读书报告

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2017年7月23日

Food Research International 97 (2017) 364–371

Contents lists available at ScienceDirect

Food Research International

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

Effects of the combination of ω-3 PUFAs and proanthocyanidins on the gut microbiota of healthy rats

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IF 3.086

ω-3 PUFAs

Eicosapentaenoic acid (EPA, 20:5, n-3)

Docosahexaenoic acid (DHA, 22:6, n-3)

Cardiovascular diseases (CVD)

EPA/DHA 1:1/1:2

→ Protein carbonylation ↓

→ Inflammation ↓

2:1 → Oxidative stress ↑

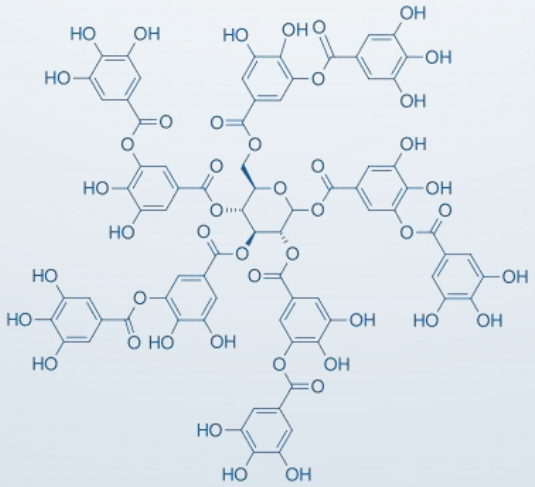
Gut dysbiosis

Polyphenols

Antioxidants can be divided into three major groups:

- **Carotenoids(** 类胡萝卜素**)**, which are discussed in greater detail in my "Basic Vitamin A Primer"
- **Allyl sulfides (** 烯丙基硫醚**)**, found in garlic and onions
- **Polyphenols (** 多酚**)**, (also known as phenolics)

Flavonoids	黄酮类
Stilbenes	芪类
Lignans	木脂素类
Phenolic acids	酚酸



抗氧化的秘密-葡萄籽中的原花青素

葡萄籽中含有丰富的抗氧化、抗衰老的营养成分-原花青素(OPC)，天然存在于各种植物中，具有多种生活活性，是目前国际上公认的清除人体内自由基最有效的植物抗氧化剂。抗氧化能力远远高于维生素C和维生素E，不仅可以延缓衰老，祛斑祛皱，还有防辐射，改善过敏等的作用。

Oligomerization **Proanthocyanidins**

Grape seed extract (GSE)

为什么需要营养补充品

ω-3 PUFAs + GSE

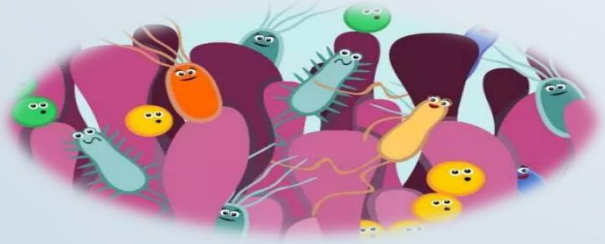


Table 1
Composition of experimental diets.

Ingredients (g)	STD	ω-3	GSE	ω-3 + GSE
Flour[a]	1000.0	1000.0	1000.0	1000.0
tert-butylhydroquinone	0.08	0.08	0.08	0.08
Porcine gelatin	25.0	25.0	25.0	25.0
Soybean lecithin	6.0	6.0	6.0	6.0
Soybean oil	17.4	–	17.4	–
ω-3 PUFAs[b]	–	17.4	–	17.4
Grajfnol®	–	–	0.88	0.88
Protein (% by weight)	16.4	16.4	16.4	16.4
Carbohydrate (% by weight)	46.6	46.6	46.5	46.5
Fat (% by weight)	6.2	6.2	6.2	6.2
Energy from protein (%)	21.3	21.3	21.3	21.3
Energy from carbohydrate (%)	60.5	60.5	60.5	60.5
Energy from fat (%)	18.2	18.2	18.2	18.2
Total energy density (kcal/g)[c]	3.1	3.1	3.1	3.1

[a] Standard flour (Teklad Global 2014).
[b] The amount of EPA/DHA was 25 mg/kg body weight.
[c] Energy density is estimated as *metabolizable energy* based on the Atwater factors, assigning 4 kcal/g to protein, 9 kcal/g to fat, and 4 kcal/g to available carbohydrate.

n = 7/group

STD, the control group;

ω-3, supplemented with EPA/DHA 1:1 (16.6 g/kg feed);

GSE, supplemented with 0.84 g GSE/kg feed;

ω-3 + GSE, supplemented with both EPA/DHA 1:1 and GSE.

Sample collection

After 0, 6, 12, 18 and 24 weeks

Plasma and urine

NO, NO2−

Fecal microbial subgroups

qRT-PCR

QIAamp® DNA Stool Mini Kit

All DNA samples were diluted to **20 ng/μL**.

qRT-PCR cycling conditions：
5 s at 95 ℃;
45 cycles of:
5 s at 95 ℃,
30 s at the primer-specific annealing temperature (Table 2),
30 s at 72 ℃ (extension)
melting curve analysis：
2 s at 95 ℃;
for 15 s at 65 ℃,
followed by a temperature gradient up to 95 ℃ at a rate of 0.11 ℃ /s, with five fluorescence recordings per ℃

Bacteroidetes and **Firmicutes** phyla,

the **Lactobacillales** and **Bifidobacteriales** orders,

the ***Lactobacillus plantarum***, ***Lactobacillus acidophilus*** and ***Bifidobacterium longum*** species

Short-chain fatty acids in feces

SCFAs (acetic acid, propionic acid, butyric acid and pentanoic acid)

HP-Innowax (30 m × 0.25 mm i.d. × 0.25 μm f.d.).

Injector : 240℃

FID : 240℃

Injection volume : 1 μL

Carrier gas : helium , 1 mL/min

The mode of injection : splitless

The oven temperature program :

50 ℃ (3 min), then slope 8 ℃ /min to 180 ℃ (0 min) and slope 50 ℃ /min to 200 ℃ (5 min).

Other conditions were: gas helium flow, 30 mL/min; hydrogen flow, 40 mL/min; and airflow 400 mL/min.

