The protection of Lactobacillus plantarum JM113 against deoxynivalenol (DON)-induced apoptosis and intestinal inflammation on the jejunum of broiler chickens and the potential roles of gut microbiota were determined. A total of 144 one-day-old male broilers (Arbor Acres) were randomly divided into 3 treatment groups consisting of 6 replicates with 8 birds per replicate, including the CON (basal diet), the DON (basal diet -10 mg/kg DON), and the DL (basal diet +10 mg/kg DON + 1 × 10^9 CFU/kg L. plantarum JM113). The DON-diet decreased (P < 0.05) the mRNA expression of mucosal defense proteins and mechanistic target of rapamycin pathway genes. Meanwhile, DON challenge significantly increased Bcl-2-associated X gene/B-cell lymphoma 2 gene (Bcl-2) in the jejunum (P < 0.05) and demonstrated proapoptosis status. In contrast, the DL group showed normal immunity-related gene expression of jejunal mucosa and manifested a superior antiapoptosis status. Adding L. plantarum JM113 significantly raised (P < 0.05) propionic acid, n-butyric acid, and total short-chain fatty acids concentrations in cecal contents of birds fed with DON diet. In addition, DON exposure altered bacterial community structure and disturbed the abundance of several bacterial phyla, families, and genera, leading to dysbiosis. Supplementation with JM113 shifted the gut microbiota composition to that of the CON group. Finally, Spearman correlation analysis suggested that most positive correlations with the mRNA expression of immunity-related and apoptosis-regulatory gene were observed within the phylum Bacteroidetes, and most negative correlations with the indicators were observed within the phylum Firmicutes. The mRNA expression of Bcl-2, TLR2, mTOR, Raptor, and RPS6KB1 (P < 0.05), which are regarded as important cell proliferation and anti-apoptosis parameters, were significantly negatively associated with the relative abundances of norank_f__Erysipelotrichaceae, Subdoligranulum, and Anaeroplasma, whereas they had a strong positive correlation with Ruminococcaceae_UCG-004, Alistipes, and Ruminococcaceae_NK4A214_group. These results implied that L. plantarum JM113 supplementation could ameliorate DON-induced apoptosis and intestinal inflammation via manipulating the bacterial community composition and could be used as a potential candidate to attenuate intestinal impairments.

**Key words:** broiler chicken, deoxynivalenol, gut microbiota, intestinal inflammation, Lactobacillus

**INTRODUCTION**

Deoxynivalenol (DON), one of the most abundant trichothecenes found on cereals, has been implicated in mycotoxicoses in both humans and farm animals (Mishra et al., 2019) and causes quantities of economic losses for animal production (Nagl and Schatzmayr, 2015). DON stability during cooking and processing explains its large presence in food and feed. DON has been shown to be involved in acute and chronic illnesses in both humans and farm animals (Girardet et al., 2011). DON has been reported to cause various adverse effects including anorexia, vomiting, gastroenteritis diarrhea, and oxidative damage (Maresca, 2013; Mishra et al., 2019). Gut, a tissue with a high protein turnover, is the primary target organ of DON in poultry (Awad et al., 2014). Earlier reports showed that consumption of DON impaired the intestinal morphology, digestion, and absorption function, intestinal barrier, and the immune response in chickens
The gut microbiota plays a crucial role in gastrointestinal health. In view of this situation, how DON optimizes the gut microbiota and how gut microbiota detoxifies DON became a popular research hotspot in the recent times. The pioneer work that DON moderately impacts on pig’s intestinal microflora can be traced to Wache et al. (2009). Subchronic exposure to DON increased in the Bacteroides/Prevotella group and decreased in Escherichia coli (Saint-Cyr et al., 2013). Recent research found that even the lowest level of dietary DON contamination had modulatory effects on chicken’s cecal bacterial microbiota composition and diversity (Annegret et al., 2018). These studies put forward a hypothesis that gut microbiota with variations could play a crucial role in DON-induced toxicity. Therefore, optimization of gut microbiota could be considered as a potential strategy to reduce the harmful impact of DON on the intestinal health.

Consulting the previous researches, Lactobacillus plantarum JM113 has high antioxidant activity and thereby has the potential to protect the integrity of the intestinal morphological structure and barrier function in broilers challenged with DON (Yang et al., 2017; Wu et al., 2018b), yet the mechanisms linking this effect to immunomodulatory properties and gut microbiota remain obscure. This study was carried out to first evaluate the potential protective effect of JM113 on the alleviation of inflammation induced by lipopolysaccharide (LPS) using spleen cells of broiler chicken by in vitro test, further investigated the beneficial effects of L. plantarum JM113 as a potential immunomodulator on the intestinal mucosal disruption of DON and discussed the possible correlation between gut microbiota and jejunal mucosal immunity-related function to propose a potential mechanism of DON-induced toxicity.

