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Author	Elick Kinara[a,1], Joseph Moturi[a,1], Abdolreza Hosseindoust[a], Jun Young Mun[a], Habeeb Tajudeen, Sang Hun Ha, Se Rin Park [a], Su Hyub Lee[b], & Jin Soo Kim[a*]
Affiliation	[1]These Authors Contributed Equally. [a]Department of Animal Industry Convergence, Kangwon National University, Chuncheon, 24341, Republic of Korea. [b]Department of Swine Science, Korean Nationational University of Agriculture and Fisheries, Jeonju 54874.
ORCID (for more information, please visit https://orcid.org)	Elick kinara (0009-0003-1848-0399) Joseph Moturi (0000-0002-0626-0255) JunYoung Mun (0000-0002-3075-7157) Abdolreza Hosseindoust (0000-0001-9191-0613) Habeeb Tajudeen (0000-0002-5623-3175) Sang Hun Ha (0000-0003-3779-1144) Se Rin Park (0009-0007-1413-5853) Su Hyub Lee (0000-0001-8996-3740) JinSoo Kim (0000-0002-9518-7917)
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4 **CORRESPONDING AUTHOR CONTACT INFORMATION**

For the corresponding author (responsible for correspondence, proofreading, and reprints)	**Fill in information in each box below**
First name, middle initial, last name	Jin Soo Kim
Email address	kjs896@kangwon.ac.kr
Secondary Email address	
Address	Department of Animal Industry Convergence, Kangwon National University, Chuncheon, 24341, Republic of Korea
Cell phone number	01025665961
Office phone number	+82-33-250-8616
Fax number	+82-33-244-4946

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Abstract

Suckling piglets face the hurdle of pathogenic inversion before the full development of their gastrointestinal tract. The provision of *Lactobacillus (L) salivarius* guarantees resilient gut health, controls pathogens, increases microbiota, and fortifies intestinal structure. We evaluated the effect of *L. salivarius* LS144 probiotic given to suckling piglets through the post-weaning stage on the gut microbiota, intestinal morphology, and growth performance. 120 three-day-old crossbred (Landrace × Yorkshire × Duroc) piglets were assigned to four dietary treatments on the basis of initial body weight. The NN group was not supplemented with the probiotic in both the suckling and post-weaning phases, the NP group was supplemented with the probiotic during the post-weaning phase, the PN group was supplemented with the probiotic only during the suckling phase, and the PP group was supplemented with the probiotic during both the suckling and post-weaning periods. Results revealed that the average daily gain was higher (p<0.05) in the PN and PP groups than in the NN and NP groups in phase 1. In the overall study (1~51 d), average daily gain was greater (p<0.05) in the PP treatment compared to all other groups. The average daily feed intake was higher (p<0.05) in the PP group (22~ 51 d) than all groups. The villus height was greater in the duodenum (p<0.05), jejunum (p<0.05), and ileum (p<0.05) in the PP compared with the NN. The pH of the intestinal digesta was higher (p<0.05) in the NN treatment than in the PN and PP treatments in the duodenum. The population of total *L.* bacteria was greater in both the PN and PP groups compared to the NN treatment in the duodenum (p<0.01), jejunum (p<0.05), ileum (p<0.01), and cecum (p<0.01). There was no significant difference in the population of total anaerobes, Clostridium, and coliform bacteria in the duodenum, jejunum, ileum, and cecum among the groups. Based on these findings, dietary supplementation with *L. salivarius* in suckling piglets continued to post-weaning could establish appropriate intestinal microbiota, improve feed intake, and increase the villus height, which translates to improved growth performance during this critical period in piglet's life.

Keywords: villus, crypt depth, *Lactobacillus*, probiotic, weanlings, stress.

1. Introduction

In intensive pig rearing, suckling and weaning stages in piglets are the most critical phases that determine their performance later in life [1,2]. A healthy gut is home to thousands of different species of microorganisms [3,4] coexisting with the pig in a symbiotic relationship making the pigs gut normal and accurate execution [5,6]. During lactation, piglets experience perturbations in the gut microbial community, which may be due to environmental ingestion of pathogenic bacteria, and stresses impacted by practices such as teeth clipping, castration, iron injection, and detailing. These enteric bacterial imbalances lead to poor digestion, absorption of nutrients, and enteric disorders resulting in low growth performance [7,8]

Weaning, on the other hand, is a very stressful moment in the life of a piglet due to abrupt separation from the mother, mixing with other litter, change of food from mother milk to solid feeds, and fighting to establish a dominance hierarchy [9]. Weaning stress negatively impact gut morphology and physiology, leading to the shortening of villi accompanied by increased width of villi and deepening of crypts, together with the disrupted activity of digestive enzymes such as maltase and lactase and increased permeability of the epithelial barrier [10,11]. A balanced gastrointestinal environment with appropriately established populations of commensal microflora, including *bifidobacteria* and lactic acid bacteria especially *lactobacilli,* is vital in protecting the animal from gut infections [12] and improving gut histomorphology and physiology [13]. To mitigate these challenges, over the years pig rearing has incorporated the prophylactic use of antibiotics to overcome diarrhea in suckling and weaned piglets and equally to promote growth [14]. However, the continued use of antibiotic growth promoters (AGPs) has given rise to the emergence of resistant strains of pathogenic bacteria, which is a health issue in both humans and animals. This led to the ban on the sub-therapeutic use of AGPs as feed additives in the European Union in 2006 accompanied by Korea. Consequently, there has been increased interest in the search for alternatives to the AGPs, thus the rise in the use of probiotics.

