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RESEARCH PAPER

Pectin supplementation ameliorates intestinal epithelial barrier function damage by modulating intestinal microbiota in lipopolysaccharide-challenged piglets

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Abstract

During weaning, infants and young animals are susceptible to severe enteric infections, thus inducing intestinal microbiota dysbiosis, intestinal inflammation, and impaired intestinal barrier function. Pectin (PEC), a prebiotic polysaccharide, enhances intestinal health with the potential for a therapeutic effect on intestinal diseases. One 21-d study was conducted to investigate the protective effect of pectin against intestinal injury induced by intraperitoneal injection of *Escherichia coli* lipopolysaccharide (LPS) in a piglet model. A total of 24 piglets (6.77±0.92 kg BW; Duroc × Landrace × Large White; barrows; 21 d of age) were randomly assigned into three groups: control group, LPS-challenged group, and PEC + LPS group. Piglets were administrated with LPS or saline on d14 and d21 of the experiment. All piglets were slaughtered and intestinal samples were collected after 3 h administration on d21. Pectin supplementation ameliorated the LPS-induced inflammation response and damage to the ileal morphology. Meanwhile, pectin also improved intestinal mucin barrier function, increased the mRNA expression of *MUC2*, and improved intestinal mucus glycosylation. LPS challenge reduced the diversity of intestinal microbiota and enriched the relative abundance of *Helicobacter*. Pectin restored alpha diversity and improved the structure of the gut microbiota by enriching anti-inflammatory bacteria and short-chain fatty acids (SCFAs)-producing bacteria, and increased the concentrations of acetate. In addition, Spearman rank correlation analysis also revealed the potential relationship between intestinal microbiota and intestinal morphology, intestinal inflammation, and intestinal glycosylation in piglets. Taken together, these results indicate that pectin enhances intestinal integrity and barrier function by altering intestinal microbiota composition and their metabolites, which subsequently alleviates intestinal injury and finally improves the growth performance of piglets.

Keywords: Pectin; Piglet; Lipopolysaccharide; Intestinal barrier function; Glycosylation; Microbiota.

1. Introduction

During weaning, piglets are prone to occur immune stress and intestinal injury due to low immune function, hypoplasia of the digestive tract, and environmental stresses, which lead to postweaning diarrhea and affect the normal growth of piglets [1–3]. Previous studies indicated that the intestinal microbiota played a very important role in host health and disease throughout life, particularly in infancy [4]. The colonization of intestinal microflora in infancy is a critical period for the formation of intestinal microflora.

Abbreviations: PEC, pectin; LPS, lipopolysaccharide; MUC2, Mucin 2; TFF3, Trefoil factor 3; SCFA, short-chain fatty acid; BW, body weight; ADFI, average daily feed intake; ADG, average daily gain; F:G, feed-to-gain ratio; VH, villus height; CD, crypt depth; VH:CD, the ratio of villus height to crypt depth; H&E, hematoxylin and eosin stain; PAS-AB, periodic acid-Schiff-Alcian Blue stain; UEA-1, Ulex europaeus agglutinin 1; ConA, concanavalin A; WGA, wheat germ agglutinin; GlcNAc, N-acetylglucosamine; FITC, Fluorescein Isothiocyanate; DAPI, 4′,6-diamidino-2-phenylindole; FUT2, fucosyltransferases 2; B4GALT1, beta-1,4-galactosyltransferase 1; B3GALT5, beta-1,3-galactosyltransferase 5; B3GNT3, beta-1,3-N-acetylglucosaminyltransferase 3; C1GALT1, core 1 synthase, glycoprotein-N-acetylgalactosamine 3-beta-galactosyltransferase 1; C1GALT1C1, C1GALT1 specific chaperone 1; GCNT3, glucosaminyl (N-acetyl) transferase 3; GCNT1, glucosaminyl (N-acetyl) transferase 1; B3GNT6, beta-1,3-N-acetylglucosaminyltransferase 6; MCP1, monocyte chemotactic protein 1; IL-, interleukin-; TNF-α, tumor necrosis factor-α; TLR4, Toll-like receptor 4.

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During this phase, the intestinal microbiota can not only promote intestinal health, maintain normal intestinal barrier functions, and escape from pathogenic bacteria, but also improve the intestinal and systemic immune system development and maturation [5–8]. It has been confirmed that intestinal dysbiosis has been implicated in the pathogenesis of certain inflammatory diseases and infections [9]. Furthermore, the intestinal microbiota influences the host nutritional metabolism and immune response as well as intestinal homeostasis, and the microbiota makeup is, in turn, regulated by the host, the environment, and inter-microbial interactions [10–12]. From this, intestinal microbiota modulation has become a new strategy to enhance intestinal health, which has been verified in some studies [13–15].

Pectin (PEC) is a complex polysaccharide, the main ingredient is an α-(1,4)-linked d-galacturonic acid (d-galUA) that is account for over 70% [16]. Pectin can modulate the intestinal microbiota and their metabolic products, thereby improving intestinal homeostasis and intestinal health [17,18]. Meanwhile, pectin can ameliorate intestinal inflammation by reducing pro-inflammatory cytokines and enhancing the mRNA expression of *MUC2* [19–21]. Our previous study also indicated that pectin exerts beneficial biological functions by regulating the intestinal microbial community [22]. At present, there are few studies about adding pectin to the diet of weaned piglets. Therefore, pectin supplementation may be an effective strategy to regulate the development of early intestinal microflora and thus maintain the long-term health of the host.

Given the interaction between intestinal health and intestinal microbiota balance, we hypothesized that pectin would restore intestinal injury by altering intestinal microbiota in weaning piglets. Therefore, we used a lipopolysaccharide (LPS) challenge to trigger intestinal inflammation and damage that post-weaning piglets generally suffered [23], to evaluate whether pectin could ameliorate LPS-induced gut microbial dysbiosis, inflammatory response, and intestinal barrier dysfunction.

2. Materials and methods

2.1. Experimental design, animals, and diets

All animal experimental procedures were performed by the Guidelines for Care and Use of Laboratory Animals of Chinese Academy of Agriculture Sciences and experiments were approved by the Animal Ethics Committee of Experimental Animal Welfare and Ethicals of Institute of Animal Science, Chinese Academy of Agriculture Sciences (IAS2019-37). Twenty-four piglets (6.77±0.92 kg BW; Duroc × Landrace × Large White; barrows; 21 d of age) with good health and similar physical condition were randomly divided into three groups: control group (CON) and LPS-challenged group (LPS) were fed with the control diet, and PEC+LPS group (PECL) was fed with the pectin diet. The adaption period was 3 d, and the experimental period was 21 d. On d14 and d21 of the entire experiment, piglets in the LPS group and PECL group were injected intraperitoneally with LPS (80 μg per kg BW, *E. coli* 055: B5, Sigma), and the piglets in the CON group were injected with the same amount of normal saline intraperitoneally. During the whole trial period, all pigs were housed in separated pens with a plastic slatted floor and the pens were cleaned daily to avoid disease occurrence. Meanwhile, all piglets were given *ad libitum* access to clean drinking water and corresponding diets (Table S1). The control diet and pectin diet are corn-soybean meal diets containing 5% cellulose or pectin, respectively. The diets had been formulated to meet the nutritional requirements suggested by NRC (2012) for pigs, among which cellulose was used to formulate equal fiber diets as previously described [17]. The amount of supplemental pectin used in this experiment was based on our reported results [22,24]. Cellulose was purchased from the Beijing Engineering Research Center of Cellulose and Its Derivatives (Beijing, China). Pectin was purchased from the Yuzhong Biotech Corporation (Zhengzhou, China). The injection method and dosage of the LPS treated model were chosen based on the results of our preliminary trials. The experimental scheme is shown in Fig. 1A.

2.2. Sample collection

After 3 h injection of LPS or normal saline on d21, all piglets were anesthetized using an intravenous injection of pentobarbital sodium (25 mg/kg of BW) and killed by exsanguination. The sections of the ileum were in situ ligated before the whole gut was removed from the abdominal cavity. An ileal segment of approximately 3 cm was preserved in 4% paraformaldehyde for routine morphological measurement. Ileal mucosa or digest was collected in 2-mL sterile tubes, immediately frozen in liquid nitrogen, and stored at -80°C for sequencing of microbial 16S genes and specific gene expression or analysis for short-chain fatty acids (SCFAs) quantification, respectively. Previous studies proved that LPS causes serious damage to the intestinal structure and barrier function within 2–6 h after injection, so the time of sample collection was carried out at 3 h after LPS or normal saline injection [25,26].

2.3. Growth performance

During the whole experiment, the daily feed intake of each replicate was accurately recorded, and the health status of piglets was observed. The piglets were weighted on the morning of the d1, d14, and d21 of the experiment, respectively. The average daily feed intake (ADFI), average daily gain (ADG), and feed-to-gain ratio (F:G) for each stage and over the whole period of the experiment was calculated.

2.4. Intestinal morphology analysis

After 24 h fixation, the ileum segment was dehydrated with gradient alcohol, made transparent with xylene, and embedded in paraffin. To determine the effects of pectin supplementation on the LPS challenge-induced damage to intestinal integrity, we evaluated the ileal morphology using hematoxylin and eosin (H&E staining kit, Solarbio, Beijing, China) staining. Five cross-sections (5 μm thick) of each ileum specimen were taken to prepare histological sections and stained, then observed on a Leica DM2000 light microscope (Leica Microsystems, Wetzlar, Germany). The images were analyzed with ImageJ v1.8.0 software. Fifteen well-oriented and intact villi and their associated crypts were taken from each segment. Villus height (VH), crypt depth (CD), and the ratio of villus height to crypt depth (VH:CD) were measured. Goblet cells were examined by periodic acid-Schiff-Alcian Blue stain (PAS-AB staining kits, Solarbio, Beijing, China). Briefly, deparaffinized slides were exposed to 3% acetic acid, followed by staining with Alcian Blue, 1% periodic acid-Shiff reagent, and sodium metabisulfite. Finally observed and analyzed on a Leica DM2000 light microscope (Leica Microsystems, Wetzlar, Germany).

