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Original Research Article

Lactiplantibacillus plantarum L47 and inulin alleviate enterotoxigenic *Escherichia coli* induced ileal inflammation in piglets by upregulating the levels of α-linolenic acid and 12,13-epoxyoctadecenoic acid

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ABSTRACT

Alternatives to antibiotics for preventing bacteria-induced inflammation in early-weaned farm animals are sorely needed. Our previous study showed that *Lactiplantibacillus plantarum* L47 and inulin could alleviate dextran sulfate sodium (DSS)-induced colitis in mice. To explore the protective effects of *L. plantarum* L47 and inulin on the ileal inflammatory response in weaned piglets challenged with enterotoxigenic *Escherichia coli* (ETEC), 28 weaned piglets were assigned into four groups, namely, CON group—orally given 10 mL/d phosphate buffer saline (PBS), LI47 group—orally given a mixture of 10 mL/d *L. plantarum* L47 and inulin, ECON group—orally given 10 mL/d PBS and challenged by ETEC, and ELI47 group—orally given 10 mL/d *L. plantarum* L47 and inulin mixture and challenged by ETEC. The results demonstrated that the combination of *L. plantarum* L47 and inulin reduced inflammatory responses and relieved the inflammatory damage caused by ETEC, including ileal morphological damage, reduced protein expression of ileal tight junction, decreased antioxidant capacity, and decreased anti-inflammatory factors. Transcriptome analysis revealed that *L. plantarum* L47 and inulin up-regulated the gene expression of phospholipase A2 group IIA (PLA2G2A) ($P < 0.05$) as well as affected alpha-linolenic acid (ALA) metabolism and linoleic acid metabolism. Moreover, *L. plantarum* L47 and inulin increased the levels of ALA ($P < 0.05$), lipoteichoic acid (LTA) ($P < 0.05$), and 12,13-epoxyoctadecenoic acid (12,13-EpOME) ($P < 0.05$) and the protein expression of Toll-like receptor 2 (TLR2) ($P = 0.05$) in the ileal mucosa. In conclusion, *L. plantarum* L47 and inulin together alleviated ETEC-induced ileal inflammation in piglets by up-regulating the levels of ALA and 12,13-EpOME via the LTA/TLR2/PLA2G2A pathway.

1. Introduction

Bacterial infection is an issue for newborn and weaned piglets (Cutler et al., 2007), which increases morbidity and mortality, resulting in massive financial losses in the global swine industry (Yang et al., 2014). Enterotoxigenic *Escherichia coli* (ETEC) can colonise the intestinal tract, increase the production of endotoxin, disrupt the intestinal barrier, and eventually cause intestinal inflammation (Fairbrother et al., 2005). Antibiotics have been routinely utilised in the pork production industry for decades to prevent numerous bacteria-induced infections and inflammation. Long-time or excessive use of antibiotics, on the other hand, cause veterinary drug residues in livestock products and the development of drug-resistant microorganisms (Bach Knudsen, 2001). As a result, researching alternatives to commonly used antibiotics is important.

As alternatives to antibiotics, probiotics and prebiotics or a combination of the two are widely preferred for bacteria-induced infections and inflammation. Probiotics have been shown to

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suppress mucosal inflammation and restore cytokine balance (Farkas et al., 2014), functional mechanisms of which may include the modulation of gut microbiota (Bin et al., 2018) or interaction with intestinal mucous to alter host metabolism (Ashaolu and Fernández-Tomé, 2021). *Lactiplantibacillus plantarum*, a type of probiotic found in both human and animal intestines, has a wide range of probiotic qualities, gastrointestinal transit tolerance, and anti-inflammatory capabilities (Fidanza et al., 2021). Prebiotics, such as inulin, stimulate the growth or activity of health-promoting bacteria and induce the production of beneficial metabolites, thus eventually improving intestinal health. *L. plantarum* L47 is a laboratory strain that was screened from the gut microbiota of healthy pigs in our lab, presenting superior probiotic properties (Zhang et al., 2021b) and favourable combination effects with inulin in in-vitro fermentation experiments (Zhu et al., 2021). Moreover, in our previous study, the combination of *L. plantarum* L47 and inulin was shown to alleviate dextran sulfate sodium (DSS)-induced mice colitis (Zhang et al., 2021a).

The ileum is a section of the small intestine that is critical for nutritional digestion and absorption, as well as immunity (Hou et al., 2021). Ileal enterocytes are functionally enriched in a range of immune-related activities, in contrast to the proximal small intestine's functional enrichment in pathways for digestion, absorption, and metabolism (Esterhazy et al., 2019). Nevertheless, the majority of inflammation still often occurs in the ileum (Petagna et al., 2020). Considering the specific characteristics in various strains, it has remained unclear whether a combination of *L. plantarum* L47 and inulin could alleviate ETEC-induced ileal inflammation in pigs and how the combination function.

The purpose of the present study was to explore the protective effects of *L. plantarum* L47 and inulin supplementation on ETEC-induced ileal inflammation in piglets, as well as the mechanisms of its effects. The study revealed that the combination of *L. plantarum* L47 and inulin could alleviate ileal inflammation in ETEC-challenged pigs by up-regulating the levels of alpha-linolenic acid (ALA) and 12,13-epoxyoctadecenoic acid (12,13-EpOME) via the lipoteichoic acid (LTA)/Toll-like receptor 2 (TLR2)/phospholipase A2 group IIA (PLA2G2A) pathway, associated with regulating the metabolism of polyunsaturated fatty acids (PUFA) rather than modulating gut microbiota. This study offers new insight into the potential mechanisms of probiotic action.

2. Materials and methods

2.1. Animal ethics statement

All animal protocols in this study followed the Chinese guidelines for animal welfare and were approved by the Animal Ethics Committee of Nanjing Agricultural University (SYXK-2021-0086).

2.2. Culture of L. plantarum L47 and preparation of mixture of L. plantarum L47 and inulin

L. plantarum L47 was previously isolated from the intestine of a healthy piglet and stored in our laboratory. The strain was cultured for 24 h in sterile De Man, Rogosa and Sharpe (MRS) liquid medium at 37 °C to reach 1.0×10^9 CFU/mL. Then, inulin was added to the *L. plantarum* L47 solution at a proportion of 1:10 (wt/vol).

2.3. Experimental design

Twenty-eight castrated piglets (Duroc × Landrace × Large White) weighing 6.80 ± 0.84 kg at 21 d old were fed in individual cages (maintained at 27 ± 2 °C) with free access to feed and water for 24 experimental days. The piglets were randomly specified into four treatment groups ($n = 7$), namely, the CON group, orally given 10 mL/d phosphate buffer saline (PBS) from d 1 to 21 of the experimental period; the LI47 group, orally given 10 mL/d *L. plantarum* L47 and inulin mixture from d 1 to 21 of the experimental period; the ECON group, orally given 10 mL/d PBS from d 1 to 21 of the experimental period and challenged by 10 mL ETEC containing 1×10^9 CFU/mL of *E. coli* K88 strain CVCC224 (China Institute of Veterinary Drugs Control, Beijing, China) at d 22 of the experimental period; and the ELI47 group, orally given 10 mL/d *L. plantarum* L47 and inulin mixture from d 1 to 21 of the experimental period and challenged by 10 mL the same ETEC culture medium as ECON group on d 22 of the experimental period as well. Conversely, the CON and LI47 group were orally given an equivalent dosage of PBS on d 22. The basal diet (Table 1) without antibiotics was designed based on the guidelines of NRC (2012). The nutrient levels (Table 1) are calculated according to the Tables of Feed Composition and Nutritive Values in China.

2.4. Sample collection

On d 24 of the experimental period, all pigs were slaughtered (after fasting for 12 h). The entire ileum of each pig was collected; from each, a middle segment of ileum (approximately 1.5 cm) was fixed in 4% paraformaldehyde solution for at least 24 h. The ileal digesta were collected and stored at −20 °C for subsequent analysis. The ileum tissue was first flushed with precooled sterile PBS, and then the ileal mucosa was collected via scraping by sterile glass microscope slides and subsequently stored at −80 °C until analysis.

2.5. Determination of ileal mucosal antioxidant property

A total of 0.1 g of ileal mucosa was homogenised with 1000 μL of precooled 0.9% normal saline. The supernatant of the homogenate was collected after centrifuging at 3,500 × *g* for 15 min at a temperature of 4 °C. The protein concentration was measured with a Bradford protein assay kit (Solarbio, Beijing, China). Finally, the concentration of total antioxidant capacity (T-AOC), total superoxide dismutase (T-SOD), glutathione peroxidase (GPX), and malondialdehyde (MDA) in the ileal mucosa was determined using kits and following the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, China).

Table 1
Experiment basal diet composition and nutrient level (%, as-fed basis).

