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Lactoferrin Attenuates Intestinal Barrier Dysfunction and Inflammation by Modulating the MAPK Pathway and Gut Microbes in Mice

Ping Hu,[1] Qiufang Zong,[1] Yahui Zhao,[1] Haotian Gu,[1] YaYa Liu,[1] Fang Gu,[1] Hao-Yu Liu,[1] Abdelkareem A Ahmed,[2,3] Wenbin Bao,[1] and Demin Cai[1]

[1]College of Animal Science and Technology, Yangzhou University, Yangzhou, PR China; [2]Department of Veterinary Biomedical Sciences, Botswana University of Agriculture and Agriculture and Natural Resources, Ebele, Gaborone, Botswana; and [3]Biomedical Research Institute, Darfur University College, Nyala, Sudan

ABSTRACT

Background: Deoxynivalenol (DON) is a major mycotoxin present in staple foods (particularly in cereal products) that induces intestinal inflammation and disrupts intestinal integrity. Lactoferrin (LF) is a multifunctional protein that contributes to maintaining intestinal homeostasis and improving host health. However, the protective effects of LF on DON-induced intestinal dysfunction remain unclear.

Objectives: This study aimed to investigate the effects of LF on DON-induced intestinal dysfunction in mice, and its underlying protective mechanism.

Methods: Male BALB/c mice (5 wk old) with similar body weights were divided into 4 groups ($n = 6$/group) and treated as follows for 5 wk: Veh [peroral vehicle daily, commercial (C) diet]; LF (peroral 10 mg LF/d, C diet); DON (Veh, C diet containing 12 mg DON/kg); and LF + DON (peroral 10 mg LF/d, DON diet). Intestinal morphology, tight junction proteins, cytokines, and microbial community were determined. Data were analyzed by 2-factor ANOVA or Kruskal–Wallis test.

Results: The DON group exhibited lower final body weight (−12%), jejunal villus height (VH; −41%), and jejunal occludin expression (−36%), and higher plasma IL-1β concentration (+85%) and jejunal *Il1b* mRNA expression (+98%) compared with the Veh group ($P < 0.05$). In contrast, final body weight (+19%), jejunal VH (+49%), jejunal occludin (+53%), and intelectin 1 protein expression (+159%) were greater in LF + DON compared with DON ($P < 0.05$). Additionally, jejunal *Il1b* mRNA expression (−31%) and phosphorylation of p38 and extracellular signal regulated kinase 1/2 (−40% and − 38%) were lower in LF + DON compared with DON ($P < 0.05$). Furthermore, the relative abundance of *Clostridium* XIVa (+181%) and colonic butyrate concentration (+53%) were greater in LF + DON compared with DON ($P < 0.05$).

Conclusions: Our study highlights a promising antimycotoxin approach using LF to alleviate DON-induced intestinal dysfunction by modulating the mitogen-activated protein kinase pathway and gut microbial community in mice. *J Nutr* 2022;152:2451–2460.

Keywords: deoxynivalenol, intestinal dysfunction, lactoferrin, MAPK pathway, gut microbial community

Introduction

Mycotoxins are invisible factors that affect food and feed safety; they pose a severe threat to the health of humans and animals and cause immense economic losses (1). Deoxynivalenol (DON), naturally produced by *Fusarium*, is a prevalent mycotoxin that contaminates staple foods, particularly cereal products. DON belongs to the trichothecene group and is chemically stable; thus, removing it during food or feed processing is challenging. Therefore, DON is the most common contaminant found in food safety evaluation and animal production systems (2, 3). Recent studies have revealed that DON adversely affects intestinal barrier function and immune balance, involving activation of the mitogen-activated protein kinase (MAPK) pathway, and disrupts intestinal homeostasis and host health (4, 5). Moreover, DON is reported to induce gut microbiota dysbiosis and promote the colonization of pathogenic bacteria, thereby triggering intestinal inflammation (6, 7). Therefore, maintaining intestinal barrier integrity, immune balance, and gut microbial homeostasis could be an attractive way to reduce DON-induced toxicity in humans and animals.

Lactoferrin (LF), a classic component of mammalian milk, has been reported to perform multiple biological functions, especially intestinal health improvement (8, 9). Garas et al. (10) demonstrated that LF alleviates the intestinal damage caused by malnutrition by upregulating tight junction protein expression and decreasing proinflammatory cytokine concentrations. Recent studies have highlighted that the anti-inflammatory

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effect of LF is mainly a result of its ability to weaken MAPK activation, thereby suppressing intestinal inflammation and maintaining intestinal integrity (9, 11, 12). In addition, LF exerts a "probiotic" effect, whereby it promotes the colonization of beneficial bacteria (e.g., *Lactobacillus* spp.) and the production of SCFAs, which can be absorbed through the intestinal lumen and display multiple biological functions (13). Given its beneficial properties, LF intervention might be an efficient approach to counteract the adverse effects caused by DON in the intestine. However, the potential protective mechanism of LF on DON-induced intestinal abnormality is little known.

Therefore, this study hypothesized that LF supplementation would attenuate DON-induced intestinal damage by maintaining intestinal epithelial barrier integrity, downregulation of proinflammatory cytokine production, and gut microbial community modulation. Therefore, we used LF as a nutritional modulator to investigate its protective effects on the intestine with its underlying mechanisms in DON-exposed mice.

Methods

Animals and experimental design

All experiments involving mice were reviewed and approved by the Animal Care and Use Committee of Yangzhou University (YZUDWSY 2017-09-06). A total of twenty-four 5-wk-old BALB/c mice (male) were purchased from Yangzhou University. Mice were housed in pairs for 1 wk for acclimation. After 1 wk, mice with similar body weights (BWs; 24.7 ± 0.32 g) were divided into 4 groups (n = 6/group, 2 mice in each cage) and were assigned to the following treatments for 5 wk: the Vehicle group (Veh group) was given a commercial diet (**Supplemental Table 1**) (Anlimao) and its members were orally administered 100 μL physiological saline by orogastric gavage each day; the LF group was given the commercial diet and its members were orally administered 100 μL LF solution (containing 10 mg LF) by orogastric gavage each day; the DON group was given the commercial diet containing 12 mg DON/kg and its members were orally administered 100 μL physiological saline by orogastric gavage each day; and the LF + DON group was given the commercial diet containing 12 mg DON/kg and its members were orally administered 100 μL LF solution (containing 10 mg LF) by orogastric gavage each day. All mice consumed the diets ad libitum. The bovine LF was separated from bovine milk and the purity was 95% (Ingradia). Diets were artificially contaminated with fungal cultures containing DON as previously described (14). DON and LF dosages were selected as previously described (15, 16). The BW and feed intake were recorded twice per week.

