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RESEARCH ARTICLE

Fucoidan Alleviates Intestine Damage in Mice Induced by LPS via Regulation of Microbiota

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ABSTRACT

Fucoidan is a popular polysaccharide known to have important biological functions. In the present research, the therapeutic effect of fucoidan on gastrointestinal illness induced by lipopolysaccharide (LPS) in mice was determined. A total of thirty ICR mice were divided into three groups, including group C, M and Y. Mice in group Y were treated with fucoidan (200 mg/kg b.w.) for 14 days, while mice in groups C and M received normal saline. On day 15, the mice in group M and Y were challenged with LPS @10mg/kg b.w. by intra-peritoneal injection. After 24 hours, the mice in all groups were euthanized and different samples were collected for analysis. Results showed that fucoidan decreased the weight loss in mice to a certain degree and alleviated the intestinal damage caused by LPS by improving ratio of villus length/crypt depth, through increasing villus length and decreasing crypt depth in mice. It was established that mice treated with fucoidan had a significantly higher levels of T-AOC (P<0.01), GSH-Px (P<0.001), IL-10 (P<0.001) and SOD (P<0.01) and a significantly lower levels of MDA (P<0.001) and TNF-α (P<0.05), indicating that fuciodan may have protective effect against oxidative damage by LPS. Microbiota analysis revealed that the number and abundance of phyla and genera found in the microbiome of mice in group Y were close to those of mice in group C, indicating that fucoidan may promote the biodiversity of microbiota in mice. Taken together, the fucoidan may have protective effect against intestinal damages in mice by regulating oxidation resistance, inflammatory response and improving microbiota.

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INTRODUCTION

Botanicals and herbs are widely used in food, health and production aspects of both humans and animals (Ghazwani *et al*., 2023; Hussain *et al*., 2023; Prakoso *et al*., 2023). In traditional Chinese medicine, many herbs are recognized as food and medicine that include *Ganoderma lucidum*, *Chinese wolfberry*, *Rhodiola* and *Brown algae* etc. Among these, *Brown algae* is an important source in global food system which is widely utilized in foods of humans and animals as biological energy sources, health care products and pharmaceuticals (Rashed *et al.,* 2022). As, it produces important bioactive compounds, more researchers are paying attention to *Brown algae* to promote its antioxidant and anti-inflammatory abilities in humans (Rashed *et al.,* 2022). Fucoidan is a long chain polysaccharide commonly reported in brown algae. This carbohydrate is also found in several seaweed and marine animals like sea cucumber, sea urchin and abalone (Van Weelden *et al.,* 2019). Recent studies showed that this indigestible polysaccharide derived from brown algae could promote

host health by regulating immune system, intestinal function, microbiota, limiting inflammatory response, and controlling lipid metabolism, obesity and diabetes (Rashed *et al.,* 2022).

There are billions of microorganisms (bacteria, funguses, protozoa, archaea and viruses) that reside in the intestine making up gut microbiota (Sittipo *et al.,* 2018; Lei *et al.,* 2023). These enteric microorganisms interact with host and play an import role in food digestion and nutrients absorption, inhibiting pathogenic growth and regulating intestinal immunity (Song *et al.,* 2021). Gut microbiota dysbiosis is commonly observed in diarrhea (Li *et al.,* 2021), nonalcoholic fatty liver disease (Fang *et al.,* 2022) and inflammatory bowel disease (Haneishi *et al.,* 2023).

Lipopolysaccharide (LPS) are essential components in gram-negative bacteria causing serious inflammatory reactions during bacterial infections (Sun and Shang 2015). The release of heat-stable LPS in host leads to serious health problems like fever, phlegmonosis, diarrhea and asthma which sometime could be fatal (Tang *et al.,* 2021). Previous reports have documented that LPS causes microbiota imbalance in mice and piglets (Xu *et al.,* 2021). However, very less information is available about the effect of fucoidan on microbiota imbalance caused by LPS. In the present study, an inflammatory reaction was induced in Mice by injecting LPS and the effect of fucoidan on gastrointestinal illness induced by LPS was investigated through several analyses, such as determination of cytokines and oxidation resistance, histpathology and microbiome analysis.

MATERIALS AND METHODS

Experimental design: Thirty (n=30) ICR (Institute of Cancer Research) mice of both sexes having four-weeks age (19.57±1.16) were purchased from experimental animal center of Yangzhou University, China. All the mice were kept on a standard 12h light/dark schedule having free access to feed and water. After three days of acclimatization, the mice were randomly and equally divided into three groups, viz. group C (non-treated/non-infected), group M (non-treated/infected) and group Y (treated/infected). Mice of group Y were treated with fucoidan @ 200 mg/kg (Boer Chemical Reagent Co., LTD, Shanghai, China) by gavage, and group C and M were given equal amount of normal saline for fourteen days. On the 15th day, the mice in group M and Y were challenged with LPS @ 10mg/Kg by intra-peritoneal injection for induction of gastrointestinal illness. After 24 hours, all the mice were euthanized by carbon dioxide method. Serum was harvested and fecal samples from ileum and rectum were taken and kept at -80 ℃ till future study.

Inflammatory cytokine and Oxidation resistance analysis: The interleukins (6, 10, 1β) and tumor necrosis factor-alpha in ICR mice were detected through ELISA kits by following manufacturer's instructions (Solarbio Science and Technology, China). The antioxidant abilities, including total antioxidant capacity, superoxide dismutase, glutathione peroxidase and malondialdehyde in mice were examined by employing assay kits (Jiancheng Bioengineering Institute Co., Ltd, Nanjing, China).