Results

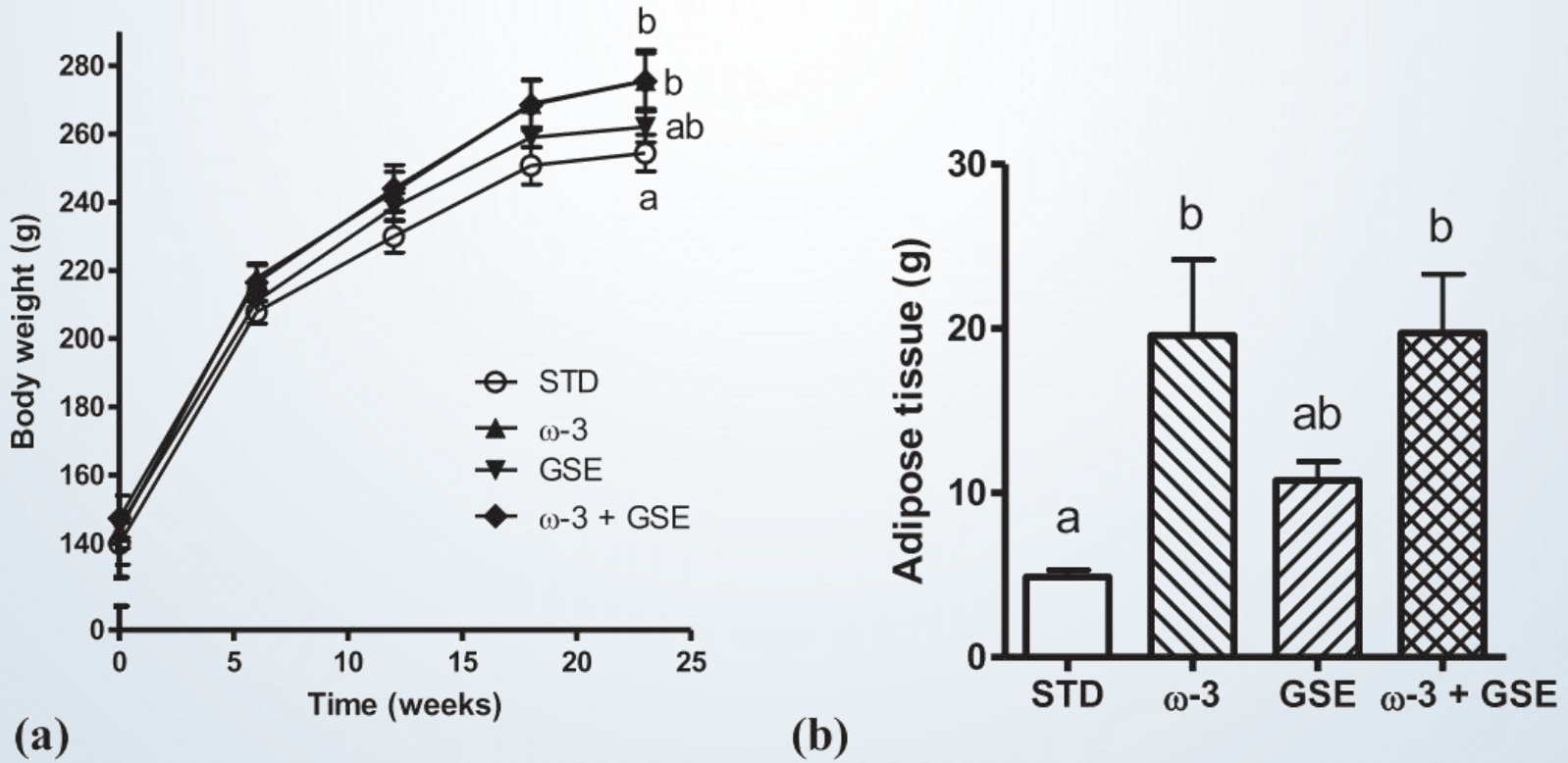


Fig. 1. Body weight (a) and perigonadal fat (b) of rats fed the different diets for 24 weeks: STD, ○; ω-3, ▲; GSE, ▼; ω-3 + GSE, ◆. The curves corresponding to the ω-3 and ω-3 + GSE groups are superimposed. The data represent means with their standard errors. Comparisons were performed using two-way ANOVA for repeated measures (a) or one-way ANOVA followed by Tukey's post-hoc test (b). Means with different letters differ, $P < 0.05$.

Table 3
CDV risk factors in rats supplemented with ω-3 PUFAs and/or GSE for 24 weeks.

	STD		ω-3		GSE		ω-3 + GSE	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Systolic pressure (mmHg)	123.5	4.0	123.2	2.4	114.8	3.6	117.2	3.1
Diastolic pressure (mmHg)	84.0	4.0	96.2	7.0	94.6	7.4	91.3	2.1
Plasma cholesterol (mmol/L)	4.7^{a}	0.2	3.8^{bc}	0.1	4.4^{ab}	0.1	3.5^{c}	0.2
Plasma HDLc (mmol/L)	1.5	0.1	1.6	0.1	1.5	0.1	1.5	0.1
Plasma LDLc (mmol/L)	0.4^{a}	0.1	0.4^{a}	0.0	0.7^{b}	0.0	0.6^{c}	0.0
Plasma triglycerides (mmol/L)	1.7	0.1	1.7	0.1	1.9	0.1	1.6	0.1
Plasma adiponectin (μg/mL)	21.7^{a}	2.9	30.0^{ab}	2.1	22.0^{a}	2.8	32.1^{bc}	1.5
Urine nitrites (ng/mL)	1.3	0.3	2.5	0.9	1.5	0.1	2.1	0.2

Means with different letters differ, $P < 0.05$. Comparisons were performed using one-way ANOVA and Tukey's post-hoc tests.

