SYNBIOTICS REDUCE SYMPTOMS IN UC MICE THROUGH THE REGULATION OF INTESTINAL MICROBIOTA AND SHORT-CHAIN FATTY ACIDS

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ABSTRACT

To see if the simultaneous combination of Pediococcus acidilactici (PA) with inulin could alter the gut microbial community and the levels of short-chain fatty acids (SCFAs) in the cecum of the mouse with ulcerative colitis (UC) an experiment was laid out under the Completely Randomized Design (CRD). A total of 60 SGPF mice were randomly assigned to 6 experimental arms, 10 mice to each arm. These were an untreated normal control group (NC), a dextran sulfate sodium model group (DSS), a PA-only group, and three combined-treatment groups of PA with low-dose (PA.IN.L), intermediate-dose (PA.IN.M), and high-dose inulin (PA.IN.H). Colitis was induced by providing the animals free access to drinking water that was supplemented with 2.5% DSS. The experimental schedule consisted of 14 days of preventive administration and 7 days of disease-inducing period. Depending on the amount of inulin used, different responses of the SCFA were observed. Acetate concentration increased significantly ($P \leq 0.05$), and overall SCFA concentration increased very significantly ($P \leq 0.001$) in intermediate supplementation. The lower dose, in contrast, resulted in significant increases in butyrate ($P \leq 0.05$) and the total SCFAs ($P \leq 0.001$). The microbial diversity was further analysed and revealed PA. IN. M lowered the Shannon index values and Simpson index values compared to the DSS-induced colitis group ($P \leq 0.05$). Compositional assessment showed that PA.IN.M administration enhanced the relative prevalence of *Erysipelotrichaceae* ($P \leq 0.05$). Furthermore, it prompted a significant elevation in the proportions of *Akkermansiaceae* (P=0.01), while concurrently diminishing those of *Muribaculaceae* and *Tannerellaceae* (P=0.01). At the genus level, a notable enrichment of *Akkermansia* was observed in both the low- and medium-dose synbiotic groups compared to the DSS cohort ($P \leq 0.05$). Additionally, the medium- and high-dose treatments resulted in a substantial rise in *Candidatus_Saccharimonas* ($P \leq 0.001$). *Limosilactobacillus* positively correlated with SCFAs ($P \leq 0.05$). In summary, this synbiotic preparation alleviates DSS-induced ulcerative colitis. Its therapeutic effect is closely associated with the modulation of gut microbial composition, including the enrichment of beneficial bacteria and the enhancement of SCFA production, which may collectively contribute to the restoration of intestinal homeostasis.

Keywords: Inulin; *Pediococcus acidilactici*; ulcerative colitis; intestinal flora; SCFAs

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INTRODUCTION

One of the most prevalent diseases of animals is intestinal disease, and the pathogenesis of intestinal diseases is connected with microbial dysbiosis. Ulcerative colitis (UC) is linked with a defective intestinal barrier and derangement of the gut microbiota. This is specifically characterized by microbial imbalance, mainly in the form of pathogenic germs that have proliferated, and friendly bacteria (Wasim et al., 2025) that have diminished. Thus, it is important to keep the gut flora in balance for host health.

A diverse class of probiotics known as lactic acid bacteria (LAB) includes genera like *Lactococcus*, *Lactobacillus*, and *Pediococcus*. One particular species, *Pediococcus acidilactici* (PA) from the Pediococcus genus, facilitates the regulation of gastrointestinal microbiota and sustains microecological equilibrium via its synthesis of lactic

acid (Fu et al., 2022). In addition, LABs can competitively inhibit pathogenic bacteria and produce antimicrobial substances that help to maintain the balance of the gastrointestinal tract, thereby enhancing the barrier function and stability of the gastrointestinal tract microecology (Deng et al., 2021).

Prebiotics are often found in dietary fiber, which confer health benefits. Bacteria in the gut degrade fibre to form SCFAs (Quigley et al., 2019). Inulin is a proven prebiotic. This action helps to maintain gut microbiota homeostasis and is associated with host health (Bastard et al., 2020). Moreover, inulin exerts some effects on host local and systemic metabolism, mostly due to the generation of SCFAs as a result of its fermentation process (Tawfick et al., 2022). Further, inulin has shown promise in reducing the production of inflammatory cytokines (Qin et al., 2023), highlighting its potential health benefits. Inulin is an important nutrient source for probiotics, enhancing their growth and microbial balance, while beneficial to host health (Holscher et al., 2017). Studies indicate that inulin intake can enhance intestinal immunity and help maintain a healthy gut environment (Song et al., 2020). Consequently, inulin has been used to manage gut microbiota-related conditions, including enteritis (Pascale et al., 2018; Song et al., 2019; Chambers et al., 2019). While our previous work established that this synbiotic combination improves antioxidant status and mitigates inflammation in mice—notably by repairing severe mucosal damage and lowering serum IL-1β, TNF-α, and IL-6 levels (Wang et al., 2024)—its synergistic impact on intestinal SCFAs and the intestinal microbiome within a DSS-induced colitis model had not been examined.

Building on our prior findings, this study aims to elucidate the principles by which this inulin-based synbiotic alleviates colitis, focusing on its impact on SCFAs production and gut microbial homeostasis. We hypothesise that inulin dosage affects SCFA synthesis and that it can do so by altering the gut microbiota. The experimental findings will offer a theoretical foundation for figuring out the ideal inulin dosage for treating intestinal inflammation in real-world settings.

MATERIALS AND METHODS

Animal Care: All experimental protocols followed animal welfare guidelines and were authorized by the Yanbian University Institutional Animal Care and Use Committee (Approval No. SYXK2020-0009). This study was completed in June 2023 at the College of Agriculture, Yanbian University. The study utilized sixty specific-pathogen-free (SPF) male C57BL/6 mice (6-8 weeks old; 18-22 g), sourced from the university's Experimental Animal Center. These mice were maintained in individually Ventilated Caging Systems under controlled SPF conditions, featuring a regulated temperature (23 ± 2°C), humidity (55–65%), and a 12-hour light/dark cycle. Throughout the experiment, unrestricted access to purified water was provided.

