Effects of Lactobacillus casei on Growth Performance and Gut Microbiota of Beijing Black Pigs during the Nursery Stage: Postprint

Authors: Wang Sixin, Ji Haifeng, Shi Guohua, Liu Hui, Wang Hongwei, Zhang Dongyan, Wang Jing, Zhang Wei, Wang Yamin

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Abstract

This experiment aimed to investigate the effects of dietary supplementation of *Lactobacillus casei* on growth performance and intestinal microbiota in Beijing black pigs during the nursery stage. A total of 120 Beijing black piglets aged (35±2) days with a body weight of (7.53±0.21) kg were selected and randomly divided into 3 groups: control group, *Lactobacillus casei* group, and chlortetracycline group, with 4 replicates per group and 10 piglets per replicate. Pigs in the control group were fed a basal diet (without *Lactobacillus casei* and chlortetracycline); pigs in the *Lactobacillus casei* group were fed a diet supplemented with *Lactobacillus casei* live freeze-dried preparation at an effective viable count of 4.0×10^9 CFU per kilogram of feed; pigs in the chlortetracycline group were fed a diet supplemented with chlortetracycline premix at a concentration of 75 mg chlortetracycline per kilogram of feed. The experimental period lasted 30 days, and fresh fecal samples were collected on day 28 of the experiment for sequencing of the V3-V4 region of 16S rRNA. The results showed: 1) Compared with the control group, the average daily gain in the *Lactobacillus casei* group and chlortetracycline group increased by 12.71% ($P<0.05$) and 8.58% ($P<0.05$), respectively, and the feed-to-gain ratio decreased by 7.34% ($P<0.05$) and 4.52% ($P>0.05$), respectively. 2) Analysis of the ACE index, Chao1 index, Shannon index, and Simpson index of fecal microbiota revealed that the richness and diversity of intestinal microbiota in the *Lactobacillus casei* group were higher than those in the control group and chlortetracycline group. 3) The fecal samples from the three groups contained a total of 17 phyla and 206 genera, with the control group, *Lactobacillus casei* group, and chlortetracycline group containing 198, 200, and 197 genera, respectively. These results indicate that dietary supplementation of *Lactobacillus casei* during the nursery stage in Beijing black pigs can improve the intestinal microbiota structure of piglets, thereby enhancing growth performance.

Full Text

Effects of Lactobacillus casei on Growth Performance and Intestinal Microbiota of Beijing Black Pigs in Nursery Stage

WANG Sixin[1], JI Haifeng[1]*, SHI Guohua[2], LIU Hui[1], WANG Hongwei[2], ZHANG Dongyan[1], WANG Jing[1], ZHANG Wei[1], WANG Yamin[1]

[1]Institute of Animal Husbandry and Veterinary Medicine, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China
[2]Beijing Heiliu Animal Husbandry Science and Technology Ltd. Co., Beijing 102211, China

*Corresponding author, professor, E-mail: jhf207@126.com

Abstract

This experiment was conducted to investigate the effects of dietary Lactobacillus casei supplementation on growth performance and intestinal microbiota of Beijing black pigs during the nursery stage. A total of 120 Beijing black piglets at (35±2) days of age with an average body weight of (7.53±0.21) kg were randomly allocated into three groups: control group, Lactobacillus casei group, and chlortetracycline group. Each group consisted of four replicates with ten piglets per replicate. Piglets in the control group were fed a basal diet without Lactobacillus casei or chlortetracycline. Those in the Lactobacillus casei group received the basal diet supplemented with lyophilized Lactobacillus casei preparation at 4.0×10 CFU viable bacteria per kilogram of feed. Piglets in the chlortetracycline group were fed the basal diet supplemented with chlortetracycline premix at 75 mg per kilogram of feed. The experimental period lasted 30 days, and fresh fecal samples were collected on day 28 for sequencing of the V3-V4 region of the 16S rRNA gene. The results showed that: (1) Compared with the control group, the average daily gain of piglets in the Lactobacillus casei and chlortetracycline groups increased by 12.71% ($P<0.05$) and 8.58% ($P<0.05$), respectively, while feed-to-gain ratio decreased by 7.34% ($P<0.05$) and 4.52% ($P>0.05$), respectively. (2) Analysis of ACE index, Chao1 index, Shannon index, and Simpson index revealed that the Lactobacillus casei group exhibited higher intestinal microbiota richness and diversity compared with both the control and chlortetracycline groups. (3) A total of 17 phyla and 206 genera were identified across all fecal samples, with the control, Lactobacillus casei, and chlortetracycline groups containing 198, 200, and 197 genera, respectively. These findings indicate that dietary supplementation with Lactobacillus casei during the nursery stage improves intestinal microbiota structure and consequently enhances growth performance in Beijing black pigs.