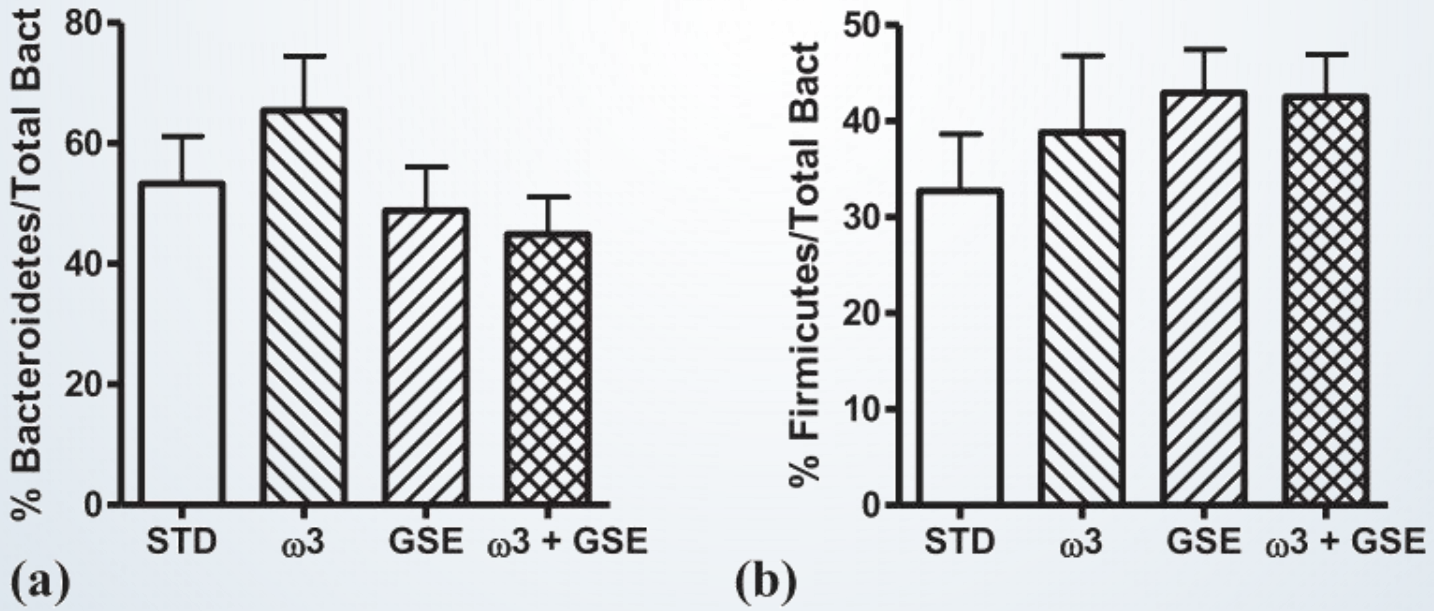
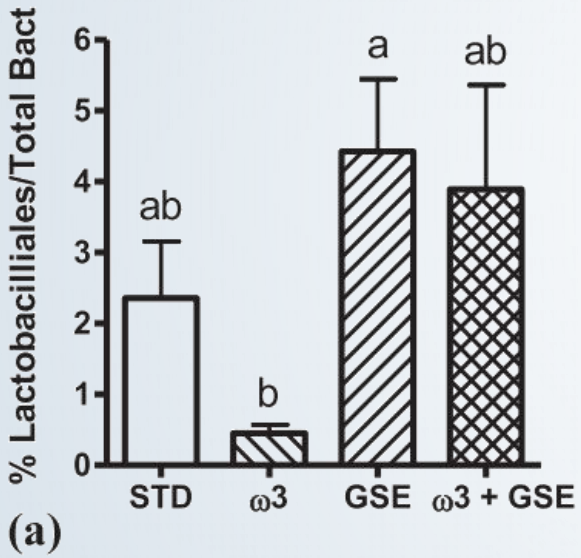
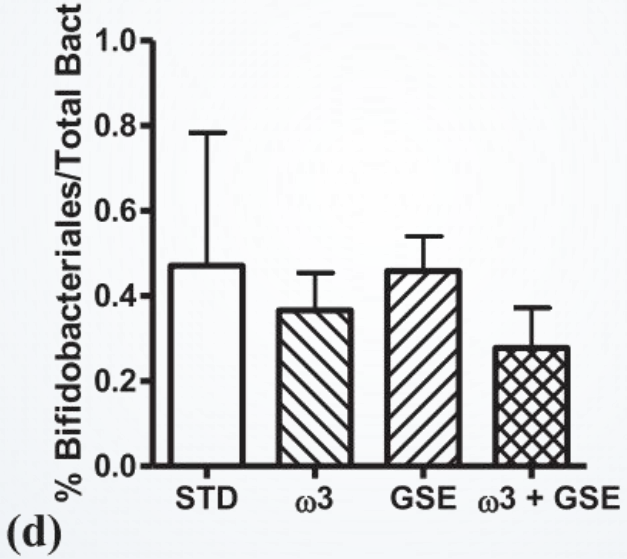
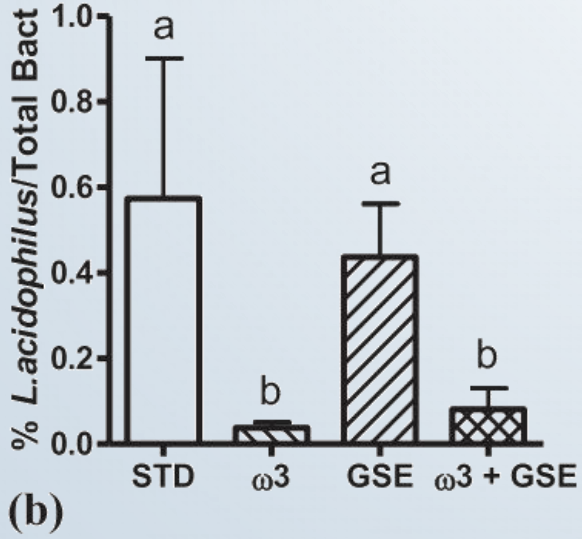
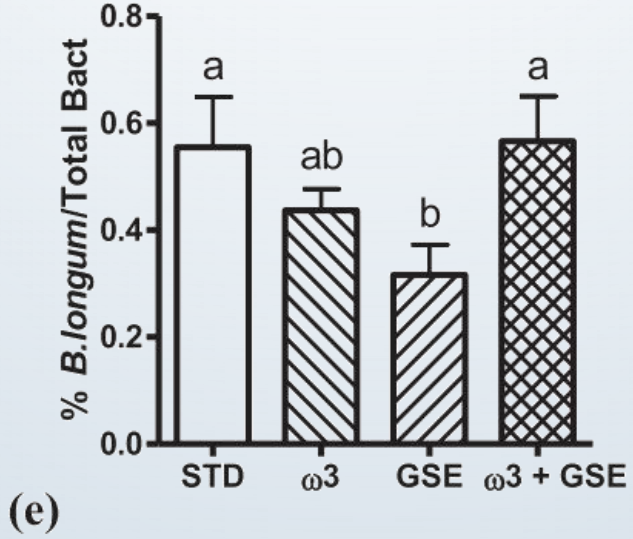


Fig. 2. Bacteroidetes (a) and Firmicutes (b) in fecal samples from rats fed the different diets (STD, ω-3, GSE, or ω-3 + GSE) for 23 weeks. The data represent means with their standard errors. Comparisons were performed using one-way ANOVA followed by Tukey's post-hoc test.


% Lactobacilliales/Total Bact
6
5
4
3
2
1
0
ab
b
a
ab
STD
ω3
GSE
ω3 + GSE
(a)


% Bifidobacteriales/Total Bact
1.0
0.8
0.6
0.4
0.2
0.0
STD
ω3
GSE
ω3 + GSE
(d)


% L.acidophilus/Total Bact
1.0
0.8
0.6
0.4
0.2
0.0
a
b
a
b
STD
ω3
GSE
ω3 + GSE
(b)


% B.longum/Total Bact
0.8
0.6
0.4
0.2
0.0
a
ab
b
a
STD
ω3
GSE
ω3 + GSE
(e)

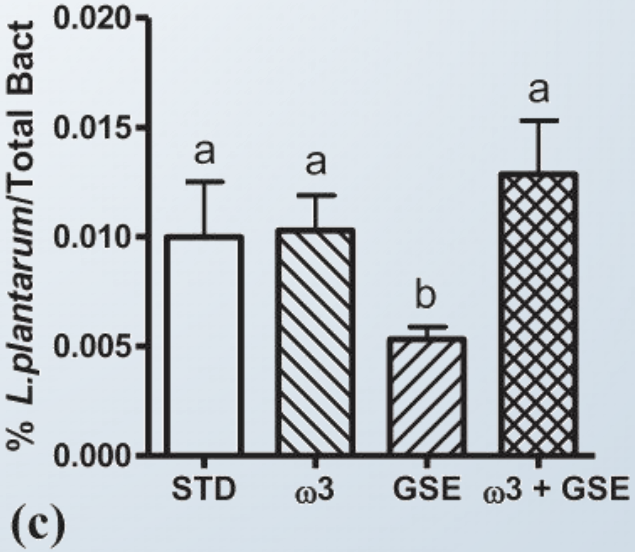

% L.plantarum/Total Bact
0.020
0.015
0.010
0.005
0.000
a
a
b
a
STD
ω3
GSE
ω3 + GSE
(c)

Table 4
Short-chain fatty acids determined in feces from rats supplemented with ω-3 PUFAs and/or GSE for 23 weeks.

	STD		ω-3		GSE		ω-3 + GSE	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Acetic acid	5.54[a]	1.26	9.10[b]	1.36	6.03[a]	1.62	4.48[a]	1.89
Propionic acid	1.18	0.43	1.47	0.54	1.11	0.51	1.24	0.63
Butyric acid	0.26	0.07	0.31	0.11	0.30	0.14	0.24	0.14
Valeric acid	0.15[ab]	0.07	0.21[a]	0.08	0.09[b]	0.05	0.10[b]	0.02

SCFA content is expressed as mmol of caproic acid equivalents/g dry feces. Means with different letters differ, $P < 0.05$. Comparisons were performed using one-way ANOVA and Tukey's post-hoc tests.

