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Ethics approval and consent to participate	The experimental protocol for this experiment was reviewed and approved by the Institutional Animal Care and Use Committee of Chungnam National University, Daejeon, South Korea (approval #202203A-CNU-061).

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Abstract

This study evaluated the effects of dietary probiotic *Lactococcus lactis* on growth performance, frequency of diarrhea, immune responses, and intestinal health of weaned pigs. In a randomized complete block design (block = initial body weight), 48 newly weaned pigs were assigned to two dietary treatments (4 pigs/pen; 6 replicates/treatment; 4-week trial): a basal diet based on corn and soybean meal (CON) and CON supplemented with 0.02% *L. lactis* (LL). Growth performance, frequency of diarrhea, systemic immune responses and serum biochemical parameters, intestinal morphology, and ileal gene expression of tight junction proteins and inflammatory cytokines were measured. No differences were found in growth performance and serum biochemical parameters between CON and LL. However, the LL group tended to show lower the frequency of diarrhea during the first two weeks after weaning ($p = 0.092$), hematocrit levels on day 14 ($p = 0.093$), and serum cortisol concentrations on day 7 ($p = 0.096$) than the CON group. The LL decreased concentrations of serum tumor necrosis factor-α (TNF-α) on day 7 ($p < 0.05$), interleukin-6 (IL-6) on days 7 and 14 ($p < 0.05$), transforming growth factor-β1 (TGF-β1) on day 14 ($p = 0.060$), and interleukin-1β (IL-1β) on day 14 ($p = 0.082$) and day 28 ($p = 0.072$) compared with the CON. Pigs fed LL diet had higher villus area and the number of goblet cells in the small intestine ($p < 0.05$) and had a tendency to increase villus height in the duodenum ($p = 0.084$) compared with those fed CON diet. Furthermore, the LL upregulated ($p < 0.05$) gene expressions of claudin-1, claudin-4, and tight junction protein-1 in the ileum and downregulated ($p < 0.05$) gene expressions of TNF-α, TGF-β, IL-1β, IL-6, and interleukin-8 compared with the CON. Consequently, the dietary probiotic *L. lactis* tended to alleviate post-weaning diarrhea; this may be correlated with improved anti-inflammatory responses, intestinal morphology, and gut barrier functions of weaned pigs.

Keywords: Cytokines, Intestinal health, Probiotics, Weaned pigs

Introduction

Weaning is a critical developmental stage in pigs, and characterized by an abrupt transition from a milk-based diet to solid feed. This process often triggers physiological stress and substantial alterations in the intestinal microbiota, commonly resulting in post-weaning diarrhea (PWD), which has detrimental effects on growth performance, health, and survival [1,2]. During this period, beneficial gut bacteria such as *Lactobacillus* spp. decrease, whereas pathogenic strains such as *Escherichia coli* proliferate, exacerbating these issues [3,4]. Weaning compromises intestinal integrity by increasing gut permeability, facilitating the translocation of pathogens and toxins into the bloodstream, and triggering systemic inflammation [5]. This disruption is largely attributed to the alteration of tight junction proteins, including claudins and occludin, which are key components of the intestinal epithelial barrier [6]. Weakening of this barrier increases the risk of infection and impairs nutrient absorption, thus, hindering piglet growth during this vulnerable stage.

Probiotics, defined as live microorganisms that confer health benefits when administered in adequate amounts [7]. Previous studies have demonstrated that probiotics can modulate the gut microbiota, enhance intestinal health, and positively influence immune responses [8–11]. Probiotic strains such as lactic acid bacteria, including species previously classified as *Lactobacillus*, and *Lactococcus* spp. have been shown to stabilize the gut microbiota, improve intestinal barrier function, and reduce the incidence of PWD [12]. *Lactococcus lactis* is an attractive probiotic bacterium for animal feed because of its acid resistance, which allows it to survive in the gastrointestinal tract [13,14]. In particular, because the effects of probiotics vary depending on the characteristics of the strain used, further research is needed to investigate the physiological effects and potential mechanisms of *L. lactis*. Although the effects of *Lactobacillus*- and *Bifidobacterium*-based probiotics have been extensively studied, research on the effects of *L. lactis* on intestinal health and inflammatory responses induced by rapid changes and stress after weaning remains limited. Therefore, in this study, we aimed to evaluate the effects of dietary *L. lactis* supplementation on growth performance, systemic immune responses, and intestinal health in weaned pigs.

