**Lactiplantibacillus plantarum** ZJ316–fermented milk ameliorates dextran sulfate sodium–induced chronic colitis by improving the inflammatory response and regulating intestinal microbiota

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**ABSTRACT**

The pathogenesis of inflammatory bowel disease may be related to local inflammatory damage and disturbances in intestinal microecology. Probiotic therapy is a safe and effective therapeutic approach. Considering that fermented milk is accepted and enjoyed by many people as a daily dietary intervention strategy, its potential to alleviate dextran sulfate sodium (DSS)–induced chronic colitis in mice needs to be explored. In this study, we evaluated the therapeutic effects of *Lactiplantibacillus plantarum* ZJ316–fermented milk by establishing a mouse model of DSS-induced chronic colitis. The results showed that the disease severity and colonic lesions of inflammatory bowel disease were effectively alleviated by ingestion of fermented milk. At the same time, the expression of proinflammatory cytokines (TNF-α, IL-1β, and IL-6) effectively decreased, and the expression of antiinflammatory cytokines (IL-10) increased. Results based on 16S rRNA gene sequencing indicated that the structure and diversity of intestinal microorganisms changed markedly by intake of *L. plantarum* ZJ316–fermented milk, and fermented milk reduced the abundance of harmful bacteria (*Helicobacter*) while promoting the growth of beneficial bacteria (*Faecalibacterium*, *Lactiplantibacillus*, and *Bifidobacterium*). Additionally, the levels of short-chain fatty acids (acetic acid, propionic acid, butyric acid, pentanoic acid, and isobutyric acid) were also increased. In conclusion, the intake of *L. plantarum* ZJ316–fermented milk can alleviate chronic colitis by suppressing the inflammatory response and regulating intestinal microbiota.

**Key words:** *Lactiplantibacillus plantarum* ZJ316, fermented milk, dextran sulfate sodium–induced chronic colitis, inflammation, short-chain fatty acids

**INTRODUCTION**

Inflammatory bowel disease (IBD) is an inflammatory disease of the colon with an unclear pathogenesis that is often characterized by recurrent and repeated relapses (Zuo and Ng, 2018; Liang et al., 2021). It can be divided into Crohn's disease and ulcerative colitis (Baumgart and Carding, 2007). In recent years, there has been a growing number of patients with colitis worldwide. Colitis has evolved into one of the most difficult diseases in the world due to its incurability and recurrent nature, substantially impairing patients’ life quality and placing a significant financial burden on their families and society as a whole (Kaplan, 2015).

Although the etiology and pathogenesis of colitis have not been precisely determined, a growing number of studies have found that colitis is caused by genetic, immune, environmental, microecological, infectious, dietary, psychological stress, and many other factors (Jeong et al., 2019). The currently recognized pathogenic mechanism is based on the premise of genetic factors and the role of environmental factors (O’Toole and Korzenik, 2014) or dietary factors (Fitzpatrick et al., 2022) constantly induced by the occurrence of infection. This, in turn, activates the intestinal specific immune system, resulting in abnormalities in the immune system, disruption of the intestinal physiological barrier, and the opportunity for pathogenic bacteria to invade the intestine and change its microecology, ultimately leading to the development of intestinal inflammatory diseases (Fitzpatrick et al., 2022). Anti-inflammatory drugs, immune system suppressants, and biologic agents are commonly used in clinical treatment (Danese et al., 2020). Among them, 5-aminosalicylic acid (5-ASA, mesalamine) inhibits the expression of proinflammatory cytokines such as IL-1β and TNF-α, inhibits the lipoxygenase pathway, scavenges free radicals and oxidants, and also inhibits NF-κB activation (Danese et al., 2020). It should be noted that while treatments for IBD offer relief, they do not guarantee a complete cure. While surgery may be a more extensive
form of treatment, postoperative medication remains necessary to minimize the likelihood of recurrence. Emerging research provides promising indications for dietary interventions and treatment with probiotics in mitigating symptoms associated with IBD (Liang et al., 2021). As a result, a large number of researchers have turned their attention to probiotic-based dietary interventions as therapeutic tools.

