JAST (Journal of Animal Science and Technology) TITLE PAGE
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ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research article
Article Title (within 20 words without abbreviations)	A commensal Lactobacillus-based probiotic consortium isolated from healthy pig feces alleviates weaning stress in nursery pigs via modulation of gut microbiota and metabolites
Running Title (within 10 words)	Commensal *Lactobacillus* Consortium in Weaned Pigs
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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 the National Research Foundation of Korea (NRF) grants funded by the Korean government (MEST; NRF-2021R1A2C3011051) and by Basic Science Research Program through the 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: Kim Y, Lee WJ. Data curation: Lee WJ, Park J. Formal analysis: Lee WJ, Park J, Kang A. Methodology: Kim Y, Song M, Lee WJ. Software: Lee WJ, Park J, Kang A. Validation: Kim Y, Song M, Lee WJ, Jang KB. Investigation: Lee WJ, Park J. Writing - original draft: Lee WJ, Jang KB. Writing - review & editing: Kim Y, Lee WJ, Jang KB.
Ethics approval and consent to participate	The protocol for weaning pig experiment was reviewed and approved by the Institutional Animal Care and Use Committee of Chungnam National University, Daejeon, Korea (approval: 202203A-CNU-063).

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A commensal *Lactobacillus*-based probiotic consortium isolated from healthy pig feces alleviates weaning stress in nursery pigs via modulation of gut microbiota and metabolites

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ABSTRACT

This study aimed to evaluate the effects of a commensal *Lactobacillus*-based consortium (CLC) on growth performance, gut microbiota, and metabolite profiles in weaned pigs. A total of 48 newly weaned pigs (7.32 ± 0.96 kg body weight) were randomly allotted to one of two dietary treatments based on a randomized complete block design with initial body weight as the blocking criterion. Dietary treatments were a basal nursery diet based on corn–soybean meal (CON) and CON + 0.02% CLC (10^{10} CFU/kg of diet) containing *Lactobacillus amylovorus, L. johnsonii, L. mucosae, L. reuteri, and L. ruminis*. Pigs fed with the CLC exhibited significantly improved body weight gain and feed efficiency compared to controls. Although hematological parameters remained unaffected, serum levels of AST were reduced by CLC supplementation, with trends toward decreased ALT and LDH. Lipid profiles improved with elevated HDL and reduced LDL, alongside significant reductions in serum malondialdehyde (MDA) and cortisol levels. Gut microbiota analysis revealed that CLC supplementation increased the abundance of *Lactobacillus* and Prevotellaceae, with a reduced abundance of Clostridiaceae. Metabolomic profiling showed that CLC supplementation enriched short-chain fatty acids, particularly lactate and acetate, as well as elevated levels of amino acids (methionine, glycine, alanine, threonine, and valine) and key intermediates such as malate, succinic acid, and conjugated linoleic acid. Taken together, dietary supplementation with CLC enhanced growth performance and systemic health in weaned pigs, potentially by mitigating oxidative stress and cortisol responses while promoting beneficial shifts in the gut microbiota and metabolome during the critical post-weaning period.

Keywords: Weaning stress, nursey pigs, commensal *Lactobacillus*-based consortium, Microbiota, Metabolomic profiling, stress biomarker

Introduction

Weaning represents a critical transition in swine production, exposing the immature gastrointestinal tract of nursery pigs to a multitude of stressors that frequently result in growth retardation and post-weaning diarrhea. During the post-weaning period, nursery pigs undergo abrupt nutritional changes and environmental challenges, which destabilize the gut microbiota and increase susceptibility to pathogen colonization [1]. Thus, weaning stress disrupts physiological homeostasis and provokes inflammatory and oxidative stress responses, ultimately impairing immunity and heightening the risk of infection in early-life pigs [2]. One of the significant outcomes of weaning stress is disruption of the gut microbial balance, where dysbiosis is closely related to colonization resistance and impairs the intrinsic protective functions in the intestine [3]. This condition makes nursery pigs more susceptible to pathogenic bacteria, intestinal inflammation, and infection [4, 5]. These health concerns have driven growing interest in nutritional strategies aimed at alleviating dysbiosis during the post-weaning period.

Dietary supplementation with probiotics has shown promise in improving intestinal health in nursery pigs [6-8]. Dietary probiotics in starter feeds could enhance nutrient metabolism, including amino acid and energy pathways, as well as intestinal barrier function of nursery pigs during the post-weaning period [9-11]. *Lactobacillus* species are widely recognized for their beneficial roles in maintaining gut homeostasis and host health via mucosal adhesion, production of organic acids and antimicrobial compounds, and modulation of immune responses [9]. Interestingly, *Lactobacillus* species are one of the predominant beneficial microbes in the gut environment of neonatal pigs around the weaning period, where they play a pivotal role in stabilizing the intestinal ecosystem and conferring health benefits [12, 13]. Our previous research employing genomic and culturomic-based analysis identified *Lactobacillus* species consistently enriched in fecal samples from nursery pigs fed a multi-strain probiotic after weaning. These species were associated with enhanced intestinal barrier function after weaning [14]. Furthermore, in vitro studies by Lee et al. [15] demonstrated that these commensal *Lactobacillus* strains produce methionine and branched-chain amino acids, thereby indicating their metabolites potentially benefit intestinal barrier integrity and host metabolic support. Furthermore, previous studies have shown that the use of probiotics derived from commensal microbes, which are well-adapted to the host gut microbiota due to their higher colonization capacity, better immunological compatibility, enhanced ecosystem recovery, and stronger competitive exclusion of pathogens [16, 17].

Based on these findings, we hypothesized that supplementation with a consortium of commensal *Lactobacillus* strains would mitigate the adverse effects of weaning stress by modulating the gut microbiota, reducing dysbiosis-related stress responses, and improving growth performance in nursery pigs after weaning. To test this hypothesis, the objective of the present study was to evaluate the effects of a multi-strain probiotics formulation, composed of anaerobically cultivable commensal *Lactobacillus*-based consortium (CLC), on growth performance, blood parameters, oxidative stress indicators, gut microbiota, and fecal metabolomic profiles in nursery pigs after weaning.

Materials and Methods

Probiotics strains

Lactobacillus strains used in this study were isolated from fecal samples of healthy piglets. Briefly, 10 g of fecal sample were diluted in 45 mL L-cysteine solution (8.5 g NaCl, 0.5 g L-cysteine, 1 L distilled water) and plated on de Man, Rogosa, and Sharpe, with 0.004% bromocresol purple (MRS-BCP) agar. The plates were incubated anaerobically at 37 °C for 48 h using an anaerobic glove box (Coy Laboratory Products, Grass Lake, MI, USA). Single colonies were selected randomly, sub-cultured in MRS broth, and re-streaked onto MRS agar to confirm purity. Following assessment of probiotics properties (unpublished data), five strains were selected for formulation of the commensal *Lactobacillus*-based probiotic consortium: *Lactobacillus amylovorus* SLAM_LAA04, *L. johnsonii* SLAM_LAJ06, *L. mucosae* SLAM_LAM08, *L. reuteri* SLAM_LAR09, and *L. ruminis* SLAM_LAR12. The selected strains were propagated in a farm-scale fermentation medium consisting of 1% glucose, 1% molasses, 0.2% sea salt, and 0.2% yeast extract at 37 °C. Following cultivation, strains were individually harvested and stored at 4 °C before consortium preparation. Equal volumes of each strain were combined to formulate the probiotic consortium and incorporated into the experimental diet to achieve a final concentration of 1×10^{10} CFU/kg of feed, as previously described [14].

Experimental design, animal, and diets

The protocol for animal experiments was reviewed and approved by the Institutional Animal Care and Use Committee of Chungnam National University, Daejeon, Korea (approval: 202203A-CNU-063). The animal experiment was conducted in accordance with the guidelines for the care and management of animals at research facilities in Chungnam National University. A total of 48 weaned pigs [Landrace x Yorkshire × Duroc; 7.32 ± 0.96 kg; 28 days old] were allotted to one of two treatment groups based on a randomized complete block design with initial BW (heavy and light) as a blocking criterion. Each treatment group had 6 replicates with 4 pigs per pen. Dietary treatments were a basal nursery diet based on corn–

soybean meal (CON) and CON + 0.02% CLC, replacing an equal portion of corn in the CON diets. All experimental diets (Table 1) were formulated to meet or exceed the nutrient requirements described in NRC (2012). The experimental period was for 4 weeks. All pigs were housed in environmentally controlled pens set at 28℃ ~ 30℃. They were allowed access to feed and water *ad libitum* during the study. Body weight (BW) and feed intake were measured weekly to evaluate growth performance, including average daily gain (ADG), average daily feed intake (ADFI), and gain-to-feed ratio (G:F).