Probiotics are nutritional supplements comprised of live microorganisms which upon ingestion in adequate amounts, colonize, and modify microbiota in the gastrointestinal tract (GIT) provoking health benefits above basic nutrition [15,16]. Among the microorganisms extensively used, the *Lactobacillus (L)* genus has been found to inhibit the activity of pathogenic microorganisms, participate in food fermentation in the gut, improve mineral and nutrient absorption, synthesize vitamins, and stimulate immunological responses [17]. The *L. salivarius* is a gram-positive bacterium and a member of lactic acid-forming bacteria which has exhibited the potential to participate in glucose

fermentation, inhibit the activity of pathogenic bacteria and modulate gut morphology and physiology [18,19]. In the previous study by Moturi et al. [13], *L. salivarius* supplementation in suckling and weanling piglets has was shown to modulate the intestinal microbiota, improve gut morphology, and enhance growth performance. Similarly, Sayan et al. [20] demonstrated that oral administration of *L. salivarius* to suckling piglets during the first 10 days of life could significantly decrease the pH of the duodenum, indicating improved gut health. Nevertheless, it has been found to positively influence the immune response, intestinal morphology, and gut microbiota composition in suckling piglets in a study conducted by Wang et al. [21]. Thus, the objective of this study was to assess the potential of oral supplementation of *L. salivarius* LS144 in suckling and weaning piglets in modulating gastrointestinal microbiota, gut morphology, and growth performance.

2. **Materials and methods**

2.1. Animal care

The research was conducted with proper ethical standards and according to the institutional protocol approved by the Kangwon National University Animal Care and use committee (KW-210503-6), in the Republic of Korea.

2.2. Animals, experimental designs, and diets

The experiment was conducted at a commercial pig farm in Gangneung in the Republic of Korea. Standard farm management and husbandry practices were routinely carried out by the farm staff. In this study, a total of 120 three-day-old, crossbred piglets (Duroc × Yorkshire × Landrace) with initial BW 1.50±0.05 kg of mixed sex were randomly allotted to four treatments. Each dietary treatment consisted of 3 replicates of 10 piglets each (n=10, from 12 sows). Cross-fostering was not done throughout the experimental period. During the suckling phase each experiment litter was housed individually with the dam in individual stainless-steel pens with reinforced plastic floors, the ambient temperature was kept at 28^{o}C. Piglets had *ad libitum* access to sow milk and water through self-feeder and nipple drinker. The treatments comprise of; NN (no supplementation in both suckling and post-weaning phases), NP (unsupplemented in the suckling phase but supplemented with *L. salivarius* LS144 probiotic in post-weaning phase), PN (supplemented with *L. salivarius* LS144 probiotic during suckling phase but not in the post-weaning phase), and PP (supplemented with *L. salivarius* LS144 probiotic during both the suckling and post-weaning phases). The screened *L. salivarius* LS144 used was acquired from Kangwon National University microbiology laboratory and stored at 4^{o}C

in individualized centrifugal tubes. At weaning the piglets were transferred to a weaning pen measuring 3 m × 4 m with reinforced slatted plastic floors, equipped with 2 feed troughs and a nipple waterer. The sows were provided with corn and soybean meal diet while they were nursing their piglets. The piglets had two different diets: a milk formula that was similar to sow milk during suckling phase, and weaner pellets during post-weaning phase. An experimental basal diet was formulated to provide all the nutrients as per the National Research Council [22] requirement for weanling pigs (Table 1).

2.2. Isolation and identification of *Lactobacillus salivarius*

The *L. salivarius* strains were obtained from fecal specimens of rapidly growing piglets during the weaning phase, the isolated *Lactobacilli* were subjected to testing against *Salmonella* spp. a prevalent pathogenic bacterium responsible for inducing *Lactobacilli* intestinal disorders in swine to evaluate their anti-pathogenic attributes. After the screening procedure, the identification of the *L. salivarius* strain was accomplished through the utilization of species-specific primer sets targeting relevant genes, alongside 16S rRNA sequencing. The specific strains, *L. salivarius* 144 (accession no. PRJNA669977). The Genomic DNA was extracted from 300 mg of each fecal sample using the NucleoSpin Soil kit (Macherey–Nagel, Duren, Germany) following the manufacturer's recommendations. The 16S ribosomal (rRNA) V4 region was then amplified from the extracted genomic DNA using Takara Ex-Taq DNA polymerase (Takara Bio, Shiga, Japan) and specific primer sets (forward: 5′-GGACTACHVGGGTWTCTAAT-3′, reverse: 5′-GTGCCAGCMGCCGCGGTAA-3′). The amplification process involved one cycle at 94°C for 180 seconds, followed by 30 cycles at 94°C for 45 seconds, 55°C for 60 seconds, and 72°C for 90 seconds, with a final extension cycle at 72°C for 10 minutes. Amplicons were separated and purified using agarose gel electrophoresis and the QIAquick gel extraction kit (Qiagen, Valencia, CA, USA), respectively. Subsequently, the DNA library was sequenced on an Illumina MiSeq platform, generating paired-end sequence reads. These reads were quality-trimmed and de-multiplexed using in-house Perl scripts. Filtered reads were then analyzed for microbial community diversity and richness indices using Quantitative Insights Into Microbial Ecology (QIIME 1.9.1). Each read was assigned as an Operating Taxonomic Unit (OTU) when it exhibited 97% sequencing identity with the Greengenes 13_8 database. Finally, OTUs were normalized to 40,000 reads per sample through single rarefaction, and Principal Coordinate Analysis (PCoA) was performed. The isolated *L.* strain was grown at 30°C under anaerobic conditions in a custom medium containing protease and yeast extract.

