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Treatment of inflammatory bowel disease: Potential effect of NMN on intestinal barrier and gut microbiota

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ABSTRACT

Nicotinamide mononucleotide (NMN) exerts physiological effects in mammals through its conversion to nicotinamide adenine dinucleotide (NAD^+). In this study, we established experimental models of colitis by mixing drinking water of C57BL/6J mice with dextran sodium sulphate (DSS), and then fed them with the same concentration of NMN or at the same time. After NMN treatment, we observed improved morphology of inflamed intestines, slightly restored length of colon, improved barrier function and reduced proinflammatory factors expression in serum. Also, significant alterations in the composition and abundance of intestinal flora in IBD mice were found. The abundance of *Firmicutes*, *Verrucomicrobia*, *Akkermansia* and *Lactobacillus*, considered as beneficial bacteria, increased, while *Bacteroidetes* and *Muribaculaceae* unclassifiably decreased. Taken together, these results suggest that NMN may improve intestinal inflammation, reduce intestinal mucosal permeability and repair gut flora dysbiosis in IBD.

1. Introduction

Nonspecific chronic inflammation of the intestines with multiple predisposing factors is termed as inflammatory bowel disease (IBD) which include genetic factors, immune disorders, intestinal barrier dysfunction and altered gut microbiota. Furthermore, environmental factors like altered diet, antibiotic use and smoking also play a crucial role in increasing IBD incidence (Lee et al., 2015). It consists of two main subtypes: Crohn disease (CD) and ulcerative colitis (UC). In terms of characteristics of IBD, recurrent episodes of pain in abdomen, diarrhea, mucopurulent and bloody stools, with tenesmus and a long course of illness have been reported, while its causative factors and pathogenesis have not been fully explained (Cohen et al., 2019). IBD can impair intestinal epithelial barrier, intestinal morphology and gastrointestinal microflora (Ananthakrishnan, 2015). Therefore, unearthing efficient drugs for IBD treatment to alleviate the physical damage caused by the disease is of vital importance to the study of intestinal health.

Nicotinamide adenine dinucleotide (NAD^+), referred to as coenzyme I, is an oxidative co-enzyme which is essential for several physiological and biochemical processes. In mammals, NAD^+ is the only substrate for NAD^+-dependent ADP ribosyltransferases, which performs different physiological functions in various cells, e.g., activating NAD^+-only protein type III lysine deacetylases (Sirtuins), maintaining cellular redox status and regulating apoptosis (Garten et al., 2009; Imai, 2009). An important metabolite for normal NAD^+ biosynthesis in mammals is NMN, which is the most significant precursor for the synthesis of NAD^+, wherein its function is reflected by regulatory effects of NAD^+ levels (X. Wang, Hu, Yang, Takata and Sakurai, 2016). Previous studies have found that NAD^+ produced through supplementation of NMN in organisms can prevent, treat and repair the damage of cardiac and brain,

Abbreviations: NAD^+, Nicotinamide adenine dinucleotide; IBD, Inflammatory bowel disease; NMN, Nicotinamide mononucleotide; DSS, Dextran sodium sulphate.

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metabolic diseases, aging and its related degenerative diseases (Poddar et al., 2019). Many studies have also demonstrated that NMN can attenuate age-related Alzheimer's disease, ischemia/reperfusion injury and type 2 diabetes through increasing intracellular NAD^+ levels (X. Wang et al., 2016; Yamamoto et al., 2014; Yoshino et al., 2011). In addition, NMN maintains homeostasis of intestines through regulation of intestinal microbiota, which potentially protected the mucosa of intestines (Huang et al., 2021).

In summary, metabolism of NAD^+ has been investigated in various cells, tissues and organ system, however, few researchers have analyzed the relationship between metabolism of NAD^+ in the intestine and IBD. Therefore, considering the relationship of NMN with NAD^+, we assessed NMN function in treating and improving IBD by constructing colitis model through DSS with NMN gavage and observation of its metabolism in intestinal tract in order to unearth novel therapeutic options for IBD.

2. Materials and methods

2.1. Animals grouping and colitis model establishment

Protocols of the entire experiments were followed after the Ethics Committee of Jiangsu University (JU) for Animal Experiments have approved the study. Laboratory of Diseases Research Center at JU supplied 32 C57BL/6J (male, 8 weeks old) mice with similar physiological status. The mice were acclimatized for 1 week before they were randomized into four groups, namely normal control (CON), disease (dextran sodium sulphate-DSS), treatment during early stage (N + D), and treatment during challenge groups (D + N). Eight animals in each group received daily treatment for 3 weeks. In the first week (day1 to day7), three groups were treated with drinking water containing 3% DSS solution (Meilunbio, MB5535, Mw: 36000-50000). NMN (1 mg/g of body wight, Sigma, SLBT9580) was administered via gavage, wherein it was divided into full 21 days of dosing (N + D) and last 14 days of dosing (D + N), but both them were used to observe the therapeutic effects of NMN, accordingly. Standard chow was provided during the experimental period. The mice in all the groups were fed freely and kept in the same conditions (temperature: 20 ± 2 °C, humidity: 50%~60%) (Huang et al., 2021).

2.2. Examination of inflammatory indicators and tissue processing in mice

Weight of mice, fecal properties and occult blood were observed daily. Fecal occult blood was detected using occult blood reagent (OB, Leagene, 1216A20). Three weeks later, we withdrew blood from eye frames of mice, before 12 min of centrifugation at 4 °C and 3000×*g* to obtain serum. Afterwards, all the mice were executed by cervical dislocation method, wherein the whole colon was removed, prior to measurement of colonic length of mice (in each group) and photographing. Part of the colon was stored in the refrigerator at −80 °C, while others was fixed with 4% paraformaldehyde (PFA, Biosharp, 21351381) to prepare the following experiment (Huang et al., 2021). Later, we collected the feces in the intestine before frozen (at −80 °C) for 16S-rDNA sequencing at a later stage.

