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The Effect of an Early-Life *Lactiplantibacillus plantarum* LPJZ-658 Intervention on Performance and Gut Microbiota in Suckling Piglets

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Simple Summary: The nutritional supplementation of piglets during the neonatal period has become one of the most important factors affecting the profitability of pork production. This study examines the effects of early-life supplementation with *Lactiplantibacillus plantarum* LPJZ-658 on growth and the gut microbiota in piglets. Twelve one-day-old piglets were assigned to two groups: control (C) and LPJZ-658 (LP), receiving saline or 1.0×10^{10} cfu of LPJZ-658 daily for 28 days. Fecal samples were collected at days 7 and 28 for 16S rRNA sequencing. LPJZ-658 supplementation significantly improved body weight and daily gain. Microbial diversity and composition differed between groups, with LPJZ-658 reducing harmful genera like Clostridium and increasing beneficial ones like Ruminococcus. KEGG analysis suggested improved amino acid metabolism. In conclusion, early LPJZ-658 supplementation enhances growth performance by modulating the gut microbiota in piglets.

Abstract: This study aimed to investigate the effects of early-life supplementation with *Lactiplantibacillus plantarum* LPJZ-658 on the growth performance and gut microbiota of newborn piglets. Twelve one-day-old suckling piglets were randomly divided into two groups: the control group (C) was orally administered saline and the LPJZ-658 group (LP) was gavaged with 1.0×10^{10} cfu LPJZ-658. The supplementation was carried out once daily for 28 days. Fresh fecal samples were collected at 7 and 28 days, respectively. The microbiota composition (16S rRNA gene amplicon sequencing) and its predicted functions (PICRUSt2) were analyzed. The body weight and average daily weight gain were significantly increased in the LP group. Statistically significant differences were observed in bacterial diversity and composition of the gut microbial community between the C and LP groups. The predominant bacterial phylum in the piglets changed from Firmicutes, Bacteroidetes, and Proteobacteria at day 7 to Firmicutes, Bacteroidetes, and Spirochaetota at day 28 in both the C group and LP group. We found that LPJZ-658 supplementation suppressed a significant decrease in the relative abundance of Bacteroidota in suckling piglets at 28 days. At the genus level, LPJZ-658 reduced the relative abundance of pathogenic genera such as Clostridium sensu stricto 1, and increased the relative abundance of beneficial genera such as Ruminococcus, Christensenellaceae_R-7_group, Turicibacter, and UCG-002. KEGG metabolic pathway analysis showed that LPJZ-658 may improve amino acid metabolism by regulating the intestinal microbiota of suckling piglets. In summary, the early-life LPJZ-658

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interventions significantly improve the growth performance of suckling piglets by the modulation of the gut microbiota.

Keywords: suckling piglets; *Lactiplantibacillus plantarum* LPJZ-658; growth performance; gut microbiota

1. Introduction

Pork is one of the most popular meats in the world and, due to its rapid economic returns, pig farming has become one of the most profitable business concepts. The nutritional supplementation of piglets during the neonatal period has become one of the most important factors affecting the profitability of pork production [1]. Since the gastrointestinal tract of newborns is "bacteria-free" at birth, the microbiota begins to accumulate and stabilize over time [2,3]. During this process, microorganisms from the living environment and the vagina, skin, and feces of the mother sow are dynamic colonizers [4]. The gut microbiota influences the development and maturity of the gastrointestinal tract, metabolic homeostasis, and immune defense of the newborn piglets [5–7]; in particular, it is less stable and susceptible to environmesntal stress, weaning stress, and various pathogens, leading to intestinal dysfunction [8,9]. Therefore, considering the fact that the microbial community in the gastrointestinal tract of piglets plays a key role in newborn piglet health and growth, early probiotic intervention may be possible to improve the gut microbial colonization as well as the growth, development, and disease resistance of the animals in the early postnatal period.

