1 **TITLE PAGE**

ARTICLE INFORMATION	**Fill in information in each box below**
Article Type	Research article
Article Title (within 20 words without abbreviations)	*Bacillus*-based probiotics improve growth and intestinal health of weaned pigs vaccinated against porcine reproductive and respiratory syndrome
Running Title (within 10 words)	*Bacillus* probiotics in vaccinated weaned pigs
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Competing interests	Lea Hübertz Birch Hansen, Shirley Mok, and In Sun Yu are employees of Novonesis, the manufacturer of the probiotic product (Solvens) evaluated in this study and the funder of this work. The remaining authors declare that they have no competing interests.
Funding sources **State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available.**	This work was supported by Novonesis. The animal trial, laboratory analyses, and statistical analyses were conducted independently by Chungbuk National University.
Acknowledgements	We acknowledge Novonesis for providing Solvens for this study.
Availability of data and material	All data generated or analyzed during this study are included within the article. Raw 16S rRNA gene sequencing data are available upon reasonable request from the corresponding author.
Authors' contributions **Please specify the authors' role using this form.**	[#] *These authors contributed equally to this work* Conceptualization: Hansen LHB, Mok S, Yu IS, Cho JH Data curation: Kim H, Jeon KH Formal analysis: Kim H, Jeon KH Methodology: Song DC, Hansen LHB, Mok S, Yu IS, Cho JH Validation: Hansen LHB, Mok S, Yu IS Software: Yang JM, Yu HH Investigation: Kim H, Jeon KH, Song DC, Yang JM, Yu HH Writing - original draft: Kim H, Jeon KH Writing - review & editing: Kim H, Jeon KH, Song DC, Yang

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	JM, Yu HH, Hansen LHB, Mok S, Yu IS, Cho JH
Ethics approval and consent to participate	The animal experimental protocols used in this study were approved by the Institutional Animal Care and Use Committee of Chungbuk National University, Republic of Korea (Approval number: CBNU-25-0110-01), and the study was conducted in strict compliance with Korean national animal welfare legislation.
Declaration of generative AI	During the preparation of this manuscript, the author(s) used Claude (Anthropic) solely for translation of the text into English. The author(s) reviewed and edited the output and take(s) full responsibility for the content of the publication.

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Abstract

In the Republic of Korea, weaned pigs on commercial farms are routinely vaccinated against porcine reproductive and respiratory syndrome virus at weaning. This study evaluated whether dietary supplementation with a *Bacillus*-based probiotic improves growth performance, nutrient digestibility, immune indices, and intestinal health in pigs managed under this routine vaccination program. A total of 96 crossbred weaned pigs (28 days of age; initial body weight 8.22 ± 1.27 kg) were allotted to two dietary treatments in a randomized complete block design with two pigs per pen and 24 replicate pens per treatment: a basal diet (Vac) and the basal diet supplemented with 0.04% of a *Bacillus*-based probiotic containing *Bacillus licheniformis* DSM 5749, *Bacillus subtilis* DSM 5750, and *Bacillus subtilis* DSM 27273 (Vac+Pro). All pigs were vaccinated intramuscularly with a modified live porcine reproductive and respiratory syndrome virus vaccine on d 0, and the trial lasted 28 d. Compared with Vac, Vac+Pro increased body weight on d 28, increased average daily gain and improved the feed conversion ratio from d 7 to 28 and from d 0 to 28, and lowered the fecal score from d 0 to 7 and from d 0 to 28. In Vac+Pro compared with Vac, apparent total tract digestibility of dry matter and crude protein was higher on d 7, 14, and 28, and apparent ileal digestibility of crude protein, gross energy, and organic matter was higher on d 28. Serum immunoglobulin G and serotonin were higher, whereas tumor necrosis factor-alpha, interleukin-6, and cortisol were lower in Vac+Pro on d 7 and 28. Villus height and the villus height to crypt depth ratio were greater and crypt depth was lower in Vac+Pro. In Vac+Pro, fecal acetate and propionate were higher and fecal *Escherichia coli* counts were lower on d 28. Because no unvaccinated control group was included, these results represent the efficacy of the probiotic under the vaccination program used in commercial practice, where it may be applied as a functional feed additive to support growth, digestive capacity, intestinal integrity, and immune balance in weaned pigs.

Keywords: *Bacillus*-based probiotics, Growth performance, Gut health, Nutrient digestibility, Porcine reproductive and respiratory syndrome virus, Weaned pigs

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INTRODUCTION

Porcine reproductive and respiratory syndrome (PRRS) is one of the most economically significant infectious diseases affecting commercial swine production worldwide, causing substantial losses due to respiratory issues, reproductive failures, and increased mortality [1]. For over three decades, modified live virus (MLV) vaccines have been utilized to control PRRS virus (PRRSV) and are administered to nursery and growing pigs [2, 3].

Weaned pigs receive vaccination at a developmental stage characterized by immature immunity, reduced feed intake, and compromised gut barrier function [4]. This makes them particularly susceptible to the additional immunological stress introduced by vaccination. Immunization is known to induce immune stress, activating the hypothalamic-pituitary-adrenal (HPA) axis, increasing cortisol levels, and leading to lymphocyte apoptosis, which can suppress growth [5]. PRRSV MLV vaccination is no exception. Jayaraman et al. [1] reported that, during disease outbreaks, nursery pigs in large-scale commercial herds vaccinated with a full dose of PRRSV MLV experienced significantly higher mortality (8.84 percentage points; 95% confidence interval: 4.70–12.98) compared to those receiving a half dose. Furthermore, vaccinating 3-week-old pigs with PRRSV MLV resulted in reduced body weight at wk 3 and lower average daily gain during wk 2 to 3 compared to unvaccinated pigs [6]. Additionally, PRRSV MLV vaccination has been shown to increase levels of haptoglobin, a primary acute phase protein in pigs, with concentrations peaking around 3 days post-vaccination [7]. Collectively, these findings indicate that the immune activation triggered by vaccination imposes a measurable physiological burden on weaned pigs. Because vaccination is an essential disease-control measure in commercial pig production, a practical strategy is to improve the overall resilience of weaned pigs during the period in which vaccination is routinely administered; nutritional approaches evaluated under these routine conditions have received little attention.

Probiotics, particularly *Bacillus*-based probiotics, have been studied as a nutritional strategy to enhance growth performance and immune function in livestock [8, 9]. The *Bacillus* genus offers several advantages over other probiotics, including tolerance to acid and bile, intestinal adherence, anti-pathogenic activity, and support for the mucosal barrier [10, 11]. *Bacillus* supplementation has been shown to alleviate immune challenges by strengthening intestinal barrier integrity in weaned pigs [12–14]. Notably, a triple-strain *Bacillus* probiotic (comprising *Bacillus licheniformis* and *Bacillus subtilis*) downregulated the expression of ileal tumor necrosis factor-α (TNF-α) and interleukin-4 (IL-4) while upregulating the tight junction protein occludin in weaned pigs [15]. These findings indicate that *Bacillus*-based probiotics can modulate cytokine responses and reinforce the gut barrier in weaned pigs.

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On commercial farms in the Republic of Korea, PRRSV MLV vaccination is routinely administered to pigs at weaning; a nutritional intervention for this stage should therefore be evaluated under this condition. We hypothesized that dietary supplementation with a *Bacillus*-based probiotic (Solvens) would improve growth performance, nutrient digestibility, immune indices and intestinal health in weaned pigs receiving routine PRRSV MLV vaccination. Vaccination was a standardized management condition applied identically to both dietary treatments.

MATERIALS AND METHODS

The animal experimental protocols used in this study were approved by the Institutional Animal Care and Use Committee of Chungbuk National University, Republic of Korea (Approval number: CBNU-25-0110-01), and the study was conducted in strict compliance with Korean national animal welfare legislation.

