



Research article

Effects of deep-bedding floor and fermented feed supplementation on growth performance, intestinal morphology, and gut microbiome in Thai native crossbred pigs

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Abstract

The gut microbiota is crucial for maintaining the nutritional, physiological, and immunological functions of pigs and plays an important role in pig productivity. Native crossbred pigs are particularly important to smallholder farmers in many Southeast Asian countries. These pigs are often raised in deep litter or deep bedding pen (DP) systems, and their traditional feed resources frequently include fermented agricultural by-products such as banana stem. However, knowledge of the gut microbiota of these native pigs remains limited. Breed, bedding system, and fermented feed may influence gut microbial composition and result in differences from the gut microbiota of conventional pigs. This study characterized the cecal microbiota of Thai native crossbred pigs reared under different bedding systems and dietary treatments, while pig manure samples from the DP system were analyzed separately to characterize the microbial community associated with the bedding environment. The objective of the study was to investigate the effects of bedding type and fermented feed supplementation on growth performance, intestinal morphology, and gut microbiota in Thai native crossbred pigs. Thirty-two Thai native crossbred pigs were assigned to a 2 × 2 factorial experiment in a completely randomized design. Factor A was bedding type: DP or concrete flooring (C). Factor B was diet type: concentrate feed supplemented with fresh banana stem (BS) or fermented banana stem (FBS). Cecal content samples were collected after 120 days for bacterial DNA extraction and 16S rRNA gene sequencing. In addition, pig manure samples mixed with bedding material were collected from DP to characterize the microbial community associated with the manure composting environment. Sequencing of the V3-V4 region of the 16S rRNA gene was used to profile the gut microbiota, and analyses were performed using QIIME2. For the cecal microbiota, bedding type, diet type, and their interaction had no significant effects on alpha diversity indices (Chao1, Shannon, and Simpson) or beta diversity based on unweighted UniFrac distances. In cecal content samples, Bacteroidetes, Firmicutes, and Spirochaetes were the three most abundant phyla across all treatments. Pigs reared in the DP system had higher relative abundances of *Solitalea*, *Paraprevotella*, *Sphingobacterium*, and *Phascolarctobacterium*, but a lower relative abundance of *Lactobacillus*, than pigs reared in the C system ($P < 0.05$). However, the average daily gain and final body weight of pigs raised in the C system were significantly higher than those of pigs raised in the DP system ($P < 0.05$). In conclusion, this study suggests that raising native crossbred pigs in a DP system with FBS supplementation may reduce environmental impact and feed costs while potentially improving the microbial community associated with pig manure composting.

Keywords: Deep bedding system, Fermented banana stem, Gut microbiota, Thai native crossbred pig

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Article history; received manuscript: 29 April 2026,
revised manuscript: 6 June 2026,
accepted manuscript: 17 August 2026,
published online: 25 August 2026,

Academic editor; Korakot Nganvongpanit

INTRODUCTION

Pig production is an economically important global industry (Woonwong et al., 2020). Pork is an important source of animal protein and is in high demand worldwide (Ottosen et al., 2021). In current pig husbandry, native pigs are crucial to pig production in many parts of the world. In particular, they are important to smallholders and highland communities in many Southeast Asian countries, such as Vietnam, Laos, the Philippines, Cambodia, and Thailand (Deka et al., 2014; Delsart et al., 2020; Chaiwang et al., 2021). In highland communities, pigs play an important role not only as a source of nutrition but also as a major source of family income and as a financial reserve for specific needs, such as children's tuition, cultural activities, and debt repayment (Deka et al., 2014). Most native pigs are considered hardy, resistant to various diseases and internal parasites, and exceptionally well adapted to hot and humid climates (Nidup et al., 2010; Charoensook et al., 2013). In addition, native pigs are able to survive on low-quality feed, which is usually made from agricultural by-products (Charoensook et al., 2013; Arjin et al., 2020). Despite these advantages, native purebred pigs nevertheless perform poorly in terms of growth compared with commercial pig breeds. To enhance pig productivity and provide smallholder farmers with opportunities to generate additional income, programs have been developed to crossbreed native pigs with commercial pigs, using exotic germplasm for enhanced productivity and indigenous germplasm for better adaptability (Arjin et al., 2020; Chaiwang et al., 2021; Sringarm et al., 2022). Interestingly, native crossbred pigs are often raised in deep litter or deep bedding (DP) systems (Arjin et al., 2020; Chaiwang et al., 2021; Mitchaonthai and Srikijkasemwat, 2022). DP systems are an inexpensive way to mitigate slurry accumulation and improve pigs' thermal comfort (Kunde Correa et al., 2009). A more comfortable lying surface is another way in which this system enhances animal welfare, consequently reducing joint lesions (Grevenhof et al., 2011). The majority of the bedding is made of rice husks, which provide pigs with a steady source of manipulable material that promotes rooting and other natural behaviors (Morrison et al., 2007). Most importantly, this system effectively promotes environmental conservation by implementing efficient water management strategies that minimize water consumption during pen cleaning and limit the release of barn wastewater into the environment (Pérez et al., 2022), while also generating minimal odor and facilitating manure recycling (Chaiwang et al., 2021).

In highland pig production systems, the main traditional feed resources are agricultural by-products and non-conventional forages that grow naturally in forests, along stream banks, and in cropping areas; however, feed availability is insufficient during the dry season, which is one of the primary obstacles to pig productivity. Feed is limited in both quantity and quality, which severely restricts productivity in smallholder pig production systems (Phengsavanh et al., 2010). Banana is native to tropical Southeast Asia (Hazarika et al., 2014) and is considered of great socioeconomic importance in countries throughout the region (Persley and De Langhe, 1987). Banana stem is a widely available by-product of harvesting field-grown bananas. It is therefore considered suitable for use as a low-cost pig feed and can be stored for use during periods of feed scarcity. In terms of nutrition, although banana stem has a low nutritive value, with crude protein of 6.5% and lipid content of 1.5% on a dry matter basis, it contains high levels of lignocellulose (60%-85%) and cellulose (50%) (Huy et al., 2018). However, banana stem can be improved by fermentation with other ingredients to enhance its nutritive value. Furthermore, producing fermented feed from banana stems has the additional benefit of increasing the digestibility of fibrous components for pigs (DuPonte et al., 2016). Arjin et al. (2020) reported that supplementing a low-protein diet with fermented banana stem (FBS) increased villus height and width in native crossbred pigs, thereby enhancing nutrient absorption. Fermented feed also has the potential to improve growth performance and nutrient absorption capacity while maintaining

gastrointestinal health in pigs (Cho et al., 2011). Therefore, DP systems and effective feed resource management should provide a sustainable solution for smallholder pig farming. These approaches are critically important for achieving food security and alleviating poverty among smallholder farmers while reducing environmental impact.