2.5. Intestinal glycosylation analysis

The expression of intestinal glycosylation oligosaccharides was examined using FITC-conjugated lectins: Ulex europaeus agglutinin 1 (UEA-1; fucose), concanavalin A (ConA; mannose), and wheat germ agglutinin [WGA; N-acteylglucosamine (GlcNAc); Vector Laboratories, Burlingame, CA] were used as previously described [27]. Briefly, sections were deparaffinized, blocked with Carbohydrate blocking solution (Vector Laboratories, Burlingame, CA, USA), and stained with FITC-labeled lectin (5 μg/mL, added 0.05% Tween 20) for 1 h at room temperature. Sections were then washed three times in PBS (added 0.05% Tween 20), counterstained with DAPI Mounting medium (Vector Laboratories, Burlingame, CA), and analyzed by confocal laser scanning microscopy (Leica LSM Confocal TCS SP8; Leica, Germany). Eight animal samples from each treatment group were analyzed. Positively stained cells were counted. A minimum of 1,000 cells were counted for each sample of each experiment. The data were then normalized to the control.

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

Total RNA was isolated from the ileal mucosa using the RNeasy Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Took 1 μg of total RNA and quantified it with an Ultramicro Protein Nucleic acid analyzer (BioDrop-μLite, UK) to ensure that the detection value of the RNA sample was A260/280=1.8∼2.0. cDNA was transcribed using a PrimeScript RT reagent kit with gDNA Eraser (Takara, Kusatsu, Shiga, Japan). qRT-PCR was performed according to the TB Premix Ex Taq instructions (Takara, Kusatsu, Shiga, Japan), and conducted on ABI Q7 Flex Real-time PCR System (ABI, Singapore). The amplification procedure was 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 60°C for 30 s. β-*actin* and *GAPDH* were used as the internal reference for relative expression of each gene in ileal mucosa, and gene expression was calculated by $2^{-\triangle\triangle CT}$ method [28]. All the above operations are completed on the clean bench. All primer sequences were provided in Table S2.

2.7. Western blotting analysis

The total protein of ileum tissue was extracted using RIPA (Thermo Fisher Scientific Inc., MA, USA) and preserved at -80°C for subsequent analysis. Then the concentration of total protein was quantified using BCA assays (Thermo Fisher Scientific Inc., MA, USA), separated them using SDS-PAGE, and then transferred to membranes for western blotting. To determine the effects of pectin supplementation on the LPS challenge-induced damage to intestinal barrier integrity, we evaluated the protein expression of tight junction protein using western blotting. Occludin (Thermo Fisher Scientific Inc., MA, USA, #40-4700, 1:500), Claudin-1 (Thermo Fisher Scientific Inc., MA, USA, #51-9000, 1:500), and β-actin antibody (Proteintech, Chicago, USA,

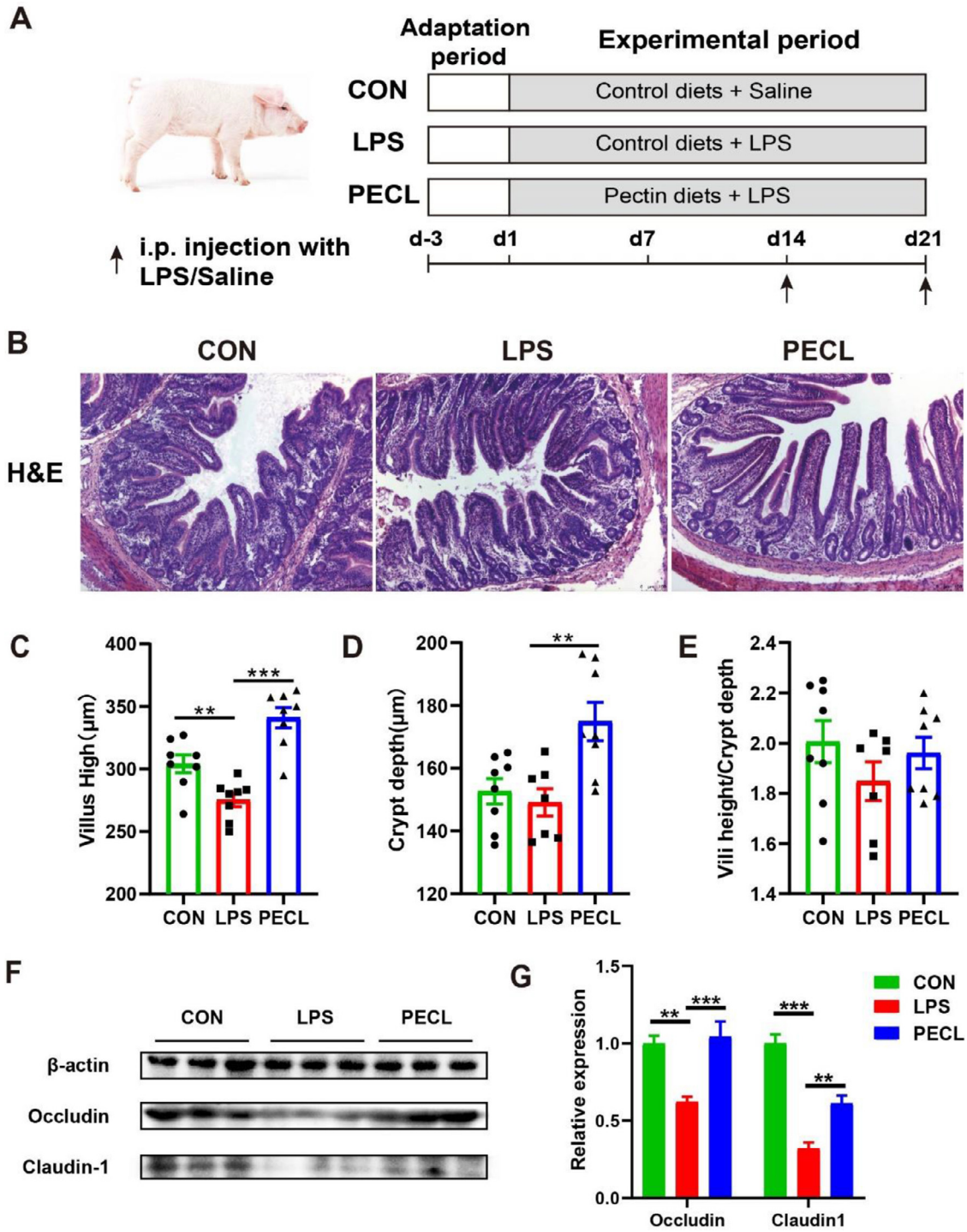

Fig. 1. Changes in intestinal barrier function of piglets in different groups. (A) The schematic of the experimental timeline. (B) Representative H&E -stained ileum sections (C) Villus height. (D) Crypt depth. (E) Villus height/crypt depth. Scale bar 100 μm; (F and G) Western blot analysis of tight junction proteins Occluding and Claudin-1. Signification is presented as * $P<.05$, ** $P<.01$, and *** $P<.001$; data are presented as the mean ± SE ($n=8$).

#20536-1-AP, 1:1,000) were used as primary antibodies and incubated with membranes overnight at 4°C. The membranes were then incubated with the HRP-labeled goat anti-rabbit secondary antibodies (Abcam, Cambridge, UK, #ab6721, 1:5000). The experiments were repeated three times and the band density of the target protein was quantified after normalization to β-actin using Image lab software.

2.8. The 16S rRNA sequencing and analysis of ileal microflora

DNA extraction, PCR amplification, DNA quantification, and Illumina MiSeq sequencing by the standardized protocol of Shanghai Majorbio Bio-pharm Technology (Shanghai, China). Briefly, DNA was extracted from the mucosa using the Qiagen DNA isolation kit (Qiagen, Hilden, Germany) and followed by the provided protocol. The V3-V5 hypervariable region of the 16S bacterial rRNA was amplified using primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The amplicons were sequenced on the Illumina HiSeq sequencing platform, as described before [22]. Raw data obtained from gut microbiota were analyzed on the free online platform of Majorbio Cloud Platform (www.majorbio.com) to understand the gut microbial diversity and composition. To gain insights into how the function alteration responded to the structural shift of intestinal microbiota, we investigated the intestinal microbial function using the PICRUST2 method.

Table 1
Growth performance of piglets in different groups*

Parameter and period (days)	Piglet group			*P-value*
	CON	LPS	PECL	
Body Weight				
Initial BW	6.73±0.38	6.93±0.96	6.60±0.69	.76
d14 BW	10.06±0.41	10.42±0.32	10.38±0.41	.77
d21 BW	11.57±0.45	11.24±0.33	12.07±0.35	.32
D1–14				
ADFI (g)	378.88±11.98	402.75±15.51	390.13±42.76	.83
ADG (g)	238.12±6.66	248.93±8.25	269.64±26.36	0.40
F:G	1.60 ± 0.06^{a}	1.62 ± 0.04^{a}	1.43 ± 0.03^{b}	<.05
D15–21				
ADFI (g)	350.11 ± 17.86^{B}	199.02 ± 16.06^{C}	482.79 ± 43.40^{A}	<.01
ADG (g)	215.71 ± 16.47^{A}	116.43 ± 9.26^{B}	241.07 ± 17.05^{A}	<.01
F:G	1.64 ± 0.07^{b}	1.73 ± 0.09^{ab}	2.01 ± 0.13^{a}	<.05

In the same row, values with the same or no letter superscripts mean no significant difference ($P>.05$), while with different small letter superscripts mean significant difference ($P<.05$), and with different capital letters superscripts mean significant difference ($P<.01$).
* Values are means ± SE.