Animals, treatments, and experimental design

This study was conducted as two independent trials using identical experimental methods (dietary treatments and procedures). A total of 96 crossbred weaned pigs [(Yorkshire × Landrace) × Duroc; 48 pigs per trial] at 28 days of age, with an initial body weight (BW) of 8.22 ± 1.27 kg, were used. Pigs were assigned to a randomized complete block design, in which pigs were stratified into blocks by initial BW prior to treatment assignment to minimize the contribution of initial size variation to experimental error. There were two pigs per pen and 24 replication pens per treatment. Pigs were allotted to one of two dietary treatments: Vac, an antibiotic- and organic acid-free corn-soybean meal-based basal diet; Vac+Pro, Vac supplemented with 0.04% *Bacillus*-based probiotics at an inclusion rate of 400 mg/kg. All pigs were vaccinated with Ingelvac PRRS MLV (VR-2332 strain; Boehringer Ingelheim, Ingelheim am Rhein, Germany) via intramuscular injection (2 mL per pig, 10^5 50% tissue culture infective doses ($TCID_{50}$)/mL) on d 0, reproducing the vaccination program routinely applied to weaned pigs on commercial farms in the Republic of Korea. Vaccination was therefore a standardized management condition common to both treatments, not an experimental treatment or an immune challenge, and the experiment was not designed to quantify the effect of vaccination itself. *Bacillus*-based probiotics (Solvens, Novonesis, Kongens Lyngby, Denmark) provided a viable count of 1.30×10^8 colony-forming units (CFU)/g, comprising three characterized probiotic strains: *Bacillus licheniformis* DSM 5749, *Bacillus subtilis* DSM 5750, and *Bacillus subtilis* DSM 27273. Basal diets were formulated to meet or exceed the nutritional requirements for weaned pigs as specified by the National Research Council [16], and the composition and nutrient levels of the basal diet are

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presented in Table 1. All diets were provided in mash form throughout the experimental period. Incorporation of *Bacillus*-based probiotics into the Vac+Pro diet was performed using a mixer to ensure homogeneous distribution of the probiotic additive throughout the diet matrix. The feeding program comprised two dietary phases: Phase 1 (d 0 to 7), corresponding to the immediate post-weaning adaptation phase, and Phase 2 (d 8 to 28), corresponding to the early growth phase.

Animals, housing, and husbandry

Pigs were housed in pens measuring 2.2 m^2 fitted with fully slatted floor systems to facilitate fecal passage and hygienic management. Each pen was equipped with a single nipple drinker providing ad libitum access to potable water throughout the experimental period. All pigs received a standardized baseline vaccination program at 21 days of age, prior to experimental entry, comprising vaccines against *Mycoplasma hyopneumoniae* and porcine circovirus type 2 (PCV2); this program was part of routine commercial management and was identical for both treatments. Environmental conditions, including temperature and ventilation, were maintained within age-appropriate thermocomfort ranges throughout the trial duration. Feed was provided ad libitum.

Growth performance and fecal score

Pigs were weighed individually on d 0, d 7, and d 28. Feed intake was recorded daily as the difference between the amount of diet supplied and the amount remaining. Average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were calculated for each interval from d 0 to 7, d 7 to 28, and d 0 to 28. The fecal scores were individually recorded at 08:00 and 17:00 by the same person throughout the experimental period, and were summarized for d 0 to 7, d 8 to 28, and d 0 to 28. The fecal scoring system described by Chang et al. [17] was used, and scores were assigned as follows: 0, normal feces; 1, soft feces; 2, mild diarrhea; 3, severe diarrhea.

Nutrient digestibility

Chromium oxide (Cr_2O_3, 2 g/kg) was added to the diets as an indigestible marker for digestibility [18]. Pigs were fed diets mixed with chromium oxide for 4 consecutive days before each collection (d 4 to 7, d 11 to 14, and d 25 to 28), and fresh feces were collected by rectal massage on the last day of each marker period (d 7, 14, and 28), after 3 d of marker feeding. Feces collected within each treatment and trial were pooled across pens to give

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three composite samples per treatment per trial (six per treatment). The same indigestible marker, inclusion level, and 4-d marker feeding period were applied in a previous nutrient digestibility study in weaned pigs [17]. Fecal samples for apparent total tract digestibility (ATTD) were collected on d 7, 14, and 28, whereas ileal digesta for apparent ileal digestibility (AID) were collected on d 28 only. For ileal digesta sampling on d 28, one pig per pen, the heavier of the two pigs, was anesthetized with carbon dioxide gas after blood sampling and euthanized by exsanguination. Immediately upon confirmation of death, the abdominal cavity was opened, and sections of the ileum were isolated, comprising the terminal 80 cm of the ileum. Ileal digesta from these pigs were likewise pooled across pens to give three composite samples per treatment per trial. At the same time, 24 replications of these feed samples were collected. Fresh fecal, ileal digesta, and feed samples were immediately stored at −20°C until further processing. Feed, fecal, and ileal digesta samples were all dried at 65°C for 72 h and ground through a 1-mm screen prior to analysis. Dry matter (DM; method 930.15), organic matter (OM; method 942.05), and ether extract (EE; method 920.39) were determined according to AOAC methods [19]. The gross energy (GE) of diets, feces, and ileal digesta was analyzed using an oxygen bomb calorimeter (6400 Automatic Isoperibol Calorimeter, Parr Instrument, Moline, IL, USA). The crude protein (CP) content was analyzed using the Dumas combustion method (Rapid MAX N-Exceed, Elementar, Langenselbold, Germany). Dried samples were ashed at 550°C for 6 h for Ca and P analysis. The Ca concentration (method 968.08) was analyzed according to AOAC methods. The P concentration was determined colorimetrically using a wet ash procedure, as described by Sands et al. [20]. Chromium levels were determined via UV absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan) according to the method of Williams et al. [21]. For calculating the ATTD and AID of the nutrients, the following equation was used: digestibility (%) = [1 − (Nf × Cd) / (Nd × Cf)] × 100, where Nf = concentration of nutrient in fecal or ileal digesta, Nd = concentration of nutrient in the diet, Cd = concentration of chromium in the diet, and Cf = concentration of chromium in the fecal or ileal digesta.

Fecal *Escherichia coli*

To analyze fecal *Escherichia coli* (*E. coli*), fresh feces were collected using the rectal massage method from all pigs in each pen for each treatment group on d 0, d 7, and d 28. Bacterial colonies were counted by the pour plate method. One gram of each fecal sample was diluted with 9 mL of sterile distilled water and homogenized. Samples were used for measuring the number of viable cells by plating serial 10-fold dilutions. *E. coli* was cultured at 37°C for 24 h using MacConkey agar (KisanBio, Seoul, Republic of Korea), and beta-hemolytic colonies were

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enumerated after culture at 37°C for 24 h on blood agar (KisanBio, Seoul, Republic of Korea). The number of *E. coli* and beta-hemolytic colonies was counted immediately after the plates were removed from the incubator. Colonies showing beta-hemolysis on blood agar were enumerated as such; their species identity was not confirmed by further biochemical or molecular testing, and they are therefore reported as beta-hemolytic colonies rather than as hemolytic *E. coli*. The number of microbial colonies was log-transformed prior to statistical analysis.

Blood profile

Blood samples were collected by jugular venipuncture from all pigs on d 0, d 7, and d 28. At the time of collection, blood samples were collected into nonheparinized tubes for serum analysis. Serum samples were centrifuged at 3,000×g for 20 min at 4°C and subsequently stored at −20°C in a freezer until further analysis. Serum concentrations of immunoglobulin G (IgG) and immunoglobulin A (IgA) were determined with an automatic biochemistry blood analyzer (Hitachi 747, Hitachi, Tokyo, Japan). Interleukin-6 (IL-6) and TNF-α concentrations were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Quantikine, R&D Systems, Minneapolis, MN, USA), and the absorbance was measured at 450 nm. Serum cortisol levels were assessed using a radioimmunoassay kit (Coat-A-Count Cortisol, cat. no. TKCO5, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). Concentrations of serotonin in serum were analyzed using a commercially available ELISA kit (Cloud-Clone Corp., Katy, TX, USA) according to the manufacturer's protocol.

Intestinal morphology

At the end of the experiment, one pig per pen, the heavier of the two pigs, was anesthetized with carbon dioxide gas after blood sampling and euthanized by exsanguination. After euthanasia, intestinal tissue segments of approximately 5 cm in length were harvested from the ileum (close to the ileocecal junction) and immediately fixed in 10% neutral buffered formalin (Sigma-Aldrich, St. Louis, MO, USA) for morphological analysis. Fixed tissues were embedded in paraffin, sectioned at a thickness of 5 μm, deparaffinized, and rehydrated. Sections were mounted on slides and stained with hematoxylin and eosin. The slides were examined using an Olympus IX51 inverted phase-contrast microscope (Olympus, Tokyo, Japan). Intestinal morphological measurements included villus height (VH), crypt depth (CD), and villus height to crypt depth ratio (VH:CD).

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Fecal short-chain fatty acids

Fresh fecal samples were collected into sterile conical tubes from all pigs on d 0 and d 28. Aliquots of approximately 1 g were suspended in 9 mL of ultrapure water and then centrifuged at 12,000×g and 4°C for 15 min. A sample of each collected supernatant (1 mL) was mixed with meta-phosphoric acid (0.2 mL) and then stored at −20°C until further use. For analysis, thawed samples were passed through a 0.22-μm filter membrane and centrifuged at 12,000×g for 5 min. A 1-μL aliquot of the supernatant was injected into a GC-TRACE1300 gas chromatograph (Thermo Fisher Scientific, Waltham, MA, USA) using a micro sampler. Fecal concentrations of acetic acid, propionic acid, and butyric acid were measured.