Despite the documented benefits of DP systems and fermented feed described above, both factors may also be beneficial to the pig gut microbiota. In pig production, the population of gut microbes, or microbiota, plays key roles in maintaining the nutritional, physiological, and immunological functions of the pig (Lee and Mazmanian, 2010; Brestoff and Artis, 2013; Fohse et al., 2016). It also plays an important role in pig productivity (Wang et al., 2018). Previous studies have reported that flooring and bedding systems are among the major environmental factors that directly affect the developmental dynamics of microbial communities in pigs (Megahed et al., 2019). In pre-weaned piglets, indoor-raised piglets exhibited significantly greater ileal mucosa-adherent microbial diversity and richness than outdoor-raised piglets (Mulder et al., 2009; Schmidt et al., 2011). Similarly, in DP systems, pig waste is able to ferment in situ, resulting in a complex microbial community. For example, microbes are closely associated with waste fermentation efficiency (Yin et al., 2015), which may influence gut microbiota composition. Regarding fermented feed, various fermented feeds have been reported to have beneficial effects on the pig gut microbiota. Kiarie et al. (2011) suggested that feeding weaned pigs yeast-fermented products tended to increase bacterial richness and diversity in ileal digesta. Tajima et al. (2010) also found that fermented liquid feed improved lactobacilli populations in the ileum of weaned pigs. Arjin et al. (2020) reported that FBS supplementation affected nutrient digestibility, productive performance, and intestinal morphology in crossbred pigs. However, the effects of DP systems and fermented feed supplementation, as well as their interaction, on the microbial composition and diversity of native crossbred pigs remain unclear. Therefore, the present study extends previous work by characterizing the cecal microbiota and manure-associated microbial communities under different bedding systems and banana stem supplementation strategies using 16S rRNA sequencing. The main objective of this study was to determine the effects of bedding type (DP and C) and diet type (concentrate supplemented with either BS or FBS) on growth performance, intestinal morphology, and gut microbiota profiles in Thai native crossbred pigs.

MATERIALS AND METHODS

Feed and bedding preparation

For FBS, banana stems were weighed and sliced into 1- to 2-cm lengths. Each 50-kg batch was placed in a polyethylene tank, and 2 kg of sugar and 500 g of salt (NaCl) were added. The stems were compressed to remove air, and the tank was sealed to create anaerobic conditions. The tanks were stored indoors for 21 days away from direct sunlight before being used in pig diets. Every morning, BS were sliced into 1- to 2-cm pieces before diet preparation. The experimental diets were prepared by mixing the concentrate mixture with either BS (BS diet) or FBS (FBS diet) at a 1:1 ratio on a weight basis. Thus, the BS diet contained 50% concentrate mixture and 50% BS, whereas the FBS diet contained 50% concentrate mixture and 50% FBS. The ingredient composition and chemical composition of the experimental diets are shown in Table 1. Two bedding systems were examined in this study. Rice husk was used to prepare the DP, which had dimensions of 2 × 3 × 1.5 m, with a bedding depth of 90 cm for absorbing pig manure. The C system comprised an dimensions of 2 × 3 × 1.5 m. Pens were cleaned daily with high-pressure water throughout the experiment.

Table 1 Ingredients and chemical composition of experimental diets

Ingredients %	BS	FBS
Corn	20.5	20.5
Broken rice	20.5	20.5
Rice bran	2.5	2.5
Soybean meal	4	4
Fish meal	1.25	1.25
DCP	1	1
Salt	0.125	0.125
Vitamin Premix	0.125	0.125
Fermented banana stem	-	50
Fresh banana stem	50	-
Total	100	100
ME (Mcal/kg)	3.75	3.82
CP	13.34	13.52
CF	8.36	8.87
EE	4.21	4.38
NDF	42.47	43.10
ADF	22.12	21.24
ADL	31.72	32.80
Lysine	0.54	0.53
Methionine	0.16	0.17

BS = Concentrate supplement with fresh banana stems; FBS = Concentrate supplement with fermented banana stems; ME = Metabolizable Energy; CP = Crude protein; CF = Crude fiber; EE = Ether extract; NDF = Neutral detergent fiber; ADF = Acid detergent fiber; ADL = Acid detergent lignin.

Experimental animals and management

In total, 32 crossbred pigs (25% Thai native × 25% Meishan × 50% Duroc) were used in this study, with an average initial body weight of 13.71 ± 2.82 kg. The experiment was conducted as a 2 × 2 factorial arrangement in a completely randomized design. The factors evaluated were bedding type (factor A: DP or C) and diet type (factor B: concentrate feed supplemented with BS or FBS). Each replicate consisted of two pigs per pen, resulting in eight pigs per treatment group (n = 4). The pigs were provided ad libitum access to feed and water for 120 days. Throughout the experimental period, all pigs were observed daily for general health status, clinical signs of disease, abnormal behavior, and mortality. No disease outbreaks, severe clinical illness, or mortality occurred during the study. All 32 pigs completed the experiment, with eight pigs remaining in each treatment group at the end of the trial: DPBS = 8, DPFBS = 8, CBS = 8, and CFBS = 8. Pig body weight was measured weekly, and feed intake was recorded daily to evaluate growth performance by calculating average daily gain (ADG), feed conversion ratio per pen, and average daily feed intake (ADFI). The use of animals in the present experiment was reviewed and approved by the Institutional Animal Care and Use Committee of Chiang Mai University (2561/AG-0004). Throughout the study, all animals were housed at the Faculty of Agricultural Technology, Chiang Mai Rajabhat University, Chiang Mai, Thailand.

Intestinal morphology

The analysis of intestinal morphology was conducted following the method described by Wang et al. (2026). At the end of the experiment, four pigs per treatment group were randomly selected for intestinal tissue collection. Approximately 2.5-cm segments were collected from the duodenum, jejunum, and

ileum of each selected pig. The intestinal segments were gently rinsed with normal saline and fixed in 10% neutral buffered formalin. The samples were then processed using standard histological procedures, embedded in paraffin, sectioned using a rotary microtome, and stained with hematoxylin and eosin. Villus height (VH) and crypt depth (CD) were measured at 40× magnification using a digital camera (Motic MC2000) connected to a compound microscope (CX21; Olympus Corporation, Tokyo, Japan). For each intestinal segment, 10 well-oriented villi and 10 corresponding crypts were selected from the histological sections for measurement. VH was measured from the tip of the villus to the villus–crypt junction, whereas CD was measured from the villus–crypt junction to the base of the crypt. The VH-to-CD ratio (VH:CD) was then calculated. The mean value of the measured villi and crypts for each intestinal segment within each pig was used for statistical analysis.