Experimental design and protocol: The Pediococcus acidilactici strain, sourced from the College of Agriculture at Yanbian University, was stored at -80°C. The strain was revived in the medium broth for 24 hours at 37°C for experimental use. After three subcultures, cell pellets were collected. Skim milk and distilled water (20% w/v) were mixed together, sterilised at 116°C for 10 minutes, and the cryoprotectant solution was cooled. This sterile solution was then mixed with the harvested pellet (1:10 ratio), frozen overnight at -80°C, and lyophilized to produce a concentrated lyophilized powder containing a known number of viable bacteria.

In this in vivo study, sixty male SPF C57BL/6 mice were acclimatized for a week with ad libitum food and water. After acclimatization, the experiment was set up in a Completely Randomized Design (CRD). To carry out this research, the researcher employed Zhao's method (Zhao et al., 2023) to randomly assign the mice into 6 groups (n=10 per group): Control (NC), Model (DSS), *Pediococcus acidilactici* alone (PA) (1 x 10^8 CFU/ml), and synbiotic groups receiving PA with low (5 mg/mL) (PA.IN.L), medium (10 mg/mL) (PA.IN.M), and high (15 mg/mL) (PA.IN.H) doses of inulin. The experimental group of mice was given 2.5% DSS (MP Biomedicals, Solon, OH, USA) in their drinking water continuously for 7 days (from day 21 to day 28). Each mouse received 200 µL of an appropriate treatment solution by gavage every day. The duration of the experimental period was a 14-day prevention period and a 7-day disease induction period. Specific treatment details are provided below:

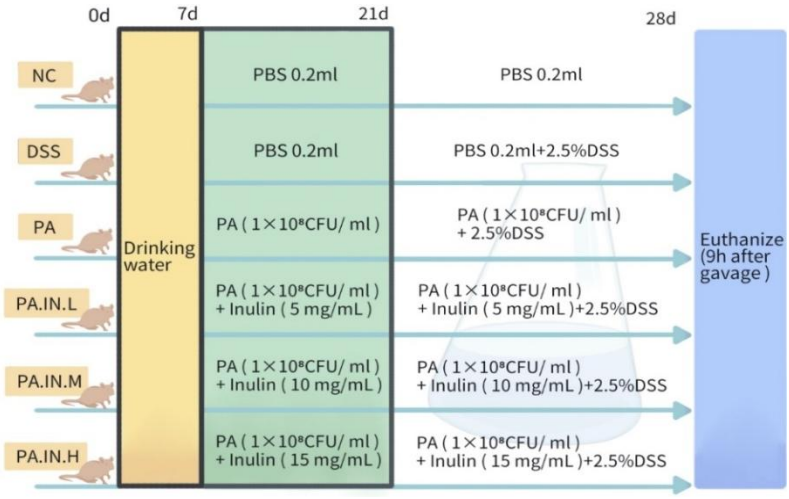


Fig. 1. Trial group design and dosing regimen

Sampling: After the experimental period, mice were fasted for 24 hours after the last gavage. Fecal samples were collected via abdominal palpation and immediately frozen, while cecal contents were collected post-euthanasia and stored at −80°C.

Determination of SCFAs in Samples: We used a previously described protocol (Li et al., 2019) to measure cecal concentrations of SCFA. The quantification was done with use of octanoic acid-1-13C (Sigma-Aldrich, St. Louis, MO, USA) and butyric-2, 2-d2 (CDN Isotopes, Pointe-Claire, QC, Canada) as internal references. SCFAs were simply extracted from the sample with an equal volume of acetonitrile/ water solution and subsequently derivatized by employing 3-nitrophenylhydrazine hydrochloride. After vortexing and centrifuging (10 min, 12, 000 rpm), the supernatant was collected and filter-sterilized (0.22 μm). The concentrations of SCFAs were analyzed using a Gas Chromatography system.

Gut microbiome profiling: After collection, fecal samples were treated for DNA extraction, and the DNA was extracted as genomic DNA for further analysis. The yield and purity of the DNA extracted from all samples were evaluated, and these samples were then diluted to 1 ng/μL. We amplified the 16S rRNA gene for each sample using Phusion® High-Fidelity PCR Master Mix with GC Buffer (New England Biolabs, USA) and barcoded primers. To check the success of the PCR, the PCR products were run on a 2% agarose gel. Library construction was carried out using the KAPA HyperPrep PCR-Free Sample Preparation Kit (Roche, Switzerland). A Quantus Fluorometer (Promega, USA) and qPCR were employed to assess the quality and quantity of the libraries. Following final validation, the libraries were subjected to sequencing on an Illumina NovaSeq 6000 instrument (USA).

The sample data was divided. The readings for each sample were concatenated using FLASH, which then extracted the original labels and carried out stringent filtering to produce clean, high-quality labels. A species annotation database (https://github.com/torognes/vsearch/) (Haas et al., 2011) was used to filter the label sequences in order to remove chimeric sequences and produce effective labels. Raw reads were demultiplexed according to their unique barcodes. Quality-filtered tags from all samples were clustered into OTUs at 97% similarity using Uparse v7.0.1001 (Edgar et al., 2013). A representative sequence was then selected for each OTU. Taxonomic classification was performed using the Mothur method against the SILVA138 SSUrRNA database (Wang et al., 2007; Quast et al., 2013), with a confidence threshold of 0.8–1.0 for phylum-, family-, and genus-level assignment.

Statistical analysis: All statistical computations were carried out using GraphPad Prism 9 and SPSS 17.0. Data are presented as mean ± standard deviation. One-way ANOVA was used to analyse group differences, and Tukey's post-hoc test was employed to compare treatment group and control group differences. Statistical significance was defined as $P \leq 0.05$.

RESULTS

Effects of the Inulin Synbiotic on Cecal SCFA Concentrations in DSS-Induced Mice: The concentrations of SCFAs in the mouse cecal contents were determined. As shown in Fig. 2, DSS administration significantly reduced the amounts of acetic acid, butyric acid, and total SCFAs compared to the healthy control (NC) group ($P \leq 0.001$ for all). Treatment with the synbiotic helped restore these levels. Specifically, the medium-dose synbiotic (PA.IN.M) significantly increased

acetic acid concentration compared to the DSS group (P≤0.05) (Fig. 2A). The low-dose synbiotic (PA.IN.L) significantly elevated butyric acid levels (P≤0.05) (Fig. 2B). While no treatment group significantly restored propionic acid (Fig. 2C), both the medium- and low-dose synbiotics significantly increased total SCFA concentrations relative to the DSS group (P≤0.05) (Fig. 2D). The data show that the synbiotic can ameliorate the DSS'-induced decline in SCFA production, with the medium- and high-dose formulations showing the most pronounced effects.