Fecal microbiome

Fresh fecal samples were collected directly into sterile conical tubes from the heavier of the two pigs in each pen at the start of the experiment (d 0), which was also the pig used for ileal digesta and intestinal morphology sampling, on d 0 and d 28 and stored at −20°C until further processing. Samples were sent to Sanigen (Anyang, Republic of Korea) for 16S rRNA gene amplicon sequencing. All raw data were converted into QIIME2 artifacts, encompassing information about data types and sources for subsequent analysis. Amplicon sequence variants (ASVs) were identified from the raw sequence data using the Divisive Amplicon Denoising Algorithm 2, a QIIME2 plugin. This algorithm corrects amplicon errors and filters out potential base errors and chimeric sequences using the Bolyen et al. [22] method. The Relative Classification Frequency Table, which shows differential abundance tests at specific taxonomic levels, was generated using collapse and feature functions within the QIIME2 plugins. Alpha-diversity indices were estimated using the QIIME2 'diversity' plugin and subsequently compared between groups using a mixed model in JMP 16.0. This microbial diversity analysis pipeline used the ASV table (a more refined alternative to the traditional operational taxonomic unit table) as input data. Species richness and evenness scores, accounting for sampling depth, were measured using Chao1, Shannon, and Simpson indices. All indices were calculated from the V3–V4 hypervariable region of the bacterial 16S rRNA gene. Relative abundance differences were analyzed by comparing average bacterial proportions and compositions at each taxonomic level. Furthermore, bacterial classification accuracy across different amplicon regions was verified by comparing the taxonomy matching rate of each ASV's taxonomy with the National Center for Biotechnology Information (NCBI) bacterial reference genome database at the phylum and genus levels.

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205 **Statistical analysis**

206 All statistical analyses were performed using JMP 16.0 (SAS Institute Inc., Cary, NC, USA), except for fecal
207 scores and beta-diversity. The pen was considered the experimental unit for all measurements except ATTD and
208 AID; for traits measured on both pigs within a pen, pig-level values were averaged to obtain a single pen-level
209 value, whereas for intestinal morphology and fecal microbiota analysis, the heavier pig in each pen was used to
210 represent the pen. For ATTD and AID, samples from several pens were pooled, and the six composite samples per
211 treatment (three per trial) were the experimental units. Data were analyzed using a mixed model with treatment
212 as a fixed effect and trial as a random effect to account for variation between experimental trials, using the
213 restricted maximum likelihood (REML) method. Normality of the residuals was assessed using the Shapiro-Wilk
214 test after model fitting, and all variables approximated a normal distribution. Measured variables included body
215 weight, apparent nutrient digestibility, intestinal morphology, gut microbiota, gut metabolites, and blood
216 parameters. Fecal scores were analyzed using the chi-square test based on the frequency distribution of each score
217 across treatment groups. Alpha-diversity indices (Shannon, Simpson, Chao1) were compared between groups
218 using a mixed model with treatment as a fixed effect and trial as a random effect in JMP 16.0. Beta-diversity was
219 visualized in MicrobiomeAnalyst [23] and tested by permutational multivariate analysis of variance
220 (PERMANOVA) on Bray–Curtis dissimilarities of genus-level relative abundances, using the adonis2 function of
221 the vegan package in R with 9,999 permutations; the R^2 and *p*-value of each test are reported. Relative abundance
222 of bacterial taxa at the phylum and genus levels was compared between treatment groups using a mixed model
223 with treatment as a fixed effect and trial as a random effect in JMP 16.0. Within each taxonomic level and time
224 point, *p*-values were adjusted for multiple testing using the Benjamini-Hochberg procedure, and both unadjusted
225 *p*-values and *q*-values are reported (Tables 9 and 10). Relative abundance data are compositional, and the mixed
226 model applied here does not account for that structure; the taxon-level comparisons are therefore treated as
227 exploratory rather than confirmatory. Data are presented as least squares means with pooled standard errors (SE).
228 Differences were considered statistically significant when $p \leq 0.05$, and as a tendency when $0.05 < p \leq 0.10$.
229

230 **RESULTS**

231 **Growth performance**

232 The effects of *Bacillus*-based probiotics on the growth performance of the PRRSV-vaccinated weaned pigs are
233 presented in Table 2. BW was significantly higher ($p < 0.05$) in the Vac+Pro group on d 28. The ADG was
234 significantly higher ($p < 0.05$) in the Vac+Pro group during d 7 to 28 and d 0 to 28. In addition, the FCR was

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significantly lower ($p < 0.05$) in the Vac+Pro group during d 7 to 28 and d 0 to 28. No significant differences were observed in ADFI.

Fecal score

The effects of *Bacillus*-based probiotics on fecal score are presented in Figure 1. Fecal score was significantly lower ($p < 0.05$) in the Vac+Pro group during d 0 to 7 and d 0 to 28.

Nutrient digestibility

The effects of *Bacillus*-based probiotics on the ATTD and AID of nutrients are presented in Tables 3 and 4, respectively. The ATTD of DM, CP, EE, and OM was significantly higher ($p < 0.05$) in the Vac+Pro group on d 7. On d 14, the ATTD of DM and CP was significantly higher ($p < 0.05$) in the Vac+Pro group. In addition, the ATTD of DM, CP, and GE was significantly higher ($p < 0.05$), and OM tended to be higher ($p = 0.09$), in the Vac+Pro group on d 28. However, the ATTD of P tended to be lower ($p = 0.08$) on d 28. The AID of CP, GE, and OM was significantly higher ($p < 0.05$) in the Vac+Pro group. In addition, the AID of DM tended to be higher ($p = 0.06$) in the Vac+Pro group.

Blood profile

The effects of *Bacillus*-based probiotics on blood immune and stress markers are presented in Table 5. On d 0, IgA tended to be lower ($p = 0.07$) in the Vac+Pro group. On d 7, IgG and serotonin were significantly higher ($p < 0.05$), while TNF-α, IL-6, and cortisol were significantly lower ($p < 0.05$) in the Vac+Pro group. IgA tended to be higher ($p = 0.07$) in the Vac+Pro group. In addition, all parameters showed significant differences ($p < 0.05$) on d 28.

Intestinal morphology

The effects of dietary *Bacillus*-based probiotics on intestinal morphology are presented in Table 6 and Figure 2. The VH and VH:CD were significantly higher ($p < 0.05$), and the CD was significantly lower ($p < 0.05$) in the Vac+Pro group.

Fecal short-chain fatty acids

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The effects of dietary *Bacillus*-based probiotics on fecal short-chain fatty acid (SCFA) profiles are presented in Table 7. On d 28, acetate and propionate were significantly higher ($p < 0.05$) in the Vac+Pro group. No significant differences were observed in butyrate.

Fecal *Escherichia coli*

The effects of dietary *Bacillus*-based probiotics on fecal *E. coli* counts are presented in Table 8. *E. coli* counts were significantly lower ($p < 0.05$) in the Vac+Pro group on d 28. Beta-hemolytic colonies were not detected in either treatment group at any time point (d 0, d 7, and d 28).

Fecal microbial community structure and taxonomy

The effects of dietary *Bacillus*-based probiotics on fecal microbiota are presented in Tables 9 and 10 and Figures 3–6. The Simpson index tended to differ ($p = 0.06$) and the Shannon index tended to differ ($p = 0.06$) among treatments on d 28 (Figure 3). Beta-diversity of the fecal microbiota did not differ between treatments (Figure 4; PERMANOVA on Bray–Curtis dissimilarity: $R^2 = 0.007$, $p = 0.971$ on d 0 and $R^2 = 0.011$, $p = 0.687$ on d 28). No significant differences were observed in the relative abundance of fecal microbiota at the phylum level (Figure 5; Table 9), and no phylum differed after Benjamini-Hochberg correction ($q \geq 0.650$ on d 0 and $q \geq 0.670$ on d 28). Before correction, the *Ruminococcus* abundance was lower (unadjusted $p = 0.032$) in the Vac+Pro group on d 28 (Figure 6; Table 10); however, after Benjamini-Hochberg correction across the 20 genera compared at this time point, no genus differed between treatments ($q = 0.640$ for *Ruminococcus*).

DISCUSSION

In this study, dietary supplementation with a triple-strain *Bacillus*-based probiotic (Solvens; *B. licheniformis* DSM 5749, *B. subtilis* DSM 5750, and *B. subtilis* DSM 27273) enhanced growth performance, intestinal health, and immune status in vaccinated weaned pigs. Supplementation with the *Bacillus*-based probiotic improved FCR and ADG from d 7 to 28, with no significant difference in ADFI between treatments. The lack of variation in ADFI indicates that the growth advantage stemmed not from increased feed consumption, but from more efficient nutrient utilization. Systemic immune activation redirects dietary amino acids from muscle protein synthesis toward the production of hepatic acute-phase proteins, which increases maintenance energy requirements and limits nutrients available for growth [24–26]. The enhanced growth performance seen in the Vac+Pro group is consistent with, although it does not demonstrate, reduced inflammatory nutrient repartitioning, preserving a

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larger proportion of absorbed nutrients for growth. ADG was improved during d 7 to 28 but not during d 0 to 7, and this later period coincided with the period of peak viremia reported after PRRSV MLV vaccination [27]. Because all pigs were vaccinated and no unvaccinated control group was included, this temporal correspondence is descriptive, and the present design cannot separate responses to vaccination from those to weaning; the improvements observed here may equally reflect a general improvement in the resilience of weaned pigs during the post-weaning period, and a 2 × 2 design including unvaccinated groups would be required to distinguish the two. Similar growth-promoting effects of *Bacillus*-based probiotics have been observed in lipopolysaccharide-challenged pigs [28] and in weaned pigs during the post-weaning period [29, 30].