Ingredients	Contents	Nutrient level	Contents
Corn	46.40	Digestible energy[2], Mcal/kg	3.57
Soybean meal	20.00	Crude protein	20.61
Extruded soybean	10.00	Calcium	0.94
Fish meal	10.00	Available phosphorus	0.51
Whey powder	5.00	Lysine	1.62
Soybean oil	4.00	Methionine	0.47
L-Lysine	0.50	Methionine + cysteine	0.72
DL-Methionine	0.05	Threonine	0.91
L-Threonine	0.05	Tryptophan	0.27
Premix[1]	4.00		
Total	100.00		

[1] The premix provided the following per kilogram of diet: vitamin A, 9600 IU; vitamin D_3, 3,200 IU; vitamin E, 20 mg; vitamin K_3, 0.6 mg; vitamin B_1, 1.6 mg; vitamin B_2, 2.4 mg; vitamin B_6, 1.2 mg; vitamin B_{12}, 0.02 mg; niacin, 20 mg; pantothenic acid, 12 mg; folic acid, 0.6 mg; biotin, 0.08 mg; choline chloride, 500 mg; Fe (from ferrous sulfate), 120 mg; Cu (from copper sulfate), 60 mg; Mn (from manganese sulfate), 12 mg; Zn (from zinc sulfate), 100 mg; I (from calcium iodate), 0.4 mg; Se (from sodium selenite), 0.3 mg.
[2] The digestible energy was calculated according to the Tables of Feed Composition and Nutritive Values in China.

2.6. Ileal morphology

The fixed ileum segment of each pig was embedded in paraffin and divided into 5-μm slices, which were separately stained with haematoxylin and eosin (H&E) and Alcian Blue and periodic acid–Schiff (AB-PAS). The values of villus height (VH), crypt depth (CD), and number of ileal goblet cells were determined using six well-oriented crypt-villus systems using Image-Pro Plus software 6.0 (Media Cybernetics, Rockville, MD, USA).

2.7. Ileal microbial metabolites

The contents of short-chain fatty acids (SCFA) in the ileal digesta were evaluated by gas chromatography. A sample of 0.4 g digesta was homogenized into 6-fold double distilled water. The mixture was mixed and centrifuged (13,400 × *g*, 4 °C, 10 min) in order to obtain supernatants. One milliliter supernatant and 200 μL of 25% (wt/vol) metaphosphoric acid were mixed and kept overnight at −20 °C. Then, the supernatant was centrifuged (13,400 × *g*, 4 °C, 10 min) and filtered with a 0.22-μL filter, and then measured with gas chromatograph (Shimadzu GC - 14 A, Kyoto, Japan).

2.8. 16S rRNA sequencing

The DNA of ileal digesta was extracted with a DNA extraction kit (Omega Bio-Tek, Norcross, GA, USA) as per the manufacturer's instructions. The V4–V5 region of the 16S rRNA gene was amplified with the primers 515F and 907R. Thereafter, the pooled DNA product was used to generate sequencing following Illumina's procedure. Then, the library was paired-end sequenced using the Illumina MiSeq platform. QIIME (version 1.70) was performed to identify sequences. The sequences were clustered as one operational taxonomic unit (OTU) at ≥97% similarity. The data of OTU abundance was refined to perform alpha diversity.

2.9. Ileal mucosal transcriptome analysis

The total RNA of the ileal mucosa was extracted with TRIzol Reagent (Invitrogen, CA, USA, 15596018). Subsequently, RNA quantity was tested using a 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA) and quantified using an ND-2000 NanoDrop Technologies (Thermo Scientific, Wilmington, DE, USA), respectively. The isolated mRNA was fragmented using fragmentation buffer. Following the instructions of the TruSeqTM RNA sample preparation kit (RS-122-2101, Illumina, San Diego, USA), cDNA libraries were created from reverse transcribed mRNA fragments. Afterwards, sequencing was performed via the Illumina NovaSeq 6000 sequencing (Shanghai BIOZERON Co., Ltd). *P*-values < 0.05 were used as criteria to identify differentially expressed genes (DEG). Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed utilizing Kobas (http://Kobas.cbi.pku.edu.cn/home.do).

2.10. Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from ileal tissue using a Steadypure RNA Kit (AG21024, Accurate, Hunan, China), and the purity and concentration of the RNA were then assessed using a Nano-Drop spectrophotometer (Thermo Fisher Scientific, Wilmington, USA). All samples were kept at a constant RNA concentration of 500 ng/μL using enzyme-free water. PCR was carried out with the use of the Roche SYBR Green PCR Kit (Q221-01, Vazyme, Nanjing, China). Table 2 lists the primer sequences used in this investigation. Finally, using the $2^{-\Delta\Delta Ct}$ method, the relative expression of the target genes was assessed.

2.11. Enzyme linked immunosorbent assay (ELISA)

The levels of interleukin (IL)-6, IL-1β, IL-8, IL-10, tumour necrosis factor-α (TNF-α), secretory immunoglobulin A (sIgA), lipopolysaccharide (LPS), LTA, and peptidoglycan (PG) in the ileal mucosa were measured using ELISA kits (Nanjing Jiancheng Bioengineering Institution, Nanjing, China) according to the manufacturer's instructions. The levels of arachidonic acid (AA), linoleic acid (LA), ALA, 12,13-EpOME, and 20-hydroxyeicosatetraenoic acid (20-HETE) in the ileal mucosa were also measured with ELISA kits (Nanjing Angle Gene Biotechnology, Nanjing, China) according to manufacturer's instructions.

2.12. Western blot

The expression of ileal mucin 2 (MUC2), tight junction protein 1 (ZO-1), occludin, claudin-1, TLR2, and Toll-like receptor 4 (TLR4) was identified using Western blot analysis. The primary antibodies were MUC2 (1:1,000, Proteintech, 27675-1-AP), ZO-1 (1:1,000, Proteintech, 21773-1-AP), occludin (1:1,000, Proteintech, 27260-1-AP), claudin-1 (1:1,000, Bioss, bs-10008 R), TLR2 (1:1,000, Proteintech, 66645-1-Ig), TLR4 (1:1,000, Proteintech, 66350-1-Ig), and β-actin (1:1,000, Proteintech, 81115-1-RR). The secondary antibodies used included horseradish peroxidase-conjugated Affini-Pure goat anti-mouse IgG (H + L) (1:5,000, Proteintech, SA00001-1) or goat anti-rabbit IgG (H + L) (1:5,000, Proteintech, SA00001-2). The intensities of the protein bands were quantified using ImageJ software (NIH, Bethesda, MD, USA).

2.13. Statistical analysis

The growth performance was examined by the Student's *t*-test. Data pertaining to the morphology, tight junction proteins, antioxidant properties, mucosal immunity, microbial metabolites, gene expressions, PUFA levels and Toll-like receptor were analyzed by using two-way ANOVA with the General Linear Model (GLM) procedure of SPSS as a 2 (LI47) × 2 (ETEC) factorial design. Tukey's multiple range test was used based on the analysis of ANOVA, which showed a significant difference. Different microorganisms were compared using the Mann–Whitney U test, with multiple comparisons adjusted for the Benjamini–Hochberg false discovery rate. All data were analyzed by SPSS 20.0 (SPSS Inc., Chicago, IL, USA) and expressed as means with standard error of the mean (SEM). Subsequently, graphs were generated via GraphPad Prism 8.0.2 (La Jolla, CA, USA). *P*-values < 0.05 represent a significant difference and *P*-values between 0.05 and 0.1 show a significant trend.

3. Results

3.1. Growth performance

During the 21 d, *L. plantarum* L47 and inulin administration did not affect the body weight, average daily gain, average daily feed intake, ratio of feed to gain, and diarrhoea rate of the pigs from d 1–21 of the experimental period (Table 3).

3.2. Ileal morphology

As shown in Fig. 1, ileal morphology was examined using H&E staining to determine the effects of the combination *L. plantarum* L47 and inulin treatment on pigs. Additionally, AB-PAS was used to quantify ileal goblet cell density (Liu et al., 2019). The data of the ileal morphology and goblet cells are presented in Table 4 and Fig. 1C. Compared with the CON group, ETEC challenges significantly decreased the VH and increased the CD of the ileum

Table 2
Primer sequences of RT-PCR.

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)
LOC110259329	GACCTACACCTCCTATGACAATG	GACCTACACCTCCTATGACAATG
CYP3A29	CCCCAGCCAGTCCATCATCAC	GAGAGACAAACAAGCACC
PLA2G2A	GTACGACAAGTCCCTACAGTTTG	AACACTTCCACCTCAGCCTTC
PLB1	CACAACAACTGCGAGAAGGA	CCGTTGATAAGCGTCAGGAT
β-Actin	TTTGCGTCAGTGTCATCG	TGCTCTGCCTTGGGTAAT

LOC110259329 = docosahexaenoic acid omega-hydroxylase CYP4F3-like isoform ×1; *CYP3A29* = cytochrome P450 3A29; *PLA2G2A* = phospholipase A2 group IIA; *PLB1* = phospholipase B1.

Table 3
Effects of *Lactobacillus plantarum* L47 and inulin on the growth performance of piglets from d 1 to 21 ($n = 7$).

Items	CON[1]	LI47[2]	SEM	*P*-value
First week				
Average daily feed intake, g/d	310.49	274.40	81.183	0.18
Average daily gain, kg	0.21	0.17	0.136	0.32
Weight, kg	8.08	7.79	1.240	0.24
Second week				
Average daily feed intake, g/d	348.79	370.76	75.766	0.41
Average daily gain, kg	0.22	0.23	0.129	0.83
Weight, kg	9.63	9.41	1.695	0.73
Third week				
Average daily feed intake, g/d	478.98	488.65	84.679	0.38
Average daily gain, kg	0.33	0.32	0.151	0.58
Weight, kg	11.91	11.66	2.179	0.78
Three weeks, total				
Average daily feed intake, g/d	379.42	377.94	62.200	0.59
Average daily gain, kg	0.25	0.24	0.091	0.35
Feed conversion ratio	1.85	1.83	0.989	0.83
Diarrhoea rate, %	33.33	32.31		

PBS = phosphate buffer saline; SEM = standard error of the mean.
[1] CON, piglets orally given 10 mL/d PBS from d 1 to 21 of the experimental period.
[2] LI47, piglets orally given 10 mL/d *L. plantarum* L47 and inulin mixture from d 1 to 21 of the experimental period.

