

JAST (Journal of Animal Science and Technology) TITLE PAGE

Upload this completed form to website with submission

ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research article
Article Title (within 20 words without abbreviations)	Dietary <i>Lactiplantibacillus plantarum</i> modulated immune responses and intestinal barrier-related gene expression of weaned pigs
Running Title (within 10 words)	Efficacy of dietary probiotics on weaned pigs
Author	Kyeong Il Park1#, Jinmu Ahn1#, Hansol Hwang1, Yujeong Oh2, Sangwoo Park2, Younghoon Kim3, Jin Ho Cho4, Hyeun Bum Kim5, Yanhong Liu6, Hyunjin Kyoung1,6*, Minho Song1* #These authors contributed equally to this work as the first author *Corresponding authors
Affiliation	1Department of Animal Science and Biotechnology, Chungnam National University, Daejeon, Korea 2Division of Animal Bioscience and Integrated Biotechnology, Gyeongsang National University, Jinju, Korea 3Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Science, Seoul National University, Seoul, Korea 4Department of Animal science, Chungbuk National University, Cheongju, Korea 5Department of Animal Biotechnology, Dankook University, Cheonan, Korea 6Department of Animal Science, University of California, Davis, CA, USA
ORCID (for more information, please visit https://orcid.org)	Kyeong Il Park (https://orcid.org/0000-0002-3590-3993) Jinmu Ahn (https://orcid.org/0009-0005-1490-2974) Hansol Hwang (https://orcid.org/0009-0000-9698-5710) Yujeong Oh (https://orcid.org/0009-0003-2634-8240) Sangwoo Park (https://orcid.org/0000-0003-2288-1374) Younghoon Kim (https://orcid.org/0000-0001-6769-0657) Jin Ho Cho (https://orcid.org/0000-0001-7151-0778) Hyeun Bum Kim (https://orcid.org/0000-0003-1366-6090) Yanhong Liu (https://orcid.org/0000-0001-7727-4796) Hyunjin Kyoung (https://orcid.org/0000-0001-5742-5374) Minho Song (https://orcid.org/0000-0002-4515-5212)
Competing interests	No potential conflict of interest relevant to this article was reported.
Funding sources State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available.	This work was supported by research fund of Chungnam National University and the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (RS-2025-25418389).

Acknowledgements	Not applicable.
Availability of data and material	Upon reasonable request, the datasets of this study can be available from the corresponding author.
Authors' contributions Please specify the authors' role using this form.	Conceptualization: Song M. Data curation: Park KI, Kyoung H, Song M. Formal analysis: Ahn J, Hwang H, Oh Y, Kyoung H. Methodology: Song M. Software: Park KI, Kyoung H. Validation: Park S, Kim Y, Cho JH, Kim HB, Liu Y. Investigation: Park S, Kim Y, Liu Y. Writing - original draft: Park KI, Ahn J. Writing - review & editing: Park KI, Ahn J, Hwang H, Oh Y, Park S, Kim Y, Cho JH, Kim HB, Liu Y, Kyoung H, Song M.
Ethics approval and consent to participate	The experimental protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee of Chungnam National University, Daejeon, Republic of Korea (approval: #202103A-CNU-079).
Declaration of generative AI	No AI tools were used in this article.

4

5 **CORRESPONDING AUTHOR CONTACT INFORMATION**

For the corresponding author (responsible for correspondence, proofreading, and reprints)	Fill in information in each box below
First name, middle initial, last name	Hyunjin Kyoung Minho Song
Email address – this is where your proofs will be sent	hjkyoung@cnu.ac.kr mhsong@cnu.ac.kr
Secondary Email address	
Address	Department of Animal Science and Biotechnology, Chungnam National University, Daejeon 34134, Korea; Department of Animal Science, University of California, Davis, CA, USA Department of Animal Science and Biotechnology, Chungnam National University, Daejeon 34134, Korea
Cell phone number	
Office phone number	+82-42-821-7857 +82-42-821-5776
Fax number	

6

7

8 Abstract

9 This study was conducted to evaluate the effects of probiotic *Lactiplantibacillus plantarum* (*L.*
10 *plantarum*) on growth performance, blood profiles, serum immune responses, serum biochemical
11 parameters, intestinal morphology, and ileal gene expression of weaned pigs. A total of 90 weaned pigs
12 (initial body weight: 7.91 ± 0.83 kg) were assigned to two dietary treatments (5 pigs/pen; 9
13 replicates/treatment). The two dietary treatments consisted of a commercial corn and soybean meal-
14 based nursery diet (CON) and the same diet supplemented with 0.02% of dietary probiotic *L. plantarum*
15 (PLA). Pigs fed PLA tended to have higher average daily gain (ADG; $p = 0.066$), while G:F was higher
16 ($p < 0.05$) during days 1 to 7. The PLA group tended to have lower ($p = 0.078$) diarrhea frequency
17 during days 1 to 14. Pigs fed PLA tended to have lower ($p = 0.071$) HCT levels on day 7 and higher (p
18 < 0.05) HCT levels on day 28. Serum IL-10 concentrations tended to be higher ($p = 0.054$) in the PLA
19 group. Dietary PLA decreased crypt depth ($p < 0.05$) and increased villus height to crypt depth ratio
20 (VH:CD; $p < 0.05$) in the duodenum. Also, the ileal VH:CD ratio tended to be greater ($p = 0.093$) with
21 PLA supplementation. Goblet cell counts tended to be greater in the duodenum ($p = 0.086$) and were
22 greater in the jejunum ($p < 0.05$) in pigs fed PLA. On day 28, dietary PLA upregulated ($p < 0.05$) the
23 mRNA expression of *CLDN3* and *OCN* genes, while downregulating ($p < 0.05$) *TNFA*, *TGFB*, *IFNA*,
24 *IL1A*, *IL1B*, *IL6*, and *IL8* in the ileal mucosa. The present study suggested that dietary supplementation
25 with *L. plantarum* may support intestinal health of weaned pigs, as indicated by improved intestinal
26 morphology and altered expression of tight junction-related genes. In addition, probiotic *L. plantarum*
27 may have modulated systemic immune response and intestinal expression of inflammatory cytokine
28 genes.