Additionally, the improvement in fecal scores for the Vac+Pro group suggests improved intestinal function and reduced enteric dysbiosis during the critical post-weaning period. Weaning-associated diarrhea is a complex disorder caused by a combination of factors, including villous atrophy, decreased gastric acid production, transient hypersensitivity to dietary antigens, and the proliferation of opportunistic enteropathogens in a destabilized gut ecosystem [31–33]. Intestinal permeability during this period can be further increased through mechanisms such as mast cell degranulation and corticotropin-releasing factor-mediated disruption of tight junction complexes [34], resulting in a vicious cycle of luminal antigen translocation, mucosal inflammation, and secretory diarrhea. *Bacillus* spore-forming probiotics counteract these processes through three key mechanisms: 1) *B. subtilis* produces lipopeptide antimicrobials, such as surfactin, iturin, and fengycin, that disrupt the membranes of gram-negative bacteria, leading to lysis [35]; 2) germinating *Bacillus* spores compete with pathogens for mucosal adhesion by utilizing fibronectin-binding proteins and exopolysaccharides, thereby excluding harmful bacteria [36]; and 3) they promote goblet cell differentiation and mucin 2 expression, which enhances the mucus barrier against luminal contents [11]. Collectively, these mechanisms may have contributed to the improved fecal scores observed in the Vac+Pro group, suggesting that *Bacillus*-based probiotic supplementation may help maintain intestinal barrier function in weaned pigs.

Bacillus-based probiotic supplementation consistently improved nutrient digestibility throughout the experimental period. In line with our study, Lee et al. [37] reported enhanced CP digestibility and preserved villus morphology in weaned pigs supplemented with *B. subtilis*. These findings suggest that epithelial structural integrity plays a crucial role in determining digestibility outcomes. Correspondingly, the higher villus height in the Vac+Pro group likely increased the absorptive surface area and the density of mature enterocytes that express apical membrane peptidases and amino acid transporters [31, 38].

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Gastric pH in weaned pigs typically exceeds the optimal threshold for pepsinogen activation during the early post-weaning period, limiting endogenous proteolytic capacity [39]. *B. licheniformis* and *B. subtilis* produce extracellular proteases that may help overcome this limitation, facilitating dietary protein hydrolysis beyond the capabilities of endogenous enzymes alone [40]. AID excludes hindgut microbial fermentation; thus, the significant improvements in AID of CP, GE, and OM observed on d 28 are likely attributable to small intestinal enzymatic hydrolysis and transporter-mediated absorption [41]. Deng et al. [42] and Lee et al. [37] reported improvements in nutrient digestibility and VH:CD in weaned pigs supplemented with *B. subtilis* under post-weaning stress conditions. The present study extends these observations to weaned pigs receiving routine PRRSV MLV vaccination. The enhanced nutrient absorption in the Vac+Pro group likely provided the nutritional basis for the improved FCR and ADG documented in this study.

TNF-α, IL-6, IgG, cortisol, and serotonin differed significantly between treatments on d 7 and 28, whereas IgA was higher in Vac+Pro on d 28 and tended to be higher on d 7. In the Vac+Pro group, higher serum IgG levels were associated with lower TNF-α and IL-6 concentrations. This suggests that probiotic supplementation enhanced the humoral immune response while reducing the systemic inflammatory response measured in these pigs. Residual effects of the *Mycoplasma hyopneumoniae* and PCV2 vaccines given 7 d before entry on these immune measurements cannot be excluded, although they would have applied equally to both treatments. *Bacillus* spores translocate across microfold cells into Peyer's patches, where they activate dendritic cells and macrophages and thereby stimulate immunoglobulin production by B cells [9, 43]. Previous studies have reported that probiotic-associated SCFAs suppress nuclear factor kappa B-mediated cytokine transcription, leading to reductions in TNF-α and IL-6 concentrations [44, 45], which supports the findings of the present study. Tang et al. [11] reviewed concurrent improvements in serum immunoglobulins and reductions in pro-inflammatory cytokines in *Bacillus*-supplemented weaned pigs, consistent with the immune response observed in our study. Furthermore, TNF-α and IL-6 redirect dietary amino acids from muscle accretion toward hepatic acute-phase protein synthesis, thereby reducing nutrient utilization for growth [5, 24, 25]; the lower concentrations of these cytokines in Vac+Pro may therefore have contributed to the improved FCR observed in this study. IgA-committed plasma cells activated in Peyer's patches secrete IgA locally at intestinal mucosal sites, with a proportion entering systemic circulation and measured as serum IgA [43]. Secretory IgA at the intestinal surface was not measured, and serum IgA cannot be used to infer mucosal secretion; elevated serum IgA may also reflect increased translocation of mucosal IgA across a compromised epithelium. The higher serum IgA in Vac+Pro is therefore reported as a systemic observation only.

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Intestinal enterochromaffin cells synthesize serotonin from tryptophan and are the primary source of peripheral serotonin, accounting for approximately 95% of whole-body production [46]. Improved CP digestibility in the Vac+Pro group may have increased luminal tryptophan availability, supporting enterochromaffin cell serotonin synthesis. However, pro-inflammatory cytokines are known to divert tryptophan toward the kynurenine degradation pathway at the expense of serotonin biosynthesis [47]. The elevated serum serotonin in the Vac+Pro group, therefore, suggests that cytokine attenuation preserved tryptophan flux toward serotonin synthesis. Since peripherally released serotonin does not cross the blood-brain barrier, the higher serum serotonin in this group likely reflects reduced inflammatory tryptophan catabolism rather than a direct change in central serotonergic tone [48]. Reduced cortisol concentrations indicate diminished HPA axis activation in the Vac+Pro group. Elevated cortisol levels during immune stress have been associated with suppression of antiviral immunity and disruption of the intestinal barrier in pigs [34, 49]. Similarly, the reduction in cortisol levels in the Vac+Pro group was consistent with the preservation of villus morphology.

Villus height was significantly greater in the Vac+Pro group, which may provide a structural basis for the improved nutrient digestibility observed in this study. Mature villus-tip enterocytes are the primary sites for apical surface digestive enzyme activity and transporter-mediated nutrient uptake [31, 38]. The reduced crypt depth in the Vac+Pro group suggests a lower rate of enterocyte replacement, which may indicate less epithelial cell loss [50]. The CD reflects the rate of epithelial cell proliferation necessary to replace damaged or lost enterocytes; thus, the reduced CD in the Vac+Pro group suggests that the ileal epithelium sustained less damage than in the Vac group, aligning with the decreased concentrations of TNF-α and IL-6 observed in the blood profile [51]. The improved VH:CD is consistent with the lower fecal scores observed during d 0 to 7 and d 0 to 28, as a more intact mucosal barrier limits paracellular permeability and reduces the secretory responses that contribute to diarrhea [52]. Previous studies have shown that *Bacillus*-based probiotics help preserve intestinal morphology by enhancing tight junction protein expression, stimulating mucosal restitution factors, and suppressing epithelial apoptosis [42]. Xue et al. [15] found that weaned pigs supplemented with a triple-strain *Bacillus* probiotic exhibited upregulated occludin expression, which was associated with enhanced villus morphology, indicating that tight junction reinforcement is one mechanism through which *Bacillus* probiotics protect villus architecture. Similarly, Lee et al. [37] and Deng et al. [42] reported improvements in villus height and VH:CD in *B. subtilis*-supplemented weaned pigs under standard post-weaning conditions. In this study, the morphological data suggest that the nutritional benefits of the *Bacillus*-based probiotic, including improved protein and organic matter

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digestibility, may be partly due to its ability to preserve intestinal absorptive capacity under the conditions of this study.