($P < 0.05$), whereas the *L. plantarum* L47 and inulin pretreatment increased the VH and decreased the CD in the ETEC-challenged pigs. Additionally, ETEC challenges decreased the villus height-to-crypt depth ratio (VCR) of the ileum ($P < 0.05$), whereas the *L. plantarum* L47 and inulin pretreatment increased the VCR upon ETEC challenges. The interaction between ETEC challenge and *L. plantarum* L47 and inulin supplementation was found in the CD and VCR ($P < 0.05$). No interaction was found between ETEC challenge and *L. plantarum* L47 and inulin supplementation in the VH ($P > 0.05$). Interestingly, the number of goblet cells was highest in the LI47 group compared with the other three groups (Fig. 1C, $P < 0.05$). However, there were no significant differences in the protein expression of MUC2 secreted by goblet cells across the four groups (Fig. 2E, $P > 0.05$).

3.3. Ileal tight junction proteins

A significant decrease in the protein expression of claudin-1, occludin, and ZO-1 in the ileal mucosa was revealed in the groups with ETEC challenges compared to that in the CON group (Fig. 2A–C, $P < 0.05$), while a trend with higher protein expression of ZO-1 was demonstrated in the administration of the *L. plantarum* L47 and inulin group compared with that in the ECON group ($P = 0.09$, Fig. 2D). No interaction was found between ETEC challenge and *L. plantarum* L47 and inulin supplementation in the protein expression of claudin-1, occludin, and ZO-1 ($P > 0.05$).

3.4. Ileal antioxidant properties

As shown in Table 5, ETEC challenges significantly decreased the mucosal T-SOD and GPX concentrations in piglets compared with the CON group ($P < 0.05$). However, the administration of

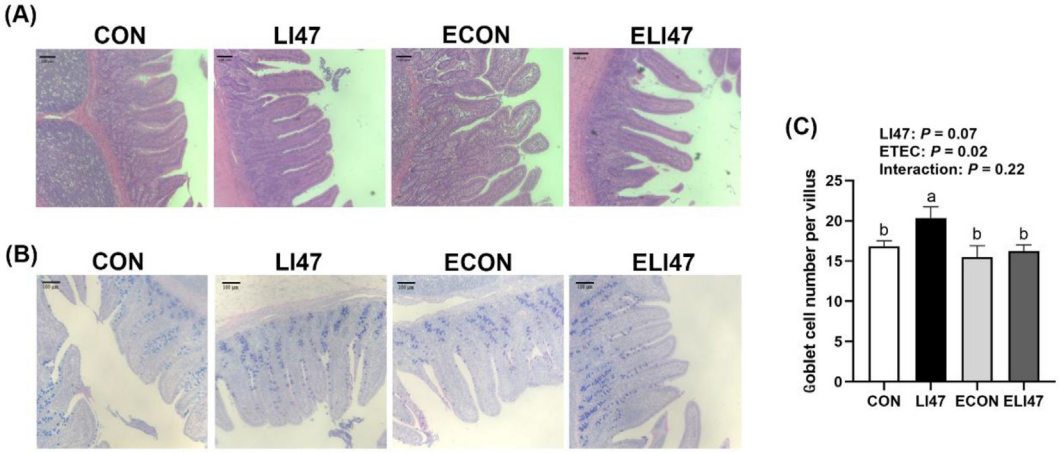

Fig. 1. Representative pictures of H&E staining (morphology) and AB-PAS staining (goblet cells) in the ileum tissues of piglets. (A) H&E staining of the ileum tissues. (B) AB-PAS staining of the ileum tissues. (C) Number of goblet cells per villus in the ileum ($n = 7$). Scale bar, 100 μm. Data are presented as mean ± SEM ($n = 7$). Bars without a common letter (a, b) denote statistically significant differences ($P < 0.05$). CON, piglets orally given 10 mL/d PBS from d 1 to 21 of the experimental period; LI47, piglets orally given 10 mL/d *L. plantarum* L47 and inulin mixture from d 1 to 21 of the experimental period; ECON, piglets orally given 10 mL/d PBS from d 1 to 21 of the experimental period and challenged by 10 mL ETEC containing 1×10^9 CFU/mL of *E. coli* K88 strain CVCC224 at d 22 of the experimental period; ELI47, piglets orally given 10 mL/d *L. plantarum* L47 and inulin mixture from d 1 to 21 of the experimental period and challenged by 10 mL the same ETEC culture medium as ECON group on d 22 of the experimental period. PBS = phosphate buffer saline; ETEC = enterotoxigenic *Escherichia coli*; H&E = haematoxylin and eosin; AB-PAS = Alcian Blue and periodic acid–Schiff.

Table 4
Effects of *L. plantarum* L47 and insulin supplementation on the intestinal morphology of piglets challenged by ETEC.

Items	Treatments				SEM	*P*-value		
	CON[1]	LI47[2]	ECON[3]	ELI47[4]		LI47	ETEC	Interaction
Villus height, μm	336.56^{b}	399.38^{c}	269.71^{a}	410.20^{c}	75.751	<0.01	0.16	0.55
Crypt depth, μm	198.75^{a}	209.34^{a}	244.78^{b}	190.69^{a}	32.743	0.04	0.18	<0.01
VCR	1.73^{b}	2.06^{c}	1.20^{a}	2.30^{c}	0.520	<0.01	0.22	<0.01

ETEC = enterotoxigenic *Escherichia coli*; VCR = villus height-to-crypt depth ratio.
Data are presented in mean ± SEM ($n = 7$). Within a row, means without a common superscript are statistically significant different ($P < 0.05$).
[1] CON, piglets orally given 10 mL/d PBS from d 1 to 21 of the experimental period.
[2] LI47, piglets orally given 10 mL/d *L. plantarum* L47 and inulin mixture from d 1 to 21 of the experimental period.
[3] ECON, piglets orally given 10 mL/d PBS from d 1 to 21 of the experimental period and challenged by 10 mL ETEC containing 1×10^9 CFU/mL of *E. coli* K88 strain CVCC224 at d 22 of the experimental period.
[4] ELI47, piglets orally given 10 mL/d *L. plantarum* L47 and inulin mixture from d 1 to 21 of the experimental period and challenged by 10 mL the same ETEC culture medium as ECON group on d 22 of the experimental period.

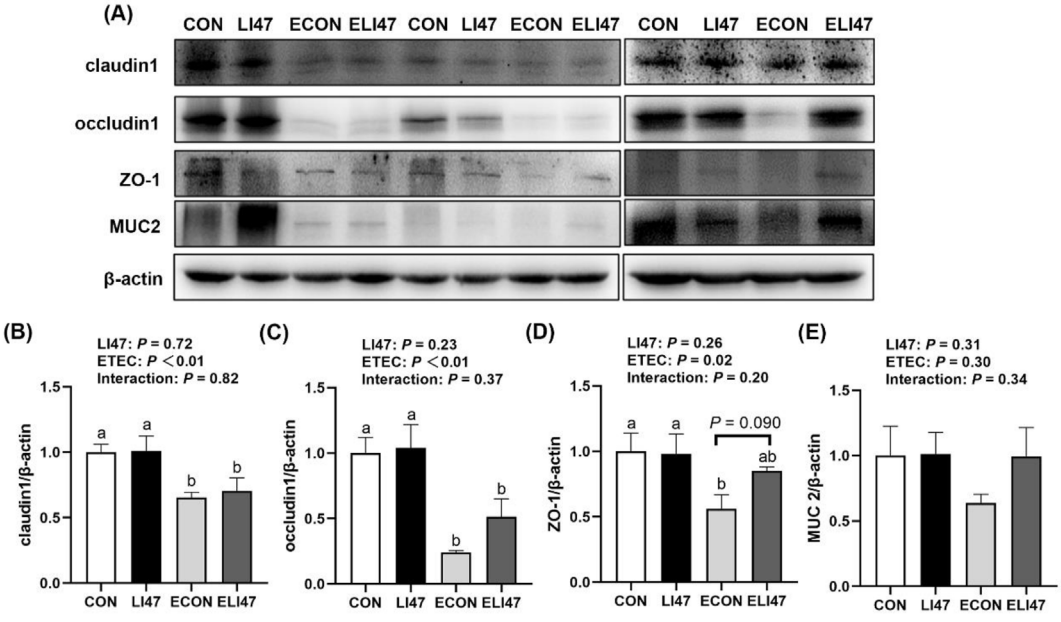

Fig. 2. Effects of *L. plantarum* L47 and inulin supplementation on the tight junction proteins and MUC2 protein expressions in the ileal mucosa of piglets challenged with ETEC. (A) Expressions of tight junction proteins and MUC2. (B to E) Protein expressions of claudin-1, occludin, ZO-1 and MUC2 in the ileal mucosa ($n = 3$). Data are presented in mean ± SEM. Bars without a common letter (a, b) denote statistically significant differences ($P < 0.05$). CON, piglets orally given 10 mL/d PBS from d 1 to 21 of the experimental period; LI47, piglets orally given 10 mL/d *L. plantarum* L47 and inulin mixture from d 1 to 21 of the experimental period; ECON, piglets orally given 10 mL/d PBS from d 1 to 21 of the experimental period and challenged by 10 mL ETEC containing 1×10^9 CFU/mL of *E. coli* K88 strain CVCC224 at d 22 of the experimental period; ELI47, piglets orally given 10 mL/d *L. plantarum* L47 and inulin mixture from d 1 to 21 of the experimental period and challenged by 10 mL the same ETEC culture medium as ECON group on d 22 of the experimental period. PBS = phosphate buffer saline; ETEC = enterotoxigenic *Escherichia coli*; MUC2 = mucin 2; ZO-1 = tight junction protein 1; SEM = standard error of the mean.