Materials and Methods

Animal ethics statement

The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Chungnam National University, Daejeon, Republic of Korea (approval: #202203A-CNU-061).

Experimental animals, design, and diets

A total of 48 weaned pigs ([Landrace × Yorkshire] × Duroc; 6.49 ± 0.97 kg of average initial body weight [BW]; 28 days of age) were used in this study. Pigs were assigned to two dietary treatments in a randomized complete block design in which pigs were blocked by their initial BW to minimize variation. Each treatment consisted of 6 replicates with 4 pigs per pen. Dietary treatments were a basal weaner diet based on corn and soybean meal (CON) and CON supplemented with 0.02% *L. lactis* (LL). The probiotic product (IDCC 2301; 1.0×10^{10} CFU/g) obtained from a commercial supplier (ILDONG Bioscience) was isolated from homemade cheese [15]. The basal diet was formulated to meet the nutritional requirements of the weaned pigs as estimated by the National Research Council [16] (Table 1). The experimental period was 28 days after weaning. All pigs were housed under the same environmental conditions, with the ambient temperature maintained between 28–30°C, and the relative humidity ranging from 50% to 60%. Feed and water were provided *ad libitum*, and the pens were uniformly sized at 232 × 175 cm (width × length).

Data and sample collection

The BW of each pig was individually recorded on days 1, 7, 14, and 28 to assess growth performance over the experimental period. The average daily gain (ADG), average daily feed intake (ADFI), and gain to feed

ratio (G:F) were calculated from individual BW and feed intake data. The fecal score was monitored daily for the first two weeks of the experiment using a scoring system ranging from 1 to 5 (1 = normal hard feces, 2 = slightly soft feces, 3 = soft partially formed feces, 4 = semi-liquid feces, and 5 = watery diarrhea). Frequency of diarrhea was calculated as the number of diarrhea cases (fecal scores ≥ 4) divided by the total number of pen days [17]. Blood samples were collected from the jugular vein of pigs with a median BW (one pig per pen) on days 1, 7, 14, and 28 using two types of tubes (Becton Dickinson): 10 mL ethylenediaminetetraacetic acid (EDTA)-coated tubes for whole blood analysis and 10 mL serum tubes for serum analysis. Serum samples were separated from the non-EDTA tubes by centrifugation at 3,000 × g for 15 min at 4°C (1580R, LaboGene) and stored at -80°C for further immune response analysis [18]. At the end of the experimental period, the pigs were anesthetized intramuscularly with 2 mL of suxamethonium chloride (Succicholine Inj., Il Sung IS). After euthanasia by exposure to carbon dioxide, tissue samples (approximately 5 cm) were collected from the middle of each part of the small intestine. The intestinal samples were washed with distilled water and fixed in 10% neutral-buffered formalin (BBC Biochemical) in 50 mL conical tubes for subsequent morphological analysis. After washing the other ileal segments with distilled water, the mucosal samples were carefully scraped using a sterile slide. The scraped mucosal samples were placed in 1.5 mL microtubes containing RNAlater reagent (QIAGEN GmbH) and stabilized at room temperature for 24 h before being stored at -80°C for gene expression analysis.

Hematology, immune response, and biochemical analysis

Whole blood samples collected in EDTA tubes were analyzed using an automated hematology analyzer specifically calibrated for porcine blood (scil Vet abc hematology analyzer; scil Animal Care), measuring total white blood cell (WBC) count and hematocrit (HCT). Serum samples were analyzed to determine biochemical parameters using a clinical autoanalyzer (Toshiba Acute Biochemical Analyzer-TBA-40FR; Toshiba Medical Instruments) and specific kits (Wako Pure Chemical Industries) [19]. The biochemical markers included alanine aminotransferase, aspartate aminotransferase, total protein, albumin, creatinine, blood urea nitrogen, glucose, total cholesterol, triglycerides, calcium, inorganic phosphorus, and magnesium. Serum samples were also analyzed for concentrations of cortisol, tumor necrosis factor-α (TNF-α), transforming growth factor-β1 (TGF-β1), and interleukin-1β (IL-1β) and IL-6 using porcine-specific enzyme-linked immunosorbent assay kits (R&D Systems) according to manufacturer's protocol. To quantify the concentrations, the absorbance was measured at 450 nm using a microplate reader (Epoch microplate spectrophotometer, BioTek Instruments Inc.)[20].