SCFAs serve as an energy source, are effectively absorbed by the intestinal mucosa, and play a role in regulating host immunity and intestinal health [53]. The increase in acetate and propionate production by d 28 suggests enhanced hindgut fermentation. Given that the aerobic nature of *B. licheniformis* and *B. subtilis* limits their capacity for direct anaerobic SCFA synthesis, this increase likely reflects cross-feeding interactions with indigenous fermentative bacteria stimulated by *Bacillus* supplementation [32]. Butyrate production in the porcine hindgut primarily depends on obligate anaerobes such as *Faecalibacterium* and *Roseburia* [54]. The lack of a difference in butyrate production in this study indicates that these butyrogenic bacteria were not significantly activated by *Bacillus* supplementation. Among the elevated SCFAs, propionate has been shown to suppress pro-inflammatory cytokine secretion by activating free fatty acid receptor 3 and inhibiting histone deacetylases in immune cells [44, 45]. Therefore, the elevated fecal propionate concentrations on d 28 align with the reduced serum TNF-α and IL-6 found in the blood profile analysis. These findings suggest that hindgut-derived propionate may have contributed to the systemic anti-inflammatory phenotype observed in the Vac+Pro group. Previous studies have also indicated that SCFAs may attenuate HPA axis reactivity through vagal afferent signaling [55]. The increased fecal acetate and propionate concentrations on d 28 may have contributed to the lower cortisol levels in the Vac+Pro group [56]. Hu and Kim [29] reported that elevated fecal SCFA concentrations were associated with improved growth performance in *B. subtilis*-supplemented weaned pigs, which is consistent with the observations in the present study.

Fecal *E. coli* counts were significantly lower in the Vac+Pro group on d 28. Kim et al. [50] reported reduced fecal *E. coli* counts in *B. subtilis*-supplemented weaned pigs under an enterotoxigenic *E. coli* challenge, which supports the antibacterial outcomes documented in this study. Both *B. licheniformis* and *B. subtilis* may have exerted additional antimicrobial pressure through lipopeptide production. Surfactin, iturin, and fengycin have been shown to suppress coliform growth in related *Bacillus* strains [35, 36]. Additionally, the elevated fecal acetate and propionate concentrations in the Vac+Pro group on d 28 may have contributed to a lower luminal pH, allowing the resulting undissociated organic acids to penetrate gram-negative bacterial membranes and dissipate the proton motive force required for coliform energy metabolism [50, 57]. Beta-hemolytic colonies were not detected in either treatment group at any time point.

On d 28, alpha-diversity tended to be lower in the Vac+Pro group, which may reflect selective rather than broad

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modulation of the microbial community, although no individual taxon differed after correction for multiple testing. This finding aligns with previous *Bacillus* supplementation studies in weaned pigs, where alpha-diversity indices either remained unchanged or showed a trend toward reduction, despite positive compositional outcomes [8]. *Bacillus*-based probiotics have been reported to enrich beneficial taxa, such as *Lactobacillus* and *Bifidobacterium*, while competitively excluding opportunistic bacteria [11]. This mechanism can lead to a decrease in overall community evenness without necessarily increasing richness.

The post-weaning period is marked by rapid microbiome restructuring due to abrupt dietary changes and environmental exposure [52, 54]. By d 28 of the trial, the microbial communities in both treatment groups may have approached a stable compositional equilibrium, leading to reduced detectable differences in alpha-diversity indices.

At the genus level, the abundance of *Ruminococcus* was significantly lower in the Vac+Pro group on d 28, but this difference did not survive correction for multiple testing ($q = 0.640$) and is therefore reported as an exploratory, hypothesis-generating observation. Because 16S rRNA gene profiling at the genus level cannot resolve species, and because this genus contains taxa with divergent functions — from the pro-inflammatory *R. gnavus*, which activates toll-like receptor 4 signaling through glucorhamnan polysaccharides [58], to the polysaccharide-degrading *R. bromii* [59] — no functional interpretation is attached to this change. Together with the absence of differences in beta-diversity and at the phylum level, the microbiota data indicate that the probiotic did not reorganize the fecal community over 28 d.

CONCLUSION

Dietary supplementation with the *Bacillus*-based probiotic at 0.04% for 28 days improved growth performance in weaned pigs vaccinated against PRRSV, as evidenced by enhanced feed conversion ratio and average daily gain during d 7 to 28. These performance gains were supported by significant improvements in nutrient digestibility, including apparent total tract digestibility of dry matter and crude protein and apparent ileal digestibility of crude protein, gross energy, and organic matter, along with enhanced ileal morphology characterized by greater VH, reduced CD, and an improved VH:CD ratio. Probiotic supplementation was also associated with higher serum IgG and serotonin and lower serum TNF-α, IL-6, and cortisol concentrations. Additionally, hindgut fermentation was enhanced, with increased fecal acetate and propionate on d 28, alongside reduced fecal *E. coli* counts. Collectively, these findings suggest that *Bacillus*-based probiotic supplementation supports growth, intestinal absorptive capacity, and humoral immune responses in weaned pigs vaccinated against PRRSV. Because no

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unvaccinated control group was included, these benefits are reported as the efficacy of the probiotic under a routine vaccination program and are not attributed to mitigation of vaccination-induced immune stress. Under these conditions, this *Bacillus*-based probiotic may serve as a useful functional feed additive in commercial swine production settings in which weaned pigs are routinely vaccinated.

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

Items	Phase 1	Phase 2
Ingredients, %		
EP corn	46.25	60.95
Soybean meal	10.00	16.00
Rice	4.00	4.00
Soy protein concentrate	2.50	2.50
Fermented soybean meal	4.00	3.00
Meal protein fat (whey, oil, etc.)	17.50	5.00
Fish meal	1.50	2.00
Plasma protein	4.00	
Soybean oil	2.40	2.50
Lactose	4.00	
Ca-formate	1.30	1.50
Coconut	1.50	1.40
Vitamin premix[1]	0.20	0.20
Mineral premix[2]	0.25	0.25
Methionine	0.10	0.10
L-Lysine	0.40	0.50
Threonine	0.10	0.10
Total	100.00	100.00
Calculated value		
NE, kcal/kg	2,600.00	2,600.00
CP, %	19.20	17.77
Lysine, %	1.40	1.21
Methionine, %	0.40	0.40
Ca, %	0.50	0.49
P, %	0.45	0.39

[1]Provided per kg of complete diet: vitamin A, 9,000 IU; vitamin D_3, 1,620 IU; vitamin E, 35 IU; vitamin B_{12}, 40 μg; vitamin B_2, 6 mg; vitamin B_6, 3 mg; vitamin K_3, 2 mg; vitamin C, 100 mg; niacin, 50 mg; ca-pantothenate, 20 mg; biotin, 0.2 mg.
[2]Provided per kg of complete diet without zinc: Mn, 35 mg; Zn, 30 mg; Fe, 150 mg; Cu, 50 mg; I, 0.5 mg; Co, 0.15 mg; and Se, 0.2 mg.
Abbreviation: EP corn, extruded-pelleted corn; NE, net energy; CP, crude protein.

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Table 2. Effects of *Bacillus*-based probiotics on growth performance in weaned pigs

Items	Vac	Vac+Pro	SE	*p*-value
BW, kg				
d 0	8.22	8.22	0.262	0.989
d 7	11.31	11.23	0.297	0.564
d 28	24.24	24.95	0.616	0.025
ADG, g				
d 0 to 7	440.57	430.48	13.498	0.564
d 7 to 28	615.74	653.13	17.392	0.002
d 0 to 28	571.95	597.47	13.971	0.025
ADFI, g				
d 0 to 7	507.52	511.76	14.020	0.779
d 7 to 28	892.04	897.64	22.795	0.604
d 0 to 28	795.89	801.21	19.398	0.559
FCR				
d 0 to 7	1.18	1.21	0.032	0.342
d 7 to 28	1.45	1.38	0.016	0.002
d 0 to 28	1.39	1.35	0.015	0.039

Abbreviation: Vac, control vaccinated with porcine reproductive and respiratory syndrome virus modified live virus vaccine; Vac+Pro, Vac supplemented with 0.04% *Bacillus*-based probiotics; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio; SE, standard error.

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Table 3. Effects of *Bacillus*-based probiotics on apparent total tract digestibility in weaned pigs

Items, %	Vac	Vac+Pro	SE	*p*-value
d 7				
DM	80.90	81.92	0.571	0.002
CP	71.53	72.99	0.306	0.003
GE	82.94	83.20	0.407	0.243
EE	74.55	75.81	0.505	0.047
OM	82.83	83.52	0.676	0.006
Ca	78.35	78.68	0.693	0.739
P	83.05	83.37	0.701	0.730
d 14				
DM	81.32	81.73	0.342	0.011
CP	72.24	73.20	0.676	0.001
GE	82.00	81.84	0.393	0.519
EE	75.89	76.31	0.464	0.480
OM	84.35	84.05	0.289	0.149
Ca	78.98	78.38	0.875	0.373
P	83.11	81.72	1.238	0.224
d 28				
DM	82.90	83.36	0.221	0.014
CP	75.33	76.33	0.768	<0.001
GE	82.79	83.33	0.455	0.029
EE	81.62	81.94	0.405	0.456
OM	85.11	85.43	0.261	0.087
Ca	82.17	81.82	0.784	0.502
P	85.67	84.54	0.502	0.081

Abbreviation: Vac, control vaccinated with porcine reproductive and respiratory syndrome virus modified live virus vaccine; Vac+Pro, Vac supplemented with 0.04% *Bacillus*-based probiotics; DM, dry matter; CP, crude protein; GE, gross energy; EE, ether extract; OM, organic matter; Ca, calcium; P, phosphorus; SE, standard error.