Effects of the Inulin Synbiotic on Gut Microbiota Diversity: The petal diagram presented in Fig. 3A illustrates the overlap in microbial composition among the different sample groups. This visualization revealed a core of 356 Operational Taxonomic Units (OTUs) that were shared across all cohorts. In contrast, the quantities of OTUs unique to each group were as follows: NC (64), DSS (45), PA (87), PA.IN.L (77), PA.IN.M (230), and PA.IN.H (37). A key observation was that the DSS group possessed fewer OTUs than the NC group. In contrast, all administration groups except the PA.IN.H cohort exhibited a greater number of OTUs compared to the NC group. All the above indicate that the inulin-containing synbiotic has a modulatory effect on the gut microbial composition in mice.

The alpha diversity of each group was calculated. As shown in Fig. 3B, the Shannon index was significantly increased in the DSS group (P⩽0.01) as well as in the PA. IN. The L group (P⩽0.05) had a greater difference compared to the NC control. The PA.IN.M group, however, showed a notable decrease in Shannon diversity compared to the DSS group (P≤0.05), while the PA.IN.L group's index was significantly higher than that of the PA group (P≤0.01). The Simpson index analysis (Fig. 3C) also revealed significant changes. The PA.IN.H group's index was higher than the NC group's (P≤0.05), and the PA.IN.M group's was lower than the DSS group's (P≤0.05). Furthermore, relative to the PA group, the Simpson index was significantly greater in both the PA.IN.L (P⩽0.05) and PA.IN.H (P⩽0.01) cohorts. In contrast, no significant disparities were apparent for the ACE and Chao1 richness indices across any groups (Fig. 3D and 3E; P>0.05). The α-diversity of the murine gut microbiota was influenced by the inulin-based synbiotic, as reflected in our results.

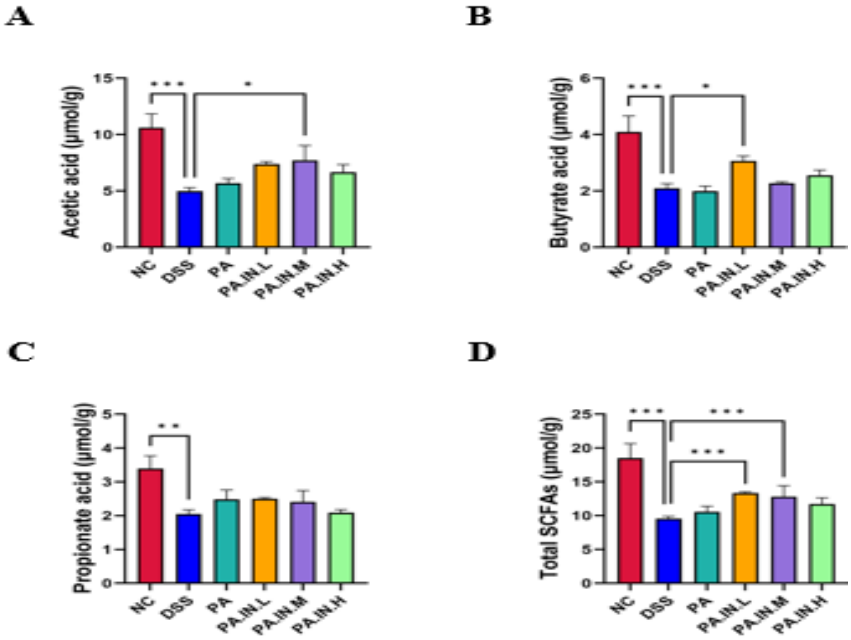


Fig. 2. Effects of *Pediococcus acidilactici* and inulin combined with *Pediococcus acidilactici* on mouse intestinal SCFAs

Note: Data are mean ± standard deviation (n=10 per group) (*P≤0.05, **P≤0.01, and ***P≤0.001.)

We examined beta diversity to compare community structures among experimental groups. Two analyses (Principal Coordinates Analysis (PCoA) and Non-Metric Multi-Dimensional Scaling (NMDS) were performed using Weighted Unifrac distances to display inter-group variability. Distinct clustering on the PCoA plot (Fig. 3F) and NMDS ordination (Fig. 3G) showed that the overall composition of gut microbiota was significantly different among the six groups. These structural changes were reflected in a quantitative comparison of Weighted Unifrac distances (Fig. 3H). The community dissimilarity of the DSS group was significantly greater than that of the NC group (P≤0.05). However, this difference was greatly reduced in each treatment group compared to the DSS group. The decrease was the largest in

the PA, PA.IN.M, and PA.IN.H. These findings demonstrate that the inulin-based synbiotic can substantially reduce the β-diversity shifts induced by DSS in the mouse gut microbiome.