Histopathology: Ileum tissues from all mice groups were collected and fixed in four percent paraformaldehyde for over two days. Then, the tissues were embedded with paraffin embedding technique and subjected to hematoxylin-eosin staining (Zhu *et al.,* 2022a). Pathological analysis was carried out under piloting Olympus SZ61 microscope (Olympus, Japan).

Microbiome analysis: FastPure microbiome DNA isolation kits (Vazyme, China) were used for extraction of genomic DNA from rectum tissues of mice (n=5) in all groups and then concentration and quality of those products were checked by NanoDrop one Ultra microspectrophotometer (Thermo Scientific, USA). 16S rRNA gene (V3-4 targeting region) of microbiome was amplified by employing primers of 338F/806R (Xia *et al.,* 2022) and the amplified products were also checked for determination of concentration and quality. Those products were used to generate sequencing libraries through Hieff NGS (Yeasen, China), followed by sequencing of amplicons via Illumina NovaSeq platform (Wuhan, China).

The raw data filed in QIIME2 (Rai *et al.,* 2021) and then used to generate amplicon sequence variants and taxonomy table (Harbuzov *et al.,* 2022; He *et al.,* 2023). The alpha diversity of ICR mice' microbiota was examined by QIIME2 as mentioned to explore the microbial diversity within an individual mouse (Palmieri *et al.,* 2022). Microbiota structural variation between different ICR mice groups was calculated by analyzing of nonmetric multidimensional scaling, partial least squares discriminant and principal coordinate according to previous studies (Mekadim *et al.,* 2022). The microbiome evolutionary analysis was conducted through ggtree in R package (Yu *et al.,* 2018). Distinguished species between different ICR animal groups were revealed by employing LEfSe and DEseq2 (Eriksson *et al.,* 2022). The function differences of ICR mice microbiota among the groups was analyzed by targeting KEGG via BLAST search with databases of MetaCyc and ENZYME.

Statistical analysis: The statistical difference of values among the ICR groups was analyzed by considering $P<0.05$ as level of significance through chi-square test, ANOVA and Dunn test by using BM SPSS Statistics (SPSS 22.0).

RESULTS

Effect of fucoidan on mice body weight: The changes in the daily body weight were found in ICR mice in this study (Fig. 1a). The average body weight in mice of group Y were slightly higher than that in those of other groups. With the induction of LPS in mice, the body weight dropped significantly in group M (6.31%) and Y (2.58%) compared to the weight of mice in group C (2.01%) (Fig. 1b).

Protective effect of fucoidan on the intestinal damages in mice induced by LPS: Pathological analysis showed that LPS significantly damaged intestine villi, whereas treatment with fucoidan alleviated the damages caused by LPS (Fig. 1c). The villus length/crypt depth ratio and villus length in group M was smaller than those in group

C (P<0.0001), while the crypt depth was longer in group C (P<0.0001) (Fig. 1c). Moreover, fucoidan increased villus length/crypt depth ratio and villus length in ICR mice whereas, crypt depth was decreased.

Effect of Fucoidan on the levels of inflammatory factors and antioxidant abilities: As shown in Fig. 2, a significantly lower level of T-AOC (P<0.001), SOD (P<0.0001), GSH-Px (P<0.001) and IL-10 (P<0.001) in mice of group C was observed, while a higher level of MDA (P<0.0001) and TNF-α (P<0.01) were observed in this group. Interestingly, the mice supplemented with fucoidan exhibited lower MDA (P<0.001) and TNF-α (P<0.05) levels, and higher T-AOC (P<0.01), SOD (P<0.01), GSH-Px (P<0.001) and IL-10 (P<0.001) levels.

Sequencing data analysis: The average raw and filtered data in current ICR mice were 91954 and 84786 in group C, 97801 and 91181 in group M, and 80644 and 74731 in group Y (Table 1). The filtered data was aligned to 2171 ASVs, among which 218 ASVs were shared in three groups (Fig. 3a). Alpha diversity shows that chao1 (P<0.01) and observed features (P<0.01) in group C were remarkably increased than group M. Shannon entropy was higher in group C (P<0.001) and Y (P<0.05) (Fig. 3b).