Sample collection

Blood samples were collected from the jugular vein of one pig with a median BW per pen using tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA) with or without ethylenediaminetetraacetic acid (EDTA) at the end of the animal experiment. Collected blood samples were kept at room temperature for 2 h, centrifuged at 3,000 × g for 15 min to collect serum samples, and stored at -80℃. Fecal samples were also collected from the pigs randomly selected from each dietary treatment by rectal palpation after blood sampling. The samples in tubes were stored at -80°C until further analysis.

Hematological profiles and serum biochemical properties

Whole blood samples from EDTA tubes were measured to analyze the blood profiles of total white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), and platelet (PLT), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) using an automated hematology analyzer calibrated for porcine blood (Scil Vet Animal Blood Counter, Scil Animal Care Company, Altorf, France). Serum biochemical analysis was performed using the DRI-CHEM NX600V automated clinical chemistry analyzer (Fujifilm, Tokyo, Japan). Lactate dehydrogenase (LDH), glucose (GLU), triglycerides (TG), and high-density lipoprotein (HDL) levels were measured according to the manufacturer’s instructions.

Furthermore, to evaluate the regulation of stress-related biomarkers, the levels of advanced oxidation protein products (AOPP) and malondialdehyde (MDA) were quantified using a commercial AOPP Kit (ab242295; Abcam, Cambridge, UK) and Lipid Peroxidation (MDA) Kit (ab118970; Abcam). For MDA assay, serum samples were mixed with 42 mM H_2SO_4 (2 N sulfuric acid, Duksan, Seoul, Korea) and phosphotungstic acid solution to precipitate lipids. Glacial acetic acid (Sigma-Aldrich) was used to prepare Developer VII/TBA Solution. In addition, serum cortisol concentration was quantified using Porcine Cortisol ELISA kit (E-EL-0159; Elabscience). All procedures were carried out according to the manufacturer's instructions.

Gut microbiota analysis using Illumina sequencing

Fecal DNA was isolated using the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) according to the manufacturer's protocol and a previously reported method [18] with minor adjustments. In short, fecal material was disrupted by vortexing for 2 min with sterile zirconia beads (0.1 mm; BioSpec, Cat. No. 11079101z). DNA yield and purity were evaluated by spectrophotometric absorbance readings at 230, 260, and 280 nm using a SpectraMax ABS Plus (Molecular Devices, San Jose, CA, USA). The V3-V4 hypervariable region of the 16S rRNA gene was amplified using the primer pair: forward 5′-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG GTG CCA GCM GCC GCG GTA A-3′ and reverse 5′-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGG ACT ACH VGG GTW TCT AAT-3′. Sequencing was conducted on the Illumina NextSeq platform (Sanigen, Gyeonggi-do, South Korea).

Raw sequence data were processed using QIIME2 (version 2024.10) implemented on a Linux server accessed via PuTTY. Reads were quality-filtered and denoised using the DADA2 plugin to obtain high-resolution amplicon sequence variants (ASVs), with removal of chimeric and low-quality sequences. Taxonomic assignment was performed against the SILVA 138 reference database trained on the V3–V4 region. To characterize microbial diversity, alpha diversity indices (Chao1, Shannon, and Simpson) were calculated, and beta diversity was evaluated using Bray–Curtis dissimilarity and weighted/unweighted UniFrac distances. Community dissimilarities were visualized by principal coordinates analysis (PCoA), and group differences were tested by PERMANOVA with false discovery rate (FDR) correction.

Profiling of metabolome networks

The assay for fecal metabolome analysis was conducted as previously described in Kang et al. [19]. Fecal samples were preserved at –80 °C until subjected to metabolomic profiling. For extraction, each sample was weighed and suspended in methanol to yield a concentration of 20 mg/mL, followed by vortexing on ice for 5 min. The suspensions were centrifuged at 15,000 × g for 5 min at 4 °C, and the resulting supernatants were passed through a 0.2 µm polyvinylidene fluoride (PVDF) syringe filter. Filtered extracts (200 µL) were evaporated to dryness using a vacuum concentrator and stored at –81 °C until derivatization and gas chromatography–mass spectrometry (GC–MS) analysis. For derivatization, 30 µL of methoxyamine hydrochloride (20 mg/mL in pyridine; Sigma, St. Louis, MO, USA) was added to the dried extract and incubated at 30 °C for 90 min. Subsequently, 50 µL of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA; Sigma) was introduced and maintained at 60 °C for 30 min. Fluoranthene was included as the internal standard. GC–MS was performed using a Thermo Trace 1310 gas chromatograph coupled with a Thermo ISQ LT single quadrupole mass spectrometer (Waltham, MA, USA). Separation was achieved on a DB-5MS column (60 m length, 0.20 mm internal diameter, 0.25 µm film thickness; Agilent, Santa Clara, CA, USA). Samples were injected at 300 °C with a split ratio of 1:60 and a helium split flow of 90 mL/min. Chromatographic separation was carried out under a constant helium flow of 1.5 mL/min, with an oven temperature program of 50 °C (2 min hold), ramped to 180 °C at 5 °C/min (8 min hold), to 210 °C at 2.5 °C/min, and finally to 325 °C at 5 °C/min (10 min hold). Mass spectra were acquired in electron impact ionization mode with an ion source temperature of 270 °C, scanning a mass-to-charge ratio (m/z) range of 35–650 at a rate of five spectra per second. Data processing was performed using Thermo Xcalibur software with automated peak detection. Metabolite identification was achieved by matching mass spectra and retention indices against the NIST Mass Spectral Search Program (version 2.0, Gaithersburg, MD, USA). Detected metabolite intensities were normalized to the internal standard fluoranthene.

Metabolomics data were first processed using MetaboAnalyst 6.0 (www.metaboanalyst.ca) for normalization, transformation, and partial least squares–discriminant analysis (PLS-DA). In addition, further analyses were conducted in R Studio (version 4.3.2). Specifically, correlation networks between metabolites were constructed using the igraph and Hmisc packages, visualized with *ggraph*. Radar charts were generated with the *fmsb* package to display group-wise metabolite profiles. Functional associations between metabolites were evaluated by mapping to KEGG pathways using the *KEGGREST* and *clusterProfiler* packages.

Statistical analysis

For the pig experiment, data were analyzed using the Mixed procedure of SAS (SAS Institute Inc., Cary, NC, USA) under a randomized complete block design, with the initial body weight (BW) used as a blocking factor. The pen was considered the experimental unit. The statistical model for analyzing growth performance and blood profiles included dietary treatment as the fixed effect and initial BW as a random effect. All data were presented as mean ± SEM. In the results, $p < 0.05$ was considered significant, whereas $0.05 \leq p < 0.10$ was considered a tendency. Statistical analyses for microbiota and metabolomic data were performed in Ubuntu 24.04.1 LTS, GraphPad Prism (version 9.0; GraphPad Software, CA, USA) and R Studio (version 2024.12.0+467, "Kousa Dogwood" Release) on Windows 11 (x64). Nonparametric tests (Mann–Whitney U or Kruskal–Wallis test) were used when the data did not meet the normality assumptions.

Results

Growth performance of nursing pigs with supplemented with a commensal *Lactobacillus*-based probiotic consortium

In preliminary screening, approximately 5,000 anaerobic *Lactobacillus* strains were isolated from fecal samples of healthy piglets and evaluated for probiotic potential based on acid and bile tolerance, intestinal adhesion capacity, and resistance to pathogenic infection using *Caenorhabditis elegans* as an *in vivo* model (unpublished data). Among them, five strains including *L. amylovorus* SLAM_LAA04, *L. johnsonii* SLAM_LAJ06, *L. mucosae* SLAM_LAM08, *L. reuteri* SLAM_LAR09, and *L. ruminis* SLAM_LAR12, were selected and formulated into a commensal *Lactobacillus*-based consortium used in this study.