Keywords: Beijing black pigs; growth performance; intestinal microbiota; Lac-

tobacillus casei; chlortetracycline

Introduction

With scientific advancement and social progress, feed antibiotics are gradually being banned in animal production, making the search for antibiotic alternatives and replacement technologies a subject of considerable interest. Probiotics can improve intestinal microbial balance, produce beneficial substances, promote animal health, and enhance production performance, thus holding great promise. Lactobacillus casei is a type of probiotic approved for use in livestock by the Ministry of Agriculture' s "Feed Additive Catalogue" (2013). Previous studies by Xian Yihan et al. [1], Zhang Weiwei et al. [2], and Wang Qiheng et al. [3] demonstrated that feeding Lactobacillus casei to piglets (Large White × Landrace and Duroc × Landrace × Large White) promoted intestinal villus and mucosal epithelial cell development, increased feed intake and daily gain, and reduced feed-to-gain ratio. Regarding the effects of probiotics and antibiotics on piglet intestinal microbiota, Zhang et al. [4] observed that oral administration of Lactobacillus reuteri or chlortetracycline to piglets (Landrace × Large White) resulted in increased ACE and Chao1 indices in the jejunum of the Lactobacillus reuteri group compared with the chlortetracycline group, indicating enhanced microbial richness. Looft et al. [5] found that dietary antibiotic supplementation increased the relative abundance of Proteobacteria in intestinal contents. However, Poole et al. [6] reported no significant changes in fecal microbiota structure or diversity in piglets (Duroc × Large White) fed low-dose chlortetracycline (50 g/t). Li et al. [7] replaced antibiotics with Enterococcus faecalis in piglet (Duroc × Landrace × Large White) diets and observed similar changes in intestinal microbiota as those seen with antibiotic supplementation.

Regarding differences in intestinal (fecal) microbiota among pig breeds, Yang Liu et al. [8], Xiao Wenping [9], Yang Lina [10], and Yang Weiping [11] investigated microbiota in Landrace, Large White, and Duroc pigs, as well as in Tibetan, Rongchang, Bama, Erhualian, and Meishan pigs. Their findings revealed substantial differences in microbial species/genera and quantities among different breeds, with domestic breeds exhibiting greater intestinal (fecal) microbiota diversity than imported lean-type breeds.

Beijing black pig is a characteristic breed independently cultivated in China. Compared with introduced breeds such as Duroc, Landrace, Large White, or their crossbreeds, Beijing black pigs exhibit superior meat quality, tolerance to roughage, and disease resistance, making them increasingly popular among consumers. However, no studies have reported the application of Lactobacillus casei in Beijing black pigs. Investigating the effects of Lactobacillus casei on growth performance and intestinal microbiota of Beijing black pigs during the nursery stage will provide technical support for scientific breeding and green development of this breed.

Materials and Methods

1.1 Experimental Materials The lyophilized Lactobacillus casei preparation (viable count: 2.0×10^{1} CFU/g) was prepared by the Institute of Animal Husbandry and Veterinary Medicine, Beijing Academy of Agriculture and Forestry Sciences. The 15% chlortetracycline premix was purchased from Jinhe Group.

1.2 Experimental Design and Animal Management A total of 120 Beijing black piglets in the nursery stage at (35±2) days of age with an average body weight of (7.53±0.21) kg were randomly divided into three groups: control group, Lactobacillus casei group, and chlortetracycline group. Each group comprised four pens (replicates) with ten piglets per pen. Piglets in the control group were fed a basal diet without Lactobacillus casei or chlortetracycline. Those in the Lactobacillus casei group received the basal diet supplemented with lyophilized Lactobacillus casei preparation at 4.0×10 CFU viable bacteria per kilogram of feed. Piglets in the chlortetracycline group were fed the basal diet supplemented with chlortetracycline premix at 75 mg per kilogram of feed. The composition and nutrient levels of the basal diet are presented in Table 1 .