2.9. Short-chain fatty acids (SCFAs) analysis

Quantification of SCFAs was analyzed using gas chromatography (GC) based on our previous studies [29]. Briefly, the digesta were dissolved in and extracted with distilled water. The extracted samples were obtained by centrifuge at 9,000 g. Metaphosphoric acid (25%, w/v) was added to the extracts at a ratio of 1:9. After centrifugation at 10,000 g, the supernatant was subjected to SCFAs analysis with Agilent 6890N GC (Palo Alto, CA, USA).

2.10. Statistical analysis

Each animal is the experimental unit. One-way ANOVA of the data on growth performance (BW, ADG, ADFI, and F:G), intestinal morphology and glycosylation, SCFAs, qRT-PCR (cytokines, *MUC2, TFF3*, and glycosyltransferase), WB (Claudin-1 and Occludin) and bacterial alpha diversity indices (Sobs, Shannon, Simpson, ACE, Chao, and Coverage) was performed using the JMP software (JMP 10.0.0, SAS Institute, Cary, NC, USA) for Windows. Statistical differences among the treatments were separated by the "least-squares means Student t" method and significant differences among various groups are represented as $^{*}P<.05$, $^{**}P<.01$, and $^{***}P<.001$. $P<.05$ was regarded as statistically significant, while $.05<P<.1$ was set as a significant trend. In addition, the R package "ggalluvial" was used to profile microbial communities and Spearman's correlation analysis was applied using the R package "pheatmap".

3. Results

3.1. Growth performance

Pectin supplementation significantly decreased the feed-to-gain ratio (F:G) of piglets from d1–14 ($P<.05$; Table 1) . From d15–21, the LPS challenge, compared with that in the CON group, significantly decreased the average daily feed intake (ADFI) and average daily gain (ADG) of piglets and increased the F:G by 5.49% ($P<.05$). Pectin supplementation significantly increased ADFI and ADG of piglets compared with the LPS group ($P<.05$), and F:G tended to be increased ($P=.05$).

3.2. Intestinal barrier function

LPS challenge caused fever, diarrhea, anorexia, shivering, and inactivity within 1 h in all piglets. LPS challenge damaged the ileal morphology (Fig. 1B) and decreased the VH (Fig. 1C), compared with that in the CON group ($P<.05$). Pectin supplementation significantly increased the VH (Fig. 1C), compared with the LPS group ($P<.05$). Pectin supplementation significantly increased the CD ($P<.05$), but the VH: CD showed no significant difference (Fig. 1C–E). Moreover, our results showed that the LPS challenge reduced the protein expression of Claudin-1 and Occludin ($P<.05$). The protein expression of Occludin and Claudin-1 in the PECL group was significantly elevated compared with that in the LPS group (Fig. 1F and G).

3.3. Intestinal glycosylation

LPS challenge increased the number of PAS-positive goblet cells in the ileal villus, compared with the CON group ($P<.05$). Pectin supplementation significantly restored the number of PAS-positive goblet cells in the ileal villus ($P<.05$; Fig. 2B). Additionally, Pectin supplementation significantly increased the number of PAS/AB-positive goblet cells in the ileal crypt ($P<.05$; Fig. 2C). The mRNA expression of *MUC2* and *TFF3* was decreased after the administration of LPS compared with the CON group, while pectin supplementation significantly restored the level of *MUC2* ($P<.05$; Fig. 2D and E). Gut mucus oligosaccharides N-acetyl-galactosamine (GlcNAc), galactose (Gal), GalNAc, fucose (Fuc), N-acetylneuraminic acid (NeuNAc), and mannose (Man) [30,31] are attached to mucin glycoproteins and can be used by bacteria to maintain their relative niches. The result showed that there are some differences in the expression of these oligosaccharides in each group (Fig. 3A). The LPS group exhibited increased levels of GlcNAc and decreased levels of Fuc and Gal, compared with the CON group ($P<.05$). Pectin supplementation significantly restored the level of GlcNAc, Fuc, and Gal ($P<.05$). No changes in intensity were observed in mannose levels between the groups and we do not detect GalNAc expression in the ileum (Fig. 3B). Moreover, LPS challenge significantly decreased the mRNA expression of terminal glycosylation transferase including fucosyltransferases2 (*FUT2*) and galactosyltransferase (*B4GALT1* and *B3GALT5*) but increased the mRNA expression of GlcNAc transferase (*B3GNT3*) in the ileum of piglets compared with that in the CON group ($P<.05$), and pectin supplementation restored those shifts. Furthermore, LPS challenge significantly decreased the mRNA expression of the *C1GALT1C1* and *GCNT3* and increased the mRNA expression of *GCNT1* and *B3GNT6* compared with the CON group ($P<.05$). Pectin supplementation decreased the mRNA expression of *C1GALT1* and *GCNT1*, but further decreased the mRNA expression of *GCNT3* and increased the mRNA expression of *B3GNT6* ($P<.05$; Fig. 3D and E). In all, the LPS challenge changed the proportion of core1 and core2 glycan, and pectin supplementation restored these changes to some extent and promoted the shifts of core1, 2, and 4 glycans to core3 glycans in the mass.

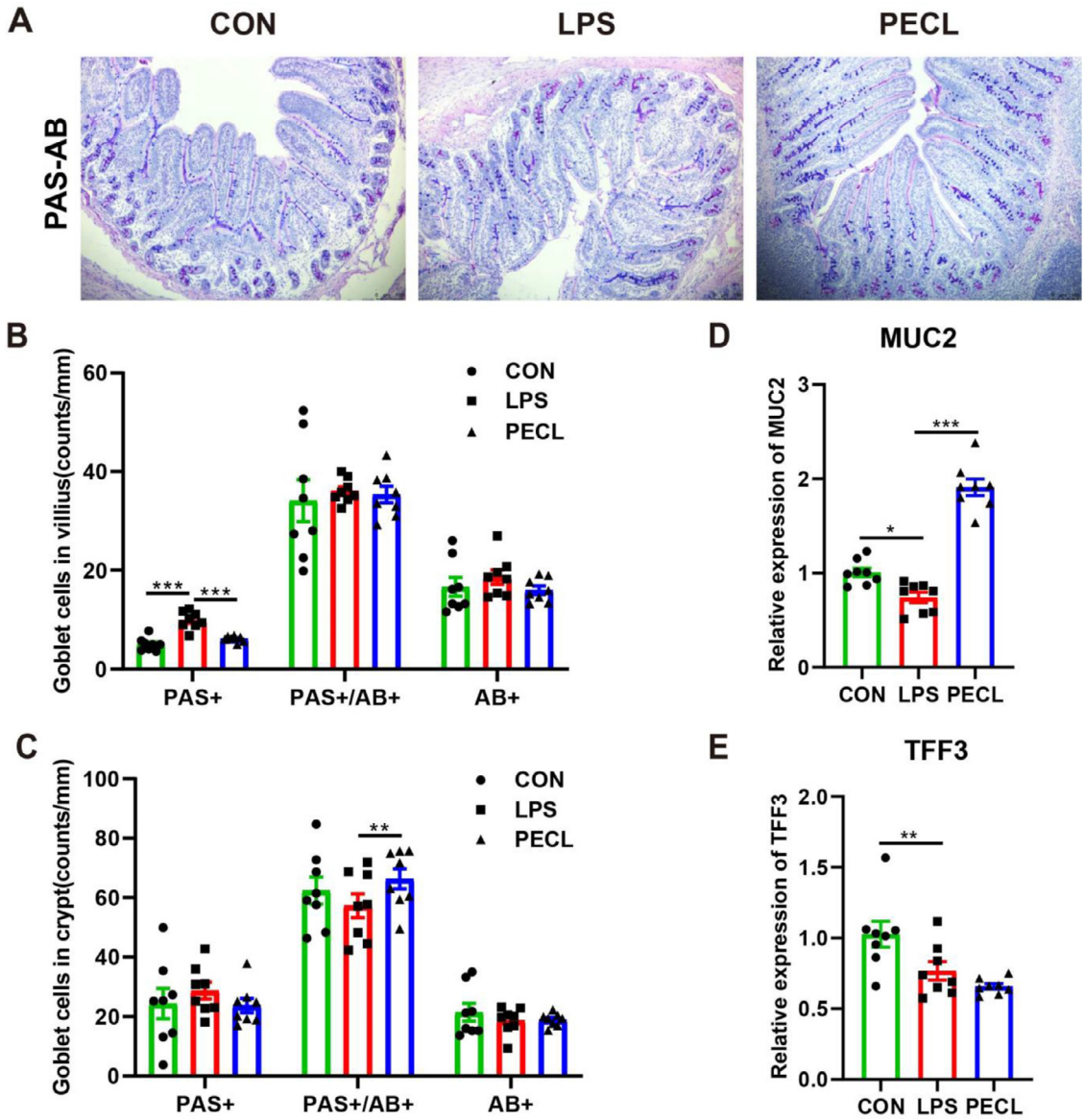

Fig. 2. Changes in intestinal mucus barrier function of piglets in different groups. (A) Representative PAS-AB -stained ileum sections. The positive goblet cell in (B) ileum villus and (C) crypt (counts/mm). Scale bar 100μm; The fold change in mRNA expression relative to *GAPDH* and *β-actin* for their marker (D) *MUC2* and (E) *TFF3* is shown. Signification is presented as * $P<.05$, ** $P<.01$, and *** $P<.001$; data are presented as the mean ± SE ($n=8$).