29 **Keywords:** Growth performance, Intestinal health, Immune responses, *Lactiplantibacillus plantarum*,
30 Tight junction, Weaned pigs

Introduction

Weaning is a stressful event for piglets characterized by stressors such as separation from the sow, the transition from liquid to solid feed, and struggles for social dominance. These stressors reduce feed intake and increase local inflammatory responses [1,2]. Furthermore, because the piglets' digestive and immune systems are not yet fully developed [3,4], these challenges contribute to gastrointestinal tract (GIT) dysfunction. This dysfunction is characterized by decreased enzyme activation, an imbalance between epithelial cell proliferation and apoptosis, increased intestinal permeability, and reduced mucin production due to impaired cell differentiation [5,6]. Consequently, GIT dysfunction contributes to growth retardation and post-weaning diarrhea (PWD), leading to substantial economic losses in the swine industry. Furthermore, increasing restrictions on antibiotic growth promoters (AGPs) worldwide have stimulated the development of nutritional strategies to support intestinal health and growth performance in weaned pigs [7].

Recently, the concept of gut health in swine research has expanded beyond the traditional focus on nutrient digestion and absorption to include an integrated system of physical, chemical, immunological, and microbial barriers that protect the host from luminal antigens and pathogens [8]. This multi-layered defense system is essential for maintaining intestinal homeostasis and preventing the translocation of harmful toxins and bacteria into the systemic circulation [9]. Disruption of these barriers during the post-weaning period can impair nutrient utilization, exacerbate intestinal inflammatory responses, and increase susceptibility to GIT disorders. Therefore, nutritional interventions that enhance GIT barrier function have received considerable attention as strategies to improve growth and health in weaned pigs.

Lactiplantibacillus plantarum (*L. plantarum*) is a Gram-positive lactic acid bacterium widely recognized for its probiotic potential to enhance the health and growth of weaned pigs. *L. plantarum* has been reported to improve animal health via modulating immune responses and regulating gene expression [10–12]. Specifically, *L. plantarum* modulates production of inflammatory cytokines by interacting with specific cell wall components and regulating key signaling pathways, including toll-

like receptors (TLRs) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) [13]. Furthermore, *L. plantarum* has been shown to enhance intestinal integrity by regulating the gene expression of tight junction protein genes including the claudin (*CLDN*) family and occludin (*OCLN*), and certain strains of *L. plantarum* stimulate mucin secretion, thereby strengthening the intestinal chemical barrier against pathogenic infiltration [10]. However, despite these documented benefits, the effects of *L. plantarum* SKO-001 on inflammatory responses and intestinal barrier integrity in weaned piglets remain unclear. We hypothesized that dietary supplementation with *L. plantarum* SKO-001 would enhance growth performance and health in weaned pigs by modulating inflammatory responses and improving intestinal barrier functions. Therefore, this study evaluated the effects of dietary supplementation with *L. plantarum* SKO-001 on growth performance, systemic and local immune responses, and intestinal health of weaned pigs.

Materials and methods

Experimental design, animals, diets, and housing

On the day of weaning, a total of 90 crossbred piglets [(Landrace $\times$ Yorkshire) $\times$ Duroc; average initial body weight (BW) = 7.91 ± 0.83 kg; 28 days of age] were allocated to two dietary treatment groups with nine replicates of five pigs per pen in a randomized complete block design with initial BW as a block. The two dietary treatments were a commercial nursery diet based on corn and soybean meal with fish meal and spray-dried plasma (CON) and the CON supplemented with 0.02% of dietary probiotic *L. plantarum* (PLA). The commercial nursery diets were formulated to meet/exceed the nutritional requirements of weaned pigs as recommended by the National Research Council [14] (Table 1). The probiotic product supplied by Kolmar BNH, contained 1×10^{10} CFU/g of *L. plantarum* SKO-001. The strain was isolated from the root of *Angelica gigas* Nakai and deposited in the Korean Collection for Type Cultures (KCTC) at the Korea Research Institute of Bioscience and Biotechnology under accession number KCTC 14816BP. All treatments were fed *ad libitum* for four weeks in the mash form.

All pigs were housed in the same sized pens (232 × 175 cm; width × length) with plastic floor and automatic stainless-steel waterer. During the whole study, the climate of the pens was automatically ventilated and heated by the temperature controller with a setting of 25 to 28°C. The lighting was maintained on a 12 h light/dark cycle.

Data and sample collection

The BW of all pigs and feed consumption were recorded on day 1, 7, 14, and 28 and used to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F, feed efficiency) [15]. For the first two weeks after weaning, fecal condition was visually assessed in each pen by two independent evaluators to estimate diarrhea frequency. Fecal scores were assigned on a scale from 1 to 5 (1 = hard and dry feces, 2 = soft feces, 3 = moist feces, 4 = mild diarrhea, and 5 = watery severe diarrhea). The frequency of diarrhea was calculated as a percentage by counting a number of pen days with a fecal score higher than 3 [16]. On days 1, 7, 14, and 28, whole blood was obtained from one randomly selected pig in each pen using 10 mL ethylenediaminetetraacetic acid (EDTA)-containing blood tubes (BD Vacutainer Systems) for complete blood count analysis. Additional blood samples were collected from the same pigs on days 1 and 28 using non-EDTA tubes for serum analysis. Serum was separated by centrifugation (1580R; LaboGene) at $3,000 \times g$ for 15 min at 4°C and stored at -80°C until analysis [17]. Before euthanasia for necropsy, one pig per pen was randomly selected and anesthetized using 2 mL suxamethonium chloride (Succicholine Inj.; ILSUNG Pharmaceuticals) via intramuscular injection. Small intestinal samples were collected from pigs that were euthanized by exposure to carbon dioxide after anesthesia. Duodenal, jejunal, and ileal tissues of about 3 cm were collected, digests were removed, washed with distilled water, fixed in a 50 mL conical tube containing 10% neutral buffered formalin solution (BBC Biochemical), and stored until microscopy analysis. Other ileal tissues were stabilized in 1.5 mL microtube with RNAlater reagent (QIAGEN) for 24 hours at room temperature and stored at -80°C until gene expression analysis [18]. For intestinal morphology and ileal gene expression analyses, samples from six pigs per treatment were used as a subset of the pigs selected for necropsy.