115 2.3. Animal feeding and management

116 During the suckling phase, Fresh milk formula was provided, two times daily (0800 h. and 1400 h.) to all groups. The
117 diets were reconstituted at 500 g dry milk formula diet in 1L of warm water at 40ºC. 10ml of the probiotic cultures *L.*
118 *salivarius* LS144 was added to the PN, and PP treatments. The viable probiotic cultures were stored at 4°C as
119 confirmed by the manufacturer, containers of the lyophilized probiotic. The post-weaning period (22~51 d) involved
120 feeding the piglets with the basal diet of weaner pellets mixed with 2 g/kg of *L. salivarius* probiotics for NP and PP
121 treatment groups. Before the beginning of the experiment (day 1) and at the end of the experiment in phase 1 (day 21),
122 each piglet weight was recorded for calculation of weight gain and average daily gain (ADG). At the end of the study
123 on day 51, two piglets from each treatment were euthanized by approved anesthetic, and exsanguination and tissue
124 samples from duodenum, jejunum, and ileum were harvested for analysis.

125 2.4. Sample collection and analyses.

126 2.4.1. Growth performance

127 All the experimental animals were weighed individually on day one of the experiment, at weaning (d 21), end of the
128 second week post-weaning (d 36), and at the end of the experiment (d 51). Feed consumption was also determined at
129 the end of the second and fourth weeks after weaning. This was used to calculate the ADG, FCR, and average daily
130 feed intake (ADFI).

131 2.4.2. Intestinal histomorphology

132 Mucosal and histological tissue samples were collected from; the duodenum, jejunum, and ileum then frozen in liquid
133 nitrogen and stored at -80°C for intestinal histomorphology analysis. The duodenal, jejunal and ileal samples were cut
134 approximately 5cm, fixed in neutral buffered 10% formalin for 24 h, then transferred into a 70% ethanol solution and
135 embedded in wax, sectioned, and stained with hematoxylin and eosin. Finally, the slices were each mounted on slides
136 for analysis as previously described by Tsirtsikos et al. [23]. To measure the intestinal morphology, five well-defined
137 villi and crypts from each section were identified. The villus height (VH), measured from the villi tip up to the villi-
138 crypt junction was recorded along with the crypt depth (CD), measured from the villi base as the lowest point of the
139 crypt. Intestinal sample slides were read using Olympus Vanox-S Microscope (Olympus Corporation, Lake Success,
140 NY) and then analyzed using SPOT basic imaging software (Diagnostic Instruments, Sterling Heights, MI)

141 2.4.3. Intestinal digesta bacterial population and pH determination

142 Digesta samples were obtained from the stomach, duodenum, jejunum, ileum, and cecum by puncturing, then collected

in sterile plastic bottles for pH and polymerase chain reaction microbial population analysis. These samples were immediately placed on ice and taken to the laboratory for analysis. One gram of samples (intestinal digesta) was transferred into 9 mL of sterile peptone PBS (0.1%) and mixed thoroughly. 1 mL of digesta suspension was transferred into a second tube containing 9 mL sterile PBS. A serial of 10-fold dilution was made from 10-3 to 10-8. Thereafter, one ml of each solution was duplicated and transferred to a sterile agar plate then topped up with a freshly made sterile agar and spread plate. The culture media for total bacteria, clostridia, lactobacilli, and coliform counts, including culture conditions were PCA incubated for 48 hours at 37°C; violet red bile agar (VRB, Merck co., Ltd, Germany) incubated for 24 hours at 37°C; MRS agar incubated in carbon dioxide incubator for 72 hours at 37°C, respectively. Dilution plates with colony numbers ranging from 15 to 150 colonies were recorded (Bacteriological Analytical Manual, 2001) [24]. The average of duplicate plates was calculated and expressed as log CFU/mL. The proximate pH values of the; duodenum, jejunum, and ileum digesta were recorded by a hand-held (PB-11, Sartorius, UK) pH meter.

2.5. Statistical analyses

All the results were expressed as mean ± standard error of the mean, statistical analyses were done using unpaired t-test for; growth performance, intestinal pH, intestinal digesta and fecal microbial abundance, and total blood cell count. The data were analyzed as a randomized complete block design. Litter were blocked by initial body weight with the pen as the experimental unit. Differences of (p<0.05), and (p<0.01) were considered statistically significant using the mixed procedure of SAS, 2002 [25].

3. Results

3.1. Growth performance

The growth performance of piglets during the various phases of the study is presented in Table 2. The ADG was higher (p<0.01) in the PN and PP groups compared to that in NN and NP in phase 1, whereas in phase 2, ADG was greater (p<0.05) in the PP than in the NN and NP groups; however, it was not different from that in the PN group. Moreover, in phase 3, ADG was the highest (p<0.05) in the PP group and during the overall 1 (1~51 d) of the study (p<0.01) compared to the rest of the treatments. During post-weaning (22~51 d), the ADG was greater (p<0.05) in the PP group than in the NN and NP groups, although it was not different from the PN group in phase 2. The ADFI was higher (p<0.05) in both phases 2 and 3 of the PP group than in the NN and NP groups; however, it did not differ significantly from the PN group. In the overall postweaning period, the ADFI was greater (p<0.01) in the PP group than in the other groups. The feed conversion ratio did not differ among treatments throughout the experimental period.

3.2. Intestinal morphology

The VH was higher in the duodenum (p<0.01), jejunum (p<0.05), and ileum (p<0.05) in the PP group than that in the NN group, although it was not different from that in the PN group. The CD and VH:CD ratios in the duodenum, jejunum, and ileum did not differ among treatments (Table 3).

3.3. Intestinal digesta pH

The pH of the duodenal digesta was lower (p<0.05) in the PN and PP groups than in the NN group (Table 4). There was no difference in the pH of the intestinal digesta between the jejunum and ileum.

