes/Bacteroidetes ratio due to the reduced RA of the phylum Firmicutes compared with Group Con. The ratio of Firmicutes/Bacteroidetes closely related to the energy metabolism. A high value of this ratio indicates an acceleration of energy harvest from food and increment of energy storage in adipose tissue of host (Angelakis, Armougom, Milion, & Raoult, 2012), which would further suppressed the production of fasting-induced adipose factor (Fiaf). The suppression led to a higher storage of triacylglycerols in adipose tissue and lower release of satiety hormones (Crovesy, Ostrowski, Ferreira, Rosado, & Soares-Mot, a M. , 2017). Hence, a lower value of this ratio will indicate a better performance on body weight control, which is supported by our result. Despite only a weak dose-dependent effect existed between FG content and the RA of some specific bacteria, the FG intervention still lowered the RA of *Roseburia*, Unclassified_*Clostridiales*, Unclassified_*Peptostreptococcaceae*, *Lactobacillus*, *Ruminococcus*, *Oscillospira*, Unclassified_*[Mogibacteriaceae]*, *[Ruminococcus],* Unclassified_*Coriobacteriaceae* and *Turicibacter*, all of which belong to the phylum Firmicutes, and further resulted in body weight and body fat loss. In addition, more FG in feed also enhanced the RA of *Clostridium*, Unclassified_*Enterobacteriaceae*, *Sutterella*, Unclassified_*YS2*, *Veillonella* and Unclassified_*Burkholderiales*, which may also relate to body weight loss and even the degradation of FG. Especially for the genus *Clostridium*, which belonged to the phylum Firmicutes, presented a positive

Table 5
OTU at phylum level and indices of alpha-diversity in each group.

	OTU	Alpha-diversity			
		Chao1	ACE	Shannon	Simpson
Group FG_H	0867.33 ± 087.52^{b}	0927.37 ± 111.61^{cc}	0962.22 ± 125.27^{b}	5.60 ± 0.63^{b}	0.89 ± 0.06^{b}
Group FG_M	1035.00 ± 091.79^{a}	1093.19 ± 082.14^{ab}	1117.51 ± 088.44^{a}	6.60 ± 0.70^{a}	0.94 ± 0.04^{a}
Group FG_L	1034.50 ± 098.40^{a}	1187.65 ± 155.31^{aa}	1203.85 ± 144.84^{a}	6.86 ± 0.33^{a}	0.96 ± 0.02^{a}
Group Con	0887.50 ± 122.94^{b}	0939.26 ± 153.12^{bc}	0945.90 ± 146.72^{b}	6.83 ± 0.32^{a}	0.96 ± 0.01^{a}

OTU: Operational taxonomic units; ^{abc}: different letters in the same column indicated significant differences were found. Group FG_H: the group consuming a diet supplemented with 30% flaxseed gum after obese model built; Group FG_M: the group consuming a diet supplemented with 20% flaxseed gum after obese model built; Group FG_L: the group consuming a diet supplemented with 10% flaxseed gum after obese model built; Group Con: the group consuming a standard diet after obese model built.