Sample collection and bacterial DNA extraction

FBS samples were randomly collected from the plastic tanks after 21 days of fermentation. At the end of the experiment, pig manure samples consisting of a mixture of rice husks and pig feces were randomly collected from the DP to assess differences in bacterial communities between DP fed with BS (DPBS) and FBS (DPFBS). Cecal contents were collected immediately after slaughter. All samples were stored in microcentrifuge tubes at -80°C until DNA isolation. Total genomic DNA was extracted using the QIAamp® Fast DNA Stool Mini Kit (Qiagen Inc., Germany) according to the manufacturer's instructions. DNA purity and concentration were measured using a NanoDrop 2000 UV-VIS spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

16S rRNA gene sequencing of gut microbiota

The V3-V4 region of the 16S rRNA gene was amplified using the downstream primer 806R (5'-GGACTACHVGGGTWTCTAAT-3') and the upstream primer 338F (5'-ACTCCTACGGGAGGCAGCAG-3'). The PCR amplification protocol consisted of an initial denaturation at 95°C for 3 min; 28 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 45 s; followed by a final extension at 72°C for 10 min. Library preparation for next-generation sequencing and Illumina NovaSeq sequencing were performed at Macrogen, Inc. (Seoul, South Korea) according to the manufacturer's instructions. Library quality was assessed using a Qubit 2.0 Fluorometer (Thermo Scientific, Waltham, MA, USA) and an Agilent Bioanalyzer 2100 system. The DNA libraries were then multiplexed and loaded onto an Illumina NovaSeq instrument according to the manufacturer's instructions (Illumina, San Diego, CA, USA). Raw images were generated by the Illumina sequencer using sequencing control software for system control and base calling through the integrated primary analysis software RTA (Real Time Analysis). The BCL (base calls) files were converted to FASTQ format using the Illumina software package bcl2fastq.

Bioinformatic data processing

All paired-end raw FASTQ sequencing reads were quality-filtered and analyzed using Cutadapt (<https://journal.embnnet.org/index.php/embnnetjournal/article/view/200>) and FastQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). Sequence reads were filtered according to the following criteria: no ambiguous bases, sequence length ≥ 200 bp, and mean quality score ≥ 20. The sequence reads were subsequently processed using the QIIME2 and DADA2 pipelines to remove chimeras, dereplicate DNA reads, and identify operational taxonomic units (OTUs). Briefly, OTUs were clustered at 97% sequence similarity, and centroid sequences were used for taxonomic assignment against the SILVA version 138 database. Alpha diversity indices (Chao1, Shannon, and Simpson) were calculated using the Phyloseq package in R software (version 4.3.1) (McMurdie and Holmes, 2013).

Differences between deep-bedding samples were assessed using the Mann-Whitney U test. Rank-transformed analysis of variance (ANOVA) (Rayner and Best, 2013) was used to analyze differences in alpha diversity among bedding types, diet types, and their interactions in the animal trial using R software. UpSet plots and Venn diagrams were used to evaluate the distribution of OTUs among different groups. Principal coordinates analysis (PCoA) plots were generated in QIIME2 based on unweighted UniFrac distance metrics. The statistical significance of beta diversity among treatments was determined using permutational multivariate ANOVA (PERMANOVA) with the *adonis* function in the *vegan* package in R software (Anderson, 2001). For comparisons of microbial taxa between bedding types and within diets for each bedding type, microbial abundance data were transformed using $\log_{10}(x + 1)$ to improve normality (Jin et al., 2016). A general linear model was fitted with bedding type and diet type as the main effects and their interaction using R software. Finally, linear discriminant analysis effect size (LEfSe) (Segata et al., 2011) was performed to identify taxa that were differentially abundant between bedding types ($\alpha = 0.05$ and linear discriminant analysis score > 3.0). The significance level for microbiome analyses was set at 0.05.

Statistical analysis

The effects of bedding type, diet type, and their interaction on growth performance were evaluated as a 2×2 factorial experiment in a completely randomized design using R software (version 4.3.1). Data were analyzed with the cage as the experimental unit. Results are presented as means with the standard error of the mean, and $P < 0.05$ was considered statistically significant.

RESULTS

Sequencing data

The V3–V4 region of the 16S rRNA gene was sequenced from 14 samples using the Illumina MiSeq sequencing platform. The samples consisted of two fermented feed samples, four pig manure samples collected from DP (two samples each from DP and DPF), and eight cecal content samples collected from the four animal treatment groups (two samples each from DPBS, DPFBS, CBS, and CFBS). After removal of low-quality and chimeric sequences, a total of 372,040 high-quality reads were generated. An average of 26,574 sequences per sample was obtained, with an average read length of 301 bp. The total numbers of identified OTUs in fermented feed, pig manure, and cecal samples were 213, 570, and 296, respectively, based on a 97% sequence similarity threshold for species-level classification.

Microbial ecosystem structure of FBS

Four major phyla and 10 genera (mean relative abundance of $>1\%$ of the community) were observed in FBS after 21 days of fermentation. The predominant bacterial phyla in FBS were Bacteroidetes (78.84%), Firmicutes (10.87%), Proteobacteria (1.56%), and Spirochaetes (1.52%) (Figure 1a), accounting for 92.79% of the total bacterial community in the fermented feed. The dominant genera were *Prevotella* (62.21%), *Paludibacter* (2.37%), *Ethanoligenens* (2.17%), *Barnesiella* (1.98%), *Paraprevotella* (1.81%), *Olivibacter* (1.69%), *Treponema* (1.50%), *Succiniclasticum* (1.33%), *Cytophaga* (1.25%), and *Bacteroides* (1.04%).