MATERIALS AND METHODS

Assays for Interleukin-10 and Interleukin-12 in Vitro

Preparation methods of spleen mononuclear cells described by Feng et al. (2016) were used. Spleen cells were cultured in triplicate, at a density of $1 \times 10^6$ cells/mL of RPMI (Roswell Park Memorial Institute)-1640 medium without penicillin-streptomycin, in 24-well tissue culture plates. L. plantarum JM113 cells were centrifuged at 8,000 g for 5 min, and the pellets were resuspended in RPMI-1640 medium containing spleen cells to a final concentration from $10^6$ to $10^9$ CFU/mL. Lipopolysaccharide (10 µg/mL, Sigma, St Louis, MO) was used as a positive control. After 48 h incubation, interleukin-10 (IL-10) and interleukin-12 (IL-12) produced in the culture supernatants were analyzed.

Cytokines were measured using an enzyme-linked immunosorbent assay method. The 96-well Immuno-Maxisorp plates (Nunc) were coated with polyclonal antibodies (Feng et al., 2016) for IL-10 and IL-12 (1: 1,000) in coating buffer (0.05 M carbonate buffer, pH 9.6) overnight at 4°C. Plates were blocked and washed. Culture medium was added to the plates, and they were incubated at 37°C for 2 h. Plates were then washed again, and biotinylated anti-rabbit and horseradish peroxidase-conjugated streptavidin (1: 20,000) were added, followed by incubation at 37°C for 1 h. The chromogenic reactions were developed with 3, 3′, 5′, 5′-tetramethylbenzidine substrate at 37°C for 30 min. The reactions were terminated with 50 µL 2 mol/L H₂SO₄, and the absorbance at A450 nm was measured. Equivalent levels of IL-10 and IL-12 were calculated by comparison with reference curves generated using IL-10 and IL-12 standards. The results were expressed as the concentration of the cytokines in culture medium (ng/mL).

Preparation of Toxins and Bacterial Strains

According to the procedures described by Yu et al. (2018), DON was manufactured by inoculating rice with Fusarium graminearum ACCC 37687 and then was prepared into powder. The contaminated diets were prepared by adding the powder of DON into the basic diet directly. The powder of L. plantarum JM113 was prepared according to the procedures described by Yang et al. (2017).

Experimental Design, Birds and Diets

The study was approved by Institutional Animal Care and Use Committee of Northwest A&F University ( Permit Number: NWAFAC 1008).

One hundred forty-four hatched 1-day-old healthy (Arbor Acres) male broilers with similar BW were obtained from a commercial hatchery. The chicks were randomly divided into 3 groups consisting of 6 replicates with 8 birds per replicate. The 3 feeding groups were as follows: basal diet (CON), basal diet experimentally contaminated with 10 mg DON/kg diet (DON), and basal diet incorporated with 10 mg DON/kg feed and freeze-dried L. plantarum JM113 at 1 $\times 10^9$ CFU/kg diet (DL). The ingredients and composition of the experimental diets are shown in Table 1. The birds had free access to feed and water throughout the 42 D feeding trial. All chicks were reared in two layers metal cage, and the brooding temperature was maintained at 35°C for the first week and gradually decreased to 27°C in the third week. Diet analyses for DON were conducted using HPLC following the method of Waśkiewicz et al. (2014). The assay results indicated that the dietary concentration of DON was lower than 500 µg/kg in the basal diets of two stages. Meanwhile, in the DON-contaminated starter and grower feeds, the concentrations of DON were 10.268 ± 0.090 mg/kg and 10.764 ± 0.090 mg/kg, respectively. Moreover, the viability assay of probiotic in feed by using plate counting method discovered that the viability of probiotic was greater than $8 \times 10^8$ CFU/kg in feed of the DL group and was not detected in the basal diet.
Nutrient composition
cyte proliferation group)/OD570 (control group).

peripheral blood T and B lymphocyte were calculated using the following equation: SI
medium. Subsequently, the number of proliferation

Acid, 25 mg; pantothenic acid, 240 mg; niacin, 1,000 mg; Vitamin B2, 120 mg; vitamin B12, 0.50 mg; vitamin E, 600 IU; biotin, 0.65 mg; folic acid, 50,000 IU; vitamin K3, 53 mg; vitamin B1, 40 mg; vitamin D, 80 mg, Zn (as zinc sulfate) 75 mg, I (as potassium iodide) 0.40 mg, Se (as sodium selenite) 0.30 mg.