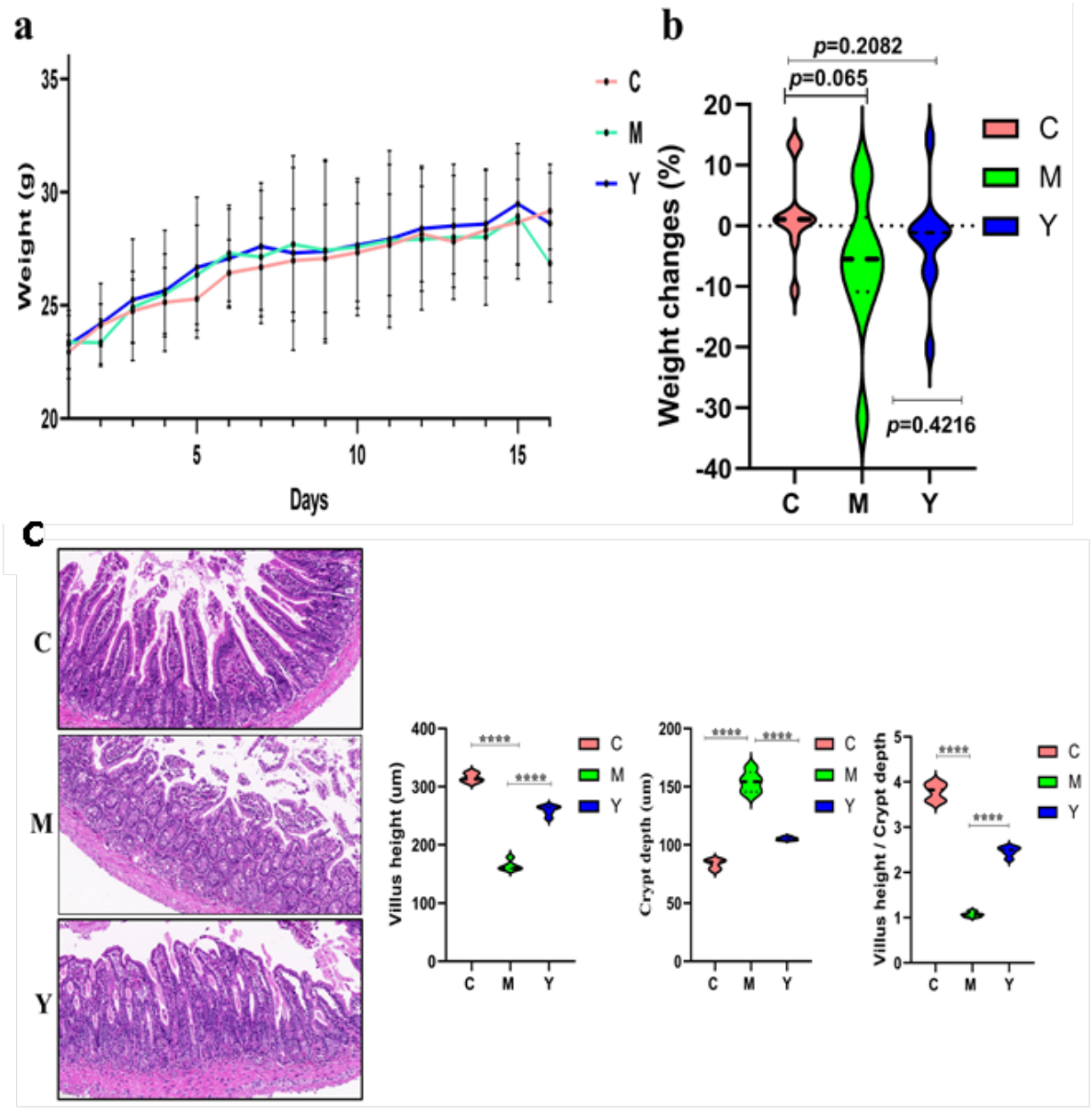

Fig. 1: The body weights and histopathological findings of mice. The results were presented as Mean ± SEM, (n=10). (a) Average daily body weights, (b) Body weight changes from day 15 to day 16, (c) histopathology.

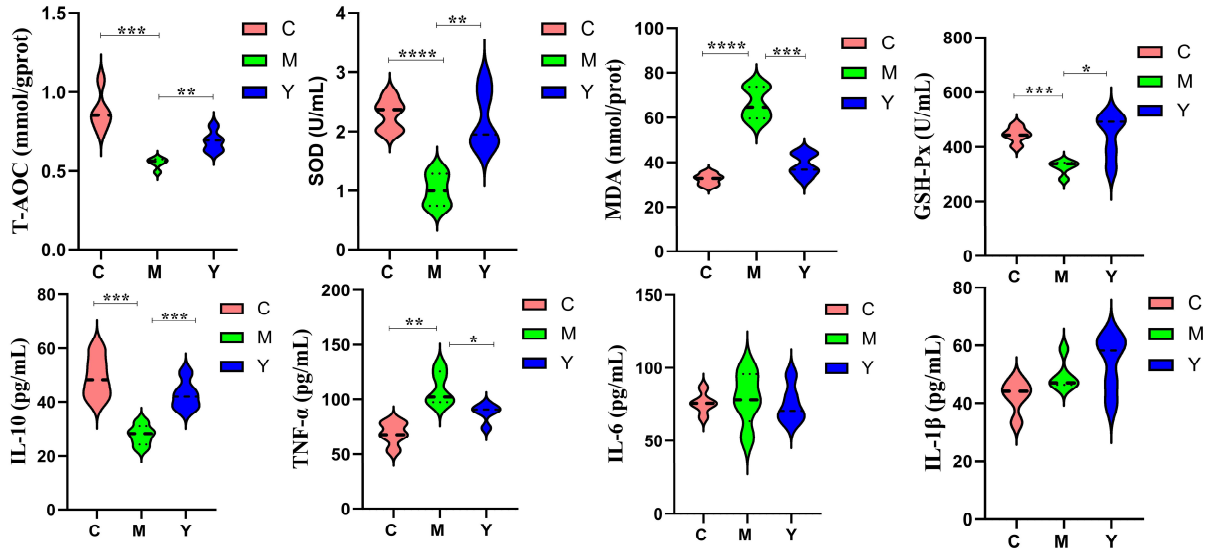

Fig. 2: The effect of Fucoidan on inflammatory factor and antioxidant abilities in ICR mice. The results were described as Mean ± SEM, (n=5). *P<0.05, **P<0.01, ***P<0.001, ***P<0.0001.