The use of probiotics has received widespread attention since the 1970s. Several studies have demonstrated that adding probiotics to swine diets significantly improves growth performance, increases nutrient digestibility, and improves piglet gut health [10–13]. Furthermore, the nutritional supplementation of neonatal piglets with probiotics at birth substantially alters the structure of the gut microbial community in piglets [14], decreases diarrhea incidence, and improves immune status in newborn piglets [15], which provides a potential strategy to address weaning stress. *Lactiplantibacillus plantarum*, previously known as *Lactobacillus plantarum,* a bacterium used as a probiotic and also a type of lactic acid bacterium, is found in a variety of ecological niches in the mammalian gastrointestinal tract, dairy products, and vegetables. *Lactiplantibacillus plantarum* possesses several probiotic properties including fermentation of a wide range of plant carbohydrates, high-density growth, tolerance to bile salts and low pH, and intestinal pathogen antagonistic potential [16,17]. Previous studies have demonstrated that *Lactiplantibacillus plantarum* improves the growth performance of weaned piglets, possibly by increasing the relative abundance of beneficial bacteria and increasing the expression levels of genes related to intestinal metabolism [18]. The addition of *Lactiplantibacillus plantarum* to weaned piglet diets not only alters the microbial composition of the host, but also mediates tryptophan metabolism and fat digestion and absorption in the gut, which, in turn, affects host health [19]. Mixed *Lactiplantibacillus plantarum* metabolite combinations improved growth performance and increased intestinal lactic acid bacteria populations and fecal short-chain fatty acid concentrations in weaned piglets [20]. Although *Lactiplantibacillus plantarum* strains are good growth promoters for post-weaning piglets, few studies have demonstrated the effects of *Lactiplantibacillus plantarum* as an additional additive for newborn lactating piglets.

In this study, LPJZ-658 is a strain of *Lactiplantibacillus plantarum* isolated and preserved by our group. We have confirmed the safety and probiotic properties of LPJZ-658 [21]. In our previous study, we demonstrated that LPJZ-658 can enhance the egg-laying capac-

ity of laying hens, increase the growth performance of broilers, and improve the meat quality and the regulation of microbiota in broilers [22]. Furthermore, we found that LPJZ-658 mitigated the development of non-alcoholic steatohepatitis (NASH) and alleviated metabolic dysfunction-associated steatotic liver disease (MASLD) by modulating the gut microbiota [23,24]. These studies suggest that LPJZ-658 can be used as a potential probiotic candidate for human and animal applications. Therefore, this study aimed to evaluate the effects of LPJZ-658 on growth performance and intestinal microflora structure in early-life piglets by determining body weight and fecal microbiota composition.

2. Materials and Methods

2.1. Experimental Settings

The probiotic used in this study was *Lactiplantibacillus plantarum* LPJZ-658, isolated and extracted in our laboratory. Twelve Duroc×Long White suckling piglets with similar body weight (1.45 ± 0.07 kg) were randomly divided into two groups: the Control group (C) and the LPJZ-658 treatment group (LP), and the experimental period was 28 d. The suckling piglets in both groups were breastfed ad libitum. The LP group received a daily gavage of LPJZ-658 at a dose of 1.0×10^{10} CFU, while the C group was given a daily gavage of the same dose of physiological saline. The health status of the suckling piglets was monitored daily, and all suckling piglets remained healthy during the experimental period.

2.2. Growth Performance

The average daily gain (ADG) was determined by measuring the weight of piglets individually at birth, during the second week, and at the fourth week. ADG = (final weight − initial weight)/feeding days.

2.3. Sample Collection and Microflora Analysis

Feces were collected from all suckling piglets on days 7 and 28 and stored at −80 °C. The samples were sent to Novogene Co., Ltd. (Beijing, China) for 16S rRNA. In brief, the total genomic DNA from the samples was extracted using the CTAB method. DNA concentration and purity was monitored on 1% agarose gel. The V3–V4 hypervariable region of the 16S rDNA gene was targeted by PCR reaction. PCR products were purified with a Qiagen Gel Extraction Kit (Hilden, Germany). DNA libraries were prepared using the TruSeq DNA PCR-free Sample Preparation Kit (San Diego, CA, USA), and sequencing was performed on an Illumina NovaSeq platform. Paired-end reads were merged with FLASH (V1.2.7) [25], and quality filtering was performed on the raw sequences using QIIME (V1.9.1) quality control protocols [26]. Sequence analysis was performed with Uparse software (v7.0.1001) [27], where operational taxonomic units (OTUs) were clustered. The quality of the samples was ensured through rigorous quality control processes, including removing low-quality sequences and chimeric reads during preprocessing. Alpha diversity indices were calculated with QIIME (Version 1.7.0) and displayed with R software (Version 2.15.3). Beta diversity analyses were calculated by QIIME software (Version 1.9.1). The analysis was carried out using the Novogene Magic Cloud platform (https://magic.novogene.com, accessed on 14 March 2024), which includes 16S rRNA sequencing data processing and functional prediction through PICRUSt.

2.4. Statistical Analysis

All data were analyzed by multifactor ANOVA using GraphPad Prism 10. The results are expressed as mean ± standard error of the mean (SEM), with $p < 0.05$ being significant, $p < 0.01$ being highly significant, and $p < 0.001$ being extremely significant.