109

110 ***Hematological, immunological, and biochemical parameters***

111 White blood cell (WBC) count, red blood cell (RBC) count, and hematocrit (HCT) were determined
112 using an automated hematology analyzer (scil Vet abc; scil animal care company) [19]. Serum
113 concentrations of tumor necrosis factor- α (TNF- α), transforming growth factor- β 1 (TGF- β 1), and
114 interleukin-10 (IL-10) were determined using enzyme-linked immunosorbent assay (ELISA) kits from
115 R&D Systems, while C-reactive protein (CRP) was measured using a kit from Aviva Systems Biology
116 and immunoglobulin G (IgG) and A (IgA) were measured using kits from Bethyl Laboratories. ELISA
117 procedures followed the manufacturers' protocols, and absorbance was determined with a microplate
118 reader (Epoch microplate spectrophotometer; BioTek Instruments) [20]. Serum biochemical parameters
119 were analyzed using an automated biochemical analyzer (Accute TBA-40FR; Toshiba) and purchased
120 reagents (Wako Pure Chemical Industries) for total protein, aspartate transaminase, alanine
121 transaminase, blood urea nitrogen, calcium, inorganic phosphorus, magnesium, total cholesterol,
122 triglyceride, glucose, albumin, and creatinine [21,22].

123

124 ***Intestinal morphology***

125 For the microscopic analysis, all fixed intestinal tissues were stained with hematoxylin and eosin (H&E)
126 and stored in slides with cover glass after deparaffinization and rehydration. The H&E slides were
127 examined by selecting ten intact villi and their associated crypts to estimate villus height, width, area,
128 crypt depth, villus height to crypt depth ratio (VH:CD), and the number of goblet cells. The slides were
129 scanned by fluorescence microscope (TE2000; Nikon). Measurements were performed using image
130 processing and analysis software (NIS-Elements BR software; Nikon).

131

132 ***Ileal gene expression***

133 Total RNA was extracted from the ileal tissues using HiGene Total RNA Prep kit (BIOFACT) and cDNA

was synthesized by using QuantiTect Reverse Transcription kit (QIAGEN). Quantitative real-time polymerase chain reaction analysis was performed on a StepOnePlus real-time PCR system (Applied Biosystems) with gene-specific designed primers (Table 2). The relative gene expression level was determined by the $2^{-\Delta\Delta C_t}$ method [23]. β -actin was used as an internal control to calculate target gene level. The encoded target genes, tight junction proteins and inflammatory cytokines, are as follows: (claudin-1 [*CLDN1*], claudin-2 [*CLDN2*], claudin-3 [*CLDN3*], claudin-4 [*CLDN4*], occludin [*OCLN*], tight junction protein-1 [*TJP1*], tumor necrosis factor- α [*TNFA*], transforming growth factor- β [*TGFB*], interferon- α [*IFNA*], interferon- γ [*IFNG*], interleukin-1 α [*IL1A*], interleukin-1 β [*IL1B*], interleukin-6 [*IL6*], and interleukin-8 [*IL8*]) [18].

Statistical analyses

Data were analyzed using the general linear model procedure of SAS (SAS Institute) in a randomized complete block design (block = initial BW). Experimental unit was the pen. Statistical model included dietary treatment as a main effect, initial BW as a covariate. Dietary treatment means were determined using the LSMEANS statement and compared using the PDIF option. Chi-square test was used to analyze the frequency of diarrhea. Statistical significance and tendency were considered at $p < 0.05$ and $0.05 \leq p < 0.10$, respectively.

Results

Growth performance and frequency of diarrhea

Pigs fed PLA tended to have higher ADG ($p = 0.066$) and had higher G:F ($p < 0.05$) than those fed CON during days 1 to 7. (Table 3). However, growth performance did not differ during the overall experimental period. The PLA diet tended to reduce ($p = 0.078$) the frequency of diarrhea during the first two weeks after weaning compared with the CON diet.

Hematological, biochemical, and immunological parameters

Pigs in the PLA group tended to have lower ($p = 0.071$) HCT levels on day 7 and higher ($p < 0.05$) HCT levels on day 28 than those in the CON group (Table 4). Serum IL-10 concentrations tended to be higher ($p = 0.054$) when dietary PLA was fed on day 28 (Table 5). However, no differences were observed in serum biochemical parameters (Table 6).

Intestinal morphology

Dietary PLA decreased ($p < 0.05$) crypt depth and increased ($p < 0.05$) VH:CD in the duodenum (Table 7). Also, ileal VH:CD tended to be higher ($p = 0.093$) when dietary PLA was fed. The PLA diet tended to increase ($p = 0.086$) the number of goblet cells in the duodenum and increased ($p < 0.05$) the number of goblet cells in the jejunum.

Ileal gene expression

On day 28, dietary PLA upregulated ($p < 0.05$) the mRNA expression of *CLDN3* and *OCLN* in the ileal mucosa (Figure 1). Pigs in the PLA group downregulated ($p < 0.05$) mRNA expression of cytokine genes, including *TNFA*, *TGFB*, *IFNA*, *IL1A*, *IL1B*, *IL6*, and *IL8* on day 28 compared with those in the CON group.

Discussion

In the swine industry, alleviating the reduction in productivity caused by weaning stress is considered a primary issue. Weaning stress, along with temporary anorexia, weakens the intestinal health of piglets and increases inflammatory cytokine production. Previous studies reported that while

these changes typically returned to normal within a short period after weaning, certain factors can have long-term effects [24,25]. In the present study, dietary PLA tended to increase ADG in the early weaned period, and tended to reduce the frequency of PWD for the first two weeks after weaning. These results suggest that dietary PLA may help alleviate critical changes during the immediate post-weaning period. These findings may provide a potential mechanism by which PLA impacts intestinal health and the immune system. Furthermore, previous studies reported that supplementation of *L. plantarum* in nursery diet improves growth performance and reduces frequency of diarrhea of weaned pigs. These results are consistent with our hypothesis [11,26]. Taken together, these findings partially support our hypothesis and suggest that *L. plantarum* may enhance the adaptation of pigs to weaning stress.