24-wk supplementation
Female Wistar Kyoto rats

ω-3 Fish Oil
(EPA/DHA 1:1)

↓ Cholesterol & ↑ Adiponectin
but
↓ Lactobacillus & ↑ SCFA

Grape Seed Extract
(Proanthocyanidins)

↓ Bifidobacteria

COMBINATION

↓ Cholesterol & ↑ Adiponectin
and
Equilibrated microbiota
Standard levels of SCFA

Mol. Nutr. Food Res. 00, 0, 2017, 1601082 DOI 10.1002/mnfr.201601082 (1 of 14) 1601082

RESEARCH ARTICLE

Grape seed proanthocyanidin extract ameliorates inflammation and adiposity by modulating gut microbiota in high-fat diet mice

IF 4.323

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[2] Laboratory of Endocrinology and Metabolism, Institute of Health Sciences, Shanghai Institutes for Biological Sciences (SIBS), Chinese Academy of Sciences (CAS) & Shanghai Jiao Tong University School of Medicine (SJTUSM), Shanghai, China

Grape seed proanthocyanindin extract (GSPE)

Anti-inflammatory

Induced obesity

Identified the contribution of the gut microbiota

Materials and methods

7 weeks

Male 8-week-old C57BL/6

4 weeks of acclimatization

Three groups with four cages in each group (2–3 mice per cage, n = 10–12 in each group)

NCD+P (BS)

HFD+P (BS)

HFD+GS (PE)

daily gavage with 300 mg/kg body weight GSPE

Treated with antibiotics Abx, 1.0 g/L ampicillin [Sigma, USA] and 0.5 g/l neomycin [Sigma, USA] in drinking water three days

Oral gavage

GSPE (HFD+GSPE+Abx)

PBS (HFD+PBS+Abx)

Glucose tolerance test

2 g/kg body weight by intraperitoneal injection;

Glucose levels were measured with whole blood from the tail vein at 0, 15, 30, 60, and 120 min using a glucose meter (LifeScan, USA).

Insulin resistance test

After 6h of food deprivation;

Intraperitoneal injection

1 IU/kg body weight human insulin (Novolin R, Denmark)

Morphological studies

Epididymal white adipose tissue (**eWAT**)

4% neutral buffered formalin

Anti-F4/80 primary antibody (Abcam, British)； Immunohistochemical staining

Biochemical analyses

TNF-α , MCP-1 and IL-6 ELISA kit (Millipore, USA)

Gut microbiota qPCR quantification

QIAamp Fast DNA stool Mini Kit (Qiagen, Germany)

QuantSutdio Real-time PCR Instrument (Applied Biosystems, USA)

SYBER Green Supermix (Takara, Japan)

10 μL reactions（2× SYBER Green Supermix, 10 μM of each primer and 40 ng of genomic DNA）

2−ΔΔCT method

RNA isolation and real-time PCR

Supporting information Table1. Primers for quantitative real-time PCR.

Target	Forward primer sequence (5'-3')	Reverse primer sequence (3'-5')
Proteobacteria	TCG TCA GCT CGT GTY GTG A	CGT AAG GGC CAT GAT G
Roseburia spp.	AAG CGA CGA TCA GTA GCC GA	TTC TTC TTC CCT GCT GAT AGA G
Roseburia inulinivorans	TCT GAC CGG ACA GTA ATG TG	CGC TGG CTA CTG GGG ATA AG
Universal bacteria	GTG STG CAY GGY YGT CGT CA	ACG TCR TCC MCN CCT TCC TC
mF4/80	CTTTGGCTATGGGCTTCCAGTC	GCAAGGAGGACAGAGTTTATCGTG
mCd68	CTTCTGCTGTGGAAATGCAA	AGAGGGGCTGGTAGGTTGAT
mMcp1	CAGCCAGATGCAGTTAACGC	GCCTACTCATTGGGATCATCTTG
mG6pc	CGACTCGCTATCTCCAAGTGA	GTTGAACCAGTCTCCGACCA
mPck1	CAGGATCGAAAGCAAGACAGT	AAGTCCTCTTCCGACATCCAG
m36b4	GAAACTGCTGCCTCACATCCG	GCTGGCACAGTGACCTCACACG

Protein preparation and western blot

Target proteins： Akt, p-Akt (Ser473), p-JNK, JNK, IkB-α.

Internal control： Heat shock protein 90 (HSP90).