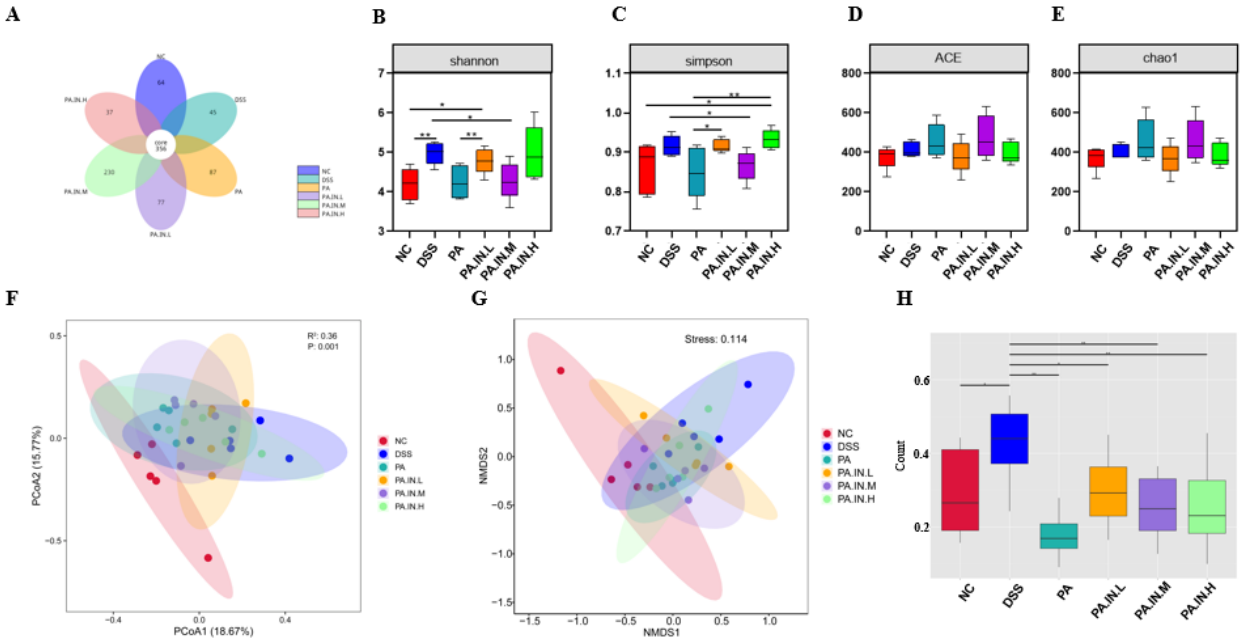


Fig. 3. Effects of the inulin synbiotic on gut microbiota diversity

(A) Venn diagram showing OTU overlap and uniqueness across groups. (B-E) Alpha diversity metrics: (B) Shannon, (C) Simpson, (D) ACE, and (E) Chao1 indices. (F) PCoA and (G) NMDS plots based on weighted UniFrac distances. (H) Boxplot of weighted UniFrac distances, quantifying beta diversity between groups. Group definitions are the same as in Figure 2. Data are mean ± standard deviation (n=10 per group). Statistical significance is denoted as *P≤0.05, **P≤0.01, and ***P≤0.001.

Effect of Inulin Synbiotic on Gut Microbiota Abundance in Mice

Effect of inulin synbiotic on the phylum level of the cecal Microbial community in DSS-induced mice: *Firmicutes* and *Bacteroidota* were the dominant phyla in the gut microbiota of all mouse groups (Fig. 4A, top 10). In Fig. 4B, *Firmicutes*, *Desulfobacterota*, *Verrucomicrobiota* (P=0.003), and *Actinobacteria* (P≤0.05) were significantly reduced in the DSS group, while *Bacteroidota* was markedly elevated (P≤0.001) relative to the NC group. Compared with the DSS group, all four treatment groups (PA, PA.IN.L, PA.IN.M, and PA.IN.H) demonstrated a sharply increased *Firmicutes* proportion (P≤0.001) alongside a markedly decreased relative abundance of *Bacteroidota* (P≤0.01). Among them, the inulin synbiotics in the PA.IN.L and PA.IN.M groups could significantly increase the proportion of *Verrucomicrobiota* (P≤0.05); *Pediococcus acidilactici*, as well as the inulin synbiotic in the PA.IN.H group, produced a significant increase in *Desulfobacterota* relative abundance (P≤0.05). In summary, the inulin synbiotic notably modified the phylum-level profile of the cecal microbiota in mice with DSS-induced colitis.

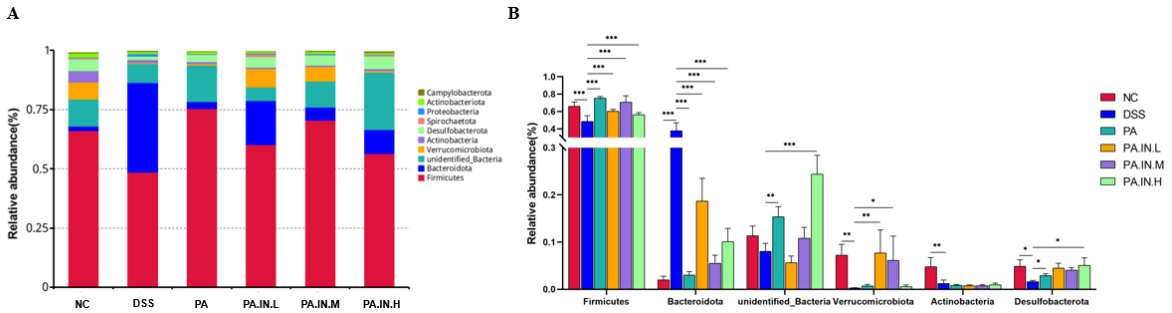


Fig. 4. Phylum-level relative abundance of the mouse cecal microbiota.

A: Stacked bar chart showing the composition of the top 10 most abundant phyla in each group. B: Statistical comparison of key phylum abundances across all groups.

Note: Data are mean ± standard deviation (n=10 per group). (*P≤0.05, **P≤0.01, and ***P≤0.001.)

Effect of inulin synbiotic on the family level of the cecal bacterial community in DSS-induced mice: From Fig. 5A, it can be seen that at the Top 10 family level, the dominant families in several experimental groups were *Erysipelotrichaceae*, *Lachnospiraceae* (phylum *Firmicutes*), *Muribaculaceae* (*Bacteroidetes*), and *Saccharimonadaceae*. Further difference analysis found that compared to the control group (NC), the DSS group had a highly significantly decreased relative abundance of *Erysipelotrichaceae* and *Akkermansiaceae* (P=0.003), a significantly decreased proportion of *Lachnospiraceae* (P=0.02) and *Saccharimonadaceae* (P=0.04), and a highly significantly decreased relative abundance of *Lactobacillaceae* (P≤0.001). Significant increases in abundance were detected for *Muribaculaceae* (P≤0.001) and *Tannerellaceae* (P=0.01). Compared to the DSS group, the proportion of *Erysipelotrichaceae* was also significantly increased in the PA, PA.IN.M, and PA.IN.H groups (P≤0.05); the relative abundance of *Muribaculaceae* was substantially decreased in the PA, PA.IN.L, PA.IN.M, and PA.IN.H groups (P≤0.001); the proportion of *Saccharimonadaceae* was markedly increased in the PA, PA.IN.M (P=0.02), and PA.IN.H groups (P≤0.05); *Tannerellaceae* abundance was significantly reduced across the PA, PA.IN.L, PA.IN.M, and PA.IN.H groups (P≤0.05); the proportion of *Akkermansiaceae* was significantly increased in the PA.IN.L (P=0.001) and PA.IN.M groups (P=0.01). Furthermore, we examined the fold changes of *Akkermansiaceae* and discovered that DSS treatment considerably decreased their abundance. *Akkermansiaceae* was 24.36 times more abundant in the NC group than in the DSS group. As well, the PA.IN.L and PA.IN.M groups had *Akkermansiaceae* abundances that were 26.1 and 20.77 times higher than those of the DSS group, respectively. A significant modulatory effect of the inulin synbiotic on fecal bacterial communities was observed at the family level in DSS-colitis mice.