3.4. Intestinal microbial population (Duodenum, Jejunum, Ileum and Cecum)

The populations of total anaerobic bacteria, *Clostridium*, and coliforms in the duodenum, jejunum, ileum, and caecum sections of the intestinal gut were not significantly different among the groups. However, the total population of *L. salvarius* was significantly higher (p<0.01) in the duodenum, jejunum, ileum, and caecum of the PN and PP treatment groups than in the NN group (Table 5).

4. Discussion

Piglets are exposed to stressors during and post weaning period which hinder their growth [1]. This stress can be relieved through the supplementation of *L. salivarius* LS144 during and after weaning to promote the growth of piglets [15,26]. The *L. salivarius* LS144 is a probiotic gram-positive bacterium belonging to the genus *Lactobacillus*, that can confer health benefits to the host when consumed in adequate amounts [18,2]. Herein and previous study` reports, it was shown to have beneficial effects on the growth performance and intestinal health of piglets [3,27-29].

In this study, the administration of *L. salivarius* LS144 to piglets, both at birth and after weaning, increased ADG and ADFI throughout the experimental period in the PP and NP groups at different phases. The possible mechanisms underlying these effects include adapting to the piglet GIT, enhancing colonization and adhesion to the intestinal epithelium [6], exerting antimicrobial activity against enteric pathogens [4], producing enzymes and organic acids that facilitate digestion and absorption of nutrients and immunoglobulins in colostrum milk, enabling better viability and minor losses of piglets particularly in the initial days of life [12,30], stimulating intestinal development and immunity, and intestinal disorders [7,31]. This may help to form a protective barrier against pathogenic bacteria and modulate the piglet immune system [5,30]. The *L. salivarius* LS144 may also enhance the digestibility and utilization of nutrients from solid feed, as it can produce organic acids including lactic acid and acetic acid. This lowers the pH of the GIT and activating digestive enzymes that can break down the feed components into smaller and more bioavailable

molecules, resulting in increased ADG [32,33]. The improved ADG of neonatal piglets receiving *L. salivarius* LS144 was consistent with a meta-analysis by Zhu et al. [34], who reported improved ADG upon *Lactobacillus* spp. supplementation in piglets. Similarly, Lessard [35] and Kyriak et al. [36] reported improved growth rates, immune responses, and feed intake in piglets supplemented with *Lactobacillus*. The increased growth rate in *L. salivarius* LS144 recipient piglets may have been due to the increased VH:CD in the GIT, particularly in the ileum, which is a marker for improved absorption area accompanied by a thinner lamina propria in this section of the intestinal gut where nutrient absorption takes place [11]. Similarly, the increased number of *L*. bacterium LS144 could have a pronounced beneficial effect on digestive enzyme activities, thereby improving digestion. Fuller et al. [15], Lidbeck et al. [37], and Roselli et al. [38] suggested that improving nutrient utilization and high concentrations of organic acids in the gut may also impart antibacterial effects against enteropathogenic bacteria. This study established that early supplementation in neonatal piglets was critical for the establishment of a stable gut microbiota dominated by commensal bacteria, especially *L*. bacteria. Furthermore, continued supplementation during the postweaning period maintained this balance and exerted an additive effect.

Weaning stress combined with anorexia results in tremendous changes in the intestinal architecture, especially in the VH and CD [9]. A previous study by Kelly et al. [39] and Pluske et al. [40] reported villus atrophy and crypt hyperplasia in piglets. In our study, dietary supplementation with *L. salivarius* LS144 significantly increased the VH in all four segments of the intestinal tract (duodenum, jejunum, ileum, and caecum). However, no significant differences were observed in VH:CD between the supplemented groups (PN, PP) and the unsupplemented group. This could be because the probiotics did not colonize the intestinal mucosa or did not affect the intestinal epithelial cell proliferation and differentiation owing to their dependence on the strain, dose, duration, and timing of administration [8,41]. The improvement in VH by the probiotic is due to its ability to produce short-chain fatty acids such as lactic acid and acetic acid, which stimulate the proliferation of epithelial cells, enterocytes, and colonocytes, as established by previous research by Zhang et al. [42]. Similar results were also obtained by Liu et al. [43] in weaned piglets supplemented with *Lactobacillus* fermentum. Improved VH translates to higher nutrient absorption in the intestine, leading to improved growth performance.

Intestinal digesta pH is an indicator of microbial activity and stability [12,44,45]. However, an appropriate pH is rarely maintained during weaning. This could be due to the changes that occur constraining the gastric gland to produce

insufficient HCl, leading to a high gastric pH [46]. Low pH in the stomach inhibits the proliferation and passage of pathogens through the stomach to the intestines. Furthermore, acidic pH facilitates pepsin activity, thereby enhancing protein digestion. The results of our study showed that *L. salivarius* LS144 supplementation lowered the pH of the duodenum, potentially killing pathogens transiting through the stomach. Lactic acid bacterial probiotics can ferment glucose via the glycolysis pathway, producing organic acids that lower the pH in the gut [47].

Probiotics are included in animal diets to provide health benefits beyond basic nutrition [13,16,48]. Living organisms that constitute probiotics should possess several desirable attributes including the ability to withstand acidic pH in the stomach and move on to colonize the intestines [1] and the ability to adhere to the intestinal walls, and competitively exclude pathogenic bacteria from the intestines [49,50]. This study reveals the positive attributes of *L. salivarius* LS144 as a potential candidate for use in nursery piglets. When given early at birth, it was able to colonize piglet gut and boost the population of commensal bacteria, as depicted in this study by the increased population of *Lactobacillus*. Consistent with our findings, Moturi et al. [13] observed higher *Lactobacillus* population in suckling piglets supplemented with *L. salivarius* probiotic. In our study, throughout the four segments of the intestine, the population of *Lactobacillus* was significantly higher in the *L. salivarius* LS144-treated groups. This effect was replicated in both the PN treatment group, where supplementation was discontinued at weaning, and the PP group, where supplementation continued post-weaning, unlike the NN and NP groups, which did not receive the probiotic early in the suckling stage. This points to the essence of probiotic supplementation in early life, as it influences colonization with symbiotic bacteria at the expense of pathogens.