2.3. HE staining and morphological analysis

Washing of colon tissues that have been fixed with PFA (4%) was accomplished with phosphate buffer solution (PBS) before dehydration with anhydrous ethanol and ethanol (70%, 80% and 90%). We cut thick sections of tissues (4 μm) after they have been made transparent through xylene treatment and subsequently embedded in paraffin. Hematoxylin-eosin (H&E) staining (SolarBio, G1120) of the sections was performed, before dehydration with gradient ethanol, and sealing with neutral gel to observe histopathological changes in the intestinal mucosa under the light microscope (Shen et al., 2019).

2.4. Immunohistochemistry

Immunohistochemistry was performed using anti-claudin-1 (polyclonal, rabbit, 1:20 00, 13255S; CST, Massachusetts, USA), anti-E-Ca (polyclonal, rabbit, 1/100, 65–8103, Invitrogen), anti-Occluding (polyclocnal, rabbit, 1/100, 30–7559, Invitrogen) and secondary goat anti-rabbit Alexa fluor-488 antibody (1/2000, Invitrogen). Overnight incubation of paraffinized sections was accomplished with primary antibodies at 4 °C. Afterwards, accompanying secondary antibodies were utilized to incubate the sections for 20 min at 37 °C. Later, nuclei staining with H&E was carried out after DAB incubation. We then examined the sectioned tissues with light microscopic technique before quantitative analysis of relative protein. The quantitative analysis was conducted by image J.

2.5. Alcian blue staining experiment

After dehydration, embedding and sectioning into thick sections (4 μm), we stained the colonic tissues with Alcian Blue (Leagene, DG0041) for 30 min at room temperature to observe the pathological changes of colonic mucosal epithelial cells under light microscope (Huang et al., 2021).

2.6. Intestinal mucosal permeability assay [(including mouse live imaging and serum fluorescein isothiocyanate (FITC)-Dextran assay)]

Twelve (12) hours before the end of the experiment, we chose four mice from each group and gave them FITC-dextran 4 kda (FD4: 500 mg/kg, Sigma, BCCD8787). The mice were then anesthetized with chloral hydrate for live imaging 1 h later. We sampled orbital blood 4 h later and separated the serum to detect concentration of FD4 with a fully automated infinite M200 ELISA (Tecan, Austria, Ex 485 nm/Em 525 nm).

2.7. Enzyme-linked immunosorbent assay (ELISA)

Measurement of serum concentrations of inflammation-related factors, viz., interleukin (IL)-4, 6, 10, tumor-necrosis factor-alpha (TNF-α) and NAD^+ in mouse was carried out with ELISA kit (Peng et al., 2019). After completion of all the steps according to relevant operation instructions of the kit, we immediately measured the OD value at 450 nm with an enzyme standardizer (Bio-rad 680), and calculated the corresponding sample concentration by plotting the standard curve through the standards.

2.8. 16S-rDNA fragment amplification, sequencing and intestinal flora analysis

After executing the mice, we squeezed the samples while removing the colorectum and immediately freezing and storing it at −80 °C refrigerator. Later, they were transported to Hangzhou LC-Bio, Zhejiang, China for gut microbial analysis and sequenced on Illumina NovaSeq platform. After completion of sequencing, we obtained the raw data. Later, splicing of the double-end data with overlap was done, while we obtained high quality clean data through quality control and filtering of chimera. Afterwards, we obtained the feature table and sequences for further analysis of diversity, species taxonomic annotation and difference analysis, etc. via the construction of class operational taxonomy units (OTU) table by the concept of Amplicon Sequence Variants (ASVs).

2.9. Statistical analysis

Expression of data was carried out with mean and SD (M. Li et al., 2022; Shang et al., 2022). One-way analysis of variance (ANOVA) was utilized to compare the group differences, while Kruskal-Wallis test was applied to assess data sets involving more than two pools. Dunnett's method was used for multiple comparisons between some groups, while

correlations were analyzed using Spearman Correlation Coefficient. GraphPad Prism 8.02, Photoshop and PowerPoint were used for analysis and plotting. Statistically, consideration was given to $P < 0.05$ as acceptable level of significance.

3. Results

3.1. NMN increases concentration of NAD^+ in serum

Serum NAD^+ concentration of mice in the DSS group was insignificantly different from those in CON group. Meanwhile, mice in N + D and D+N groups demonstrated substantial increased serum NAD^+ concentration compared with DSS group (Fig. 1A).

3.2. The effect of NMN treatment on body weight of mice

During the first 7 days, the body weight of mice in DSS and the NMN intervention groups demonstrated a decreased trend. Among them, the DSS group declined maximally and at faster rate. After completion of the modelling (8~21 days), the weight of mice in DSS group continued to decrease, while that of mice in other groups began to increase. The most obvious increase was seen among mice in CON group, followed by D + N group, with N + D group also increasing slightly (Fig. 1B and C).

3.3. The effect of NMN treatment on colonic length and fecal occult blood in mice

Compared with CON group, colonic length of mice in DSS group decreased markedly ($P < 0.01$). After NMN intervention, the colon length of mice in the D + N ($P < 0.01$) and N + D groups recovered slightly (Fig. 1H and I). Except for the CON group, the fecal occult blood was detected in mice of other groups from next day and then continued to exist till the end of the study. The intensity of the occult blood also increased gradually (Fig. 1J).

3.4. NMN alleviates symptoms of enteritis in mice

After blood sampling, centrifugation and collection of supernatants, we ascertained serum concentrations of inflammation-related mediators, viz., TNF-α and IL-6, as well as markers of anti-inflammation like IL-4 and IL-10 in mice via ELISA. Compared with CON group, concentration of TNF-α and IL-6 in DSS group markedly increased ($P < 0.01$). Meanwhile, IL-4 concentration did not show any significant difference within the aforementioned groups. Also, we observed substantial decrease in levels of IL-6 and TNF-α in N + D and D + N groups compared with DSS batch ($P < 0.05$ or $P < 0.01$), albeit insignificant difference in IL-4 and IL-10 concentrations (Fig. 1D–G).