3. Results

3.1. Growth Performance

The results of the suckling piglet's weight changes are shown in Figure 1A. The differences between the two groups on day 1 and day 14 were not significant. Compared with those in the C group, the suckling piglets in the LP group had significantly higher body weights on day 28. The results of the average daily weight gain of the suckling piglets are shown in Figure 1B. Compared with the C group, the suckling piglets in the LP group showed no significant difference on day 14, while they were significantly higher on day 28 and between day 14 and 28. The results indicated that supplementation with LPJZ-658 significantly improved the growth performance of the suckling piglets.

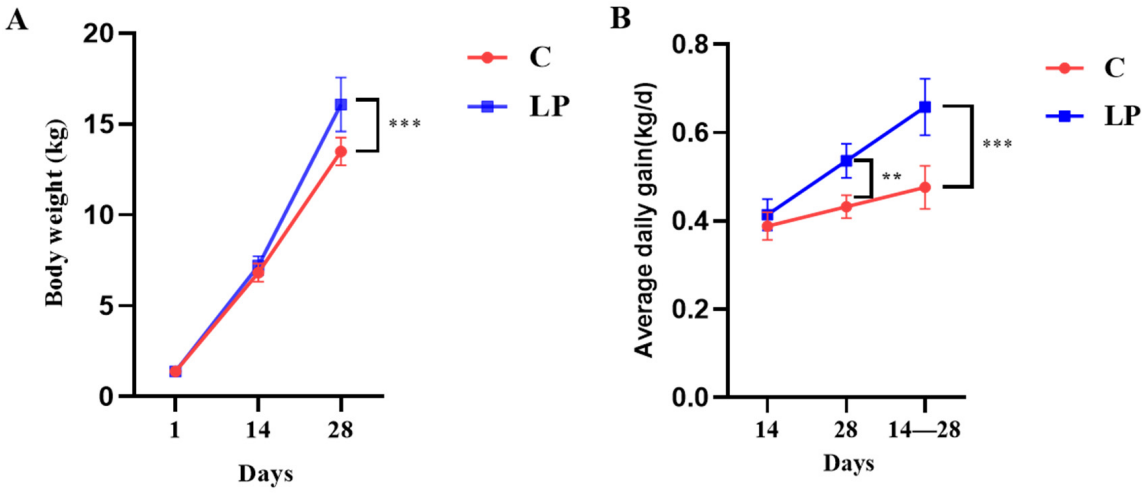
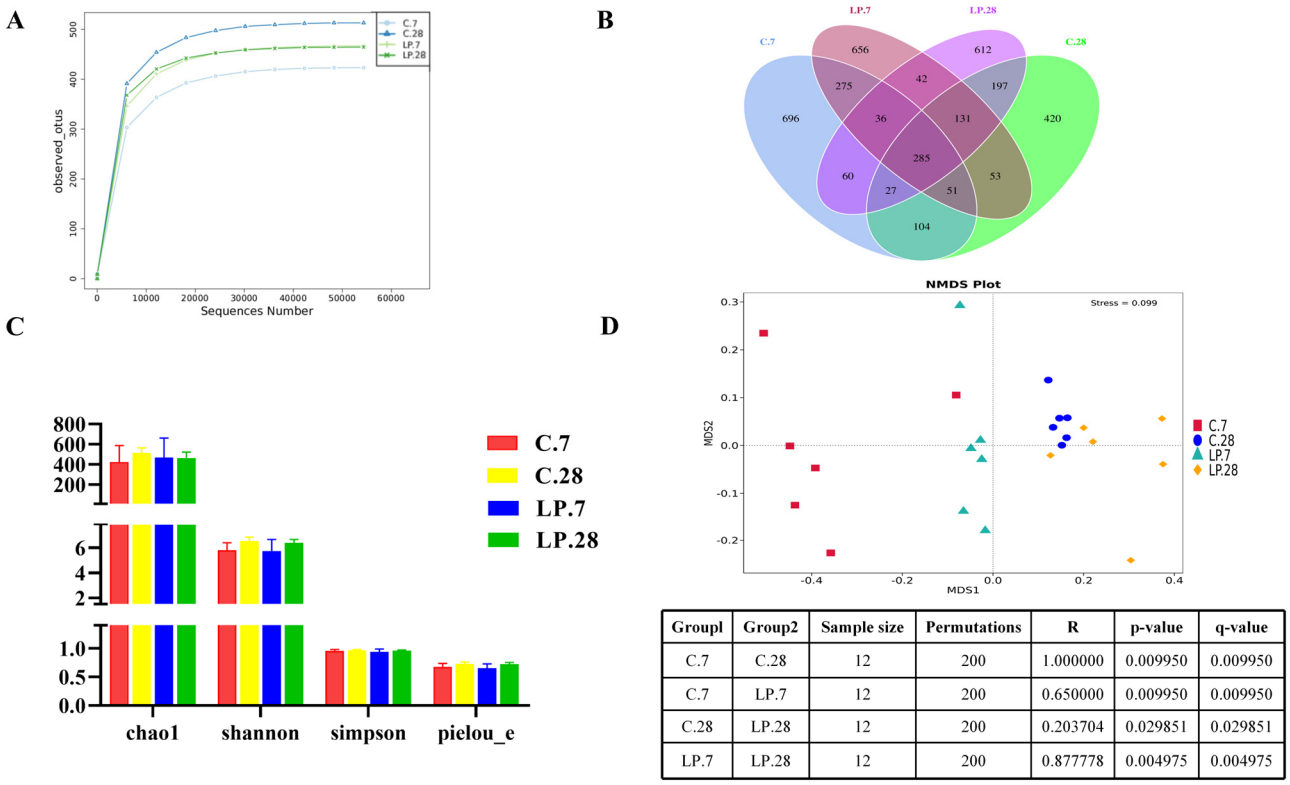