Intestinal morphology analysis

The fixed intestinal samples were deparaffinized, rehydrated, and stained with hematoxylin and eosin. Stained tissue sections were mounted on glass slides for further analysis. The slides were examined by

selecting 10 well-oriented villi per section. Intestinal morphological analysis was performed using a fluorescence microscope (TE2000, Nikon) equipped with a charge-coupled device camera (DS-Fi1, Nikon), and image-based analysis was performed using NIS-Elements software (version 3.00, NIS Elements, Nikon). Intestinal morphological parameters, including villus height, crypt depth, villus area, and the number of goblet cells were measured.

Gene expression analysis

Gene expression analysis of the ileal mucosa was conducted to evaluate the relative expression levels of tight junction protein genes and inflammatory cytokine genes using quantitative real-time polymerase chain reaction (qRT-PCR). Target genes included claudin (CLDN; CLDN1, CLDN2, CLDN3, and CLDN4), occludin (OCLN), and tight junction protein 1 (TJP1), as well as inflammatory markers such as TNF-α, TGF-β, IL-1α, IL-1β, IL-6, IL-8, interferon (IFN; IFN-α and IFN-γ). Total RNA was extracted from the ileal samples using TRIzol Reagent (Invitrogen), and RNA concentration and purity were measured using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies). Reverse transcription was performed using the SuperScript IV cDNA synthesis kit (Invitrogen) to generate cDNA for subsequent qRT-PCR analyses. qRT-PCR was performed using a StepOnePlus Real-Time PCR System (Applied Biosystems) with SFCgreen I (BIOFACT) and gene-specific primers designed by Bioneer Inc. Primer sequences were designed based on our previous study [9] using Primer Express Software v 3.0.1 (Applied Biosystems) and is shown in Table 2. The samples were normalized using the average cycle threshold (Ct) for β-actin. Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method [21]. Each sample contained three technical replicates for each gene.

Statistical analyses

Data for growth performance, serum biochemical parameters, immunological, and morphological indices were analyzed using the PROC GLM of SAS (version 9.4, SAS Institute Inc.) in a randomized complete block design (block: initial BW). The experimental unit was the pen for growth performance, whereas the individual pig was considered the experimental unit for other measurements. The statistical model for growth performance and biochemical, immunological, and morphological measurements included dietary treatment as a fixed effect. The Chi-square test was used to determine the frequency of diarrhea. The *t*-test was used to analyze the expression levels of tight junction protein genes and inflammatory cytokine genes in the ileum to compare the dietary treatments. Statistical significance and tendency were defined at $p < 0.05$ and $0.05 \leq p < 0.10$, respectively.

Results

Growth performance and serum biochemical parameters

The effects of dietary treatments on the growth performance and biochemical parameters of weaned pigs are shown in Table 3 and 4. No differences were observed in ADG, ADFI, and G:F, or biochemical parameters between the CON and LL groups throughout the experimental period.

Frequency of diarrhea and serum immune responses

Frequency of diarrhea, total WBC counts, HCT levels, and serum immune responses are presented in Table 5. Pigs fed LL tended to reduce the frequency of diarrhea from days 8 to 14 ($p = 0.056$) and 1 to 14 ($p = 0.092$) compared with those fed CON. There were no differences in the number of WBC between dietary treatments during the experimental period. However, pigs fed LL tended to have lower ($p = 0.093$) HCT levels on day 14 than those fed CON. In the systemic immune responses, pigs in the LL group had lower ($p < 0.05$) serum concentrations of TNF-α and IL-6 on day 7 and tended to have lower ($p = 0.096$) cortisol level on day 7 than those in the CON group. On day 14, pigs fed LL had lower ($p < 0.05$) serum IL-6 level and tended to have lower levels of serum TGF-β1($p = 0.060$) and IL-1β ($p = 0.082$) than those fed CON. Additionally, concentration of serum IL-1β on day 28 tended to be lower ($p = 0.072$) in the LL group than in the CON group.