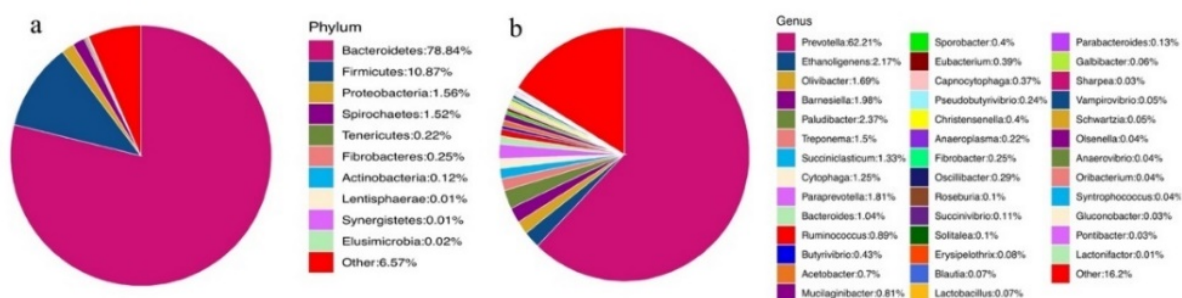


Figure 1 Microbial taxonomic profiles of the fermented banana stem at the (a) phylum and (b) genus levels at the 21-day fermentation period.

Microbial ecosystem structure and diversity of pig manure

Figure 2a shows that the total number of OTUs in group DP was higher than that in group DPF. In addition, 163 OTUs were shared between the two groups. The alpha diversity indices of pig manure samples are presented in Figure 2b. The Chao1, Shannon, and Simpson indices did not differ significantly between the DP and DPF groups ($P > 0.05$). PCoA of bacterial OTUs based on unweighted UniFrac distance metrics revealed clear separation of the microbial communities between the two groups (Figure 2c). At the phylum level (Figure 3a), Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria were the dominant phyla in group DPF, accounting for 96.44% of the total bacterial community. In group DP, the dominant phyla were Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria, accounting for 89.61% of the total bacterial community. At the genus level (Figure 3b), *Lactobacillus* was the most abundant genus (47.02%) among the 119 genera detected in group DPF. Other genera with relative abundances greater than 1% included *Acinetobacter* (1.78%), *Leuconostoc* (1.49%), and *Klebsiella* (1.30%). In group DP, the most abundant genera (>1%) were *Streptococcus* (7.94%), *Flavobacterium* (3.68%), *Acinetobacter* (2.73%), *Parabacteroides* (1.97%), *Clostridium* (1.69%), and *Lactobacillus* (1.27%), among the 188 genera identified in this group.

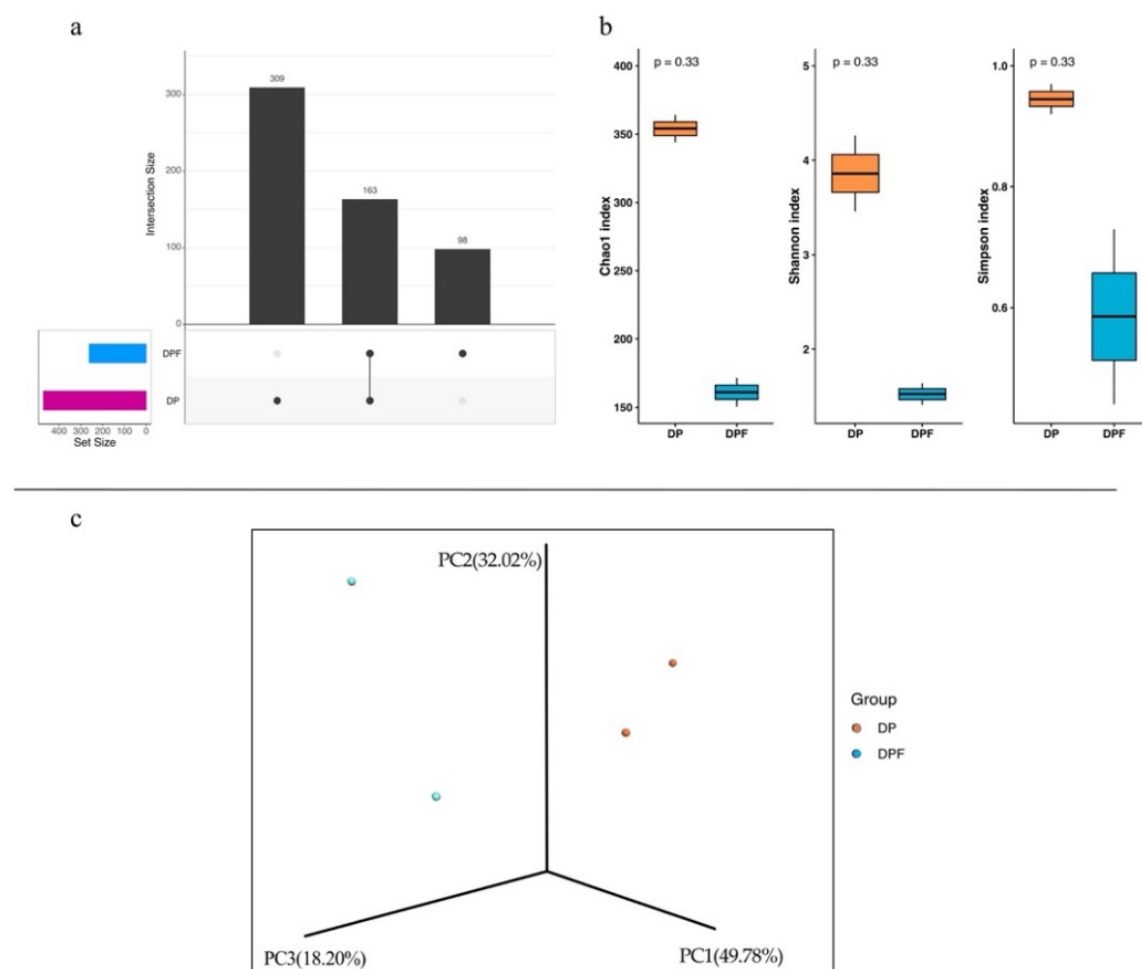


Figure 2 Alpha and beta diversity analysis of pig manure of crossbred pigs fed with BS (DP) or FBS (DPF). (a) Up Set plot for bacterial OTUs. (b) Alpha diversity indices. (c) Beta diversity measures using principal coordinates analysis (PCoA) based on unweighted UniFrac distance matrix. PC1, PC2, and PC3 explained 49.78%, 32.02%, and 18.20% of the total variation, respectively.

Effects of bedding and diet types on growth performance, intestinal morphostructural characteristics, and gut microbiota

Growth performance

The effects of bedding and diet types on the growth performance of crossbred pigs are shown in [Table 2](#). Pigs raised under different bedding systems and dietary treatments showed no significant differences in initial body weight, ADFI, or feed conversion ratio ($P > 0.05$). Nevertheless, C resulted in significantly higher final body weight and ADG than did DP ($P < 0.05$). No interactions between bedding and diet types were observed ($P > 0.05$).