The experiment was conducted in the same nursery pig house with elevated pen housing. Piglets had ad libitum access to feed and water. The house temperature was maintained at 24–28°C with relative humidity of 60%–70%. Pig management followed the unified technical protocols of the farm, and the experimental period lasted 30 days.

Table 1 Composition and nutrient levels of the basal diet (air-dry basis)

Items	Content
Ingredients	
Corn	
Wheat bran	
Soybean meal	
Fish meal	
Premix[1]	
Total	
Nutrient levels[2]	
Moisture	
Crude protein (CP)	
Calcium (Ca)	
Total phosphorus (TP)	

[1]The premix provides the following per kilogram of diet: Cu (as copper sulfate)

150 mg, Fe (as ferrous sulfate) 150 mg, Zn (as zinc sulfate) 140 mg, Mn (as manganese sulfate) 40 mg, Se (as sodium selenite) 0.3 mg, I (as potassium iodide) 0.6 mg, VA 8,000 IU, VD 1,800 IU, VE 24 IU, VK 1.8 mg, VB 1.6 mg, VB 6 mg, VB 3.2 mg, VB 0.05 mg, biotin 0.3 mg, folic acid 1.2 mg, nicotinic acid 24 mg, pantothenic acid 18 mg, choline chloride 0.4 g, L-lysine salt 2.2 g, threonine salt 0.4 g, calcium 5 g, total phosphorus 3 g.

[2]Moisture, crude protein, calcium, and total phosphorus are measured values.

1.3 Growth Performance Measurement Body weight was measured after overnight fasting at the beginning and end of the experiment. Daily feed consumption was recorded to calculate average daily gain, average daily feed intake, and feed-to-gain ratio.

1.4 Fecal Sample Collection, Total DNA Extraction, and 16S rRNA Sequencing Fecal sample collection: On day 28 of the experiment, fresh fecal samples were collected before morning feeding. Two samples were collected per pen, totaling eight samples per group and 24 samples overall. Sterile stainless steel spoons were used to collect naturally excreted feces (20 g per piglet) into sterile tubes, which were placed on dry ice, transported to the laboratory, and stored at -80°C for total DNA extraction.

Total DNA extraction: Total bacterial DNA was extracted from pig feces using the TIANamp Microbial DNA Kit [Tiangen Biotech (Beijing) Co., Ltd.] following the manufacturer' s instructions. The extracted DNA was purified using a DNA purification kit, and its concentration and purity were assessed using a spectrophotometer and agarose gel electrophoresis. The purified DNA was stored at -20°C for subsequent analysis.

16S rRNA gene amplification and sequencing: Total bacterial DNA from Beijing black pig feces was used as template for PCR amplification of the V3-V4 hypervariable region of the bacterial 16S rRNA gene. The amplification system contained 4 L of 5× FastPfu Buffer, 2 L of 2.5 mmol/L dNTPs, 0.8 L each of forward and reverse primers (5 mol/L), 0.4 L of FastPfu DNA Polymerase, and 10 ng of DNA template in a final volume of 20 L. Universal bacterial primers 338F (5' -ACTCCTACGGGAGGCAGCA-3') and 806R (5' -GGACTACHVGGGTWTCTAAT-3') were used. PCR conditions were as follows: initial denaturation at 95°C for 3 min; 27 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 45 s; final extension at 72°C for 10 min. Three replicates were prepared for each sample. PCR products from the same sample were pooled and detected by 2% agarose gel electrophoresis. The PCR products were purified using the AxyPrep DNA Gel Extraction Kit (Axygen) and eluted with Tris-HCl. After detection by 2% agarose gel electrophoresis and preliminary quantification based on electrophoresis results, the PCR products were quantified using the QuantiFluor™-ST Blue Fluorescence Quantification System (Promega). Samples were then mixed in

appropriate proportions according to sequencing requirements and subjected to paired-end 250 bp (PE250) sequencing on the Illumina MiSeq platform.

1.5 Data Analysis Reads with quality scores below 20 at the tail were filtered using a 50 bp sliding window. If the average quality value within the window was below 20, the downstream bases from the window were truncated. Reads shorter than 50 bp after quality control were removed. Paired-end reads were merged into single sequences based on overlap relationships, with a minimum overlap length of 10 bp. The maximum mismatch ratio allowed in the overlap region of merged sequences was 0.2, and sequences not meeting this criterion were filtered out. Samples were distinguished based on barcodes and primers at both ends of the sequences, with sequence orientation adjusted. The barcode mismatch allowance was 0, and the maximum primer mismatch was 2. Operational taxonomic units (OTUs) were clustered from non-redundant sequences (excluding singletons) at 97% similarity, with chimeras removed during clustering to obtain representative OTU sequences. All optimized sequences were mapped to OTU representative sequences, and those with 97% similarity to representative sequences were selected to generate OTU tables. Taxonomic classification of each OTU was performed using the RDP classifier Bayesian algorithm, and microbial composition of each sample was statistically analyzed at various taxonomic levels.