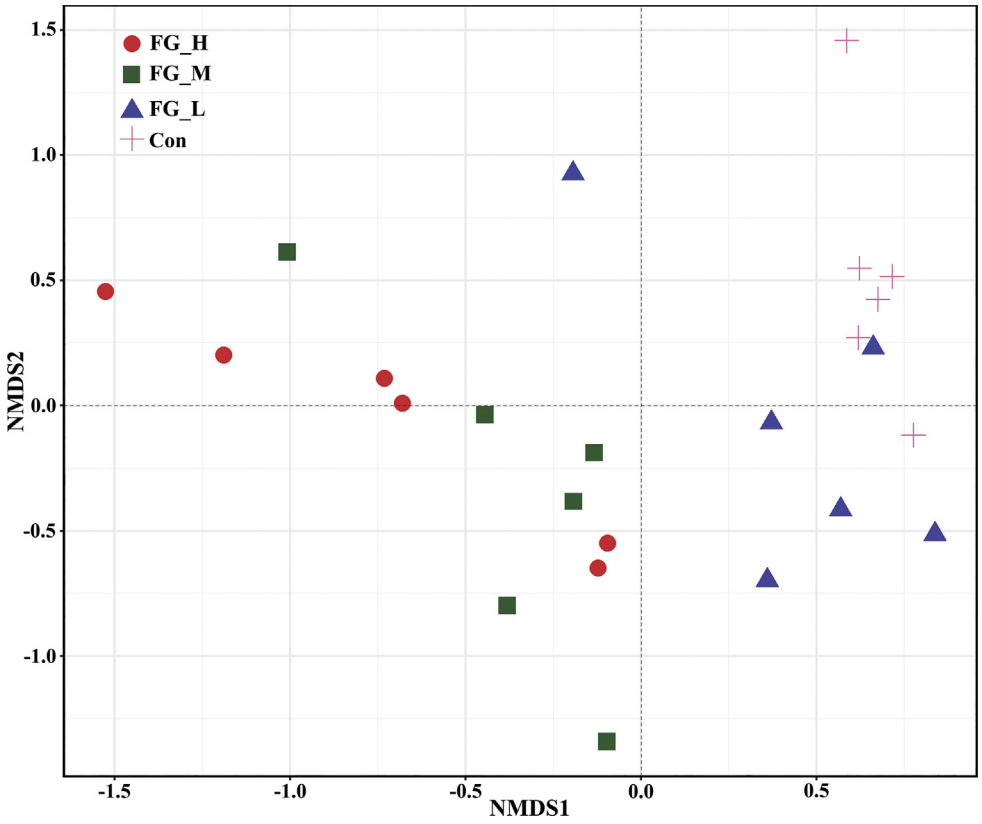

Fig. 1. Plots of Nonmetric Multidimensional Scaling (NMDS) of gut microbiota in each group. Group FG_H: the group consuming a diet supplemented with 30% flaxseed gum after obese model built; Group FG_M: the group consuming a diet supplemented with 20% flaxseed gum after obese model built; Group FG_L: the group consuming a diet supplemented with 10% flaxseed gum after obese model built; Group Con: the group consuming a standard diet after obese model built.

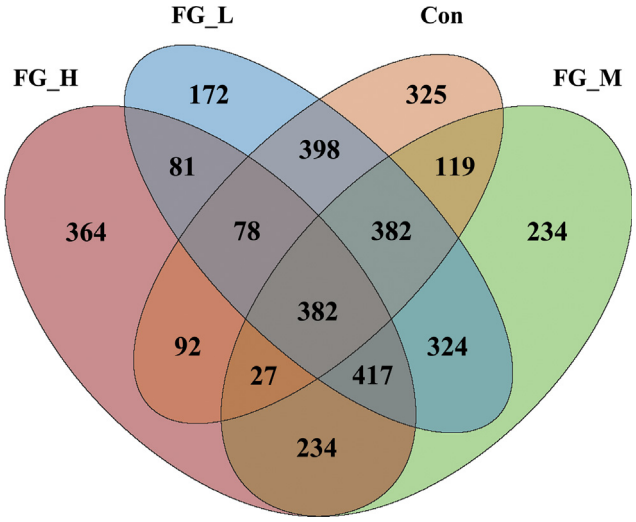

Fig. 2. Shared OTU among groups. Group FG_H: the group consuming a diet supplemented with 30% flaxseed gum after obese model built; Group FG_M: the group consuming a diet supplemented with 20% flaxseed gum after obese model built; Group FG_L: the group consuming a diet supplemented with 10% flaxseed gum after obese model built; Group Con: the group consuming a standard diet after obese model built.

dose-dependence with FG in diet. This result indicated *Clostridium* was the key genus for the degradation of FG. Actually, *Clostridium* has already been found in a high amount in mice which fed another type of gum (Takagi et al., 2016), which implied the ability of *Clostridium* in gum utilization. Of course, the function of these specific bacteria must be further investigated and confirmed in the future research.

Surprisingly, the contents of short-chain fatty acid (SCFA), an important microbial metabolite which exerted multi-organ effects on host energy metabolism (Canfora, 2015), among all groups except Group FG_H were similar. We inferred this result was attributed to different SCFA-producing microbes in various groups. The bacterial community compositions of Group Con and FG_L were somewhat similar (Fig. 1), and the genus *Lactobacillus*, *Ruminococcus*, *Oscillospira* and *Roseburia* in these two groups were responsible for high SCFA content because they all are SCFA-producing bacteria (Gophna, Konikoff, & Nielsen, 2017; Song et al., 2017; Zhao, Nian, Kwok, Sun, & Zhao, 2017). Different from these two groups, the higher RA of genus *Prevotella* and unclassified_*Elusimicrobiaceae* should be responsible for the high SCFA content in Group FG_M (de la Cuesta-Zuluaga et al., 2017; Geissinger et al., 2009). In addition, an extreme low content of butyrate were observed in Group FG_H, which might be attributed to tympanites and very little feeds consumption since we only took some sticky liquid with only a little solid when collecting samples. No dose-dependent effect was found between SCFA production and FG content. Therefore, the body weight controlling effect of FG was not achieved by SCFAs, even though they had been reported to have a putative role in reducing appetite and stimulating secretion of satiety hormones (Fetissov, 2017).

It should also be noted that, the 30% flaxseed gum replacement resulted in the extreme lowest content of SCFAs and reducing biodiversity in rat's cecum. Together with the highest RA of Proteobacteria, a phylum to which most of the pathogen belonged (Stecher, 2015), we believed that over amount of FG in diets could lead to undesirable effects, such as tympanites. The tympanites could be also responsible for body weight increment when rats consumed a diet containing 30% of FG.