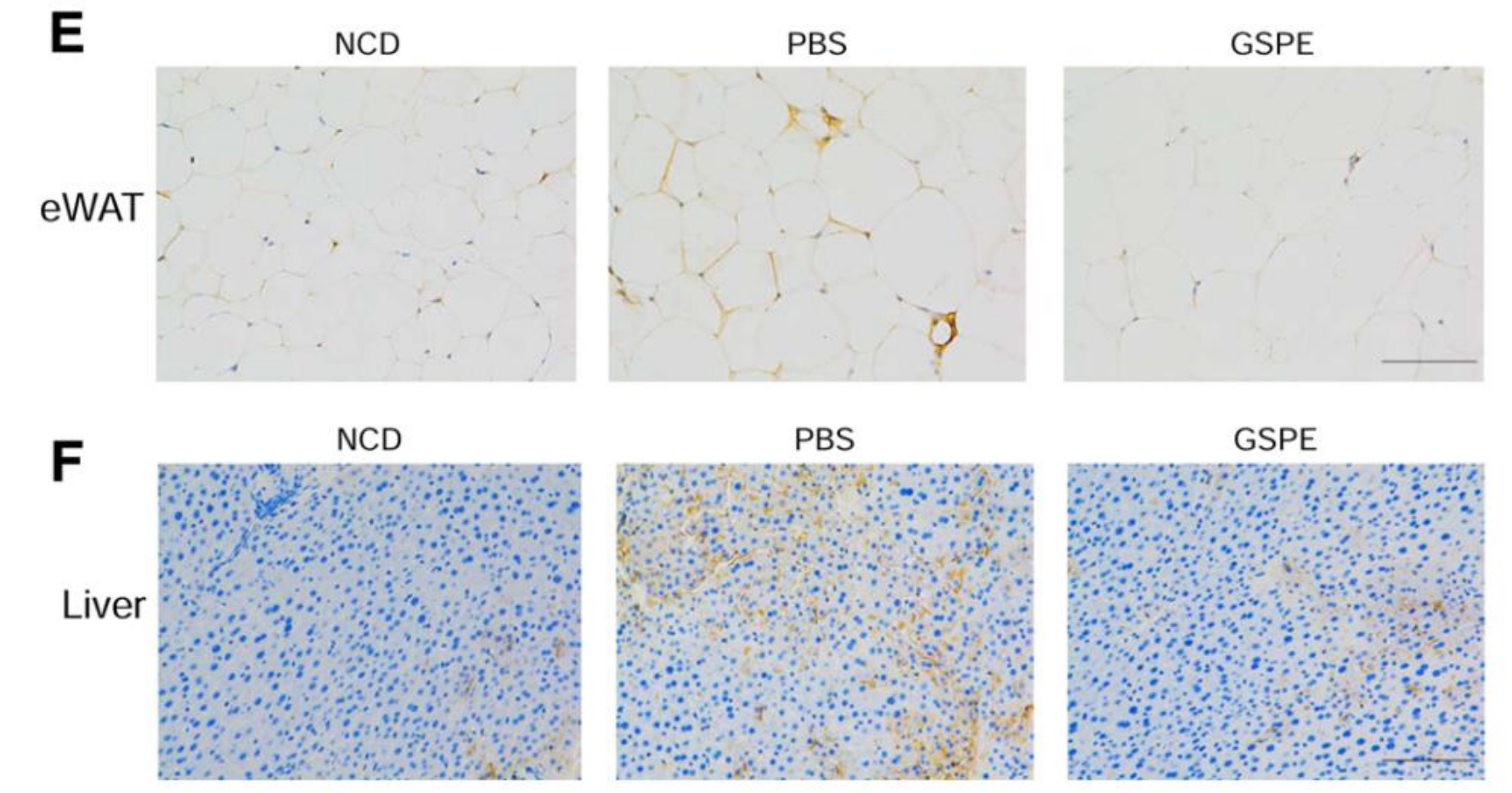
16S rDNA gene sequencing and analysis

Illumina MiSeq platform

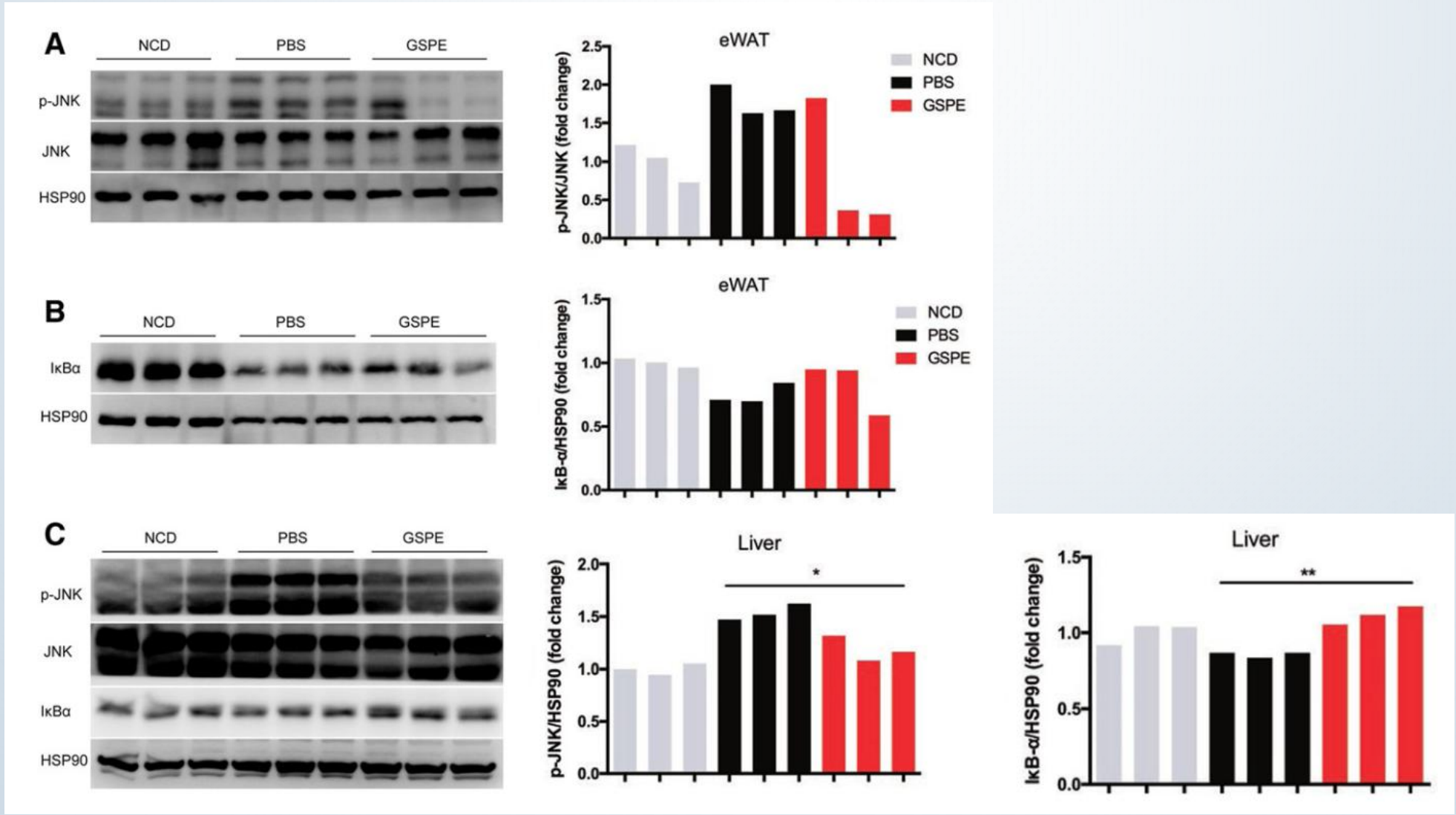
341F (5'-CCTACGGGRSGCAGCAG-3') and 806R (5'-GGACTACVSGGGTATCTAAT-3')

Results

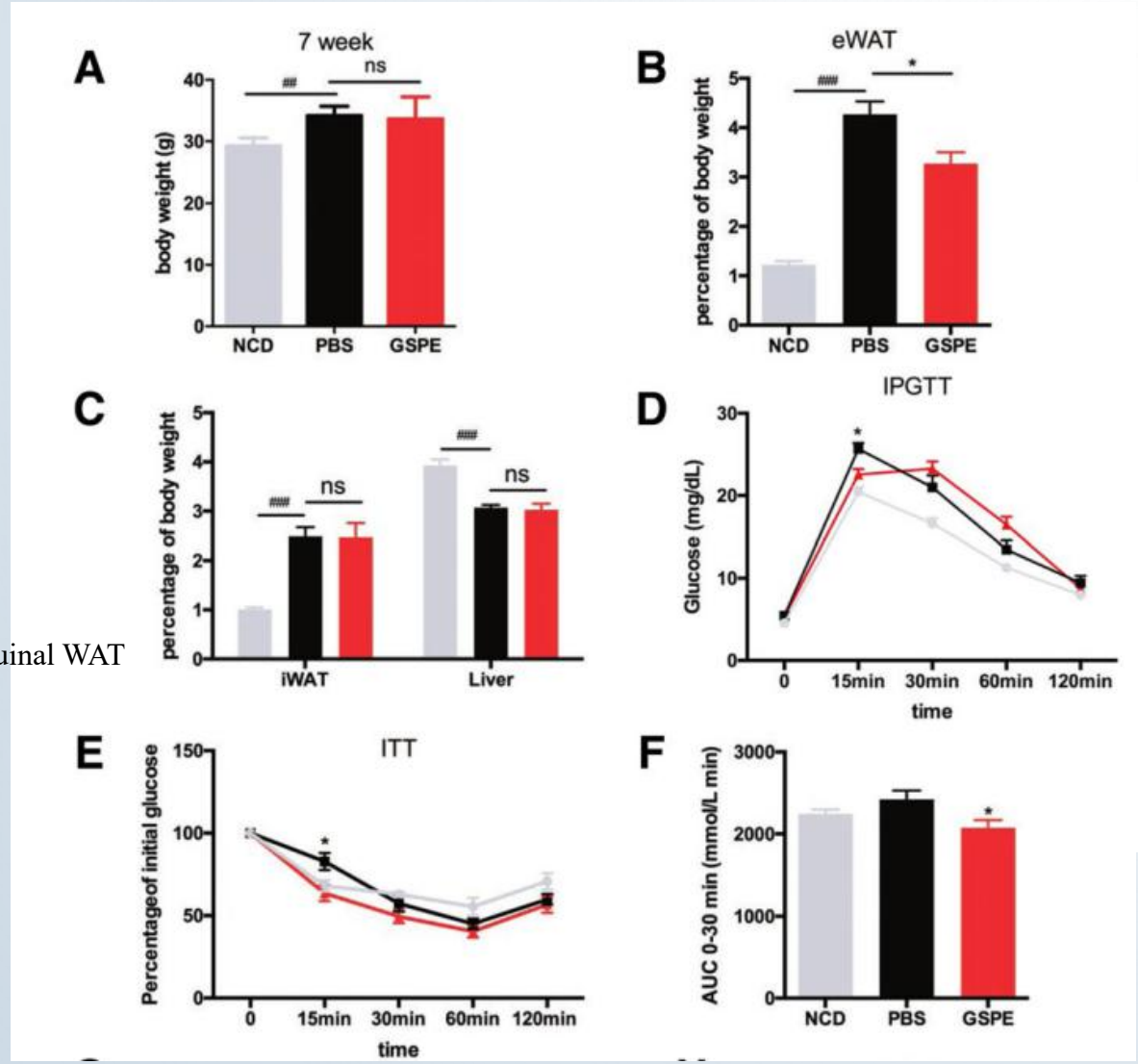
1. GSPE reduces inflammation in HFD-fed mice