Figure 1. Effects of LPJZ-658 on growth performance of suckling piglets. (**A**) Body weight and (**B**) average daily gain of suckling piglets. Data presented indicate the mean ± SEM (** $p < 0.01$, and *** $p < 0.001$).

3.2. Summary of Bacterial Community Richness, Biodiversity, and β-Diversity

A comparison was made of the microbial communities in suckling piglet feces between the two groups via high-throughput sequencing on days 7 and 28, respectively. The dilution curve (Figure 2A) showed that the number of OTUs no longer increased significantly with the increase in sequencing volume, and the dilution curve showed a tendency to flatten, which indicated that the results of this sequencing were credible and could be analyzed in the next step. The microbiota in feces is shown in the Venn diagram (Figure 2B), and 696 OTUs, 420 OTUs, 656 OTUs, and 612 OTUs were divided among C.7, C.28, LP.7, and LP.28 groups, respectively. The number of OTUs shared by the four experimental groups was 285. An alpha diversity analysis of the two groups on days 7 and 28 showed that there was no significant difference in the Shannon, Simpson, Chao1, or pielou_e indices (Figure 2C). Beta diversity was calculated by analyzing the species composition among the sample composition and abundance information to reflect the relationship between samples, based on the Jaccard distance algorithm, to obtain the NMDS plot. Stress = 0.099 implies that the low-dimensional representation of NMDS has a good fit with the original data. ANOSIM was used to determine the significance of the differences between groups observed on the NMDS plot, and by analyzing the R-value and *p*-value, it was clear that there were significant differences both within and between groups (Figure 2D).



Groupl	Group2	Sample size	Permutations	R	p-value	q-value
C.7	C.28	12	200	1.000000	0.009950	0.009950
C.7	LP.7	12	200	0.650000	0.009950	0.009950
C.28	LP.28	12	200	0.203704	0.029851	0.029851
LP.7	LP.28	12	200	0.877778	0.004975	0.004975

Figure 2. Effects of LPJZ-658 on structure of gut microbiota. (**A**) Bacterial rarefaction curves based on observed_otus index; (**B**) Venn diagram; (**C**) alpha diversity measurements; (**D**) Non-metric Multidimensional Scaling (NMDS) based on Jaccard distance.

3.3. Characterization of the Microbiota of Suckling Piglets

The microbial composition of the intestinal contents of the suckling piglets was analyzed, and the 10 most abundant phyla are shown in Figure 3A. At day 7, Firmicutes, Bacteroidetes, and Proteobacteria were the dominant phyla in the C and LP groups, accounting for more than 90% of the total bacterial abundance. As the suckling piglets grow, the dominant phyla change to Firmicutes, Bacteroidetes, and Spirochaetota by day 28. As shown in Figure 3B, the relative abundance of Spirochaetota and Euryarchaeota was significantly higher in both the C group and LP group at day 28 compared to day 7. It is noteworthy that the relative abundance of Bacteroidota was significantly lower on day 28 compared to day 7 in the C group, but did not change significantly in the LP group.