Intestinal morphology and ileal gene expression

The intestinal morphology and relative gene expression levels are shown in Figure 1. In the duodenum, pigs fed LL tended to have increased villus height ($p = 0.084$) compared with those fed CON. Pigs fed diet supplemented with LL had greater ($p < 0.05$) villus area and the number of goblet cells in the duodenum, jejunum, and ileum than the CON diet. Regarding the relative expression levels of tight junction protein genes in the ileum, pigs fed LL had upregulated ($p < 0.05$) expression of CLDN1, CLDN4, and TJP1 genes compared with those fed CON. By contrast, the LL group had downregulated ($p < 0.05$) relative expression of TNF-α, TGF-β, IL-1β, IL-6, and IL-8 genes compared with the CON group.

Discussion

Our findings indicate that *L. lactis* supplementation did not improve growth performance, as evidenced by the lack of significant differences in BW, ADG, ADFI, and G:F between the LL and CON groups. This result is consistent with previous research showing that *L. lactis* did not affect growth performance [15]. The stable health conditions and absence of environmental stressors in the present study may have limited their potential effects on growth performance. In contrast, probiotics have exhibited beneficial effects on growth and health when animals are challenged with pathogens [22], suggesting that their efficacy varies depending on factors such as strain specificity and environmental conditions [23,24]. Interestingly, unlike the typical outcomes observed with other probiotic strains [25], the LL group showed no significant changes in serum biochemical parameters compared with the CON group in our study. Although these changes were

not significant, they may indicate subtle metabolic shifts attributable to the probiotics. Elevated blood urea nitrogen levels may suggest enhanced protein metabolism, possibly due to improved nutrient absorption in the gut, whereas increased glucose levels may reflect alterations in energy metabolism [26]. The enhanced mucosal surface area and nutrient transport efficiency resulting from this modulation may improve amino acid and glucose availability.

Dietary probiotics have been proposed to have a primary mode of action of beneficial impacts on weaned pigs by modulating the gut microbiota to promote the growth and health of animals [27,28]. However, the effects of dietary probiotics on PWD vary depending on the beneficial strain used, and the results of previous studies have been inconsistent [8–11,29]. The efficacy and potential mechanisms of action of dietary probiotics vary depending on the beneficial strain used. In the present study, supplementation of dietary *L. lactis* IDCC 2301 tended to alleviate PWD in weaned pigs. This may be attributed to its ability to enhance the intestinal epithelial barrier integrity and modulate the gut ecosystem during the critical post-weaning period. Similarly, previous studies have reported the therapeutic potential of *L. lactis* in improving recovery from infectious diarrhea, pathogen colonization, and viral infections [14,30]. This may be related to the suppression of pathogenic bacterial colonization through the production of antimicrobial substances by the probiotic LL [14,31]. Additionally, a decreasing trend in HCT levels was observed in the LL group at two weeks post-weaning. HCT is closely related to the hydration status, and excessive fluid loss is a common challenge during the post-weaning period. These aspects may be associated with changes in the hydration status related to reduced fluid loss and improved intestinal recovery. This point appears to reflect a recovery phase rather than a peak stress period, when supplement differences become more apparent. These findings suggest that dietary *L. lactis* supplementation may contribute to alleviating PWD by supporting intestinal health and maintaining physiological stability during the post-weaning period.

The decreasing trend in serum cortisol concentrations observed on day 7 supports the stress-mitigating effects of dietary LL after early weaning. Cortisol, a hormone regulated by the hypothalamic pituitary adrenal axis, is an indicator of physiological stress and is commonly elevated after a stressful weaning phase [32]. Previous studies suggested that probiotics may influence host stress responses via gut-associated pathways [33,34]. Supporting these observations, the modulation of systemic inflammatory cytokine profiles was also observed in pigs fed *L. lactis*. These changes suggest that dietary LL exerts systemic anti-inflammatory effects, particularly during acute weaning stress. A previous study reported that the probiotic *L. lactis* IDCC 2301 exhibited anti-inflammatory effects by modulating nuclear factor-kappa B and mitogen-activated protein kinase signaling pathways [35]. Although this study did not directly investigate these mechanisms, the observed responses may be consistent with reported properties. Collectively, the results of this study suggest that dietary *L. lactis* may alleviate physiological stress and modulate systemic inflammatory responses during the early post-weaning period.