Table 1.

| Items, g/kg diet | Growing phases |
|------------------|----------------|
|                  | Starter (0–21 D) | Grower (21–42 D) |
| Ingredients      |                |
| Corn             | 572.7          | 609.9          |
| Soybean meal     | 352.0          | 300.0          |
| Cottonseed meal  | 20.0           | 40.0           |
| Soybean oil      | 20.0           | 20.0           |
| Sodium chloride  | 3.6            | 3.5            |
| Limestone        | 20.0           | 17.0           |
| Calcium hydrogen phosphate | 3.0 | 3.0 |
| Choline chloride | 0.5            | 0.5            |
| L-Lysine•HCl     | 1.4            | 0.6            |
| Minerals premix1 | 3.0            | 3.0            |
| Phytase          | 1.0            | 1.0            |
| DL-Methionine    | 2.5            | 1.2            |
| Vitamin premix2  | 0.3            | 0.3            |
| Nutrient composition
| Metabolism energy, MJ/kg | 12.34 | 12.74 |
| Crude protein    | 219.0 | 190.0 |
| Ca               | 9.7   | 9.1  |
| Total P          | 6.2   | 6.0  |
| Available P      | 4.0   | 4.0  |
| Lys              | 12.1  | 10.5 |
| Met              | 5.7   | 4.4  |
| Met + Cys        | 9.0   | 7.5  |

1The element premix provided per kilogram of diets: Cu (as copper sulfate) 10 mg, Fe (as ferrous sulfate) 80 mg, Mn (as manganese sulfate) 80 mg, Zn (as zinc sulfate) 75 mg, I (as potassium iodide) 0.40 mg, Se (as sodium selenite) 0.30 mg.
2The vitamin premix provided per kilogram of diets: vitamin A, 250,000 IU; vitamin D, 50,000 IU; vitamin K3, 53 mg; vitamin B12, 0.50 mg; vitamin B1, 40 mg; vitamin B2, 120 mg; vitamin B12, 0.50 mg; vitamin E, 600 IU; biotin, 0.65 mg; folic acid, 25 mg; pantothenic acid, 240 mg; nisin, 1,000 mg.

Lymphocyte Isolation and Preparation

At 21 D and 42 D of age, 1 bird randomly from each replicate group was fasted for 12 h. Heparinized blood samples were collected from the wing vein. Lymphocytes were isolated from peripheral blood using lymphocyte density-gradient centrifugation medium (HaoYang Biological Manufacture Co. Ltd., Tianjin, China) and centrifuged at 1100 × g for 30 min. The lymphocyte fraction was collected from the interface and washed three times with RPMI-1640 culture medium (Invitrogen Corp., Grand Island, NY). After the final washing, cells were re-suspended in RPMI-1640 complete culture medium supplemented with 5% (vol/vol) heat inactivated fetal calf serum, 100 U penicillin/mL, 100 μg streptomycin/mL, and 24 mM Hepes buffer (Amresco 0511; Amresco Inc., Cleveland, OH). Cell viability was determined by the trypsin blue exclusion method. Cell concentration was adjusted to 1 × 10⁷ cells/mL culture medium. Subsequently, the number of proliferation peripheral blood T and B lymphocyte were calculated using the following equation: SI = OD570 (T/B lymphocyte proliferation group)/OD570 (control group).

Assay of Disaccharidase in Jejunal Mucosa

The birds were euthanized by an intraperitoneal injection of sodium pentobarbitone (30 mg per kg BW) after the blood samples were collected. The jejunal mucosa was scraped from 10 cm of the jejunum (5 cm proximal to the Meckel’s diverticulum). A portion of mucosa was homogenized in ice-cold 50 mM isotonic physiological buffer (pH 7.4) and centrifuged at 3,000 × g for 20 min at 4°C. The second portion of the sample was snap frozen in liquid nitrogen and then stored at −80°C for later mRNA relative expression analysis. Mucosal disaccharidase (maltase and sucrase) activities and protein content of mucosal homogenates were measured using commercial kits (Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer’s instructions.