Table 5
Effects of *L. plantarum* L47 and inulin supplementation on the antioxidant properties in the ileal mucosa of ETEC-challenged piglets.

Items	Treatments				SEM	*P*-value		
	CON[1]	LI47[2]	ECON[3]	ELI47[4]		LI47	ETEC	Interaction
T-AOC, U/mgprot	3.42	4.10	2.00	4.04	1.572	0.02	0.20	0.24
T-SOD, U/mgprot	13.51^{b}	13.59^{b}	10.41^{a}	14.18^{b}	2.988	0.06	0.22	0.07
GPX, U/mgprot	26.38^{b}	40.20^{c}	14.99^{a}	38.37^{c}	13.753	<0.01	0.08	0.20
MDA, nmol/mgprot	1.56	0.95	1.25	1.20	0.623	0.18	0.91	0.25

ETEC = enterotoxigenic *Escherichia coli*; T-AOC = total antioxidant capacity; T-SOD = total superoxide dismutase; GPX = glutathione peroxidase; MDA = malondialdehyde.
Data are presented in mean ± SEM ($n = 7$). Within a row, means without a common superscript are statistically significant different ($P < 0.05$).
[1] CON, piglets orally given 10 mL/d PBS from d 1 to 21 of the experimental period.
[2] LI47, piglets orally given 10 mL/d *L. plantarum* L47 and inulin mixture from d 1 to 21 of the experimental period.
[3] ECON, piglets orally given 10 mL/d PBS from d 1 to 21 of the experimental period and challenged by 10 mL ETEC containing 1×10^9 CFU/mL of *E. coli* K88 strain CVCC224 at d 22 of the experimental period.
[4] ELI47, piglets orally given 10 mL/d *L. plantarum* L47 and inulin mixture from d 1 to 21 of the experimental period and challenged by 10 mL the same ETEC culture medium as ECON group on d 22 of the experimental period.

the combination of *L. plantarum* L47 and inulin significantly increased the T-SOD and GPX concentrations in the ileal mucosa of the ETEC-challenged pigs ($P < 0.05$). Moreover, the GPX concentrations were significantly higher in the LI47 group than that in the CON group ($P < 0.05$). No interaction was found between ETEC challenge and *L. plantarum* L47 and inulin supplementation in the T-SOD and GPX ($P > 0.05$). There were no significant differences in T-AOC and MDA across the four groups ($P > 0.05$).

3.5. Ileal mucosal immunity

ETEC challenges elevated the expression levels of inflammatory cytokines, such as IL-1β, IL-6, and TNF-α in the ileal mucosa, whereas the *L. plantarum* L47 and inulin pre-treatment significantly decreased their expression levels in the ileal mucosa (Fig. 3A–C, $P < 0.05$). Moreover, *L. plantarum* L47 and inulin significantly increased the expression levels of IL-10 and TGF-β in the ileal mucosa of the ETEC-challenged pigs (Fig. 3D–E, $P < 0.05$). The expression level of sIgA was found to be significantly higher in the ELI47 group than in the ECON group, and the expression level of sIgA was significantly higher in the LI47 group than in the CON group ($P < 0.05$). The interaction between ETEC challenge and *L. plantarum* L47 and inulin supplementation was found in the expression levels of IL-1β, IL-6, IL-10 and TGF-β ($P < 0.05$). No interaction was found between ETEC challenge and *L. plantarum* L47 and inulin supplementation in the expression levels of TNF-α and sIgA ($P > 0.05$).

3.6. Analysis of ileal microbiota and microbial metabolites

The α diversity index, which includes the Shannon, Simpson, Ace, and Chao indices, was not significantly different among the four groups, as shown in Fig. 4A and B. At the phylum level, Firmicutes, Proteobacteria, and Actinobacteriota are the dominant phyla (Fig. 4C). The relative abundance of Methylomirabilota and Nitrospirota in the ECON group was significantly increased compared with the CON group (Fig. 4D, $P < 0.05$). The relative abundance of Chloroflexi, MBNT15, Nitrospirota, Sva0485, and unclassified phyla in the ELI47 group significantly decreased ($P < 0.05$) compared with the ECON group (Fig. 4D). At the genus level, *Lactobacillus*, *Limosilactobacillus*, *Bradyrhizobium*, *Terrisporobacter*, *EscherichiaShigella*, and *Actinobacillus* were the abundant genera (Fig. 4E). Compared with the CON group, the relative abundance of *Lactobacillus* in the ECON group tended to decrease (Fig. 4F, $P = 0.06$); the relative abundance of *Roseburia*, *Blautia*, and *Faecalibacterium* significantly decreased; and the relative abundance of *Actinobacillus, Klebsiella, and Bacteroidetes_vadinHA17_norank* significantly increased (Fig. 4F–G, $P < 0.05$). Compared with the ECON group, the relative abundance of *Fournierella* in the ELI47 group tended to increase (Fig. 4F, $P = 0.06$), and the relative abundance of *Roseburia* significantly increased, while the relative abundance of *Howardella*, *Aminicenantales_norank*, and *Bacteroidetes_vadinHA17_norank* significantly decreased (Fig. 4F–G, $P < 0.05$). Moreover, the relative abundance of *Parabacteroides* significantly increased in the LI47 group compared with the CON group (Fig. 4F, $P < 0.05$).

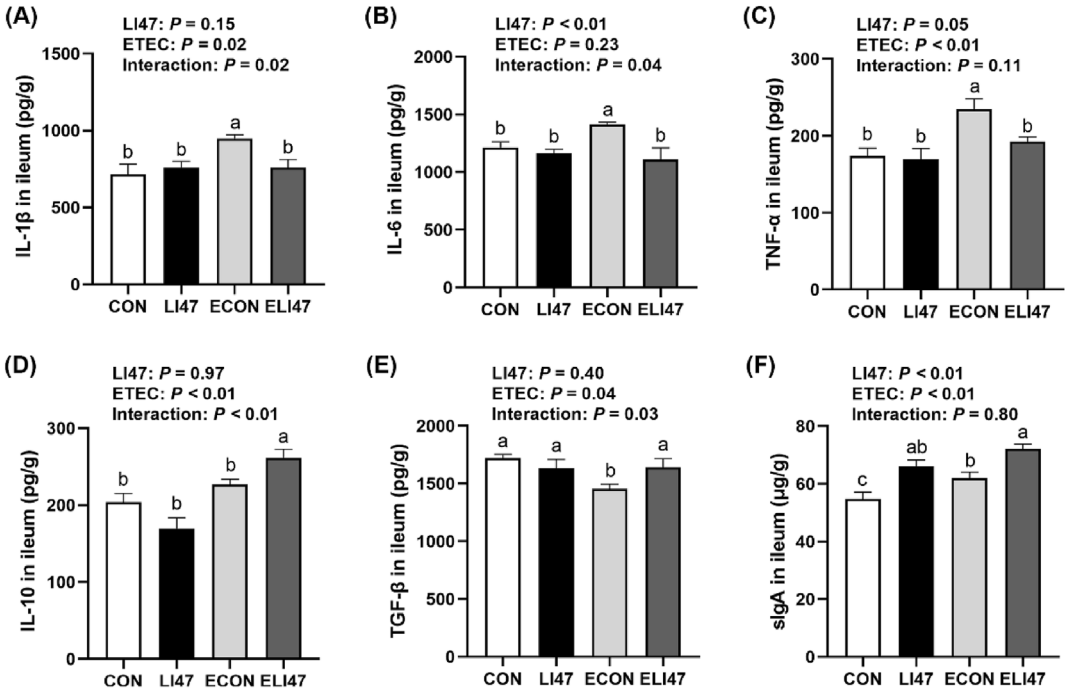

Fig. 3. Effects of *L. plantarum* L47 and inulin supplementation on the cytokines in the ileal mucosa of piglets challenged with ETEC. Concentration of (A) IL-1β, (B) IL-6, (C) TNF-α, (D) IL-10, (E) TGF-β and (F) sIgA. Data are presented in mean ± SEM ($n = 7$). Bars without a common letter (a, b) denote statistically significant differences ($P < 0.05$). CON, piglets orally given 10 mL/d PBS from d 1 to 21 of the experimental period; LI47, piglets orally given 10 mL/d *L. plantarum* L47 and inulin mixture from d 1 to 21 of the experimental period; ECON, piglets orally given 10 mL/d PBS from d 1 to 21 of the experimental period and challenged by 10 mL ETEC containing 1×10^9 CFU/mL of *E. coli* K88 strain CVCC224 at d 22 of the experimental period; ELI47, piglets orally given 10 mL/d *L. plantarum* L47 and inulin mixture from d 1 to 21 of the experimental period and challenged by 10 mL the same ETEC culture medium as ECON group on d 22 of the experimental period. PBS = phosphate buffer saline; ETEC = enterotoxigenic *Escherichia coli*; IL = interleukin; TNF-α = tumour necrosis factor-α; TGF = transforming growth factor; sIgA = secretory immunoglobulin A; SEM = standard error of the mean.