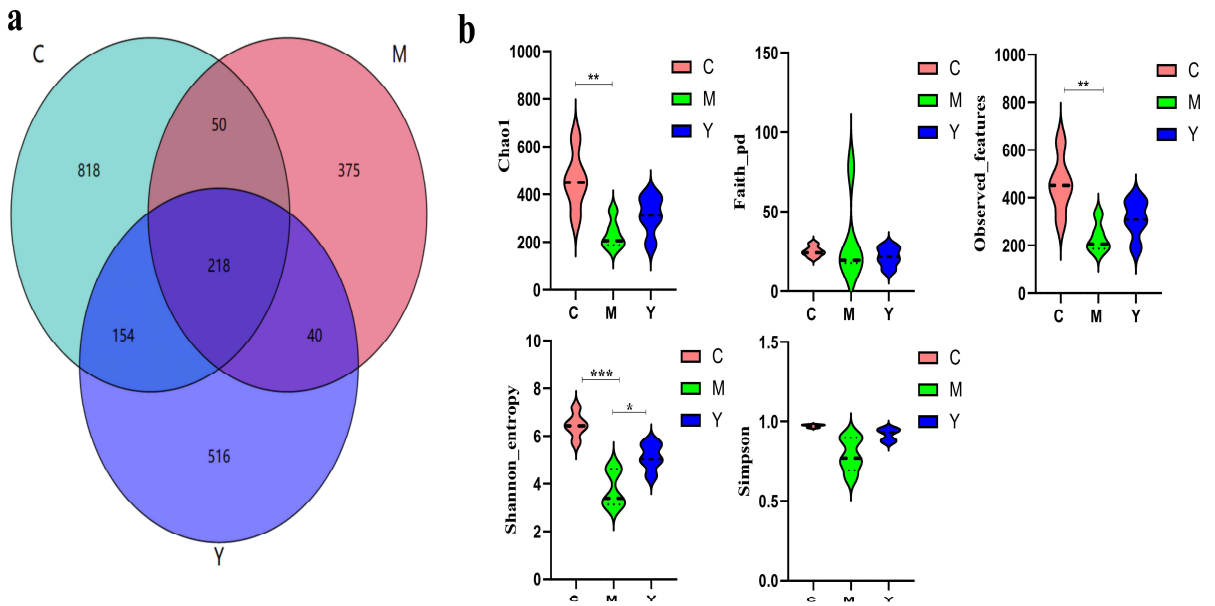

Fig. 3: Annotation findings through ASV venn map (a), and Alpha diversity index (b).

Table 1: Statistical analysis of the generated sequence data in ICR mice

Samples	input	filtered	percentage of input passed filter	denoised	merged	percentage of input merged	non-chimeric	percentage of input non-chimeric
C1	85371	78813	92.32	77932	74627	87.41	69126	80.97
C2	90674	83238	91.80	82564	80003	88.23	76685	84.57
C3	82386	76494	92.85	75419	71007	86.19	67044	81.38
C4	91866	84393	91.87	83602	80493	87.62	68435	74.49
C5	109473	100996	92.26	99773	92940	84.90	80220	73.28
M1	101609	94375	92.88	93768	91700	90.25	80703	79.43
M2	79649	74414	93.43	73589	68793	86.37	54486	68.41
M3	106026	98992	93.37	97878	90796	85.64	70490	66.48
M4	103170	95745	92.8	94742	89821	87.06	71666	69.46
M5	98552	92380	93.74	90806	83003	84.22	60953	61.85
Y1	89050	83219	93.45	82852	81906	91.98	80744	90.67
Y2	63068	57939	91.87	57510	56642	89.81	55267	87.63
Y3	54874	50782	92.54	50437	49789	90.73	49501	90.21
Z4	103739	96296	92.83	95848	95119	91.69	94236	90.84
Y5	92492	85422	92.36	85009	84483	91.34	80882	87.45

Comparative microbiome analysis: Bacteroidetes, Firmicutes and Proteobacteria were the main phyla in group C (50.91%, 38.72% and 4.71%), M (20.50%, 18.75% and 58.51%) and Y (38.50%, 26.98% and 18.96) (Fig. 4a). At the class level, *Bacteroidia* (50.81%) and *Clostridia* (16.94%) and *Bacilli* (16.64%) were the primary classes found in the GIT of mice in group C. *Gammaproteobacteria* (56.71%), *Bacteroidia* (20.37%) and *Bacilli* (17.26%) were the dominant classes in mice of group M, while *Bacteroidia* (38.50%), *Bacilli* (20.74%) and *Gammaproteobacteria* (17.10%) were the staple classes in group Y (Fig. 4b). At order level, *Bacteroidales* (50.81%), *Clostridiales* (16.94%) and *Lactobacillales* (12.92%) were the major classes in group C whereas, *Bacteroidales*, *Enterobacteriales* and *Enterobacteriales* were the prime classes in M (20.37%, 56.63% and 17.08%) and Y (38.50%, 17.10% and 20.36%) (Fig. 4c). At the family level, S24_7 (43.08%), *Lactobacillaceae* (12.69%) and *Lachnospiraceae* (5.48%) were mainly present in group C, while S24_7, *Enterobacteriaceae* and *Lactobacillacea* were the staple families in group M (12.01%, 56.63% and 16.66%) and Y (30.41%, 17.10% and 20.20%) (Fig. 4d). The dominating genera in group C were *Lactobacillus* (12.69%), *Allobaculum* (4.96%) and *Prevotella* (4.19%), in group M were *Lactobacillus* (16.66%), *Enterobacter* (15.00%) and *Escherichia* (10.05%), and in group Y were *Lactobacillus* (20.20%), *Akkermansia* (10.17%) and *Enterobacter* (5.76%) (Fig. 4e).