The relative abundance of the microbiota of suckling piglets at the genus level (top 30) is shown in Figure 3C, and the microorganisms that differed at the genus level are shown in Figure 3D. Noteworthy is that, after 7 days of LPJZ-658 treatment, the relative abundance of UCG-002 and Turicibacter were significantly increased, and the relative abundance of Muribaculaceae was markedly decreased in the LP group compared with the C group. Furthermore, compared with day 7, the relative abundance of Alistipes was significantly lower, whereas Rikenellaceae_RC9_gut_group, Turicibacter, and Familly_XIII_AD3011_group were significantly higher on day 28 in the C group. In particular, the relative abundance of the Familly_XIII_AD3011_group, Clostridium_sensu_stricto_1, and Turicibacter were significantly decreased on day 28 in the LP group compared to the C group. Moreover, the relative abundance of Ruminococcus, Treponema, and Christensenellaceae_R-7_group compared to day 7 was significantly higher on day 28 in the LP group, but no significant change was observed in the C group.

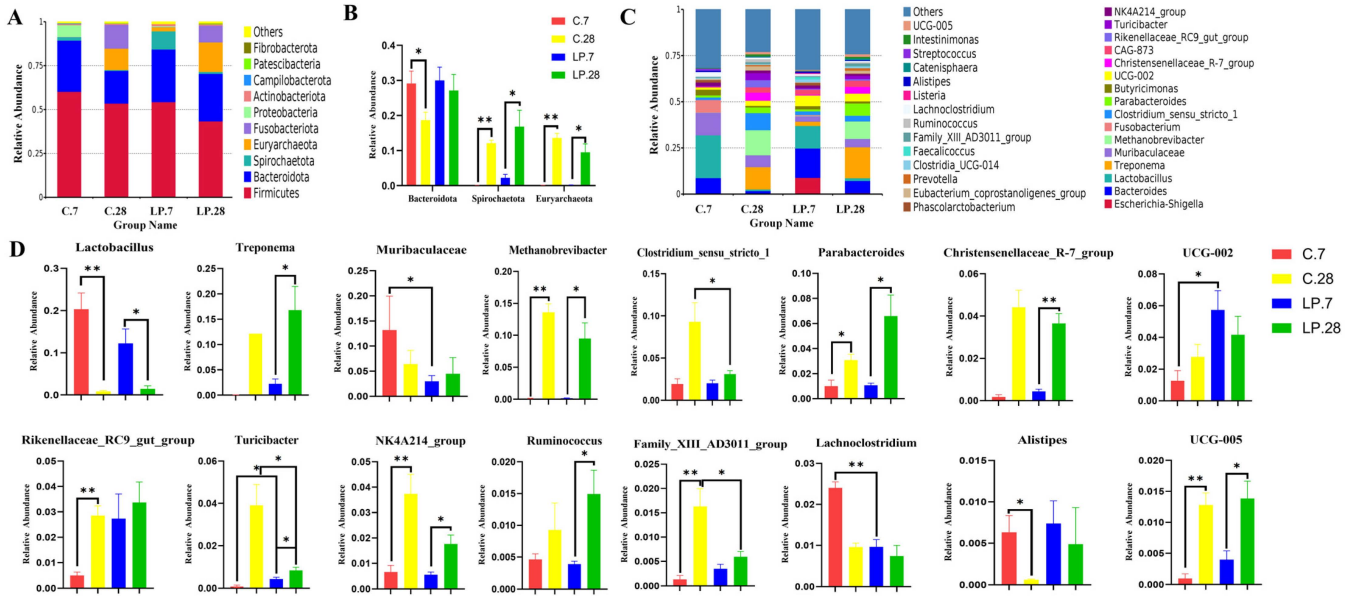


Figure 3. Effect of LPJZ-658 on gut microbiota composition in suckling piglets. (**A**) Relative abundance of the top 10 phylum-level microbiota; (**B**) relative abundance of differential flora at the phylum level; (**C**) relative abundance of the top 30 genus-level microbiota; (**D**) relative abundance of differential flora at the genus level. Data presented indicate the mean ± SEM (* $p < 0.05$ and ** $p < 0.01$).

3.4. Intestinal Bacterial Community Differences and Metabolic Pathways

LEfSe (LDA effect size) was used to analyze the species (biomarkers) that differed significantly at different levels (LDA > 4) between the two groups on days 7 and 28, respectively. The histograms of the distribution of the LDA values showed the presence of 17 different species in the C.7 group, 15 species in the C.28 group, 9 species in the LP.7 group, and 11 species in the LP.28 group (Figure 4A,B).