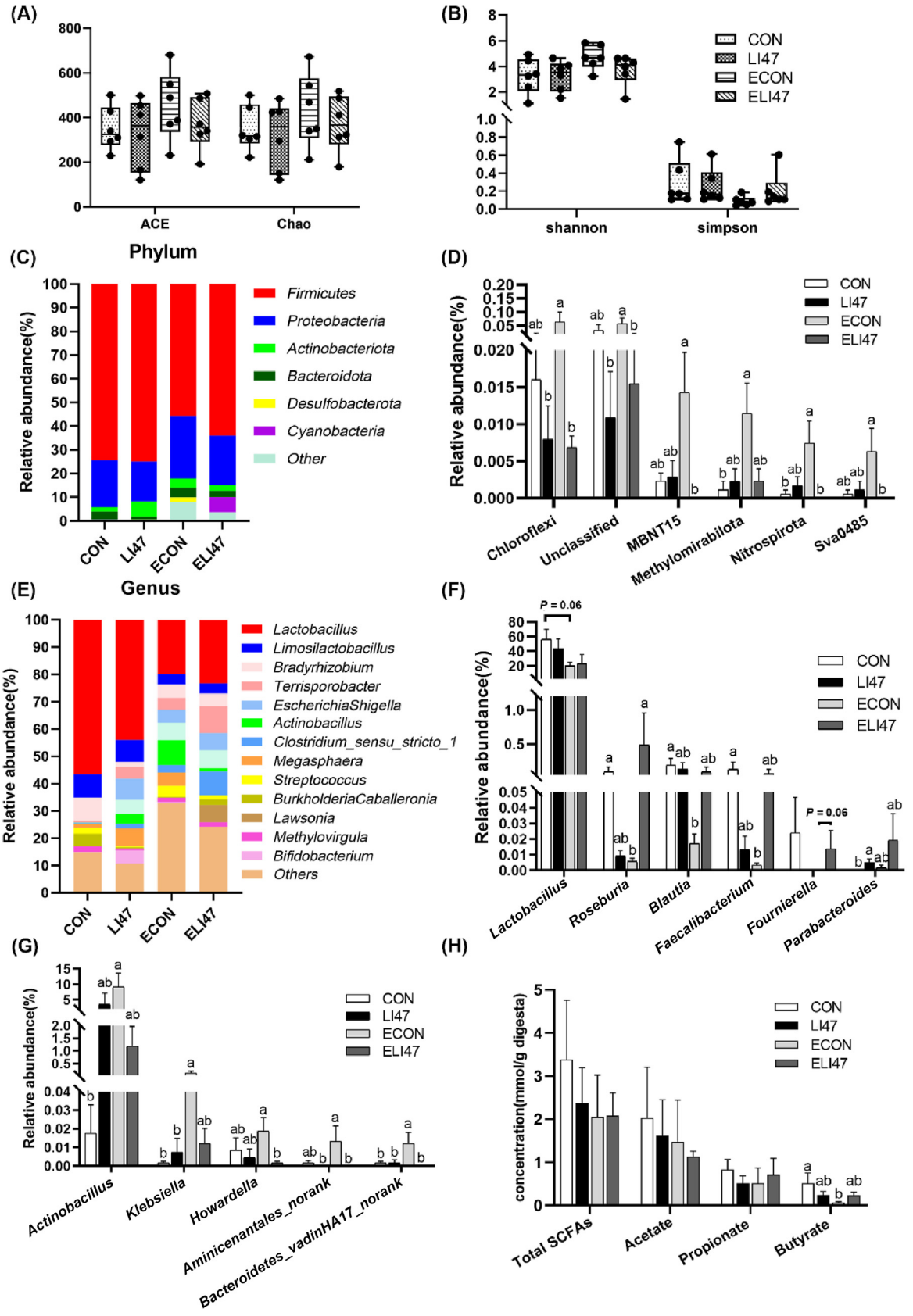

Fig. 4. Effects of *L. plantarum* L47 and inulin supplementation on the microbial composition and SCFA in the ileal digesta of ETEC-challenged piglets. (A) ACE and Chao index of different groups. (B) Shannon and Simpson index of different groups. (C) Relative abundance of the microbial phylum and (D) different phyla. (E) Relative abundance of the microbial genus and (F–G) different genera. (H) Concentrations of SCFA. Data are presented in mean ± SEM ($n = 6$). Bars without a common letter (a, b) denote statistically significant differences ($P < 0.05$). CON, piglets orally given 10 mL/d PBS from d 1 to 21 of the experimental period; LI47, piglets orally given 10 mL/d *L. plantarum* L47 and inulin mixture from d 1

No significant differences were observed in the concentrations of acetate, propionate, and total SCFA among the four groups. It was only revealed that the concentrations of butyrate significantly decreased in the ECON group compared with the CON group (Fig. 4H, $P < 0.05$).

3.7. Ileal transcriptome

Compared with the CON group, 1,169 DEG (457 up-regulated and 712 down-regulated) were identified in the ECON group (Fig. 5A). KEGG pathway analysis of DEG showed enrichment in a series of signalling pathways. Several pathways were related to the metabolism of PUFA, including ALA metabolism, LA metabolism, and AA metabolism (Fig. 5B). Compared with the ECON group, 862 DEG (558 up-regulated and 304 down-regulated) were identified in the ELI47 group (Fig. 5C). KEGG pathway analysis showed that ALA metabolism and LA metabolism were enriched in the ELI47 group (Fig. 5D). Compared with the CON group, 920 DEG (429 up-regulated and 491 down-regulated) were identified in the LI47 group (Fig. 5E). KEGG pathway analysis showed that AA metabolism and LA metabolism were enriched in the ELI47 group (Fig. 5F).

3.8. Ileal gene expressions and PUFA levels

Based on the RNA-seq data, we performed qRT-PCR to detect the DEG related to the metabolism of PUFA. Compared with the CON group, ETEC challenges showed a trend towards the increased transcription expression of *LOC110259329* (Fig. 6C, $P = 0.09$), lower transcription expression of *PLA2G2A*, and higher transcription expression of *PLB1*, whereas the *L. plantarum* L47 and inulin pretreatment enhanced the transcription expression of *PLA2G2A* and *CYP3A29* upon ETEC challenges (Fig. 6A–B and D, $P < 0.05$). No interaction was found between ETEC challenge and *L. plantarum* L47 and inulin supplementation in the transcription expression of *PLA2G2A*, *PLB1*, *LOC110259329* and *CYP3A29* ($P > 0.05$). We also investigated the levels of PUFA including AA, LA, ALA, 12,13-EpOME, and 20-HETE in the ileum mucosa. Compared with the CON group, ETEC challenges significantly increased the mucosal 20-HETE concentration in the piglets (Fig. 6F, $P < 0.05$). However, the administration of *L. plantarum* L47 and inulin significantly increased the ALA, LA, and 12,13-EpOME concentrations in the ileal mucosa of the ETEC-challenged pigs (Fig. 6G–I, $P < 0.05$). Moreover, the ALA concentrations were significantly higher in the LI47 group than in the CON group (Fig. 6G, $P < 0.05$). The interaction between ETEC challenge and *L. plantarum* L47 and inulin supplementation was found in the expression levels of 12,13-EpOME ($P < 0.05$). No interaction was found between ETEC challenge and *L. plantarum* L47 and inulin supplementation in the expression levels of AA, LA, ALA, and 20-HETE ($P > 0.05$). There were no significant differences in AA across the four groups (Fig. 6E, $P > 0.05$).

3.9. Microorganism-associated molecular patterns and toll-like receptors

The *L. plantarum* L47 and inulin pretreatment presented a low concentration of LPS in the ETEC-challenged pigs (Fig. 7A, $P < 0.05$). Notably, the LTA concentration was significantly higher (Fig. 7C, $P < 0.05$) and the protein expression of TLR2 trended to be increased (Fig. 7E, $P = 0.05$) in the ELI47 group compared to the ECON group. The interaction between ETEC challenge and *L. plantarum* L47 and inulin supplementation was found in the expression levels of LPS ($P < 0.01$). No interaction was found between ETEC challenge and *L. plantarum* L47 and inulin supplementation in the expression levels of LTA and PG ($P > 0.05$). There were no significant differences in PG and TLR4 across the four groups (Fig. 7B and F, $P > 0.05$).