Major bacteria species in the microbiota of experimental mice: Beta diversity analyses of mice' microbiota indicated that samples in group Y were found taxonomically closer to group C, while the distance between group M and C was analyzed as farther via PCA (Fig. 5a), PCoA (Fig. 5b), NMDS (Fig. 5c) and Qiime 2β (Fig. 5d). LEfSe analysis indicated that the abudancy of phyla TM7 was higher in C (P<0.01) and that of *Proteobacteria* was higher in M (P<0.05) (Fig. 6a). At the genus level, the abudancies of *Ruminococcaceae* (P<0.05), *Lactobacillus* (P<0.05), *Rikenella* (P<0.05), *Mycobacterium* (P<0.05), *Ruminococcus* (P<0.05), *Odoribacter* (P<0.05), *Prevotellaceae* (P<0.05), *Coprococcus* (P<0.05), *Actinomycetales* (P<0.05), *Prevotella* (P<0.05), *Oscillospira* (P<0.05) and *Mycobacteriaceae* (P<0.05) were memorably distant in group C, while the abundancies of *Leuconostocaceae* (P<0.05), *Sphingobacteriaceae* (P<0.05), *Pasteurellales* (P<0.05), *Achromobacter* (P<0.05), *Sphingobacteriia* (P<0.05), *Aggregatibacter* (P<0.05), *Sphingobacteriales* (P<0.05), *Sphingobacterium* (P<0.05), *Bacteroidaceae* (P<0.05), *Gammaproteobacteria* (P<0.05), *Pasteurellaceae* (P<0.05), *Bacteroides* (P<0.05) and *Weissella* (P<0.05) were higher in group M. *Alcaligenaceae* (P<0.01), *Burkholderiales* (P<0.01), *Betaproteobacteria* (P<0.01) and *Sutterella* (P<0.01) were enriched in group Y (Fig. 6b). DESeq2 volcano plot indicated that compared with phyla in C, the abudancies of *Proteobacteria* (P<0.0001) in M, *Spirochaetes* (P<0.001) and *Fusobacteria* (P<0.01) in group Y were lower, while TM7 (P<0.0001) and Tenericutes (P<0.01) in C and *Actinobacteria* (P<0.05) in group Y were found enriched (Fig. 7a). At the genus level, the abundancies of *Escherichia* (P<0.0001), *Enterobacter* (P<0.0001),

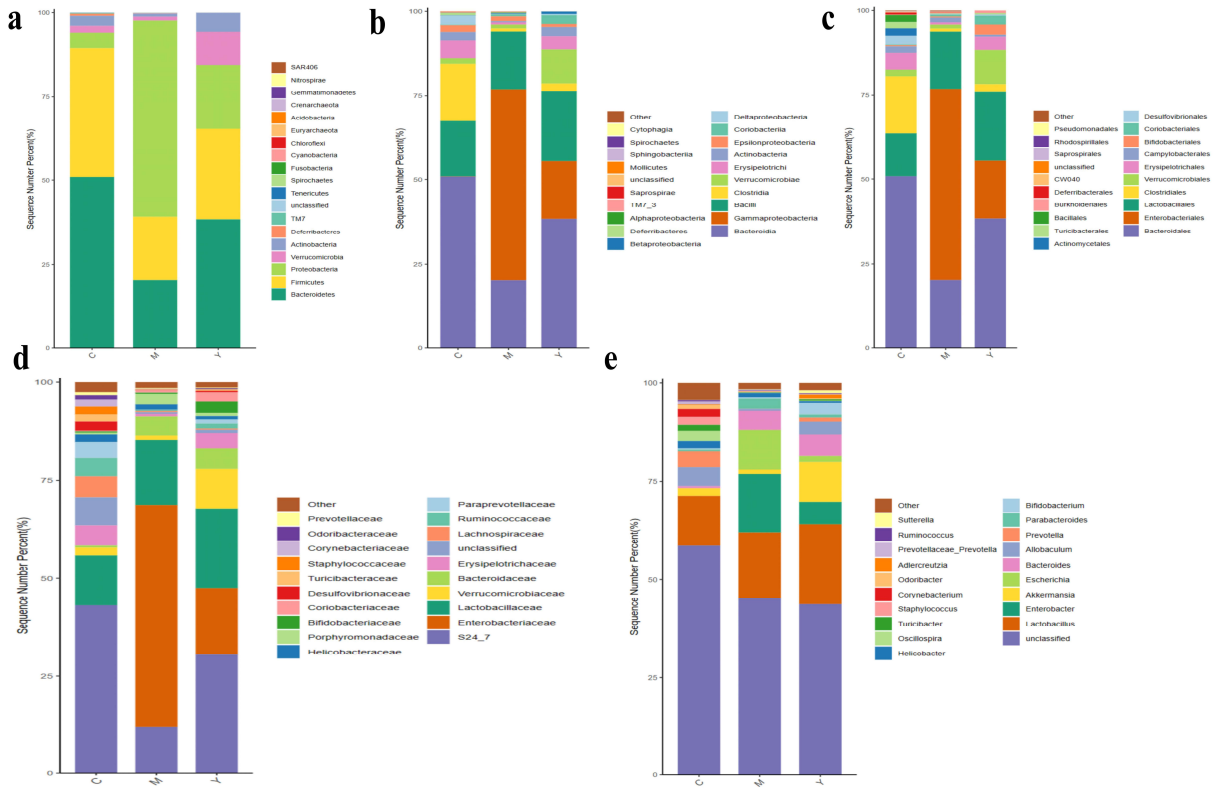

Fig. 4: Comparison of microbiota taxa among various groups. (a) Phylum, (b) Class, (c) Order, (d) Family, (e) Genus.