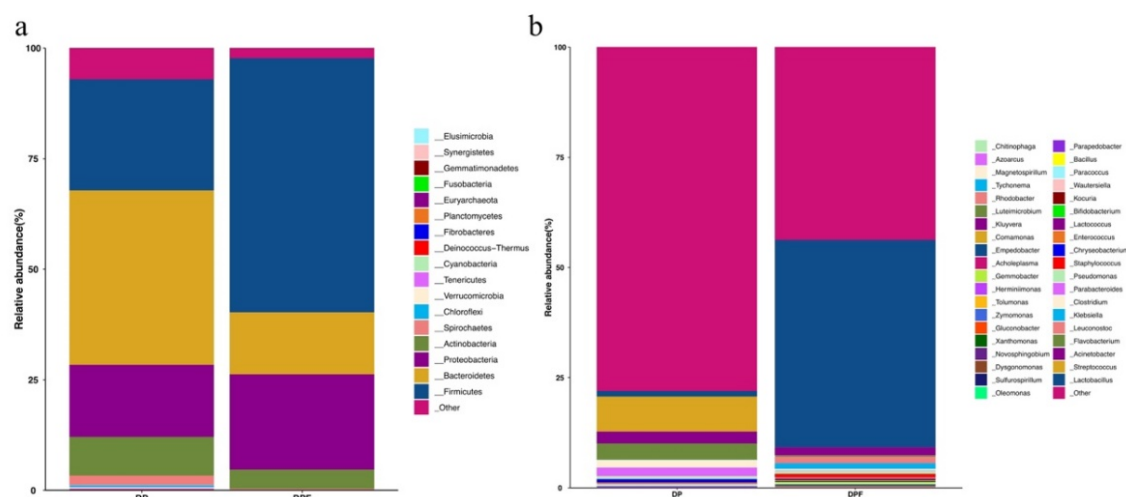


Figure 3 Microbial taxonomic profiles of the pig manure of crossbred pigs fed with BS (DP) or FBS (DPF) diets at the phylum (a) and genus (b) levels.

Table 2 Effects of bedding and diet types on the growth performance of crossbred pigs

Items	Deep ¹		Concrete ²		SEM	P-value ³		
	DPBS	DPFBS	CBS	CFBS		B	D	B x D
Initial weight (kg)	13.89	13.78	13.84	13.76	0.352	0.96	0.891	0.974
Final weight (kg)	66.08	65.18	69.32	69.49	0.797	0.025	0.821	0.740
ADFI (kg/day)	2.68	2.70	2.73	2.79	0.023	0.155	0.435	0.637
ADG (kg/day)	0.435	0.429	0.462	0.464	0.008	0.046	0.886	0.79
FCR	6.25	6.39	5.96	6.02	0.117	0.172	0.670	0.876

ADFI = Average daily feed intake; ADG = Average daily gain; FCR = feed conversion ratio, expressed as kg feed/kg body weight gain. 1 DPBS = Deep bedding with BS diet; DPFBS = Deep bedding with FBS diet. 2 CBS = Concrete bedding with BS diet; CFBS = Concrete bedding with FBS diet. 3 B = Effect of bedding type; D = Effect of diet type; B x D = Interaction between bedding and diet type.

Intestinal morphostructural characteristics

The effects of bedding and diet types on the intestinal morphology of crossbred pigs are presented in Table 3. VH in the duodenum, jejunum, and ileum was not significantly affected by the treatments ($P > 0.05$). Similarly, CD in all intestinal segments was not affected by bedding or diet type ($P > 0.05$). In addition, no significant differences in the VH:CD ratio were observed among treatments in any segment of the small intestine ($P > 0.05$).

Table 3 Effects of bedding and diet types on the intestinal morphology of crossbred pigs

Items	Deep ¹		Concrete ²		SEM	P-value ³		
	DPBS	DPFBS	CBS	CFBS		B	D	B x D
Duodenum								
VH (µm)	807.88	727.62	815.39	801.72	13.71	0.131	0.083	0.217
CD (µm)	210.43	193.21	201.49	183.68	7.33	0.534	0.239	0.984
VH:CD	4.24	4.14	4.34	4.53	0.14	0.385	0.871	0.596
Jejunum								
VH (µm)	728.31	783.12	794.23	789.50	14.55	0.217	0.391	0.308
CD (µm)	184.23	217.73	185.37	196.58	7.31	0.494	0.129	0.447
VH:CD	4.53	3.87	4.61	4.29	0.16	0.423	0.123	0.589
Ileum								
VH (µm)	590.86	624.69	617.00	636.96	17.18	0.583	0.442	0.843
CD (µm)	203.85	221.43	204.46	195.58	7.16	0.121	0.303	0.103
VH:CD	3.21	2.67	3.04	3.43	0.10	0.099	0.696	0.101

DPBS = Deep bedding with BS diet; DPFBS = Deep bedding with FBS diet. 2 CBS = Concrete bedding with BS diet; CFBS = Concrete bedding with FBS diet. 3 B = Effect of bedding type; D = Effect of diet type; B x D = Interaction between bedding and diet type. VH = villus height; CD = crypt depth; VH:CD = villus height-to-crypt depth ratio.

Alpha and beta diversity

In total, 188 OTUs were shared among the four groups, whereas only 14, 18, 17, and 14 OTUs were unique to the CBS, CFBS, DPBS, and DPFBS groups, respectively (Figure 4). The average Good's coverage was 98% for all groups, indicating that the true number of OTUs was adequately represented. The alpha diversity indices describing richness, diversity, and evenness are shown in Figure 5a. A rank-transform ANOVA was fitted with bedding and diet types as the main effects and their interaction. The richness estimator (Chao1) and diversity indices (Simpson and Shannon) showed no significant differences between bedding and diet types. Similarly, the interaction between bedding and diet types did not differ significantly among groups. PCoA based on unweighted UniFrac distance metrics of bacterial OTUs was used to assess beta diversity. The first three dimensions of the unweighted UniFrac dissimilarity matrix (Figure 5b) revealed no significant differences among groups. PERMANOVA indicated that bedding type, diet type, and their interaction did not contribute significantly to the observed variation.

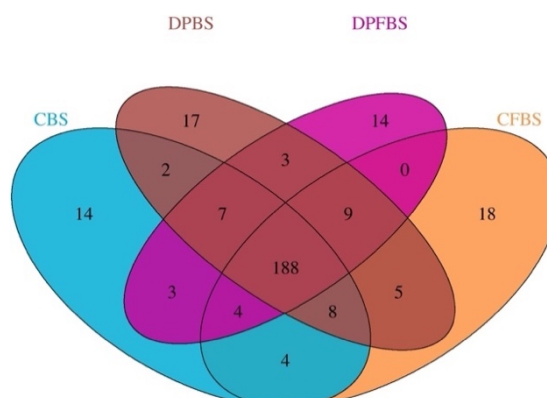


Figure 4 Venn diagram showing the number of shared OTUs among cecal content samples from the four animal treatment groups: DPBS, DPFBS, CBS, and CFBS.