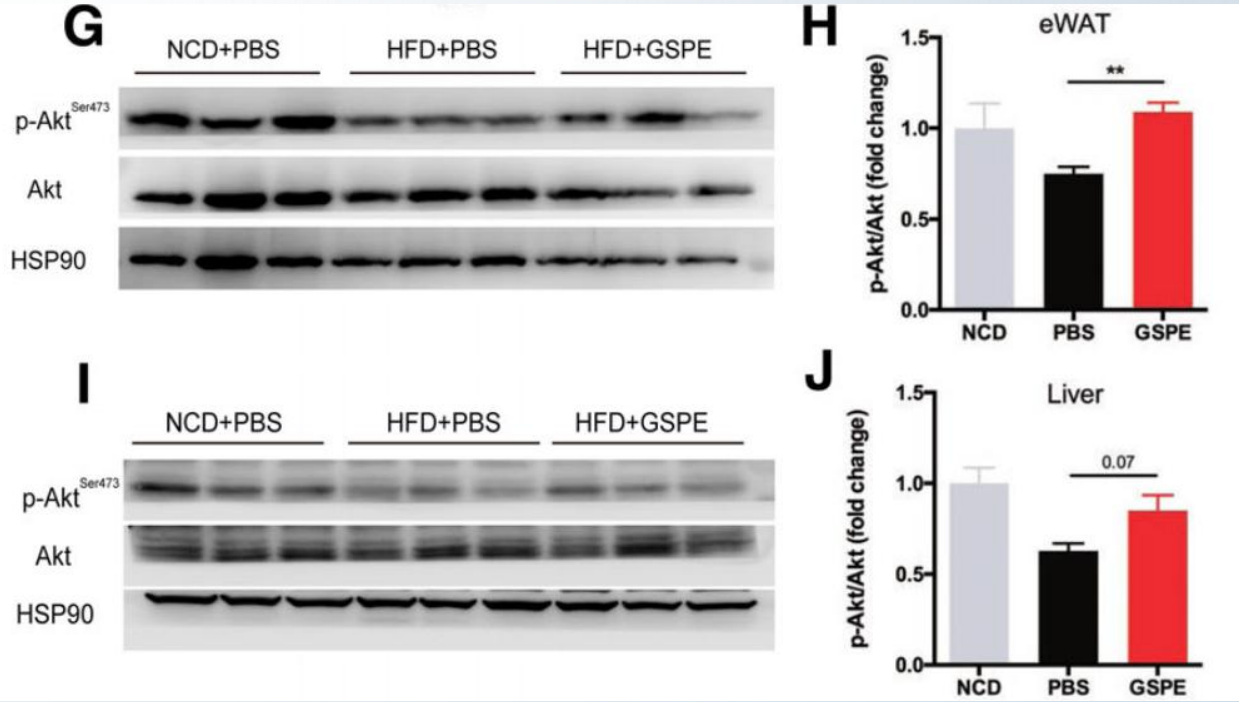
2. GSPE inhibits JNK and NF-kB signaling pathways and ameliorates inflammation



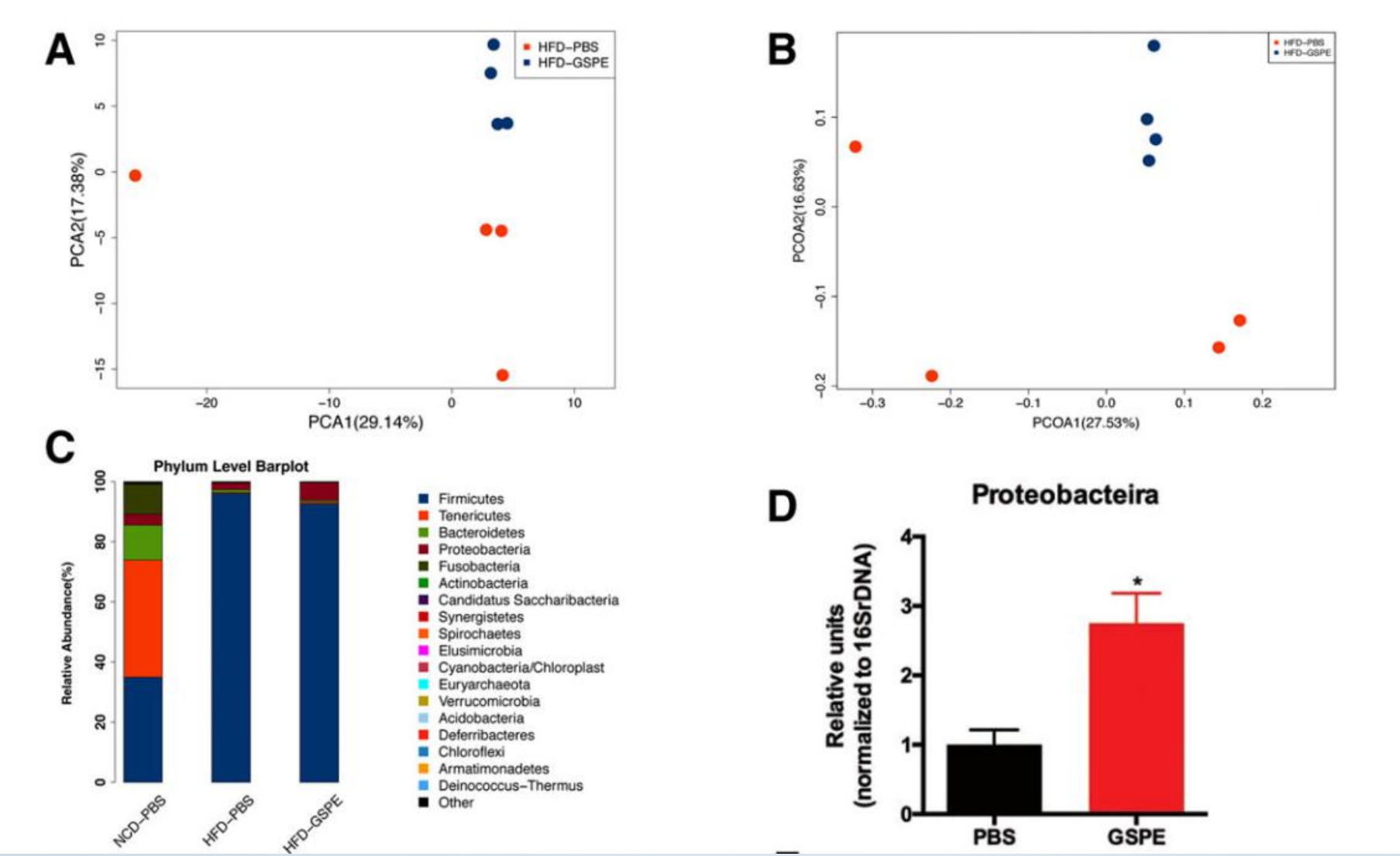
3. GSPE improves HFD-induced metabolic disorders