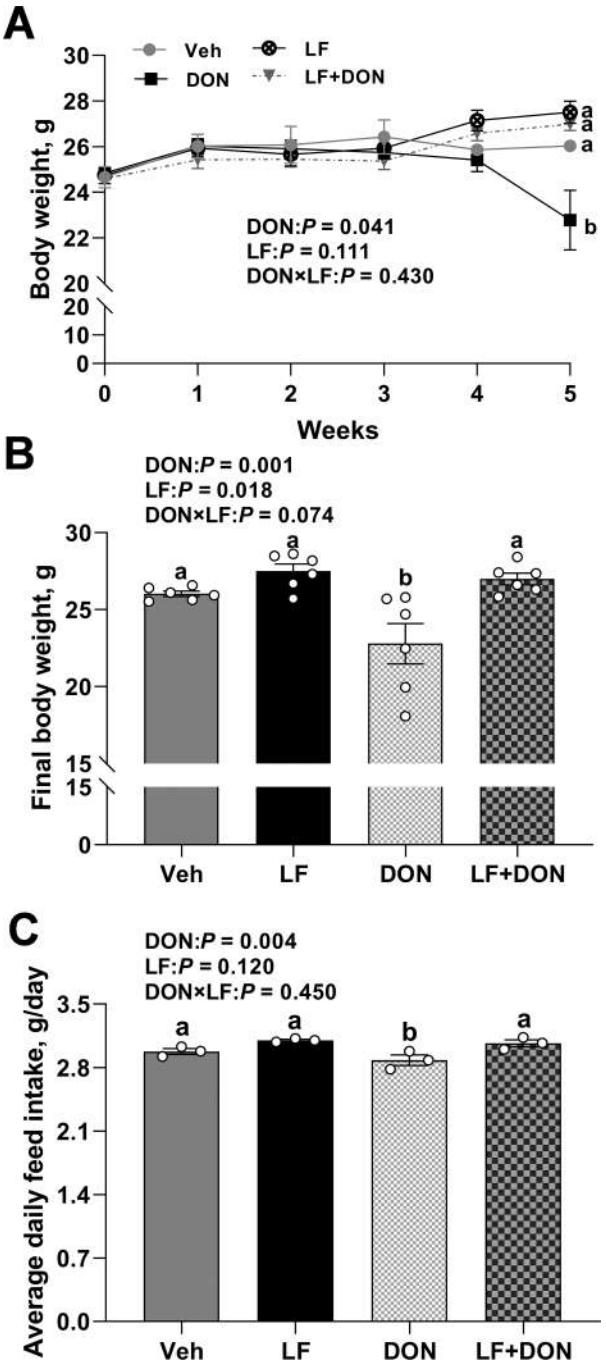

FIGURE 1 Effects of DON and LF on the growth performance of male BALB/c mice. (A) Body weight of mice during 5 wk of the experiment. (B) Final body weight of mice at week 5. (C) Average daily feed intake of mice. Values are means ± SEMs, n = 6 for panels A and B, n = 3 (2 mice per cage) for panel C. Labeled means without a common letter are significantly different from each other, $P < 0.05$. DON, deoxynivalenol; LF, lactoferrin; Veh, vehicle.

Sampling

After 5 wk, all mice were killed by anesthesia using tribromoethanol, followed by cervical dislocation. Blood samples were collected via cardiac draw, and plasma was obtained by centrifugation at 1500 × g for 15 min at 4°C and then stored at −80°C until analysis. Tissues (0.5 cm) of the duodenum, jejunum, and ileum were obtained and fixed in 4% paraformaldehyde for further analysis. Jejunal mucosa and colonic digesta were obtained and stored at −80°C until further analysis.

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Supplemental Tables 1 and 2 and Supplemental Figures 1 and 2 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn/.
Address correspondence to DC (e-mail: demincai@yzu.edu.cn).
Abbreviations used: ADFI, average daily feed intake; BW, body weight; C, commercial (diet); CD, crypt depth; DAO, diamine oxidase; DON, deoxynivalenol; ERK1/2, extracellular signal regulated kinase 1/2; H&E, hematoxylin and eosin; ITLN1, intelectin 1; LF, lactoferrin; MAPK, mitogen-activated protein kinase; p-, phosphorylated; SPSS, Statistical Package for the Social Sciences; Veh, vehicle; VH, villus height; VW, villus width.