As a kind of probiotic, lactic acid bacteria have physiological functions such as promoting nutrient absorption, improving nutrient utilization, lowering blood lipids, lowering blood pressure, and enhancing host immunity (Ali, 2010; Alard et al., 2016; Zhang et al., 2018; Liu et al., 2020). Studies have shown that lactic acid bacteria can enter the intestinal tract through layers of barriers, combine with flora in the extremely complex digestive tract microenvironment, and then regulate the intestinal flora by colonizing resistance and ecological occupancy, producing antibacterial substances, and restoring the host resistance (Liu et al., 2022; Wu et al., 2023). Due to better patient acceptance and safety, probiotic-fermented milk has become a method of dietary intervention to provide the host with a large number of probiotics and active substances beneficial to human body and rarely any side effects. Probiotics and probiotic-fermented milk have been tried for clinical relief and prevention of inflammation. A potentially promising solution to ease medical conditions within the digestive system is found in the form of Bifidobacterium lactis Probi-M8 fermented milk. Recent studies suggest it may offer relief to ailments such as alcoholic liver injury in rats (He et al., 2022).

In addition, fermented milk including Lactiplantibacillus (L. paracasei CNCM I-1518 and I-3689, L. rhamnosus CNCM I-3690) strains effectively help Helicobacter pylori patients recover by changing the intestinal flora structure, inhibiting potential pathogens and promoting short-chain fatty acid (SCFA) production (Guillemard et al., 2021). In addition, fermented milk was used to prevent inflammation. Zhang et al. (2021) found that Bacillus subtilis–fermented milk was capable of inhibiting inflammatory response, promoting mucosal barrier reconstruction, and regulating intestinal flora, and thus alleviating DSS-induced IBD. In addition, it has been determined that fermented milk utilizing Lactococcus lactis may exhibit the potential for mitigating symptoms of colitis in mice (Saraiva et al., 2015). Another research study suggested that fatty acids derived from fermented milk can attenuate the inflammatory response by inhibiting the expression of inflammatory factors such as IL-6 and TNF-α. Additionally, these fatty acids may obstruct the phosphorylation of the JNK/MAPK signaling pathway (Lao et al., 2022).

Lactiplantibacillus plantarum ZJ316, a strain of Lactiplantibacillus, is derived from infant feces and has demonstrated considerable capability to survive various environmental stresses and human gastrointestinal tract conditions, as evidenced by its genome and antimicrobial activity potential (Li et al., 2013, 2016). Lactiplantibacillus plantarum ZJ316 also has better environmental tolerance than Lactiplantibacillus rhamnosus GG and broad-spectrum antibacterial properties in vitro. In addition, reduced H. pylori adhesion could prevent and treat gastric mucosal inflammation induced by H. pylori in C57BL/6 mice (Zhou et al., 2021b; Wu et al., 2023). Natural L-phenyl lactic acid purified from L. plantarum ZJ316 showed antibacterial activity against Salmonella enterica Typhimurium and alleviates Salmonella Typhimurium–induced colitis (Zhou et al., 2020, 2021a). Moreover, it has the potential to mitigate ulcerative colitis by restraining inflammation and balancing SCFA levels and microbiota of the gut in a mouse model (Gu et al., 2023). This study discusses whether dietary interventions, particularly L. plantarum ZJ316–fermented milk, have beneficial effects on chronic colitis by establishing a DSS-induced model of chronic colitis in mice and provides a basis for further exploration of the host immunomodulatory properties of Lactiplantibacillus machine metabolites.

MATERIALS AND METHODS

Preparation of L. plantarum ZJ316–Fermented Milk

Lactiplantibacillus plantarum ZJ316 (China Center for Type Culture Collection [CCTCC] no. M 208077) was grown overnight in de Man, Rogosa, and Sharpe (MRS) broth (Hopebio, Qingdao, China; pH 6.5 ± 0.1), fermented at 42°C for 8 to 12 h until pH 4.2 ± 0.2 and the viable count was 10^9 cfu/mL by sterilized skim milk. Seven grams of sucrose (Sugarman, Guangzhou, China) and 6 mL of activated L. plantarum ZJ316 (1 × 10^7 cfu/mL) were inoculated into 100 mL of sterilized skim milk (Bright-dairy, Shanghai, China; pH 6.5 ± 0.1), fermented at 42°C for 8 to 12 h until pH 4.2 ± 0.2 and the viable count was 10^9 cfu/mL, and then preserved at 4°C.

Animals

All procedures were performed by all experiments according to the rules approved by the Institutional Animal Care and Use Committee, ZJCLA with approval number of ZJCLA-IACUC-20140001. Eight-week-old male BALB/c mice (16–20 g) were purchased from male BALB/c mice (16–20 g) were purchased from...
Shanghai SIPPR-Bk Laboratory Animals Ltd. (Shanghai, China).