4. Discussion

4.1. Growth performance and diarrhea

Many studies have reported the positive effects of synbiotic on improving animal growth performance and decreasing the diarrhoea rate (Hashem et al., 2021; Zhou et al., 2023). For example, broilers supplemented with a synbiotic were revealed an increase of average daily gain and a reduction ratio of feed to weight gain (Cheng et al., 2017). However, some researchers found that synbiotic could not promote the growth performance and reduce diarrhoea rate in piglets (Mair et al., 2010; Daniels et al., 2021). In the present study, the *L. plantarum* L47 and inulin pretreatment did not affect body weight, average daily gain, average daily feed intake, ratio of feed to gain, and diarrhoea rate of piglets from d 1 to 21 of the experimental period, when compared with the CON group. The characteristics of synbiotics, the toxic of ETEC, animal model and the number of involved animals, even the growth environment might explain the discrepancies, which probably implies that the great efforts should be made to explore a new synbiotics formula based on our previous study and bacterial isolations, focus on improving the beneficial role of synbiotics in the gut-intestine, such as the regulation of microbiota, strength the mucosal barrier and immunity.

4.2. Intestinal integrity and immunity

Intestinal morphology is a crucial factor in the maintenance of intestinal health and integrity (Costa et al., 2019). ETEC challenges can damage intestinal integrity (Clarke, 2001) as well as cause various morphological changes in the gut (Wu et al., 2021), such as the decrease of VH and increase of CD. In this study, the increased VH and VCR in the LI47 group may result from the promotion of intestinal development supplemented by *L. plantarum* L47 and inulin. The lower CD and higher VH and VCR in the ELI47 group compared with the ECON group suggested the protective effects of *L. plantarum* L47 and inulin on ETEC-induced injury. Tight junction proteins are essential for maintaining intestinal integrity (Chelakkot et al., 2018). Decreased protein expressions of claudin-1, occludin, and ZO-1 in the ECON group indicated that ETEC challenges impaired the intestinal barrier integrity, while the administration of *L. plantarum* L47 and inulin tended to increase the protein expression of ZO-1, suggesting that *L. plantarum* L47 and inulin play a role in the prevention of ETEC injury. The antioxidant activities of intestinal mucosa are also crucial for the healthy intestinal function (Huang et al., 2022). We found that *L. plantarum* L47 and inulin elevated the GPX and T-SOD concentrations in the ileal mucosa of ETEC-challenged pigs, indicating an improved antioxidant capacity of *L. plantarum* L47 and inulin.

To explore whether impaired ileal morphology was associated with an inflammatory response, the concentration of cytokines was detected in the ileal mucosa. Proinflammatory cytokines, including TNF-α, IL-1β, and IL-6, are generally expressed at high levels in intestinal inflammatory sites (Han et al., 2021), and the severity of

to 21 of the experimental period; ECON, piglets orally given 10 mL/d PBS from d 1 to 21 of the experimental period and challenged by 10 mL ETEC containing 1×10^9 CFU/mL of *E. coli* K88 strain CVCC224 at d 22 of the experimental period; ELI47, piglets orally given 10 mL/d *L. plantarum* L47 and inulin mixture from d 1 to 21 of the experimental period and challenged by 10 mL the same ETEC culture medium as ECON group on d 22 of the experimental period. PBS = phosphate buffer saline; ETEC = enterotoxigenic *Escherichia coli*; SCFA = short chain fatty acids; SEM = standard error of the mean.

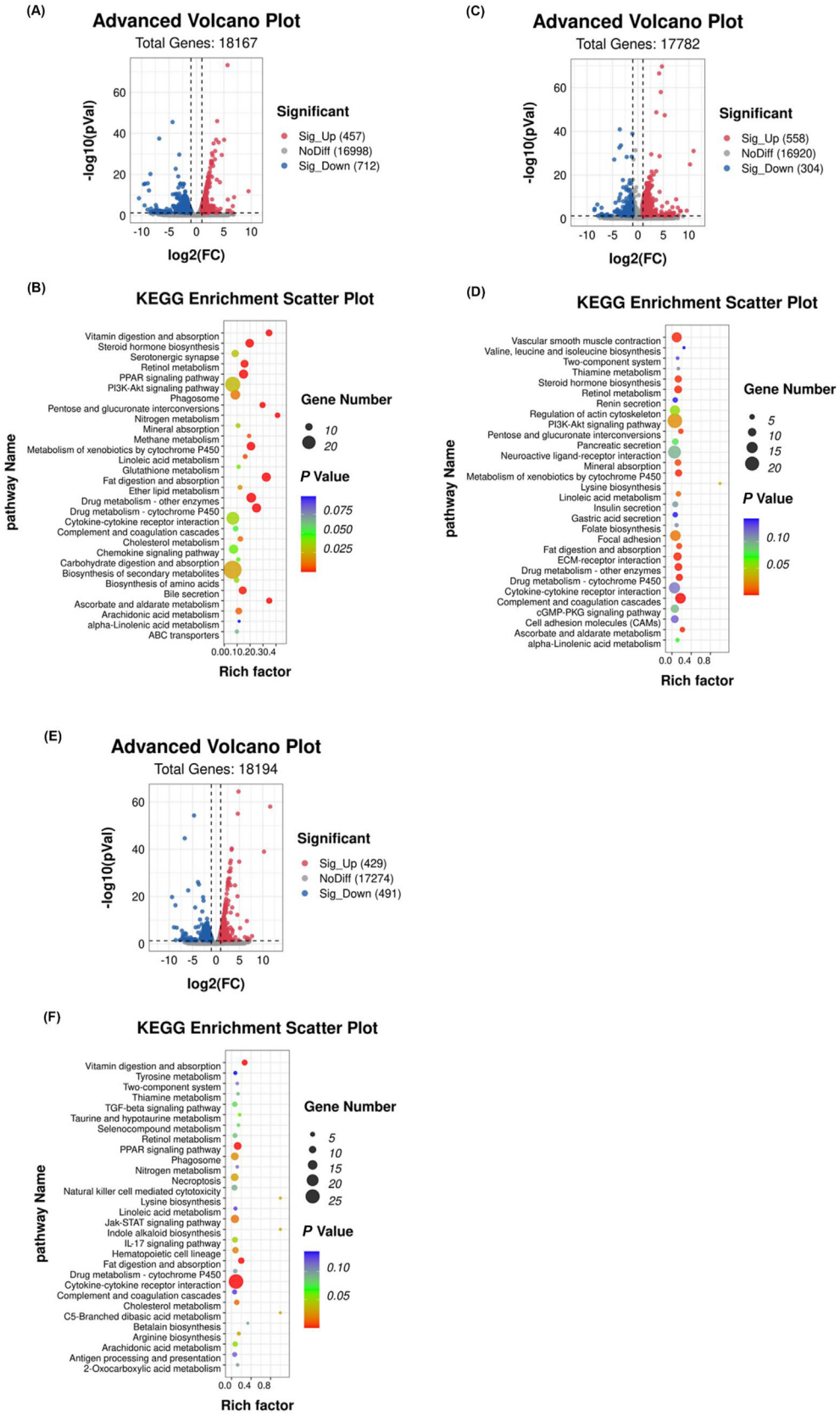

Fig. 5. Ileal mucosal transcriptome. (A) Volcano plot of DEG in ECON and CON group. (B) KEGG pathway enrichment analysis of DEG in ECON and CON groups. (C) Volcano plot of DEG in ELI47 and ECON groups. (D) KEGG pathway enrichment analysis of DEG in ELI47 and ECON groups. (E) Volcano plot of DEG in LI47 and CON groups. (F) KEGG pathway enrichment analysis of DEG in LI47 and CON groups. Up-regulation, none difference, and down-regulation are represented by red, grey, and blue dots, respectively ($n = 4$). The size and colour of the circles represent the number of enriched genes and the *P*-value. CON, piglets orally given 10 mL/d PBS from d 1 to 21 of the experimental period; LI47, piglets orally given 10 mL/d *L. plantarum* L47 and inulin mixture from d 1 to 21 of the experimental period; ECON, piglets orally given 10 mL/d PBS from d 1 to 21 of the experimental period and