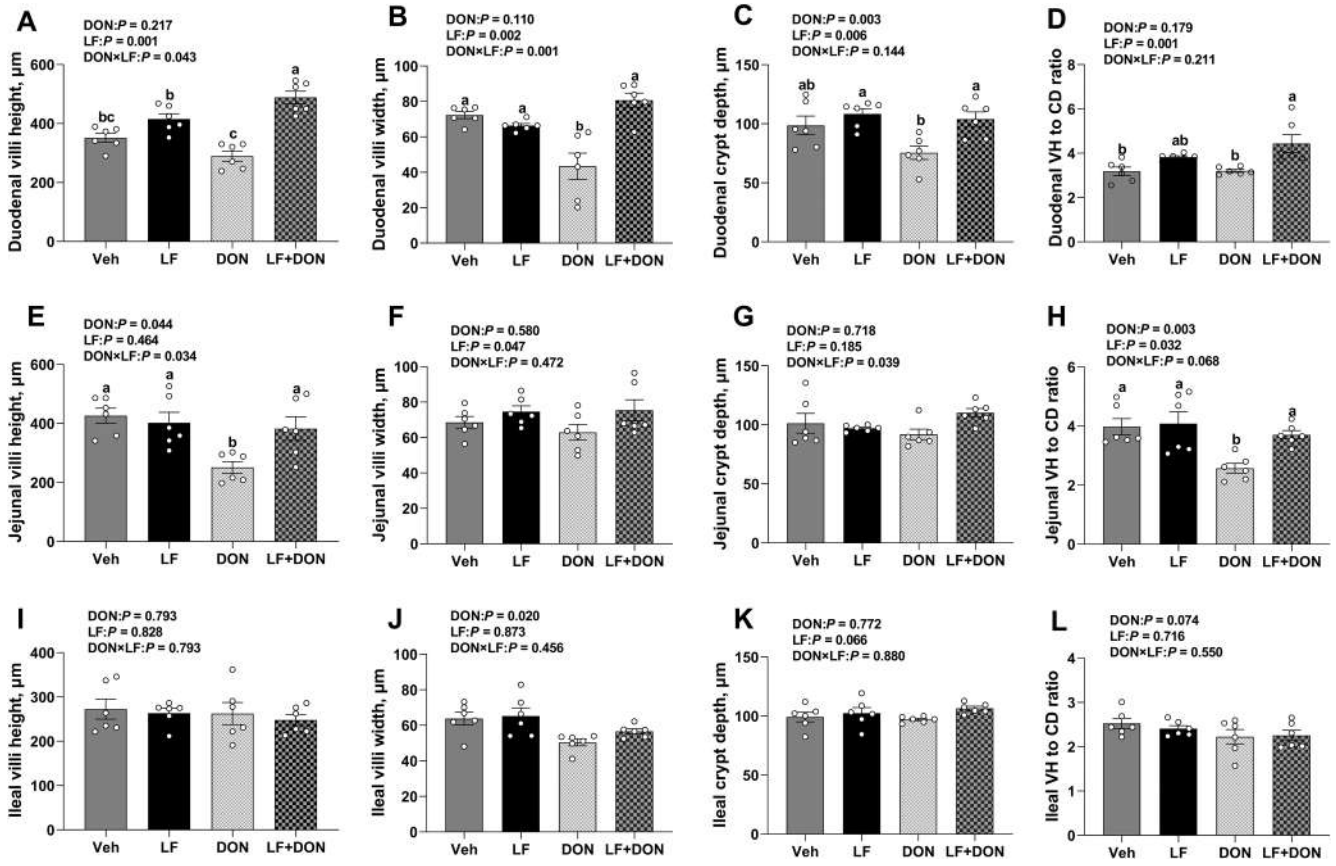

FIGURE 2 Effects of DON and LF on the intestinal morphology of male BALB/c mice. (A) Duodenal VH, (B) duodenal villus width, (C) duodenal CD, (D) duodenal VH to CD ratio, (E) jejunal VH, (F) jejunal villus width, (G) jejunal CD, (H) jejunal VH to CD ratio, (I) ileal VH, (J) ileal villus width, (K) ileal CD, and (L) ileal VH to CD ratio. Values are means ± SEMs, $n = 6$. Labeled means without a common letter are significantly different from each other, $P < 0.05$. CD, crypt depth; DON, deoxynivalenol; LF, lactoferrin; Veh, vehicle; VH, villus height.

Intestinal histomorphology analysis

For slide preparation, tissues (0.5 cm) of the duodenum, jejunum, and ileum ($n = 6$) were taken and fixed in 4% paraformaldehyde for 24 h at room temperature (25°C) and then embedded in paraffin blocks before hematoxylin and eosin (H&E) staining. The paraffin blocks were sectioned into 5-μm slices and stained using H&E for microscopic examination. Quantitative measurements of villus height (VH, in micrometers), villus width (VW, in micrometers), and crypt depth (CD, in micrometers) were made using 8 to 10 well-oriented crypt-villus units in the duodenum, jejunum, and ileum using an image analysis system.

Enzymatic analyses of cytokine and diamine oxidase in the plasma

The plasma IL-1β, IL-6, IL-8, IL-10, TNF-α, and diamine oxidase (DAO) concentrations were measured using mice ELISA kits (R&D Systems) according to the manufacturer's instructions.

Mucosal RNA extraction and real-time PCR

Total RNA was extracted from the jejunum using TRIzol Reagent (Invitrogen). The total RNA (1 μg) was reverse-transcribed to cDNA using PrimeScript RT Reagent Kit with genomic DNA eraser (TaKaRa Biotechnology) according to the manufacturer's instructions. The primers used for *Il1b*, *Il6*, *Il8*, *Il10*, and *Tnfa* are shown in **Supplemental Table 2**.

Western blotting

Protein extraction and immunoblot procedures were performed according to a method previously described (17). Cellular protein concentrations were measured using a BCA Protein Assay Kit (Beyotime). The following primary antibodies were used: anti-occludin (1:1000; Abcam), anti-claudin1 (1:1000; Abcam), anti-intelectin 1 (ITLN1; 1:1000; Abcam), anti-extracellular signal regulated kinase 1/2 (anti-ERK1/2) (137F5; 1:1000; Cell Signaling Technology), anti-p38 (D13E1; 1:1000; Cell Signaling Technology), anti-phospho-ERK1/2 (Thr202/Tyr204; 1:1000; Cell Signaling Technology), and anti-phospho-p38 (Thr180/Tyr182; 1:1000; Cell Signaling Technology). GAPDH was used as the internal reference protein. Western blot bands of each protein are shown in **Supplemental Figure 1**.

DNA extraction, PCR amplification, and Illumina MiSeq sequencing

Genomic DNA was extracted from the contents of the colon (0.2 g) using the bead-beating method with a mini bead beater (Biospec Products). The DNA concentration was determined using a NanoDrop 1000 spectrophotometer (Thermo Scientific Inc). Forward primer 515F (GTGCCAGCMGCCGCGGTAA) and reverse primer 806R (GGACTACNNGGGTATCTAAT) were used to amplify the V4 region of the bacterial 16S ribosomal RNA gene (95°C for 3 min, followed by 30 cycles at 95°C for 45 s, 56°C for 45 s, 72°C for 45 s, and a final extension at 72°C for 10 min). Purified amplicons were paired-end sequenced on an Illumina MiSeq platform according to a standard protocol. Bacterial diversity was estimated for the observed species [ACE (abundance-based coverage estimator), Chao, Shannon, and Simpson indexes] using the mothur program (version 1.35.0). The 16S sequencing data in the present study were uploaded to GenBank at the National Center for Biotechnology Information with accession number PRJNA815919.