Throughout the period, all mice were housed in a standard pathogen-free animal housing facility, where a 12-h light-dark cycle was maintained at an ambient temperature of 21 ± 2°C and relative humidity of 45 ± 10%. Mice were given food and water ad libitum. All animals were acclimated and fed for 1 wk before the start of the trial.

Construction of IBD Model and Experimental Design

According to the method of Zhai et al. (2019) and Fan et al. (2021), the DSS-induced model of chronic colitis was modified and developed. Thirty mice were randomly divided into 6 groups of 5 animals each. The experimental design is displayed in Figure 1. Upon completion of the adaptation period, the mice underwent randomization to form 6 distinct groups (n = 5). In addition to the control group (CK), the remaining 25 mice were provided with water containing 2.5% (wt/vol) DSS (MP Biomedicals, Illkirch, France) for 3 cycles (7 d on 2.5% DSS and 7 d on sterile water). After successful induction of chronic colitis by DSS for 6 wk, mice in the DSS model group (DSS) were killed. Mice in the fermented milk group (FM), milk group (M), normal group (N), and 5-ASA group (5-ASA) were intragastrically gavaged with *L. plantarum* ZJ316–fermented milk, milk, sterile water, and 30.25 mg/mL 5-ASA (200 μL/10 g) every other day, respectively. After treatment, the mice were killed for future serological tests.

![Figure 1](image_url)

**Figure 1.** Experimental design (n = 5). DSS: dextran sulfate sodium (DSS)–induced chronic colitis model group; N: received sterile water after successful induction of chronic colitis by DSS; FM: received *Lactiplantibacillus plantarum* ZJ316–fermented milk after successful induction of chronic colitis by DSS; M: received milk after successful induction of chronic colitis by DSS; 5-ASA: received 5-aminosalicylic acid after successful induction of chronic colitis by DSS; CK: received sterile water without DSS.

Throughout the course of the experiment, mice were orally administered at predetermined intervals, and their respective BW were documented on a weekly basis. Each mouse was weighed 3 times and averaged as the BW for the day and observed for growth and activity.

**Histopathology**

After the mice were killed, the colon was extracted from the abdominal cavity. The contents of the colon were cleared with the use of scissors and forceps, and subsequently flushed with a PBS solution. Utilizing a Vernier caliper, the colon length was measured and this was followed by an assessment of the colon’s weight, which was recorded.

The distal colon was taken for 1 cm and fixed in 10% formalin for 16 to 18 h. The colon samples were subjected to thorough wash and dehydration in standard procedures comprising sterile water and gradient ethanol. Subsequently, the samples were embedded in paraffin and sectioned at 4 μm thickness, followed by hematoxylin and eosin (H&E) staining. The stained tissue sections were carefully observed under a Nikon Eclipse Ci light microscope. The H&E-stained colon tissue sections were then scored for tissue damage including 4 indices of the degree of inflammation, depth of lesion, crypt destruction and extent of lesion, and the specific criteria. Histological scoring and identification criteria were 0, no significant change; 1, mild inflammation, destruction of one-third of the crypts, 1 to 25%
of lesion extent; 2, moderate inflammation, destruction of two-thirds of crypts, 26 to 50% of lesion extent; 3, severe inflammations, loss of all crypts, 51 to 75% of lesion extent; 4, all crypt and intestinal epithelium damage and lesions.

**Real-Time Quantitative PCR**

Total RNA was extracted from colon tissue using TRIzol reagent (Takara, Shiga, Japan). The cDNA was reversely transcribed from 1 μg of RNA using the PrimeScript 1st Strand cDNA Synthesis Kit (Takara, Japan).

Relative gene expression levels were determined by real-time quantitative PCR (RT-qPCR) on a Bio-Rad Real-time System using TB Green Premix Ex Taq (Tli RNaseH Plus; Takara, Japan) and specific primers. The RT-qPCR temperature program was 95°C for 30 s, 95°C for 10 s, 55°C for 10 s, and 72°C for 30 s for a total of 40 cycles. The data were normalized to the GAPDH control and analyzed by the 2^−ΔΔCt method. The primer sequences are shown in Table 1.

**Short-Chain Fatty Acids**

Changes in SCFA in the colon of mice in each intervention group were determined using GC-MS. Fifty milligrams of fecal samples was homogenized with 1.0 mL of 6% phosphoric acid solution, all transferred to a 20-mL headspace injection vial, and 1 mL of internal standard solution was added and sealed. Analysis was carried out by GC.

The GC conditions were as follows: DB-FFAP capillary column (30 cm × 0.25 μm × 0.25 mm; Agilent Technologies), injection temperature 200°C, detector temperature 250°C, injection volume 2 μL, hydrogen as carrier gas, split injection (split ratio 10:1, flow rate 1 mL/min).