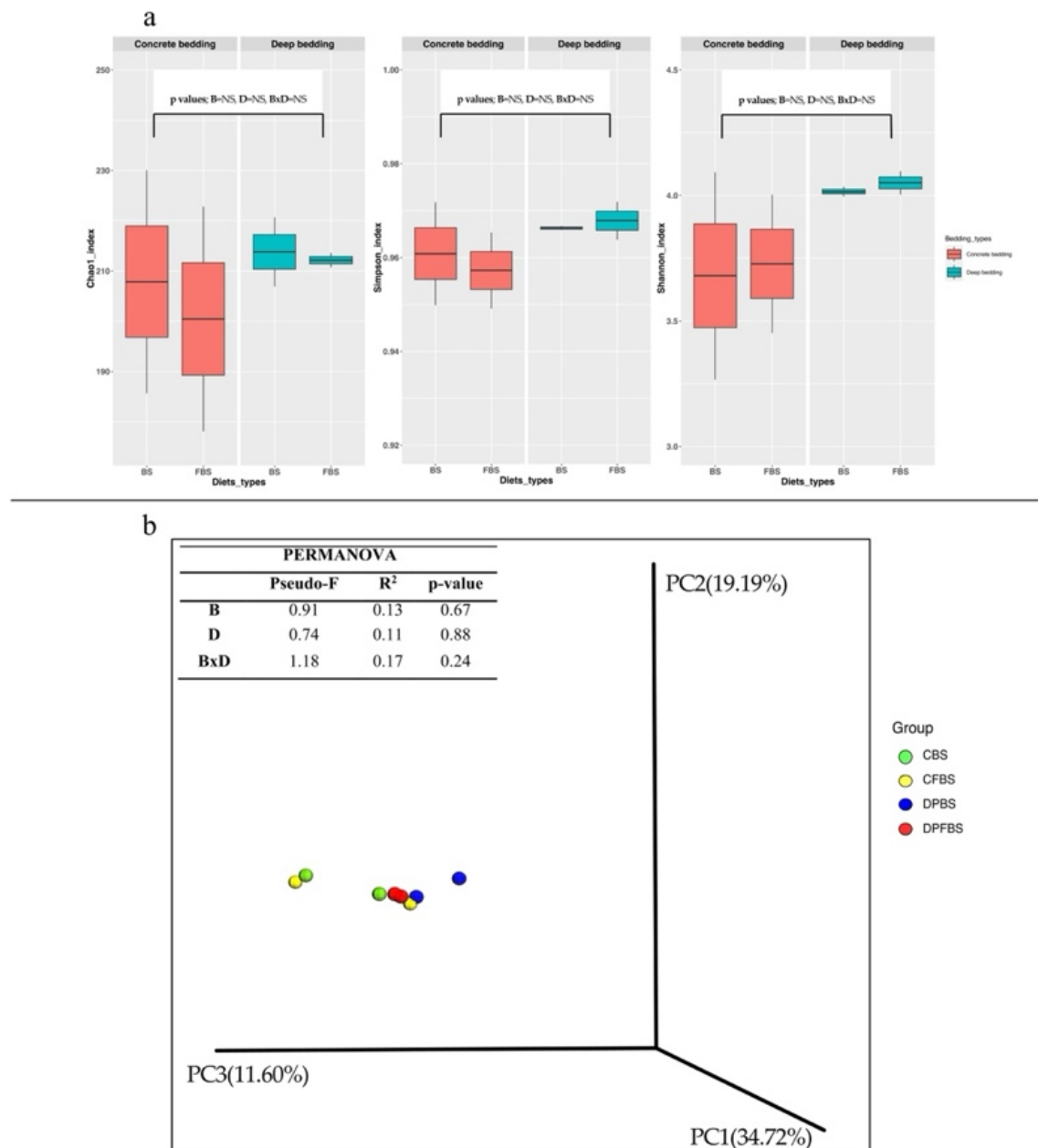


Figure 5 Alpha and beta diversity analysis of the cecal microbiota of crossbred pigs from the four animal treatment groups: DPBS, DPFBS, CBS, and CFBS. (a) Box-and-whiskers plot for estimated alpha diversity indices. (b) Beta diversity measures using principal coordinate analysis (PCOA) based on unweighted-UniFrac distance matrix. The axis of principal component 1 (PC1) described 34.72% principal component 2 (PC2) described 19.19%, and principal component 3 (PC3) described 11.60 of total data variability. NS = Not Significant; B = Effect of bedding type; D = Effect of diet type; B × D = Interaction between bedding and diet types.

Microbial ecosystem structure

A total of 18 phyla, 19 classes, 110 families, and 261 genera were identified from cecal samples of crossbred pigs. The two dominant bacterial phyla, Firmicutes and Bacteroidetes, had relative abundances of 39.43%–51.40% and 35.84%–43.12%, respectively. Spirochaetes was the third most abundant phylum,

with relative abundances ranging from 8.87% to 16.88% (Figure 6a). At the genus level (Figure 6b), the three dominant genera were *Parabacteroides*, *Prevotella*, and *Treponema*, accounting for 10.53%–19.01%, 8.58%–11.39%, and 5.66%–16.80% of the total bacterial community, respectively. When the relative abundance of each genus was compared across groups, several genera showed significant differences between deep and concrete bedding types, whereas diet type and the bedding type $\times$ diet type interaction had no significant effect on genus-level relative abundance. Heatmap hierarchical clustering (Figure 6c) showed that the relative abundances of *Solitalea*, *Paraprevotella*, *Sphingobacterium*, and *Phascolarctobacterium* were higher in the DP group than in the C group ($P < 0.05$). However, *Lactobacillus* abundance was significantly lower in the DP group than in the C group ($P < 0.05$).