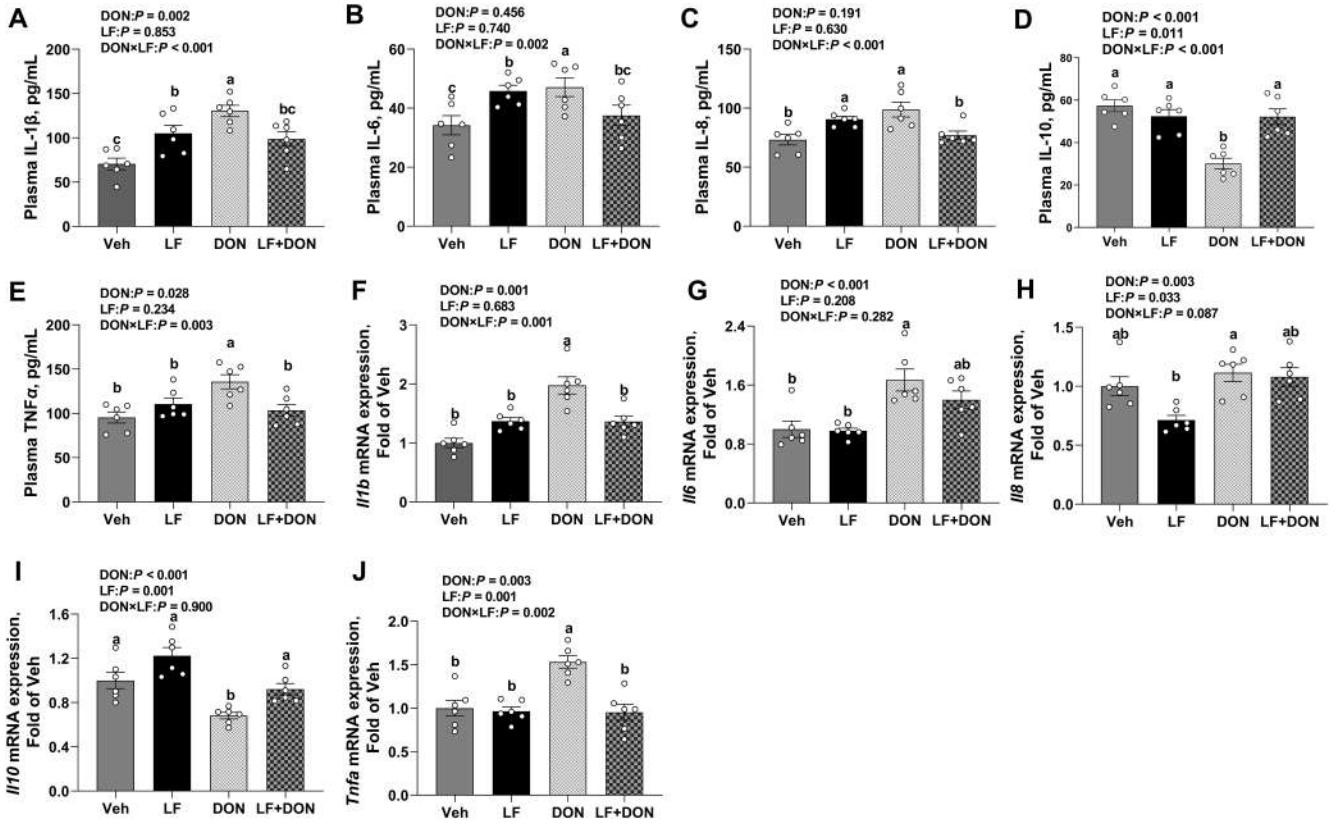

FIGURE 3 Effects of DON and LF on cytokine protein concentrations in the plasma and cytokine mRNA abundances in the jejunum of male BALB/c mice. (A–E) Protein concentrations of IL-1β, IL-6, IL-8, TNF-α, and IL-10 in the plasma. (F–J) Relative mRNA abundances of *Il1b*, *Il6*, *Il8*, *Tnfa*, and *Il10* in the jejunum. Values are means ± SEMs, $n = 6$. Labeled means without a common letter are significantly different from each other, $P < 0.05$. DON, deoxynivalenol; LF, lactoferrin; Veh, vehicle.

Measurement of microbial metabolites

The SCFA concentrations in the colonic digesta were measured by GC using a GC-14B system (Shimadzu; capillary column 30 m × 0.32 mm × 0.25 μm film thickness; column temperature 110°C; injector temperature 180°C; and detector temperature 180°C).

Statistical analyses

Data were analyzed by the Statistical Package for the Social Sciences (SPSS) version 22.0 (IBM) and expressed as means ± SEMs. The data were analyzed as a 2 × 2 factorial with the general linear model procedures of the SPSS. The model included the fixed effects of DON and LF and their interaction. Repeated-measures 2-factor ANOVA with the Duncan multiple-range post hoc test was used for BW analysis. All other data were analyzed by 2-factor ANOVA, and post hoc testing was conducted using Duncan multiple comparison tests when a significant effect of DON, LF, or their interaction ($P < 0.05$) was observed. The Kruskal–Wallis test was used as the nonparametric method for independent samples to analyze the variable (microbial composition), which showed a nonnormal distribution (*Ruminococcus* relative abundance). A value of $P < 0.05$ was considered statistically significant, whereas P values between 0.05 and 0.10 were considered as a tendency. The R package Hmisc was used for calculating Spearman correlation coefficients.

Results

LF improved the growth performance in DON-exposed mice

At week 5, the BW of mice was significantly affected by DON and LF ($P < 0.05$) as shown in **Figure 1**, and the BW in the DON group was lower than that in the Veh and LF + DON groups (−12% and −19%, respectively, $P < 0.05$, Figure 1B). Additionally, the average daily feed intake (ADFI) was affected by DON, and was found to be lower in the DON group than in the LF + DON group (+6%, $P < 0.05$, Figure 1C).

LF modulated the intestinal morphology

The VH and VW of the duodenum were significantly affected by the interaction between DON and LF, as shown in **Figure 2** and **Supplemental Figure 2** ($P < 0.05$), and the VH and VW in the DON group were lower compared with those in the Veh and LF + DON groups (VH: −14% and −69%; VW: −40% and −86%, respectively, $P < 0.05$) (Figure 2A, B). The VH in the jejunum was affected by the interaction between DON and LF ($P < 0.05$), and the VH in the DON group was lower compared with that in the Veh and LF + DON groups (−41% and −49%, respectively, $P < 0.05$, Figure 2E). Additionally, the VH-to-CD ratio of jejunum was significantly affected by DON and LF, and the VH-to-CD ratio in the DON group was lower compared with that in the Veh and LF + DON groups (−35% and −44%, respectively, $P < 0.05$) (Figure 2H).

LF reduced the production of proinflammatory cytokines

Proinflammatory cytokines (IL-1β, IL-6, IL-8, and TNF-α) in the plasma were significantly affected by the interaction between DON and LF as shown in **Figure 3** ($P < 0.05$): the IL-1β, IL-8, and TNF-α concentrations in the DON group were higher compared with those in the Veh and LF + DON groups (IL-1β: +85% and +24%; IL-6: +38% and +20%; IL-8: +35% and +22%; TNF-α: +42% and +24%, respectively,