Quantitative RT-PCR Analysis of Gene Expression

Total RNA was extracted from snap-frozen jejunal mucosa (50 mg) using TRizol reagent (Invitrogen Company, Carlsbad, CA) according to the manufacturer’s instructions. Quantity and quality of extracted total RNA was determined using a spectrophotometer (NanoDrop-2000, Thermo Fisher Scientific, Waltham, MA) at 260 and 280 nm (Wang et al., 2016). First-strand cDNA was synthesized from 2 μg of total RNA using an EasyScript First-Strand cDNA Synthesis SuperMix kit (TransGen Biotech, Beijing, China) according to the manufacturer’s instructions. The mRNA level of the following genes were analyzed by real-time quantitative PCR: Bcl-2-associated X gene (Bax), B-cell lymphoma 2 gene (Bcl-2), mechanistic target of rapamycin (mTOR), polymeric Ig receptor (PIgR), regulatory associated protein of mTOR complex 1 (Raptor), ribosomal protein S6 kinase polypeptide 1 (RPS6KB1), Toll-like receptor 2 (TLR2), and Toll-like receptor 4 (TLR4). The primers were listed in Table 2. Expressions of the genes were quantified with a BioRad CFX96 Sequence Detection System and TransStart Top Green qPCR SuperMix (TransGen Biotech). The mRNA was analyzed for relative expression of the genes mRNA contents after normalizing for glyceraldehyde3-phosphate dehydrogenase.

Determination of Bacterial Metabolites

The cecal digesta were collected and immediately placed into plastic tubes, sealed, and frozen at −80°C for subsequent short-chain fatty acids (SCFA) analysis. The concentration of SCFA (acetic acid, propionic acid, isobutyric acid, n-butyric acid, isopentanoic acid, and n-pentanoic acid) was measured using gas chromatography (Agilent 7890A; Agilent Technologies, Santa Clara, CA) according to the method of Qaisrani et al. (2014).

Microbial DNA Extraction, 16S rRNA Gene Amplification of the V3 + V4 Region, Sequencing, and Bioinformatics Analysis

The collected cecal digesta (300 mg) from CON, DON, and DL groups of 42-day-old broilers were used for DNA
extraction using QIAamp DNA Stool Mini Kit (Qiagen, Dusseldorf, Germany). The quantity and quality of those DNA samples were further assessed by Nanodrop ND-1000 spectrophotometer (Thermo Scientific, Waltham, MA) and then stored at −280°C until sequencing analysis.

The 16S rRNA gene PCR of the V3–V4 hypervariable region using primer 341F_hmp (CCTYGGGRBGCASCAG) and 806R_hmp (GGACTACNNGGGTATCTAAT) were used to determine bacterial composition and functional prediction of the bacterial species by Illumina MiSeq sequencing at Novogene Bioinformatics Technology Co., Ltd., Beijing, China. The PCR products were purified, quantified, and sequenced using Illumina HiSeq PE250.

Raw data were merged using FLASH v1.2.7 (Liu et al., 2018). Sequencing data were analyzed according to usearch-derep_fulllength and QIIME (version 1.9.1), and having a quality score lower than 20 in the raw reads were removed. The chimera sequences were also removed by usearch-cluster_ottus and detected by the usearch-uchime_ref and the Gold database. These effective tags were clustered into the same operational taxonomic units at an identity threshold of 97% similarity using UPARSE software. For each operational taxonomic unit, a representative sequence was screened and used to assign taxonomic composition using the RDP Classifier (Version 2.2) and the Silva 16S sequence database. The Metastats was used to identify the effect of DON on gut microbiota and the effect of L. plantarum JM113 on gut microbiota when challenge with DON in the phylum, class, order, family, and genera levels. Taxonomic abundances analysis was performed by LEfSe. R software 2.15.3 were used to calculate Spearman’s rank correlation coefficients between the relative abundance of gut microbiome and the concentrations of SCFA, as well as the mRNA expression of immunity-related genes and apoptosis-regulatory genes, and exhibit the result by the heatmap.

### Statistical Analysis

The data were analyzed by one-way ANOVA with the Turkey multiple comparison tests, using SPSS statistical software (ver. 20.0 for Windows, SPSS Inc., Chicago, IL). Results were expressed as treatment means with their pooled standard error of the mean. A probability value of *P*, 0.05 was described to be statistically significant.

### RESULTS AND DISCUSSION

#### IL-10 and IL-12 Production by Spleen Cells After Stimulation With L. plantarum JM113

To determine whether L. plantarum JM113 plays a role in regulating cytokine production of spleen cells with beneficial clinical effects in DON-induced intestine toxicity, we measured the cytokine production of spleen cells co-cultured with LPS or L. plantarum JM113. In accordance with a recent study (Feng et al., 2016), we found that the cytokine levels in control group were below detectable limits. Both JM113 and LPS enhanced IL-10 production compared with the control and the co-add group (*P* < 0.05). Furthermore, there were no