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Table 4. Effects of *Bacillus*-based probiotics on apparent ileal digestibility in weaned pigs

Items, %	Vac	Vac+Pro	SE	*p*-value
DM	79.73	81.25	0.122	0.058
CP	69.01	72.91	0.183	<0.001
GE	77.00	78.87	0.321	0.020
EE	72.84	76.69	1.154	0.409
OM	83.07	85.80	0.289	<0.001
Ca	77.04	76.63	1.154	0.657
P	75.69	76.63	1.564	0.641

Abbreviation: Vac, control vaccinated with porcine reproductive and respiratory syndrome virus modified live virus vaccine; Vac+Pro, Vac supplemented with 0.04% *Bacillus*-based probiotics; DM, dry matter; CP, crude protein; GE, gross energy; EE, ether extract; OM, organic matter; Ca, calcium; P, phosphorus; SE, standard error.

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Table 5. Effects of *Bacillus*-based probiotics on blood profile in weaned pigs

Items	Vac	Vac+Pro	SE	*p*-value
d 0				
IgA, mg/mL	0.83	0.79	0.015	0.072
IgG, mg/mL	9.48	9.89	0.187	0.127
TNF-α, pg/mL	29.12	30.61	1.658	0.445
IL-6, pg/mL	71.61	74.90	4.251	0.587
Serotonin, ng/mL	179.16	178.02	3.997	0.841
Cortisol, nmol/L	86.63	86.63	2.566	0.990
d 7				
IgA, mg/mL	0.84	0.89	0.018	0.065
IgG, mg/mL	10.31	11.68	0.151	<0.001
TNF-α, pg/mL	198.71	154.11	7.129	<0.001
IL-6, pg/mL	532.13	300.21	16.900	<0.001
Serotonin, ng/mL	124.42	160.27	3.240	<0.001
Cortisol, nmol/L	121.67	97.67	4.138	<0.001
d 28				
IgA, mg/mL	1.22	1.61	0.029	<0.001
IgG, mg/mL	14.35	16.71	0.364	<0.001
TNF-α, pg/mL	54.57	36.07	2.331	<0.001
IL-6, pg/mL	142.96	86.99	5.168	<0.001
Serotonin, ng/mL	151.02	231.70	6.131	<0.001
Cortisol, nmol/L	115.33	82.22	4.194	<0.001

Abbreviation: Vac, control vaccinated with porcine reproductive and respiratory syndrome virus modified live virus vaccine; Vac+Pro, Vac supplemented with 0.04% *Bacillus*-based probiotics; IgA, immunoglobulin A; IgG, immunoglobulin G; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; SE, standard error.

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Table 6. Effects of *Bacillus*-based probiotics on intestinal morphology in weaned pigs

Items	Vac	Vac+Pro	SE	*p*-value
Villus height, μm	458.43	506.47	14.081	0.021
Crypt depth, μm	186.07	150.76	5.753	<0.001
Villus height to crypt depth ratio	2.52	3.50	0.154	<0.001

Abbreviation: Vac, control vaccinated with porcine reproductive and respiratory syndrome virus modified live virus vaccine; Vac+Pro, Vac supplemented with 0.04% *Bacillus*-based probiotics; SE, standard error.

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Table 7. Effects of *Bacillus*-based probiotics on fecal short-chain fatty acids in weaned pigs

Items, mmol/kg	Vac	Vac+Pro	SE	*p*-value
d 0				
Acetate	71.77	69.64	1.737	0.378
Propionate	56.28	58.36	3.223	0.654
Butyrate	13.74	13.03	0.750	0.504
d 28				
Acetate	34.77	48.98	2.613	<0.001
Propionate	14.84	19.36	0.687	<0.001
Butyrate	9.01	9.72	0.491	0.311

Abbreviation: Vac, control vaccinated with porcine reproductive and respiratory syndrome virus modified live virus vaccine; Vac+Pro, Vac supplemented with 0.04% *Bacillus*-based probiotics; SE, standard error.

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Table 8. Effects of *Bacillus*-based probiotics on fecal *Escherichia coli* content in weaned pigs

Items, Log CFU/g	Vac	Vac+Pro	SE	*p*-value
d 0	6.40	6.40	0.013	0.622
d 7	6.34	6.37	0.015	0.105
d 28	6.35	6.28	0.007	<0.001

Abbreviation: Vac, control vaccinated with porcine reproductive and respiratory syndrome virus modified live virus vaccine; Vac+Pro, Vac supplemented with 0.04% *Bacillus*-based probiotics; CFU, colony-forming unit; SE, standard error.

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Table 9. Effects of *Bacillus*-based probiotics on fecal microbiota at the phylum level in weaned pigs

Item, %	Vac	Vac+Pro	SE	*p*-value	*q*-value
d 0					
Firmicutes	46.16	45.36	1.997	0.776	0.804
Bacteroidota	43.34	46.20	2.070	0.325	0.650
Spirochaetota	4.93	3.42	0.821	0.200	0.650
Proteobacteria	2.61	2.41	0.565	0.804	0.804
d 28					
Firmicutes	53.51	56.14	2.786	0.513	0.670
Bacteroidota	38.21	36.60	2.614	0.670	0.670
Spirochaetota	3.23	2.82	0.459	0.524	0.670
Proteobacteria	0.95	1.24	0.331	0.535	0.670

Abbreviation: Vac, control vaccinated with porcine reproductive and respiratory syndrome virus modified live virus vaccine; Vac+Pro, Vac supplemented with 0.04% *Bacillus*-based probiotics; SE, standard error. q-value, Benjamini-Hochberg adjusted p-value within each taxonomic level and sampling day.

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Table 10. Effects of *Bacillus*-based probiotics on fecal microbiota at the genus level in weaned pigs

Item, %	Vac	Vac+Pro	SE	*p*-value	*q*-value
d 0					
Clostridium_sensu_stricto_1	13.24	12.32	1.143	0.573	0.966
Muribaculaceae	6.13	5.63	0.983	0.725	0.966
Rikenellaceae_RC9_gut_group	4.11	4.03	0.559	0.923	0.966
Prevotella_9	3.54	4.02	0.479	0.490	0.966
uncultured	3.38	3.33	0.562	0.949	0.966
Treponema	2.88	3.17	0.437	0.642	0.966
Prevotellaceae_UCG_003	2.36	2.89	0.400	0.338	0.966
Terrisporobacter	2.70	1.67	0.510	0.163	0.966
Phascolarctobacterium	1.37	2.59	0.768	0.244	0.966
UCG_002	1.72	2.21	0.252	0.184	0.966
Clostridia_UCG_014	1.54	1.64	0.497	0.877	0.966
Alloprevotella	1.51	1.64	0.558	0.872	0.966
Lactobacillus	1.52	1.46	0.566	0.937	0.966
Prevotella	0.85	1.82	0.551	0.218	0.966
Megasphaera	1.46	1.15	0.281	0.445	0.966
HT002	1.30	1.14	0.294	0.690	0.966
Agathobacter	0.99	1.19	0.353	0.691	0.966
Ruminococcus	0.73	1.38	0.497	0.362	0.966
Eubacterium_coprostanoligenes_group	1.11	0.99	0.269	0.751	0.966
UCG_010	1.05	1.03	0.296	0.966	0.966
d 28					
Clostridium_sensu_stricto_1	10.35	11.72	2.917	0.744	0.939
Muribaculaceae	11.67	9.17	1.178	0.144	0.913
Rikenellaceae_RC9_gut_group	7.75	8.02	0.961	0.845	0.939
Prevotella_9	2.53	3.57	0.589	0.223	0.913
uncultured	2.93	3.12	0.271	0.627	0.939
Treponema	2.64	2.46	0.431	0.772	0.939
Prevotellaceae_UCG_003	2.65	2.30	0.452	0.586	0.939
Terrisporobacter	2.43	2.39	0.712	0.973	0.973
Phascolarctobacterium	2.36	2.33	0.455	0.970	0.973
UCG_002	2.38	2.20	0.352	0.718	0.939
Clostridia_UCG_014	2.07	2.42	0.413	0.556	0.939
Alloprevotella	2.11	1.82	0.427	0.639	0.939
Lactobacillus	1.67	2.07	0.420	0.509	0.939
Prevotella	1.79	1.91	0.395	0.828	0.939
Megasphaera	1.46	2.17	0.511	0.327	0.934
HT002	1.76	1.44	0.377	0.551	0.939
Agathobacter	1.07	1.59	0.285	0.202	0.913
Ruminococcus	1.54	1.04	0.161	0.032	0.640
Eubacterium_coprostanoligenes_group	1.37	1.12	0.168	0.274	0.913
UCG_010	1.40	0.96	0.253	0.232	0.913

Abbreviation: Vac, control vaccinated with porcine reproductive and respiratory syndrome virus modified live virus vaccine; Vac+Pro, Vac supplemented with 0.04% *Bacillus*-based probiotics; SE, standard error. q-value, Benjamini-Hochberg adjusted p-value within each taxonomic level and sampling day.