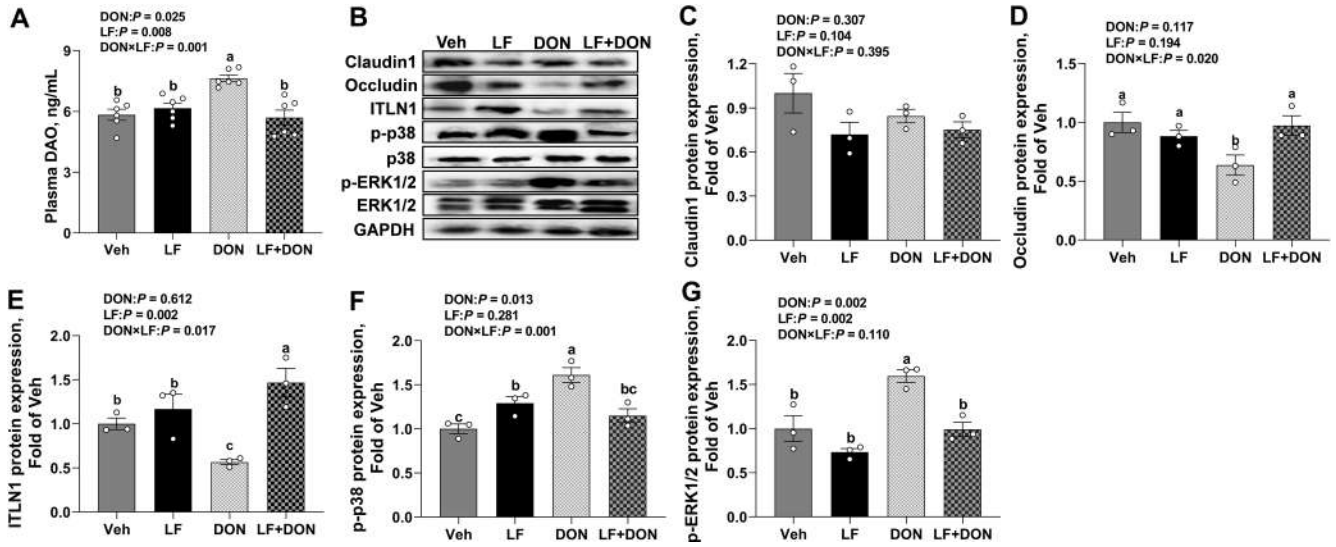

FIGURE 4 Effects of DON and LF on intestinal permeability, ITLN1 expression, and MAPK pathway protein expression in the jejunum of male BALB/c mice. (A) Concentration of DAO in the plasma. (B) Representative western blot images of claudin1, occludin, ITLN1, p-p38, p38, p-ERK1/2, ERK1/2, and GAPDH. Protein expression level of (C) claudin1, (D) occludin, and (E) ITLN1. Phosphorylation level of (F) p38 and (G) ERK1/2. Values are means ± SEMs, $n = 6$ for panel A, $n = 3$ for panels C–G. Labeled means without a common letter are significantly different from each other, $P < 0.05$. DAO, diamine oxidase; DON, deoxynivalenol; ERK1/2, extracellular signal regulated kinase 1/2; ITLN1, intelectin 1; LF, lactoferrin; p-, phosphorylated; Veh, vehicle.

$P < 0.05$) (Figure 3A–C, E). Furthermore, the plasma anti-inflammatory cytokine IL-10 concentration was significantly affected by the interaction between DON and LF: the IL-10 concentration was lower in the DON group compared with the Veh and LF + DON groups (−48% and −74%, respectively, $P < 0.05$, Figure 3D).

The *Il1b* and *Tnfa* mRNA expression levels in the jejunum were significantly affected by the interaction between DON and LF ($P < 0.05$, Figure 3F, J): *Il1b* and *Tnfa* mRNA expression levels were higher in the DON group compared with those in the Veh and LF + DON groups (*Il1b*: +98% and +31%; *Tnfa*: +53% and +38%, respectively; $P < 0.05$, respectively, Figure 3F, J). Moreover, the *Il10* mRNA expression levels were affected by the DON and LF treatment; the *Il10* mRNA expression was downregulated in the DON group compared with the Veh and LF + DON groups (−32% and −35%, respectively, $P < 0.05$; Figure 3I).

LF reduced the intestinal permeability by enhancing the expression of jejunal tight junction proteins

The plasma DAO concentration was significantly affected by the interaction between DON and LF: the DAO concentration in the DON group was higher compared with that in the Veh and DON + LF groups as shown in **Figure 4**A (+31% and +26%, respectively, $P < 0.05$). Jejunal occludin protein expression was affected by the interaction between DON and LF, being significantly decreased in the DON group compared with the Veh and LF + DON groups (−36% and − 53%, respectively, $P < 0.05$, Figure 4B, D).

LF enhanced the protein expression of ITLN1 and inhibited the activation of the MAPK pathway

The jejunal ITLN1 protein expression level was affected by the interaction between DON and LF as shown in Figure 4, being lower in the DON group than in the Veh and LF + DON groups (−43% and −159%, respectively, $P < 0.05$) (Figure 4B, E). Moreover, the p38 phosphorylation level was affected by the interaction between DON and LF, being higher in the DON group compared with the Veh and LF + DON groups (+61% and +40%, respectively, $P < 0.05$, Figure 4B, F). DON treatment significantly upregulated the ERK1/2 phosphorylation level, which was higher in the DON group than in the Veh and LF + DON groups (+60% and +38%, respectively, $P < 0.05$, Figure 4B, G).

LF modulated the colonic microbial community in DON-exposed mice

The Shannon and Simpson indices were affected by the interaction between DON and LF as shown in **Figure 5**. The DON group had a lower Shannon index and higher Simpson index compared with the Veh and LF + DON groups (Shannon: −11% and −10%; Simpson: +107% and +46%, respectively, $P < 0.05$; Figure 5D, E). Additionally, principal coordinates analysis of the colonic microbiota community revealed 4 separate microbial clusters (Figure 5C).

At the phylum level, *Bacteroidetes* was the most dominant phylum in the colon, and the relative abundances of *Bacteroidetes* and *Firmicutes* were significantly affected by the DON treatment ($P < 0.05$, Figure 5F–H). At the genus level, *Clostridium* XIVa and *Lactobacillus* were the dominant bacteria (**Figure 6**A). The relative abundance of *Clostridium* XIVa was significantly affected by the interaction between DON and LF, with the relative abundance of *Clostridium* XIVa in the DON group being lower compared with the Veh and LF + DON groups (−66% and −181%, respectively, $P < 0.05$) (Figure 6B), and the relative abundance of *Ruminococcus* being higher compared with the Veh and LF + DON groups (+168% and +86%, $P < 0.05$) (Figure 6C). Moreover, the DON treatment reduced the relative abundances of *Alistipes* (−47%) and *Odoribacter* (−37%) compared with the Veh treatment ($P < 0.05$) (Figure 6D, E).