Growth performance data were expressed as mean ± standard error. Data processing and analysis were performed using one-way ANOVA in SPSS 19.0 statistical software, with Duncan' s multiple comparison test used for post-hoc analysis. Differences were considered significant at $P<0.05$.

Results

2.1 Growth Performance As shown in Table 2 , dietary supplementation with Lactobacillus casei and chlortetracycline improved average daily gain and reduced feed-to-gain ratio in Beijing black pigs during the nursery stage. Compared with the control group, average daily gain in the Lactobacillus casei and chlortetracycline groups increased by 12.71% ($P<0.05$) and 8.58% ($P<0.05$), respectively, while feed-to-gain ratio decreased by 7.34% ($P<0.05$) and 4.52% ($P>0.05$), respectively. Although the Lactobacillus casei group showed improved average daily gain and feed-to-gain ratio compared with the chlortetracycline group, the differences were not significant ($P>0.05$).

Table 2 Effects of Lactobacillus casei on growth performance of Beijing black pigs in nursery stage

Items	Control group	Lactobacillus casei group	Chlortetracycline group
Initial weight (kg)	7.57±0.24	7.57±0.18	7.45±0.20
Final weight (kg)	17.01±0.55	18.21±0.44	17.70±0.47
Average daily gain (g)	314.67±11.95	354.67±10.58	341.67±10.86
Average daily feed intake (g)	557.60±40.58	581.66±46.42	578.56±50.23
Feed-to-gain ratio	1.77±0.12	1.64±0.20	1.69±0.11

In the same row, values with different small letter superscripts indicate significant differences ($P<0.05$).

2.2.1 Sequence and OTU Statistics A total of 24 fresh fecal samples were collected from the control, Lactobacillus casei, and chlortetracycline groups. Analysis yielded 1,439,917 high-quality sequences with an effective length of 634,742,973 bp and an average length of 440.82 bp. The control, Lactobacillus casei, and chlortetracycline groups generated 467,327, 488,949, and 483,641 sequences, respectively, with lengths of 206,235,445 bp, 215,290,320 bp, and 213,217,208 bp, respectively.

OTU clustering was performed at 97% similarity on non-redundant sequences (excluding singletons) with chimeras removed, yielding 871,056 representative OTU sequences and 14,390 OTUs across all 24 fecal samples. The control, Lactobacillus casei, and chlortetracycline groups contained 4,782, 4,920, and 4,688 OTUs, respectively. As shown in Figure 1 [Figure 1: see original paper], the three groups shared 823 OTUs, with the control, Lactobacillus casei, and chlortetracycline groups possessing 5, 29, and 3 unique OTUs, respectively.

C: control group; **L:** Lactobacillus casei group; **A:** chlortetracycline group. The same applies to subsequent figures.

Fig.1 The OTU Venn diagram of fecal samples among three groups

2.2.2 Intestinal Microbiota Diversity As shown in Table 3 , the sequencing coverage of fecal samples from the control, Lactobacillus casei, and chlortetracycline groups ranged from 99.68% to 99.72%. Among the three groups, the control group exhibited the lowest ACE and Chao1 indices, while the Lactobacillus casei group showed the highest ACE, Chao1, and Shannon indices. The chlortetracycline group had the highest Simpson index and lowest Shannon index. Comparison of ACE, Chao1, Shannon, and Simpson indices among the three groups revealed that the Lactobacillus casei group possessed higher intestinal microbiota richness and diversity than both the control and chlortetracycline groups.