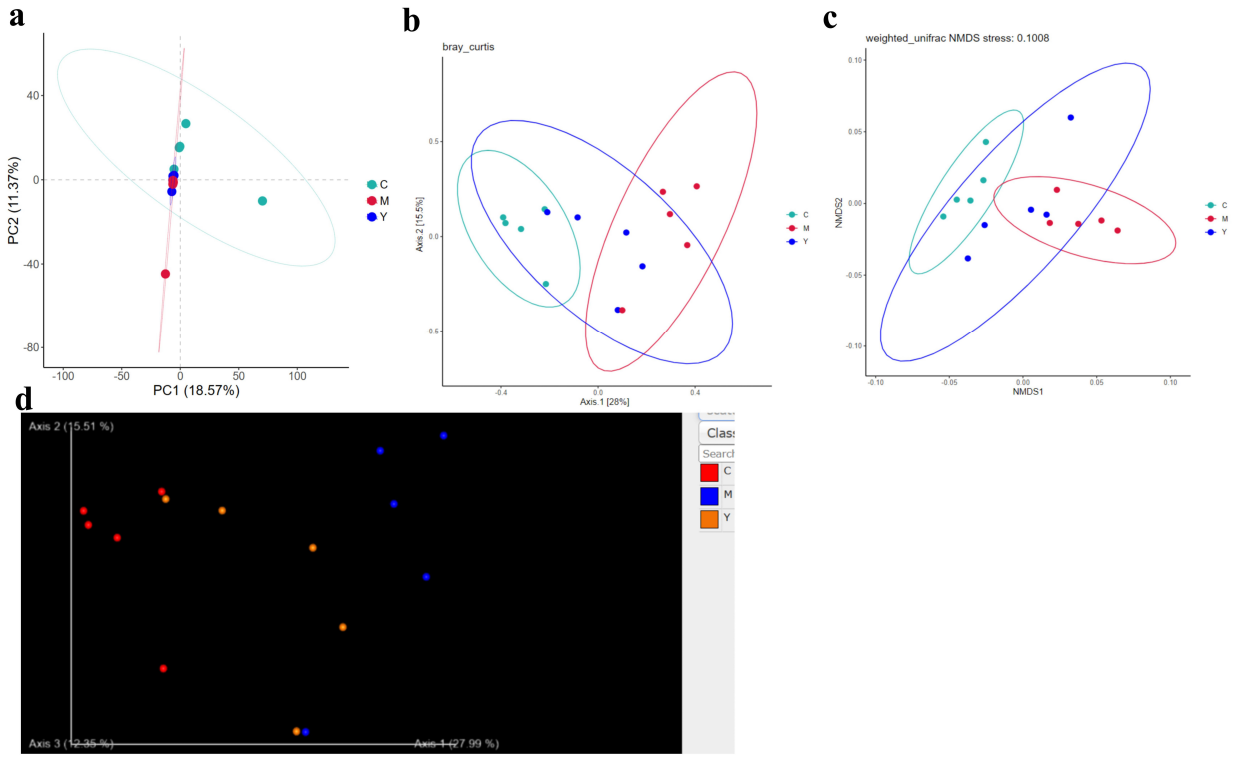

Fig. 5: Beta diversity analysis among ICR mice groups. (a) PCA, (b) PCoA, (c) NMDS, (d) Qiime 2β.

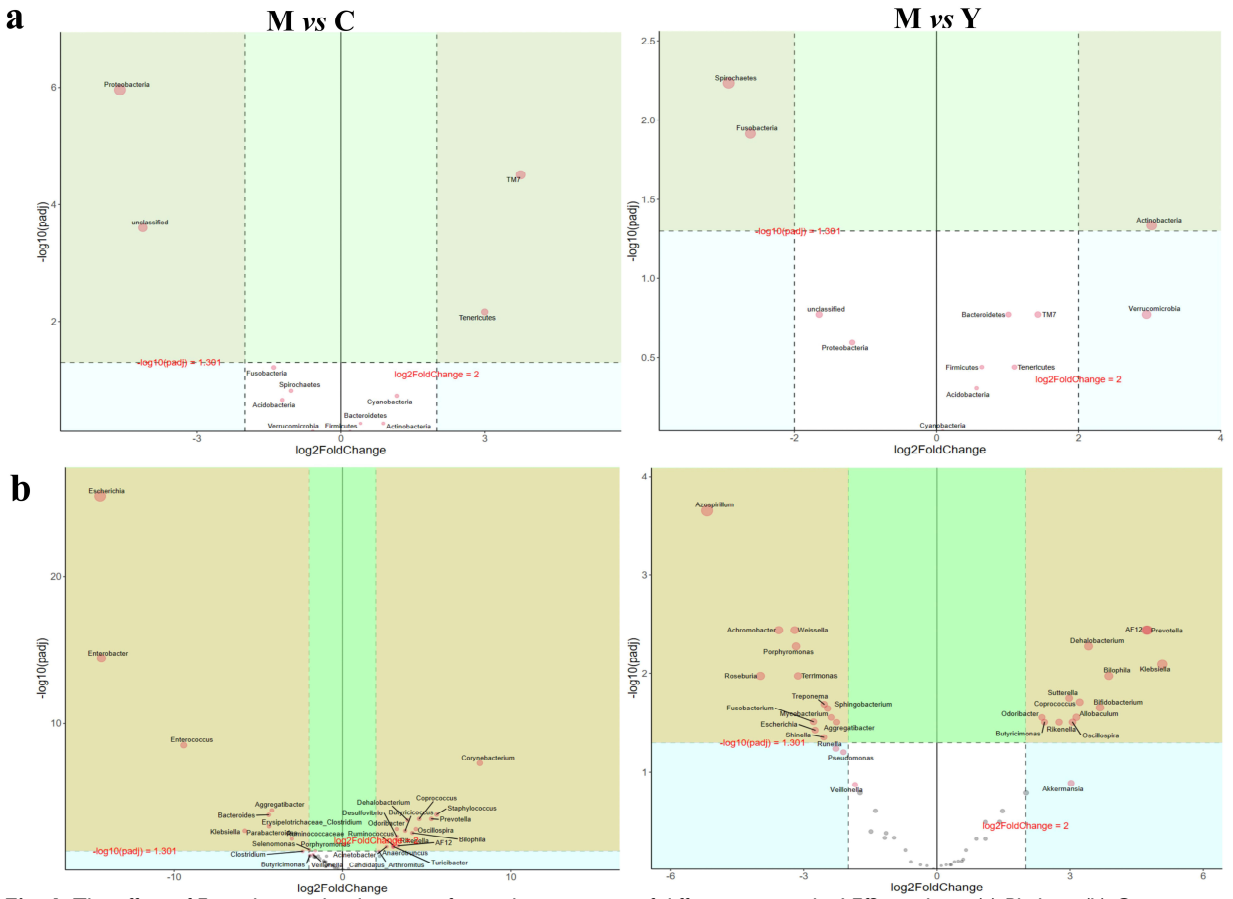

Fig. 6: The effect of Fucoidan on the diversity of microbiota in mice of different groups by LEfSe analysis. (a) Phylum, (b) Genus.