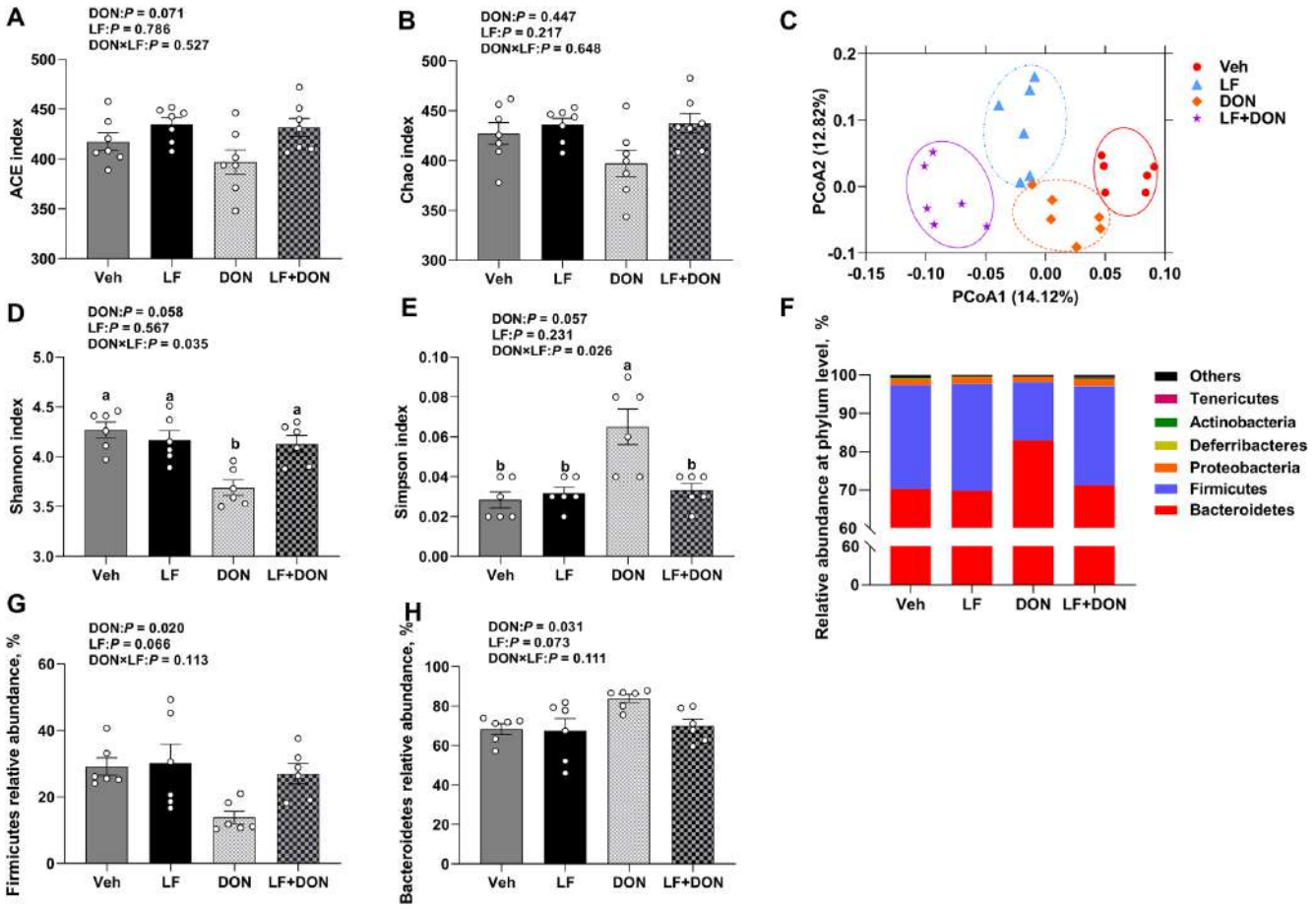

FIGURE 5 Effects of DON and LF on the diversity and relative abundance of colonic microbiota in male BALB/c mice. (A) ACE index, (B) Chao index, (C) principal coordinates analysis (PCoA), (D) Shannon index, (E) Simpson index, (F) average relative abundance of bacterial taxa at the phylum level, (G) relative abundance of *Firmicutes*, and (H) relative abundance of *Bacteroidetes*. Values are means ± SEMs, $n = 6$. Labeled means without a common letter are significantly different from each other, $P < 0.05$. ACE, abundance-based coverage estimator; DON, deoxynivalenol; LF, lactoferrin; Veh, vehicle.

LF promoted the colonic microbial metabolites in DON-exposed mice

The colonic propionate concentration was affected by the interaction between DON and LF as shown in Figure 6G, being lower in the DON group compared with the Veh and LF + DON groups (−54% and −97%, respectively, $P < 0.05$, Figure 6G). Additionally, the DON treatment reduced the butyrate concentration ($P < 0.05$), which was lower in the DON group compared with the Veh and LF + DON groups (−35% and −53%, respectively, $P < 0.05$, Figure 6H).

Correlation analysis of cytokines and DAO concentrations in the plasma, microbiota, and SCFAs in the colon

The correlation analysis of cytokines, DAO, SCFA concentrations, and the relative abundance of bacterial taxa is shown in Figure 7. The IL-1β concentrations displayed a strong positive correlation with the concentrations of IL-8, TNF-α, and DAO, and the relative abundance of *Ruminococcus* ($P < 0.05$), and a negative correlation with the IL-10 and propionate concentrations, and the relative abundance of *Clostridium* XIVa ($P < 0.05$). The IL-10 concentrations showed a positive correlation with the colonic propionate concentrations. The butyrate concentrations showed a positive correlation with the relative abundances of *Alistipes*, *Clostridium* XIVa, and *Odoribacter* ($P < 0.05$).

Discussion

DON contamination is a common problem in food safety evaluation and animal production, which adversely affects intestinal and host health (18). LF is expressed by epithelial cells, and its highest concentrations are detected in milk, with lower concentrations found in tears, nasal fluids, saliva, pancreatic, gastrointestinal, and reproductive tissue secretions (19, 20). Human colostrum and mature milk contain 5.0 and 2.1 g LF/L, respectively (21). Additionally, LF is present in bovine milk, with a mean concentration of 30 and 485 mg/L in mature milk and colostrum respectively (22). It is a pivotal nutrient to support host homeostasis in the form of an antibacterial and antiviral agent (9). The present study investigated the effects of DON on intestinal health, and evaluated the potential protective effects of LF on the parameters of intestinal health, including intestinal barrier integrity, inflammatory responses, and microbial community changes. First, the growth performance (BW and ADFI) of DON-exposed mice was found to have decreased, but was restored by LF. The growth performance could be attributed to the intestinal morphology alterations, because villus-crypt architecture directly influences nutrient digestion and absorption (23). Our data showed that DON shortened the duodenal VH and VW and reduced the jejunal VH and VH-to-CD ratio, which contributed to a smaller intestinal surface area and lower ability for nutrient digestion and absorption in