3.5. NMN improves the structure of the damaged colon

As presented in Fig. 2A, the colon structure of the CON group mice was intact with clear mucosa and submucosa as well as neatly arranged glands, coupled with no obvious inflammatory cell infiltration. The mice in DSS cohort showed plentiful neutrophils and lymphoplasma cell infiltrations involving in mucosa and submucosa, while the lamina propria mucosa was damaged. Moreover, crypt structure was deformed and goblet cells was lost. After NMN intervention, the N + D and D + N groups showed some improvement compared to DSS batch. Also, the

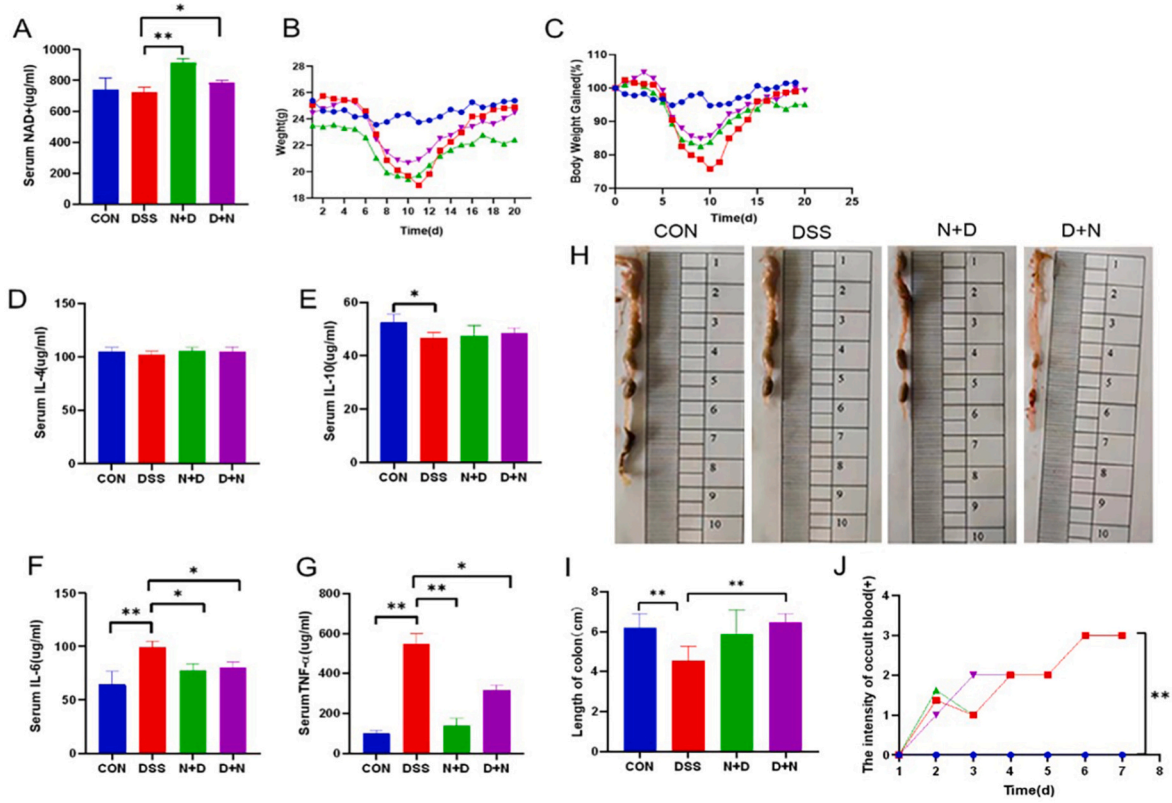

Fig. 1. The NMN effect on the body weight of mice, colon length of mice, concentration of NAD^+ and occult blood in the feces with colitis. A: Concentration of NAD^+ in mice in all the groups. N+D vs. DSS ($P = 0.0079$); D + N vs. DSS ($P = 0.0159$). B: The body weight of mice in all the groups during NMN administration period. C: Rate of weight loss in mice in all the groups during the administration period. D–G: Serum concentrations of IL4, IL-6. IL-10 and TNF-α in colitis mice before and after NMN treatment, E: CON vs. DSS ($P = 0.0286$), F: CON vs. DSS ($P = 0.00760$), N + D vs. DSS ($P = 0.0357$), D + N vs. DSS ($P = 0.0357$), G: CON vs. DSS ($P = 0.0043$), N + D vs. DSS ($P = 0.0079$), D + N vs. DSS ($P = 0.0159$). H–I: The NMN effect on the colon length of mice with colitis. CON vs. DSS ($P = 0.0061$); D + N vs. DSS ($P = 0.0095$). J: Occult blood in the feces of the mice during modeling. $P = 0.0047$. *:0.01<P < 0.05, **:0.001<P < 0.01 and ***:P < 0.001.