A static headspace injection was used with an incubation temperature of 85°C, an incubation time of 30 min, an injection needle temperature of 95°C, and an injection volume of 1 mL. The column temperature was raised from 80°C to 220°C at 8°C/min.

**Microbiota 16S rRNA Gene Sequencing**

The genomic DNA of feces was extracted using Zymo Research Corp. kit. The V3–V4 hypervariable region of bacterial 16S rRNA genes was amplified and sequenced on the Illumina NovaSeq platform of Beijing Nuokezhiyuan Bioinformatics Co., Ltd. (Beijing, China). The UCLUST algorithm is used to cluster the selected sequences into operational taxonomic units, and the similarity threshold is 97%.

**Statistical Analysis**

Statistical analyses were performed using SPSS version 22.0 and analyzed by 1-way ANOVA. The α diversity (Shannon index, Chao1 index, ACE index), β diversity (principal coordinate analysis, PCoA; Bray-Curtis distance), and linear discriminant analysis effect size were performed using the Biozeron Cloud Platform (http://www.cloud.biomicroclass.com/CloudPlatform). The graphics were generated by GraphPad Prism version 9.0.

**RESULTS**

**L. plantarum ZJ316–Fermented Milk Mitigated Disease Severity in Mice With DSS-Induced Chronic Colitis**

To evaluate the efficacy of *L. plantarum* ZJ316-fermented milk in providing relief from IBD symptoms, we established the DSS-induced chronic colitis mouse model. The severity of the disease was assessed by monitoring the growth activity profile and BW dynamics of the subjects. At the end of the modeling period, all mice showed positive fecal occult blood tests and visible blood in the feces, indicating that the model was successfully established. Then we assessed the relief of IBD symptoms by ingesting milk, fermented milk, and 5-ASA without altering the normal diet of the mice, and sterile water was ingested as a control (Figure 2). Throughout the experiment, the weight of all mice showed an upward trend. During the modeling period,
the weight of mice induced by DSS dropped rapidly. However, the weight rose after returning to a normal diet. It was obvious that the weight of IBD mice induced by DSS was much lighter than that of the control group ($P < 0.01$). In addition, after 5 wk of dietary intervention, the BW of mice in all treatment groups showed a significant increase ($P < 0.01$). The weight of mice in the FM group and 5-ASA group returned to normal levels. However, a notable contrast persisted between the N and M groups and the CK group, despite the fact that the latter demonstrated recuperation.

L. plantarum ZJ316–Fermented Milk Mitigated Colon Lesions in Mice With DSS-Induced Chronic Colitis

The administration of DSS resulted in noteworthy lesions in the colon of mice, with reduced colon lengths and weights (Figure 3). Compared with the control group, the mice in the DSS group exhibited a noteworthy decrease in colon length and a reduction in colon weight of 79% ($P < 0.01$), and this trend of colon lesions was prevented to varying degrees by each of the intervention groups. Figure 3A and Figure 3B clearly showed that fermented milk significantly maintained the length of the colon ($P < 0.01$) and was not significantly different from the CK group ($P > 0.05$). Although the other treatment groups had similar effects, they were much less effective than L. plantarum ZJ316–fermented milk.

Furthermore, the histological analysis of colon sections demonstrated that the administration of DSS effectively fostered the progression of ulcerative and chronic colitis in mice, with the magnitude of lesions extending to roughly 40%. The lumen of the intestine was slightly dilated and the shedding of epithelial cells was visible. After a period of intervention with gavage, the histology scores were lower much than the DSS group, indicating that the symptoms of mice with chronic colitis were alleviated in each treatment group. However, there were still varying degrees of inflammation present in each intervention group compared with the control group. Taken together, these results suggested that L. plantarum ZJ316–fermented milk had potential in protecting mice from the development of chronic colitis.

L. plantarum ZJ316–Fermented Milk Reduced Inflammation Levels in Mice With DSS-Induced Chronic Colitis

To evaluate the effect of L. plantarum ZJ316–fermented milk on inflammation levels in mice with DSS-induced chronic colitis, we monitored the mRNA levels of proinflammatory cytokines (TNF-α, IL-1β, and IL-6) and antiinflammatory cytokines (IL-10) present in the colon tissue of the subjects. According to the Figure 4, the N group and the M group did not significantly improve the immune response of mice ($P > 0.05$). It is worth noting that the mRNA expression of IL-6 was observed to be higher in the N group when compared with the other groups. This might be due to the spatial and temporal interval between the time and site at which transcription and translation of eukaryotic gene expression occurs.