5. Conclusion.

In conclusion, the timing of the initial introduction of *Lactobacillus* is crucial because it influences the development and function of the GIT and immune system. The results demonstrated that probiotic supplementation produced lactic acid, lowered the intestinal pH, inhibited pathogenic bacteria, and modulated the immune system. All these led to positive effects on the growth performance and intestinal health of weaned piglets, especially when *Lactobacillus* was administered both before and after weaning. We suggest that probiotic supplementation can be used as an alternative to antibiotics to improve piglet productivity.

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6. References

1. Choudhury R, Middelkoop A, Boekhorst J, Gerrits WJ, Kemp B, et al. Early life feeding accelerates gut microbiome maturation and suppresses acute post-weaning stress in piglets. Environ Microbiol. 2021; 23:7201-13. https://doi.org/10.1111/1462-2920.15791

2. Jensen BB. The impact of feed additives on the microbial ecology of the gut in young pigs. J Anim Feed Sci. 1998; 7:45-64. https://doi.org/10.22358/jafs/69955/1998

3. Wang M, Radlowski EC, Monaco MH, Fahey Jr GC, Gaskins HR, Donovan SM. Mode of delivery and early nutrition modulate microbial colonization and fermentation products in neonatal piglets. J Nutr. 2013; 143:795-03. https://.doi.org/10.1096/fasebj.23.1

4. Leser TD, Amenuvor JZ, Jensen TK, Lindecrona RH, Boye M, Møller K. Culture-independent analysis of gut bacteria: the pig gastrointestinal tract microbiota revisited. App Environ Microbio. 2002; 68:673-90. https://doi.org/10.1128/AEM.68.2.673-690.2002

5. Willing BP, Malik G, Van Kessel AG. Nutrition and gut health in swine. Sust Swine Nutr. 2013; 4:197-213. https://doi:10.1002/9781118491454

6. Lee S, Hosseindoust A, Goel A, Choi Y, Kwon IK, Chae B. Effects of dietary supplementation of bacteriophage with or without zinc oxide on the performance and gut development of weanling pigs. Ital J Anim Sci. 2016; 2;15:412-8. https://doi.org/10.1080/1828051X.2016.1188676

7. Armstrong DG. Gut-active growth promoters. Control and Manipulation of Animal Growth: Proceedings of Prev Easter Sch Agric Sci. 2016: 22;21.

8. Choi Y, Hosseindoust A, Goel A, Lee S, Jha PK, Kwon IK, Chae BJ. Effects of Ecklonia cava as fucoidan-rich algae on growth performance, nutrient digestibility, intestinal morphology and caecal microflora in weanling pigs. Asian-Australasian J Anim Sci. 2017; 30:64. https://doi: 10.5713/ajas.16.0102

9. Hötzel MJ, de Souza GP, Dalla Costa OA, Machado Filho LC. Disentangling the effects of weaning stressors on piglets' behaviour and feed intake: Changing the housing and social environment. App Anim Behav Sci. 2011; 135:44-50. https:// doi.org/10.1016/j.applanim.2011.09.003

10. Spreeuwenberg MA, Verdonk JM, Gaskins HR, Verstegen MW. Small intestine epithelial barrier function is compromised in pigs with low feed intake at weaning. J Nutr. 2001; 131:1520-7

11. Boudry G, Péron V, Le Huerou-Luron I, Lalles JP, Seve B. Weaning induces both transient and long-lasting modifications of absorptive, secretory, and barrier properties of piglet intestine. J Nutr. 2004; 134:2256-62

12. Berg RD. The indigenous gastrointestinal microflora. Tre Microbiol. 1996; 4:430-5. https://doi: 10.1016/0966-842x(96)10057-3

13. Moturi J, Kim KY, Hosseindoust A, Lee JH, Xuan B, Park J, Kim EB, Kim JS, Chae BJ. Effects of *Lactobacillus salivarius* isolated from feces of fast-growing pigs on intestinal microbiota and morphology of suckling piglets. Scie Rpt. 2021; 11:6757. https://doi: 10.1038/s41598-021-85630-7

14. Hosseindoust AR, Lee SH, Kim JS, Choi YH, Noh HS, et al. Dietary bacteriophages as an alternative for zinc oxide or organic acids to control diarrhoea and improve the performance of weanling piglets. Vet Med. 2017; 62:53-61. https://doi.org/10.17221/7/2016-VETMED

15. Fuller R. Probiotic in man and animals. J Appl Bacteriol. 1989; 66:131-9. https://doi:10.1079/BJN2002628

16. Lata J, Juránková J, Doubek J, Příbramská V, Frič P, Dítě P, Kolář M, Scheer P, Kosáková D. Labelling and content evaluation of commercial veterinary probiotics. Acta Vet Brno. 2006; 75:139-44. https://doi.org/10.2754/avb200675010139

17. Jacela YJ, De Rouchey JM, Tokach MD, Goodband RD, Nelssen JM, et al. Feed aditives for swine:fact sheets-prebiotics. Swine H Prod. 2010; 18:132.