Table 3 The microflora diversity indexes of fecal samples among three groups

Groups	Abundance indexes	Diversity indexes
	ACE index	Chao1 index
Control group		
Lactobacillus casei group		
Chlortetracycline group		

2.2.3 Intestinal Microbiota Composition At the phylum level, microbiota composition is presented in Figure 2 [Figure 2: see original paper] and Figure 3 [Figure 3: see original paper]. A total of 17 phyla were identified across the three groups, with Bacteroidetes, Firmicutes, Proteobacteria, and Spirochaetae being the dominant phyla, accounting for 99.02%–99.07% of relative abundance. The relative abundances of dominant phyla differed among groups. In the chlortetracycline group, Bacteroidetes accounted for 51.28% of relative abundance, while Firmicutes, Proteobacteria, and Spirochaetae represented 44.94%, 2.38%, and 0.43%, respectively. The Lactobacillus casei and control groups showed similar relative abundances of dominant phyla, with Bacteroidetes at 47.30% and 47.30%, Firmicutes at 47.89% and 46.86%, Proteobacteria at 3.01% and 3.89%, and Spirochaetae at 0.82% and 1.02%, respectively. Notably, the Lactobacillus casei group uniquely harbored Verrucomicrobia at a relative abundance of 0.00069%.

Bacteroidetes: Bacteroidetes phylum; **Firmicutes:** Firmicutes phylum; **Proteobacteria:** Proteobacteria phylum; **Spirochaetae:** Spirochaetae phylum; **Others:** Others.

Fig.2 Distribution of dominant flora at phylum level

Spirochaetae: Spirochaetae phylum; **Proteobacteria:** Proteobacteria phylum; **Tenericutes:** Tenericutes phylum; **Cyanobacteria:** Cyanobacteria phylum; **Actinobacteria:** Actinobacteria phylum; **Firmicutes:** Firmicutes phylum; **Bacteroidetes:** Bacteroidetes phylum; **Synergistetes:** Synergistetes phylum; **Bacteria_unclassified:** Unclassified bacteria; **Lentisphaerae:**

Lentisphaerae phylum; **Fibrobacteres:** Fibrobacteres phylum; **Fusobacteria:** Fusobacteria phylum; **Chlamydiae:** Chlamydiae phylum; **Verrucomicrobia:** Verrucomicrobia phylum.

Fig.3 Heatmap of microbiota at phylum level

At the genus level, microbiota composition is shown in Figure 4 [Figure 4: see original paper] and Figure 5 [Figure 5: see original paper]. A total of 206 genera were identified across the three groups, with the control, Lactobacillus casei, and chlortetracycline groups containing 198, 200, and 197 genera, respectively. Thirty genera had relative abundances greater than 1%, collectively accounting for 83.28%-86.02% of total abundance. The relative abundances of genera differed among groups. In the chlortetracycline group, *Prevotella_9* showed higher relative abundance at 27.90%, while the Lactobacillus casei and control groups exhibited similar levels at 19.87% and 20.27%, respectively. The relative abundance of *Prevotellaceae_NK3B31_group* in the chlortetracycline group (5.12%) was lower than in the Lactobacillus casei and control groups (7.31% and 7.22%, respectively). Additionally, the control group showed higher relative abundances of *Streptococcus* (5.31%) and *Veillonellaceae_uncultured* (3.94%). The chlortetracycline group exhibited higher relative abundances of *Megasphaera* (6.47%) and *Lactobacillus* (3.16%). The Lactobacillus casei group showed higher relative abundances of *Lachnospiraceae_unclassified* (5.76%) and *Anaerovibrio* (3.49%).

Prevotella_9: *Prevotella* genus 9; **Prevotellaceae_NK3B31_group:** *Prevotellaceae* NK3B31 group; **Megasphaera:** *Megasphaera* genus; **Lachnospiraceae_unclassified:** Unclassified *Lachnospiraceae*; **Streptococcus:** *Streptococcus* genus; **Anaerovibrio:** *Anaerovibrio* genus; **Prevotella_2:** *Prevotella* genus 2; **Prevotella_1:** *Prevotella* genus 1; **Prevotellaceae_uncultured:** Uncultured *Prevotellaceae*; **Veillonellaceae_uncultured:** Uncultured *Veillonellaceae*; **Lactobacillus:** *Lactobacillus* genus; **Alloprevotella:** *Alloprevotella* genus; **Ruminococcaceae_UCG-002:** *Ruminococcaceae* UCG-002; **Prevotellaceae_unclassified:** Unclassified *Prevotellaceae*; **Succinivibrio:** *Succinivibrio* genus; **Ruminococcaceae_UCG-005:** *Ruminococcaceae* UCG-005; **Selenomonas:** *Selenomonas* genus; **Prevotella_7:** *Prevotella* genus 7; **Ruminococcaceae_UCG-014:** *Ruminococcaceae* UCG-014; **Phascolarctobacterium:** *Phascolarctobacterium* genus; **Prevotellaceae_UCG-003:** *Prevotellaceae* UCG-003; **Ruminococcaceae_NK4A214_group:** *Ruminococcaceae* NK4A214 group; **Dialister:** *Dialister* genus; **Mitsuokella:** *Mitsuokella* genus; **Others:** Others. The same applies to Figure 5.