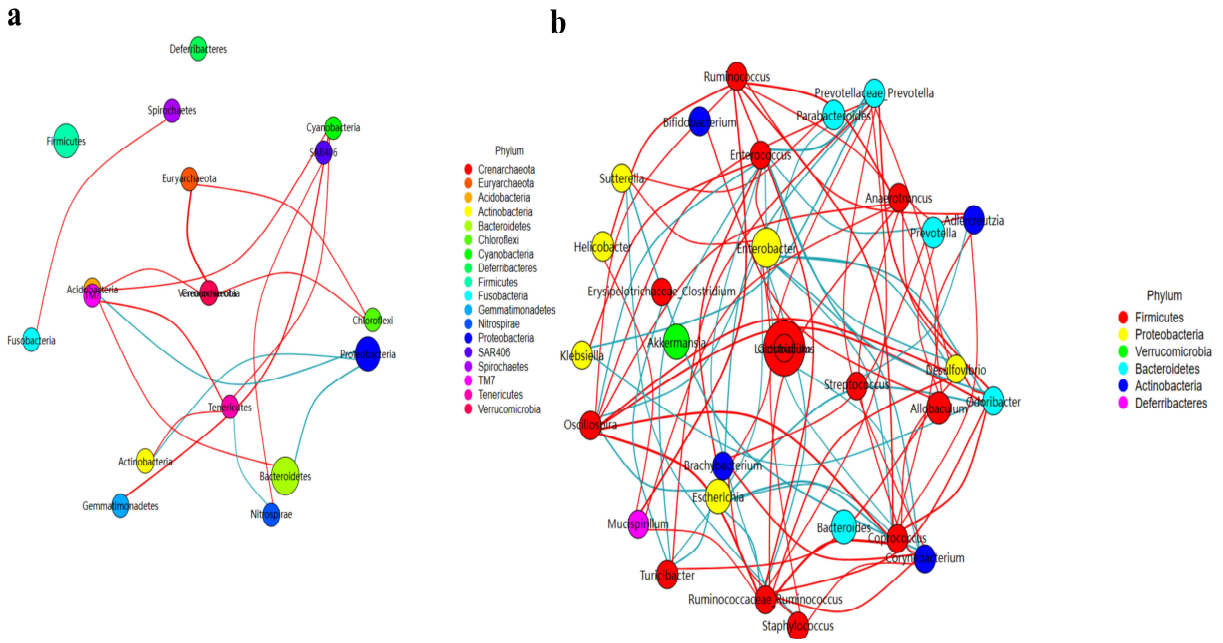

Fig. 7: The effect of Fucoidan on diversity of microbiota in the three groups via DESeq2 volcano plot. (a) Phylum, (b) Genus.

Enterococcus (*P*<0.0001), *Aggregatibacter* (*P*<0.0001), *Bacteroides* (P<0.001), *Erysipelotrichaceae_Clostridium* (P<0.01), *Klebsiella* (*P*<0.01), *Parabacteroides* (*P*<0.01), *Selenomonas* (*P*<0.05) and *Porphyromonas* (*P*<0.05) were lower in mice of group C compared to that in mice of group M, whereas *Corynebacterium* (*P*<0.0001), *Staphylococcus* (*P*<0.001), *Prevotella* (*P*<0.001), *Coprococcus* (*P*<0.001), *Dehalobacterium* (*P*<0.001), *Odoribacter* (*P*<0.01), *Oscillospira* (*P*<0.01), *Butyricicoccus* (*P*<0.01), *Bilophila* (*P*<0.01), *Desulfovibrio* (*P*<0.01), *Rikenella* (*P*<0.01), *Ruminococcaceae_Ruminococcus* (*P*<0.05), AF12 (*P*<0.05), *Turicibacter* (*P*<0.05), *Acinetobacter* (*P*<0.05) and *Anaerotruncus* (*P*<0.05) were enriched in group C. Compared with mice in group M, the abundancies of *Azospirillum* (*P*<0.001), *Weissella* (*P*<0.01), *Achromobacter* (*P*<0.01), *Porphyromonas* (*P*<0.01), *Terrimonas* (*P*<0.05), *Roseburia* (*P*<0.05), *Treponema* (*P*<0.05), *Sphingobacterium* (*P*<0.05), *Mycobacterium* (*P*<0.05), *Fusobacterium* (*P*<0.05), *Aggregatibacter* (*P*<0.05), *Escherichia* (*P*<0.05) and *Shinella* (*P*<0.05) were significantly lower in group Y while, *Prevotella* (*P*<0.01), AF12 (*P*<0.01), *Dehalobacterium* (*P*<0.01), *Klebsiella* (*P*<0.01), *Bilophila* (*P*<0.05), *Sutterella* (*P*<0.05), *Coprococcus* (*P*<0.05), *Bifidobacterium* (*P*<0.05), *Allobaculum* (*P*<0.05), *Odoribacter* (*P*<0.05), *Butyricimonas* (*P*<0.05), *Rikenella* (*P*<0.05) and *Oscillospira* (*P*<0.05) were enriched in this mice group (Fig. 7b).