Lactiplantibacillus plantarum ZJ316–fermented milk significantly downregulated the transcript levels of IL-
1β, IL-6, and TNF-α (P < 0.05), almost returning to normal levels compared with the CK group (P > 0.05). Moreover, the transcript level of IL-10 was also significantly upregulated (P < 0.01). On the other hand, 5-ASA had no significant effect on TNF-α, although it significantly reduced the transcript levels of IL-1β and IL-6 and improved the expression of IL-10. Overall, L. plantarum ZJ316–fermented milk reduced inflammation levels in the mice with DSS-induced chronic colitis.

**L. plantarum ZJ316–Fermented Milk Modulated the Gut Microbiota Diversity and Structure in Mice With DSS-Induced Chronic Colitis**

To ascertain the modulatory effect of L. plantarum ZJ316–fermented milk on the diversity and structure of intestinal flora induced by DSS in mice, we examined the composition of the mouse intestinal microbiota employing 16S rRNA gene sequencing (Figure 5). Based on a similarity level above 97%, mean operational taxonomic unit values were observed in 6 groups (Figure 5A).

Gut microbiota diversity and richness was conducted utilizing the Chao1, abundance-based coverage estimator, and Shannon indices, respectively (Figure 5C-E). In comparison with the control group, the DSS group displayed a considerable decrease in richness and diversity, indicating that the induction of DSS could potentially disrupt the gut microbiota of the host. In contrast, all intervention groups were able to increase microbial richness to some extent except the 5-ASA group. Among them, the L. plantarum ZJ316–fermented milk intervention was significantly increased, indicating that the intake of fermented milk increased the abundance of intestinal microorganisms.
According to the operational taxonomic unit level, PCoA (Bray distance) was conducted to a magnitude of differences between samples in each treatment group (Figure 5B). When species abundance was considered, individual differences from the same group were large. It is apparent that discernible variances persisted in the colonic microbial communities among the various groups. Illustratively, the figure demonstrates that the microbiota structure of the DSS group diverged markedly from that of the control group. Conversely, the other treated groups shared some overlap with the control group in the PCoA score plots, implying that the microbiota of the mice subjected to the dietary intervention exhibited similarities with those of the control group. This demonstrated that the microbiota of the mice was significantly improved by the dietary intervention and may have contributed to a gradual return of the gut microbiota structure from the DSS-induced disorder to a normal level similar to that of the control group.

**L. plantarum ZJ316–Fermented Milk Modulated the Gut Microbiota Composition in Mice With DSS-Induced Chronic Colitis**

Linear discriminant analysis effect size and linear discriminant analysis were deployed to determine the effect of individual species abundance on differential effects to discern significant groups or species responsible for sample delineation (Figure 6A and B). Significant differences were noted regarding the gut microbiota amid the 6 distinct groups. It showed *Prevotellaceae* and *Alloprevotella* were identified as the dominant microbiota in the DSS group. The F group showed *Faecalibacterium* and *Agathobacter* as the dominant microbiota, whereas *Lachnospiraceae*, *Desulfovibrio*, and *Butyricicoccus* were
identified as the dominant microbiota in the 5-ASA group.

Figure 6C and D reveal discernible variations in the intestinal microbiota of mice, characterized by a significant prevalence of top 10 phylum and genus at their respective levels of taxonomic classification. In DSS-induced mice, fermented milk may exert a moderating effect compared with the inhibitory effect of 5-ASA on intestinal microbial growth. At the phylum level, the intestinal microbiota of each group of mice consisted mainly of Firmicutes, Bacteroidetes, and Proteobacteria. Of these, Firmicutes and Bacteroidetes were the most dominant groups, accounting for nearly 50% of the samples. Compared with the CK group, there was a notable increase in the relative abundance of Bacteroidetes and Proteobacteria, and a concurrent decrease in that of Firmicutes. After supplementation with the L. plantarum ZJ316–fermented milk, Firmicutes and Bacteroidetes remained the predominant flora. At the gene level, DSS induction led to a significant rise in the prevalence of Bacteroides and Helicobacter, whereas L. plantarum ZJ316–fermented milk was found to act as a viable solution in reducing this effect. It was also noteworthy that it increased the proportion of Faecalibacterium, Lactiplantibacillus, Bifidobacterium, and Alistipes in the intestinal microflora. In addition, 5-ASA was
more effective in killing pathogenic bacteria such as *Bacteroides*, but at the same time a number of other beneficial bacteria were also killed. Compared with 5-ASA, *L. plantarum* ZJ316–fermented milk not only inhibited the multiplication of pathogenic bacteria, but also had more advantages in regulating the beneficial intestinal bacteria.