Supplemental probiotic LL had positive effects on intestinal structural changes observed in weaned pigs. Our results showed that pigs in the LL group tended to have an increased duodenal villus height and improved duodenal, jejunal, and ileal villus areas, but the duodenal crypt depth was reduced compared with pigs in the CON group. These changes indicate that *L. lactis* may have contributed to alleviating villus atrophy and excessive crypt proliferation, which can be induced by weaning stress, and maintaining a more stable villus to crypt structural balance. In general, increased villus height and decreased crypt depth are associated with improved intestinal nutrient absorption and enhanced tissue repair capabilities [36,37]. This suggest that *L. lactis* affects the turnover dynamics of intestinal epithelial cells and maintains the structural stability of the intestinal mucosa. In addition, the number of goblet cells increased in the duodenum, jejunum, and ileum of the LL-fed pigs. Goblet cells serve as a chemical barrier to the intestinal mucosa by secreting mucins that inhibit the adhesion of pathogenic bacteria to the intestinal epithelial cells [38,39]. The increase in goblet cells suggests that *L. lactis* may stimulate the mucus secretion system to enhance mucosal defense and contribute to the stabilization of the intestinal environment, which is vulnerable to weaning. These results suggest that the probiotic *L. lactis* alleviates the damage to the intestinal structure and mucosal barrier function during weaning stress.

Gene expression results showed that the probiotic LL upregulated CLDN1, CLDN4, and TJP1 in the ileum of weaned pigs. This suggests that dietary *L. lactis* may affect the adhesion between intestinal epithelial cells by increasing the expression of tight junction protein genes. The upregulated expression of tight junction protein genes is crucial for maintaining intestinal integrity and plays a key role in ensuring intestinal environmental stability and immune homeostasis [40]. Simultaneously, the downregulated expression of inflammatory cytokine genes such as TNF-α, TGF-β, IL-1β, IL-6, and IL-8 indicates that the dietary LL contributed to mitigating excessive immune responses in the intestinal mucosa and maintaining immune balance. These strengthened barrier function and immunomodulatory effects may have contributed to maintaining the structural stability of the intestinal epithelium, which may also be related to the improvements in intestinal structure and the increase in mucus cells observed in the present study. Our results may partially explain the reduced frequency of diarrhea and changes in systemic inflammatory cytokine levels. Dietary probiotics are known to improve intestinal health through the competitive exclusion of pathogens, modulation of intestinal barrier function, immune system regulation, and neurotransmitter production [41]. Lactic acid-producing probiotics directly and indirectly regulate the intestinal environment by producing lactic acid and short-chain fatty acids [42]. Collectively, this study suggests that supplementation of dietary *L. lactis* to weaned pigs may contribute to physiological changes that enhance intestinal barrier structure and immune functions.

Conclusions

Dietary supplementation with 0.02% probiotic *L. lactis* IDCC 2301 tended to alleviate post-weaning diarrhea in weaned pigs. This probiotic improved the intestinal barrier function by upregulating tight junction protein genes (*CLDN1*, *CLDN4*, and *TJP1*) and enhanced intestinal morphology, including increased villus height, villus area, and goblet cell numbers. Furthermore, *L. lactis* supplementation modulated both local and systemic immune responses by downregulating pro-inflammatory cytokine genes (*TNF-α*, *TGF-β*, *IL-1β*, *IL-6*, and *IL-8*) in the ileum and reducing serum TNF-α and IL-6 concentrations. These findings suggest that *L. lactis* is an effective dietary probiotic that support intestinal health and immune function during the critical post-weaning period in pigs.