Generally, HCT levels serve as a reliable physiological indicator of hydration status because they directly reflect the proportion of blood volume occupied by red blood cells [27]. In the present study, the PLA group tended to have lower HCT levels on day 7 than the CON group. Meanwhile, no differences were observed in RBC counts between the treatments, suggesting that the lower HCT levels were not due to reduced RBC production. Together with the tendency toward a lower frequency of PWD during the first two weeks after weaning, these results suggest that dietary supplementation of *L. plantarum* may have attenuated dehydration associated with PWD. Because PWD is closely associated with disruption of intestinal barrier function and fluid loss, these findings suggest that dietary *L. plantarum* may be associated with improved physiological adaptation during the early post-weaning period. Conversely, HCT levels on day 28 were higher in the PLA group, while no differences were observed in other hematological parameters or serum biochemical parameters. Our findings indicate that the increased HCT levels were not accompanied by alterations in hematological and biochemical parameters. Although the physiological basis of this change is unclear, the contrasting HCT responses may indicate that the physiological effects of dietary *L. plantarum* differed according to post-weaning adaptation. Meanwhile, in the present study, serum IL-10 levels tended to be higher in pigs fed PLA on the last day of the study, suggesting that dietary *L. plantarum* may have modulated systemic immune response. These findings may indicate that beneficial effects of *L. plantarum* may have supported immune modulation during the subsequent adaptation phase by alleviating physiological stress after

weaning. A previous study reported that IL-10 plays a key role in maintaining intestinal epithelial integrity [28]. Furthermore, elevated IL-10 expression during the recovery phase has been shown to coincide with declining pro-inflammatory cytokine responses, supporting its role as an important regulator of immune functions [29]. Taken together with the intestinal health-related responses in our study, these results suggest that dietary *L. plantarum* may have supported immune homeostasis, thereby contributing to the maintenance of the intestinal barrier integrity during the post-weaning period. Morphological changes in the intestine, including the shortening of villi and expansion of crypts, occur due to weaning stress. These alterations reflect disrupted intestinal homeostasis resulting from reduced feed intake and altered cytokine secretion [6,30]. In the present study, dietary PLA decreased duodenal crypt depth compared with CON, and increased duodenal VH:CD and tended to increase ileal VH:CD. Because improved intestinal morphology is generally associated with enhanced digestive and absorptive capacity and reduced epithelial turnover, these findings suggest that dietary *L. plantarum* contributed to maintaining intestinal integrity during the post-weaning period. A previous study reported that dietary *L. plantarum* improves intestinal morphology through various mechanisms. Although gut microbiota were not evaluated in the present study, previous studies have suggested that one potential mechanism involves microbial modulation, whereby *L. plantarum* promotes beneficial bacterial populations and competitively inhibits pathogens [10]. Another proposed mechanism involves the production of probiotic-derived short-chain fatty acids, which serve as an energy source for intestinal epithelial cells and enhance barrier function [11]. Weaning stress impairs goblet cell differentiation, and consequently reduces mucin production [6]. Given that mucin serves as the primary line of defense for intestinal epithelial cells, this layered structure acts as an effective barrier against luminal contents [31]. Our findings showed that goblet cell counts tended to increase in the duodenum and were greater in the jejunum in pigs fed *L. plantarum*, suggesting a potential role in maintaining mucosal barrier integrity during the post-weaning period. Taken together, the changed intestinal morphology and increased goblet cell counts indicate that dietary *L. plantarum* may support intestinal structural adaptation during the post-weaning period. Weaning stress is accompanied by an inflammatory response, and an excessive inflammatory response is known to negatively affect the structure of the small intestine by damaging

tight junction structures, including transmembrane proteins such as claudins and occludin and their scaffolding protein zonula occludens-1 [25,32,33]. Because these proteins serve as the primary physical barrier against exogenous antigens and pathogens, their disruption induces a leaky gut and is intimately associated with the modulation of local and systemic immune systems [25,34]. Consistent with the systemic immune response in this study, gene expression changes were consistent with potential effects on barrier-related processes and attenuation of inflammatory signaling. Dietary supplementation with *L. plantarum* upregulated the expression of tight junction protein genes (*CLDN3* and *OCN*), suggesting a potential role in supporting intestinal barrier-related processes. In addition, PLA downregulated the expression of pro-inflammatory cytokine genes, consistent with attenuation of inflammatory signaling in the small intestine. However, intestinal permeability was not directly evaluated in the present study, and whether these molecular changes translate into improved intestinal barrier function remains to be determined. Downregulated expressions of pro-inflammatory cytokine genes may also contribute to the preservation of epithelial tight junctions, potentially supporting intestinal barrier integrity. Meanwhile, the PLA diet downregulated *TGFB* expression, although TGF- β is generally associated with anti-inflammatory responses. A previous study has shown that the expression of pro-inflammatory cytokine genes transiently increases during the weaning period, which subsequently induces a negative feedback loop that elevates *TGFB* [35]. Our finding of decreased *TGFB* expression in the PLA group may suggest that the inflammatory response had already been mitigated. While these results potentially reflect a resolved inflammatory response, further studies are required to clarify the precise kinetics of *TGFB* regulation during the post-weaning adaptation.

Conclusion

The present study demonstrated that dietary supplementation with *L. plantarum* SKO-001 showed beneficial effects on growth performance during the first week after weaning and potentially alleviated post-weaning diarrhea. These results were associated with improved intestinal morphology and altered expression of tight junction-related genes. Moreover, *L. plantarum* SKO-001 may have modulated

hematological profiles and serum inflammatory cytokines, while downregulating the expression of inflammatory cytokine genes in the small intestine. Collectively, these results suggest that dietary probiotic *L. plantarum* SKO-001 has potential as a functional feed additive to support physiological adaptation and intestinal health in weaned pigs. Additional investigations into gut microbiota and metabolomic profiles are warranted to further elucidate the mechanisms underlying the beneficial effects of dietary *L. plantarum* SKO-001.