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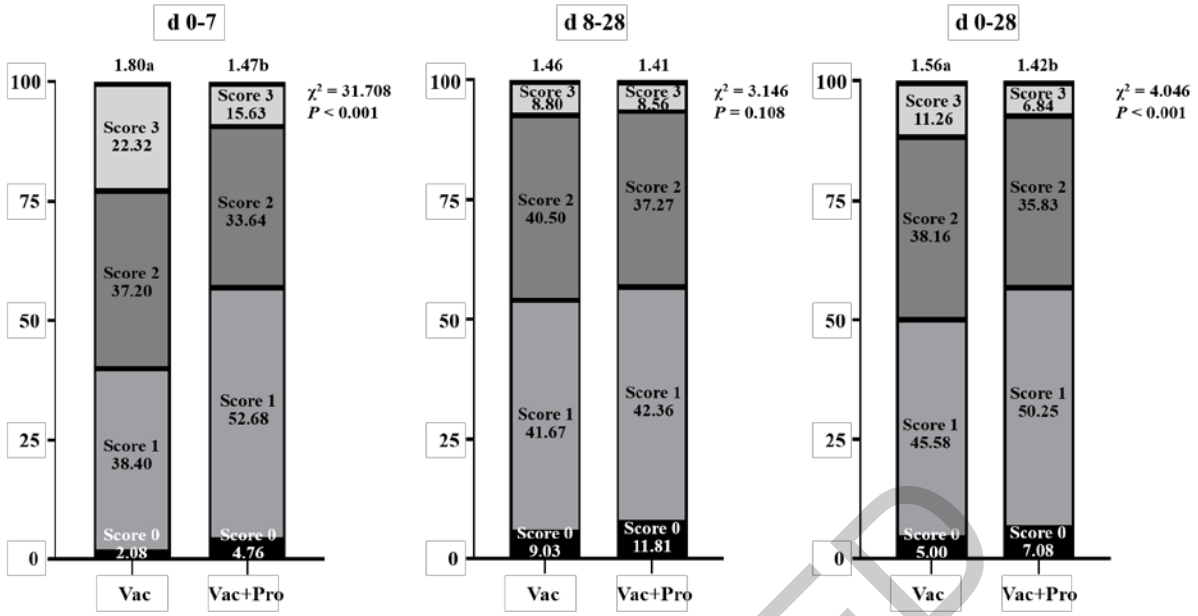


Figure 1. Effects of *Bacillus*-based probiotics on fecal score in weaned pigs vaccinated against porcine reproductive and respiratory syndrome. Vac, control vaccinated with porcine reproductive and respiratory syndrome virus modified live virus vaccine; Vac+Pro, Vac supplemented with 0.04% *Bacillus*-based probiotics. Numbers inside the bar indicate the percentage of each score out of the total (100%) as shown in the legend. 0, normal feces; 1, soft feces; 2, mild diarrhea; 3, severe diarrhea. Numbers above the bars indicate the mean fecal score of each treatment. Fecal scores were compared between treatments using the chi-square test; different letters indicate a significant difference ($p \leq 0.05$).

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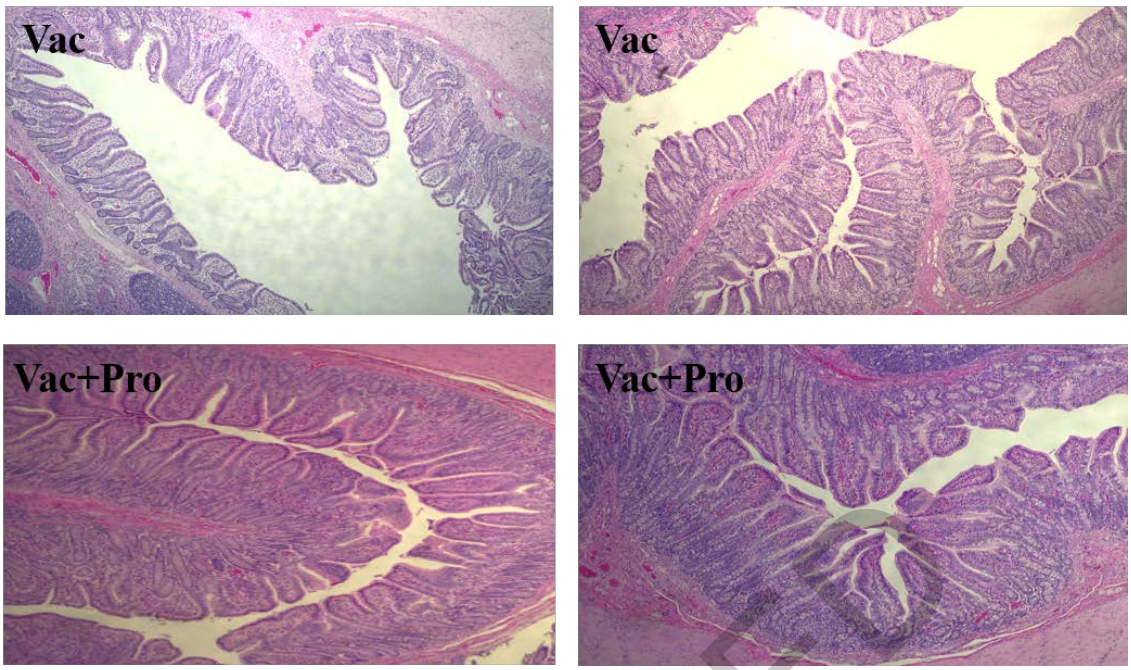


Figure 2. Effects of *Bacillus*-based probiotics on intestinal morphology in weaned pigs vaccinated against porcine reproductive and respiratory syndrome. Vac, control vaccinated with porcine reproductive and respiratory syndrome virus modified live virus vaccine; Vac+Pro, Vac supplemented with 0.04% *Bacillus*-based probiotics.

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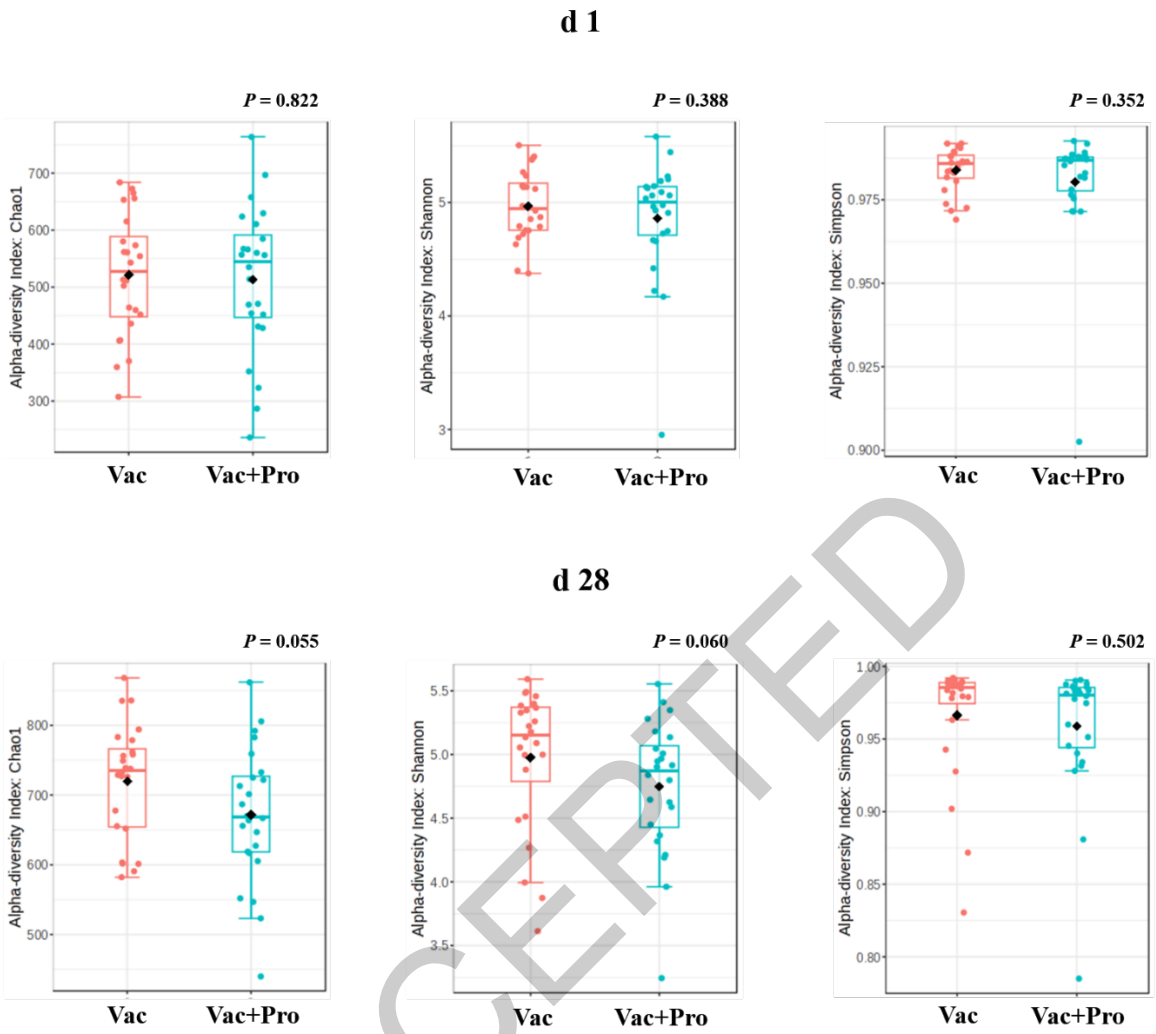