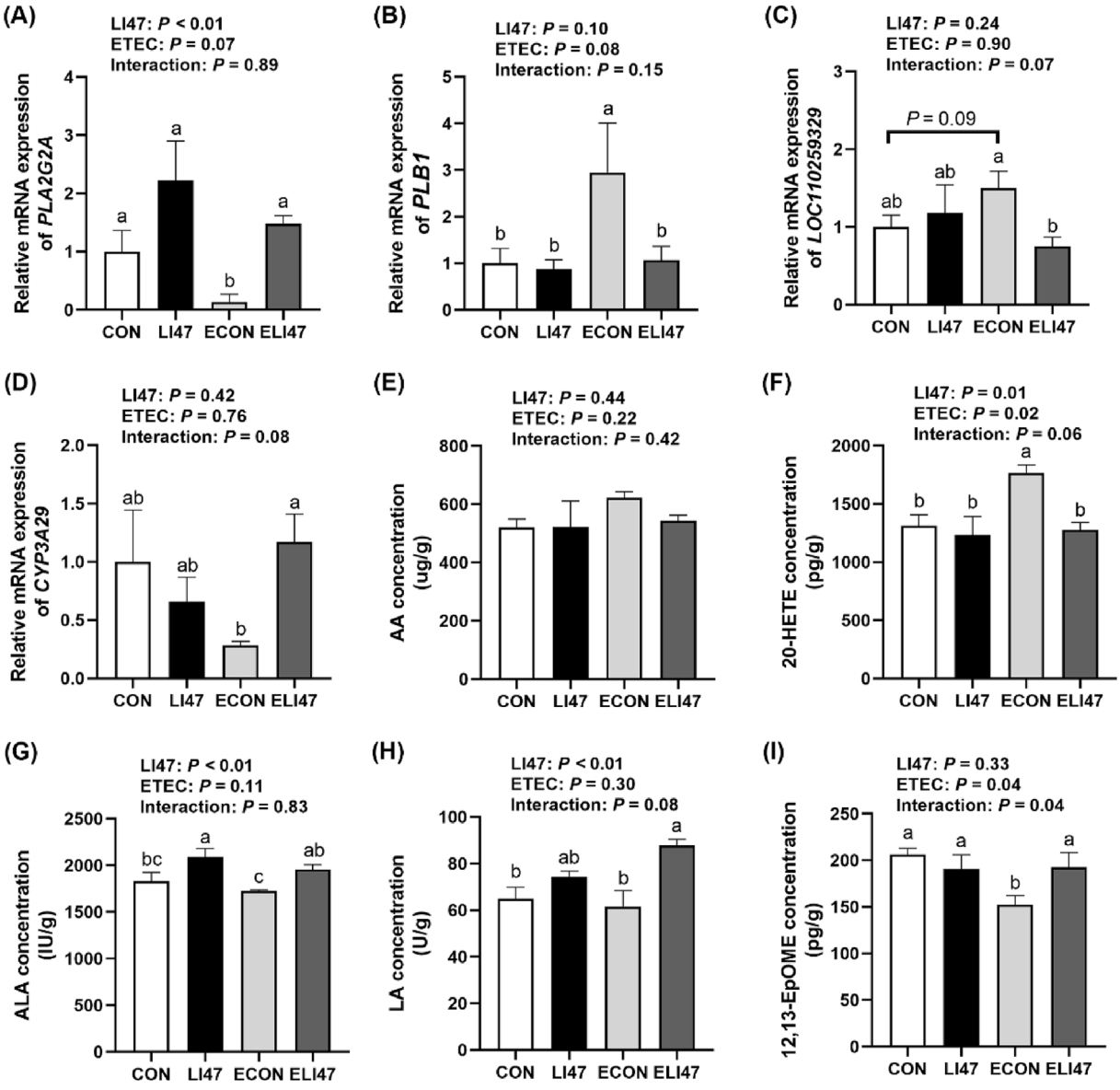

Fig. 6. Effects of *L. plantarum* L47 and inulin supplementation on gene expression and levels of PUFA in the ileal mucosa of ETEC-challenged piglets. The qRT-PCR analysis of (A) *PLA2G2A*, (B) *PLB1*, (C) *LOC110259329*, and (D) *CYP3A29*. Concentrations of (E) AA, (F) 20-HETE, (G) ALA, (H) LA, and (I) 12,13-EpOME. Data are presented as mean ± SEM ($n = 7$). Bars without a common letter (a, b) denote statistically significant differences ($P < 0.05$). CON, piglets orally given 10 mL/d PBS from d 1 to 21 of the experimental period; LI47, piglets orally given 10 mL/d *L. plantarum* L47 and inulin mixture from d 1 to 21 of the experimental period; ECON, piglets orally given 10 mL/d PBS from d 1 to 21 of the experimental period and challenged by 10 mL ETEC containing 1×10^9 CFU/mL of *E. coli* K88 strain CVCC224 at d 22 of the experimental period; ELI47, piglets orally given 10 mL/d *L. plantarum* L47 and inulin mixture from d 1 to 21 of the experimental period and challenged by 10 mL the same ETEC culture medium as ECON group on d 22 of the experimental period. PUFA = polyunsaturated fatty acids; PBS = phosphate buffer saline, ETEC = enterotoxigenic *Escherichia coli*; *LOC110259329* = docosahexaenoic acid omega-hydroxylase CYP4F3-like isoform ×1; *CYP3A29* = cytochrome P450 3A29; *PLA2G2A* = phospholipase A2 group IIA; *PLB1* = phospholipase B1; AA = arachidonic acid; 20-HETE = 20-hydroxyeicosatetraenoic acid; ALA = α-linolenic acid; LA = linoleic acid; 12,13-EpOME = 12,13-epoxyoctadecenoic acid; SEM = standard error of the mean.

intestinal inflammation is typically assessed by measuring the levels of these proinflammatory cytokines (Sanmarco et al., 2022). Our findings observed that ETEC challenges up-regulated these pro-inflammatory cytokines in the intestinal mucosa of the piglets, while *L. plantarum* L47 and inulin significantly decreased their expression levels. Moreover, the ELI47 group presented higher levels of IL-10 and TGF-β, regarded as the main anti-inflammatory cytokines (Yu et al., 2020), implying that *L. plantarum* L47 and inulin reduced inflammatory responses and therefore relieved the inflammatory damage.

4.3. Ileal microbiota and microbial metabolites

A number of studies have indicated that probiotics might ameliorate intestinal inflammation by modulating gut microbiota (Bin et al., 2018). We first hypothesised that the combination of

challenged by 10 mL ETEC containing 1×10^9 CFU/mL of *E. coli* K88 strain CVCC224 at d 22 of the experimental period; ELI47, piglets orally given 10 mL/d *L. plantarum* L47 and inulin mixture from d 1 to 21 of the experimental period and challenged by 10 mL the same ETEC culture medium as ECON group on d 22 of the experimental period. PBS = phosphate buffer saline; ETEC = enterotoxigenic *Escherichia coli*.

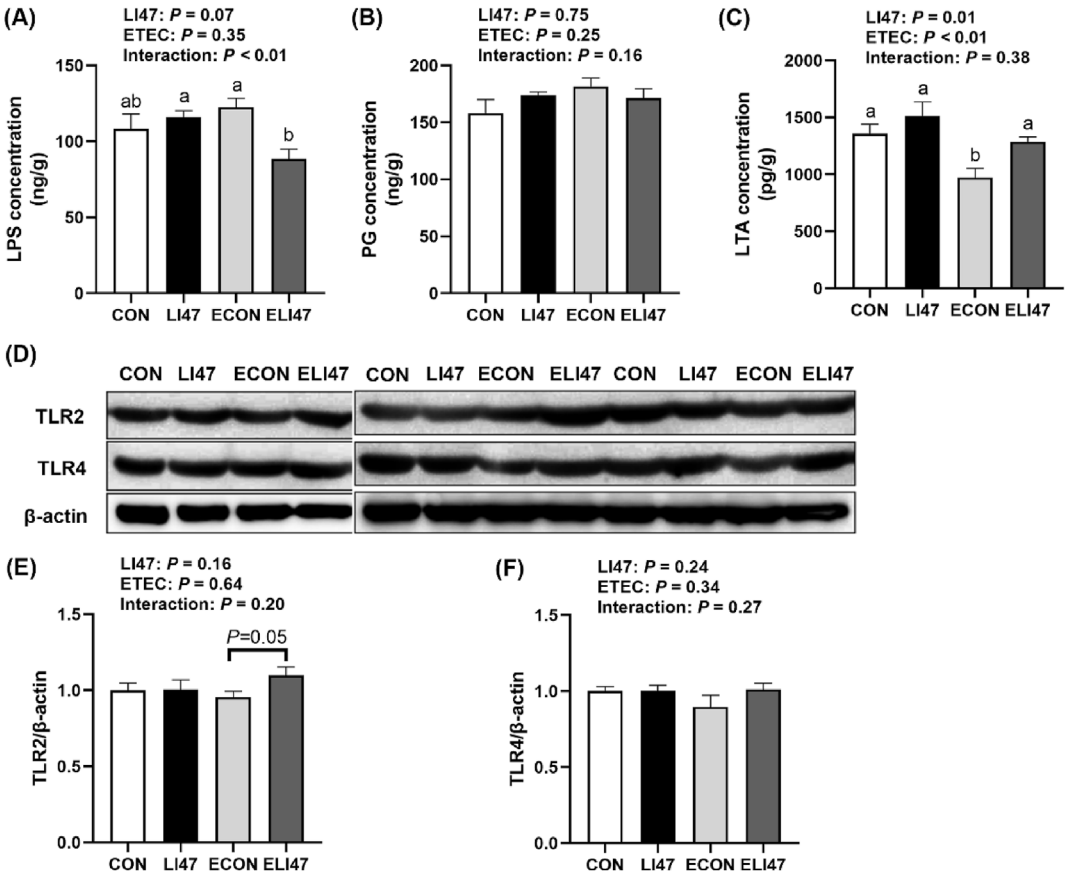

Fig. 7. Concentrations of (A) LPS, (B) PG, and (C) LTA in the ileal mucosa ($n = 7$). (D) Expression of Toll-like receptors. Protein expression of (E) TLR2 and (F) TLR4 in the ileal mucosa ($n = 3$). Data are presented as mean ± SEM. Bars without a common letter (a, b) denote statistically significant differences ($P < 0.05$). CON, piglets orally given 10 mL/d PBS from d 1 to 21 of the experimental period; LI47, piglets orally given 10 mL/d *L. plantarum* L47 and inulin mixture from d 1 to 21 of the experimental period; ECON, piglets orally given 10 mL/d PBS from d 1 to 21 of the experimental period and challenged by 10 mL ETEC containing 1×10^9 CFU/mL of *E. coli* K88 strain CVCC224 at d 22 of the experimental period; ELI47, piglets orally given 10 mL/d *L. plantarum* L47 and inulin mixture from d 1 to 21 of the experimental period and challenged by 10 mL the same ETEC culture medium as ECON group on d 22 of the experimental period. PBS = phosphate buffer saline; ETEC = enterotoxigenic *Escherichia coli*; LPS = lipopolysaccharide; LTA = lipoteichoic acid; PG = peptidoglycan; TLR = Toll-like receptor; SEM = standard error of the mean.