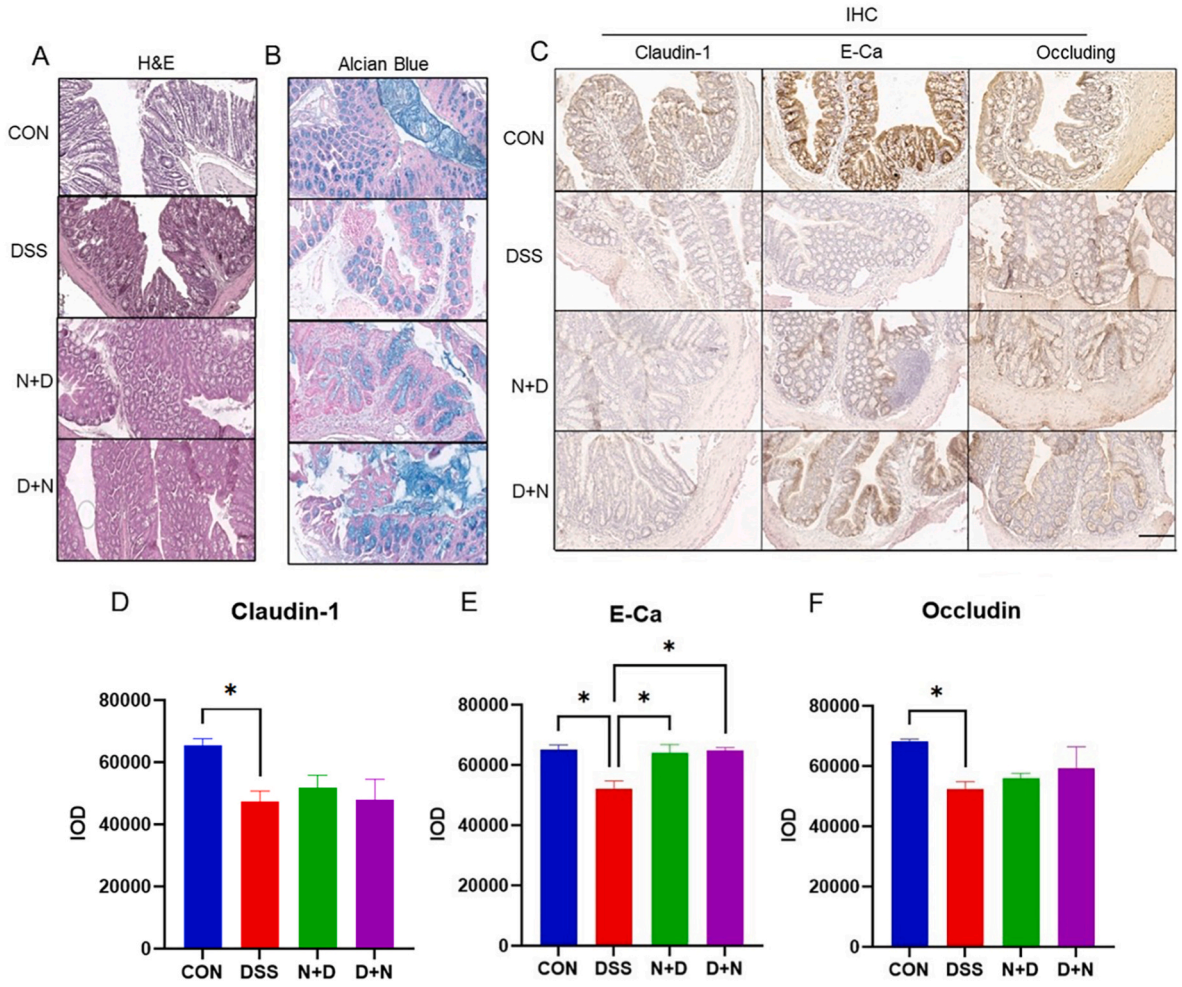

Fig. 2. The effect of NMN on the intestinal mucosal barrier. A: HE staining of colon in mice of CON, DSS, N + D and D + N groups, bar = 200 μm. B: Alcian Blue staining of intestinal mucosal in mice of CON, DSS, N + D and D + N groups, bar = 200 μm. C: Tight-junction proteins' expression (claudin-1, E-Ca and Occluding) in colon with IHC. bar = 200 μm. D-F The quantitative of the proteins. D: CON vs. DSS ($P = 0.0480$). E: CON vs. DSS ($P = 0.0118$), DSS vs. N + D (P = 0.0170), DSS vs. D + N (P = 0.0135). F: CON vs. DSS ($P = 0.0482$). *:0.01<P < 0.05, **:0.001<P < 0.01 and ***:P < 0.001. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

acute and chronic inflammatory cells in lamina propria of intestinal mucosa were decreased, while the structure of intestinal wall became clear. Furthermore, the glands were arranged orderly, with the change in crypt shape and increase in shape of the cells.

3.6. NMN enhances the function of intestinal mucosal barrier

Mucus, secreted by goblet cells, can improve damaged intestinal permeability, which acts as a chemical barrier of intestinal mucosa. According to mucus staining (Fig. 2B), the production of mucus in DSS group significantly reduced compared to those in CON group, but increased substantially when we compared DSS group with N + D and D + N groups. These results showed that damaged intestinal mucosa in DSS induced colitis was improved, while the mucosal barrier function was enhanced after NMN intervention.

3.7. Effects of NMN treatment on tight-junction proteins' expression (claudin-1, E-Ca and occluding) in colon

Tight-junction protein, claudin-1, E-Ca and occluding, located between epithelial cells are principal component of mucosal barrier of intestines. As was depicted in the immunohistochemical results, the expressions of claudin-1, E-Ca, and occluding proteins in DSS group significantly diminished obviously compared to CON group, however, E-Ca in N + D and D + N groups increased compared with DSS group ($P <$ 0.05) (Fig. 2C–F).

3.8. The effect of NMN treatment on intestinal mucosa permeability

To analyze the influence of NMN treatment on the intestinal mucosa permeability, we measured the concentration of FITC glucan by fluorescence. In vivo imaging results showed FD4 leakage in DSS group increased significantly compared to other groups (Fig. 3A). In analyzing the FD4 concentration in serum, and compared with CON group, we observed significant increased FD4 concentration in DSS group compared to CON batch ($P < 0.01$) (Fig. 3B). In comparison with DSS group, a substantial ($P < 0.01$) and slight decrease ($P < 0.05$) in FD4 concentration was observed in N + D and D + N groups.

3.9. The effect of NMN treatment on diversity of the gut microbiota

To analyze whether NMN can treat the alteration of intestinal flora induced by DSS, we examined the fecal bacterial by 16S-rDNA gene sequencing. The Chao1 (Fig. 4A), Observed otus (Fig. 4C) and Shannon indexes (Fig. 4D) all depicted diversity and abundance of gut microbiota in NMN groups, which received NMN, that is D + N and N + D groups, increased compared to DSS group, while the goods coverage indexes showed no significant difference between the two groups. Evaluation of