**L. plantarum ZJ316–Fermented Milk Influenced the Concentration of SCFA in Feces**

The contents of acetic acid, propionic acid, butyric acid, pentanoic acid, and isobutyric acid in mouse feces were measured separately by GC-MS to investigate the effect of *L. plantarum* ZJ316–fermented milk on the content of SCFA in the colon of each treatment group (Figure 7A–E). The contents of 5 SCFA in the feces of mice in the DSS group were significantly reduced (*P* < 0.01), indicating that DSS induction can lead to impaired SCFA metabolism of mice. After intervention of *L. plantarum* ZJ316–fermented milk, significant changes in SCFA content were observed (*P* < 0.05). The findings indicated that consumption of fermented milk by *L. plantarum* ZJ316 was capable of regulating the gut microbiome metabolite levels in an effective manner. Noteworthily, the content of each SCFA in the N group and M group also increased to some extent, suggesting that the SCFA metabolic system may also be restored in the process of self-recovery in mice with DSS-induced chronic colitis.

**Correlation Analysis of Gut Microbiota, SCFA, and Cytokines**

To ascertain the possibility of a correlation between the alterations noted in gut microbiota, metabolite, and cytokines, a Spearman correlation analysis was carried out on the relative abundances of groups pertaining to the top 10 genera and 4 prevailing phyla, as well as SCFA and inflammatory cytokines (Figure 8). The
results revealed *Bifidobacterium* and *Actinobacteria* exhibited significant positive correlations with acetic acid, propionic acid, butyric acid, and pentanoic acid. Moreover, *Lactiplantibacillus* was positively associated with TNF-α and IL-1β while displaying negative correlations with the antiinflammatory cytokine (IL-10); further, *Helicobacter* exhibited negative correlations with isobutyric acid.

**DISCUSSION**

Inflammatory bowel disease is an idiopathic chronic disease that causes recurrent abdominal pain, diarrhea, and blood in the stool (Walentynowicz et al., 2022). Although many treatments, including anti-inflammatory drugs, immune system inhibitors, biological agents, and surgical treatment, are available in the clinic, none of these methods are currently able to eradicate inflammatory bowel disease. A large number of researchers have turned their attention to finding new substances that can provide effective relief or treatment, such as probiotics and their products (Matsuoka et al., 2018; Bian et al., 2019; Jeong et al., 2022). Fortunately, probiotics have particular advantages in protecting the host intestinal health. The refinement of DSS-induced chronic colitis by *Bifidobacterium adolescentis* was accomplished through the stimulation of a protective Treg/Th2 response and the process of gut microbiota remodeling (Fan et al., 2021). Moreover, our previous research has identified an advantageous characteristic of *L. plantarum* ZJ316 in inhibiting the proliferation of detrimental bacterial species in vitro while fostering a healthy gut microbiota (Hang et al., 2022). It has also been found that fermented milk fermented by *Bifidobacterium breve* (Matsuoka et al., 2018) and *Lactiplantibacillus rhamnosus* GG (Yoda et al., 2014)
also had the ability to improve colitis in DSS mice. Inspired by this meaningful research, we investigated the alleviating effects of *L. plantarum* ZJ316–fermented milk on DSS-induced chronic colitis in mice through the implementation of a dietary intervention strategy. Additionally, we explored the prospective mechanisms by which this intervention could operate, including potential effects on inflammation, metabolites, and intestinal microbiota.

Several research studies have consistently demonstrated that administering DSS treatment has negative consequences on the health and growth of rodents. Therefore, we induced chronic colitis in mice with DSS-sterile water circulating gavage for a period of 6 wk. By observing the growth activity of the mice, we found that mice with chronic colitis also showed significant blood in the stool and significant weight loss, which were similar symptoms to acute colitis (Li et al., 2021; Xu et al., 2021). This suggests that DSS induction had a detrimental effect on the growth of mice. Subsequently, *L. plantarum* ZJ316–fermented milk was used to investigate whether it had a palliative effect on these diseased mice. Results revealed that the BW and growth status of the mice improved after dietary intervention in these mice with chronic colitis, and that the *L. plantarum* ZJ316–fermented milk had a significant effect and could even restore the mice to normal. The milk was also able to effectively prevent the shortening of the colon caused by DSS in mice, with no significant difference compared with the control group. These indicated that the *L. plantarum* ZJ316–fermented milk has the efficacy of promoting recovery and significantly improving IBD-related symptoms in DSS-induced mice. This is similar to the results of Yoda et al. (2014) who found that *Lactiplantibacillus rhamnosus* GG fermented milk prevented the occurrence of ulcerative colitis. The