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Tables and Figures

Table 1. Composition of basal diet for weaned pigs (as-fed basis)[1]

Item	Basal diet
Ingredient (%)	
Corn (8%)	50.05
Soybean meal (44%)	21.20
Soy protein concentrate	10.00
Whey powder	12.50
Soybean oil	2.50
Limestone	1.40
Dicalcium phosphate	1.22
Vitamin-mineral premix[1]	0.30
Lysine-HCl	0.38
DL-methionine	0.30
L-threonine	0.15
Total	100.00
Calculated energy and nutrient content	
Metabolizable energy (kcal/kg)	3,490
Crude protein (%)	22.18
Calcium (%)	0.98
Phosphorus (%)	0.67
Lysine (%)	1.54

[1]Provided per kilogram of diet: vitamin A, 12,000 IU; vitamin D_3, 2,500 IU; vitamin E, 30 IU; vitamin K3, 3 mg; $_D$-pantothenic acid, 15 mg; nicotinic acid, 40 mg; choline, 400 mg; and vitamin B_{12}, 12 μg; Fe, 90 mg from iron sulfate; Cu, 8.8 mg from copper sulfate; Zn, 100 mg from zinc oxide; Mn, 54 mg from manganese oxide; I, 0.35 mg from potassium iodide; Se, 0.30 mg from sodium selenite.

Table 2. Primer sequences for qRT-PCR analysis of tight junction proteins and inflammatory cytokines

Item[1]	Forward (5'-3')	Reverse (5'-3')
CLDN1	AGAAGATGCGGATGGCTGTC	CCCAGAAGGCAGAGAGAAGC
CLDN2	TCCTCCCTGTTCTCCCTGATAG	CCTTGCAGTGGGCAGGAA
CLDN3	GATGCAGTGCAAAGTGTACGA	GTCCTGCACGCAGTTGGT
CLDN4	TATCATCCTGGCCGTGCTA	CATCATCCACGCAGTTGGT
OCLN	GGAGTGATTCGGATTCTGTCTATGCT	CGCCTGGGCTGTTGGGTTGA
TJP1	CACGTGGAGCTATACCAGAA	TCCGGTGACATCAAAGGACA
TNF-α	GCCCTGTACCCCAACTGGTA	CCCAGGAAGACGGGCTTT
TGF-β	GCGCAGCCTTGAGGATTTC	CCCAGCTACATTATCCGAATGG
IL-1α	CTTGGGTTTGGATTCCTGGAT	CTTCCCTGGCAGCCACAT
IL-1β	CCCCTGTGCCTGGGAGAT	AGGTTTCTGGAGGAAGAGAAGGA
IL-6	TGGTAGCTCTGGGAAACTGAATG	GGCTTTGCGCTGGATCTG
IL-8	GCTCTCTGTGAGGCTGCAGTTC	AAGGTGTGGAATGCGTATTTATGC
IFN-α	GTGCTCAAAACGAAGACGAACC	CATATTGCCATGCTTTTCCCAGAA
IFN-γ	GGAGCATTGAAAGAAGCA	TGACAGGTAGGACAGACGA
β-actin	CTACGTCGCCCTGGACTTC	GATGCCGCAGGATTCCAT

[1]CLDN, claudin; OCLN, occludin; TJP1, tight junction protein 1; TNF-α, tumor necrosis factor-α; TGF-β, transforming growth factor-β; IL, interleukin; IFN, interferon.

Table 3. Effects of dietary *Lactococcus lactis* on growth performance of weaned pigs[1]

Item[2]	CON	LL	SEM	*p*-value
BW (kg)				
Day 1	6.49	6.48	0.46	0.993
Day 7	7.76	7.87	0.43	0.865
Day 14	10.24	10.31	0.54	0.929
Day 28	16.48	16.63	0.62	0.876
ADG (g/d)				
Day 1 to 7	181.43	198.57	22.70	0.630
Day 8 to 14	354.29	348.57	26.13	0.896
Day 15 to 28	445.71	451.43	14.93	0.819
Overall	356.79	362.50	11.01	0.744
ADFI (g/d)				
Day 1 to 7	272.00	268.14	15.17	0.862
Day 8 to 14	426.14	422.57	24.31	0.920
Day 15 to 28	735.86	715.07	20.90	0.502
Overall	542.46	530.21	12.53	0.509
G:F (g/g)				
Day 1 to 7	0.667	0.741	0.082	0.509
Day 8 to 14	0.831	0.825	0.056	0.818
Day 15 to 28	0.608	0.633	0.030	0.579
Overall	0.658	0.684	0.023	0.472

[1]Each value is the mean of six replicates (four pigs per pen).

