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Effects of *Lactiplantibacillus plantarum* on growth performance, physiological responses, and gut microbiota composition in pigs

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Abstract

This study evaluated the effects of continuous supplementation with *Lactiplantibacillus plantarum* (*L. plantarum*) on growth performance, physiological responses, and gut microbiota composition in pigs under commercial farm conditions. A total of 200 healthy crossbred pigs [(Landrace × Yorkshire) × Duroc], LYD; 4 weeks of age; initial body weight (BW) 6.92 ± 1.88 kg] were assigned to either a control (CON) or treatment (TRT) group using a randomized complete block design (block = initial BW; 10 pens/treatment; 10 pigs/pen), with pen as the experimental unit. Pigs in the TRT received *L. plantarum* in their drinking water via a proportional dosing pump. The daily dose was 3×10^7 CFU/pig/day during the weaning period (4–8 weeks of age) and 4×10^7 CFU/pig/day during the growing and finishing period (8–23 weeks of age), respectively**.** Individual BW and pen-level feed intake were recorded to calculate average daily gain (ADG). Blood (1 pig/pen) and fecal samples (2 pigs/pen) were collected from pigs randomly selected within each pen at four defined time points: 4, 8, 12, and 23 weeks of age. Physiological responses were assessed through the evaluation of hematological and biochemical parameters. For gut microbiome profiling, the V3–V4 region of the 16S rRNA gene was sequenced using the Illumina MiSeq platform. Sequencing reads were processed using QIIME2 and analyzed using MicrobiomeAnalyst. Compared with CON, the TRT showed higher BW and ADG at 8 weeks of age, however, no significant differences were observed at 12 and 23 weeks of age. No significant differences in hematological, biochemical, or immunological parameters were observed between the groups. Analysis of fecal microbiota revealed a higher relative abundance of *Selenomonas* in the TRT group at 8 weeks of age and an enrichment of *Butyrivibrio* at 23 weeks of age. These findings suggest that continuous supplementation with *L. plantarum* supports early growth performance and promotes beneficial, SCFA-associated microbial shifts, while also supporting the possibility that *L. plantarum* contributes to stabilization of the gut microbiota.

Keywords (3 to 6):

Pig, *Lactiplantibacillus plantarum*, Growth performance, Probiotics, Gut microbiome

Introduction

With increasing restrictions and bans on the use of antibiotic growth promoters (AGPs) worldwide, interest in alternative strategies has grown. Among these alternatives, probiotics are widely used as feed additives in swine production to improve growth performance and enhance disease resistance [1, 2]. Commonly used probiotics in pig diets include lactic acid bacteria such as *Lactobacillus* spp., spore-forming *Bacillus* spp., *Enterococcus faecium*, the yeast *Saccharomyces cerevisiae*, and the butyrate-producing bacterium *Clostridium butyricum* [3]. Among these microorganisms, *Lactiplantibacillus plantarum* (*L. plantarum*) has demonstrated promising probiotic properties, including tolerance to acidic conditions and bile salts, the ability to adhere to intestinal epithelial cells, and antimicrobial activity against enteric pathogens through mechanisms such as competitive exclusion and bacteriocin production [4, 5]. Previous studies have reported that *L. plantarum* produces organic acids, including lactic and acetic acids, during fermentation and may modulate host immune responses through changes in intestinal short-chain fatty acid (SCFA) concentrations. These effects may help alleviate diarrhea in piglets and improve growth performance and feed efficiency [6, 7]. Beyond the nursery phase, *L. plantarum* supplementation has also been reported to improve growth performance in growing–finishing pigs, suggesting its potential as an alternative to AGPs [7].

However, the physiological conditions and intestinal microbial environment of pigs change substantially throughout the production cycle, particularly during the transitions from weaning to the growing and finishing phases [8]. Each production stage is characterized by distinct nutritional requirements, dietary compositions, and environmental stressors, which are accompanied by considerable changes in gut microbiota composition [8, 9]. These changes may influence nutrient utilization, microbial fermentation, intestinal barrier function, and host immune responses, all of which are closely associated with animal health and growth performance [10-13]. Such stage-related shifts in the gut microbiota may also alter the intestinal environment in which probiotics exert their effects, potentially contributing to differences in probiotic responses across production phases [14]. Because the beneficial effects of probiotics are mediated, at least in part, through interactions with the intestinal microbial ecosystem, their efficacy may vary depending on the growth stage. Therefore, evaluating probiotic

supplementation across multiple production phases may provide a more comprehensive understanding of stage-dependent responses [8].

Furthermore, probiotic efficacy in pigs may be influenced by farm-specific factors, including management practices, environmental conditions, and baseline gut microbiota composition [14, 15]. Previous studies have frequently evaluated probiotic effects over relatively short experimental periods or under controlled conditions, whereas evidence regarding continuous supplementation across multiple production phases under commercial farm conditions remains limited [14, 16]. Evaluating probiotics under commercial conditions is particularly important because such studies account for the complex environmental and management factors encountered in practical swine production [15, 17].

Accordingly, the present study investigated the effects of *L. plantarum* supplementation on growth performance, physiological responses, and gut microbiota composition across multiple production phases in pigs raised under commercial farm conditions. By examining these outcomes from weaning through finishing, this study aimed to characterize growth-stage-dependent responses to *L. plantarum* supplementation and evaluate its potential applicability as a probiotic additive in commercial swine production.

Materials and Methods

All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Dankook University (approval No. DKU-22-029).

Experimental design and animal management

This study enrolled 200 healthy commercial crossbred pigs [(Landrace × Yorkshire) × Duroc; LYD, 4 weeks of age; initial weight (BW) 6.92 ± 1.88 kg]. The pigs were raised under standard commercial farm conditions throughout the weaning, growing, and finishing phases. Pigs were randomly allotted to two treatments (CON and TRT) in a randomized complete block design (block = initial body weight), with 10 pens per treatment and 10 pigs per pen; the pen was considered the experimental unit. The TRT group received *L. plantarum* in drinking water throughout the experimental phase using a proportional dosing pump (Dosatron®, Tresses, France). The daily dose was 3×10^7 CFU/pig/day during the weaning period (4–8 weeks of age) and 4×10^7 CFU/pig/day

during the growing and finishing period (8–23 weeks of age), respectively. The dosing regimen followed the recommendation of the manufacturer, the Anseong-si Agricultural Technology Center (Anseong, Republic of Korea). All pigs were fed a standard commercial diet (Table 1) with ad libitum access to feed and water.