DISCUSSION

In the current study, LPS seriously damaged the integrity of villi in animals which is in line with previous experiments in pigs and mice (Xu *et al.,* 2021; Chen *et al.,* 2022). However, the mice supplemented with fucoidan (group Y) showed relatively intact intestinal villi with increased villus length/crypt depth ratio and villus length along with decreased crypt depth. Biomarkers like T-AOC, MDA, SOD and GSH-Px are useful to investigate the oxidative stress status in the body (Huang *et al.,* 2022; Qin *et al.,* 2022). Among them, MDA is an indicator of oxidative damage (Qin *et al.,* 2022). A high level of MDA was observed in the mice injected with LPS, indicating oxidative injury because of LPS. Interestingly, a lower level of MDA was observed in mice received fucoidan, indicating that this polysaccharide may have the ability to alleviate the oxidative damage by decreasing MDA level in mice. On the other hand, the enzymes T-AOC, SOD and GSH-Px have the ability to resist oxidative damage (Liu *et al.,* 2020). Interestingly, a high level of these enzmyes was observed in the serum of LPS induced mice treated with fucoidan, further support the effect of fucoidan on alleviating oxidative stress by LPS. The findings of our study revealed that the IL-10 values were significantly lower in LPS induced mice while TNF-α was found significantly higher which is in coroboration with previous results (Li *et al.,* 2023). The IL-10 is an anti-inflammatory cytokine, while TNF-α is a pro-inflammatory cytokine (Aggarwal *et al.,* 2019), the higher IL-10 and lower TNF-α in fucoidan treated mice indicated that this polysaccharide could attenuate the inflammatory response in LPS induced mice.

Further we performed microbiota analysis to explore the effect of fucoidan on microbiome in mice challenged with LPS. Alpha diversity showed that the mice induced by LPS had lower Shannon entropy while its higher level was observed in mice supplemented with fucoidan (group Y), indicating that fucoidan could promote the biodiversity of microbiota in mice. Comparing the mice microbiota in different taxa showed that LPS caused intestine dysbiosis, and treatment with fucoidan could partly restore intestinal homeostasis in mice.

Further, we explored the diversity and abundance of microbiota species among the three mice groups and found that following the fucoidan intervention, there were

differences in the abundance of microbiota species among the three groups of mice, corresponding to six phyla and forty genera. Fucoidan was found to suppress the growth of several species, including *Aggregatibacter, Klebsiella, Porphyromonas,* and *Escherichia*, while these species were found significantly enriched in group M. These opportunistic pathogens belonging to gram-negative genera could release LPS in intestine and cause cosiderable damage (Xu *et al.,* 2021; Chen *et al.,* 2022; Li *et al.,* 2023). *Escherichia* was found in much higher concentrations in the fecal samples of mice in group M, suggesting that LPS may have contributed to pathogen growth in these mice. On the other hand, fucoidan may have reduced the abundance of this genus in the Y group. Furthermore, a significant reduction in *Aggregatibacter* abundance was observed in group Y, which in known to cause colitis in mice (Huang *et al.,* 2021; Wan *et al.,* 2022). Moreover, it was noted that the development and cause of disorders like Crohn's disease are strongly correlated with the gastrointestinal colonization of *Klebsiella* (Rashid *et al.,* 2013). Following the fucoidan intervention, the mice in group Y also showed a decreased level of *Klebsiella*, suggesting that fucoidan may modulate intestinal inflammation by preventing *Klebsiella* from colonizing the gut. Similarly, the intestine of mice group supplied with fucoidan had fewer lesions that may be correlated with reduced Porphyromonas growth. In fact, Porphyromonas is considered as a driver for the dysbiosis in several animal model. For example, in previous study, individuals with solid tumors had increased *Porphyromonas* abundancies (Wu *et al.,* 2022). Moreover, The H1N1 virus-infected C57BL/6J mice (Li *et al.,* 2022), the mice suffering from colitis (Kemika *et al.,* 2017; Zhu *et al.,* 2022b), the mice treated with dextran sulfate sodium salt (Wu *et al.,*2023), the mice treated with antibiotics (Keerqin *et al.,* 2021), and the mice supplemented with high-fat feeds (Guo *et al.,* 2023) were previously found to have lower abundancies of *Coprococcus, Dehalobacterium, Odoribacter, Oscillospira, Bilophila,* and *Rikenella*. Nonetheless, the fucoidan-treated mice had noticeably greater abundancies of these genera, which may also have contribution in alleviating intestinal damage in mice treated with fucoidan. On the other hand, Peña-Rodríguez *et al.* (2022) reported that cholestasis wistar rats with intestinal dysfunction had a reduced abundance of the probiotic *Prevotella*. The increased abundance of this genus in mice given fucoidan supplementation suggests that fucoidan may enhance intestinal function by elevating the abundance of *Prevotella*.

According to the aforementioned, fucoidan reduces intestinal damage in mice by controlling oxidation resistance, inflammatory response, and regulating microbiota, offering an innovative treatment for inflammatory bowel disorders.

Data availability statement: Sequencing data used in this study was stored in the NCBI database under accession number: PRJNA1059517.

Ethics statement: All the procedures were approved by the ethics committee of Nanjing Agricultural University (NJAU.No20220520108).

Author contributions: KL: research idea and methodology. SP, CX, QH and KL: reagents, materials, and analysis tools. KL: writing – original draft and preparation. JX, OPC, AI, MMM, FAK, YW and KL: writing – review and editing. KL and YW: visualization and supervision. All authors know and approved the final manuscript.

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