Moreover, supplementary effects of commensal *Lactobacillus*-based consortium were on growth performance were investigated on weaned pigs. Feed intake and body weight (BW) were recorded throughout the study period. The initial BW of newly weaned pigs at the beginning was 7.3 ± 1.0 kg, and there was no difference among treatments (Table 2). By day 28, pigs in the CLC group tended to have greater BW compared to the CON group (19.42 vs. 18.82 kg; $p = 0.067$). Moreover, average daily gain (ADG) was significantly higher in pigs receiving the probiotic consortium (433 vs. 410 g/d, $p < 0.05$). However, no significant difference in gain-to-feed ratio (G:F) was observed between the two groups.

Hematological profiles and serum biochemical properties

Dietary supplementation with the commensal *Lactobacillus*-based consortium did not affect hematological parameters, including white blood cell and red blood cell counts, hemoglobin concentration, hematocrit, platelet counts, MCV, or MCHC in nursery pigs (Table 3). pigs supplemented with the consortium exhibited a significant reduction in serum AST activity compared with the pigs in the control group ((48.0 vs. 128.0 U/L, $p < 0.05$; Table 4), and a tendency for lower ALT (50.5 vs. 133.0 U/L, $p = 0.053$). Serum LDH concentration also tended to be lower in the pigs fed with commensal *Lactobacillus*-based consortium (505.0 vs. 730.5 U/L, $p = 0.084$). Dietary supplementation of commensal *Lactobacillus*-

based consortium significantly increased HDL concentration (51.0 vs. 29.5 mg/dL, $p < 0.05$) and decreased LDL concentration (73.7 vs. 89.4 mg/dL, $p < 0.05$). No significant differences were observed in glucose, triglycerides, total cholesterol, or gamma-glutamyl transferase among treatments. Importantly, stress markers were also affected by the dietary treatment. Supplementation with the commensal *Lactobacillus*-based consortium significantly reduced the serum MDA concentration compared with that in the CON group (0.50 vs. 1.15 μmol/mL, $p < 0.05$; Fig. 1A), whereas levels of AOPP remained unchanged (Fig, 1B). Additionally, serum cortisol concentrations were markedly reduced in pigs fed the commercial *Lactobacillus*-based consortium compared with the control group (27.7 vs. 9.7 ng/mL, $p < 0.05$; Fig. 1C).

Regulation of gut microbiota by supplementation of commensal *Lactobacillus*-based consortium

Dietary supplementation with the commensal *Lactobacillus*-based consortium resulted in a marked shift in gut microbial composition, favoring enrichment of *Lactobacillus* and Prevotellaceae lineages while suppressing Clostridiaceae (Fig. 2A). At day 28, the relative abundance of *Lactobacillus* was elevated in the CLC group compared to the control (18.8% vs. 12.1%), alongside increases in *Prevotella* (6.2% vs. 2.2%), Prevotellaceae (2.8% vs. 0.9%), and Butyricicoccaceae (0.4% vs. 0.1%). In contrast, Clostridiaceae abundance was notably reduced in the CLC group (9.3% vs. 20.8%). Corresponding $\log_2$ fold-changes were +0.64 (*Lactobacillus*), +1.50 (*Prevotella*), +1.57 (Prevotellaceae), +2.54 (Butyricicoccaceae), and −1.16 (Clostridiaceae), suggesting a probiotic-induced remodeling of dominant microbial taxa. Supplementation with commensal *Lactobacillus*-based consortium also increased gut microbiota diversity and evenness at day 28 (Fig. 2B, C). Shannon entropy rose from 5.29 ± 0.79 in Control (n=6) to 6.05 ± 0.35 in CLC (n=6) at day 28 ($p = 0.056$; Fig. 2B). Pielou evenness increased from 0.61 ± 0.07 to 0.68 ± 0.03 ($p = 0.035$; Fig. 2C), indicating a more uniform taxonomic distribution within the gut microbial community under the probiotic treatment.

Principal coordinate analysis (PCoA) based on Weighted and Unweighted UniFrac distances revealed distinct clustering patterns between groups. Unweighted and weighted UniFrac ordinations determined a consistent displacement of CLC group from CON group (Fig. 2D, E). Weighted UniFrac PCoA, which incorporates taxa abundance, accounted for 35.65% and 20.11% of the variation along PC1 and PC2, respectively, and demonstrated clear separation of the CLC group from controls (Fig. 2D). Similarly, unweighted UniFrac PCoA, explained 21.18% (PC1) and 13.43% (PC2) of the variation, with CLC samples diverging along PC1 from the control group (Fig. 2E). These multivariate analyses reinforce a probiotic-driven reconfiguration of the gut microbiota.

Correlation between gut microbiota and metabolites

Principal component analysis (PCA) was used to examine overall metabolic differences among the weaning, CON, and CLC groups. The first two components accounted for 66.4% of the total variance (PC1, 53.2%; PC2, 13.2%). Notably, the weaning and CON groups clustered closely, whereas the CLC group was distinctly separated along PC1 (Fig. 3A), indicating that supplementation with the commensal *Lactobacillus*-based consortium substantially altered the metabolic landscape relative to natural weaning. Partial least squares discriminant analysis (PLS-DA) further revealed that multiple metabolites contributed significantly to the observed group separation. Oxalate demonstrated the most significant coefficient, followed by 2-hydroxy-3-methylbutyric acid, valine, methionine, 2-hydroxyisocaproic acid, stearate, cholestanol, arabinose, and methylbutanoic acid. These metabolites were consistently elevated in the CLC group and suppressed in the weaning group, with the CON group showing intermediate levels, suggesting that the CLC supplementation was the primary driver of these metabolic changes during the post-weaning period. (Fig. 3B).

The heatmap provided a complementary perspective on these differences across a broader range of metabolites. A marked elevation in the levels of amino acids (proline, glycine, alanine, valine, methionine), organic acids (malate, oxalate, glycolate, butyrate), lipids and sterols (oleate, stearate, stigmasterol, conjugated linoleic acid), and several carbohydrate-related compounds (arabinose, gluconate, glucose) was observed in the CLC group. In contrast, a marked suppression in the levels of these metabolites was observed in the feces collected at weaning. The CON group exhibited a mixture of intermediate and low levels. (Fig. 3C). This coordinated elevation of amino acid, organic acid, and lipid metabolites in the CLC group indicates a broad upregulation of energy, protein, and lipid metabolism, likely reflecting enhanced microbial activity and nutrient assimilation during the post-weaning period.

Volcano plot analysis revealed pronounced differences in metabolite abundance between the CON and CLC groups. Several amino acids were significantly enriched following supplementation of commensal *Lactobacillus*-based consortium during the post-weaning period, including glycine (FC = 26,468.9; FDR = 0.0191), aspartate (FC = 7,687.5; FDR = 0.0191), serine (FC = 7,094.2; FDR = 0.0191), proline (FC = 1,071.9; FDR = 0.0191), alanine (FC = 34.2; FDR = 0.0191), threonine (FC = 13.4; FDR = 0.0191), valine (FC = 7.11; FDR = 0.00621), and 5-oxoproline (FC = 12.2; FDR = 0.0191). Methionine also showed an upward trend (FC = 2.998; FDR = 0.0805), although it did not reach statistical significance. Notably, the huge fold change observed for glycine is likely attributable to its near-zero levels in the control group. However, consistent increases across biological replicates support the biological relevance of this finding. Collectively, these results indicate that CLC supplementation enhanced amino acid biosynthesis and one-carbon metabolism, pathways that provide precursors for protein synthesis, methyl-donor chemistry, and antioxidant defense. Significant increases were also detected for lactate (FC = 66.2; FDR = 0.0172), malate (FC = 22.6; FDR = 0.0280), and conjugated linoleic acid (FC = 10.1; FDR = 0.0431). Additional metabolites, including succinic acid (FC = 5.31; FDR = 0.0870), glucose (FC = 3.35; FDR = 0.0870), cholesterol (FC = 2.02; FDR = 0.0689), methylsuccinic acid (FC = 4.15; FDR = 0.200), and 2-hydroxy-3-methylbutyric acid (FC = 1.55; FDR = 0.231), displayed upward trends but did not reach FDR < 0.05. These findings suggest that supplementation of commensal *Lactobacillus*-based consortium during the post-weaning period influenced central carbon metabolism by promoting glycolytic and TCA cycle intermediates, while also modulating redox balance and lipid turnover (Fig. 4A-C). Network analysis mapped these changes to amino acid metabolism (glycine, serine, threonine, alanine, valine, and 5-oxoproline), one-carbon pathways (glycine, serine, threonine, and methionine), and transamination reactions (aspartate, alanine, and proline). The enrichment of lactate, malate, and succinate family members pointed to enhanced glycolysis, TCA flux, and the glyoxylate/dicarboxylate cycle, reflecting higher microbial and host energy turnover. Lipid-associated intermediates, such as conjugated linoleic acid and cholesterol, are linked to membrane composition and redox control, further indicating metabolic remodeling beyond amino acids. Taken together, these findings demonstrate that *Lactobacillus*-based consortium supplementation significantly elevated multiple amino acids, increased intermediates of central carbon metabolism, and modulated lipid/redox pathways. This coordinated remodeling suggests that CLC treatment counteracted post-weaning metabolic deficiencies and supported pathways associated with growth, energy efficiency, and stress resilience (Fig. 4D).