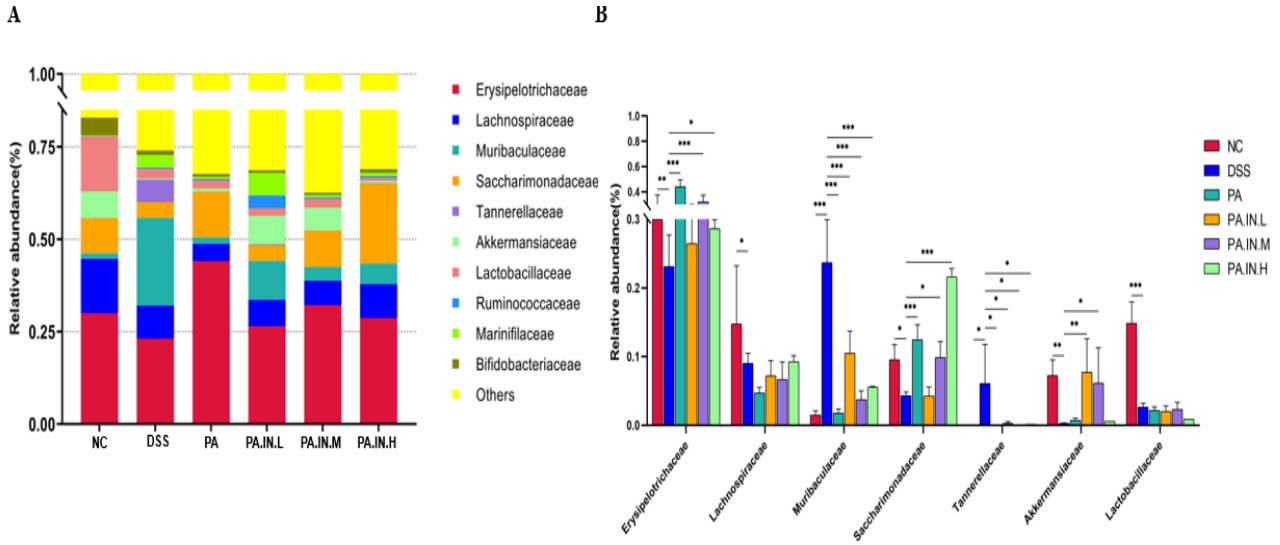


Fig. 5. Family-level relative abundance of the mouse colonic microbiota.

A: Family-level relative species abundance in the mouse cecum; B: Mouse cecal species abundance: intergroup statistical comparison at the family level. Data are mean ± standard deviation (n=10 per group). (*P≤0.05, **P≤0.01, and ***P≤0.001.)

Effect of inulin synbiotic on the genus level of the fecal bacterial community in DSS-induced mice: From Fig. 6A, it can be seen that at the genus level, the main genera were *Candidatus_Saccharimonas*, *Turicibacter*, *Parabacteroides*, and *Akkermansia*. In the DSS group, *Candidatus_Saccharimonas*, *Turicibacter*, and *Akkermansia* were significantly reduced (P≤0.001), while *Parabacteroides* was significantly elevated (P≤0.001) relative to the NC group. Compared to the DSS group, the PA group had a highly significantly increased proportion of *Turicibacter* and *Candidatus_Saccharimonas* (P≤0.001), and a highly significantly decreased abundance of *Parabacteroides* (P≤0.001). The PA.IN.L (P=0.02) and PA.IN.M groups had a highly significantly increased proportion of *Turicibacter* and *Akkermansia* (P≤0.05), and a highly significantly decreased abundance of *Parabacteroides* (P≤0.001). The PA.IN.M and PA.IN.H groups had a highly significantly increased relative abundance of *Candidatus_Saccharimonas* (P≤0.001). The PA.IN.H group had a highly significantly decreased abundance of Parabacteroides and *Turicibacter* (P≤0.001). In summary, the inulin synbiotic modulated the fecal microbiota of DSS-colitis mice by enriching beneficial genera (e.g., *Akkermansia* and *Candidatus_Saccharimonas*) while depleting *Parabacteroides*. Notably, *Turicibacter* exhibited a biphasic response, increasing at lower inulin concentrations and decreasing at higher doses.

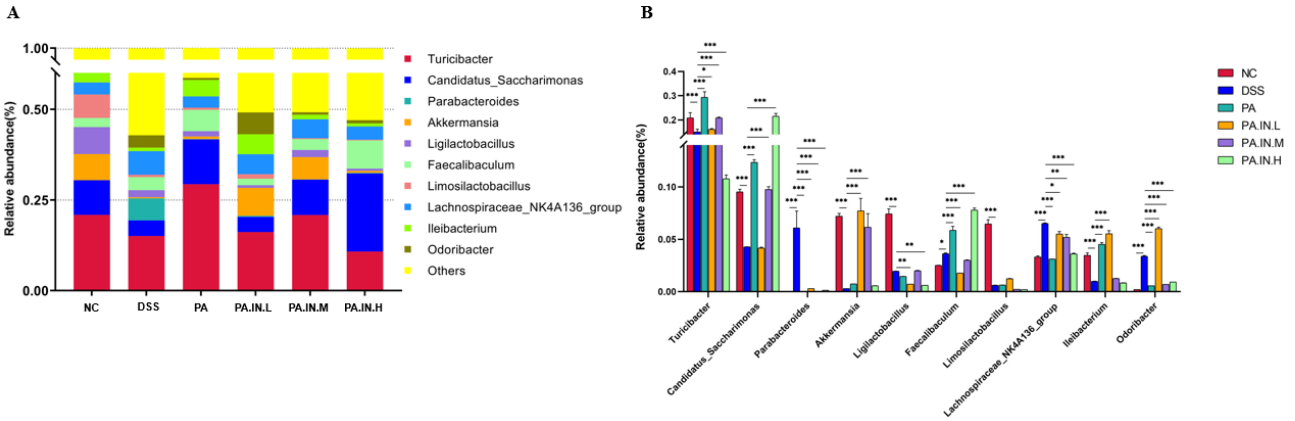


Fig. 6. Genus-level relative abundance of the mouse cecal microbiota.