Fig.4 Distribution of dominant flora at genus level

Fig.5 Heatmap of microbiota at genus level

2.2.4 Similarity Analysis Cluster tree analysis at the phylum level revealed differences in microbiota composition and relative abundance among the three

groups, as shown in Figure 6 [Figure 6: see original paper]. Compared with the control and Lactobacillus casei groups, the chlortetracycline group exhibited higher relative abundance of Bacteroidetes but lower relative abundances of Firmicutes and Proteobacteria. The control and Lactobacillus casei groups showed more similar microbiota composition and relative abundance.

Bacteroidetes: Bacteroidetes phylum; **Firmicutes:** Firmicutes phylum; **Proteobacteria:** Proteobacteria phylum; **Others:** Others.

Fig.6 Microbiota community barplot with cluster tree at phylum level

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Discussion

3.1 Effects of Lactobacillus casei on Growth Performance of Beijing Black Pigs in Nursery Stage Probiotics can enhance intestinal barrier function, increase small intestinal villus height, expand absorption area, and promote nutrient digestion, absorption, and healthy growth. Studies by Xian Yihan et al. [1], Zhang Weiwei et al. [2], and Wang Qiheng et al. [3] demonstrated that Lactobacillus casei significantly increased villus height in the small intestine of weaned piglets, elevated the villus height-to-crypt depth ratio (V/C value), increased muscular layer thickness and proliferating cell nuclear antigen (PCNA) content, effectively maintained intestinal health, alleviated damage to intestinal mucosal structure caused by *Escherichia coli*, promoted intestinal development, and improved growth performance and feed utilization efficiency in weaned piglets. In this study, supplementation with Lactobacillus casei preparation and chlortetracycline in Beijing black pigs during the nursery stage improved piglet growth performance. The Lactobacillus casei group showed a 12.71% increase in average daily gain and a 7.34% reduction in feed-to-gain ratio compared with the control group, while the chlortetracycline group exhibited an 8.58% increase in average daily gain and a 4.52% reduction in feed-to-gain ratio. Although the Lactobacillus casei group showed improved average daily gain and feed-to-gain ratio compared with the chlortetracycline group, the differences were not significant. These results indicate that dietary supplementation with Lactobacillus casei preparation during the nursery stage can significantly increase average daily gain and reduce feed-to-gain ratio in Beijing black pigs, consistent with the findings of Xian Yihan et al. [1] in piglets (Large White × Landrace). This suggests that appropriate Lactobacillus casei supplementation in nursery diets benefits both introduced and domestically cultivated pig breeds by maintaining intestinal health and improving growth performance.

3.2 Effects of Lactobacillus casei on Intestinal Microbiota of Beijing Black Pigs in Nursery Stage The animal intestine harbors a vast and complex microbial community that maintains a symbiotic relationship with the host. The host provides habitat and nutrients for the microbiota, while the microbiota assists in host metabolism. Intestinal microbiota plays a crucial role

in nutrient digestion and host health [12–14]. Researchers often assess intestinal microbiota through analysis of fresh fecal samples. In microbiota diversity analysis, higher Coverage index values indicate greater probability of sequence detection. Larger ACE, Chao1, and Shannon index values reflect higher community richness and diversity, whereas larger Simpson index values indicate lower community diversity. In this study, high-throughput sequencing of 24 fresh fecal samples from Beijing black pigs during the nursery stage achieved sequencing coverage of 99.68%–99.72%, generating 1,439,917 high-quality sequences and 14,390 OTUs. Venn diagram comparison revealed that the control, Lactobacillus casei, and chlortetracycline groups shared 823 OTUs, with the Lactobacillus casei group possessing the most unique OTUs (29) and the chlortetracycline group the fewest (3).