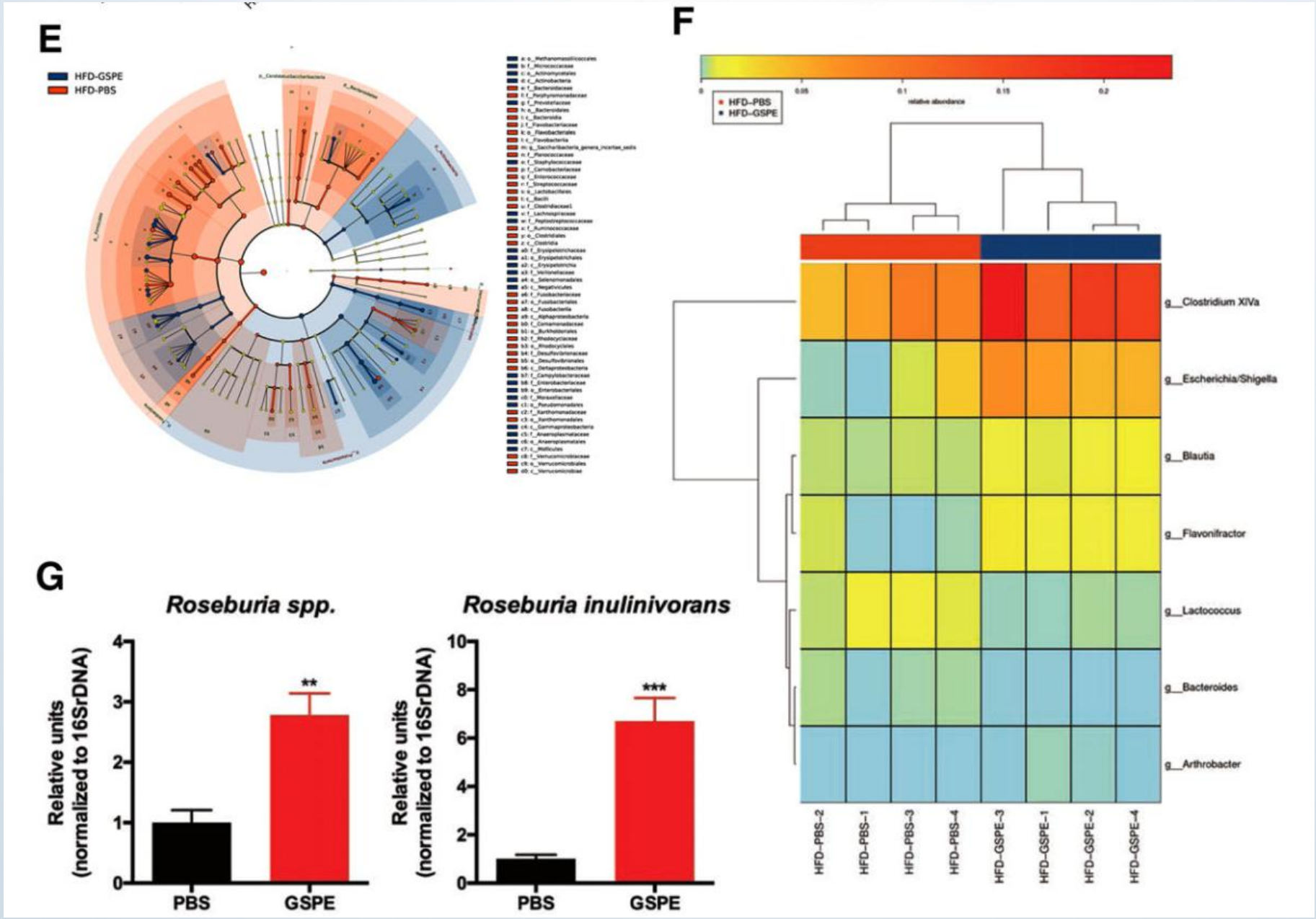


inguinal WAT



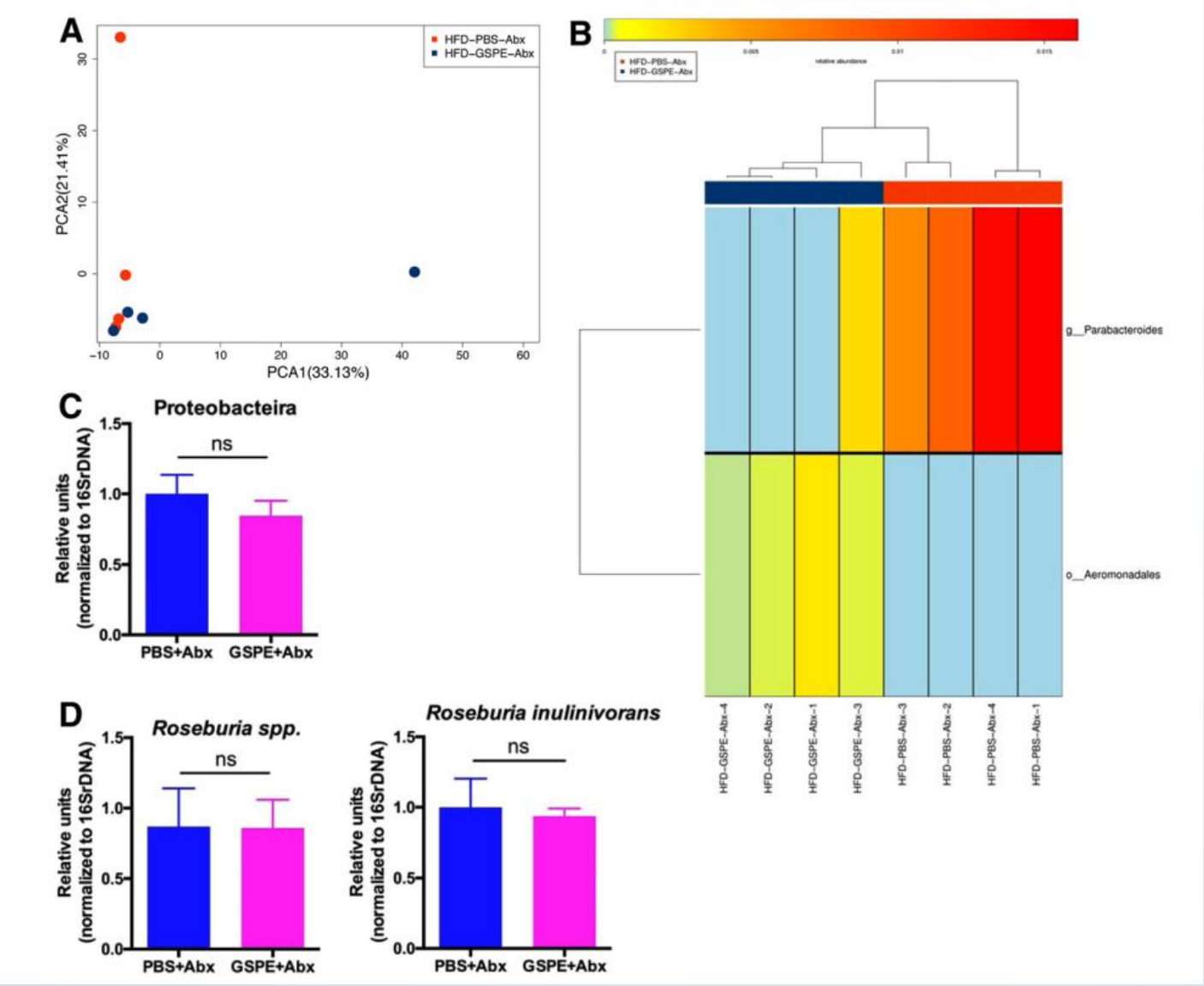
4. GSPE supplementation changes the composition of gut microbiota