18. Vasala A, Panula J, Neubauer P. Efficient lactic acid production from high salt containing dairy by-products by *Lactobacillus salivarius* ssp. salicinius with pre-treatment by proteolytic microorganisms. J Biotechnol. 2005; 117:421-31. https://doi.org/10.1016/j.jbiotec.2005.02.010

19. Jeong YD, Ko HS, Hosseindoust A, Choi YH, Chae BJ, Yu DJ, et al. Lactobacillus-based fermentation product and lactose level in the feed for weanling pigs: effects on intestinal morphology, microbiota, gas emission, and targeted intestinal coliforms. Liv Sci. 2019;227:90-6. https://doi.org/10.1016/j.livsci.2019.06.018

20. Sayan H, Assavacheep P, Angkanaporn K, Assavacheep A. Effect of Lactobacillus salivarius on growth performance, diarrhea incidence, fecal bacterial population and intestinal morphology of suckling pigs challenged with F4+ enterotoxigenic Escherichia coli. Asian-Aust J Anim Sci. 2018;31:1308. https;//doi: 10.5713/ajas.17.0746

21. Wang J, Zhang W, Wang S, Wang Y, Chu X, and Ji H. Lactobacillus plantarum exhibits antioxidant and cytoprotective activities in porcine intestinal epithelial cells exposed to hydrogen peroxide. Oxidat Med Cel Longevity, 2021;8936907. https;//doi: 10.1155/2021/8936907

22. National Research Council, Division on Earth, Life Studies, & Committee on Nutrient Requirements of Swine. Nutrient requirements of swine. 2012.

23. Tsirtsikos P, Fegeros K, Kominakis A, Balaskas C, Mountzouris KC. Modulation of intestinal mucin composition and mucosal morphology by dietary phytogenic inclusion level in broilers. Animal. 2012; 6:1049-57. https://doi.org/10.1017/S1751731111002680

24. Manual BA. BAM. Food and Drug Administration. 2001. https://metone.com/wp-content/uploads/2024/01/E-BAM-9805-Manual-Rev-H-WEB.pdf

25. SAS. SAS/SAT guide for personal computers. 9.00ed. Carry, NC, USA: SAS Inst., Inc.; 2002

26. Ross GR, Gusils C, Oliszewski R, De Holgado SC, González SN. Effects of probiotic administration in swine. J Biosci Bioeng. 2010; 109:545-9. https://doi.org/10.1016/j.jbiosc.2009.11.007

27. Song D, Lee J, Oh H, Chang S, An J, Park S, et al. Effects of probiotics on growth performance, intestinal morphology, intestinal microbiota weaning pig challenged with Escherichia coli and Salmonella enterica. J Anim Sci Technol. 2023.

28. Kim JS, Hosseindoust A, Lee SH, Choi YH, Kim MJ, Lee JH, Kwon IK, Chae BJ. Bacteriophage cocktail and multi-strain probiotics in the feed for weanling pigs: effects on intestine morphology and targeted intestinal coliforms and *Clostridium*. Animal. 2017 ; 11:45-53. https://doi.org/10.1017/S1751731116001166

29. Kim JH, Lee JH, Lee KW, Kim S H, and Kim H B. Effects of dietary supplementation of *Lactobacillus salivarius* LS144 on growth performance and intestinal health in weaned piglets. J Anim Sci Technol. 2019; 61:333-341.3

30. Abe F, Ishibashi N, Shimamura S. Effect of administration of bifidobacteria andlactic acid bacteria to newborn calves and piglets. J Dairy Sci. 1995; 78:2838-2846. https://doi:10.3168/jds.S0022-0302(95)76914-4

31. Shimauchi H, Mayanagi G, Nakaya S, Minamibuchi M, Ito Y, Yamaki K, et al. Improvement of periodontal condition by probiotics with *Lactobacillus salivarius* WB21: a randomized, double-blind, placebocontrolled study. J Clin Periodontol. 2008; 35:897-5. https://doi.org/10.1111/j.1600-051X.2008.01306

32. Lallès JP, Bosi P, Smidt H, and Stokes CR. Nutritional management of gut health in pigs around weaning. Proc Nutr Socie, 2007; 66:260-68. https://doi.org/10.1017/S0029665107005484

33. Kim JB, Kang JH, Kim HJ, Han GM, Jeong JY, Kil DY. Effects of dietary supplementation of probiotic, *Clostridium butyricum*, on growth performance, immune response, intestinal barrier function, and digestive enzyme activity in broiler chickens challenged with Escherichia coli K88. Asian-Australas J Anim Sci. 2017; 30:1205.

34. Zhu C, Yao J, Zhu M, Zhu C, Yuan L, Li Z, Cai D, Chen S, Hu P, Liu HY. A meta-analysis of Lactobacillus-based probiotics for growth performance and intestinal morphology in piglets. Fron Vet Sci. 2022; 9:1045965. https://doi.org/10.3389/fvets.2022.1045965

35. Lessard M, Brisson GJ. Effect of a *Lactobacillus* fermentation product on growth, immune response and fecal enzyme activity in weaned pigs. Ca J Anim Sci. 1987; 67:509-16. https://doi.org/10.4141/cjas87-049

36. Kyriakis SC, Tsiloyiannis VK, Vlemmas J, Sarris K, Tsinas AC, Alexopoulos C, Jansegers L. The effect of probiotic LSP 122 on the control of post-weaning diarrhoea syndrome of piglets. Res Vet Scie. 1999; 67:223-8. https://doi.org/10.1053/rvsc.1999.0308