Differences in bacterial communities between bedding types

The OTUs associated with each bedding type were compared to identify bacterial taxa that differed significantly between bedding systems. Subsequently, LEfSe with a linear discriminant analysis threshold of 3.0 was used to identify potential biomarkers. In total, 11 microbial taxa were identified as significantly discriminative between DP and C (Figure 7). Nine features were more abundant in the DP group, including five genera (*Solitalea*, *Paraprevotella*, *Sphingobacterium*, *Phascolarctobacterium*, and *Robinsoniella*). At the family level, Sphingobacteriaceae and Acidaminococcaceae were enriched in the DP group compared with the C group. At the order level, Sphingobacteriales differed between the two bedding types. At the class level, Negativicutes was significantly different between DP and C groups. Two features were less abundant in the DP group, namely *Lactobacillus* at the genus level and Lactobacillaceae at the family level.

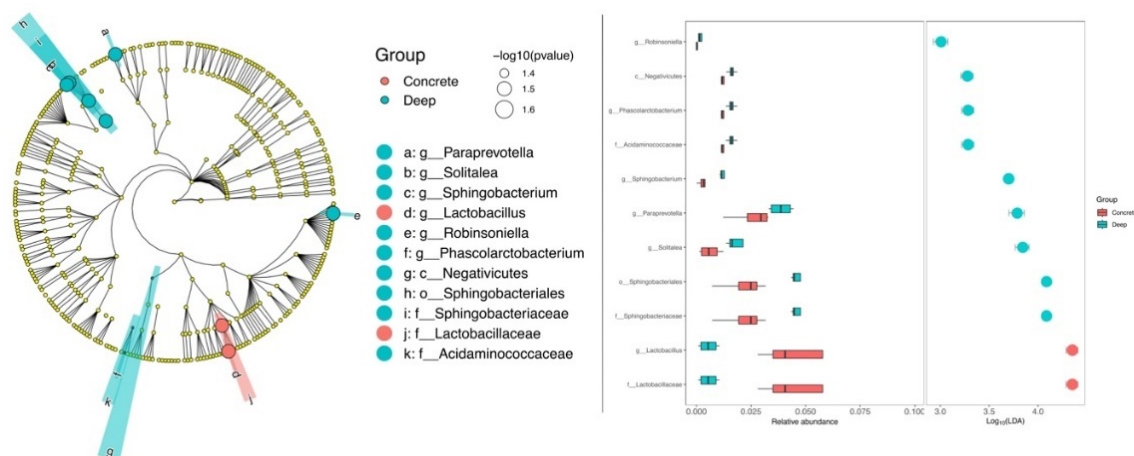


Figure 7 The cladogram and histogram show microbial taxa that differed significantly between the cecal contents of crossbred pigs reared in deep-bedding and concrete-floor systems. The cladogram shows the presence of microbial taxa (class, order, family, and genus) that are significantly different based on linear discriminant analysis (LDA) scores in the histogram (An LDA score > 3.0 was used as the threshold).

DISCUSSION

In recent years, researchers have identified a complex relationship between the gut microbiome and pig production (Gardiner et al., 2020). The microbiota is a promising target for improving pig production and increasing economic efficiency (Maltecca et al., 2020; Vasquez et al., 2022). As a dynamic ecosystem, the gut microbiome is influenced by many factors, including host genetics (Chen et al., 2018), diet (Grevenhof et al., 2011; Szabó et al., 2023), and flooring and bedding systems (Megahed et al., 2019). Although many previous studies have identified these factors as important determinants, no studies to date have examined the gut microbiome of Thai native crossbred pigs raised in DP systems and supplemented with FBS. In the present study, we investigated the association between gut microbial composition and the growth performance of Thai native crossbred pigs. To the best of our knowledge, this study represents the first attempt to evaluate differences in the gut microbiota of Thai native crossbred pigs reared in a DP system and fed a dietary program formulated as an alternative strategy to improve pig production for smallholder farming while reducing environmental impact.

In the FBS samples, Bacteroidetes was the dominant phylum and *Prevotella* was the dominant genus among the 16S rRNA gene sequences. Although plant fermentation with added sugar and salt generally favors lactic acid bacteria, particularly *Lactobacillus*, the predominance of Bacteroidetes and *Prevotella* in the present study may be related to the high fiber content of BS and the farm-scale, nonsterile fermentation conditions used without a defined starter culture. BS is a lignocellulosic plant material with a high fiber content (Huy et al., 2018), and *Prevotella* is commonly associated with the degradation and utilization of plant-derived carbohydrates. Therefore, the predominance of *Prevotella* may reflect the fibrous nature of the fermented substrate and possible microbial contributions from the raw material, handling process, equipment, and surrounding environment. However, because fermentation quality parameters such as pH and organic acid profiles were not evaluated, the functional role of *Prevotella* in FBS fermentation should be interpreted with caution.

In general, acid production during plant fermentation contributes to a decrease in pH, which can inhibit spoilage and undesirable microorganisms and thereby support feed preservation (Thierry et al., 2023). This may allow BS to be preserved for longer periods, particularly during seasons of feed scarcity. In our previous study, fermentation enhanced the dry matter and crude fiber digestibility of BS (Arjin et al., 2020). Specifically, FBS showed greater dry matter digestibility than BS (34.77% vs. 30.42%), and crude fiber digestibility increased from 17.24% to 19.54%. These improvements may be partly associated with microbial activity during fermentation, although the specific functional contributions of individual microbial taxa were not determined in the present study. In addition, fermented feed and its microbial metabolites have been reported to influence intestinal microbial balance and gut development in pigs (Yang et al., 2020; Li et al., 2022). For example, dietary fiber fermentation and microbial metabolites may support intestinal epithelial development by increasing gut mass, VH, and absorptive surface area (Agyekum and Nyachoti, 2017). This is consistent with our previous study, in which pigs fed diets containing FBS at a 50% inclusion level had greater VH in the duodenum, jejunum, and ileum than pigs fed diets containing BS at the same inclusion level (Arjin et al., 2020). In the present study, however, VH in the duodenum, jejunum, and ileum did not differ significantly among treatments. Therefore, although previous studies have suggested that fermented feed may support intestinal development, the present results do not provide evidence that FBS supplementation or bedding type significantly altered intestinal morphology in Thai native crossbred pigs. Overall, fermentation may be useful for preserving feedstuffs during the dry season and improving the digestibility and nutritional value of fibrous feedstuffs for pigs, although its effects on intestinal morphology were not significant under the conditions of the present study.