References

- 269 1. McCracken BA, Spurlock ME, Roos MA, Zuckermann FA, Gaskins HR. Weaning Anorexia May Contribute
270 to Local Inflammation in the Piglet Small Intestine. *J Nutr.* 1999;129:613–9. <https://doi.org/10.1093/JN/129.3.613>
- 271 2. Pluske JR. Invited review: Aspects of gastrointestinal tract growth and maturation in the pre- and postweaning
272 period of pigs. *J Anim Sci.* 2016;94:399–411. <https://doi.org/10.2527/JAS.2015-9767>
- 273 3. Stokes CR, Bailey M, Haverson K, Harris C, Jones P, Inman C, et al. Postnatal development of intestinal
274 immune system in piglets: implications for the process of weaning. *Anim Res.* 2004;53:325–34.
275 <https://doi.org/10.1051/ANIMRES:2004020>
- 276 4. Wijtten PJA, Meulen J Van Der, Verstegen MWA. Intestinal barrier function and absorption in pigs after
277 weaning; a review. *Br J Nutr.* 2011;105:967–81. <https://doi.org/10.1017/S0007114510005660>
- 278 5. Lallès J-P, Boudry G, Favier C, Le Floc'h N, Luron I, Montagne L, et al. Gut function and dysfunction in young
279 pigs: physiology. *Anim Res.* 2004;53:301–16.
- 280 6. Tang X, Xiong K, Fang R, Li M. Weaning stress and intestinal health of piglets: A review. *Front Immunol.*
281 2022;13:1042778. <https://doi.org/10.3389/FIMMU.2022.1042778/FULL>
- 282 7. Casewell M, Friis C, Marco E, McMullin P, Phillips I. The European ban on growth-promoting antibiotics and
283 emerging consequences for human and animal health. *J Antimicrob Chemother.* 2003;52:159–61.
284 <https://doi.org/10.1093/JAC/DKG313>
- 285 8. Pluske JR, Turpin DL, Kim JC. Gastrointestinal tract (gut) health in the young pig. *Anim Nutr.* 2018;4:187–96.
286 <https://doi.org/10.1016/J.ANINU.2017.12.004>
- 287 9. Modina SC, Polito U, Rossi R, Corino C, Di Giancamillo A. Nutritional regulation of gut barrier integrity in
288 weaning piglets. *Animals.* 2019; 9(12):1045. <https://doi.org/10.3390/ani9121045>
- 289 10. Kyoung H, Park KI, Shin S, Ahn J, Nam J, et al. Probiotic *Lactiplantibacillus plantarum* improved growth
290 performance of weaned pigs by enhancing intestinal health and modulating immune responses. *J Anim Sci*
291 *Technol.* 2026;68:541–61. <https://doi.org/10.5187/JAST.2025.E22>
- 292 11. Wang T, Teng K, Liu Y, Shi W, Zhang J, Dong E, et al. *Lactobacillus plantarum* PFM 105 promotes intestinal

development through modulation of gut microbiota in weaning piglets. *Front Microbiol.* 2019;10:437106.
<https://doi.org/10.3389/fmicb.2019.00090>

12. Rocchetti MT, Russo P, Capozzi V, Drider D, Spano G, Fiocco D. Bioprospecting Antimicrobials from
Lactiplantibacillus plantarum: Key Factors Underlying Its Probiotic Action. *Int J Mol. Sci.* 2021;22:12076.
<https://doi.org/10.3390/IJMS222112076>

13. Wang S, Ahmadi S, Nagpal R, Jain S, Mishra SP, Kavanagh K, et al. Lipoteichoic acid from the cell wall of a
heat killed *Lactobacillus paracasei* D3-5 ameliorates aging-related leaky gut, inflammation and improves physical
and cognitive functions: from *C. elegans* to mice. *GeroScience.* 2019;42:333–52. [https://doi.org/10.1007/S11357-](https://doi.org/10.1007/S11357-019-00137-4)
019-00137-4

14. National Research Council. Nutrient Requirements of Swine. 11th revised edition. Washington, D.C.: National
Academies Press; 2012. <https://doi.org/10.17226/13298>

15. Park S, Choe J, Cho JH, Jang KB, Kyoung H, Park K Il, et al. Determination of optimal energy system and
level for growing pigs. *J Anim Sci Technol.* 2024;66:514–22. <https://doi.org/10.5187/jast.2023.e63>

16. Nam J, Kim JN, Kim HB, Cho JH, Kim Y, Ahn J, et al. Effects of dietary aluminosilicate on growth
performance, frequency of diarrhea, and blood profiles of weaned pigs. *J Anim Sci Technol.* 2025;67:375–82.
<https://doi.org/10.5187/JAST.2024.E21>

17. Kyoung H, Kang Y, Ahn J, Cho JH, Seo D, Nam J, et al. Evaluation of dietary selenium sources and levels on
growth performance, carcass characteristics, selenium concentrations, and blood biochemistry of growing-
finishing pigs. *J Anim Sci Technol.* 2025;67:607–18. <https://doi.org/10.5187/JAST.2024.E53>

18. Kyoung H, Kim E, Cho JH, Lee H, Kim Y, Park K Il, et al. Dietary yeast cell wall enhanced intestinal health
of broiler chickens by modulating intestinal integrity, immune responses, and microbiota. *Poult Sci.*
2023;102:102660. <https://doi.org/10.1016/J.PSJ.2023.102660>

19. Nam J, Cho JH, Kim HB, Ahn J, Kang Y, Kim S, et al. Dietary coated copper and zinc improved growth
performance by modulating immune responses and fecal microbiota of weaned pigs. *J Anim Sci Technol.*
2025;67:1050–66. <https://doi.org/10.5187/JAST.2024.E70>

20. Ahn J, Cho JH, Kim HB, Nam J, Kang Y, Shin I, et al. Effects of dietary xylanase supplementation on growth
performance and gut health of weaned pigs fed a high non-starch polysaccharides diet. *J Anim Sci Technol.*

2026;68:841–56. <https://doi.org/10.5187/JAST.2025.E32>

21. Kim S, Cho JH, Kim HB, Song M. Evaluation of brown rice to replace corn in weanling pig diet. *J Anim Sci Technol.* 2021;63:1344–54. <https://doi.org/10.5187/JAST.2021.E112>

22. Kang Y, Kim Y, Cho JH, Kim HB, Ahn J, Kim MK, et al. Age-related changes in growth and fecal parameters, nutrient digestibility, hematology, and serum biochemistry of Beagle dogs. *J Anim Sci Technol.* 2025;67:1165–80. <https://doi.org/10.5187/JAST.2500227>

23. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2- $\Delta\Delta$ CT method. *Methods.* 2001;25:402–8. <https://doi.org/10.1006/meth.2001.1262>

24. Boudry G, Péron V, Le Huërou-Luron I, Lallès JP, Sève B. Weaning Induces Both Transient and Long-Lasting Modifications of Absorptive, Secretory, and Barrier Properties of Piglet Intestine. *J Nutr.* 2004;134:2256–62. <https://doi.org/10.1093/JN/134.9.2256>

25. Hu CH, Xiao K, Luan ZS, Song J. Early weaning increases intestinal permeability, alters expression of cytokine and tight junction proteins, and activates mitogen-activated protein kinases in pigs. *J Anim Sci.* 2013;91:1094–101. <https://doi.org/10.2527/jas.2012-5796>

26. Han J, Ding L, Zhao X, Liu J, Yin J, Wang Q, et al. Direct interaction between *Lactiplantibacillus plantarum* ZJ316-derived lipoteichoic acid and TLR2 mediates anti-inflammatory and barrier-protective effects in intestinal cells. *Food Funct.* 2025;16:6560–75. <https://doi.org/10.1039/d5fo01925g>

27. Rincker MJ, Hill GM, Link JE, Rowntree JE. Effects of dietary iron supplementation on growth performance, hematological status, and whole-body mineral concentrations of nursery pigs. *J Anim Sci.* 2004;82:3189–97. <https://doi.org/10.2527/2004.82113189x>

28. Al-Sadi R, Boivin M, Ma T. Mechanism of cytokine modulation of epithelial tight junction barrier. *Front Biosci.* 2009;14:2765–78. <https://doi.org/10.2741/3413>

29. Kruse R, Essén-Gustavsson B, Fossum C, Jensen-Waern M. Blood concentrations of the cytokines IL-1 β , IL-6, IL-10, TNF- α and IFN- γ during experimentally induced swine dysentery. *Acta Vet Scand* 2008;50:32-. <https://doi.org/10.1186/1751-0147-50-32>

30. Lallès J-P, Bosi P, Smidt H, Stokes CR. Weaning—A challenge to gut physiologists. *Livest Sci.* Elsevier;

346 2007;108:82–93.

347 31. Gustafsson JK, Johansson MEV. The role of goblet cells and mucus in intestinal homeostasis. *Nat Rev*
348 *Gastroenterol Hepatol*. 2022;19:785–803. <https://doi.org/10.1038/s41575-022-00675-x>

349 32. Wang J, Ji H. Tight Junction Proteins in the Weaned Piglet Intestine: Roles and Regulation. *Curr Protein Pept*
350 *Sci* 2019;20:652–60. <https://doi.org/10.2174/1389203720666190125095122>

351 33. Pié S, Lallès JP, Blazy F, Laffitte J, Sève B, Oswald IP. Weaning Is Associated with an Upregulation of
352 Expression of Inflammatory Cytokines in the Intestine of Piglets. *J Nutr*. 2004;134:641–7.
353 <https://doi.org/10.1093/jn/134.3.641>

354 34. Suzuki T. Regulation of intestinal epithelial permeability by tight junctions. *Cell Mol Life Sci*. 2012;70:631–
355 59. <https://doi.org/10.1007/s00018-012-1070-x>

356 35. Groot N De, Fariñas F, Cabrera-Gómez CG, Pallares FJ, Ramis G. Weaning causes a prolonged but transient
357 change in immune gene expression in the intestine of piglets. *J Anim Sci*. 2021;99:1–12.
358 <https://doi.org/10.1093/JAS/SKAB065>

359

Tables and Figures

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

Item	Basal diet
Ingredient, %	
Corn	38.90
Soybean meal, 44%	33.34
Whey permeate	20.00
Spray dried porcine plasma	1.00
Fish meal	2.18
Soybean oil	1.50
Limestone	0.83
Dicalcium phosphate	1.10
Iodized salt	0.20
Vitamin-mineral premix ¹	0.35
Lysine-HCl	0.30
DL-methionine	0.17
L-Threonine	0.13
Total	100.00
Calculated energy and nutrient contents	
Dry matter, %	90.51
Metabolizable energy, kcal/kg	3381.65
Crude protein, %	21.36
SID ² Lysine, %	1.25
SID ² Methionine, %	0.43
SID ² Cysteine + Methionine, %	0.69
SID ² Tryptophan, %	0.23
Calcium, %	0.77
Total phosphate, %	0.61
STTD ³ Phosphorus, %	0.36
ATTD ⁴ Phosphorus, %	0.32

¹ Provided per kilogram of diet: vitamin A, 12,000 IU; vitamin D₃, 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 B₁₂, 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.

366 ²SID, standardized ileal digestible.

367 ³STTD, standardized total tract digestible.

368 ⁴ATTD, apparent total tract digestible.

369

Table 2. Gene specific primers for gene expression of tight junction proteins and inflammatory cytokines of ileal tissue.

Item ¹	Forward (5'-3')	Reverse (5'-3')
<i>CLDN1</i>	AGAAGATGCGGATGGCTGTC	CCCAGAAGGCAGAGAGAAGC
<i>CLDN2</i>	TCCTCCCTGTTCTCCCTGATAG	CCTTGCAGTGGGCAGGAA
<i>CLDN3</i>	GATGCAGTGCAAAGTGTACGA	GTCCTGCACGCAGTTGGT
<i>CLDN4</i>	TATCATCCTGGCCGTGCTA	CATCATCCACGCAGTTGGT
<i>OCN</i>	GGAGTGATTCTGGATTCTGTCTATGCT	CGCCTGGGCTGTTGGGTTGA
<i>TJP1</i>	GGAGCATTGAAAGAAGCA	TGACAGGTAGGACAGACGA
<i>TNFA</i>	CTTGGGTTTGGATTCTCTGGAT	CTTCCCTGGCAGCCACAT
<i>TGFB</i>	CACGTGGAGCTATACCAGAA	TCCGGTGACATCAAAGGACA
<i>IFNA</i>	CCCCTGTGCCTGGGAGAT	AGGTTTCTGGAGGAAGAGAAGGA
<i>IFNG</i>	TGGTAGCTCTGGGAACTGAATG	GGCTTTGCGCTGGATCTG
<i>IL1A</i>	GTGCTCAAAACGAAGACGAACC	CATATTGCCATGCTTTTCCCAGAA
<i>IL1B</i>	GCCCTGTACCCCAACTGGTA	CCCAGGAAGACGGGCTTT
<i>IL6</i>	GCGCAGCCTTGAGGATTTC	CCCAGCTACATTATCCGAATGG
<i>IL8</i>	GCTCTCTGTGAGGCTGCAGTTC	AAGGTGTGGAATGCGTATTTATGC
<i>ACTB</i>	CTACGTCGCCCTGGACTTC	GATGCCGCAGGATTCCAT