37. Lidbeck A, Nord CE. *Lactobacill*i and the normal human anaerobic microflora. Clinic Infec Dise. 1993; 16:S181-

7. https://doi.org/10.1093/clinids/16

38. Roselli M, Finamore A, Britti MS, Bosi P, Oswald I, Mengheri E. Alternatives to in-feed antibiotics in pigs: Evaluation of probiotics, zinc or organic acids as protective agents for the intestinal mucosa. A comparison of in vitro and in vivo results. Anim Res. 2005; 54:203-18. https://doi.org/10.1051/animres:2005012

39. Kelly DD, Smyth JA, McCracken KJ. Digestive development of the early weaned pig: 2. Effect of level of food intake on digestive enzyme activity during the immediate post-weaning period. Brit J Nutr. 1991; 65:18-88. https://doi: 10.1079/bjn19910079

40. Pluske JR, Hampson DJ, Williams IH. Factors influencing the structure and function of the small intestine in the weaned pig: a review. Liv Prod Sci. 1997; 51:215-36. https://doi: 10.1016/S0301-6226(97)00057-2

41. Gāliņa D, Ansonska L, Valdovska A. Effect of probiotics and herbal products on intestinal histomorphological and immunological development in piglets. Vet Med Intl. 2020: 24. https://doi.org/10.1155/2020/3461768

42. Zhang J, Deng J, Wang Z, Che C, Li YF, Yang Q. Modulatory effects of *Lactobacillus salivarius* on intestinal mucosal immunity of piglets. Curr Microbiol. 2011; 62:1623-31. https://doi:10.1007/s00284-011-9906-4

43. Liu H, Zhang J, Zhang S, Yang F, Thacker PA, et al. Oral administration of *Lactobacillus* fermentum I5007 favors intestinal development and alters the intestinal microbiota in formula-fed piglets. Agric Food Chem. 2014; 62:860-6. https:// doi.org/10.1021/jf403288r

44. Nyachoti CM, Omogbenigun FO, Rademacher M Blank G. Performance responses and indicators of gastrointestinal health in early-weaned pigs fed low-protein amino acid-supplemented diets. Anim Sci. 2016; 84:125-34. https://doi:10.2527/2006.841125x.

45. Kim J, Shim Y, Ingale S L, Hosseindoust A, Lee S, Rathi P C, and Chae B. The microbial pH-stable exogenous multienzyme improved growth performance and intestinal morphology of weaned pigs fed a corn–soybean-based diet. J Appl Anim Res. 2018; 46:559-65. https://doi.org/10.1080/09712119.2017.1358628

46. Varley MA.The neonatal pig development and survival. CAB international. Wallingford, pp 99–154:1995. https://doi.org/10.1017/S0021859600077510

47. Macfarlane S, Macfarlane GT. Regulation of short-chain fatty acid production. Proc Nutr Soc. 2003; 62:67-72. https://doi:10.1079/PNS2002207

48. Klaenhammer TR. Probiotic bacteria: today and tomorrow. J Nutr. 2000; 130:415S-6S. https://doi.org/10.1093/jn/130.2.5S

49. Dunne C. Adaptation of bacteria to the intestinal niche: probiotics and gut disorder. Inflamm Bowel Dise. 2001;7:136-45. doi.org/10.1097/00054725-200105000-0001

50. Jo H, Han G, Kim EB, Kong C, Kim BG. Effects of supplemental bacteriophage on the gut microbiota and nutrient digestibility of ileal-cannulated pigs. J Anim Scie Technol. 2023. https://doi.org/10.5187/jast.2023.e96

7. Tables

Table 1. Basal diet formulation and chemical composition of the experimental diet (as-fed basis)

Item	Basal diet
Ingredient (g/kg)	
Corn	403.2
Whey powder	161.9
Fish meal (60%)	40.0
Soybean meal dehulled	263.2
Soy protein concentrate	50
Soy oil	29.9
Mono calcium phosphate	3.8
Limestone	8.0
Salt	3.0
L-lysine (98 %)	3.1
L-methionine (98 %	1.1
L-tryptophan (10 %)	2.0
L-threonine (98.5 %)	1.4
Vitamin premix[1]	2.5
Mineral premix[2]	2.5
Choline-chloride (50 %)	0.5
Phytase	0.1
Chromic oxide	2.5
Lactose	19.9
Total	1000
Calculated composition (%)	
ME (MJ/kg)	14.2
CP	22.00
Ca	0.8
Av.P	0.38
SID. Lysin	1.30
SID. Methionine	0.39
SID. Methionine + Cystein	0.71
SID. Threonine	0.76
SID. Tryptophan	0.21
Lactose	12.00

[1]Supplied per kilogram of diet: 20,000 IU vitamin A, 4,200 IU vitamin D_3, 10 IU vitamin E, 5.6 mg vitamin K_3, 2.8 mg vitamin B_1, 5.5 mg vitamin B_2, 4.2 mg vitamin B_6, 0.042 mg vitamin B_{12}, 14 mg pantothenic acid, 42 vitamin B_3, 0.105 vitamin B_7, 1.05 mg vitamin B_9.

[2]Supplied per kilogram of diet: 50 mg Fe, 0.20 mg Co, 30 mg Cu, 30 mg Mn, 20 mg Zn, 0.35 mg I, 0.3 mg Se based on the treatments.