Sample collection

Blood (1 pig/pen) and fecal samples (2 pigs/pen) were collected from pigs randomly selected within each pen at four defined time points: 4, 8, 12, and 23 weeks of age. Blood samples were collected via jugular venipuncture using 5-mL vacutainer tubes, with or without ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Whole blood samples collected in EDTA-containing tubes were stored at 4 °C and used for hematological analysis. Samples collected without EDTA were allowed to clot at room temperature for 2 h and then centrifuged at 3,000 × g for 15 min at 4 °C to obtain serum. The separated serum samples were stored at −80 °C until analysis. Fecal samples were collected by inserting a sterile swab approximately 2–3 cm into the rectum and gently rotating for about 10 s. Collected fecal samples were stored at −80 °C until use.

Growth Performance

To evaluate growth performance, pigs were individually weighed at the beginning and end of each phase; the study spanned 28 days during the weaning phase (4–8 weeks of age), 28 days during the growing phase (8–12 weeks of age), and 77 days during the finishing phase (12–23 weeks of age) The average daily gain (ADG) was calculated for each interval.

Physiological responses

Physiological responses were assessed through the evaluation of hematological and biochemical parameters in both the CON and TRT pig groups.

Whole blood samples collected in EDTA-containing tubes were used for complete blood count (CBC) analysis with an automated hematology analyzer (Scil Vet abc, Scil Animal Care Company, Altorf, France) calibrated for pig blood. The hematological parameters measured included white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb), hematocrit (HCT), lymphocyte (LYM), monocyte (MON), eosinophil (EOS), basophil (BAS), and segmented neutrophil (SEG). Serum separated from blood samples collected without EDTA was used

to quantify the concentrations of immunoglobulin A (IgA) and tumor necrosis factor-α (TNF-α) using commercial enzyme-linked immunosorbent assay (ELISA) kits (TNF-α: R&D Systems, Inc., Minneapolis, USA; IgA: Abnova Corp., Taipei City, Taiwan), according to the manufacturers' protocols.

To assess the effects of *L. plantarum* supplementation, serum biochemical parameters were analyzed at 8 weeks of age using validated commercial assay kits (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The measured indices included total protein (TP), blood urea nitrogen (BUN), creatinine (CRE), total cholesterol (TCHO), triglycerides (TG), albumin (ALB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (TBIL), calcium (Ca), inorganic phosphorus (IP), and magnesium (Mg).

Gut Microbiota

Approximately 0.11 g of each fecal sample was used for total DNA extraction using the QIAamp® Fast DNA Stool Mini Kit (Qiagen, Valencia, CA, USA), following the manufacturer's protocol. Only samples with an optical density ratio (OD260/280) between 1.80 and 2.11 were selected to ensure high-quality DNA. The V3–V4 hypervariable regions of the bacterial 16S rRNA gene were amplified by polymerase chain reaction (PCR) using universal primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3'), producing amplicons of approximately 470 bp. The PCR products were purified using the Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA).

Sequencing was performed using the Illumina MiSeq platform. Raw reads were subjected to quality control using FastQC software. Subsequently, denoising, chimera removal, and feature table construction were conducted using the Deblur algorithm implemented in the QIIME2 pipeline. Microbial diversity was evaluated by calculating α- diversity metrics such as observed operational taxonomic units (OTUs) and the Shannon diversity index. Beta diversity was assessed using both weighted and unweighted UniFrac distances and visualized through principal coordinates analysis (PCoA). Taxonomic classification was performed using the Ribosomal Database Project (RDP) Naive Bayesian Classifier version 2.13 (reference database: RDP training set v18), with a confidence threshold of 0.7, and relative abundances were compared between groups to identify differentially abundant taxa.

Statistical analysis

The experiment followed a randomized complete block design using initial BW as the block, and each pen was the experimental unit. Statistical analyses were performed using the General Linear Model procedure (PROC GLM) in SAS (version 9.0). The statistical model included dietary treatment (control vs. *L. plantarum* supplementation) as a fixed effect to evaluate its impact on growth performance and physiological responses. The statistical models for growth performance, health indicators included dietary treatment as a fixed effect.

Microbial α-diversity indices (Shannon, Simpson, observed OTUs, and Chao1) for each dietary treatment group were computed using the MicrobiomeAnalyst platform (https://www.microbiomeanalyst.ca), differences in α-diversity among groups were assessed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. Beta diversity was evaluated based on both weighted and unweighted UniFrac distance matrices, and intergroup differences were assessed using analysis of similarities (ANOSIM) [18, 19]. All statistical analyses were performed with a significance threshold of $p < 0.05$.

Results and Discussion

Growth Performance

At 8 weeks of age, the TRT group exhibited a significantly higher body weight compared to the CON group (16.13 ± 3.86 kg vs. 11.64 ± 2.79 kg, $p < 0.001$), as shown in Fig 1. In addition, ADG during the weaning phase (4-8 week) interval was significantly higher in the TRT group than in the CON group (310 ± 110 g/day vs. 160 ± 70 g/day, $p < 0.0001$). According to previous studies, probiotic supplementation administered during the weaning transition has been reported to improve body weight and ADG, thereby positively influencing the overall growth trajectory [6, 20, 21]. Wang et al. (2019) reported that supplementation with *L. plantarum* PFM105 was associated with improved ADG and a reduced incidence of diarrhea in weaned piglets [6]. In other studies, *L. plantarum* 22F and *Pediococcus acidilactici* 72N improved ADG and feed efficiency even under enterotoxigenic *Escherichia coli* challenge [20, 21]. These studies reported that *L. plantarum*-based probiotics had a positive impact on body weight gain and ADG in pigs, suggesting their potential to support growth performance. Consistent with these reports, this study indicates that *L. plantarum* supplementation during the weaning phase was associated with increased ADG during the weaning transition and higher body weight at 8 weeks of age, suggesting a beneficial effect on early growth; however, no significant differences were observed in growth performance during the growing and

finishing phases. Similarly, previous studies have reported that probiotics markedly improve growth performance and gut health during early life phases such as the weaning phase, a time when the gut microbiota is still developing and the intestinal barrier is vulnerable, while their effects during later phases have been less consistently demonstrated in the literature [22, 23]. Overall, our results and those of others indicate that the improved growth performance during the weaning period was caused by *L. plantarum* supplementation. However, we could not exclude the potential influence of other factors, such as feed intake, water consumption, or individual variation.