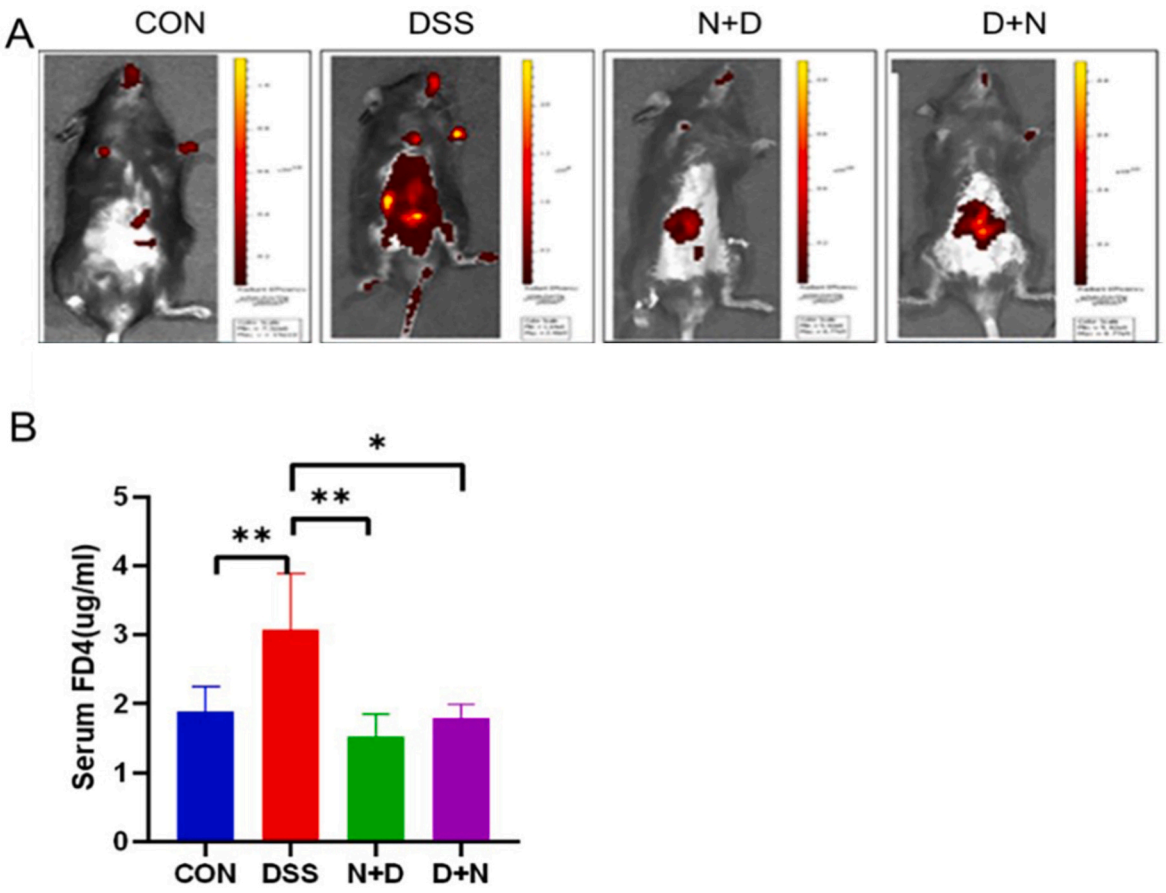

Fig. 3. A–B: The effect of NMN on intestinal mucosa permeability. A: The *in vivo* imaging in different mice groups; B: The concentration of serum FD4 in different mice groups. *:$P < 0.05$, **:$P < 0.01$ and ***:$P < 0.001$.

β diversity was accomplished with principal component analysis (PCA) (Fig. 4E) and principal coordinate analysis (PCoA, Fig. 4F), thereby exhibiting species differences in communities of various environment. Meanwhile, the results showed increase in diversity of intestinal bacterioflora after NMN treatment, which was substantially different from DSS group.

3.10. The effect of NMN treatment on abundance of the microbiota in gut of mice with IBD

Difference in gut microbiota in two groups (levels of phylum and genus) was observed after cluster analysis (Fig. 5A–B). The results showed significantly varied composition of gut microbiota in DSS group compared to NMN batch. Based on bar graphs (Fig. 5C–D), there were *Actinobateria*, *Bacteroidetes*, *Firmicutes*, *Proteobateria* and *Verrucomicrobia* et al. at phylum level both in DSS and NMN groups. Among them, *Verrucomicrobia* and *Firmicutes* abundance was significantly higher in NMN group compared to DSS batch ($P < 0.05$), while *Bacteroidetes* in NMN group decreased markedly ($P < 0.01$). At genus level, the gut microbiota in the two groups were mainly composed of *Muribaculaceae*-unclassified, *Akkermansia*, *Lactobacillus*, *Bacteroides*, *Dubosiella* and so on. Compared to DSS group, a significant increase in *Akkermansia* and *Lactobacillus* abundance in NMN group ($P < 0.01$). However, *Muribaculaceae*-unclassified abundance obviously reduced after administration of NMN ($P < 0.01$).

Circos graph was used to exhibit distribution of opportunistic flora in the gut microbiota, which presents top five abundance, coupled with the relationship between samples and species (Fig. 5E–F). The left part displayed information on the top five microbiota in abundance, while other related information in different groups was shown in the right. The wider it occupies in the picture, the richer the abundance of this flora. *Firmicutes* and *Verrucomicrobia* abundance in NMN group increased compared to DSS group at phylum level ($P < 0.05$), while *Bacteroidetes* in NMN group decreased substantially ($P < 0.01$) (Fig. 5E). Higher abundance of *Akkermansia*, *Lactobacillus*, *Dubosiella* et al. was observed in NMN group compared to DSS batch at genus level ($P < 0.05$). Contrarily, a downtrend of *Muribaculaceae*-unclassified was seen in NMN group ($P < 0.01$) (Fig. 5F).

Analysis of distribution difference with heatmap discovered that gut microbiota in two groups (Fig. 5G–H) were similar to Circos graph. Interestingly, at genus level, increase in abundance of *Dubosiella* in NMN group which existed in Circos graph was not found in the heatmap.

3.11. Difference analysis of mice gut microbiota that received NMN

Based on above results, significant difference in gut microbiota in NMN group was ascertained further. The barplot graph was applied to observe difference in gut microbiota between DSS and NMN groups (Fig. 6A–B), while LEfSe analysis was performed, with LDA score being used to identify the significant difference in various groups at genus level (Fig. 6C). The picture from inside to outside represents classification level from kingdom to species (Fig. 6D) and diameter of small circle denotes positively related abundance of bacteria. Yellow reveals insignificant differences within species in these groups, while red depicts contrary, wherein abundance of species was higher in red group. Other colors are similar with the above. These graphs revealed that compared with NMN group, the identified opportunistic floras were *Bacteroidetes* and *Candidatus_Melainabacteria* at phylum level and at genus level in DSS group were *Muribaculum* and *Odoribacter* et al., which indicates that these floras play an important role in colitic development (Fig. 6A–B).