3.4. Intestinal inflammation

LPS challenge significantly increased the mRNA expression of *TLR4, MCP1*, and pro-inflammatory cytokines *IL-1β* and *TNF-α*, but decreased the mRNA expression of anti-inflammatory cytokine *IL-10* in the ileum of piglets compared with that in the CON group ($P<.05$; Fig. 4). Pectin supplementation significantly restored the level of *TLR4, MCP1,* and *IL-1β*, decreased the mRNA expression of *TNF-α* ($P<.05$), and the mRNA expression of *IL-10* tended to increase ($P=.06$).

3.5. Structure and diversity of intestinal microflora

Among the groups, the PECL group shared more OTU with the CON group than the LPS group (Fig. 5A). This indicates that the structure of intestinal microbiota in the PECL group was more similar to that of the CON group than that of the LPS group (Fig. 5A). This was further supported by the beta diversity presented by PCoA which illustrated that the LPS group formed a distinct cluster markedly away from that of the CON group and the PECL group (Fig. 5B). LPS challenge reduced the Simpson, ACE, and Chao, but increased the Coverage compared with the CON group ($P<.05$). Pectin supplementation restored those shifts but did not reach statistical significance (Fig. 5C–H).

3.6. Composition and difference of intestinal microbiota

The relative abundance of microbiota was analyzed at the phylum level and genus level (Fig. 5I and K). The linked bar plots illustrated that LPS challenge markedly shifted the relative abundance of bacteria at different taxon levels, which were restored by pectin supplementation. In detail, at the phylum level, the ileum microbiota mainly consisted of *Firmicutes, Bacteroidetes, Actinobacteria*, and *Proteobacteria*. After the LPS challenge, compared with the CON group, the relative abundance of *Bacteroidetes* and *Actinobacteria* was significantly decreased ($P<.05$). Pectin supplementation signif-

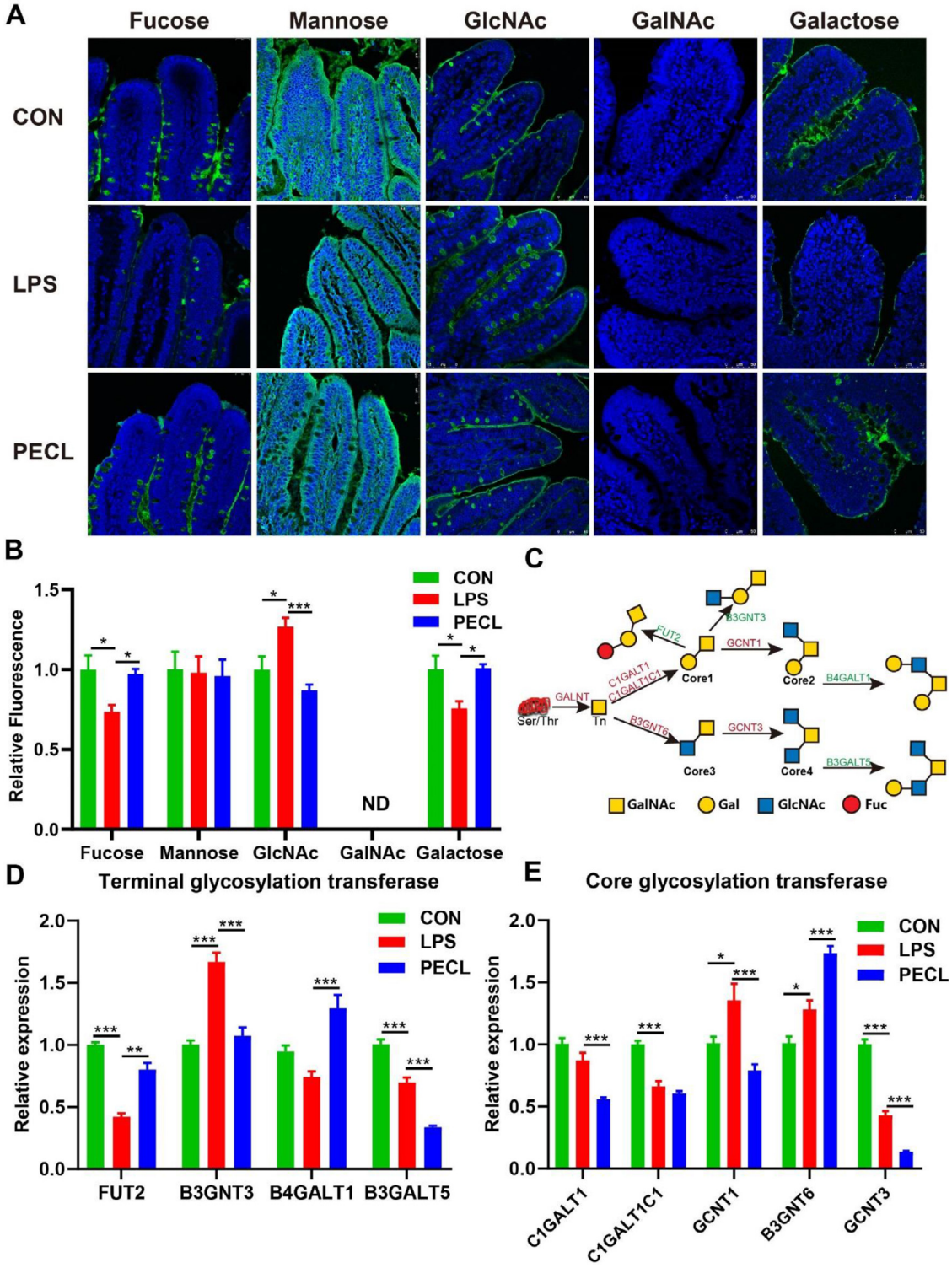

Fig. 3. The main mucin-type O-glycosylation pathways in the piglet intestine. (A) Confocal images of the ileum section stained with a panel of lectins to identify mucus oligosaccharides (green) fucose, mannose, N-acetylglucosamine, N-acetylgalactosamine, and galactose counterstained with DAPI (blue). Scale bar = 50 μM. (B) Relative fluorescence of lectin stain where fluorescence of oligosaccharide was calculated relative to CON. (C) The mucin-type O-glycosylation biosynthesis process. (D) The fold change in mRNA expression relative to *GAPDH* and *β-actin* for the intestinal (D) terminal and (E) core glycosyltransferase is shown. Only differentially abundant taxa at the genus or higher taxonomic ranks were indicated. Signification is presented as * $P<.05$, ** $P<.01$, and *** $P<.001$; data are presented as the mean ± SE ($n=8$).

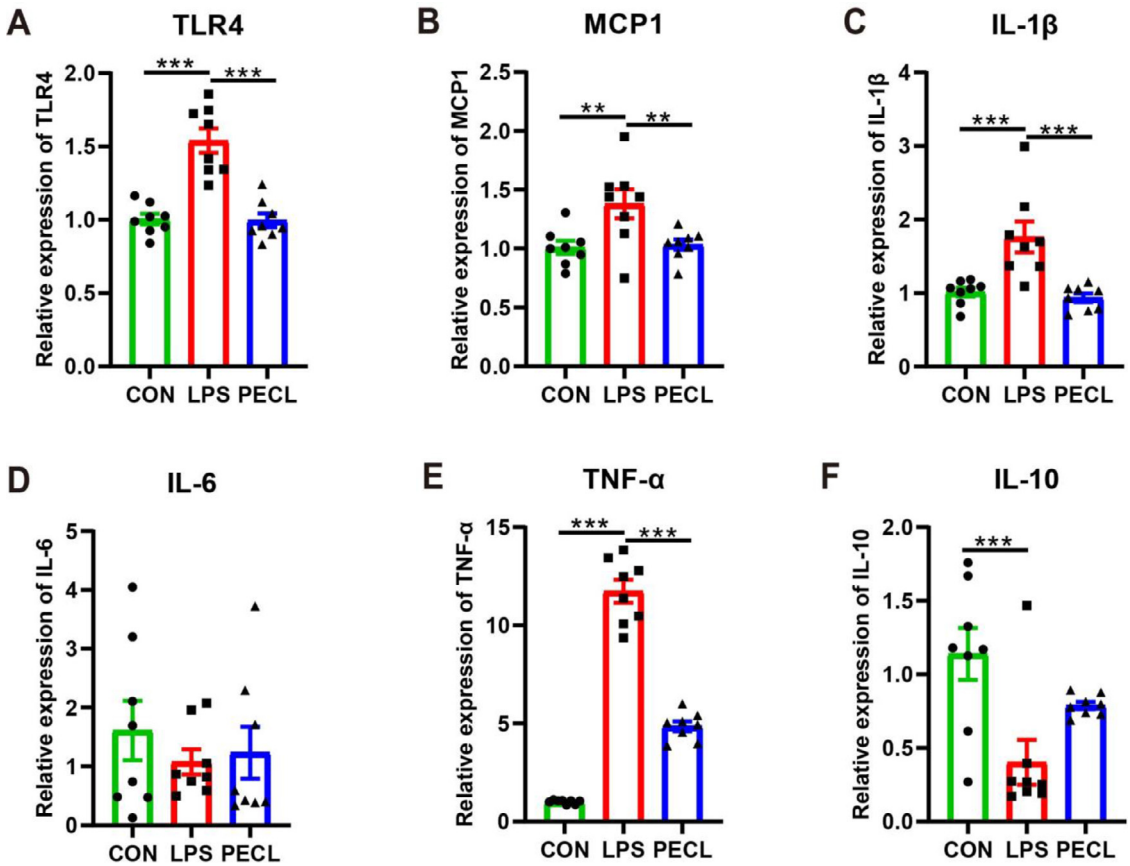

Fig. 4. The mRNA expression levels of intestinal inflammatory cytokines of piglets in different groups. The fold change in mRNA expression relative to *GAPDH* and *β-actin* for (A) *TLR4*, (B) *MCP1*, (C) *IL-1β*, (D) *IL-6*, (E) *TNF-α*, (F) *IL-10* is shown. Signification is presented as * $P<.05$, ** $P<.01$, and *** $P<.001$; data are presented as the mean ± SE ($n=8$).

icantly increased the relative abundance of *Bacteroidetes* and *Actinobacteria* ($P<.05$; Fig. 5I and J). At the genus level, the relative abundance of *Olsenella, Bacteroides, Proteus, Prevotella_9,* and *Eubacterium* were lower in ileum mucosa due to LPS challenge, whereas *Helicobacter* was more abundant, compared with the CON group ($P<.05$). The relative abundance of *Olsenella, Bacteroides, Proteus, Prevotella_9*, and *Eubacterium* was higher in the PECL group. Besides, pectin supplementation significantly decreased the relative abundance of *Helicobacter* compared with the LPS group ($P<.05$; Fig. 5K and L).