E
HFD-GSPE
HFD-PBS
F
HFD–PBS
HFD–GSPE
relative abundance
g__Clostridium XlVa
g__Escherichia/Shigella
g__Blautia
g__Flavonifractor
g__Lactococcus
g__Bacteroides
g__Arthrobacter
HFD–PBS–2
HFD–PBS–1
HFD–PBS–3
HFD–PBS–4
HFD–GSPE–3
HFD–GSPE–1
HFD–GSPE–2
HFD–GSPE–4
G
Roseburia spp.
Roseburia inulinivorans
Relative units
(normalized to 16SrDNA)
**
***
PBS
GSPE

产丁酸盐

5. Effect of GSPE on metabolic improvement partially depends on gut microbiota



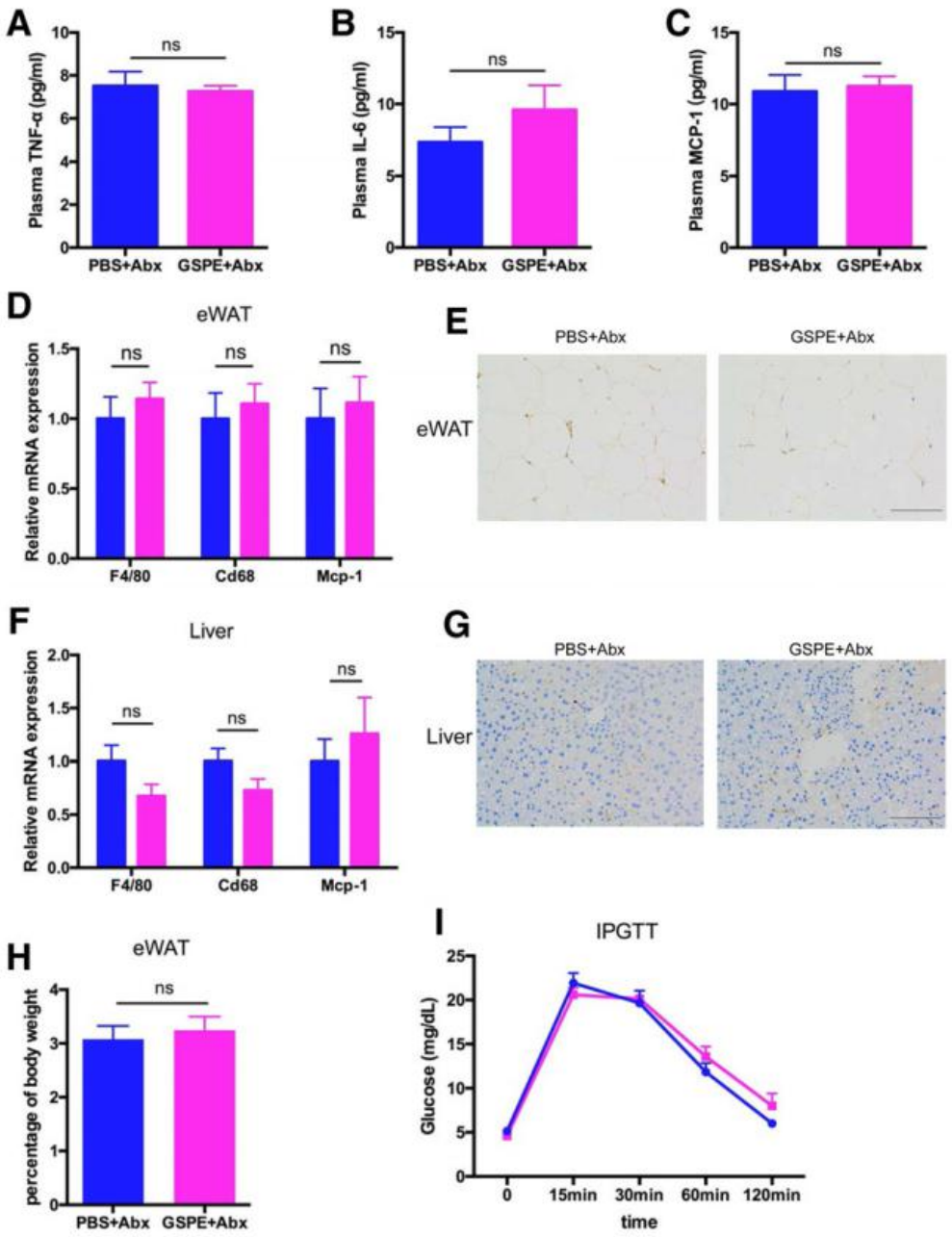


Figure 6. Depleting the microbiota counteracts the metabolic improvement of GSPE supplementation. (A) Plasma TNF-α levels (n = 10–12 per group, data were shown as mean±SEM). (B) Plasma IL-6 levels (n = 10–12 per group, data were shown as mean±SEM). (C) Plasma MCP-1 levels (n = 10-12 per group, data were shown as mean±SEM). (D) Real-time PCR results of *F4/80, Cd68,* and *Mcp-1* in eWAT. (E) Representative F4/80 immunostaining image of eWAT. (Scale bar, 10 μm; blue, nuclei; yellowish, F4/80). (F) Real-time PCR results of *F4/80, Cd68, and Mcp-1* in liver (n = 10–12 per group, data were shown as mean±SEM). (G) Representative F4/80 immunostaining image of liver. (Scale bar, 10μm; blue, nuclei; yellowish, F4/80). (H) Relative weight of eWAT (n = 10-12 per group, data were shown as mean±SEM). (I) Glucose tolerance test (n = 10–12 per group, data were shown as mean±SEM). Mice were fed a HFD supplemented with PBS (Blue) or GSPE (Rose red) under antibiotics treatment for 7 weeks.

谢 谢 ！