¹*CLDN1*, claudin-1; *CLDN2*, claudin-2; *CLDN3*, claudin-3; *CLDN4*, claudin-4; *OCN*, occludin; *TJP1*, tight junction protein-1; TNF- α , tumor necrosis factor- α ; *TGFB*, transforming growth factor- β ; *IFNA*, interferon- α ; *IFNG*, interferon- γ ; *IL1A*, interleukin-1 α ; *IL1B*, interleukin-1 β ; *IL6*, interleukin-6; *IL8*, interleukin-8; *ACTB*, β -actin.

Table 3. Effects of dietary *Lactiplantibacillus plantarum* on growth performance and frequency of diarrhea of weaned pigs¹

Item ²	CON	PLA	SEM	p-value
BW, kg				
Day 1	7.91	7.92	0.29	0.987
Day 7	8.93	9.27	0.32	0.461
Day 14	11.19	11.75	0.45	0.382
Day 28	17.21	18.31	0.66	0.254
ADG, g/d				
Day 1 to 7	145.24	193.79	17.41	0.066
Day 8 to 14	322.54	354.18	28.89	0.450
Day 1 to 14	233.89	273.15	20.74	0.199
Day 15 to 28	430.56	468.65	18.98	0.175
Day 1 to 28	332.22	371.32	16.16	0.107
ADFI, g/d				
Day 1 to 7	240.83	266.23	14.30	0.227
Day 8 to 14	429.37	459.95	23.47	0.371
Day 1 to 14	335.10	363.09	16.72	0.254
Day 15 to 28	748.46	782.67	18.00	0.198
Day 1 to 28	541.78	565.41	13.99	0.250
G:F, g/g				
Day 1 to 7	0.583	0.723	0.045	0.044
Day 8 to 14	0.750	0.765	0.042	0.796
Day 1 to 14	0.691	0.745	0.032	0.248
Day 15 to 28	0.574	0.599	0.021	0.405
Day 1 to 28	0.611	0.657	0.020	0.125
Frequency of diarrhea³, %				
Day 1 to 7	31.75	17.46		0.147
Day 8 to 14	9.52	4.76		0.334
Day 1 to 14	20.63	11.11		0.078

¹Each value is the mean value of 9 replicates (5 pigs/pen).

²CON, weaner diet based on corn and soybean meal with fish meal and spray-dried plasma; PLA, CON + 0.02% probiotic *L. plantarum*; SEM, standard error of mean; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G:F, gain to feed ratio; Frequency of diarrhea (%) = (number of fecal score higher than 3 / number of pen days) × 100.

Table 4. Effects of dietary *Lactiplantibacillus plantarum* on blood profiles of weaned pigs¹

Item ²	CON	PLA	SEM	p-value
White blood cell, ×10³/μL				
Day 1	11.95	14.67	1.62	0.263
Day 7	20.47	16.96	1.42	0.100
Day 14	23.36	23.03	2.38	0.925
Day 28	25.07	25.19	2.62	0.974
Red blood cell, ×10⁶/μL				
Day 1	5.56	4.68	0.40	0.155
Day 7	6.03	7.45	0.83	0.245
Day 14	5.09	5.63	0.59	0.525
Day 28	6.06	6.15	0.16	0.700
Hematocrit, %				
Day 1	31.53	29.15	1.37	0.248
Day 7	35.37	28.74	2.42	0.071
Day 14	30.44	25.20	2.28	0.124
Day 28	29.88	32.21	0.72	0.035

¹Each value is the mean value of 9 replicates (1 pig/pen).

²CON, weaner diet based on corn and soybean meal with fish meal and spray-dried plasma;
PLA, CON + 0.02% probiotic *L. plantarum*; SEM, standard error of mean.

Table 5. Effects of dietary *Lactiplantibacillus plantarum* on serum immune responses of weaned pigs¹

Item ²	CON	PLA	SEM	p-value
TNF-α, pg/mL				
Day 1	152.62	149.20	1.84	0.319
Day 28	130.74	101.78	15.98	0.218
TGF-β1, pg/mL				
Day 1	2,025.28	1,947.99	57.51	0.442
Day 28	2,185.99	1,859.00	209.51	0.286
IL-10, pg/mL				
Day 1	28.49	44.74	14.51	0.511
Day 28	82.31	182.53	34.06	0.054
CRP, ng/mL				
Day 1	14.41	18.39	3.13	0.463
Day 28	114.15	107.07	6.75	0.468
IgG, mg/mL				
Day 1	104.33	104.16	3.01	0.972
Day 28	300.54	298.92	4.13	0.785
IgA, mg/mL				
Day 1	0.69	0.67	0.09	0.869
Day 28	1.33	1.11	0.15	0.301

¹Each value is the mean value of 9 replicates (1 pig/pen).

²CON, weaner diet based on corn and soybean meal with fish meal and spray-dried plasma; PLA, CON + 0.02% probiotic *L. plantarum*; SEM, standard error of mean; TNF- α , tumor necrosis factor- α ; TGF- β 1, transforming growth factor- β 1; IL-10, interleukin-10; CRP, c-reactive protein; IgG, immunoglobulin G; IgA, immunoglobulin A.