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**Table 2. Primers used in real-time quantitative PCR.**

| Gene      | Primer sequence (5’-3’) | Gene bank ID |
|-----------|-------------------------|--------------|
| GAPDH     | F: AGAACATCATCCACCGGCTGC | NM_204305    |
|           | R: CGGCCAGTGAGCTGCTAAC   |              |
| Bax       | F: CTTGCGGAGGGTACTGTTT   | NM_001013611 |
|           | R: CGGGTTCGAGGATGTGTTT   |              |
| Bcl-2     | F: CTGTTTGGTCTGGTGCTT    | NM_205344    |
|           | R: CCTGCTGTTGCTGCTT      |              |
| β-Defensin-8 | F: TGTGCGGCTGGTGTTTTT    | NM_001001781 |
|           | R: TGCGTCTGTTGCTGCTT     |              |
| mTOR      | F: GATGTCAACCTCCTGCTTAG  | NM_205117.1  |
|           | R: CTGCCACCATGTTAATTC    |              |
| Pgly      | F: GGATCTGGGAGCGACCAAT   | ENSGALG0000000919 |
|           | R: GAGCCAGACCTCGTCAGA    |              |
| Raptor    | F: GCTGAGACCGCTTTCTGCT   | ENSGALG0000006938 |
|           | R: GTTCAGCCTGCGCATGACGA  |              |
| RPS6KB1   | F: TGGAGGCTGCTGGGCTCAAT  | NM_001030721 |
|           | R: GTACAGCCCACACCTCCTGAC |              |
| TLR2      | F: GATTGGGACAACATCATTGACTC | NM_001161650 |
|           | R: AGAGCTGTCTTTCAAGTTTCCC |              |
| TLR4      | F: AGTCTGAAATTGTGCTGACCTAAAT | NM_001030693 |
|           | R: GCGAGCTTTAGCCATGGAAG  |              |

**Abbreviations:** Bax, Bcl-2-associated X gene; Bcl-2, B-cell lymphoma 2 gene; F, forward; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; mTOR, mechanistic target of rapamycin; Pgly, polymeric Ig receptor; Raptor, regulatory associated protein of mTOR complex 1; R, reverse; RPS6KB1, ribosomal protein S6 kinase polypeptide 1; TLR2, Toll-like receptor 2; TLR4, Toll-like receptor 4.
statistical differences \((P > 0.05)\) in IL-10/IL-12 between JM113 and the control, whereas the co-add group significantly increased the antiinflammatory ratio IL-10/IL-12 in comparison with the others \((P < 0.05)\). IL-10 is a potent antiinflammatory cytokine for the induction of T-helper cell type 2 immune responses under inflammatory conditions. Meanwhile, IL-12 influences the development of T-helper cell type-1 cell responses (Teng et al., 2015). The balance between production of IL-10 and IL-12 in immune models has been a sensitive indicator of T-helper cell type 1 immune responses under inflammatory conditions. After 48 h of culture, IL-10 and IL-12 produced in the culture supernatants were analyzed. Each value represents the mean value ± SEM. \(^{a,c}\) Numbers with different superscripts differ statistically at \(P \leq 0.05\). Abbreviation: IL, interleukin.

### Effects of L. plantarum JM113 on Jejunal Mucosa Disaccharidase Activity of Broiler Chickens Challenged With Deoxynivalenol

To test the \textit{in vivo} protective effects of \textit{L. plantarum} JM113 on DON-induced intestinal damage, we challenged live broiler chickens with DON in their feed. The results showed that no significant differences were found in weight gain, feed intake, and feed efficiency of broiler chickens among four treatment groups over the 42-D study (data not shown).

Infection or other inflammatory insults in the small intestine often result in reduced disaccharidase enzyme levels (Solaymani-Mohammadi and Singer, 2011). The same is true in the current study. Compared with the control group, a significant decrease in jejunal mucosa disaccharidase activity \((P < 0.05)\) at day 21, as well as a decrease in jejunal mucosa sucrase and maltase activity \((P < 0.05)\) at day 42 were detected in the DON group (Table 3). Conversely, birds exposed to \textit{L. plantarum} JM113 along with DON showed normal disaccharidase activity compared with the control group, which showed a significantly increased maltase activity at day 21 and sucrase activity at day 42 in the DL group \((P < 0.05)\) compared with the DON group. Reduction in the surface area of the microvilli is linked with impaired levels of disaccharidase enzymes such as sucrase and lactase which are essential for proper digestion and absorption of sugars (Farthing, 1997). The above results verified our previous research that DON decreased villus height and \textit{L. plantarum} JM113 mitigated the damage (Yang et al., 2017). Thus, the reduction in mucosal maltase activity in the present study was because of DON challenge-induced damage to the upper villus. Additionally, we speculate that \textit{L. plantarum} JM113 supplementation could concomitantly increase mucosal maltase activity through the regulation of villus growth.

### Effects of L. plantarum JM113 on Abrogating Immune Toxicity of Deoxynivalenol on Peripheral Blood Lymphocytes Proliferation in Broiler Chickens.