Physiological responses

Across all sampling time points, most hematological indices showed no significant differences between dietary treatments (Table 2). At 8 weeks of age, the Hb in the TRT group was approximately 1 g/dL lower than that in the CON group ($p = 0.023$), but no significant differences were observed in RBC or HCT, suggesting no evidence of anemia or hemoconcentration [15]. The hematological parameters remained within the normal physiological ranges reported for pigs at each sampling time point [24, 25]. Overall, the absence of marked alterations in hematological parameters in the present study is consistent with previous reports indicating that probiotic supplementation, including *Lactobacillus* species, does not significantly alter hematological profiles in pigs under normal physiological conditions [26, 27]. In contrast, previous studies have reported that probiotic supplementation may lead to an increase in platelet counts in piglets or alterations in hematological parameters in sows [28, 29]. However, such discrepancies may be influenced by various factors, including strain characteristics, dosage, administration method, and husbandry conditions [30]. In particular, under water-based administration, individual variability in intake may result in inconsistent effective dosing, which could have partially contributed to the absence of significant changes in hematological parameters observed in the present study [31].

At 8 weeks of age, TP, BUN, creatinine, TCHO, TG, ALB, AST, ALT, TBIL, Ca, IP, and Mg showed no significant differences between CON and TRT ($p > 0.05$; Table 3). Previous studies have shown that rises in AST/ALT suggest hepatocellular injury, higher TBIL suggests cholestasis, elevated BUN/creatinine indicates renal dysfunction, dehydration, or enhanced proteolysis, and higher TCHO/TG reflect acute energy mobilization under reduced intake or stress [10, 32]. In this study, however, all biochemical parameters remained within the normal physiological ranges for pigs, and no significant differences were observed between CON and TRT at 8

weeks of age. These findings are in line with previous studies in weaning pigs, indicating that dietary supplementation with *L. plantarum* does not adversely affect hepatic or renal function, nor disrupt systemic protein and energy metabolism [33, 34]. In contrast, previous studies have reported that probiotic supplementation may reduce hepatic AST and ALT activities in pigs [35, 36]. Such variations may be attributed to differences in experimental conditions and physiological status of the animals, particularly with respect to metabolic demands and homeostatic capacity across growth phase [37]. Additionally, under water-based administration, variability in individual intake may lead to differences in actual probiotic exposure, which could have influenced the observed differences in biochemical responses [31]. Taken together, the discrepancies between the present and previous studies may be explained by differences in experimental conditions, physiological status, and probiotic administration methods.

Based on immune indices, serum TNF-α and IgA concentrations did not differ significantly between CON and TRT at any sampling time point (Fig. 2). TNF-α is a key mediator of intestinal and systemic inflammatory responses, and increased circulating TNF-α has been associated with epithelial barrier disruption and impaired nutrient absorption in weaned pigs [10]. In contrast, IgA is a major effector of mucosal immunity, and reduced IgA levels are linked to weakened mucosal defense and increased susceptibility to enteric infection [38]. However, in the present study, no TNF-α elevation or IgA reduction indicative of immune dysregulation was observed, and both markers remained within the physiological ranges reported for healthy piglets of comparable ages [32]. These findings are consistent with previous reports showing that dietary *L. plantarum* based probiotic strategies did not markedly alter serum TNF-α or immunoglobulin concentrations in weaned pigs [39]. Furthermore, no significant differences in systemic immune parameters were observed at 8 weeks of age in association with *L. plantarum* supplementation. In contrast, previous studies have demonstrated that probiotic supplementation can modulate immune responses, including reductions in TNF-α and IL-1β expression and increases in serum IgA and sIgA levels [36, 40]. These effects are typically more pronounced under conditions involving immune stimulation, such as infection, inflammation, or environmental stress [40]. In the present study, however, the absence of significant changes in TNF-α and IgA may be attributed to the lack of overt inflammatory stimuli under normal physiological conditions.

Fecal microbial diversity

Alpha-diversity indices representing microbial richness (Observed_features and Chao1) and evenness (Shannon and Simpson), showed significant differences between the CON and TRT groups at 4 weeks of age and at 8 weeks of age (Fig 3). At 4 weeks of age phase, the Observed_features index was significantly higher in the TRT group (249.6 ± 71.35) than in the CON group (191.7 ± 43.17, $p < 0.05$), and the Chao1 index was also higher in the TRT group (646.27 ± 303.2) compared with the CON group (443.53 ± 120.98, $p < 0.01$). At 8 weeks of age, the Observed_features index in the TRT group (340.45 ± 44.85) was significantly higher than in the CON group (250.6 ± 72.47, $p < 0.0001$), and the Chao1 index was also higher in the TRT group (838.04 ± 117.55) compared with the CON group (581.11 ± 164.62, $p < 0.0001$). Moreover, the Shannon index was significantly higher in the TRT group (6.55 ± 0.61) than in the CON group (5.50 ± 1.27, $p < 0.001$), and the Simpson index was also higher in the TRT group (0.955 ± 0.045) compared with the CON group (0.892 ± 0.096, $p < 0.001$). In contrast, at 12 and 23 weeks of age, none of the α-diversity indices (Observed_features, Chao1, Shannon, and Simpson indices) showed significant differences between the two groups ($p > 0.05$). Previous studies have indicated that as pigs mature, the gut microbiota becomes more stable, potentially reducing its responsiveness to probiotic supplementation [41-43]. This age-related stabilization may explain why the differences in α-diversity were not maintained at 12 and 23 weeks of age despite continuous supplementation. Furthermore, the observed changes in α-diversity should be interpreted in relation to the overall microbial community composition. Although increases in α-diversity generally reflect enhanced microbial richness and evenness, they do not necessarily indicate substantial compositional shifts in the overall microbial community [44]. Similarly, previous studies involving dietary supplementation with *L. plantarum* in pigs have reported alterations in microbial diversity indices without pronounced restructuring of the overall gut microbial community [6, 39]. In contrast, other studies have demonstrated that *L. plantarum* supplementation may result in significant shifts or restructuring of the gut microbial community [45, 46]. These discrepancies may be attributed to differences in baseline microbiota composition, dietary formulation, and growth phase-dependent responsiveness [47]. In the present study, despite the observed increase in diversity indices, no substantial changes in community structure were detected. This suggests that *L. plantarum* supplementation may have modulated the microbial composition to a limited extent, rather than inducing pronounced structural alterations in the overall community.