Table 6. Effects of dietary *Lactiplantibacillus plantarum* on serum biochemical parameters of weaned pigs¹

Item ²	CON	PLA	SEM	p-value
TP, g/dL	5.37	5.16	0.16	0.371
AST, IU/L	43.60	48.40	5.96	0.585
ALT, IU/L	30.60	31.20	3.74	0.912
BUN, mg/dL	8.37	7.87	0.77	0.659
Ca, mg/dL	9.77	10.21	0.18	0.129
IP, mg/dL	10.40	9.79	0.31	0.207
Mg, mg/dL	2.22	2.13	0.07	0.417
T-Chol, mg/dL	75.10	70.10	4.77	0.480
TG, mg/dL	37.20	46.00	7.75	0.445
GLU, mg/dL	93.00	101.80	4.46	0.201
ALB, g/dL	3.47	3.28	0.18	0.476
Crea, mg/dL	0.94	0.94	0.04	0.999

¹Each value is the mean value of 9 replicates (1 pig/pen).

²CON, weaner diet based on corn and soybean meal with fish meal and spray-dried plasma; PLA, CON + 0.02% probiotic *L. plantarum*; SEM, standard error of mean; TP, total protein; AST, aspartate transaminase; ALT, alanine transaminase; BUN, blood urea nitrogen; Ca, calcium; IP, inorganic phosphorus; Mg, magnesium; T-Chol, total cholesterol; TG, triglyceride; GLU, glucose; ALB, albumin; Crea, creatinine.

404 **Table 7.** Effects of dietary *Lactiplantibacillus plantarum* on intestinal morphology of weaned pigs¹

Item ²	CON	PLA	SEM	p-value
Duodenum				
Villus height, μm	351.51	362.49	11.70	0.506
Crypt depth, μm	298.98	224.98	16.48	0.009
VH:CD, $\mu\text{m}/\mu\text{m}$	1.19	1.63	0.08	0.002
Villus width, μm	140.69	163.99	18.76	0.383
Villus area, μm^2	50,848	54,864	4,270	0.505
Goblet cell, n	8.52	13.48	1.90	0.086
Jejunum				
Villus height, μm	400.83	418.45	19.17	0.514
Crypt depth, μm	299.83	301.49	19.67	0.952
VH:CD, $\mu\text{m}/\mu\text{m}$	1.34	1.42	0.09	0.527
Villus width, μm	168.27	166.99	9.01	0.918
Villus area, μm^2	47,278	49,771	5,374	0.740
Goblet cell, n	10.30	13.45	0.84	0.022
Ileum				
Villus height, μm	369.17	394.06	45.71	0.697
Crypt depth, μm	325.75	303.94	30.91	0.615
VH:CD, $\mu\text{m}/\mu\text{m}$	1.14	1.28	0.06	0.093
Villus width, μm	157.46	150.59	4.13	0.251
Villus area, μm^2	47,084	44,010	6,906	0.750
Goblet cell, n	16.90	14.10	2.80	0.480

405 ¹Each value is the mean value of 6 replicates (1 pig/pen).

406 ²CON, weaner diet based on corn and soybean meal with fish meal and spray-dried plasma;
 407 PLA, CON + 0.02% probiotic *L. plantarum*; SEM, standard error of mean; VH:CD, villus height to
 408 crypt depth ratio.

409

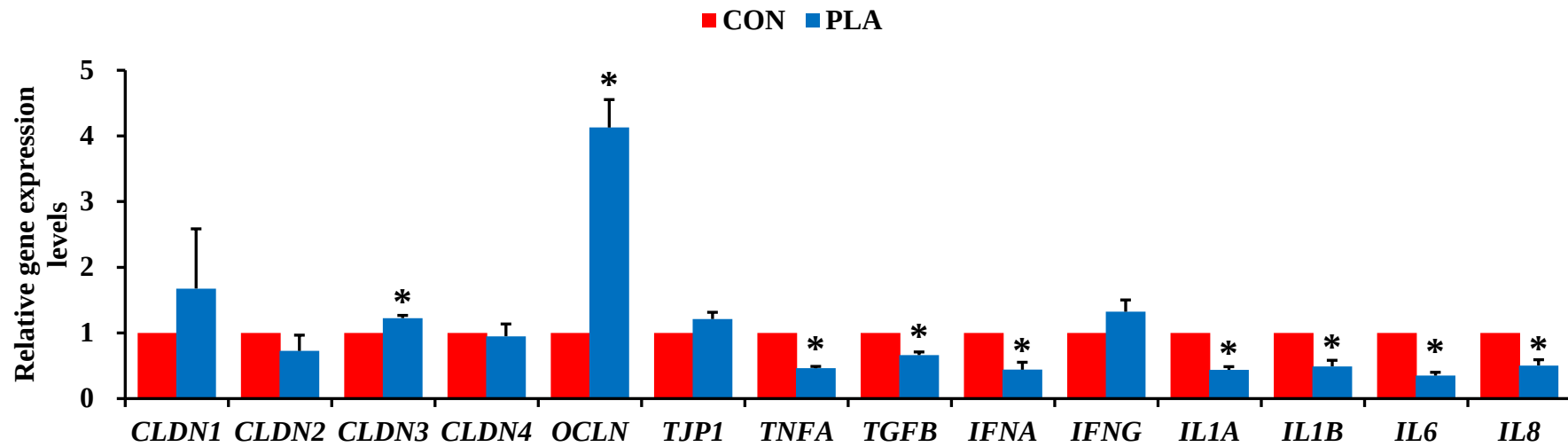


Figure 1. Effects of dietary *Lactiplantibacillus plantarum* on ileal gene expression of weaned pigs. Each value is the mean value of 6 replicates (1 pig/pen). *Statistical difference between dietary treatments ($p < 0.05$). CON, weaner diet based on corn and soybean meal with fish meal and spray-dried plasma; PLA, CON + 0.02% probiotic *L. plantarum*; *CLDN1*, claudin-1; *CLDN2*, claudin-2; *CLDN3*, claudin-3; *CLDN4*, claudin-4; *OCLN*, occludin; *TJP1*, tight junction protein-1; *TNFA*, tumor necrosis factor- α ; *TGFB*, transforming growth factor- β ; *IFNA*, interferon- α ; *IFNG*, interferon- γ ; *IL1A*, interleukin-1 α ; *IL1B*, interleukin-1 β ; *IL6*, interleukin-6; *IL8*, interleukin-8.