A: Bar chart showing the genus-level taxonomic composition of species in the murine cecum; B: Statistical comparison of genus-level abundance across mouse cecal groups. Data are mean ± standard deviation (n=10 per group). (*P≤0.05, **P≤0.01, and ***P≤0.001.)

Effects of Inulin Synbiotics on the Species Makeup of the Murine Gut Microbiota Treated with DSS: LEfSe analysis at the genus level revealed the bacterial taxa responsible for the greatest intergroup differences. LDA analysis allows for comparisons between multiple groups to find species with significantly different abundance scores (LDA scores). LEfSe analysis was performed on species with an LDA Score >2, and the results are shown in Fig. 7. Gut microbiota structure at the genus level varied across the experimental groups. Among them, in the NC group, *Lachnoclostridium*, *Eubacterium_xylanophilum_group*, *Enterorhabdus*, *Eubacterium_brachy_group*, *Clostridium_sensu_stricto_1*, *Limosilactobacillus*, *Ligilactobacillus*, and *Lactobacillus* were the main differential bacteria. In the DSS group, *Bosea*, *Parasutterella*, *Bacteroides*, *Gordonibacter*, and *Parabacteroides* were predominant. In the PA group, *Sphingomonas*, *Xanthomarina*, and *Brevundimonas* were predominant. In the (PA.IN.L) group, *Odoribacter*, *Acinetobacter*, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, *Rikenellaceae_RC9_gut_group*, *unidentified_Chloroplast*, and *Rikenella* were predominant. In the (PA.IN.M) group, *Pseudofulvimonas*, *UCG_005*, *Dietzia*, *Sulfitobacter*, *unidentified_Christensenellaceae*, *Nautella*, *Coriobacteriaceae_UCG_002*, and *Pediococcus* were predominant. In the (PA.IN.H) group, *Tuzzerella*, *Brucella*, *Alistipes*, *Allobaculum*, *Prevotellaceae_NK3B31_group*, *unidentified_Lachnospiraceae*, and *Candidatus_Saccharimonas* were the main differential taxa. In summary, in the DSS group, *Parasutterella*, as a harmful bacterium, was significantly enriched; in the inulin synbiotic groups, *Rikenellaceae_RC9_gut_group*, *Alistipes*, and *Rikenella*, as beneficial bacteria, were significantly enriched.

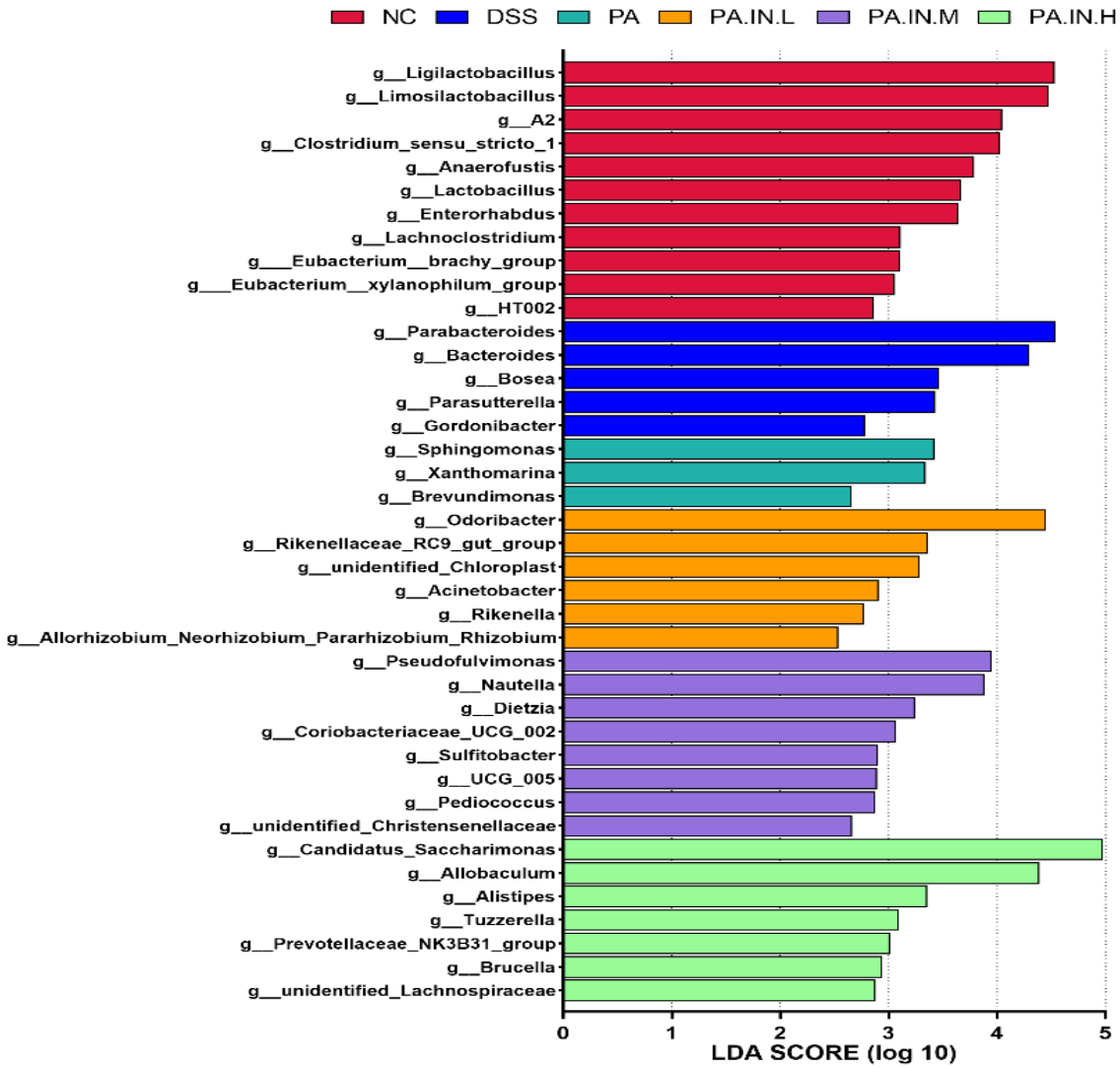


Fig. 7. Histogram of LDA value distribution from Linear Discriminant Analysis (The figure highlights species with LDA scores >2 and P≤0.05, where the bar length corresponds to the effect magnitude of each differential species); LEfSe comparison of microbiota in each group (LDA score histogram). The NC, DSS, PA, PA.IN.L, PA.IN.M, and PA.IN.H groups are identified by red, blue, dark green, yellow, purple, and light green, respectively.