3.7. Function and metabolism of intestinal microbiota

The predicted results can be enriched at three different levels of the KEGG pathways (Fig. 6A). LPS challenge significantly decreased the function of metabolic pathways, microbial metabolism in diverse environments, carbon metabolism, starch and sucrose metabolism, glycolysis/gluconeogenesis, phosphotransferase system (PTS), fructose and mannose metabolism, and galactose metabolism ($P<.05$). Pectin supplementation restored the function shifts of intestinal microbiota induced by the LPS challenge. Moreover, we also found that most feature predictions of the altered predicted bio function are related to sugar metabolisms, such as carbon metabolism, starch, and sucrose metabolism, glycolysis/gluconeogenesis, fructose, and mannose metabolism, and galactose metabolism. Among these shifted functions of the gut microbiota, the carbohydrate metabolism function is responsible for the gut microbial fermentation of carbohydrates under a strictly anaerobic environment to produce SCFAs which benefit the host [32]. Accordingly, pectin supplementation significantly increased the concentration of acetate compared with that in the LPS group ($P<.05$; Fig. 6B–H).

3.8. The correlation between the differential bacteria and the examined indices

Spearman correlations were used to further reveal the potential relationships between intestinal microbiota and ileal parameters including morphology, inflammatory cytokines, mucus makers, tight junction proteins, glycosylation, and microbial metabolite SCFAs (Fig. 7A). For ileal morphology, *Bacteroidetes, Actinobacteria, Olsenella, Prevotella_9, Acinetobacter*, and *Bifidobacterium* were positively but *Lactobacillus* was negatively correlated with VH ($P<.05$); *Actinobacteria, Olsenella* showed a significant negative correlation with CD ($P<.05$). For inflammatory cytokines, *Helicobacter* was positively correlated with *TLR4* ($P<.05$); *Lactobacillus* was positively but *Olsenella* and *Enterococcus* were negatively correlated with *IL-1β* ($P<.05$); *Eubacterium* was positively correlated with *IL-6* and *IL-10* ($P<.05$); *Proteobacteria* and *Helicobacter* were positively but *Enterococcus* and *Eubacterium* were negatively correlated with *TNF-α* ($P<.05$); For intestinal mucus, *Actinobacteria, Bacteroidetes, Olsenella, Prevotella_9*, and *Corynebacterium_1* were positively but *Lactobacillus* and *Helicobacter* were negatively correlated with *MUC2* ($P<.05$). For tight junction proteins, *Actinobacteria, Proteus*, and *Olsenella* were positively but *Lactobacillus* and *Helicobacter* were negatively correlated with Occludin ($P<.05$); *Eubacterium* was positively but *Proteobacteria* and *Helicobacter* were negatively correlated with Claudin-1 ($P<.05$).

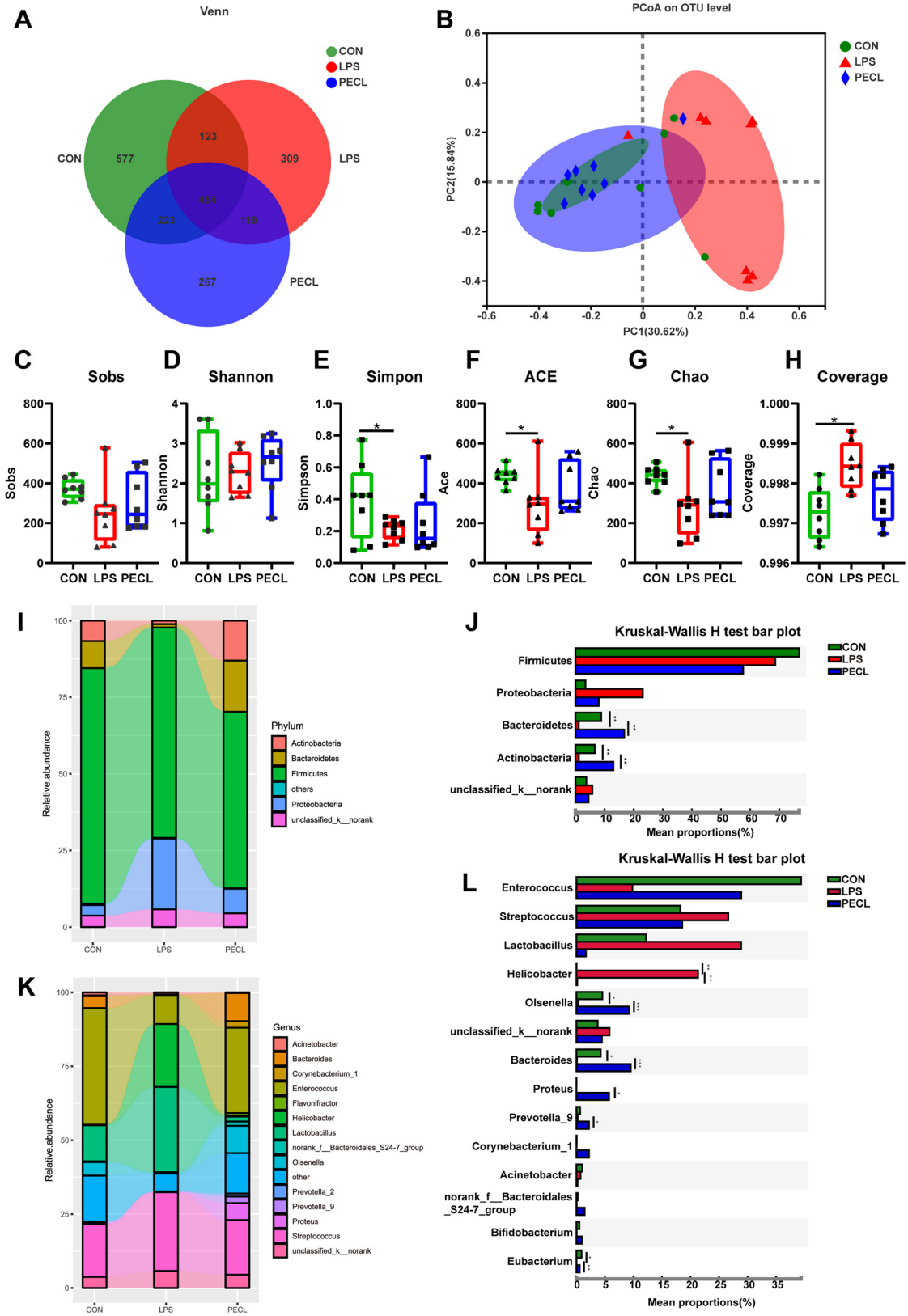

Fig. 5. Structure and diversity of intestinal microflora of piglets in different groups. The common/unique OTUs number among the groups. (B) The structure shifts (beta diversity) presented by the PCoA plot based on the OTU level. (C–H) The alpha diversity indices observed species, Chao, ACE, Shannon, Simpson, and Coverage. Relative abundance of the intestinal microbiota composition in the ileum in weaned pigs at the (I) phylum level and (K) genus level. Changes in the intestinal microbiota of piglets in different groups at the (J) phylum level and (L) genus level. Signification is presented as * $P<.05$, ** $P<.01$, and *** $P<.001$; data are presented as the mean ± SE ($n=8$).