[2]CON, a basal diet based on corn and soybean meal; LL, CON + 0.02% *Lactococcus lactis* IDCC 2301; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G:F, gain to feed ratio.

Table 4. Effects of dietary *Lactococcus lactis* on serum biochemical parameters of weaned pigs[1]

Item[2]	CON	LL	SEM	*p*-value
Alanine aminotransferase (U/L)	28.70	34.20	2.78	0.112
Aspartate aminotransferase (U/L)	37.10	31.20	3.46	0.262
Total protein (mg/dL)	5.24	5.38	0.20	0.636
Albumin (g/dL)	3.46	3.54	0.12	0.641
Creatinine (mg/dL)	0.80	0.94	0.07	0.170
Blood urea nitrogen (mg/dL)	9.22	11.68	1.11	0.159
Glucose (mg/dL)	105.20	113.30	5.67	0.154
Total cholesterol (mg/dL)	78.30	86.90	6.37	0.368
Triglyceride (mg/dL)	41.30	42.60	4.67	0.849
Calcium (mg/dL)	10.26	10.76	0.34	0.380
Inorganic phosphorus (mg/dL)	9.96	10.80	0.46	0.231
Magnesium (mg/dL)	2.60	2.84	0.23	0.479

[1]Each value is the mean of six replicates (one pig per pen).

[2]CON, a basal diet based on corn and soybean meal; LL, CON + 0.02% *Lactococcus lactis* IDCC 2301.

Table 5. Effects of dietary *Lactococcus lactis* on frequency of diarrhea and serum immune responses of weaned pigs[1]

Item[2]	CON	LL	SEM	*p*-value
Frequency of diarrhea (%)				
Day 1 to 7	23.81	19.05		0.845
Day 8 to 14	28.57	14.29		0.056
Day 1 to 14	26.19	16.67		0.092
White blood cell ($\times 10^3$/μL)				
Day 1	11.50	10.00	3.82	0.802
Day 7	18.96	17.46	1.98	0.607
Day 14	26.02	24.52	2.64	0.699
Day 28	23.81	21.19	2.35	0.845
Hematocrit (%)				
Day 1	33.10	32.80	7.07	0.768
Day 7	35.56	32.01	1.27	0.409
Day 14	35.20	30.76	1.58	0.093
Day 28	33.22	31.76	1.19	0.388
Cortisol (ng/mL)				
Day 1	54.32	51.39	4.46	0.687
Day 7	52.66	25.52	9.74	0.096
Day 14	49.82	37.61	5.09	0.141
Day 28	46.72	44.32	7.82	0.163
TNF-α (pg/mL)				
Day 1	187.81	180.79	13.59	0.750
Day 7	174.76	150.43	4.45	0.008
Day 14	106.31	96.27	4.60	0.174
Day 28	75.74	76.86	3.83	0.843
TGF-β1 (pg/mL)				
Day 1	1236.53	1165.19	151.94	0.772
Day 7	1124.18	1260.98	179.02	0.608
Day 14	1538.66	1085.14	138.43	0.060
Day 28	1336.50	1373.87	126.04	0.841
IL-1β (pg/mL)				
Day 1	36.53	39.89	6.37	0.744
Day 7	36.13	32.04	5.66	0.627

Day 14	48.31	25.41	7.44	0.082
Day 28	47.34	25.55	7.08	0.072
IL-6 (pg/mL)				
Day 1	104.81	99.55	23.53	0.889
Day 7	118.66	61.28	8.74	0.004
Day 14	129.43	80.01	11.53	0.023
Day 28	113.17	117.18	7.11	0.704

[1]Each value is the mean of six replicates (one pig per pen).

[2]CON, a basal diet based on corn and soybean meal; LL, CON + 0.02% *Lactococcus lactis* IDCC 2301; Frequency of diarrhea = (number of diarrhea score of 4 or higher / number of pen days) × 100; TNF-α, tumor necrosis factor-α; TGF-β1, transforming growth factor-β1; IL-1β, interleukin-1β; IL-6, interleukin-6.