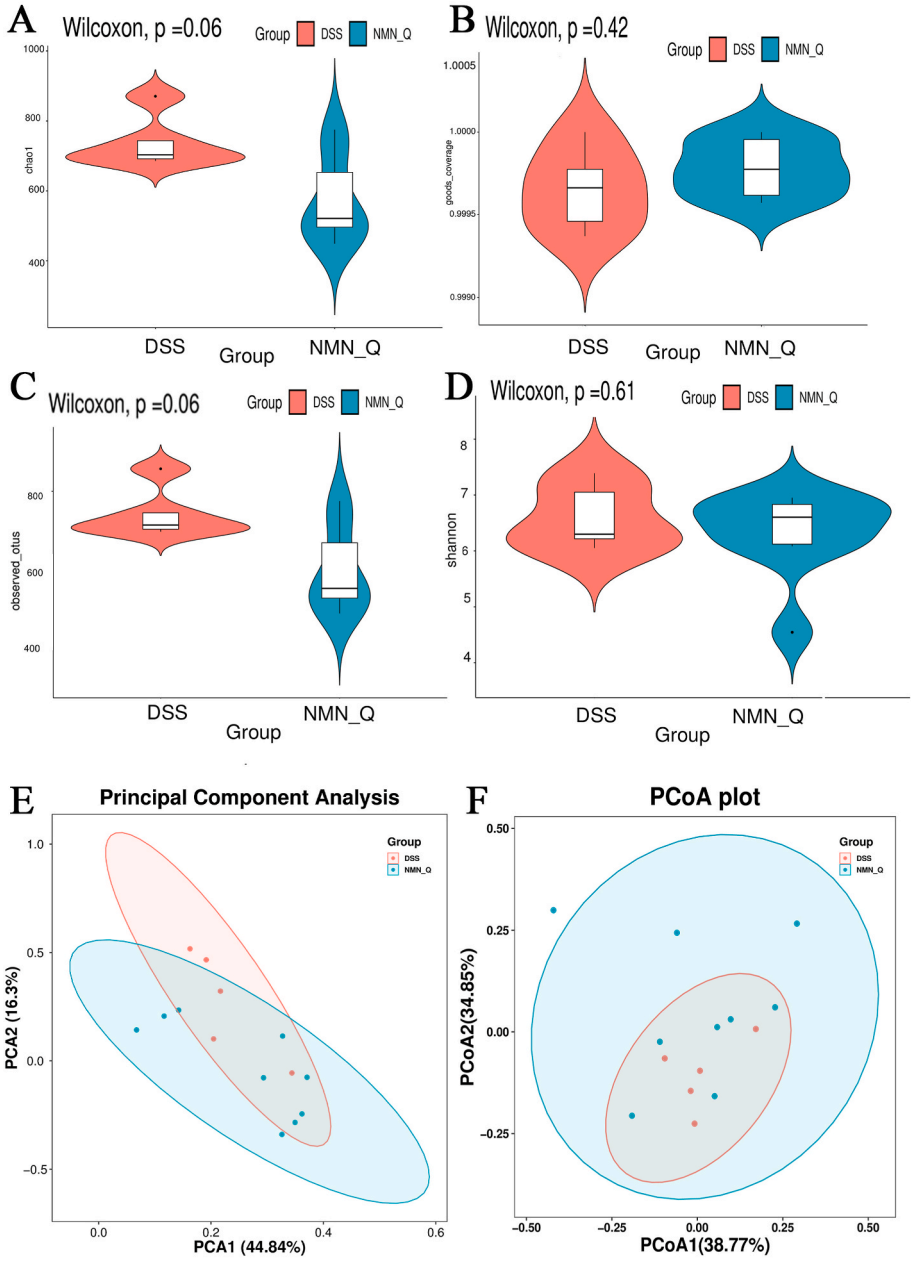

Fig. 4. A: Chao1 index, B: goods _coverage index, C: Observed_otus index, D: Shannon index, E: Principal component analysis (PCA) and F: Principal coordinates analysis (PCoA) *:$P < 0.05$.

4. Discussion

Herein, we established colitis model in mice by feeding DSS, with NMN treatment at different periods. The results on the weight, length of gut, intestinal structure and mucosa as well as the concentration of cytokines in the mice that received NMN suggested that it could relieve inflammation. Although NMN could not cure IBD completely, it could control development of disease and relieve pain induced by IBD. Evidentially, these results suggest NMN may potentially serve as treatment option for IBD.

Intestinal barriers are made of mechanical, chemical, immune and microbial barriers. Previous studies have revealed that IBD can injure intestinal mucosa, thereby exposing antigens including gut microbiota, which leads to overreaction of immune response (Green-Johnson, 2012). In this study, claudin-1, E-Ca and Occluding expression in DSS group significantly reduced, while both of them in mice that received NMN increased. Furthermore, mucus produced by goblet cells in the intestine has been proven to form the chemical barriers. According to Alcian Blue staining, the goblet cells and mucus were evidently decreased in DSS group, but increased in D + N and N + D groups. The improvement in intestinal permeability in IBD upon NMN administration was also discovered through *in vivo* imaging and FD4 detection. These findings all suggested that NMN could ameliorate DSS induced colitis by decreasing the inflammation and intestinal permeability, as well as improving function of intestinal mucosal barrier.

Intestinal microbiota plays a crucial role in completeness of intestinal epithelia and has an impact on apoptosis and renewal of gut epithelial cells coupled with expression and function of tight-junction proteins, thus strengthening the effect of intestinal barriers (Aggeletopoulou et al., 2019; Chelakkot et al., 2018). Also, the bacteria and its metabolite like SCFAs increase Treg cell number in lamina propria of intestinal mucosa which can maintain the function of immunity normal and prevent excessive immune response. The intestinal microbiota consisted of *Firmicutes*, *Bacteroidetes*, *Proteobacteria* et al., however, α