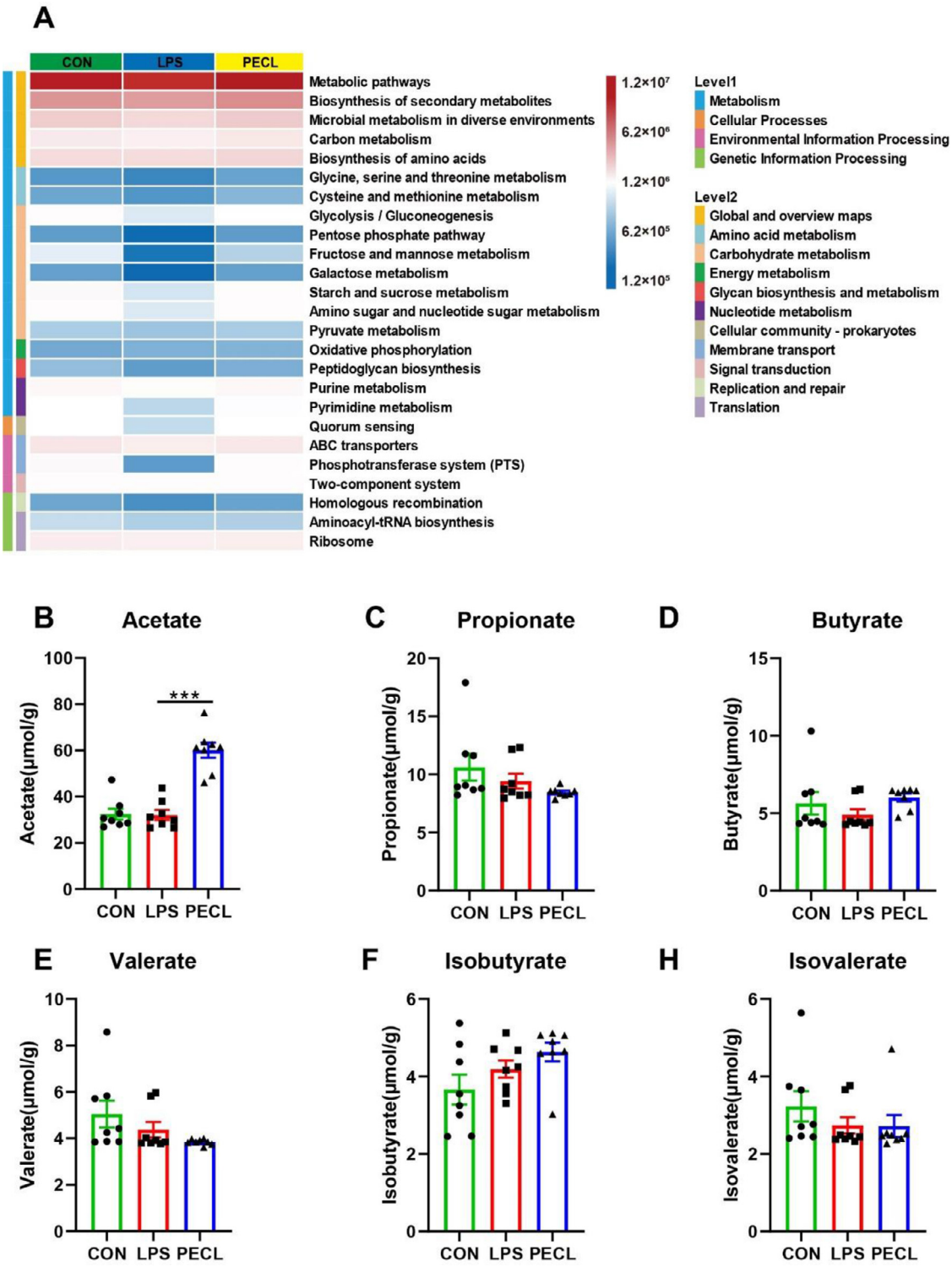

Fig. 6. Biofunction prediction of the intestinal microbial community using PICRUST2 at the KEGG pathway(A). Concentrations of SCFAs in the intestinal contents of piglets in different groups. The intestinal contents SCFAs including (B) acetate, (C) propionate, (D) butyrate, (E) valerate, (F) isobutyrate, (H) isovalerate. Signification is presented as * $P<.05$, ** $P<.01$, and *** $P<.001$; data are presented as the mean ± SE ($n=8$).

For intestinal glycosylation, *Lactobacillus* was positive but *Bacteroidetes, Actinobacteria, Olsenella, Bacteroides, Prevotella_9*, and *Eubacterium* were negatively correlated with GlcNAc ($P<.05$). For microbial metabolite SCFAs, there was a significant positive correlation between certain microbes (*Actinobacteria, Olsenella, Prevotella_9, norank_f__Bacteroidales_S24-7_group*) and acetate ($P<.05$); *Acinetobacter* and *unclassified_k__norank* had a positive correlation with propionate and butyrate, respectively ($P<.05$). Altogether, intestinal microbiota was closely associated with ileal morphology, inflammatory cytokines, mucus makers, tight junction proteins, glycosylation, and microbial metabolite SCFAs. Since SCFAs are universally considered to be beneficial for intestinal health, we also

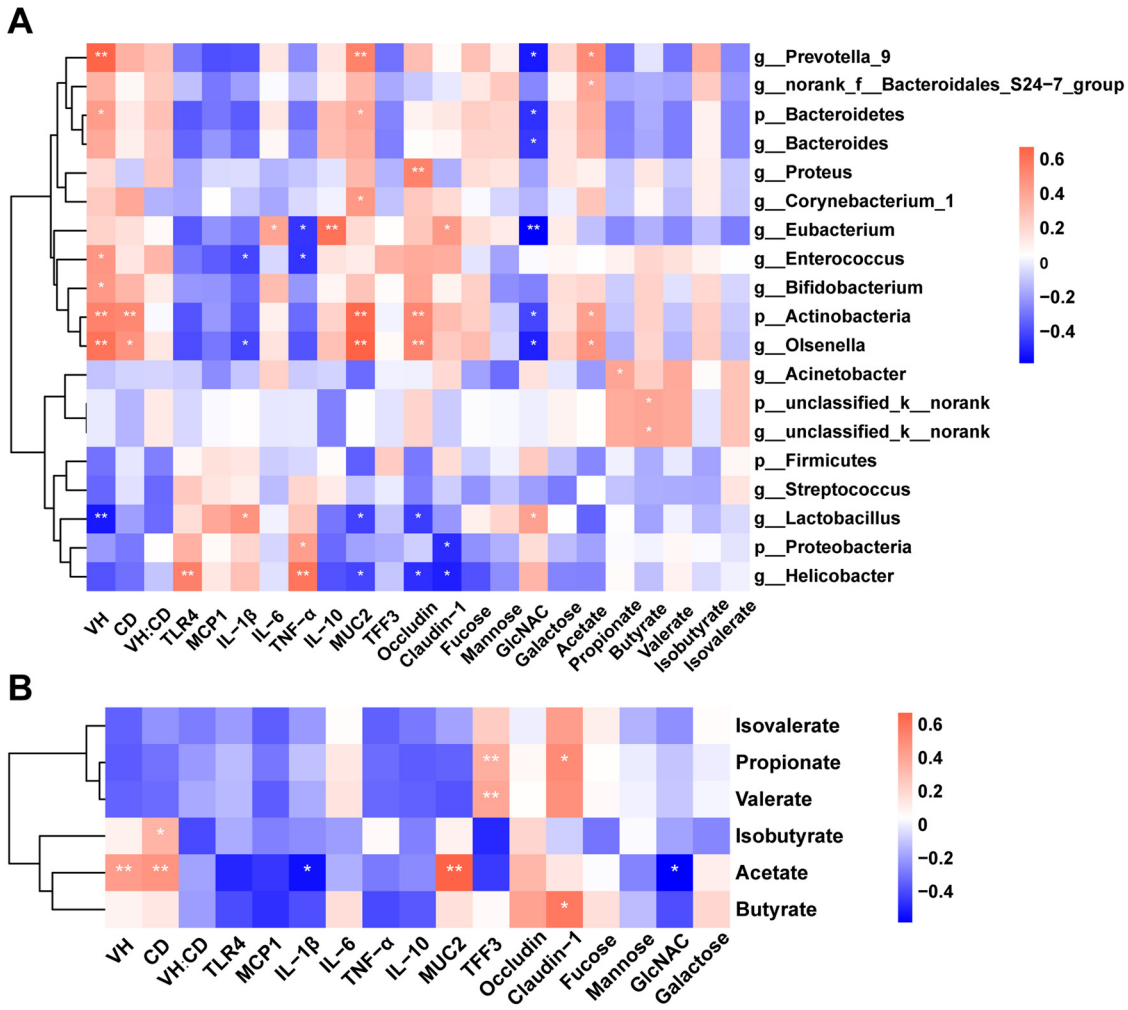

Fig. 7. (A) Heatmaps of the Spearman rank correlation coefficient and significant tests between the intestinal microbiota and ileum morphology, inflammatory cytokines, mucus makers, tight junction proteins, glycosylation, microbial metabolite SCFAs. (B) Heatmaps of the Spearman rank correlation coefficient and significant tests between the differential microbial metabolite SCFAs and ileum morphology, inflammatory cytokines, mucus makers, tight junction proteins, glycosylation. The red color represents a positive correlation, while the blue color represents a negative correlation. Signification is presented as * $P<.05$, ** $P<.01$, and *** $P<.001$; data are presented as the mean ± SE ($n=8$).

identified the correlation between SCFAs and inflammatory cytokines, mucus makers, tight junction proteins, and glycosylation (Fig. 7B). Acetate was positively correlated with VH, CD, and *MUC2*, but negatively correlated with *IL-1β* and GlcNAc ($P<.05$). Butyrate and isobutyrate were positively correlated with Claudin-1 and CD, respectively ($P<.05$). There was a significant positive correlation between propionate and *TFF3*, Claudin-1 ($P<.05$). Valerate had a positive correlation with *TFF3* ($P<.05$).

4. Discussion

It is well known that the intestine is not only an important organ for nutrient digestion, absorption, and metabolism but also the first intestinal barrier against food-derived pathogens. Thus, maintaining the integrity and functions of intestinal barrier is essential for human and animal health [13]. The weaning stress of piglets can cause immune system destruction, intestinal microbiome dysbiosis, and intestinal barrier dysfunction, then leading to intestinal injury and poor health, thus reducing the growth performance, diarrhea occurrence, and even death [2,3,33–36]. LPS is a component of the outer cell wall of Gram-negative bacteria. Previous studies showed that LPS stimulation could cause immune cells to release a large number of inflammatory cytokines, and the increase of inflammatory cytokines could activate the hypothalamus-pituitary-adrenal axis and inhibit the growth axis, resulting in growth inhibition [37–40]. Therefore, the intraperitoneal injection of LPS is widely used as a model of intestinal injury and a common tool for exploring the effects of dietary regimes [37,38]. Whether LPS challenge can completely simulate the immune stress suffered in a feeding environment is a subject of much debate. However, LPS challenge could cause immune stress and intestinal damage, which allowed a better understanding of the physiology of infection and inflammation during weaning. In the present study, LPS challenge could decrease growth performance, and induce an inflammatory response, as well as lead to intestinal barrier dysfunction, which was consistent with previous studies [11,14,26,34,41]. Thus, it is proposed that the LPS challenge model was successfully established. Dietary fiber is an important nutrient that can improve intestinal health [42,43]. In the present study, pectin supplementation significantly decreased the F: G of piglets from d 1–14 compared with the CON and LPS group, which was consistent with previous studies [1]. Previous studies found that pectin supplementation could increase the total tract apparent digestion of dry matter, total energy, total carbohydrate, and crude protein, because it can

increase the activities of digestive enzymes in the intestine [1,44]. Moreover, pectin supplementation had beneficial impacts on the intestinal health status of piglets [1,22,45]. Therefore, the improvement of growth performance is associated with a better intestinal environment and increasing intestinal digestion and absorption. Additionally, pectin supplementation can ameliorate the adverse effects of LPS challenge on growth performance in piglets. Chen et al. reported that pectin supplementation could improve the growth performance of the weaned pigs infected with rotavirus, which causes intestinal inflammation of piglets [1]. Moreover, the same results were got in other soluble dietary fibers, such as beta-glucan [41], inulin [46], and xylooligosaccharides [47]. In short, this result suggested the importance of pectin supplementation during inflammation.