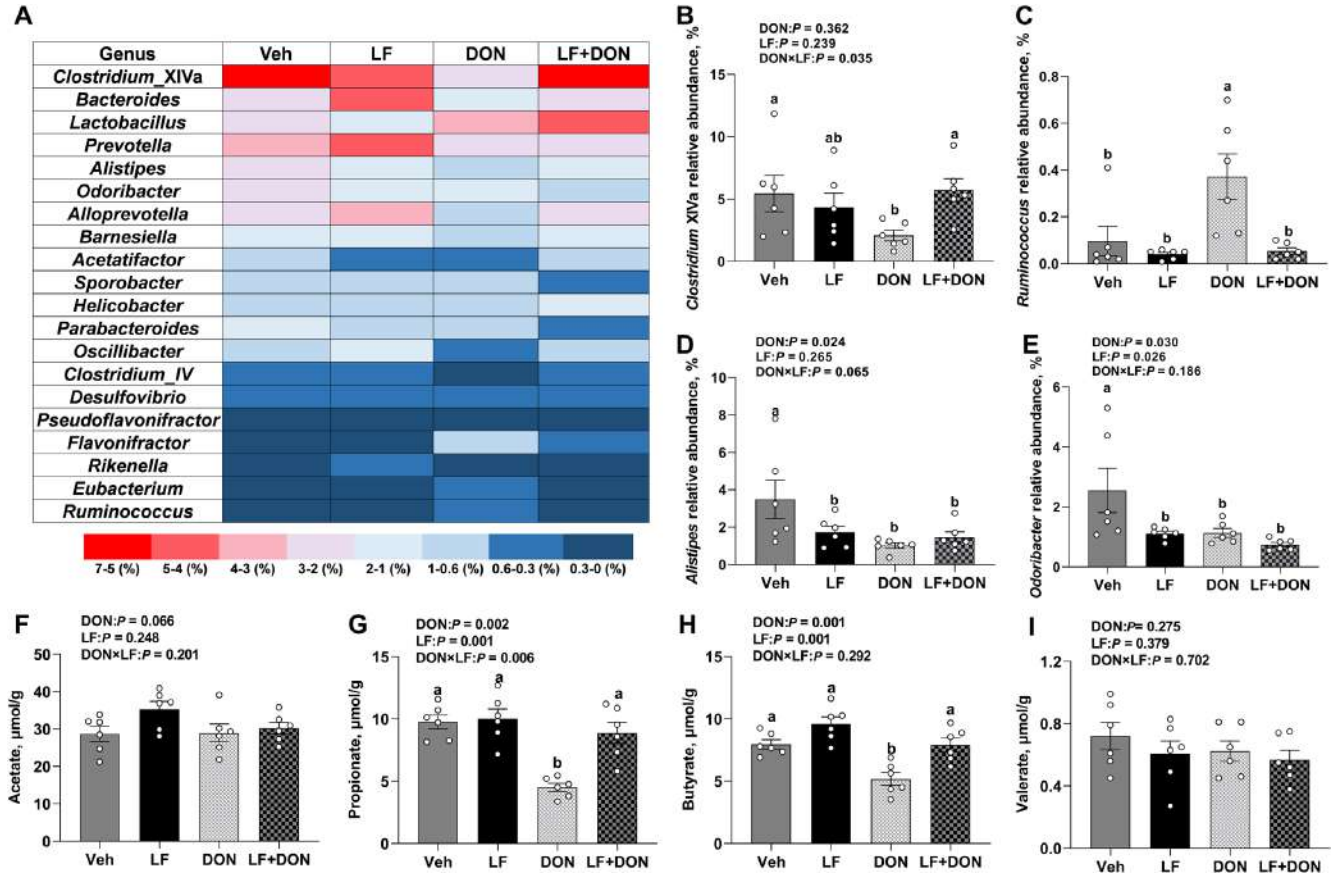

FIGURE 6 Effects of DON and LF on the abundant genera and SCFA concentrations in the colonic digesta of male BALB/c mice. (A) The 20 most abundant genera, (B–E) relative abundance of *Clostridium* XlVa, *Ruminococcus, Alistipes*, and *Odoribacter*, and (G–I) concentrations of acetate, propionate, butyrate, and valerate. Values are means ± SEMs, $n = 6$. Labeled means without a common letter are significantly different from each other, $P < 0.05$. DON, deoxynivalenol; LF, lactoferrin; Veh, vehicle.

DON-exposed mice. Similar to the present results, Zhou et al. (24) also showed decreased VH and VH-to-CD ratio in the jejunum of DON-exposed mice. However, the LF treatment maintained intestinal morphology in the DON-exposed mice, indicating improved capacity for nutrient absorption due to LF. Thus, the enhanced VH and VH-to-CD ratio of the jejunum could be one of the reasons for the improved growth performance of mice in the LF + DON group.

The intestinal mucosal barrier is the first line of defense against endotoxins and other harmful substances moving from the bowel to the intestinal epithelium. A previous study revealed that DON disrupts intestinal integrity and tight junction structure (25). Congruently, we found that DON downregulated jejunal occludin protein expression and increased plasma DAO concentration, which implies higher intestinal permeability in the DON-exposed mice (26). Higher intestinal permeability indicates damage to intestinal integrity, which could exacerbate intestinal inflammatory responses and cause intestinal diseases (27). Of note, the LF treatment restored the intestinal tight junctions by enhancing occludin expression. Notably, DON compromises the expression of intestinal tight junction protein via activation of the MAPK signaling pathway (28). The MAPK pathway is essential in regulating the cell cycle, the differentiation and growth of cells, and cell senescence, which are vital to cellular development (29). In the current study, we found that p38 and ERK1/2 phosphorylation levels were reduced after LF administration in DON-exposed mice. Similarly, studies have revealed that LF can maintain intestinal integrity by inhibiting the MAPK signaling pathway in vivo and in vitro (12, 30, 31). Altogether, these results highlight that LF maintains intestinal permeability and facilitates the expression of occludin protein in DON-exposed mice by inhibiting the activation of the MAPK pathway.