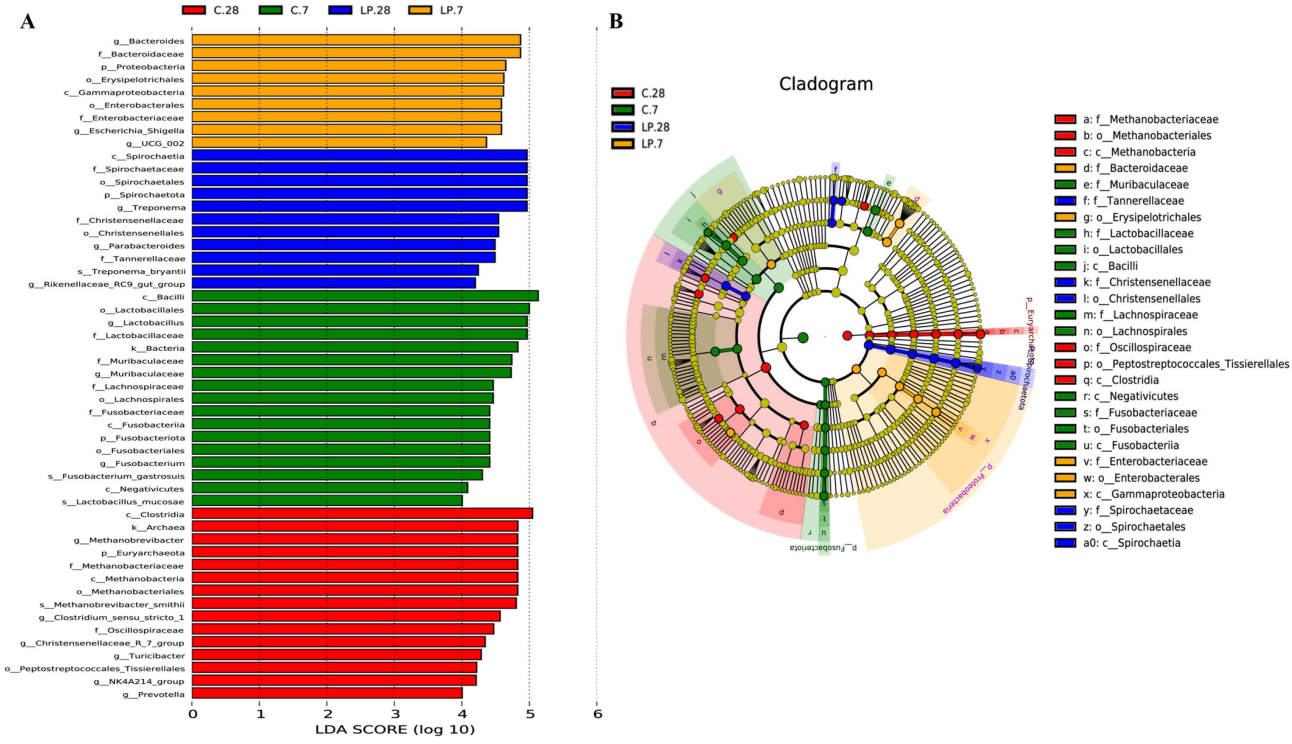


Figure 4. Effect of LPJZ-658 on bacterial community differences in suckling piglets. (**A**) Distribution histogram of LDA values (LDA score = 4); (**B**) evolutionary branching diagram.

As shown in Figure 5A, the KEGG annotation results showed that 764019964 genes were enriched in 41 metabolic pathways. Among them, membrane transport, carbohydrate metabolism, and amino acid metabolism were the three most important metabolic pathways. In addition, carbohydrate metabolism was significantly reduced in both the C group and the LP group on day 28 compared to day 7. Importantly, amino acid metabolism was significantly increased in the LP group at 28 days compared to that at 7 days, whereas no change was found in the C group (Figure 5B).