The effects of DON and \textit{L. plantarum} JM113 on peripheral blood lymphocytes proliferation were investigated. In 21-day-old broilers, DON significantly reduced \((P < 0.05)\) the number of proliferation peripheral blood T lymphocytes when compared with the control group (Table 4). Supplementation with \textit{L. plantarum} JM113 and DON synchronously could significantly increase \((P < 0.05)\) peripheral blood T lymphocyte proliferation at day 21 and the number of proliferation peripheral blood T and B lymphocytes at day 42 when compared with DON challenge group and have a similar proliferation level as the control group. T and B lymphocytes play central roles in immune response to any type of stress and/or aggression (Wu et al., 2018a). Moreover, the thymus, spleen, and bursa

#### Table 3. Effects of \textit{L. plantarum} JM113 on jejunal mucosa disaccharidase activity of broiler chickens challenged with deoxynivalenol\(^{1}\).

| Item      | CON     | DON     | DL      | SEM     | \(P\)-value |
|-----------|---------|---------|---------|---------|-------------|
| d 21      |         |         |         |         |             |
| Sucrase   | 7.02    | 7.01    | 7.12    | 0.522   | 0.997       |
| Maltase   | 21.00\(^{a}\) | 16.71\(^{b}\) | 24.08\(^{a}\) | 1.451   | 0.050       |
| d 42      |         |         |         |         |             |
| Sucrase   | 9.14\(^{a}\) | 7.58\(^{b}\) | 9.88\(^{a}\) | 0.454   | 0.022       |
| Maltase   | 49.73\(^{b}\) | 34.67\(^{b}\) | 38.64\(^{ab}\) | 3.076   | 0.002       |

\(^{a,b}\)Numbers within a row with different superscripts differ statistically at \(P < 0.05\). \(^{1}\)In = 6 per treatment group. Dietary treatments were as follows: CON = basal diet; DON = basal diet + 10 mg deoxynivalenol per kg diet; DL = basal diet +10 mg deoxynivalenol per kg diet +1 \times 10^9 cfu \textit{L. plantarum} JM113 per kg diet.

\[\text{Figure 1.}\] IL-10 and IL-12 production by spleen cells after stimulation with \textit{L. plantarum} JM113. The RPMI-1640 medium alone was used as a negative control (CON), while lipopolysaccharide (10 µg/mL) was used as a positive control (LPS). \textit{L. plantarum} JM113 \((10^6\) to \(10^9\) cfu/mL) were cultured with spleen cells. After 48 h of culture, IL-10 and IL-12 produced in the culture supernatants were analyzed. Each value represents the mean value ± SEM. \(^{a,c}\) Numbers with different superscripts differ statistically at \(P \leq 0.05\). Abbreviation: IL, interleukin.
are sites of B and T cell differentiations in broilers. Consequently, the results of peripheral blood lymphocytes proliferation in broiler chickens responded with changes in the relative weights of bursa of Fabricius in our previous research (Wu et al., 2018b). These results suggest that dietary supplementation of L. plantarum JM113 stimulates the T and B lymphocyte formations, proliferation, and the division in the immune system of broiler chickens challenged with DON.

L. plantarum JM113 could improve jejunal mucosal immunity-related and apoptosis-regulatory gene expression when challenged with deoxynivalenol.

To illuminate the roles of L. plantarum JM113 in mediating intestinal inflammation, which could counteract the effect of DON, jejunal mucosal immunity-related gene and apoptosis-regulatory gene expressions of broiler chickens were further analyzed. Polymeric immunoglobulin receptor (PIgR) and β-Defensin-8 are mucosal defense proteins of innate immune system that together function to counteract pathogen adherence and invasion (Wang et al., 2014). In 42-day-old broilers, we investigated the DON diet decreased (P < 0.05) the β-Defensin-8 and PIgR mRNA expression in the jejunum (Figure 2A). Altered expression of key function genes such as Claudins, Occludin, and Zonula occludens and gut mucosal defensive genes such as PIgR and β-Defensin-8 are primarily linked with intestinal disorders such as Crohn’s disease, ulcerative colitis, as well as other metabolic diseases (Bischoff et al., 2014). In prior study, we reported suppressed expression of Claudin-1 and Occludin induced by DON (Wu et al., 2018b), and then present study revealed significantly restrained mRNA levels of innate immune response PIgR and β-Defensin-8 genes in jejunal mucosa of broiler chickens that led to impaired barrier functions reflecting the inflammatory environment set up by DON exposure. Supplementation with L. plantarum JM113 and DON synchronously could significantly elevate (P < 0.05) jejunal β-Defensin-8 and PIgR mRNA expression when compared with the DON group. These data suggest that intestinal innate immune injury induced by DON may be repaired by L. plantarum JM113.