In addition to affecting the tight junction proteins, DON modulates immune responses by disrupting the pro- and anti-inflammatory cytokine balance (32). In the present study, DON treatment promoted the concentrations of proinflammatory cytokines (IL-1β, IL-6, IL-8, and TNF-α) and decreased the concentration of anti-inflammatory cytokine (IL-10) in the plasma, in addition to causing corresponding changes in gene expression in the jejunum. Hering et al. (33) demonstrated that LF prevents intestinal inflammation by decreasing proinflammatory cytokine expression, which is congruent with the present study where LF treatment inhibited IL-1β and TNF-α expression and promoted IL-10 expression in mice. Additionally, expression of jejunal ITLN1 protein, which is a specific LF receptor (34), was markedly decreased, whereas LF treatment restored ITLN1 expression in DON-exposed mice. ITLN1, also known as omentin 1, is involved in the gut immune defense against microorganisms and is downregulated in the model with irritable bowel syndrome, suggesting that ITLN1 could serve as an intestinal health biomarker (35, 36). Moreover, previous studies revealed that ITLN1 reduces the production of proinflammatory factors by inhibiting MAPK pathway activation (37, 38). Furthermore, Kaminska (39) showed that the attenuated MAPK signaling pathway activation

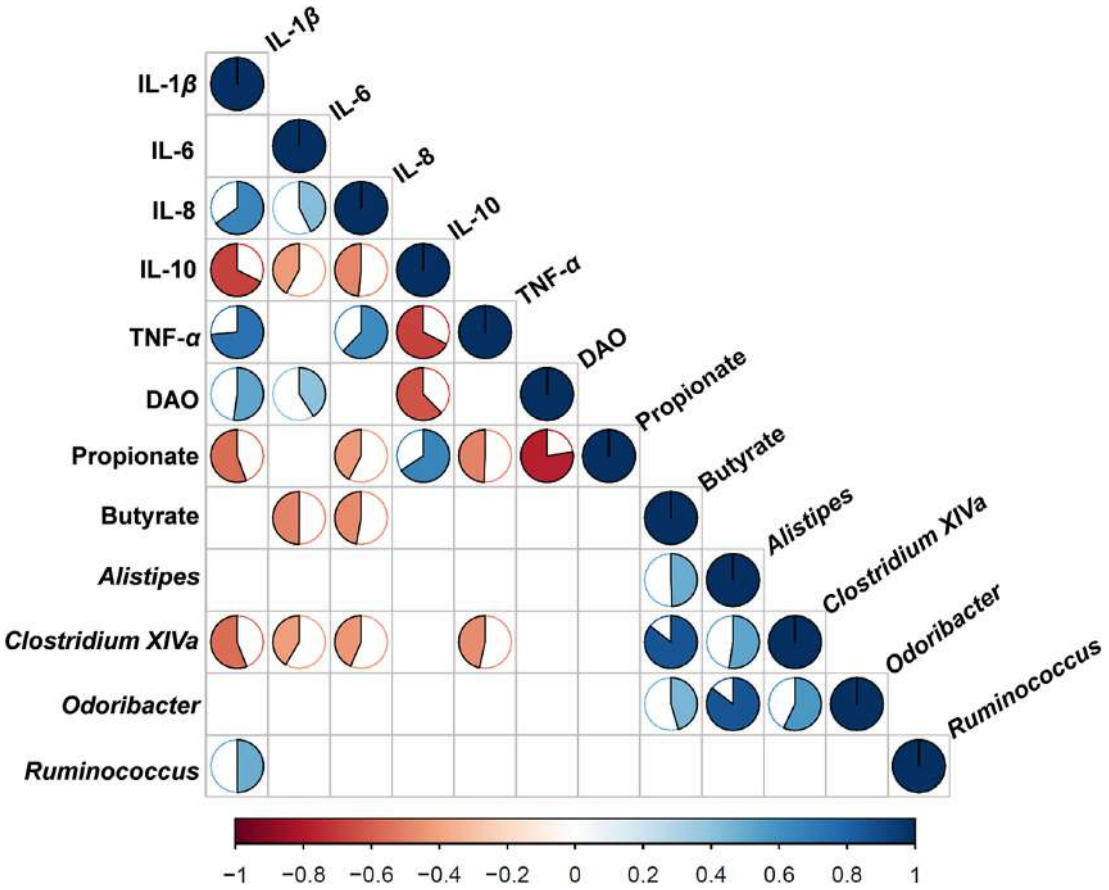

FIGURE 7 Spearman correlation coefficients of significantly differential plasma index, colonic microbiota, and SCFAs in male BALB/c mice. The R package corrplot was used for generating the heatmap. The blue represents a significant positive correlation, and the red represents a significant negative correlation. DAO, diamine oxidase.

reduced the synthesis of proinflammatory cytokines, implying that the decreased phosphorylated-p38 (p-p38) and p-ERK1/2 expression in the present study contributed to the downregulation of proinflammatory cytokine expression. Therefore, we speculate that LF inhibited the intestinal expression of proinflammatory cytokines by enhancing ITLN1 expression and attenuating the MAPK pathway activation in DON-exposed mice.

The mammalian gastrointestinal tract, especially the large intestine, is colonized by dynamic and complex communities of gut microbiota, which play a vital role in intestinal and host health (40) In the present study, a decreased α-diversity of the colonic microbiota was observed in the DON-exposed mice, which was restored by LF treatment. Generally, a high level of diversity can lead to "functional redundancy," which maintains the resistance and stability of the gut ecosystem upon perturbation, for example, by toxin exposure (41). Thus, the LF treatment increased the diversity of colonic microbiota to a certain extent to protect the mice from DON toxicity. Additionally, a decreased relative abundance of *Clostridium* XIVa was found at the genus level in DON-exposed mice, which increased after LF treatment. *Clostridium* spp., a predominant cluster of commensal bacteria in the mammalian gut, exert several salutary effects on intestinal homeostasis, such as the capacity to alleviate intestinal inflammation and microbial disorders (42). Moreover, the present correlation analysis confirmed that the relative abundance of *Clostridium* XIVa was negatively related to the proinflammatory cytokines in the plasma. Of note, the beneficial effects of *Clostridium* cluster XIVa mainly lie in its ability to produce butyrate, which is a well-known SCFA contributing to intestinal health (43). Present results also showed that the colonic butyrate concentration was increased by LF supplement in DON-exposed mice, suggesting that LF promoted their intestinal well-being. Furthermore, LF decreased the relative abundance of *Ruminococcus* in the colonic digesta of DON-exposed mice. Henke et al. (44) revealed that *Ruminococcus gnavus*, a biomarker of Crohn disease, uses intestinal mucin as a carbon source and could directly cause a breakdown in gut barrier function and immune system, implying that a higher relative abundance of *Ruminococcus* in the intestine is not conducive to intestinal health . Overall, the present results indicate that the LF treatment maintained intestinal microbial homeostasis by increasing the abundance of *Clostridium* XIVa and butyrate concentration and decreasing the relative abundance of *Ruminococcus* in DON-exposed mice.

In summary, the present study demonstrated that LF maintained intestinal health in DON-exposed mice by decreasing intestinal permeability and proinflammatory cytokine concentrations, which were associated with upregulated LF receptor (ITLN1) expression and attenuated MAPK pathway activation. Moreover, LF presented a "probiotic effect" in the colon of DON-exposed mice, including increased relative abundance of butyrate-producing bacteria (*Clostridium* XIVa) and improved concentrations of butyrate and propionate. Therefore, targeted enhancement of intestinal function by LF administration could

efficiently prevent DON-induced intestinal toxicity in humans and animals.

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Data Availability

The data that support the findings of this study are available from the corresponding author on reasonable request.

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