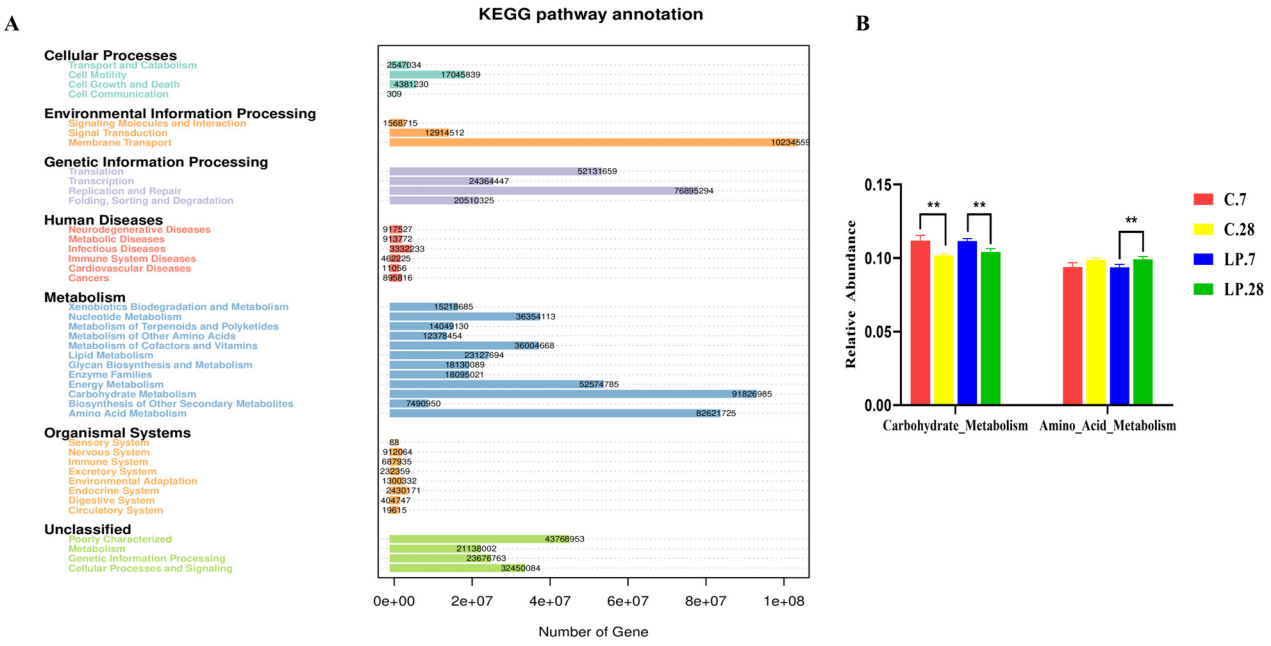


Figure 5. Effect of LPJZ-658 on metabolic pathways in suckling piglets. (**A**) PICRUSt predicted analyses; (**B**) significantly changed metabolic pathways. Data presented indicate the mean ± SEM (** $p < 0.01$).

4. Discussion

The pig is a widely farmed domestic animal characterized by fast growth and adaptability. In the contemporary era, swine farmers face unprecedented challenges as the world's population grows and the demand for pork increases. In addition, the nutritional supplementation of pigs at a young age has become one of the most important factors affecting pork production. In recent years, several studies have confirmed that probiotics can improve the growth performance of animals, such as Lactobacillus, Bacillus, and Saccharomyces [28,29]. *Lactiplantibacillus plantarum* has a long history of food applications and has proven to be valuable for development, with isolation from fermented foods being one of its main sources. In addition, *Lactiplantibacillus plantarum* has received "Qualified Presumption of Safety" (QPS) certification from the European Food Safety Authority (EFSA) and "Generally Recognized as Safe" (GRAS) certification from the U.S. Food and Drug Administration (FDA) [30]. Our group has demonstrated in previous studies that the supplementation of LPJZ-658 significantly improved the growth performance of late-laying hens and broilers [22]. This experiment was designed to evaluate the effects of LPJZ-658 on the growth performance and intestinal microbiota of suckling piglets. It provides a reference and theoretical basis for the application of LPJZ-658 in the swine breeding industry.

Several previous studies imply that the supplementation of *Lactiplantibacillus plantarum* improved the growth performance of weaning piglets [18,31]. However, few studies have confirmed the effect of *Lactiplantibacillus plantarum* on suckling piglets. In this experiment, the average daily weight gain of suckling piglets supplemented with LPJZ-658 was signifi-

cantly increased. Good health, digestion, and absorption are related to weight gain and growth performance [32]. Therefore, our result suggested that LPJZ-658 can improve the growth performance of suckling piglets.

Gut microorganisms play an important role in the digestive system of animals, and a higher diversity of gut microorganisms implies a more stable gut micro-ecosystem of the organism, which promotes growth and the absorption and utilization of nutrients [33–35]. Previous studies have reported that oral probiotics alter the structure of the intestinal microbiota and affect intestinal mucosal immunity and host metabolism [18,36]. Gut microbial colonization plays a crucial role in gut health and development and is associated with diet [37]. In particular, the gut microbiota plays a key role in intestinal development during the transition from suckling to weaning piglets. The metabolites produced by the gut microbiota of suckling and weaned piglets differ significantly, which is associated with alterations in microbiota composition [38]. The alpha diversity of the microbiota is a measure of within-sample diversity. Previous studies indicated that the alpha diversity of piglets' gut microbiota increases as they age [39,40]. It is noteworthy that those studies were concerned with changes in the alpha diversity of the microbiota of piglets before or after weaning [41,42]. In this study, none of the alpha diversity (Shannon, Simpson, chao1, and pielou_e) indices of suckling piglets supplemented with LPJZ-658 was statistically significant. This may be because we analyzed pre-weaned piglets, which are not affected by diet. However, there are significant differences in bacterial community structure (beta diversity) between groups. Thus, our results showed that LPJZ-658 had an effect on the structure of the gut microbial community in newborn piglets. Furthermore, our previous study showed that LPJZ-658 supplementation significantly improved intestinal status and modulated the intestinal microbiota in broilers [22].