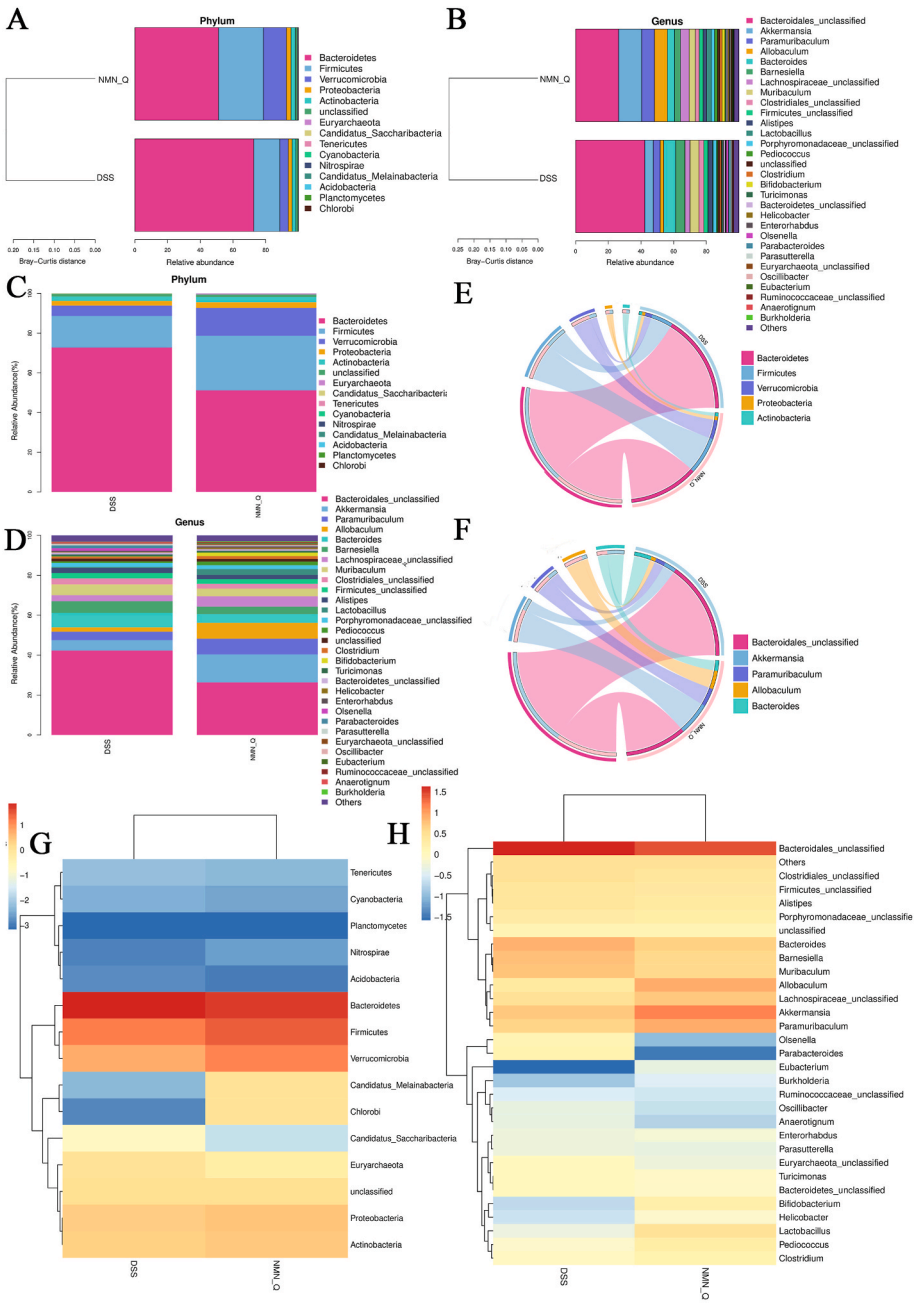

Fig. 5. A: Cluster analysis-phylum, B: Cluster analysis-genus, C: Relative abundance at phylum level, D: Relative abundance at genus level, E: Distribution of intestinal dominant flora-phylum, F: The distribution of intestinal dominant flora-genus, G: Heatmap at phylum level and H: Heatmap at genus level.

diversity of intestinal microbiota in IBD was reported to reduce, which is characteristic of increase in *Proteobacteria* and decrease in *Firmicutes* (Frank et al., 2011; Manichanh et al., 2006). And the expression of the tight junction proteins, e.g., occluding and claudin-2, can be increased by Firmicutes (B.-Y. Li et al., 2019). Analyzing the abundance of intestinal microbiota, at phylum level, NMN could up-regulate the abundance of *Firmicutes*, *Verrucomicrobia* and *Actinobacteria* but decrease that of the *Bacteroidetes*. What's more, at genus level, it could increase *Akkermansia* and *Lactobacillus* abundance, while that of *Muribaculaceae*_unclassfied was reduced. Consistently, the *Firmicutes*, *Lactobacillus* et al. which increased after treatment with NMN may play important roles in the production of SCFAs consisting of acetic, propanoic and butyric acids (Barcelo et al., 2000). Seen from the heatmap, *Akkermansia*, which is consisted of *Verrucomicrobia*, in mice with NMN treatment was significantly increased compared with CON group. According to previous studies, *Akkermansia* can regulate the differentiation of Treg and promote the production of short chain fatty acid (SCFA) (Forslund et al., 2015). Furthermore, *Akkermansia* and its outer membrane protein can weaken the infiltration of macrophage and cytotoxic lymphocyte in the colon as well as improve the depth of crypt (Moghadamrad et al., 2015). Some researchers have reported that SCFAs can provide energy for intestinal mucosa and maintain the gut homeostasis, which includes reducing pH in the gut to inhibit the growth of pathogenic bacterium, promoting mucus secretion and strengthening the intestinal mucosa barrier (Andoh et al., 2003; Barcelo et al., 2000; Goverse et al., 2017; Han et al., 2015). Also, treatment with butyric acid alone could even relieve inflammation of intestinal mucosa in UC (Andoh et al., 2003; Barcelo et al., 2000; Goverse et al., 2017; Han et al., 2015). Not only can SCFAs keep balance between probiotics and pathogenic bacterium, but also regulate congenital and adaptive immunity