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**Figure 8.** Spearman correlations between gut microbiota (top 10 genus levels and top 4 phylum levels), short-chain fatty acids, and cytokines. Red, positive correlation; blue, negative correlation. *P* < 0.05, **P* < 0.01.
results of H&E staining also revealed that \textit{L. plantarum} ZJ316–fermented milk was effective in improving colonic mucosal damage in mice with DSS colitis. In addition, in our study, mice in the N group recovered from the degree of colitis disease compared with the DSS group. It was suspected that it was probably due to the ability to recover themselves (Jialing et al., 2020). Overall, \textit{L. plantarum} ZJ316–fermented milk showed a clear advantage in alleviating disease symptoms in IBD mice, and the mechanism may be related to the capacity of promoting the host self-recovering.

Studies have shown that the pathogenesis of IBD is strongly related to an overactive and imbalanced immune response in the intestinal mucosa (Walentynowicz et al., 2022). Thus, the transcript levels of various inflammatory cytokines were detected to reflect the severity of inflammation. IL-1β and IL-6 are inflammatory cytokines that are widely involved in a variety of pathological damage processes such as tissue destruction and edema formation in animals, and they play an important role in the pathogenesis of IBD (Lopetuso et al., 2013). It has been suggested that TNF-α induces cytokine synthesis and generates a cascade response that further promotes the production of other proinflammatory cytokines, such as IL-1β, IL-6, and IL-8, ultimately leading to tissue inflammation. In contrast, IL-10 is considered to be an antiinflammatory cytokine produced by Th2 cells and monocytes and inhibits the production of proinflammatory cytokines (Gupta et al., 2016). These cytokines were considered to be important targets in the treatment of IBD. Xie et al. (2015) found that \textit{Lactiplantibacillus plantarum} NCU116 and carrot pulp fermented by this strain could improve intestinal health in mice by modulating intestinal mucosal immune responses. From our results, it is clear that DSS caused a significant upregulation of the expression of proinflammatory cytokines (IL-1β, TNF-α, and IL-6) in the mouse colon, which may therefore lead to immune disorders in the mouse colon, exacerbating inflammation and damage to colon tissue. The inflammation in the mice will not return to healthy levels without undergoing pharmacological control or receiving dietary intervention. However, supplementation of \textit{L. plantarum} ZJ316–fermented milk significantly downregulated the expression of proinflammatory cytokines and upregulated that of antiinflammatory cytokines, thus suggesting that it also has a modulatory effect on the host immune response by promoting the balance of immune responses, as reported in several reports (Santiago-López et al., 2018; Vaisberg et al., 2019).

Numerous previously conducted studies have confirmed a close association between the formation and progression of IBD and gut microbiota (Qiu et al., 2018). There was much agreement that the bacterial diversity of gut microbiota in IBD showed a similar pattern—a decrease in some beneficial taxa and an increase in potential pathogens. We therefore investigated the diversity and structure of the mice intestinal microbiota. The lowest diversity and richness of microbiota composition were found in the DSS group. After intervention, the intestinal microbiota of mice was obviously changed, especially in the FM group. That is, \textit{L. plantarum} ZJ316–fermented milk significantly increased the abundance and diversity of intestinal microbiota in mice with DSS colitis, which is consistent with the findings of Qiu et al. (2018). Furthermore, the results of PCoA showed that DSS treatment significantly disrupted the structure of the intestinal microbiota in rats, and that gavage of \textit{L. plantarum} ZJ316–fermented milk resulted in altered microbiota in the colon of mice with DSS colitis. Nonetheless, nearly all groups exhibited substantial differences within them, implying that the degree of modifications stimulated by DSS in the gastrointestinal microbiota as well as how efficiently they were reinstated through the administration of fermented milk differed from one mouse to another (Feng et al., 2022).