Available phosphorus (Av.P); metabolizable energy (ME); crude protein (CP); Calcium (Ca); standard illeal digestibility (SID)

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394 **Table 2. Effect of dietary supplementation of *Lactobacillus salivarius* LS144 on piglets' Growth performance**

Item	NN	NP	PN	PP	SEM	p-value
Phase 1 (1~21 d)						
ADG, g	217.83^{b}	216.49^{b}	241.33^{a}	241.66^{a}	3.72	0.001
Phase 2 (22 ~ 36 d)						
ADG, g	274.84^{b}	286.48^{b}	303.61^{ab}	337.13^{a}	7.58	0.005
ADFI, g	395.46^{b}	413.67^{b}	426.47^{b}	485.48^{a}	10.48	0.002
FCR	1.44	1.44	1.41	1.44	0.03	0.100
Phase 3 (37 ~ 51 d)						
ADG, g	433.73^{b}	423.75^{b}	442.78^{b}	475.57^{a}	6.40	0.007
ADFI, g	661.82^{b}	657.39^{b}	686.53^{ab}	731.19^{a}	9.46	0.005
FCR	1.53	1.55	1.55	1.54	0.01	0.436
Overall 1 (1~51 d)						
ADG, g	279.93^{c}	279.87^{c}	299.53^{b}	317.86^{a}	4.49	0.001
Overall 2 (22~51 d)						
ADG, g	354.28^{b}	355.12^{b}	373.19^{ab}	406.35^{a}	6.67	0.003
ADFI, g	528.64^{b}	535.53^{b}	556.50^{b}	608.33^{a}	13.85	0.001
FCR	1.49	1.51	1.49	1.50	0.01	0.387

Piglets from (1~51 d)
^{a,b,c} means with different superscripts within rows are significantly different at (p<0.05) or (p<0.01)
SEM- Standard error of means, ADG- Average daily gain, ADFI- Average daily feed intake,
FCR- Feed conversion ratio.
NN, Unsupplemented with the probiotic in both suckling and post-weaning phases.
NP, Unsupplemented in the suckling phase but supplemented in post-weaning phase.
PN, Supplemented with LS144 probiotic during suckling phase but not in the post-weaning phase.
PP, Supplemented with LS144 probiotic during both the suckling and post-weaning phases.

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398 **Table 3. Effect of dietary supplementation of *Lactobacillus salivarius* on piglets' Intestinal Morphology (d 51)**

Item	NN	PN	PP	SEM	p-value
Villus height					
Duodenum	549.02[b]	618.18[ab]	651.46[a]	15.91	0.008
Jejunum	512.02[b]	544.96[ab]	623.49[a]	18.43	0.019
Ileum	395.86[b]	441.82[ab]	490.34[a]	16.44	0.044
Crypt depth					
Duodenum	296.87	311.39	314.85	16.38	0.911
Jejunum	239.54	248.52	248.31	15.98	0.972
Ileum	212.22	211.33	210.34	12.93	0.999
VH/CD					
Duodenum	1.91	2.00	2.19	0.14	0.803
Jejunum	2.19	2.24	2.74	0.18	0.435
Ileum	1.93	2.17	2.40	0.13	0.376

Piglets from birth (0 ~5)
[a,b] means with different superscripts within rows are significantly different at *(p<0.05)* or *(p<0.01)*
SEM- Standard error of means.
NN, Unsupplemented with the probiotic in both suckling and post-weaning phases.
PN, Supplemented with LS144 probiotic during suckling phase but not in the post-weaning phase.
PP, Supplemented with LS144 probiotic during both the suckling and post-weaning phases
VH, villus height; CD, crepth depth.

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401 **Table 4. Effect of dietary supplementation of *Lactobacillus salivarius* LS144 on piglets' intestinal pH (d 51)**

Item	NN	PN	PP	SEM	p-value
Duodenum	6.10[b]	5.75[a]	5.73[a]	0.07	0.025
Jejunum	6.30	6.38	6.12	0.1	0.617
Ileum	6.43	6.44	6.58	0.11	0.866

Piglets on day 51.
[a,b] means with different superscripts within rows are significantly different at (p<0.05)
SEM- Standard error of means.
PN, Supplemented with LS144 probiotic during suckling phase but not in the post-weaning phase.
PP, Supplemented with LS144 probiotic during both the suckling and post-weaning phases.
NN, Unsupplemented with the probiotic in both suckling and post-weaning phases

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403 **Table 5. Effect of dietary supplementation of *Lactobacillus salivarius* LS144 on piglets' gut microbial population**

Item	NN	PN	PP	SEM	p-value
Duodenum					
Total anaerobic	8.83	8.74	8.85	0.03	0.411
Lactobacillus	9.52[b]	10.17[a]	10.29[a]	0.11	0.001
Clostridium	8.28	8.32	8.09	0.05	0.196
Coliforms	8.09	8.24	8.20	0.05	0.702
Jejunum					
Total anaerobic	8.63	8.81	8.77	0.05	0.390
Lactobacillus	9.52[b]	10.25[a]	10.31[a]	0.11	0.002
Clostridium	8.29	8.35	8.39	0.03	0.567
Coliforms	8.12	8.09	8.23	0.06	0.717
Ileum					
Total anaerobic	8.50	8.76	8.68	0.06	0.243
Lactobacillus	9.57[b]	10.35[a]	10.26[a]	0.11	0.002
Clostridium	8.11	8.18	8.23	0.07	0.827
Coliforms	8.19	8.27	8.44	0.05	0.156
Cecum					
Total anaerobic	8.62	8.69	8.49	0.04	0.197
Lactobacillus	9.62[b]	10.29[a]	10.35[a]	0.11	0.001
Clostridium	7.99	8.28	8.11	0.06	0.193
Coliforms	8.06	8.15	8.22	0.06	0.636

Piglets on day 51.
[a,b], means with different superscripts within rows are significantly different at (p<0.05) or (p<0.01)
SEM, Standard error of means.
PN, Supplemented with LS144 probiotic during suckling phase but not in the post-weaning phase.
PP, Supplemented with LS144 probiotic during both the suckling and post-weaning phases.
NN, Unsupplemented with the probiotic in both suckling and post-weaning phases

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