Intestinal morphology is an important indicator of intestinal health, including VH, CD, and VH:CD. It can be used to measure the integrity of the intestinal epithelial barrier, and the ability to digest and absorb nutrients [48]. Numerous researches have shown that LPS challenge can cause various intestinal morphological changes, such as the decrease of VH and the increase of CD [11,15,23], villus shedding, submucosal edema, epithelial vacuolation, and necrosis [37,49], and we also found intestinal hyperemia. As common indicators for estimating intestinal integrity, the VH, CD, and the VH:CD can reveal some information on gut health and the functional capacity of the intestine in piglets. The present study showed that LPS challenge decreased VH and VH:CD, indicating that LPS challenge negatively affects intestinal integrity and function in piglets. Whereas these adverse effects of LPS challenge were attenuated by pectin supplementation in piglets, which was similar to an earlier finding [50]. Tight junctions maintain gut homeostasis by physiologically functioning as the “great wall” against the penetration of luminal bacteria and harmful substances into the mucosa [51], and intestinal injury is closely associated with changes in tight junctions [52]. In this study, Claudin-1 and Occludin were decreased by the LPS challenge and restored by pectin supplementation. These data demonstrated that pectin supplementation improved intestinal epithelial barrier function and alleviated tissue injury.

The mucus layer, the first defensive line of the intestinal barrier, is composed primarily of mucins secreted by goblet cells. The mucus layer not only provides a physical barrier to protect the intestinal epithelium from the intestinal pathogenic bacteria but also provides a habitat and energy source for intestinal symbiotic bacteria [30,31]. Only by destroying the mucus layer can pathogenic bacteria invade intestinal epithelial cells and trigger an intestinal immune response. Among them, *MUC2* and *TFF3* are important markers for detecting the secretion function of goblet cells. Chen et al. reported that pectin supplementation increased the mRNA expression of *MUC2*, which is beneficial to maintain the intestinal chemical barrier of the piglet and protecting the intestinal tract from the intrusion of harmful pathogens [1]. Our previous studies found pectin supplementation could increase the number of PAS/AB-positive goblet cells in the ileum of growing pigs [44]. Consistent with these reports, we found that LPS challenge decreased the mRNA expression of *MUC2* and *TFF3*, while pectin supplementation could restore their expression. Furthermore, we found that LPS challenge could increase the number of neutral goblet cells in the villus, while pectin supplementation has no significant effect on this compared with the CON group. Based on previous studies and our study, we speculated that pectin supplementation could interact directly with epithelial tissue and promote mucin discharge from individual goblet cells accompanied by *MUC2* upregulation [20]. Besides, several bacterial taxa were positively associated with the mRNA expression of *MUC2* (*e.g., Prevotella_9, Bacteroidetes, Actinobacteria*, and *Olsenella*). The potential mechanisms of this association may be that some microorganisms can degrade mucus and use it as an energy source, and on the other hand, they can modify host glycan expression following invasion [53,54]. The results of this study indicated that pectin supplementation could promote intestinal mucus secretion and the capability to defend against harmful bacteria, thus exerting a protective effect on the intestinal epithelial structure.

In addition to serving as a barrier, the mucus serves as an interface between the bacteria and the host: the mucus provides binding sites for not only commensal but also pathogenic bacteria and bacterial energy/food sources. In addition, mucin glycosylation impacts the function of the mucus layer, as well as its ability to control microbe adherence and microbial nutrition at this key microbe-host interface [55]. The mucin-type O-glycosylation biosynthesis process was shown in Figure 3C. Gut mucus oligosaccharides GlcNAc, Gal, GalNAc, Fuc, and Man are attached to mucin glycoproteins and can be used by bacteria to maintain their relative niches [27]. Moreover, unique glycosylated structures in the mucus layer and intestinal epithelial cells have crucial roles in physiological protection and functional and immunological processes, including cell attachment and signal transduction [56]. Previous studies reported that the change of glycosylation patterns of intestinal mucins is related to intestinal diseases such as irritable bowel syndrome, idiopathic chronic diarrhea, colorectal cancer (CRC), and inflammatory bowel diseases (IBD), which are accompanied by disturbances in the intestinal microbiota [57–60]. Microbial products (such as LPSs) have been reported to influence the glycosylation of intestinal mucins, because these products may create glycan-altering environments (including the energy level, pH, and cytokine expression). Meanwhile, microbial products could be recognized by pattern recognition receptors, such as TLRs [61]. As expected, LPS challenge changed intestinal glycosylation, such as increasing GlcNAc and decreasing fucose and galactose, while pectin supplementation restored the shifts, which is consistent with the gene expression of relative transferase. Such a change in orientation might correspond to mucin maturation, which greatly affects the role of mucin in the mucus barrier. For instance, mucin in intestinal epithelial cells is generally fucosylation, which is closely related to colonization of intestinal microorganisms and microbial-host interaction. Blocking the synthesis of fucose in intestinal epithelial cells caused intestinal microbiota imbalance and aggravated the susceptibility of pathogenic bacteria in *FUT2*$^{-/-}$ mice [62]. And the increased fucosylation level can inhibit the growth of harmful bacteria and promote the growth of commensal bacteria in mice during disease state [63,64]. The abundance of GlcNAc was negatively correlated with most beneficial bacteria and acetate, which was exactly the opposite of the result for *MUC2*. Previous studies also found that the GlcNAc was associated with NF-κB activation and intestinal inflammation [65]. However, increased GlcNAc levels reverse NF-κB activation in macrophages and inhibit inflammatory responses have also been reported [66]. The discrepancies among them may attribute to a major difference in modes of GlcNAc transferase activation, duration, and site of GlcNAc modulation, type of inflammatory stimulator, cell and animal models adopted, and even the oxygen levels. Moreover, the dynamics of GlcNAc transferase-mediated GlcNAc modification are complex and remain incompletely elucidated [67–69]. Furthermore, we found that pectin supplementation increased core3 while decreased core1,2 and 4 glycans, indicating the shifts of core1,2 and 4 glycans to core3 glycans. Previous studies have shown that the core3 structure promoted mucin stability in the presence of bacterial-derived proteases. This critical function preserves mucus barrier integrity, and prevents unrestricted microbial invasion of the mucosa that would otherwise lead to spontaneous chronic inflammation [70]. Loss of core3 structure led to decreasing mucin

secretion and increasing intestinal permeability [71]. These data demonstrated the importance of core3 glycans in intestinal function. Therefore, to a certain extent, the results of this study indicated that pectin could regulate the mucus barrier by changing the structural types of core glycan, thus affecting intestinal health.

Intestinal epithelial cells can provide an immune barrier, which can maintain the delicate balance between tolerance to intestinal commensal bacteria and invasion immunity to intestinal pathogenic bacteria. *TLR4*, which is best known for recognizing LPS, and its downstream signaling molecules are key members of inflammatory signaling pathways, which eventually lead to destructive and systemic inflammatory response syndrome such as fever, shock, and even death [25]. Research has suggested that pectin supplementation attenuated endotoxin shock via suppression of Toll-like receptor signaling [72]. Similarly, some studies revealed that the upregulated the mRNA expression of *TLR4* can cause the release of related inflammatory factors, including IL-1β, IL-6, and TNF-α, and participate in the immune response against bacterial pathogens [73,74]. Consistent with this, our study found that LPS challenge increased the mRNA expression of *TLR4*, whereas pectin supplementation decreased the mRNA expression levels of *TLR4*. Furthermore, we hypothesized that pectin exerts a beneficial effect on the intestine by reducing the inflammatory response. And we did find that higher pro-inflammatory cytokines *MCP1, IL-1β*, and *TNF-α* in the ileum of LPS-challenged piglets, which might be associated with the breakdown of intestinal integrity and epithelial function [15]. Adding pectin to the diet alleviated these impacts of LPS and increased the mRNA expression of anti-inflammatory cytokines *IL-10*. Comparing well with our results, Sun et al. reported that pectin supplementation reduced the mRNA expression of *IL-1β* and *IL-6* [75]. Our previous studies found pectin supplementation reduced the mRNA expression of *IL-1β* in growing pigs and increased the mRNA expression of *IL-10* in IPEC-J2 cells [44]. Pectin probably exerts a direct regulatory effect on intestinal inflammation. Therefore, pectin supplementation was effective in alleviating intestinal inflammation in weaned piglets.