Subsequently, we further identified potential microbial taxa that respond to fermented milk supplementation. Our results indicate that the phyla \textit{Firmicutes} and \textit{Bacteroidetes} were significantly influenced by DSS induction, which were associated with aging and metabolic syndrome (Compare et al., 2016). Additionally, 5-ASA was excellent at killing pathogenic bacteria, but at the same time, some beneficial bacteria present in the mouse intestine, such as \textit{Faecalibacterium}, \textit{Lactiplantibacillus}, \textit{Bifidobacterium}, and \textit{Alistipes}, are also killed. Our past findings suggested that \textit{L. plantarum} ZJ316 is a probiotic with the ability to alleviate intestinal inflammation and has both antibacterial and antiinflammatory effects in alleviating inflammatory diseases of the gastrointestinal tract caused by \textit{Helicobacter pylori} and \textit{Salmonella}. However, by ingesting fermented milk, the multiplication of pathogenic bacteria was inhibited, whereas the number of these probiotics was significantly increased. We, therefore, suggested that a possible mechanism for the relief of IBD by \textit{L. plantarum} ZJ316–fermented milk may be by increasing the abundance of beneficial microorganisms in the gut of mice with colitis, while inhibiting the growth of some harmful microorganisms, which was a great advantage over antibiotic treatment.

In a study of the pathogenesis of ulcerative colitis, intestinal diseases would be developed, when fatty acid metabolism was impaired in the colonic mucosa of mice (Zhao et al., 2014). Short-chain fatty acids are the main energy substrate for enterocytes and signal molecules for interactions between the intestinal mi-
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crobiota and the host, and are also essential for in
testinal health because of their anti-inflammatory and
anticancer properties (van der Beek et al., 2017). It
has also been reported that the SCFA content in the
intestine of patients with ulcerative colitis became low;
in particular, acetic acid, which has an antiinflamma-
tory effect, decreased significantly (Parada Venegas et
al., 2019). In the present research, contents of these
5 SCFA increased significantly after continuous intake of
L. plantarum ZJ316–fermented milk for 5 wk, yet
5-ASA did not restore the levels of SCFA in the intesti-
tine, revealing that mesalamine can restore intestinal
inflammation through other pathways (Li et al., 2022).
It has been proposed that SCFA play a significant role
in colitis by regulating both the innate and adaptive
immune responses in tandem (Koh et al., 2016). Acetic
acid, propionic acid, and butyric acid demonstrate the
ability to decrease TNF-α secretion, a proinflammatory
cytokine, resulting in an antiinflammatory effect. Ad-
ditionally, SCFA promote the differentiation of primary
T cells into Th1 and Th17 cells upon the body’s interac-
tion with pathogens in an immune response, ultimate-
ly reinforcing the host’s immunity. Our results suggested
that L. plantarum ZJ316–fermented milk could effec-
tively promote the production of SCFA in the colon
of mice with DSS-induced chronic colitis. Therefore,
the regulation of fatty acid metabolism levels in the
gut may be a potential mechanism of action for the
role of fermented milk. Although our results are similar
to those of previous studies, there is still a limitation
because of the small number of mice. In the future, we
will continue to further investigate the wider applica-
tion of lactic acid bacteria fermented milk as a dietary
intervention in human chronic diseases.

CONCLUSIONS

This study focused on the alleviating effect of L. plan-
tarum ZJ316–fermented milk on DSS-induced chronic
colitis and explored its mechanism of action, including
inhibition of inflammatory response and regulation of
intestinal microbiota imbalance. The results showed that
ingestion of fermented milk reduced disease damage,
decreased local inflammatory response, and promoted
self-recovery in mice. Furthermore, the consumption of
fermented milk has been shown to enhance the overall
diversity and variety of the gut microbiota while restor-
ing balance in cases of chronic colitis induced by DSS.
These findings suggest that the administration of L.
plantarum ZJ316–fermented milk via oral consumption
may be an efficacious approach in treating IBD in mice,
and that L. plantarum ZJ316–fermented milk possesses
significant potential as a novel functional food for IBD
treatment. However, further exploration is necessary to
determine the specific bioactive components of ferment-
ed milk responsible for mitigating and treating IBD,
and to investigate the molecular mechanisms underly-
ning their effectiveness.

ACKNOWLEDGMENTS

This work was supported by the Chinese Academy of
Engineering Academy-Locality Cooperation Project
(Note. No. 2019-ZJ-JS-02; Hangzhou, China), National
Natural Science Foundation of China (No. U20A2066;
Beijing, China), and Major Science and Technology
Projects of Zhejiang Province (No. 2020C04002; Hang-
zhou, China), Natural Science Foundation of Zhejiang
Province (No. LR22C200005; Hangzhou, China), and
Agricultural and Social Development Project of Hang-
zhou (No. 202003A26; Hangzhou, China). The authors
have not stated any conflicts of interest.

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