Diversity analysis showed that the Lactobacillus casei group exhibited higher ACE, Chao1, and Shannon indices than the control and chlortetracycline groups, indicating greater fecal microbiota richness and diversity. The chlortetracycline group showed the highest Simpson index, suggesting the lowest microbiota diversity among the three groups. These results demonstrate that Lactobacillus casei supplementation increases intestinal microbiota richness and diversity, while chlortetracycline supplementation reduces microbiota diversity in Beijing black pigs during the nursery stage. Zhang et al. [4] investigated the effects of Lactobacillus reuteri and chlortetracycline on piglet intestinal microbiota structure and diversity, reporting increased ACE and Chao1 indices and enhanced richness in the jejunum of the Lactobacillus reuteri group compared with the chlortetracycline group, consistent with our findings. However, Li et al. [7] replaced antibiotics with Enterococcus faecalis in piglet diets and observed reduced microbiota richness and diversity compared with the control group, similar to the antibiotic group. These discrepancies suggest that different microbial strains exert varying effects on intestinal microbiota.

Numerous studies have examined the effects of dietary antibiotics and probiotics on intestinal microbiota. Kim et al. [15] reported that tylosin supplementation altered intestinal microbial populations, particularly increasing the detection frequency of *Lactobacillus*, *Sporacetigenium*, *Acetanaerobacterium*, and *Eggerthella* compared with the non-supplemented group. Looft et al. [5] investigated the effects of antibiotics (chlortetracycline, sulfamethazine, and penicillin) on swine intestinal microbiota, finding that antibiotic treatment for 14 days increased Proteobacteria relative abundance by 1%–11% compared with untreated pigs. Conversely, Poole et al. [6] observed no significant effects on intestinal microbiota structure in piglets (Duroc × Large White) fed low-dose chlortetracycline (50 g/t) for four weeks. Li et al. [7] studied Enterococcus faecalis as an antibiotic alternative in piglets (Duroc × Landrace × Large White) and found similar microbiota changes as in the antibiotic group.

This study identified Firmicutes and Bacteroidetes as dominant phyla, and *Prevotella* and *Prevotellaceae* as dominant taxa in fecal samples from Beijing black pigs during the nursery stage, consistent with findings in Duroc, Landrace, and

Large White pigs at growing-finishing stages by Pajarillo et al. [16–17], Kim et al. [18], and Zhao et al. [19]. Microbiota composition and relative abundance differed among the three groups. The Lactobacillus casei group uniquely harbored Verrucomicrobia. The chlortetracycline group showed higher relative abundances of Bacteroidetes, Actinobacteria, Fibrobacteres, Lentisphaerae, and Saccharibacteria but lower relative abundances of Firmicutes, Proteobacteria, Spirochaetae, and Fusobacteria compared with the other two groups. The control group exhibited higher relative abundance of *Streptococcus* than the Lactobacillus casei and chlortetracycline groups, suggesting that Lactobacillus casei or chlortetracycline supplementation can reduce fecal *Streptococcus* populations. Additionally, chlortetracycline supplementation did not reduce relative abundances of *Lactobacillus* and *Escherichia-Shigella*, and Lactobacillus casei supplementation did not increase *Lactobacillus* relative abundance, consistent with findings by Kim et al. [15] and Li et al. [7].

Regarding breed differences in intestinal microbiota, Yang Liu et al. [8], Xiao Wenping [9], Yang Lina [10], and Yang Weiping [11] compared intestinal (fecal) microbiota among Tibetan, Rongchang, Bama, Erhualian, and Meishan pigs, as well as Landrace, Large White, and Duroc pigs. They found substantial differences in microbial species/genera and quantities among breeds, with domestic breeds (Bama, Erhualian, and Meishan) clustering separately from imported lean-type breeds (Large White, Yorkshire, and Duroc). Domestic pig breeds exhibited greater intestinal (fecal) microbiota diversity and higher numbers of cellulolytic bacteria in the cecum than imported lean-type breeds. This study observed similar patterns in Beijing black pigs, a domestic breed characterized by good meat quality, roughage tolerance, and strong disease resistance, which showed high relative abundances of cellulolytic (or hemicellulolytic) bacteria including *Prevotella*, *Prevotellaceae*, *Ruminococcaceae*, *Lachnospiraceae*, and *Anaerovibrio* during the nursery stage.

Conclusion

Dietary supplementation with Lactobacillus casei during the nursery stage increases intestinal microbiota richness and diversity, promotes intestinal health, and consequently improves feed utilization and growth rate in Beijing black piglets.

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Note: Figure translations are in progress. See original paper for figures.