Figure 3. Effects of *Bacillus*-based probiotics on alpha-diversity indices of fecal microbiota in weaned pigs vaccinated against porcine reproductive and respiratory syndrome. Vac, control vaccinated with porcine reproductive and respiratory syndrome virus modified live virus vaccine; Vac+Pro, Vac supplemented with 0.04% *Bacillus*-based probiotics.

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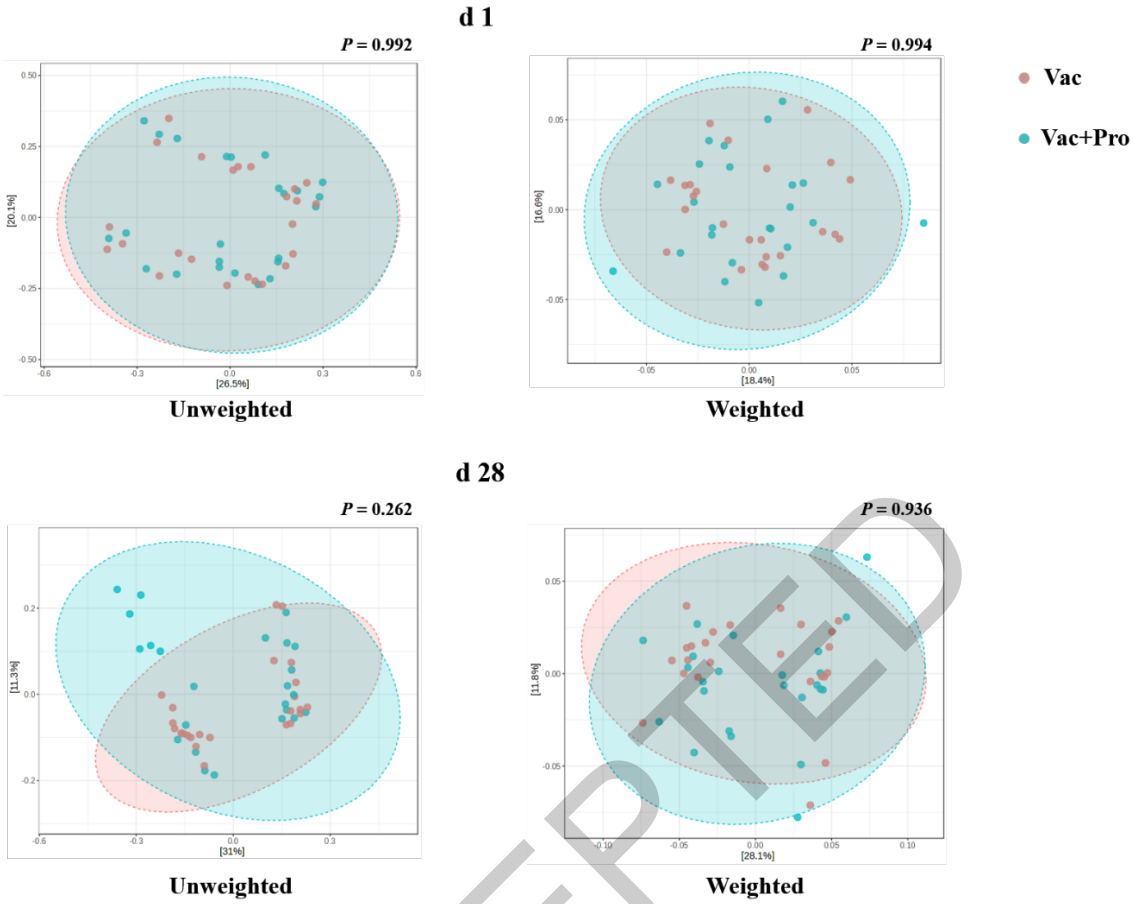
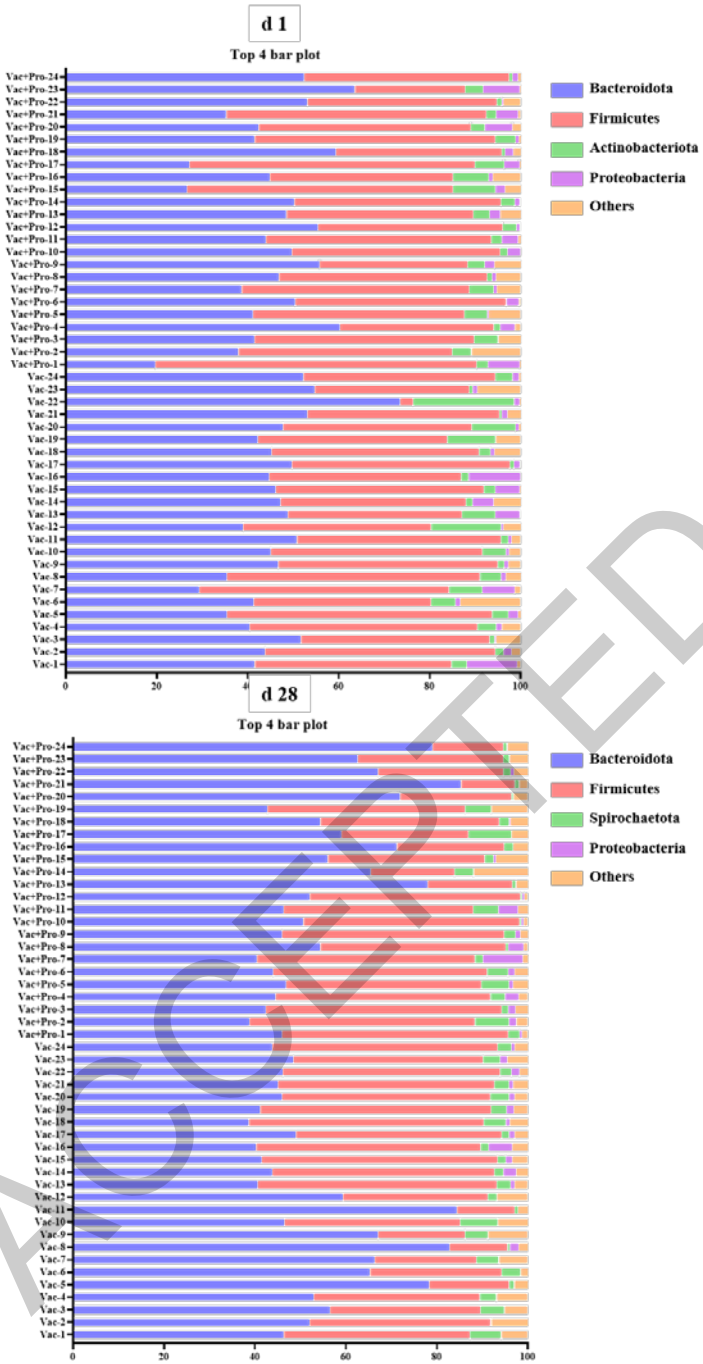


Figure 4. Effects of *Bacillus*-based probiotics on beta-diversity indices of fecal microbiota in weaned pigs vaccinated against porcine reproductive and respiratory syndrome. Vac, control vaccinated with porcine reproductive and respiratory syndrome virus modified live virus vaccine; Vac+Pro, Vac supplemented with 0.04% *Bacillus*-based probiotics.

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Figure 5. Relative abundance of fecal microbiota at the phylum level in weaned pigs vaccinated against porcine reproductive and respiratory syndrome. Vac, control vaccinated with porcine reproductive and respiratory syndrome virus modified live virus vaccine; Vac+Pro, Vac supplemented with 0.04% *Bacillus*-based probiotics.



Figure 6. Relative abundance of fecal microbiota at the genus level in weaned pigs vaccinated against porcine reproductive and respiratory syndrome. Vac, control vaccinated with porcine reproductive and respiratory syndrome virus modified live virus vaccine; Vac+Pro, Vac supplemented with 0.04% *Bacillus*-based probiotics.

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