β-diversity analysis of the microbial communities of the CON and TRT groups was compared using PCoA based on weighted and unweighted UniFrac distances (Fig. 4). At 4 weeks of age phase, both unweighted (R =

0.367, $p < 0.001$) and weighted (R = 0.100, $p = 0.012$) metrics showed significant differences. Similar patterns were also observed at 8 weeks of age, where both unweighted (R = 0.357, $p < 0.001$) and weighted (R = 0.294, $p < 0.001$) metrics were statistically significant. At 12 weeks of age, unweighted (R = 0.120, $p = 0.006$) and weighted (R = 0.047, $p = 0.047$) metrics remained statistically significant, and this significance was also maintained at 23 weeks of age for both unweighted (R = 0.144, $p < 0.001$) and weighted (R = 0.082, $p = 0.016$) metrics. However, according to the criteria proposed by Pothoven and Olds (2020) [50], the unweighted and weighted R values at 4 and 8 weeks of age indicated weak separation (R < 0.50), with substantial overlap between the two groups. Previous studies have reported that *L. plantarum* supplementation does not induce substantial changes in the overall gut microbial community structure but rather maintains its overall stability [16, 48]. In contrast, other studies have demonstrated that *L. plantarum* supplementation can significantly alter β-diversity, leading to a clear separation of microbial communities between treatment groups [16, 46, 49]. These conflicting findings may be influenced by factors such as the uniformity of actual intake depending on the mode of probiotic administration, variations in stress levels, and inter-individual differences in baseline gut microbiota composition [47, 50]. In this context, the results of the present study suggest that *L. plantarum* supplementation induce a shift in the microbial community during the early growth phase, but may have exerted only a limited effect on the overall community structure rather than inducing pronounced structural changes [51].

The relative abundances of the bacterial taxa at the phylum and genus levels are shown in Fig 5 respectively. Taxonomic analysis at the phylum level revealed that Firmicutes, Bacteroidetes, and Proteobacteria were the three most dominant phyla in both the CON and TRT groups across all sampling time points (Fig. 5A). Together, Firmicutes and Bacteroidetes comprised approximately 70–80% of the total microbial community, which aligns with previous studies describing them as major constituents of the swine gut microbiota [52, 53]. The difference in fecal microbial composition between the CON and TRT groups was observed at the genus level across all sampling time points (Fig. 5B). Genus level analysis showed that *L. plantarum* supplementation affected the relative abundance of SCFA-producing genera at specific time points, including *Selenomonas* and *Butyrivibrio* (Fig. 5C). Specifically, the relative abundance of *Selenomonas* was significantly higher in the TRT group compared with the CON group at 8 weeks of age ($p < 0.01$). Furthermore, *Butyrivibrio* showed a significantly higher abundance in the TRT group than in the CON group at 23 weeks of age ($p < 0.05$). This result is consistent with the findings of Kai Cui et al. (2019), who also reported a significant increase in *Selenomonas* following *L.*

plantarum supplementation, suggesting that changes in the intestinal metabolic environment induced by *L. plantarum* may be associated with the observed increase in *Selenomonas* [54].

Supplementation with *L. plantarum* is known to induce beneficial changes in the intestinal metabolic network, particularly by activating microbial cross-feeding [55, 56]. In this process, lactate and acetate produced by *L. plantarum* can be converted into key substrates for SCFA-producing bacteria [57-60]. Indeed, studies in piglets have demonstrated that *L. plantarum* supplementation increases the relative abundance of SCFA-producing genera such as *Anaerotruncus* and *Faecalibacterium*, along with higher butyrate concentrations [57]. Considering these previous findings, the significant enrichment of SCFA-producing genera such as *Selenomonas* and *Butyrivibrio* observed in this study may have been partially mediated by change in the intestinal environment, including increased lactate availability or localized pH shifts [61-63]. Notably, In the present study, at 8 weeks of age, when improved growth performance was observed in the TRT group, the relative abundance of *Selenomonas*, a lactate-utilizing genus associated with SCFA production, was significantly higher in the TRT group than in the CON group. Given that SCFAs serve as an energy source for intestinal epithelial cells and support intestinal barrier function [64], this microbial shift may have contributed to the improved early growth performance associated with *L. plantarum* supplementation. In contrast, previous studies have reported that probiotic supplementation does not always lead to significant changes in SCFA-producing bacteria. For example, some studies have shown that the relative abundances of key SCFA-producing genera, such as *Ruminococcus* and *Roseburia*, were not consistently altered across experimental groups despite supplementation with *Lactobacillus*-based probiotics, suggesting that their responses may depend on initial microbiota composition and growth phase rather than probiotic supplementation alone [47, 50]. Such discrepancies may be attributed to differences in probiotic administration methods [14], variability in actual intake [65], age-related differences in feeding behavior and host responsiveness [66], as well as variations in environmental conditions [14]. These findings suggest that *L. plantarum* supplementation may influence gut microbial composition, particularly through increases in the relative abundance of SCFA and organic acid–producing bacteria, although the consistency and functional implications of these changes require further investigation.