Toll-like receptors are capable of recognizing and discriminating diverse pathogen-associated molecular patterns such as LPS recognized by TLR4 and lipopeptides recognized by TLR2 (Yang et al., 2019). Toll-like receptors can induce epithelial responses including proliferation of epithelial cells, secretion of slgA into the lumen, and production of mucins, thereby promoting intestinal barrier function (Johnston and Corr, 2016). In the study, DON had no influence on the TLR2 mRNA relative expression (P > 0.05) but upregulated TLR4 mRNA relative expression (P < 0.05) in the jejunal mucosa of broiler chickens (Figure 2B), generally resulting in decreased Lactobacillus spp. count. Conversely, birds exposed to L. plantarum JM113 along with DON showed a higher (P < 0.05) TLR2 mRNA relative expression and had no statistically significant differences (P > 0.05) in TLR4 mRNA relative expression when compared with the CON group, which showed a significantly downregulated TLR4 mRNA relative expression (P < 0.05) compared with the DON group.

Deoxynivalenol, an inducer of stress responses in the ribosome and the endoplasmic reticulum, causes mitochondrial dysfunction and mitochondria-dependent apoptosis through oxidative stress in humans and animals (Wan et al., 2018). In previous study, we found that after DON treatment, the content of the product of lipid peroxidation such as malondialdehyde significantly increased, whereas the activity of superoxide dismutase and mRNA relative expression of nuclear factor erythroid 2-related factor 2 and heme oxygenase 1 in the jejunal mucosa of broiler chickens significantly decreased (Yang et al., 2017). In current study, both DON group and DL group had a significant increase (P < 0.05) in the mRNA expression of the proapoptosis gene Bax and the antiapoptosis gene Bcl-2 when compared with the CON group (Figure 2C), and DON challenge significantly increased Bax/Bcl-2 in the jejunum (P < 0.05) and demonstrated proapoptosis status. This result corroborates other studies in which DON exposure was associated with significant increases in the ratio of Bax/Bcl-2 in mouse endometrial stromal cells and in piglet hippocampal nerve cells and then caused apoptosis (Dai et al., 2017; Wang et al., 2018). Conversely, the DL group showed normal Bax/Bcl-2 when compared with the control group, which also decreased Bax/Bcl-2 of the jejunum (P < 0.05) when compared with the DON group. That is, birds exposed to L. plantarum JM113 manifested a superior antiapoptosis status, and DON-induced cell apoptosis was reverse by L. plantarum JM113.

The mTOR plays a key role in regulating cell growth and proliferation (Qi et al., 2017). A key downstream target of mTOR is RPS6KBI, a key factor in protein synthesis, which is a serine/threonine kinase regulated by mTOR in response to growth factors and nutrients to promote cell proliferation, cell growth, and cell cycle progression and has been found to be involved in a variety of human diseases ranging from diabetes, obesity,
hemangioma to cancer (Carnevalli et al., 2010; Di Conza et al., 2017). The DON decreased the relative protein expression of mTOR in the jejunal and ileal mucosa of piglets and the relative mRNA expression of mTOR on porcine oocytes (Xiao et al., 2013; Han et al., 2016). Consistent with these results, DON significantly reduced \( P, 0.05 \) mTOR, Raptor, and RPS6KB1 mRNA expression in the jejunum (Figure 2D). In contrast, the mRNA expressions of mTOR, Raptor, and RPS6KB1 in the jejunal mucosa of broiler chickens in the DL treatment were greater \( P, 0.05 \) than those in the CON and DON groups. The results confirmed the findings of our earlier research that DON decreased in jejunal villus height and the ratio of villus height to crypt depth, by contrast, when L. plantarum JM113 was added to the DON-contaminated diet, a significant increasing was observed (Yang et al., 2017). Thus, these results indicated that the mTOR/RPS6KB1 pathway activated by L. plantarum JM113 supplementation was essential to intestinal cell migration and could help to accelerate the healing of DON-induced intestinal damage and promote the recovery of tissues.