DISCUSSION

Weaning stress is a major factor that negatively affects the growth and health of nursery pigs and has been well identified as a key challenge causing substantial economic loss in the swine industry [20-22]. To alleviate the detrimental effects of this stressor, various nutritional strategies have been employed, including the use of animal-derived protein sources, dairy byproducts, functional ingredients, and dietary additives in nursery pig diets [23-25]. Despite these advances in nutritional management, growth retardation and post-weaning diarrhea remain serious concerns in swine production [26]. Understanding the mechanisms by which weaning stress impairs health and systemic metabolism is important for developing effective strategies to reduce the negative impact of weaning stress. Among nutritional interventions, probiotic supplementation is an effective dietary strategy to beneficially modulate microbial composition and metabolic functions, enhance gut barrier integrity, alleviate inflammation, and influence systemic health [27]. Recent studies have further demonstrated that probiotics have also been shown to alleviate stress by restoring microbial balance, enhancing gut health, and modulating neuroendocrine signaling through the gut–brain axis [28-30]. Similarly, recent evidence showed that *Lactobacillus* supplementation could enhance host defense with intestinal immunity by activating immune cells through NF-κB and mitogen-activated protein kinase (MAPK) signaling pathways in suckling piglets [31]. Among the diverse probiotic candidates, *Lactobacillus* species is a well-known lactic acid bacterium commonly identified in the intestine and provides multiple health benefits for the host [9, 15, 16]. *Lactobacillus* produces lactic acid to support maintaining an acidic luminal environment that suppresses the colonization of opportunistic harmful bacteria and helps microbial homeostasis [32-34]. *Lactobacillus* also positively regulates mucosal immune responses, increases IgA secretion, and reduces inflammation [35, 36]. These properties have been involved in the prevention of gastrointestinal disorders, reduced allergic responses, and favorable effects on nutrient metabolism and growth. A recent report by He et al. [37] demonstrated that a combination of *Lactobacillus* sp. and *Bifidobacterium thermacidophilum*, isolated from pigs, markedly enhanced growth performance, intestinal morphology, and systemic immunity in weaned pigs by reducing pro-inflammatory cytokines and modulating gut microbiota. Unlike single-strain supplementation, the synergistic application of two commensal species improved glycerophospholipid and cholesterol metabolism, highlighting a multi-omics mechanism that links microbial modulation to systemic metabolic remodeling. These findings support the potential of host-adapted probiotic consortia as an effective feed additive for health, which aligns with the objectives of the present study but extends the concept by focusing exclusively on supplemental effects of commensal *Lactobacillus* strains derived from pigs.

Notably, commensal *Lactobacillus* strains exhibit strong adaptability to the gut environment, supporting effective microbial colonization through producing more methionine and branched-chain amino acids, contributing to the maintenance of intestinal barrier integrity and metabolic support [15, 29]. Based on these findings, the present study was conducted to evaluate whether a consortium of the five commensal *Lactobacillus* strains could attenuate the adverse effects of weaning stress on the growth and health of nursery pigs. Our results demonstrate that dietary supplementation with the commercial *Lactobacillus*-based consortium significantly enhanced BW gain with reduced stress markers and improved HDL to LDL ratio. Importantly, these physiological improvements were accompanied by distinct shifts in fecal microbial composition, characterized by increased beneficial taxa, and elevated production of metabolites associated with amino acid, central carbon, and lipid metabolism. Therefore, this study can indicate that the commensal *Lactobacillus*-based consortium could reduce the negative impacts of weaning stress on the growth and health of nursery pigs through positive changes in gut microbiota with enhanced production of essential metabolites.

Lactobacillus populations are frequently diminished or destabilized during the post-weaning period, and recovery of their abundance through probiotic supplementation has been consistently associated with enhanced microbial diversity and improved intestinal health of nursery pigs [38, 39]. In the present study, supplementation of commensal *Lactobacillus*-based consortium distinctly altered the gut microbiota of nursery pigs after weaning. Specifically, the relative abundance of *Lactobacillus* and Prevotellaceae increased, whereas *Clostridiaceae* decreased, indicating that probiotic consortium intervention affects the bacterial community for a more favorable configuration [40]. Previous studies have demonstrated that elevated *Lactobacillus* levels are associated with improved gut morphology and enhanced barrier integrity in post-weaning pigs [36, 41]. The enrichment of *Lactobacillus* is particularly significant, as these strains are known to produce lactic acid, generate vitamins, and synthesize amino acids, thereby lowering luminal pH and conferring competitive advantages against pathogenic colonization [42]. Yie et al. [43] recently reported that *Lactobacillus* supplementation could enhance epithelial barrier function by upregulating tight junction proteins and promoting anti-inflammatory cytokine responses, indicating that host-adapted Lactobacillus species may exert outstanding mucosal protective effects under weaning stress conditions. Considering these functional traits, the decline in Clostridiaceae, a family of opportunistic harmful bacteria associated with enterocolitis, may be attributed to competition in mucosal adhesion, antimicrobial peptide production, and the production of short-chain fatty acids, particularly lactate and acetate [44]. The increased abundance of Prevotellaceae may also reflect metabolic adaptation, as this family is involved in carbohydrate fermentation and SCFA production, contributing to host energy metabolism and mucosal health [45]. These metabolites contribute to energy production and gut health. These shifts indicate that supplementation of commensal *Lactobacillus*-based consortium promoted cooperative networks of commensal taxa, reinforcing colonization resistance and metabolic stability. Significant increases in Shannon diversity and Pielou evenness indices were also observed, indicating the establishment of a more stable and functionally enriched microbial ecosystem [46]. Previous findings reported that increased microbial diversity after probiotic supplementation is associated with reduced incidence of post-weaning diarrhea and enhanced intestinal homeostasis [47, 48]. Although these findings highlight the ability of porcine gut-derived commensal *Lactobacillus* strains to beneficially shape microbial communities, a limitation of this study is that only porcine-origin strains were evaluated. Evidence from previous studies suggests that probiotics from different hosts can vary in colonization efficiency, metabolite production, and immune-modulatory capacity [49]. Therefore, further studies should directly compare porcine and non-porcine *Lactobacillus* strains to evaluate how their origin influences microbial ecology and physiological responses of nursery pigs under stress conditions.