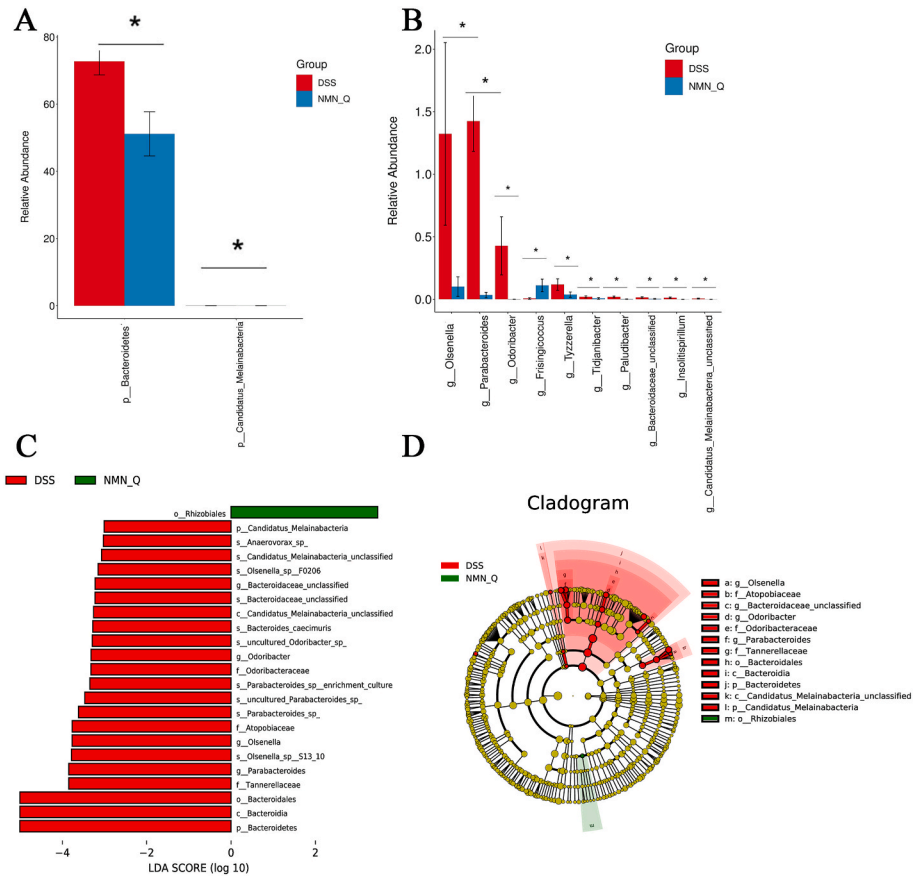

Fig. 6. A–B: Differential analysis of intestinal microbiota of mice at phylum and genus levels, C: LDA of discriminative biomarkers and D: LEfSe cladogram of intestinal flora. *:$P < 0.05$, **:$P < 0.01$ and ***:$P < 0.001$.

through G-protein coupled receptors of GPR41 and GPR43, which have an impact on maturation and differentiation of mucosal immune cells as well as can activate various immune pathways (Dasu et al., 2008; Eeckhaut et al., 2013; Nastasi et al., 2015; Rooks and Garrett, 2016; Vieira et al., 2015). *Roseburia* is an important member of *Actinobacteria*, which was discovered increased in our results, and one of the most butyric acid-producing bacteria in the gut, while *Faecalibacterium prausnitzii* generating butyric acid were discovered downtrend in CD patients, which correlated with disease activity (Pryde et al., 2002; Vetizou and Trinchieri, 2018). While the *Verrucomicrobia* and *Akkermansia* can also ease inflammation, reduce immune response in gut and mesenteric lymph nodes as well as prevent development of colitis (L. Wang et al., 2020). As mentioned above, the abundances of some bacteria were also related to the mucosal immune response in the gut, wherein they could play important roles in forming immune barriers. Intestinal flora can induce the production of IgA which the bacteria is covered with and IgA can also enter the intestinal cavity through the polyimmunoglobulin receptor of enterocyte to mediate intestinal mucosal immunity (Clavel et al., 2017; McCoy et al., 2017). *Bacteroides acidifaciens* could promote forming of germinal center and regulate the number of cells producing IgA and B (Yanagibashi et al., 2013).

NMN can protect mice from DSS induced colitis, however, it is not clear what the underlying reason is. It may result from the potential impact of NMN on intestinal microbiota, which increased bacterium production of SCFAs to protect the probiotics in the gut or the influence on the intestinal mucosal immune response to inhibit the pathogenic bacterium and immune tolerance to probiotics. Also, the existence of a "Cross-Talk" between intestinal microbiota and intestinal mucosal immune response revealed that the prognosis of IBD can't be explained only by activating or inhibiting one pathway and increasing or decreasing some kinds of bacterium (Botticelli et al., 2017; Schroeder and Bäckhed, 2016). According to our findings, it is not difficult to conclude that NMN have influence on DSS induced colitis. However, detailed investigations on the gut microbiota and intestinal mucosal immune response are needed to explain its explicit mechanism of action, which plays important roles in occurring, developing and treatment of IBD.

5. Conclusion

In conclusion, NMN may ease inflammation of DSS induced colitis mice, repair the mucosal barriers and improve the disorder intestinal microbiota. Taken together, these findings provide some evidences about the effect of NMN on treating IBD, which may have a significant impact on clinical application.

CRediT authorship contribution statement

Pan Huang: conceived and designed the experiments. **Xuxin Wang:** Writing – original draft, conducted the experiments, and, drafted the manuscript. All authors have read and agreed to the published version of the manuscript, collected the samples and analyze the data. **Siyu Wang:** Formal analysis, collected the samples and analyze the data, drafted the manuscript. All authors have read and agreed to the published version of the manuscript. **Zhipeng Wu:** Writing – original draft, Formal analysis, drafted the manuscript. All authors have read and agreed to the published version of the manuscript, collected the samples and analyze the data. **Zhengrong Zhou:** conceived and designed the experiments. **Genbao Shao:** and, conceived and designed the experiments. **Caifang Ren:** and, conducted the experiments. **Meiqian Kuang:** Formal analysis,

collected the samples and analyze the data. **Yan Zhou:** conducted the experiments. **Anqi Jiang:** conducted the experiments. **Weihong Tang:** conducted the experiments. **Jianye Miao:** Formal analysis, and, collected the samples and analyze the data. **Xin Qian:** Formal analysis, collected the samples and analyze the data. **Aihua Gong:** conceived and designed the experiments. **Min Xu:** conceived and designed the experiments.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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