The gut microbiota of mammals has numerous roles benefiting the host, such as digestion and fermentation of carbohydrates, maintenance of intestinal barrier functions, increasing the expression of tight junctions, regulating mucin biosynthesis and catabolism, regulating the immune responses, and protection from pathogenic bacteria [2,76]. Several epidemiological and experimental studies have shown that the health benefits of dietary fibers were related to the composition and function of the intestinal microbiota [42,77]. Thus, we speculated that pectin supplementation might affect the microbiota composition in the ileum of LPS-challenged piglets. As expected, our data showed that LPS challenge profoundly shifted the structure of gut microbiota, and reduced the OUT number and alpha diversity. Pectin supplementation restored these LPS-induced shifts of intestinal microbiota, including the structure of the intestinal microbiota and alpha diversity. In detail, we found that fewer *Bacteroidetes* and *Actinobacteria* abundance in the ileum of LPS-challenged piglets, whereas pectin supplementation had the reverse effects. Although they represent only a small percentage, however, they are pivotal in the maintenance of gut homeostasis. As a main contributor of SCFAs, *Bacteroidetes* promote the balance of intestinal microbiota and play an important role in many major metabolic activities involving fermentation of carbohydrates, utilization of nitrogenous substances, and prevention of pathogen colonization [78,79]. A higher abundance of *Bacteroidetes* in the ileum suggested that the pectin supplementation might change bacterial fermentation. *Actinobacteria* have been supposed to the modulation of gut permeability, immune system, metabolism, and the gut-brain axis. An unbalanced abundance has been evidenced in several pathological conditions as a recent review indicated [80]. Furthermore, the phylum *Proteobacteria* contains many potential opportunistic pathogens, including *Escherichia, Salmonella, Campylobacter*, and *Helicobacter*, and its increase can be considered a potential indicator of intestinal diseases [81,82], and this is also evidenced by a correlation between *TNF-α* and Claudin-1 and *Proteobacteria* in this study. Our study also found that *Proteobacteria* abundance was observed to increase in LPS-challenged piglets, while pectin supplementation decreased *Proteobacteria* abundance, consistent with an earlier study by Wu et al. [22]. Therefore, we speculated that this might be a mechanism of pectin regulating intestinal health by regulating the abundance of *Bacteroidetes, Actinobacteria*, and *Proteobacteria* to improve intestinal barrier dysfunction and inhibit intestinal endotoxin-mediated inflammation.

LPS or pectin also alters microbiota composition at the genus level. We found the boom of *Helicobacter* after LPS challenge, while pectin supplementation decreased it. *Helicobacter* belongs to the phylum *Proteobacteria*, and harmful effects on the intestine were mentioned. Specifically, previous studies showed that *Helicobacter* may be one of the causes of inflammatory bowel disease (IBD) by inducing alterations in intestinal permeability or by causing immunological derangements resulting in absorption of antigenic material and autoimmunity via various immunological pathways [22,83,84]. Our study also found that a decrease in the abundance of *Helicobacter* organisms in intestinal mucosa was accompanied by the exacerbation of intestinal inflammation and damage to the intestinal barrier. In addition, *Olsenella, Bacteroides, Proteus, Prevotella_9*, and *Eubacterium* abundance were observed as the major up-regulated microbes by pectin. *Olsenella* and *Prevotella_9* are generally considered beneficial to intestinal function [85], and *Prevotella* is one of the predominant fiber fermenters and SCFAs producers in the intestinal tracts of pigs [86]. In this study, pectin supplementation significantly increased the abundance of *Olsenella* and *Prevotella_9*, which may help to provide energy for intestinal cells and protect the intestinal barrier. Another study showed that the long-term diet was strongly associated with the gut microbial composition, that is, those who ate plenty of carbohydrates, particularly dietary fiber, had *Prevotella* as the dominant species [86,87], which is similar to our findings. Moreover, *Bacteroides* can break down complex plant polysaccharides [88] and the proportion of *Bacteroides* in the intestinal microbiota is dependent on the intestinal inflammatory status [89]. In ulcerative colitis patients, the proportion of *Bacteroides* is markedly decreased [90], indicating *Bacteroides* were important beneficial players in the intestinal microbiota. Similar alterations were also found in other studies [91]. *Proteus* is the most common opportunistic pathogen [92], which has been significantly increased in the PECL group. In addition, correlation analysis showed that *Proteus* had a positive correlation with the expression of Occludin, indicating that pectin supplementation is potentially beneficial to the intestinal barrier. Previous studies have already suggested that *Eubacterium* was a specialist pectin-degrading *Firmicutes* species that has the potential function to deliver anti-inflammatory activity by promoting the production of *IL-10* by epithelial cells [91]. Our results were consistent with this founding. In the present study, the abundance of *Eubacterium* in the ileum of piglets was increased after the addition of pectin, and *Eubacterium* had a positive correlation with the production of *IL-10*. Thus, pectin supplementation may play an important role in reducing intestinal inflammation and injury to maintain intestinal health by modulating intestinal microbiota composition at different taxonomic levels, such as increasing the concentration of anti-inflammatory bacteria and SCFAs-producing bacteria.

SCFAs, a group of saturated aliphatic organic acids that are comprised of no more than six hydrocarbons, closely related to the composition of intestinal microbiota, are produced through fer-

mentation of polysaccharides by anaerobic intestinal microbiota [93–95]. Growing evidence has demonstrated that SCFAs regulate immune response and could potentially function as therapeutic agents to prevent various inflammatory diseases [96]. SCFAs also play a crucial role as energy sources for intestinal epithelial cells, maintaining intestinal cell function and serving as an important link connecting intestinal cell metabolism, microflora, and gene expression regulation [97–99]. Zacharias et al. reported that pectin supplementation increased the digestibility of pectin and the concentration of total SCFAs and acetate, and speculated that pectin might be beneficial to the development of fermentative processes [100]. Similarly, fibers containing uronic acids such as pectin induced the production of acetate by inoculating pig fecal *in vitro* [101]. These are consistent with the present study, where pectin supplementation increased the concentrations of acetate in the ileum, presumably due to the increase in the abundance of certain acetate-producing bacteria, including *Bacteroidetes* and *Prevotella_9* [32,102]. In particular, they produce a high concentration of acetate that can protect the host from enteropathogenic infections. This may help improve intestinal integrity and reduce pro-inflammatory cytokines expression. Correlation analysis and significance test further demonstrated that acetate contributed significantly to improving intestinal injury caused by LPS. Consistently, earlier research reported that acetate can reduce LPS-induced TNF-α release from human blood-derived neutrophils, and also inhibit TNF-α mediated activation of the downstream inflammatory signaling pathways in the human colon adenocarcinoma cell line [103]. It could be seen that the effect of pectin on the intestinal microflora of piglets was closely related to the changes in SCFAs composition, which was beneficial to the improvement of intestinal function and growth performance of the host by improving the composition of intestinal microflora and its metabolites.

To better comprehend the functional roles of the intestinal microbiota, we used PICRUST2 to investigate the functional profiles. Carbohydrate metabolism was the primary function of piglets in each group, which might be because the feed components and substrate types fermented by dominant microorganisms were carbohydrates [11]. LPS challenge significantly decreased the function of metabolic pathways, microbial metabolism in diverse environments, carbon metabolism, starch, and sucrose metabolism, glycolysis/gluconeogenesis, phosphotransferase system (PTS), fructose and mannose metabolism, and galactose metabolism, while pectin supplementation restored the function shifts. Therefore, pectin supplementation may help to regulate the abnormal function of intestinal microflora caused by stress and thus maintain homeostasis of the intestinal tract during weaning. Moreover, we also found that most changes in predicted bio function caused by LPS or pectin are related to sugars metabolisms, such as carbon metabolism, starch, and sucrose metabolism, glycolysis/gluconeogenesis, fructose and mannose metabolism, and galactose metabolism. The altered sugars metabolism-related functional pathway provides the possibility for pectin to regulate intestinal glycosylation through microbiota, because intestinal flora is closely related to intestinal glycosylation [58,61,104], and this is consistent with the intestinal glycosylation results.

From correlation analysis, we could conclude that LPS challenge caused intestinal microflora disorder, altered mucus glycosylation, and permeability, and upregulation of pro-inflammatory cytokine expression, which triggers the impaired intestinal barriers, intestinal inflammation, and damaged intestinal morphology, ultimately reducing the growth performance of piglets. This conclusion was in line with the previous study [11], and the process could be reversed by the addition of pectin. Specifically, pectin supplementation could regulate the intestinal microbiota structure, increase the relative abundance of acetate-producing bacteria and the content of acetate, improve mucus glycosylation and permeability, then reduce the expression of intestinal pro-inflammatory cytokines, thus improving intestinal morphology, maintaining intestinal barrier function, alleviating the inflammatory reaction, and eventually improving intestinal health and growth performance of piglets.

In summary, our results revealed that pectin supplementation had beneficial impacts on the improvement of intestinal integrity, intestinal injury, and ultimately growth performance of piglets. We speculated that the improvement of growth performance of piglets challenged by LPS may be attributed to the beneficial effects of pectin supplementation on intestinal microflora and SCFAs, thus improving intestinal barrier function, reducing the inflammatory response, and enhancing body health. These findings provide a new perspective for the development of pectin as a functional food ingredient and strong support for regulating the intestinal health and growth performance of piglets via dietary pectin. Our data also provide insights for studying the role of pectin in regulating human infant intestinal health.

Declaration of competing interests

The authors declare no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jnutbio.2022.109107.

CRediT authorship contribution statement

Xiaobin Wen: Conceptualization, Methodology, Software, Writing – original draft. **Ruqing Zhong:** Writing – original draft, Resources. **Guoqi Dang:** Methodology, Formal analysis. **Bing Xia:** Writing – review & editing. **Weida Wu:** Conceptualization, Writing – review & editing. **Shanlong Tang:** Writing – review & editing, Visualization. **Lixin Tang:** Software, Visualization. **Lei Liu:** Resources. **Zhengqun Liu:** Formal analysis. **Liang Chen:** Conceptualization, Writing – review & editing, Supervision. **Hongfu Zhang:** Conceptualization, Writing – review & editing, Supervision.

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