The fecal metabolite profile provided additional mechanistic insight into how supplementation of the commensal *Lactobacillus*-based consortium could be related to the alleviation of weaning stress. Notably, there was a significant enrichment of SCFAs, particularly lactate and acetate, which are well-established for their roles in enhancing epithelial barrier integrity and attenuating intestinal inflammation [50, 51]. These metabolite shifts were strongly consistent with the microbial composition changes, as increased abundance of *Lactobacillus* and Prevotellaceae related to enhanced fermentation of carbohydrate and protein sources and SCFAs production [14]. Network analysis further highlighted lactate as a central hub metabolite, connected with glucose and amino acid metabolism, supporting its role as a key mediator of microbial shifts to systemic metabolic remodeling [52]. SCFAs provide available energy substrates for enterocytes and promote nutrient absorption efficiency [53], which may explain the enhanced feed utilization and body weight gain in the probiotic-supplemented group. Moreover, SCFAs have been reported to regulate host lipid metabolism [54], an observation that complements the improved HDL/LDL profile found in this study. Changes in lipid-associated metabolites, such as phospholipid and bile acid derivatives, indicates that commensal *Lactobacillus*-based consortium can affect host physiology outcomes with reduced hepatic stress and improved energy production, consistent with both the observed decline in liver stress markers [55]. Thus, the results suggest that restructuring of the community directly contributed to systemic metabolic and health improvements. In addition, increased levels of amino acid intermediates, including methionine, glycine, alanine, threonine, and valine, were consistent with the metabolic capacity of commensal *Lactobacillus* strains [14]. The enrichment of these metabolites is well aligned with the genomic and culture-based findings from our previous work, which showed that the five *Lactobacillus* species isolated from pigs were capable of synthesizing methionine and BCAAs [9]. These AA metabolites could be utilized for epithelial proliferation and repair, which is supported by the previous findings of improved gut barrier integrity following probiotic supplementation [52]. Although such metabolite enrichment may also reflect interactions with other feedstuffs, it also raises the intriguing question of whether supplementation with the *Lactobacillus*-based consortium could partially substitute for the use of synthetic amino acids or protein-based feedstuffs, warranting further investigation in future studies. As microbial restructuring, SCFAs, amino acid derivatives, and lipid-related metabolites are central mediators related to the changes in gut microbiota, including *Lactobacillus* and Prevotellaceae, and the reduction of Clostridiaceae, leading to improvements in health and growth of nursery pigs after weaning [56].

Weaning stress is also recognized to disrupt systemic physiology, often manifesting as altered hematological and biochemical parameters indicative of inflammation, oxidative stress, and impaired metabolic regulation in nursery pigs [57]. Previous studies have shown that elevated hepatic enzyme activities (AST and ALT) and systemic stress biomarkers (MDA and cortisol) in pigs after weaning [58, 59]. Dysregulation of lipid metabolism, typically characterized by reduced HDL and elevated LDL, has also been linked to microbial dysbiosis during this critical period [60, 61]. The results in this study also show that pigs supplemented with the *Lactobacillus*-based consortium had significantly lower AST and a trend for reduced ALT and LDH. These results indicate that the *Lactobacillus*-based consortium could alleviate stress response that is commonly related to systemic inflammation and oxidative damage during the post-weaning period. A increased HDL/LDL ratio implies enhancing lipid utilization and promoting a healthy status [62]. Improvements in serum profiles and liver function following microbiome-targeted interventions have also been reported in previous clinical trials showing fecal microbiota transplantation (FMT) in adolescents with obesity resulted in reduced systemic inflammation and higher HDL concentrations [63]. Similar reductions in hepatic enzyme activity have been reported following supplementation of *L. casei* or *L. plantarum*, further supporting the role of probiotics in alleviating liver injury [64, 65]. Marked reduction in serum MDA further highlights the capacity of probiotics to suppress lipid peroxidation, indicating a reduction in systemic oxidative stress caused by weaning [47]. These outcomes are strongly supported by the changes in fecal metabolites, which increased AA, leading to improved one-carbon metabolism and glutathione synthesis, key pathways in redox balance [66]. In addition, increases in key lipid- and energy-related metabolites, such as conjugated linoleic acid, lactate, malate, succinate, and glucose, indicating alterations in central carbon metabolism, redox pathways, and lipid turnover that further supported systemic metabolic stability [60]. Therefore, this study found that dietary supplementation with a commensal *Lactobacillus*-based consortium effectively mitigates the systemic physiological disruptions associated with weaning stress. However, as this study focused on gut microbial composition, metabolite profiles, and systemic health parameters, it remains uncertain whether the beneficial outcomes were mediated by enhanced intestinal function, or whether modulation of stress responses via the microbes–gut–brain axis signaling also contributed. Future studies integrating intestinal tissue analyses with neuroendocrine and behavioral assessments will be essential to explain the mechanistic pathways underlying the observed benefits, including host–microbe interactions, intestinal regulation, and neuroendocrine signaling pathways.

The beneficial effects of live *Lactobacillus* supplementation in swine diets have been well established. Recent evidence also highlights that postbiotic approaches using heat-killed *Lactobacillus* strains can bring immune and metabolic advantages [67], suggesting that further investigations into such strategies could further enhance feed utilization and stability with their health benefits. In addition, modulation of the gut–lung axis by the commensal *Lactobacillus*-based consortium may help alleviate ammonia-induced stress and reduce ammonia gas emission by improving nitrogen metabolism and microbial balance in the intestine [68]. Cheng and Kim [69] also recently demonstrated that microorganisms, including bacteria, can serve as nutritional and functional feedstuffs for pigs, suggesting sustainable alternatives to conventional protein supplements. These microbial ingredients are rich in high-quality protein, amino acids, fatty acids, and bioactive metabolites that improve growth performance, feed efficiency, and intestinal health by modulating immune responses and microbial balance in the gut [69]. Recent literature collectively suggests that a wide range of further studies are being conducted to explore the potential of probiotics, including *Lactobacillus*, with their value not only as feed additives but also as sustainable nutrient resources that enhance the growth and health of pigs.

CONCLUSION

In conclusion, dietary supplementation with a commensal *Lactobacillus*-based consortium appears to confer tangible benefits to nursery pigs by enhancing growth performance and systemic health. These effects are likely mediated through attenuation of oxidative stress and stress-related responses, alongside improvements in gut microbial diversity and metabolite profiles during the post-weaning period. Although these findings highlight the potential of commensal *Lactobacillus*-based consortium in mitigating weaning-associated challenges, the underlying biological mechanisms remain to be fully elucidated. Moreover, as probiotic effects may differ according to strain origin, potentially showing variation in efficacy, further comparative evaluations will be valuable. Integrative multi-omics approaches that connect microbial and metabolic alterations with host physiological and neuroendocrine responses will provide deeper mechanistic insight into the systemic benefits of probiotic consortium during the post-weaning period.

Acknowledgments

This work was supported by the National Research Foundation of Korea (NRF) grants funded by the Korean government (MEST; NRF-2021R1A2C3011051) and by the Basic Science Research Program through the NRF funded by the Ministry of Education (RS-2025-25418389).

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

Item	Basal diet
Feedstuff, %	
Corn, yellow	50.05
Soybean meal, 44% CP	21.20
Soy protein concentrate	10.00
Whey powder	12.50
Soybean oil	2.50
Limestone	1.40
Dicalcium phosphate	1.22
Vitamin-mineral premix1)	0.30
L-Lys HCl	0.38
DL-Met	0.30
L-Thr	0.15
Total	100.00
Calculated energy and nutrient contents	
Dry matter, %	90.01
Metabolizable energy, kcal/kg	3,490
Crude protein, %	22.18
SID2) Lysine, %	1.41
Calcium, %	0.98
STTD3) Phosphorus, %	0.42

1)Vitamin-mineral premix provided the following quantities of vitamins and minerals per kilogram of complete 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.

2)SID, standardized ileal digestible.

3)STTD, standardized total tract digestible.

Table 2. Growth performance of nursery pigs fed with commensal *Lactobacillus*-based consortium[1)]

Item[2)]	Control	CLC	SEM	*p*-value
BW, kg				
d 0	7.33	7.30	0.82	0.920
d 7	8.58	8.58	0.86	0.987
d 14	11.00	11.14	0.87	0.672
d 21	14.58	14.92	0.74	0.403
d 28	18.82	19.42	0.60	0.067
ADG, g/d				
d 0 to 7	179	183	6	0.531
d 7 to 14	344	366	13	0.263
d 14 to 21	513	539	23	0.339
d 21 to 28	605	643	25	0.297
Overall	410	433	9	0.037
ADFI, g/d				
d 0 to 7	285	297	8	0.317
d 7 to 14	525	564	17	0.129
d 14 to 21	676	688	21	0.652
d 21 to 28	870	880	22	0.634
Overall	589	607	10	0.180
G:F				
d 0 to 7	0.63	0.62	0.03	0.712
d 7 to 14	0.66	0.65	0.04	0.905
d 14 to 21	0.76	0.79	0.05	0.565
d 21 to 28	0.70	0.73	0.04	0.513
Overall	0.70	0.71	0.03	0.549

[1)] Each value is the mean value of 6 replicates (4 pigs/pen). SEM indicates standard error of the mean. CON indicates pigs fed diets without commensal *Lactobacillus*-based consortium; CLC indicates pigs fed diets with commensal *Lactobacillus*-based consortium at 0.02%;

[2)] BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G:F, gain to feed ratio.