By analyzing the abundance of phyla-level microbiota, we found that the abundance of Bacteroidota in the C group decreased significantly with the growth of the piglets, which was prevented by the supplementation of LPJZ-658. During the first three weeks after weaning, the piglets with the highest relative growth rate were more robust and had a higher relative abundance of Bacteroidota [43]. This phenomenon explains the elevated body weight and ADG of the suckling piglets, and can also hypothesize the increased growth performance and disease resistance of piglets after weaning.

At the genus level, LPJZ-658 supplementation for 7 days significantly increased the abundance of UCG-002 and Turicibacter and significantly decreased the abundance of Muribaculaceae. UCG-002 is a major member of the Ruminococcaceae family; in addition, a higher abundance of the Ruminococcaceae family may enable greater energy harvesting in suckling piglets [44]. Turicibacter benefits the increase in metabolic pathways of the gastrointestinal tract health of piglets and is also an important bacterium for increasing piglet weights [45,46]. Additionally, we also found that supplementing with LPJZ-658 for 28 days significantly reduced the abundance of Family_XIII-AD3011_group and Clostridium_sensu_stricto_1, which are associated with inflammation [47]. Alistipes have been reported to produce butyrate, which is involved in energy metabolism [48] and may help maintain colon health by modulating regulatory T cell differentiation [49]. In this experiment, Alistipes was significantly decreased in the control piglets at 28 days, while there was no tendency of decreasing in the LPJZ-658 group; thus, LPJZ-658 could effectively inhibit the decrease of beneficial bacteria in the intestine of piglets. Compared to the control group, the piglets fed LPJZ-658 for 28 days showed a significant reduction in the relative abundance of certain pathogenic bacteria, such as Clostridium_sensu_stricto_1, which is generally considered to be pathogenic and is associated with inflammation and damage to the colonic mucosa [50,51]. After 28 days of treatment with LPJZ-658, the relative abundance of the Family_XIII-AD3011_group decreased significantly. Family XIII-AD3011 is

believed to be associated with increased disease resistance in Tibetan pigs, but the exact mechanism of resistance still needs more research [52].

We utilized PICRUSt inferred macroeconomics reflecting the metabolic activity of the microbiota used to study functional differences in the piglet microbiota to determine probiotic-induced metabolic changes. There was no significant difference in membrane transport between the C group and the LP group. In contrast, amino acid metabolism was more abundant in the LP group compared to the control group. Previous studies have shown that inadequate amino acid supply may lead to reduced growth performance in pigs [53]. Amino acid levels have a strong influence on the intestinal development of piglets, and amino acid deficiencies can lead to significant changes in intestinal structure and function [54]. Furthermore, the weaning of piglets causes stress and the requirements for amino acids are significantly higher [55,56]. Improved amino acid metabolism during the weaning phase further improves gut barrier function and immunity in piglets [57]. Therefore, LPJZ-658 gut-microbiota-targeted approaches can be potentially used to improve the weaning transition of piglets [58]. These results indicate that LPJZ-658 can regulate the intestinal microbiota of suckling piglets and improve their growth performance by increasing amino acid metabolism.

5. Conclusions

In conclusion, the present study investigated the growth performance and gut microbiota responses of suckling piglets fed with LPJZ-658. Our study showed that supplementation with LPJZ-658 significantly improved piglet growth performance and the microbial composition of suckling piglets. These alterations may help us to understand the beneficial impacts of LPJZ-658 as an ideal additive for suckling piglets.

Author Contributions: Conceptualization: Z.C.; methodology: Z.C., Z.L., and C.C.; software: Z.C. and Z.L.; formal analysis: Y.F.; investigation: H.W.; resources: C.Z.; data analysis: Z.C., C.C., and C.Z.; writing—original draft preparation: Z.C.; writing—review and editing: J.Z. and L.L.; supervision: L.L.; project administration: L.L. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study are available in the article.

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