Conclusion

Overall, the results of this study suggest that dietary supplementation with *L. plantarum* may have a positive influence on the growth performance of pigs. Specifically, *L. plantarum* supplementation was associated with increased ADG and higher body weight during the weaning period, potentially contributing to improved growth efficiency. Most hematological, immunological, and biochemical parameters showed no significant differences across the sampling time points, and all measured values remained within the physiological reference ranges for pigs, suggesting that *L. plantarum* supplementation did not adversely affect systemic health. At the microbial level, *L. plantarum* administration modulated the gut microbiota composition by increasing the relative abundances of SCFA-producing genera such as *Selenomonas* and *Butyrivibrio*. Taken together, these findings indicate that continuous administration of *L. plantarum* may support improved growth performance while promoting balanced gut microbiota in pigs under commercial production conditions.

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Not applicable.

Abbreviations

ADG: Average Daily Gain

ALB: Albumin

ALT: Alanine Aminotransferase

ANOSIM: Analysis of Similarities

AST: Aspartate Aminotransferase

BAS: Basophil

BW: Body Weight

BUN: Blood Urea Nitrogen

Ca: Calcium

CBC: Complete Blood Count

CON: Control

CRE: Creatinine

EDTA: Ethylenediaminetetraacetic Acid

ELISA: Enzyme-Linked Immunosorbent Assay

EOS: Eosinophil

Hb: Hemoglobin

HCT: Hematocrit

IACUC: Institutional Animal Care and Use Committee

IgA: Immunoglobulin A

IP: Inorganic Phosphorus

L. plantarum: *Lactiplantibacillus plantarum*

LYM: Lymphocyte

Mg: Magnesium

MON: Monocyte

OTU: Operational Taxonomic Unit

PCoA: Principal Coordinates Analysis

PCR: Polymerase Chain Reaction

RBC: Red Blood Cell Count

RDP: Ribosomal Database Project

SCFA: Short-Chain Fatty Acid

SEG: Segmented neutrophils

SEM: Standard Error of the Mean

TCHO: Total Cholesterol

TBIL: Total Bilirubin

TG: Triglycerides

TNF-α: Tumor Necrosis Factor-A

TP: Total Protein

TRT: Treatment

WBC: White Blood Cell Count

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets analyzed for this study can be found in the National Center for Biotechnology Information (NCBI) database under Sequence Read Archive (SRA) with accession number PRJNA1274389.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Kim S, Kim HB conceptualized and structured the manuscript. Chae Y, Kim H, Kwak J, Kim S and Kim HB prepared the original draft. Kim S and Kim HB reviewed and edited the manuscript. All authors have read and approved the final manuscript.

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Table and Figures

Table 1. Composition of the basal diets.

Item	Weaning period (4-8 weeks)	Growing period		Finishing period	
		Phase 1 (8-10 weeks)	Phase 2 (10-12 weeks)	Phase 1 (12-18 weeks)	Phase 2 (18-23 weeks)
Ingredients (%)					
Corn	50.17	58.19	65.52	69.89	75.1
Soybean meal (44%)	20.00	26.00	31.35	26.97	22.3
Whey powder	15.00	7.50	-	-	-
Soy protein concentrate	8.93	3.82	-	-	-
Soybean oil	2.48	1.42	0.55	0.73	0.57
Monocalcium phosphate	1.16	1.08	0.37	0.30	0.23
Calcium carbonate	0.88	0.76	0.90	0.81	0.74
Sodium chloride	0.40	0.40	0.50	0.50	0.50
L-Lysine HCl	0.37	0.32	0.28	0.30	0.17
L-Threonine	0.11	0.09	0.09	0.10	0.05
DL-Methionine	0.17	0.12	0.13	0.10	0.04
L-Valine	0.02	-	0.01	-	-
L-Tryptophan	0.01	-	-	-	-
Vitamin and mineral premix1[a]	0.30	0.30	-	-	-
Vitamin and mineral premix2[b]	-	-	0.30	0.30	0.30
Total	100	100	100	100	100
Calculated energy and nutrient					
ME (kcal/kg)	3,468	3,343	3,241	3,257	3,251
CP (%)	21.26	20.37	19.54	17.97	16.52
Total Lys (%)	1.53	1.36	1.30	1.11	0.95

[a]Vitamin and mineral premix1: Supplied 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; vitamin B12, 20 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).

[b]Vitamin and mineral premix2: Supplied per kilogram of diet: vitamin A, 4,594 IU; vitamin D, 525 IU; vitamin E, 37.5 IU; vitamin K, 2.25 mg; riboflavin, 8.25 mg; niacin, 42 mg; pantothenic acid, 20.25 mg; vitamin B12, 0.04 mg; Cu, 12 mg (as copper sulfate); I, 0.28 mg (as potassium iodate); Fe, 160 mg (as ferrous sulfate); Se, 0.30 mg (as sodium selenate); Zn, 160 mg (as zinc sulfate)

596 **Table 2. Effects of *L. plantarum* on hematological parameters of pigs.**

Item	CON	TRT	SEM	*p*-value
WBC, $10^3/\mu\ell$				
4W	18.11	16.42	1.87	0.530
8W	24.87	25.19	1.57	0.885
12W	22.51	23.73	2.12	0.687
23W	16.08	22.06	2.79	0.114
RBC, $10^6/\mu\ell$				
4W	5.99	6.23	0.24	0.473
8W	6.59	6.09	0.25	0.178
12W	6.04	6.20	0.20	0.585
23W	5.54	6.94	0.40	0.080
Hb, g/dL				
4W	12.11	12.52	0.48	0.556
8W	11.78	10.85	0.26	0.023
12W	9.74	10.74	0.51	0.180
23W	9.61	12.36	1.18	0.296
HCT, %				
4W	42.36	41.89	1.60	0.838
8W	51.17	49.12	2.1	0.498
12W	37.29	39.43	1.76	0.402
23W	30.34	40.41	3.38	0.087
LYM, %				
4W	44.99	42.13	6.48	0.759
8W	14.63	14.77	5.70	0.887
12W	51.78	47.16	2.63	0.231
23W	35.69	32.69	1.67	0.215
MON, %				
4W	12.07	9.86	3.47	0.658
8W	16.02	13.83	4.78	0.986
12W	2.50	3.47	0.94	0.477
23W	57.36	60.86	0.61	0.446
EOS, %				
4W	1.63	1.87	0.50	0.739
8W	2.79	3.50	1.36	0.750
12W	4.81	6.97	0.96	0.127
23W	3.96	3.30	0.25	0.623
BAS, %				
4W	0.05	0.08	0.02	0.382
8W	0.18	0.12	0.09	0.717
12W	0.21	0.26	0.09	0.693
23W	2.72	2.97	0.66	0.427
NEU, %				
4W	41.11	46.06	4.14	0.409
8W	66.38	67.78	6.86	0.653
12W	40.70	42.14	2.78	0.719
23W	32.69	35.69	1.68	0.213