Table 3. Effects of commensal *Lactobacillus*-based consortium on hematological profiles of nursery pigs[1)]

Item[2)]	Control	CLC	SEM	*p*-value
White blood cells, x10^3/µL	18.2	18.2	1.5	0.987
Red blood cells, x10^6/µL	5.1	5.8	0.5	0.321
Hemoglobin, g/dL	10.1	10.3	0.3	0.481
Hematocrit, %	32.6	35.2	2.3	0.332
Platelet, x10^3/µL	254.3	331.0	36.2	0.168
MCV, µm^3	67.2	60.8	4.1	0.298
MCHC, g/dL	32.6	29.2	2.8	0.407

[1)] Each value is the mean value of 6 replicates (4 pigs/pen). SEM indicates standard error of the mean. CON indicates pigs fed diets without commensal *Lactobacillus*-based consortium; CLC indicates pigs fed diets with commensal *Lactobacillus*-based consortium at 0.02%; Blood samples were collected on d 28 after weaning

[2)] MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration.

Table 4. Effects of commensal *Lactobacillus*-based consortium supplementation on serum biochemical profile and stress markers of nursery pigs[1)]

Item	Control	CLC	SEM	*p*-value
AST, Aspartate aminotransferase, U/L	128.0	48.0	12.4	<0.001
ALT, Alanine aminotransferase, U/L	133.0	50.5	35.0	0.053
LDH, Lactate dehydrogenase, U/L	730.5	505.0	82.1	0.084
GGT, Gamma-glutamyl transferase, U/L	40.3	36.5	5.1	0.607
Glucose, mg/dL	79.3	82.2	4.0	0.632
TG, Triglycerides mg/dL	62.8	60.8	6.8	0.835
TCHO, Total cholesterol, mg/dL	131.5	136.8	4.7	0.438
HDL, High-density lipoprotein, mg/dL	29.5	51.0	1.7	<0.001
LDL, Low-density lipoprotein, mg/dL	89.4	73.7	3.4	0.010

[1)] Each value is the mean value of 6 replicates (4 pigs/pen). SEM indicates standard error of the mean. CON indicates pigs fed diets without commensal *Lactobacillus*-based consortium; CLC indicates pigs fed diets with commensal *Lactobacillus*-based consortium at 0.02%.

Figure legends

Figure 1. Serum stress markers of nursery pigs fed with commensal *Lactobacillus*-based consortium. Serum samples were collected at day 28 after weaning. CON indicates pigs fed diets without commensal *Lactobacillus*-based consortium. CLC indicates pigs fed diets supplemented with commensal *Lactobacillus*-based consortium at 0.02%. (A) serum malondialdehyde (MDA) concentration. (B) serum advanced oxidation protein products (AOPP) concentration. (C) serum cortisol concentration. Bars represent the mean ± SEM. Statistical significance between groups indicates ***$p < 0.05$.

Figure 2. Gut microbiota of nursery pigs fed with commensal *Lactobacillus*-based consortium. (A) Genus/family-level composition. Stacked bars show the average of relative abundance for gut microbiota at weaning and d 28 after weaning of the pigs fed control diets (Control) or diets with commensal *Lactobacillus*-based consortium (CLC). (B) Pielou evenness and (C) Shannon index at day 28. Statistical significance was determined using Welch's student's t-test for Pielou evenness and Shannon diversity. (D) Principal coordinate analysis (PCoA) based on weighted UniFrac distance at day 28. (E) Unweighted UniFrac distances at day 28. (F) Log2 fold-change of selected genera in response to the consortium supplementation. (G) Volcano plot showing log2 fold change and statistical significance for each bacterium.

Figure 3. Comparative fecal metabolomic analysis among weaning, control, and commensal *Lactobacillus*-based consortium groups. (A) Principal component analysis (PCA) score plots of metabolites in the feces of pigs between the MSP and control groups. **(B)** The sparse PLS-DA (sPLS-DA) algorithm represents selected metabolites for a given component. The absolute values of their loadings rank metabolites. **(C)** Heatmap showing significantly differentially abundant fecal metabolites among treatment groups based on normalized GC/MS peak intensities. Principal component analysis (PCA), variable importance plot (VIP), and hierarchical clustering heatmap were generated using MetaboAnalyst (www.metaboanalyst.ca). Data were log-transformed and auto-scaled before metabolite data analysis. CON

indicates pigs fed diets without commensal *Lactobacillus*-based consortium. CLC indicates pigs fed diets supplemented with commensal *Lactobacillus*-based consortium at 0.02%. Statistical significance among groups was determined using one-way ANOVA followed by Tukey's post hoc test, and differences were considered significant at $p < 0.05$.

Figure 4. Comparison of fecal metabolite composition between control diet- and commensal *Lactobacillus*-based consortium-fed nursery pigs. (A) Volcano plot showing log2 fold change and statistical significance for each metabolite. (B) Relative fold change of representative amino acid–related metabolites and lipid/energy/redox-related metabolites. (C) Network analysis considering significantly altered metabolites to functional pathways. All metabolite intensities were log-transformed and auto-scaled before analysis. Volcano plots and network analysis were generated using MetaboAnalyst (www.metaboanalyst.ca) and independently verified in Python (pandas, scipy, numpy). One-way ANOVA determined group differences and *p*-values were corrected for multiple testing using the Benjamini–Hochberg false discovery rate (FDR). Fold change (FC) was calculated as the ratio of mean abundance in CLC relative to control. CON indicates pigs fed diets without commensal *Lactobacillus*-based consortium. CLC indicates pigs fed diets supplemented with commensal *Lactobacillus*-based consortium at 0.02%. Metabolites with FDR < 0.05 were considered statistically significant, whereas those with higher FDR but consistent directional shifts were indicated as trends. In bar plots, significance is denoted by $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

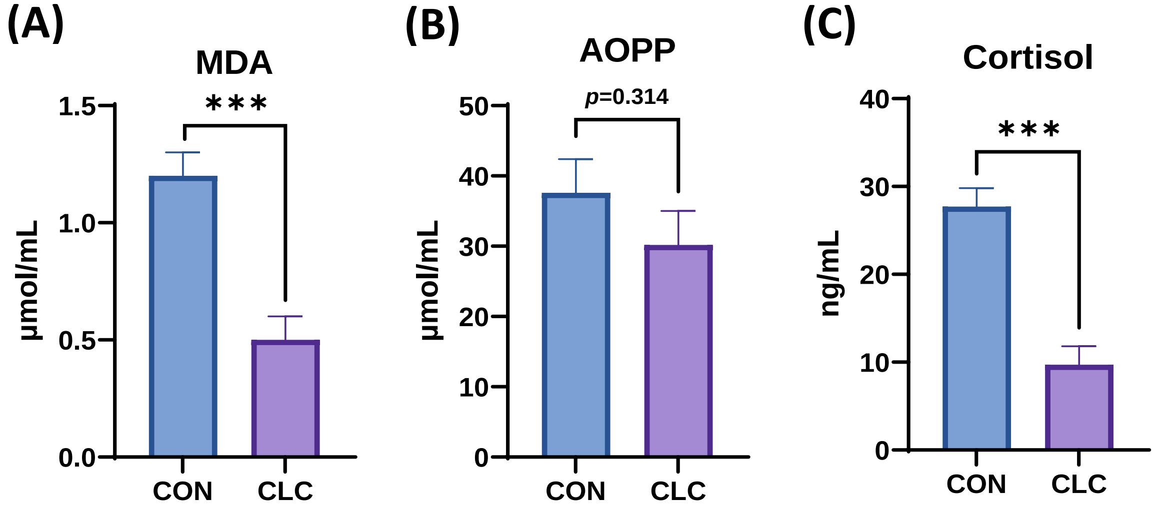
(A)
MDA
***
1.5
1.0
0.5
0.0
μmol/mL
CON
CLC
(B)
AOPP
p=0.314
50
40
30
20
10
0
μmol/mL
CON
CLC
(C)
Cortisol
***
40
30
20
10
0
ng/mL
CON
CLC

Figure 1.