5. Conclusions

A proper FG consumption could lower the body weights, body fat and total triacylglycerols, which could be attributed to the regulation of FG on gut microbiota by decreasing Firmicutes/Bacteroidetes ratio and altering some specific bacteria. Especially, the altered genus *Clostridium* was supposed to be the key one for flaxseed gum degradation. Moreover, SCFA may not be involved in rat's body weight loss.

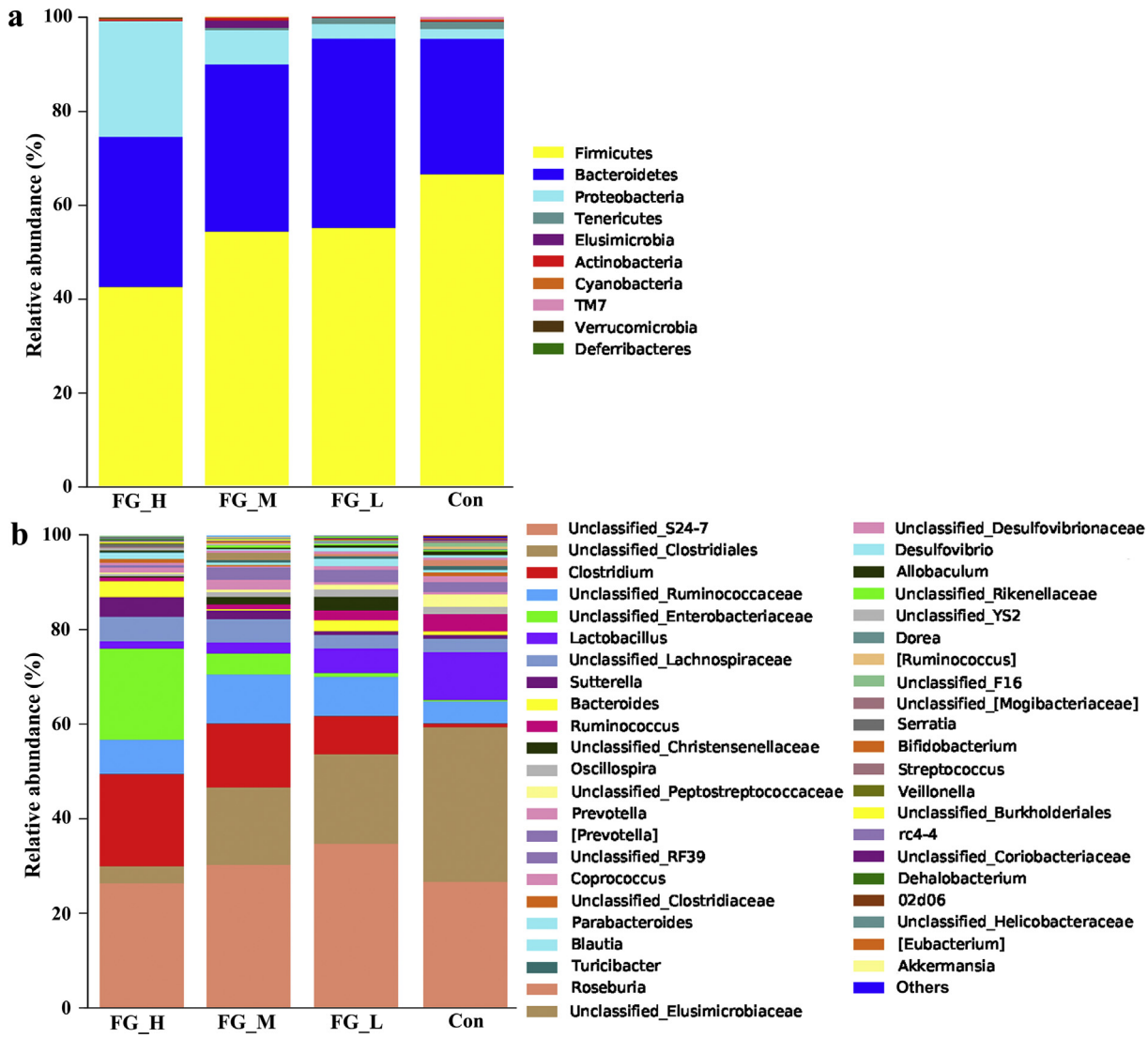

Fig. 3. The bar charts of relative abundances in each group on (a) phylum and (b) genus level. Group FG_H: the group consuming a diet supplemented with 30% flaxseed gum after obese model built; Group FG_M: the group consuming a diet supplemented with 20% flaxseed gum after obese model built; Group FG_L: the group consuming a diet supplemented with 10% flaxseed gum after obese model built; Group Con: the group consuming a standard diet after obese model built.

Conflict of interest

None.

Ethics statement

I have read and adhere to the Publishing Ethics.

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