A Study that Looks at the Relationship Between the Amount of SCFAs in Mouse Poop and the Ten Most Common Bacteria at the Genus Level: Further, Pearson correlation analysis was conducted between the mouse cecal SCFA content and the top ten differential bacteria at the genus level. *Ligilactobacillus* positively correlated with propionic acid and total SCFAs (P=0.019; Fig. 8). *Limosilactobacillus* strongly correlated with propionate (P=0.007) and significantly with acetate, butyrate, and total SCFAs (P≤0.05). Other bacteria and SCFAs had no significant correlation. In summary, the changes in SCFAs induced by DSS and the inulin synbiotic are closely related to the homeostasis of the gut microbiota.

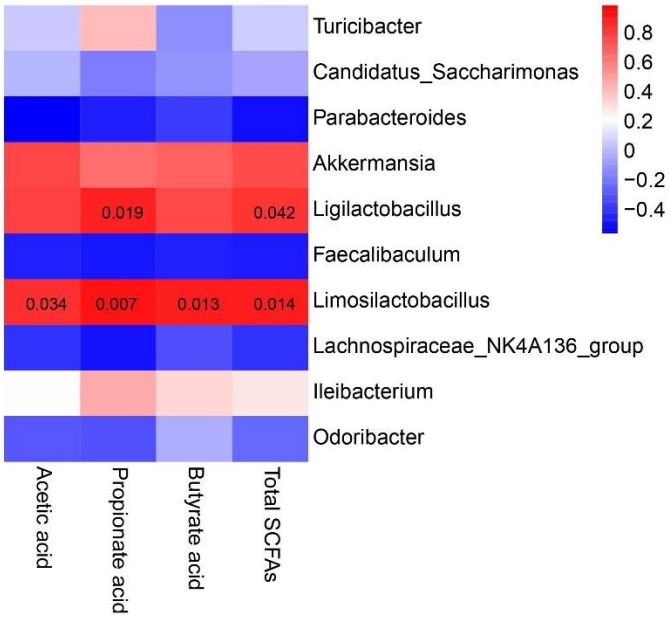


Fig. 8. Correlation analysis between genus-level differential bacteria and short-chain fatty acids

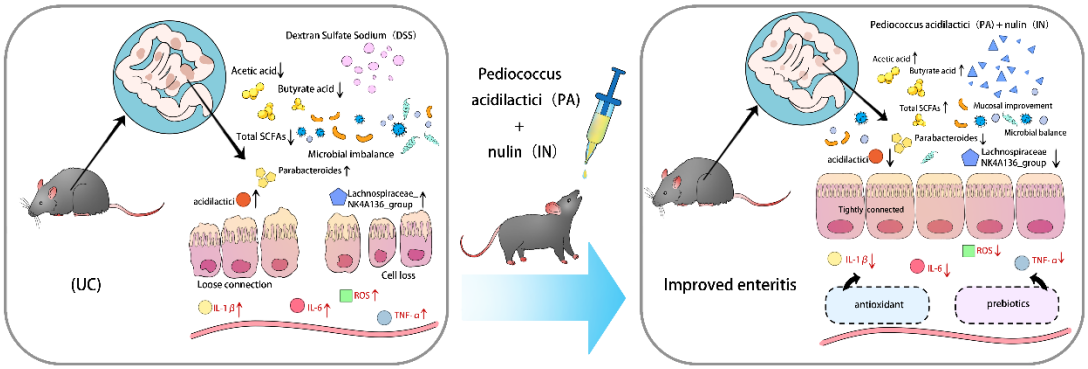


Fig. 9. Schematic representation of the inulin synbiotic-mediated protection against DSS-induced colonic inflammation in mice. Note: Red: previous research results; Black: current research results

DISCUSSION

Intestinal inflammatory diseases are a major clinical challenge and, as a result of their complex pathogenesis, there are a limited number of therapeutic options. This, therefore, emphasizes the need for innovative therapeutic strategies. Our latest study showed that inulin synbiotics had a profound effect on gut microbiota and SCFAs in mice with colitis.

Specifically, through the fermentation of dietary fibre, gut microorganisms create SCFAs, which mostly consist of propionic acid, acetic acid, and butyric acid (Shin et al., 2023). They are key regulators of metabolic processes, affecting various host physiological processes (McLoughlin et al., 2017; Koh et al., 2016). Beyond providing energy for microbial growth, SCFAs are integral to various signal transduction pathways. By upregulating tight junction proteins (claudin-1, claudin-7, ZO-1, ZO-2), studies have demonstrated that butyrate ingested by colon cells can improve barrier integrity (Hung et al., 2018). Moreover, butyric acid can decrease inflammation by preventing macrophages from activating HDAC112 and NF-κB (nuclear factor κB). (Kasubuchi et al., 2015). According to our findings, the PA.IN.L group's butyric acid levels were considerably raised by inulin synbiotics, which reduced inflammation. This is in line with our earlier research showing that inulin synbiotics dramatically lowered mice's blood levels of TNF-α, IL-6, and IL-1β (Wang et al., 2024). According to studies, acetic acid can strengthen the intestinal barrier by encouraging the production of IgA in the gut (Wu et al., 2017; Deleu et al., 2023). Inulin, a type of dietary fiber, is metabolized by the gut microbiota into SCFAs. Consequently, inulin can help regulate the gut microbiota, protect the gut epithelium, reduce

inflammatory responses, and promote gut barrier repair (Beisner et al., 2021; Uerlings et al., 2020; Tawfick et al., 2022). Previous studies support these roles. For instance, Riley L. Hughes (Hughes et al., 2022) demonstrated that inulin effectively ameliorated DSS-induced colon inflammation and contributed to the restoration of gut SCFA production. According to our findings, the PA.IN.M group's inulin synbiotics considerably reversed the DSS-induced drop in acetic acid. Therefore, we speculate that inulin synbiotics may exert a protective effect against enteritis by increasing the production of SCFAs in the cecum.