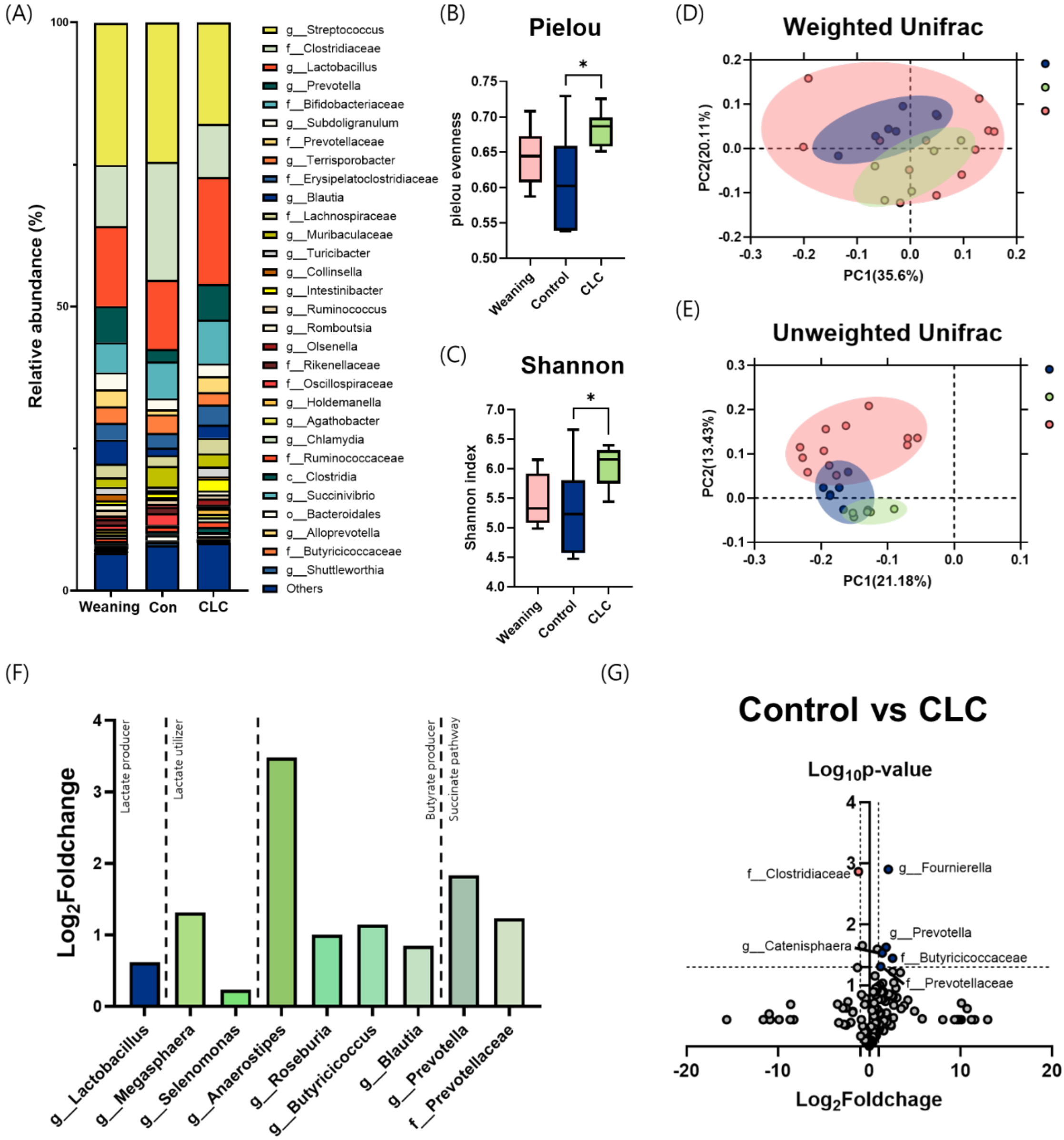
(A)
Relative abundance (%)
100
50
0
Weaning
Con
CLC
g__Streptococcus
f__Clostridiaceae
g__Lactobacillus
g__Prevotella
f__Bifidobacteriaceae
g__Subdoligranulum
f__Prevotellaceae
g__Terrisporobacter
f__Erysipelatoclostridiaceae
g__Blautia
f__Lachnospiraceae
g__Muribaculaceae
g__Turicibacter
g__Collinsella
g__Intestinibacter
g__Ruminococcus
g__Romboutsia
g__Olsenella
f__Rikenellaceae
f__Oscillospiraceae
g__Holdemanella
g__Agathobacter
g__Chlamydia
f__Ruminococcaceae
c__Clostridia
g__Succinivibrio
o__Bacteroidales
g__Alloprevotella
f__Butyricicoccaceae
g__Shuttleworthia
Others
(B)
Pielou
pielou evenness
0.75
0.70
0.65
0.60
0.55
0.50
*
Weaning
Control
CLC
(C)
Shannon
Shannon index
7.0
6.5
6.0
5.5
5.0
4.5
4.0
*
Weaning
Control
CLC
(D)
Weighted Unifrac
PC2(20.11%)
PC1(35.6%)
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.2
-0.1
0.0
0.1
0.2
Control
CLC
Weaning
(E)
Unweighted Unifrac
PC2(13.43%)
PC1(21.18%)
0.3
0.2
0.1
0.0
-0.1
-0.3
-0.2
-0.1
0.0
0.1
Control
CLC
Weaning
(F)
$Log_2Foldchange$
4
3
2
1
0
Lactate producer
Lactate utilizer
Butyrate producer
Succinate pathway
g__Lactobacillus
g__Megasphaera
g__Selenomonas
g__Anaerostipes
g__Roseburia
g__Butyricicoccus
g__Blautia
g__Prevotella
f__Prevotellaceae
(G)
Control vs CLC
Log_{10}p-value
4
3
2
1
f__Clostridiaceae
g__Fournierella
g__Prevotella
g__Catenisphaera
f__Butyricicoccaceae
f__Prevotellaceae
-20
-10
0
10
20
Log_2Foldchage

Figure 2.