As regards bacterial community dynamics in pig manure, the microbial fermentation bed system, also known as the deep-litter system, breeding pigs on litter, or in situ decomposition of pig manure, comprises deep-bedded structures in which animals are raised on a thick layer of litter (Morrison et al., 2007). Pig feces are mixed with the litter and undergo decomposition without waste discharge or odor emissions (Menalled et al., 2005; Rashad et al., 2010; Karadag et al., 2013). From an ecological perspective, composting is a dynamic process driven by the combined activity of a wide variety of microbial populations, such as bacteria and fungi, which play pivotal roles in pig feces degradation and the composting process (López-González et al., 2015). In the current study, the four dominant phyla in pig manure from both groups were partially consistent with the findings of Chen et al. (2017), who reported that Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidetes were the four dominant bacterial phyla in pig manure from pigs raised on mattress materials comprising spent mushroom substrate, mash, and rice husks. Chen et al. (2021) observed the predominance of Proteobacteria, Actinobacteria, Bacteroidetes, and Firmicutes during composting of spent mushroom substrates and rice husks with pig manure. Similarly, Ma et al. (2018) reported that Firmicutes, Proteobacteria, Bacteroidetes, and Actinobacteria were the predominant phyla during composting of wheat straw with pig manure. These phyla, identified in the gut microbiome of Thai native crossbred pigs (Figure 6a) and in FBS (Figure 1), may represent important sources of the bacterial communities detected in pig manure. Firmicutes are thought to play a major role in lignocellulose degradation (Pankratov et al., 2011). By contrast, Proteobacteria and Bacteroidetes are involved in organic matter degradation and carbon cycling (Wang et al., 2018), whereas Proteobacteria also play important roles in sulfur and nitrogen cycling and sewage degradation (Zhong et al., 2017; Wei et al., 2018). Actinobacteria play a major role in the degradation of lignin and recalcitrant cellulose (Su et al., 2015). At the genus level, our results were partially consistent with those of Lim et al. (2018), who found that *Lactobacillus*, *Streptococcus*, and *Clostridium* were among the predominant bacterial genera in pig manure. Interestingly, the proportion of *Lactobacillus* was higher in group DPF than in group DP. This result corresponds to the higher abundance of Firmicutes in group DPF because *Lactobacillus* belongs to this phylum. *Lactobacillus* is one of the major lactic acid bacteria and has been detected in manure and silage crops. It plays important roles in composting processes. A lactic acid bacteria community comprising mainly *L. fermentum*, *L. plantarum*, and *L. paracasei* efficiently accelerated straw fermentation, thereby improving rice straw utilization and reducing environmental disposal problems (Gao et al., 2008).

Regarding growth performance, the final body weight and ADG of Thai native crossbred pigs reared on DP were lower than those of pigs reared on C. This result is consistent with Hötzel et al. (2009), who reported that pigs reared on DP with rice husks had lower final body weight and ADG than pigs reared on C. One possible explanation is that pigs raised on DP may exhibit greater physical activity and exploratory behavior, resulting in increased energy expenditure (Hötzel et al., 2009). In addition, feed wastage or feed contamination with bedding material may reduce actual feed consumption, even when recorded ADFI appears similar between housing systems (Chaiwang et al., 2021). The differences in cecal microbial composition between pigs reared on DP and C may be partly explained by differences in environmental microbial exposure and housing conditions. The gut microbiome is influenced by environmental conditions, including housing and bedding systems (Megahed et al., 2019), as well as host genetics and dietary composition (Cotillard et al., 2013; Pajarillo et al., 2015). In the DP system, pigs were continuously exposed to rice husk bedding mixed with feces and urine, where in situ fermentation and accumulation of organic matter may have created a complex environmental microbial reservoir. By contrast, C pens were cleaned daily with high-pressure water, which may have reduced microbial accumulation in the pen environment and altered microbial exposure. Moreover, rooting and

exploratory behavior in DP may have increased direct contact with bedding material, thereby influencing microbial intake from the environment and contributing to differences in cecal microbial composition. In the present study, *Lactobacillus* was significantly more abundant in pigs reared on C than in those reared on DP. *Lactobacillus* spp. have been associated with gut microbial balance, intestinal barrier function, nutrient utilization, immune modulation, and growth-related traits in pigs (Yang et al., 2014; Yang et al., 2020; Lan et al., 2023). Therefore, the higher relative abundance of *Lactobacillus* in pigs reared on C may be one microbial factor associated with the greater final body weight and ADG observed in this group. However, this association should be interpreted cautiously because the present study does not establish a direct causal relationship between specific bacterial taxa and growth performance. Growth performance is influenced by multiple factors, including feed intake, feed wastage, physical activity, environmental exposure, and host physiological responses. By contrast, *Solitalea*, *Paraprevotella*, *Sphingobacterium*, and *Phascolarctobacterium* were more abundant in pigs reared on DP. These genera may reflect the more complex microbial exposure associated with the DP environment, where bedding material, feces, urine, and organic matter undergo in situ fermentation. However, the functional roles of these genera in nutrient metabolism, gut health, and growth performance in Thai native crossbred pigs remain unclear. Further studies integrating metagenomic functional prediction, metabolite profiling, and controlled environmental sampling are required to clarify whether these microbial differences directly contribute to host performance.

CONCLUSIONS

Collectively, the data presented in this study support the notion that raising native crossbred pigs on a DP system with FBS supplementation may provide a sustainable approach to pig production for smallholder farming by lowering environmental impact, reducing feed costs, and improving the microbial community associated with pig manure composting. Moreover, raising pigs on DP systems significantly affected the abundance of *Solitalea*, *Paraprevotella*, *Sphingobacterium*, and *Phascolarctobacterium* in the gastrointestinal tract. Future studies should investigate the functional roles of these genera in nutrient metabolism and immune function and their potential implications for pig health and welfare.

ACKNOWLEDGEMENTS

This research was partially supported by Chiang Mai University, Chiang Mai Thailand.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Patipan Hnokaew: Software; Formal analysis; Data curation; Writing-original draft preparation.

Korawan Sringarm: Conceptualization; Validation; Resources; Supervision; Project administration; Funding acquisition; Writing-review and editing.

Phongsakorn Chuammitri: Software; Formal analysis.

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Niraporn Chaiwang: Methodology; Resources; Writing-review and editing.

Watcharapong Wattanakul: Conceptualization; Resources; Writing-review and editing.

Marninphan Thongkham: Investigation.

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All authors have read and agreed to the published version of the manuscript.

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How to cite this article;

Patipan Hnokaew, Korawan Sringarm, Phongsakorn Chuammitri, Apinya Satsook, Niraporn Chaiwang, Watcharapong Wattanakul, Marninphan Thongkham, Kulisara Marupanthorn, Chompunut Lumsangkul and Chaiwat Arjin. Effects of deep-bedding floor and fermented feed supplementation on growth performance, intestinal morphology, and gut microbiome in Thai native crossbred pigs. *Veterinary Integrative Sciences.* 2027; 25(1): e2027002-1-20.