597

CON, control group; TRT, *L. plantarum*-supplemented group; SEM, standard error of the mean; W, weeks of age; WBC, white blood cell count; RBC, red blood cell count; Hb, hemoglobin; HCT, hematocrit; LYM, lymphocytes; MON, monocytes; EOS, eosinophils; BAS, basophils; SEG, Segmented neutrophils.

602 **Table 3. Effects of *L. plantarum* on biochemical parameters of pigs.**

Item	CON	TRT	SEM	*p*-value
TP, g/dL	5.57	5.23	0.08	0.078
BUN, mg/dL	10.09	9.01	1.51	0.358
CRE, mg/dL	0.85	0.88	0.06	0.332
TCHO, mg/dL	72.90	71.70	2.03	0.809
TG, mg/dL	56.80	52.90	3.72	0.651
ALB, g/dL	3.33	3.31	0.16	0.699
AST, IU/L	38.90	42.04	3.71	0.460
ALT, IU/L	37.50	37.40	0.16	0.886
TBIL, mg/dL	0.01	0.01	0.01	0.938
Ca, mg/dL	8.54	9.60	0.84	0.522
IP, mg/dL	8.62	9.25	0.51	0.288
Mg, mg/dL	2.30	2.30	0.15	0.963

603 CON, control group; TRT, *L. plantarum*-supplemented group; SEM, standard error of the mean; TP, total
604 protein; BUN, blood urea nitrogen; CRE, creatinine; TCHO, total cholesterol; TG, triglycerides; ALB, albumin;
605 AST, aspartate aminotransferase; ALT, alanine aminotransferase; TBIL, total bilirubin; Ca, calcium; IP,
606 inorganic phosphorus; Mg, magnesium.

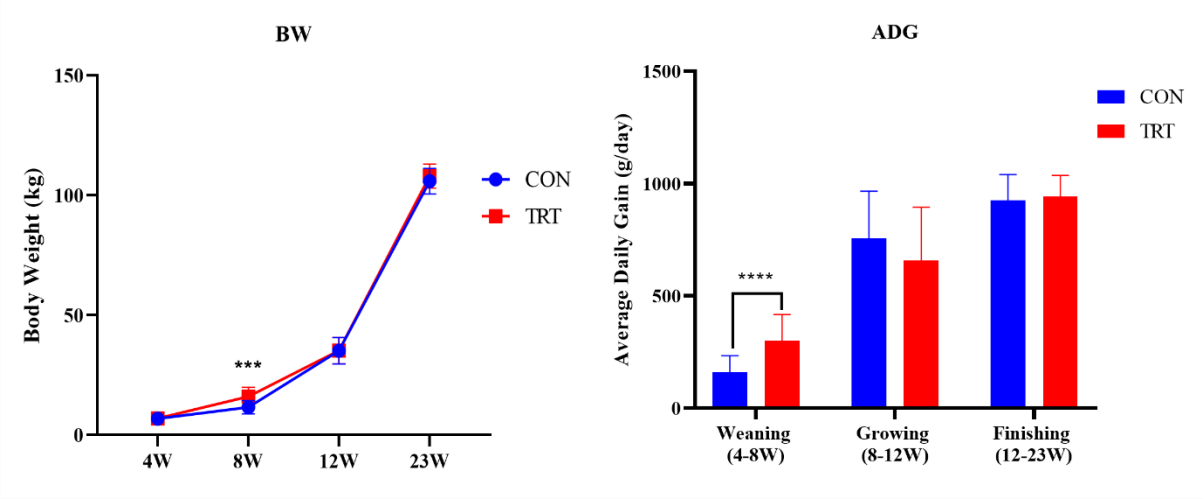


Fig 1. BW and ADG of pigs at each sampling time point. One-way ANOVA followed by Tukey's multiple comparisons test was performed, and significance levels are indicated as *$p < 0.05$, **$p < 0.005$, ***$p < 0.001$, ****$p < 0.0001$. CON, control group; TRT, *L. plantarum*-supplemented group; BW, body weight; ADG, average daily gain; W, weeks of age; 4–8W, weaning; 8–12W, growing; 12–23W, finishing.