**Effects of L. plantarum JM113 on Cecal Microbiota and Bacterial Metabolites of Broiler Chickens Challenged With Deoxynivalenol**

Adding L. plantarum JM113 significantly raised \( P, 0.05 \) propionic acid, n-butyric acid, and total SCFA concentrations in cecal contents of birds fed DON diet (Figure 3). Diet-related changes in cecal SCFA concentrations reflected the alterations in the bacterial community composition. The microbial distribution of each group at the phylum (Figure 4A) and family (Figure 4B) levels was depicted. Across all treatment groups, Firmicutes (85.40%) and Ruminococcaceae (56.99%) were the most abundant phyla and family in cecal digesta, respectively. Furthermore, the exposure to DON significantly increased percent of Ruminococcaceae abundance compared with the CON and DL groups. Percent of Bacteroidetes and Rikenellaceae were significantly increased in the DL group \( P, 0.05 \) compared with the CON and DON groups. Bacterial community richness (Chao1) and diversity (Shannon and Simpson) index values were compared for cecal digesta of 42-day-old broilers fed diets with deoxynivalenol and with or without L. plantarum JM113.
JM113. The exposure to DON decreased ($P < 0.05$) the cecal species Chao 1 index compared with CON (Figure 4C). The decreased bacterial richness suggested that certain taxa may have immunoregulation function as a result of the DON exposure which may have decreased the quantity of species. Although the Shannon index showed no statistical differences ($P > 0.05$) among the CON, DON, and DL groups (Figure 4D), birds fed DL-diet had a significant decrease ($P < 0.05$) in the Simpson indices (Figure 4E). As is known, high species diversity may reflect a more stable microbiota to prevent intestinal disease (Han et al., 2017). The results showed that the DL birds had a more stable microbiota to keep intestinal health.

To clarify specialized communities in samples, statistical analysis by the LEfSe tool was performed only from the phylum to the genus level (Figures 4F, 4G). In CON, two groups of bacteria were significantly enriched, namely Erysipelotrichia (from class to genus) and NB1_n (from order to genus), and other four genera,
including Ruminococcaceae_UCG_007, Faecalitalea, and Peptococcus were also significantly enriched. These enriched bacteria are mainly related to the production of SCFA (De Maeschalck et al., 2014).

**Correlation Analysis**

Spearman correlation analysis was used among the top 50 genera to characterize associations of bacterial abundances with SCFA concentrations and predicted metabolic functions in cecal digesta. In total, we had built 30 significant associations between genus abundance and SCFA concentrations, and 14 genera were significantly correlated with at least a SCFA indicator (Figure 5). Most positive correlations were observed within the phylum Bacteroidetes; in contrast, most negative correlations were observed within the phylum Firmicutes. Both Alistipes and Ruminococcaceae_UCG-005 positively were correlated with acetic acid, propionic acid, and total SCFA ($P < 0.05$), respectively. Meanwhile, the relative abundances of norank_f__Ruminococcaceae, Subdoligranulum, Erysipelotrichaceae, and

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**Figure 5.** Correlation analysis between short-chain fatty acid (SCFA) concentrations and relative abundance of the top 50 genera in cecal digesta of 42-day-old broilers fed diets with deoxynivalenol and with or without *L. plantarum* JM113. Colors refer to the degree of correlation. *0.01 < $P \leq 0.05$, **0.001 < $P \leq 0.01$, ***$P \leq 0.001$.**
[Eubacterium]_hallii_group, and Blautia were significantly negatively associated with the concentrations of acetic acid, propionic acid, n-butyric acid, and total SCFA \((P < 0.05)\), and unclassified_f__Bacillaceae, Eisenbergiella, and Coprococcus_1 were negatively correlated with isobutyric acid \((P < 0.05)\). These results indicate that the composition differences of bacterial metabolites reflect the changes of microbiota.

To study the role of gut microbiota in deoxynivalenol-induced apoptosis and intestinal inflammation in broiler chicken, we also examined the relationship between the relative abundance of gut microbiome and the mRNA expression of immunity-related genes and apoptosis-regulatory genes. In total, 30 significant correlations between bacterial genera and the mRNA expression could be established, and 18 genera were significantly correlated with at least an indicator (Figure 6). Similar to above, most positive correlations were observed within the phylum Bacteroidetes, and most negative correlations were observed within the phylum Firmicutes. The mRNA expression of Bcl-2, TLR2, mTOR, Raptor, and RPS6KB1 \((P < 0.05)\), which are regarded as important cell proliferation and antiapoptosis parameters, were significantly negatively associated with the relative abundances of norank_f__Erysipelotrichaceae, Subdoligranulum, and Anaeroplasma, while they had a strong positive correlation with the relative abundances of Ruminococcaceae_UCG-004, Alistipes, and Ruminococcaceae_NK4A214_group. It is recognized that commensal microorganisms and microbial metabolites impact host gene expression in the gastrointestinal tract to influence the disease progression (Levy et al., 2015;
CONCLUSIONS

Above all, these results implied that *L. plantarum* JM113 supplementation could ameliorate DON-induced apoptosis and intestinal inflammation via manipulating the bacterial community composition and could be used as a potential candidate to attenuate intestinal impairments.

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