L. plantarum L47 and inulin could modulate gut microbiota to cope with ETEC challenges. However, the results demonstrated that no significant difference was observed in α diversity across the four groups. Although the relative abundances of some genera were found to have significantly changed, those genera accounted for only a very small portion of microbial communities. Moreover, the SCFA, as mainly functional metabolites of gut microbiota fermentation (Rauf et al., 2022), did not show significant differences across the groups except for the lower butyrate level in the ECON group compared with the CON group. It seems that in this study, *L. plantarum* L47 and inulin did not present a considerable impact on ileal microbiota and its fermentation metabolites. Thus, we have speculated that the inflammation-alleviating effects of *L. plantarum* L47 and inulin were not associated with the modulation of ileal microbiota.

4.4. PUFA metabolism

Various previous studies have suggested that microbial effectors are capable of modulating host cell metabolism (Ashaolu and Fernández-Tomé,2021). The transcriptome technology was performed to determine the effects of *L. plantarum* L47 and inulin on ileal mucosa. According to the results of the KEGG enrichment analysis, *L. plantarum* L47 and inulin relieved ileal inflammation in the ETEC-challenged pigs mainly by affecting LA metabolism and ALA metabolism, products of which have anti-inflammatory properties (Tortosa-Caparros et al., 2017). To validate the transcriptome analysis results, we performed qRT-PCRs to detect the transcription expressions of four DEG (*PLA2G2A, PLB1, LOC110259329*, and *CYP3A29*) and their metabolites (AA, LA, ALA, 20-HETE, and 12,13-EpOME). PLA2G2A hydrolyses the ester bond at the sn-2 position of glycerophospholipids (Leidy et al., 2006), releasing lysophospholipids and free fatty acids (AA, LA, ALA, etc.) (Dore et al., 2022), which may contribute to the higher levels of LA and ALA observed in the LI47 group compared with the ECON group. LA was then converted to the 12,13-EpOME under the action of CYP3A29 (Nissen et al., 1998). Numerous studies have observed that ALA and 12,13-EpOME exert anti-inflammatory properties (Singh et al., 2016; Sala-Vila et al., 2022). There were no significant differences in the concentrations of AA among the four groups in the present study, which could be explained by the up-regulation *PLB1* in the ECON group, which led to the release of free fatty acids from phospholipids (Noverr et al., 2003). AA was converted to the 20-HETE under action of *LOC110259329* (Senol et al., 2016), which had higher transcription expression in the ECON group. Furthermore, it has been demonstrated that 20-HETE promotes inflammation by activating mitogen activated protein kinases (MAPK)/extracellular signal regulated kinase (ERK) and nuclear factor-kappa B (NF-kB) signalling pathways (Ishizuka et al., 2008; Senol et al., 2016).

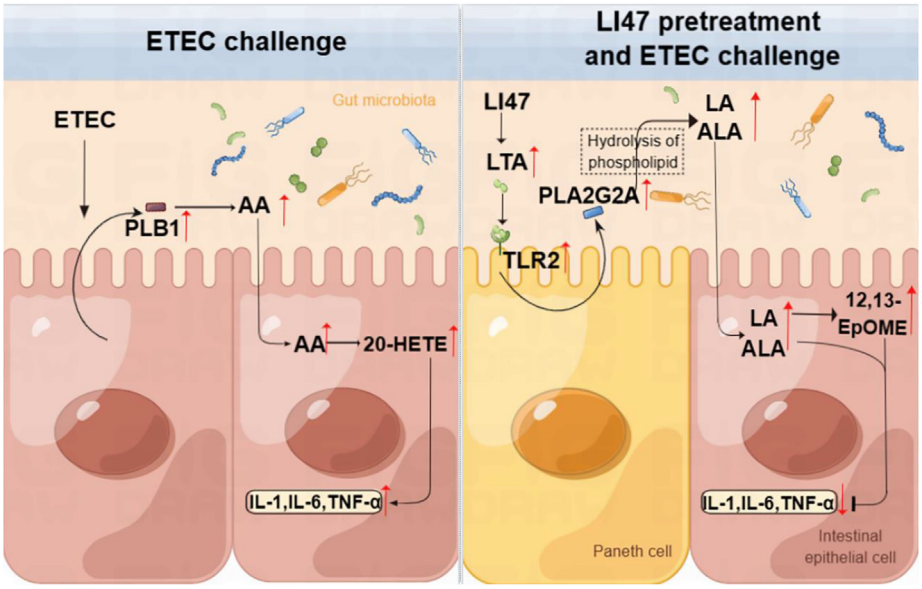

Fig. 8. Effects of *L. plantarum* L47 and inulin supplementation on the ileal enterocytes of piglets challenged by ETEC (by Figdraw). LI47 = *L. plantarum* L47 and inulin; ETEC = enterotoxigenic *Escherichia coli*; PLA2G2A = phospholipase A2 group IIA; PLB1 = phospholipase B1; AA = arachidonic acid; 20-HETE = 20-hydroxyeicosatetraenoic acid; ALA = α-linolenic acid; LA = linoleic acid; 12,13-EpOME = 12,13-epoxyoctadecenoic acid; TLR = Toll-like receptor; IL = interleukin; TNF-α = tumour necrosis factor-α.

4.5. Activation of PLA2G2A

Compared with the ECON group in the present study, there was higher transcription expression of *PLA2G2A* in the ELI47 group, which may play an important role. Studies have shown that PLA2G2A controls inflammatory reactions by enlisting inflammatory cells and boosting the production of inflammatory mediators (Dore et al., 2022), both actions taking place either through its enzymolysis or its receptor-mediated effects (Dore and Boilard, 2019). However, both direct and indirect evidence have confirmed that PLA2G2A has anti-inflammatory properties. When DSS was administered to C57BL/6 mice (whose *PLA2G2A* gene was naturally damaged) and BALB/C mice (whose *PLA2G2A* gene was intact), it was found that the BALB/C mice immediately cured the DSS-induced inflammation and significantly down-regulated the expression of inflammatory cytokines, whereas the C57BL/6 animals maintained chronic inflammation (Melgar et al., 2006). Several studies on the gastric inflammation of mice expressing a wildtype allele of *PLA2G2A* have sufficiently shown that *PLA2G2A*$^{+}$ mice possessed resistance to bacteria-induced gastric inflammation when compared to *PLA2G2A*$^{-}$ C57BL/6 mice (Huhtinen et al., 2004). Laine et al. (2000) have demonstrated that transgenic mice expressing human PLA2G2A had enhanced host resistance to experimental *E. coli* infection. In this study, it appears that PLA2G2A exerted anti-inflammatory properties to cope with ETEC-induced ileal inflammation in the piglets.

Then the new question is that which stimulator promote the production of PLA2G2A. Probiotics may improve Paneth cell function (Liu et al., 2020), causing them to secrete a variety of antibacterial compounds (Cray et al., 2021), such as antimicrobial peptides (Kamioka et al., 2022), in response to pathogens. PLA2G2A belongs to the family of antimicrobial peptides and is expressed by secretory glands such as Paneth cells in the intestine (Dore and Boilard, 2019). The microorganism-associated molecular patterns of probiotics can be identified by pattern recognition receptors such as Toll-like receptors, which activate dendritic cells to defend the gut epithelial barrier (Liu et al., 2020). Therefore, we speculated that *L. plantarum* L47 and inulin provided some substances that stimulated the production of PLA2G2A through Toll-like receptors. LTA and PG are the major components of the *L. plantarum* cell wall (Pasquina-Lemonche et al., 2020), also known as microorganism-associated molecular patterns (Akira and Takeda, 2004). In relation to our findings, the higher levels of LTA and the tendency towards high protein expression levels of TLR2 in the ELI47 group compared to those in the ECON group suggested that LTA may induce PLA2G2A through TLR2.

5. Conclusion

The current study indicated that the combination of *L. plantarum* L47 and inulin supplementation could alleviate ETEC-induced ileal inflammation in piglets. The mechanisms may be closely linked with the up-regulation of PLA2G2A, as well as the subsequent production of ALA and 12,13-EpOME, which were induced by LTA through TLR2 (Fig. 8).

Author contributions

Leihong Cui: Conceptualization, Investigation, Formal analysis, Writing - Original Draft. **Hui Zeng**: Investigation. **Meixin Hou**: Investigation. **Chunlong Mu**: Formal analysis. **Zhongxin Li**: Formal analysis. **Weiyun Zhu**: Funding acquisition. **Suqin Hang**: Conceptualization, Writing - Review & Editing, Funding acquisition.

Data availability statement

The datasets of 16S rRNA gene sequence and transcriptome sequence presented in this research have been uploaded to the online repositories of NCBI. The accession numbers of 16S rRNA gene sequence and transcriptome sequence are PRJNA913632 and PRJNA908900 respectively.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately

influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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