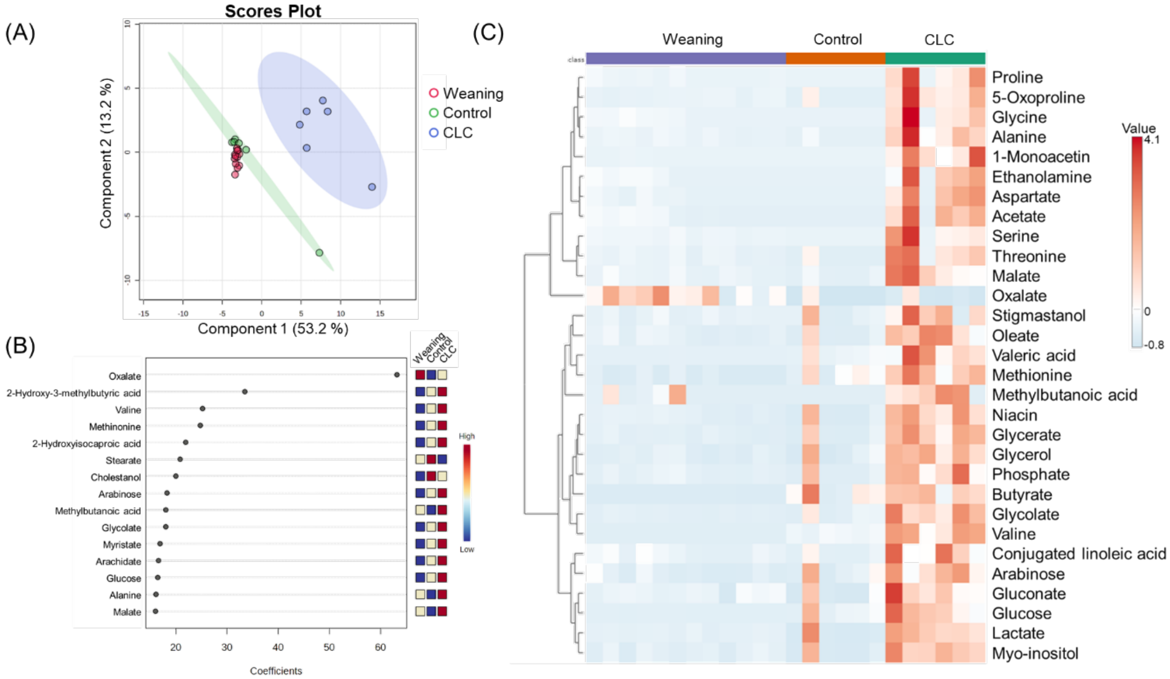
(A)
Scores Plot
Component 2 (13.2 %)
Component 1 (53.2 %)
10
5
0
-5
-10
-15
-10
-5
0
5
10
15
Weaning
Control
CLC
(B)
Oxalate
2-Hydroxy-3-methylbutyric acid
Valine
Methinonine
2-Hydroxyisocaproic acid
Stearate
Cholestanol
Arabinose
Methylbutanoic acid
Glycolate
Myristate
Arachidate
Glucose
Alanine
Malate
20
30
40
50
60
Coefficients
Weaning
Control
CLC
High
Low
(C)
Weaning
Control
CLC
class
Proline
5-Oxoproline
Glycine
Alanine
1-Monoacetin
Ethanolamine
Aspartate
Acetate
Serine
Threonine
Malate
Oxalate
Stigmastanol
Oleate
Valeric acid
Methionine
Methylbutanoic acid
Niacin
Glycerate
Glycerol
Phosphate
Butyrate
Glycolate
Valine
Conjugated linoleic acid
Arabinose
Gluconate
Glucose
Lactate
Myo-inositol
Value
4.1
0
-0.8

Figure 3.

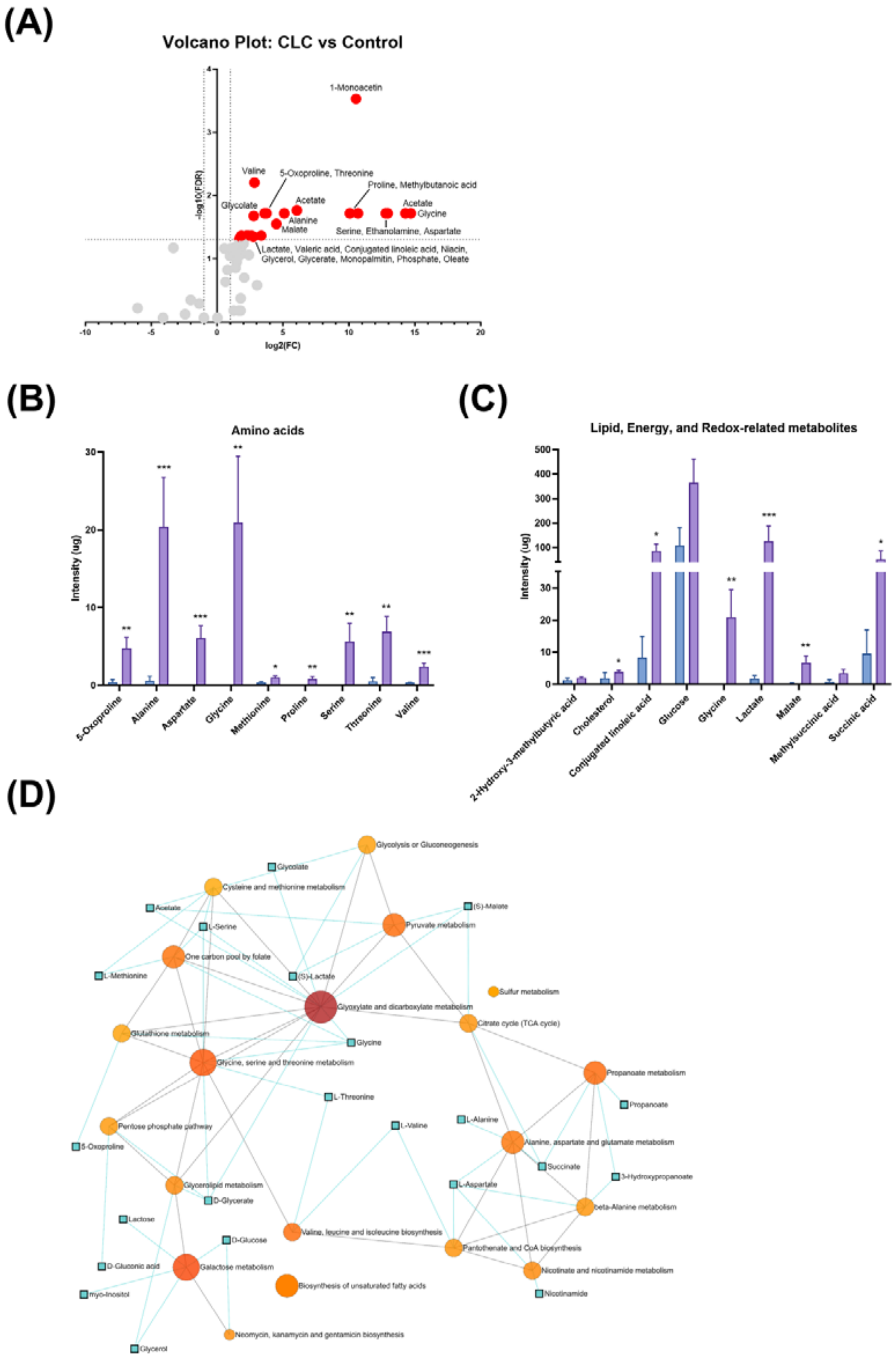
(A)
Volcano Plot: CLC vs Control
-log10(FDR)
log2(FC)
-10
-5
0
5
10
15
20
1
2
3
4
1-Monoacetin
Valine
5-Oxoproline, Threonine
Proline, Methylbutanoic acid
Glycolate
Acetate
Acetate
Glycine
Alanine
Malate
Serine, Ethanolamine, Aspartate
Lactate, Valeric acid, Conjugated linoleic acid, Niacin,
Glycerol, Glycerate, Monopalmitin, Phosphate, Oleate
(B)
Amino acids
Intensity (ug)
0
10
20
30
5-Oxoproline
Alanine
Aspartate
Glycine
Methionine
Proline
Serine
Threonine
Valine
**
***
***
**
*
**
**
**
***
(C)
Lipid, Energy, and Redox-related metabolites
Intensity (ug)
0
10
20
30
100
200
300
400
500
2-Hydroxy-3-methylbutyric acid
Cholesterol
Conjugated linoleic acid
Glucose
Glycine
Lactate
Malate
Methylsuccinic acid
Succinic acid
*
*
**
***
**
*
(D)
Glycolysis or Gluconeogenesis
Glycolate
Cysteine and methionine metabolism
Acetate
(S)-Malate
L-Serine
Pyruvate metabolism
One carbon pool by folate
L-Methionine
(S)-Lactate
Sulfur metabolism
Glyoxylate and dicarboxylate metabolism
Glutathione metabolism
Citrate cycle (TCA cycle)
Glycine
Glycine, serine and threonine metabolism
Propanoate metabolism
L-Threonine
Propanoate
L-Alanine
L-Valine
Pentose phosphate pathway
5-Oxoproline
Alanine, aspartate and glutamate metabolism
Succinate
3-Hydroxypropanoate
Glycerolipid metabolism
L-Aspartate
D-Glycerate
beta-Alanine metabolism
Lactose
Valine, leucine and isoleucine biosynthesis
D-Glucose
Pantothenate and CoA biosynthesis
D-Gluconic acid
Galactose metabolism
Nicotinate and nicotinamide metabolism
Biosynthesis of unsaturated fatty acids
myo-Inositol
Nicotinamide
Neomycin, kanamycin and gentamicin biosynthesis
Glycerol

Figure 4.