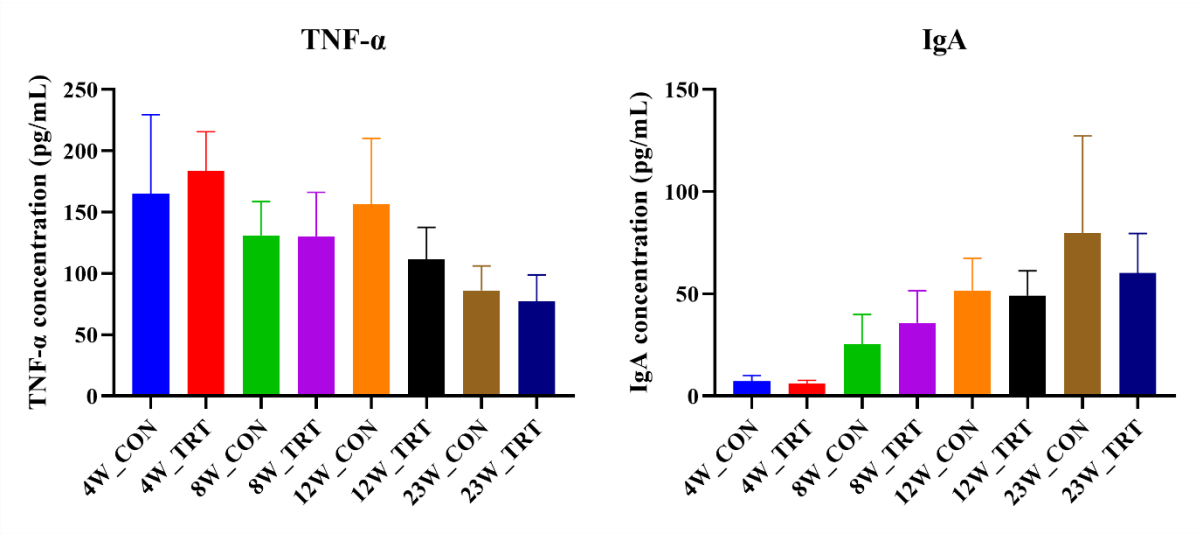


Fig 2. Effects of *L. plantarum* on serum immune status of pigs. Data were analyzed by one-way ANOVA followed by Tukey's multiple comparisons test. Statistical comparisons were performed between the CON and TRT groups at each sampling time point (4, 8, 12, and 23 weeks of age). The *p*-values were as follows: TNF-α, 4 weeks ($p = 0.994$), 8 weeks ($p > 0.999$), 12 weeks ($p = 0.591$), and 23 weeks ($p > 0.999$); IgA, 4 weeks ($p > 0.999$), 8 weeks ($p = 0.993$), 12 weeks ($p > 0.999$), and 23 weeks ($p = 0.810$). CON, control group; TRT, *L. plantarum*-treated group; W, weeks of age; TNF-α, tumor necrosis factor-alpha; IgA, immunoglobulin A.

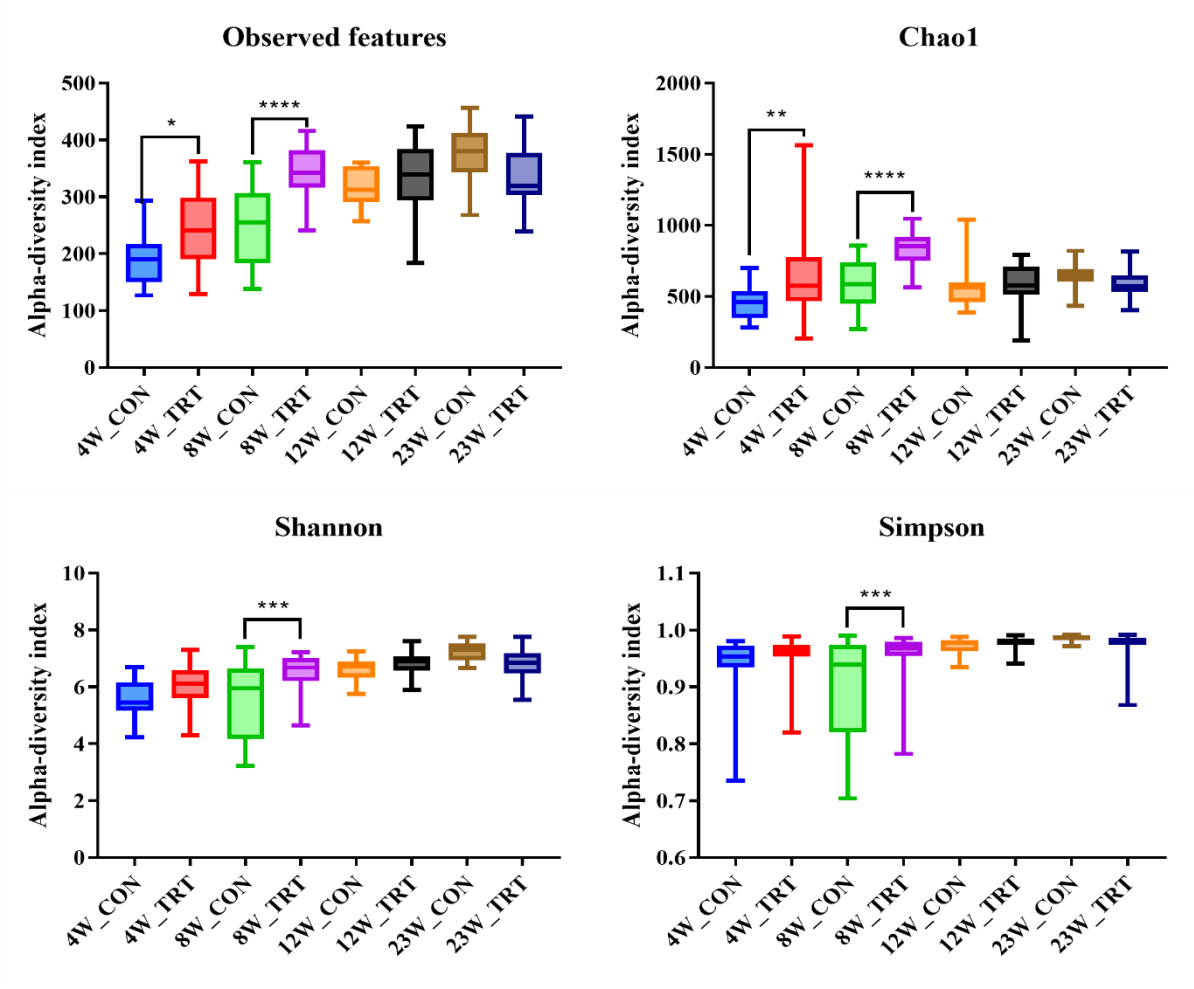


Fig 3. Alpha diversity indices of the fecal microbiota in pigs at each sampling time point in the CON and TRT groups. Data were analyzed by one-way ANOVA followed by Tukey's multiple comparisons test, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. CON, control group; TRT, *L. plantarum*-treated group; W, weeks of age.