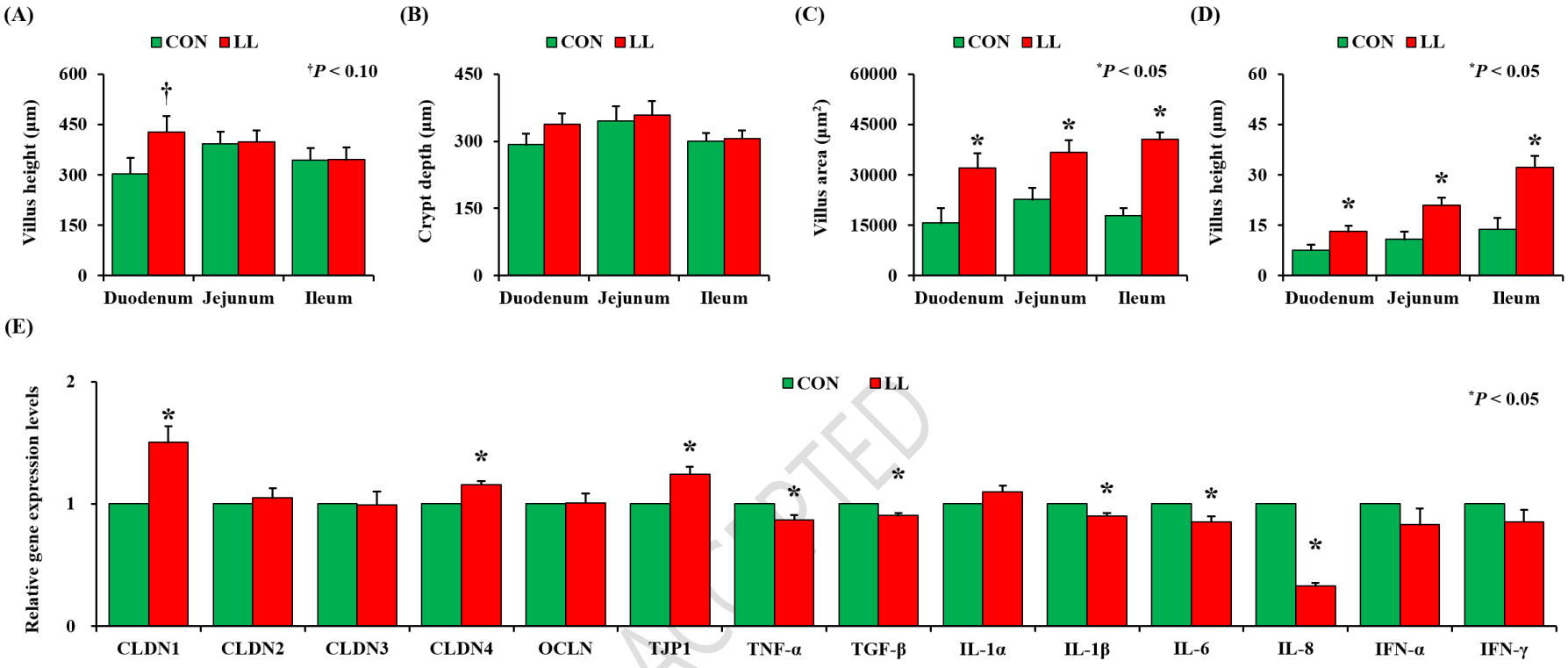

Figure 1. Effects of dietary *Lactococcus lactis* on intestinal morphology and ileal gene expression of weaned pigs: (A) villus height, (B) crypt depth, (C) villus area, (D) the number of goblet cells, and (E) relative gene expression of tight junction proteins and inflammatory cytokines in the ileum. Each value is the mean of six replicates (one pig per pen). *, †Statistical difference and tendency between dietary treatments, respectively. CON, a basal diet based on corn and soybean meal; LL, CON + 0.02% *Lactococcus lactis* IDCC 2301; CLDN, claudin; OCLN, occludin; TJP1, tight junction protein 1; TNF-α, tumor necrosis factor-α; TGF-β, transforming growth factor-β; IL, interleukin; IFN, interferon.