Second, the gut microbiota is known to critically influence the pathogenesis and therapeutic response of UC. Mucosal barrier integrity is compromised by gut dysbiosis, leading to bacterial translocation and the subsequent activation of an inflammatory immune response. Probiotics and prebiotics have been shown in numerous studies to play a regulating role in the gut flora. In accordance with earlier studies, our results demonstrate that inulin synbiotics change the composition of the gut microbiota and can restore its complexity and diversity. For example, Yingjie Guo (Guo et al., 2021) discovered that inulin improved the intestinal barrier function and reduced inflammation in mice, which was accompanied by an increase in microbial diversity. Chang (Chang et al., 2023) found that inulin administration to APOE4 female mice increased α-diversity while significantly downregulating Escherichia coli and inflammation-related pathways. The DSS group's Shannon index was higher than the NC group's in this trial. The fact that the DSS treatment enhanced the quantity and diversity of harmful microbes is one likely explanation. However, by generating gut-beneficial substances, certain bacteria can reduce intestinal inflammation brought on by DSS. The phylum Firmicutes encompasses numerous beneficial microorganisms that produce antimicrobial compounds, thereby inhibiting pathogen colonization and maintaining gut health. Our study further showed that enteritis correlated with decreased *Firmicutes* and increased *Bacteroidota*. Conversely, the inulin-based synbiotic reversed this trend, reducing *Bacteroidota* and increasing *Firmicutes* abundance. As key degraders of dietary fiber, *Firmicutes* are crucial for maintaining gut microbial balance (Mukherjee et al., 2020; Briggs et al., 2021). These bacteria can depolymerize a wide array of dietary fibers to produce beneficial metabolites like butyrate (Ndeh et al., 2018). Akkermansiaceae colonizes the mucus layer of the host's intestine and produces SCFAs by breaking down mucin. Research has demonstrated that Poria cocos polysaccharide supplementation can reduce DSS-induced dysbiosis by increasing the number of beneficial bacteria, Akkermansiaceae, in the gut of DSS-treated mice (Wan et al., 2024). The addition of inulin synbiotics considerably reversed the decline in Akkermansiaceae abundance brought on by DSS therapy in this investigation, indicating that inulin synbiotics may alleviate enteritis by boosting Akkermansiaceae abundance. Research has found a close correlation between Akkermansia and the generation of SCFAs (Wu et al., 2021). Supporting this, Wang Y-N (Wang et al., 2019) reported that a synbiotic containing Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium lactis, and inulin increased the abundance of Akkermansia in mice with DSS-induced UC. In our study, treatment with the inulin synbiotics led to significantly higher abundances of Akkermansia. Limosilactobacillus, as a probiotic, can alleviate colitis caused by DSS, promote the proliferation of beneficial bacteria Akkermansia, and restore the balance of gut microbiota (Yue et al., 2025). Using the LEfSe analysis method, we further examined the differentially expressed bacteria at the genus level and discovered that there was a significant increase in Parabacteroides in the DSS group, Odoribacter in the PA.IN.L group, and Candidatus_Saccharimonas in the PA.IN.H group. Odoribacter has been shown to be involved in the fermentation of polysaccharides in the diet to produce butyric acid, a key component in regulating intestinal homeostasis and host immune responses (Li et al., 2025). Limosilactobacillus and *Ligilactobacillus*, two key lactic acid bacteria, were also increased in the control group, signifying a healthy digestive and metabolic condition. Overall, the results suggest that inulin synbiotics could potentially help to reduce DSS-induced enteritis by modulating the amount of gut microbiota that produce SCFAs.

To further explore the relationship between the abundance of gut microorganisms and SCFAs. Significant relationships were found between cecal SCFA levels and the top 10 most abundant genera. Both *Ligilactobacillus* and Limosilactobacillus, two families of lactic acid-producing bacteria (family Lactobacillaceae, phylum *Firmicutes*), were highly correlated with the production of SCFAs. In particular, the numbers of *Ligilactobacillus* were positively correlated with propionic acid and total SCFAs. Limosilactobacillus showed a high positive correlation with propionic acid and a significant positive correlation with acetate, butyrate, and total SCFAs. There was no significant correlation between other prominent microorganisms and SCFAs. The metabolites of Limosilactobacillus reuteri are known to contain acetic acid (Yang et al., 2015; Thomas et al., 2012) that is able to restore intestinal permeability and improve barrier function. This correlation was positive between the presence of Lactobacillaceae and the production of acetate, suggesting the importance of these bacteria in the health of the intestine. Lactobacillaceae as members of *Firmicutes* group have a number of enzymes that can break down carbohydrates, allowing them to ferment complex and indigestible polysaccharides such as inulin in the production of SCFAs (Gong et al., 2021). Finally, inulin synbiotics might be used to help reduce the Relative Abundance of bacteria that are involved in SCFA production, thereby preventing or reducing DSS-induced colitis.

The aforementioned results indicate that this study has a number of limitations. First, the induction period of the illness in the experiment was short (seven days) and will be longer in future studies. Second, it is yet unknown how PA and inulin work. Targeted metabolomics and functional metagenomics will be used in future studies to investigate the exact mechanism of action. Finally, future research will focus on reducing the inulin concentration gradient, as the one used in this experiment was too high.

To conclude, the use of a synbiotic mixture of inulin and *Pediococcus acidilactici* (PA) was effective in reducing the effects of DSS-induced colitis in mice. This effect of cure was through two main mechanisms: the increase in cecal SCFA production and the correction of gut dysbiosis. There is a substantial correlation between the production of SCFAs and *Ligilactobacillus* and Limosilactobacillus. The synbiotic intervention was able to induce modulation of gut microbiota composition with a higher relative abundance of beneficial commensals such as Akkermansia, Candidatus_Saccharimonas, etc., and a lower relative abundance of potentially harmful taxa. All these changes together contributed to the alleviation of clinical symptoms of DSS-induced enteritis. In this trial, the PA.IN.L group demonstrated the greatest improvement in intestinal inflammation at the optimal dose of inulin (5 mg/mL). These findings have theoretical implications for the use of this synbiotic as a potential therapeutic approach to enteritis.

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Author Contributions: Mingbo Li and Longzhou Zhang performed the experiments and analyzed the short-chain fatty acids and tissue samples. Mingbo Li also performed the analysis and wrote the paper. Chunri Yan and Xin Jin provided technical support for the mouse experiments. Yuanming Jin analyzed the data. Changguo Yan and Lianhua Cui conceived and designed the research experiments and provided full supervision throughout the experiment. All authors interpreted the data, revised the manuscript critically for important academic content, and approved the final version.

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