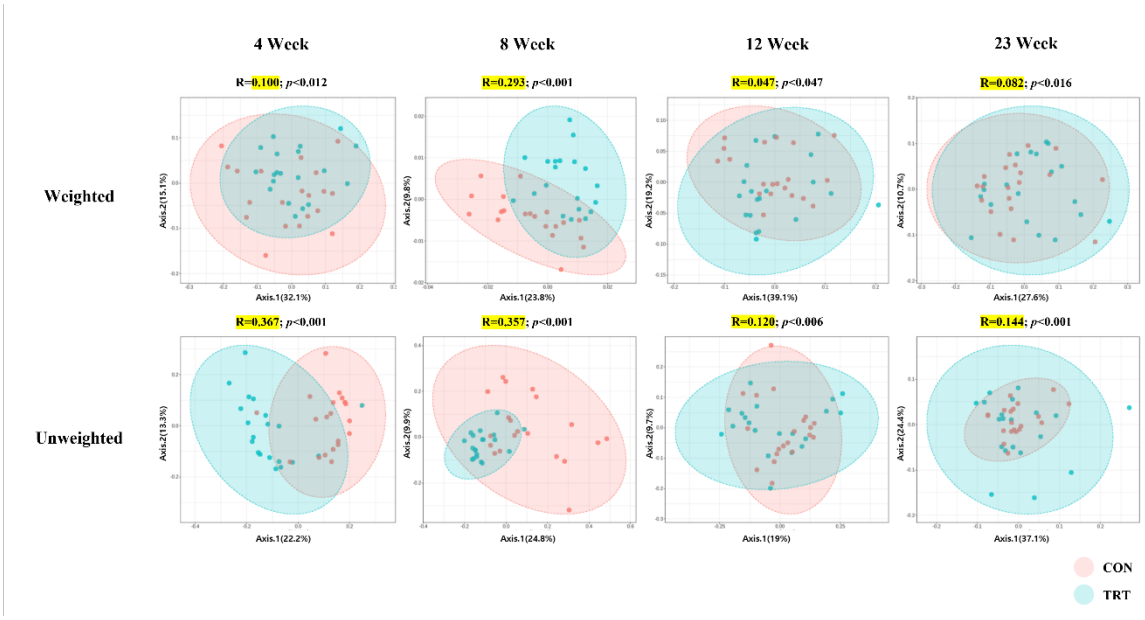


Fig 4. Beta diversity of the fecal microbiota in pigs at each sampling time point in the CON and TRT groups. Data were analyzed by PCoA using weighted and unweighted UniFrac, with ANOSIM results. CON, control group; TRT, *L. plantarum*-treated group; W, weeks of age; PCoA, principal coordinate analysis; R, ANOSIM statistic.

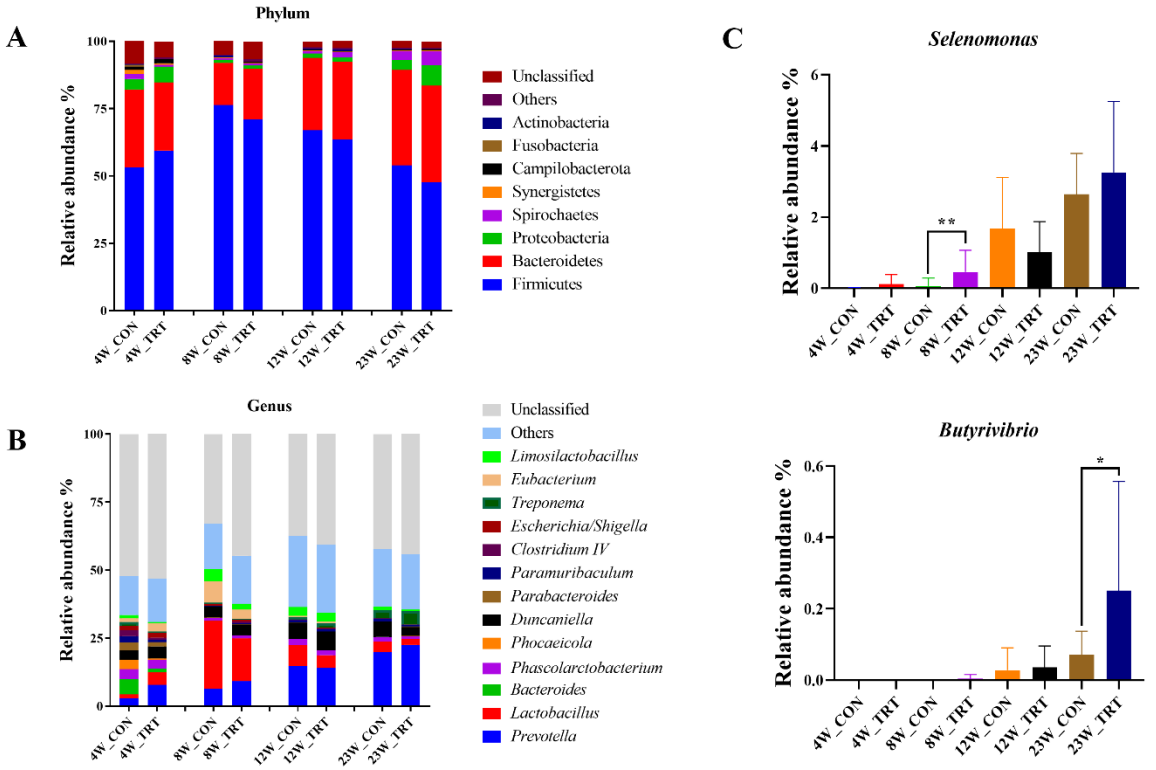


Fig 5. Relative abundance of the fecal microbiota at the phylum and genus levels in pigs at each sampling time point in the CON and TRT groups. Relative abundances of the sequences at the phylum level (A), Relative abundances of the sequences at the genus level (B), Relative abundances of *Selenomonas* and *Butyrivibrio* (C). One-way ANOVA, Tukey's multiple, *$p<0.05$, **$p<0.005$. CON, control group; TRT, *L. plantarum*-treated group; W, weeks of age.