Effects of *Lactobacillus plantarum* and Non-Starch Polysaccharide Complex Enzyme on Growth Performance, Fecal Microbiota, and Serum Indices in Weaned Piglets (Postprint)

Authors: Chai Jianmin, Wei Ronggui, LIU Xifeng, Liu Jinlan, Cui Kai, Diao Qiyu, Zhang Naifeng

Date: 2017-10-10T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of dietary supplementation with Lactobacillus plantarum and non-starch polysaccharide (NSP) complex enzyme on growth performance, fecal microbiota, and serum indices of weaned piglets, thereby achieving the purpose of replacing antibiotics. A single-factor completely randomized design was adopted. A total of 192 healthy weaned piglets at 28 days of age with an average body weight of (7.15 ± 0.36) kg were selected and randomly divided into 4 groups, with 6 replicates per group and 8 piglets per replicate. The control group (CT group) was fed a basal diet supplemented with 40 mg/kg zinc bacitracin; the experimental groups were fed the basal diet supplemented with 1 g/kg Lactobacillus plantarum (PT group), 1 g/kg NSP complex enzyme (ZT group), and 1 g/kg Lactobacillus plantarum + 1 g/kg NSP complex enzyme (PZT group), respectively. The experimental period lasted 35 days. The results showed that: 1) There were no significant differences in final body weight, average daily gain, average daily feed intake, and feed-to-gain ratio among the groups ($P>0.05$). 2) There were no significant differences in the counts of Lactobacillus, Escherichia coli, and the Lactobacillus/Escherichia coli ratio in feces among the groups ($P>0.05$). However, compared with the control group, the combined Lactobacillus plantarum and NSP complex enzyme group showed an increasing trend in fecal Lactobacillus count and Lactobacillus/Escherichia coli ratio (0.05 $P<0.10$), and a decreasing trend in Escherichia coli count (0.05 $P<0.10$). 3) There were no significant differences in serum biochemical indices and immune indices among the groups ($P>0.05$). It can be concluded that dietary supplementation with Lactobacillus plantarum and NSP complex enzyme can promote intestinal microflora balance, benefit the healthy growth of piglets, and thereby achieve the purpose of replacing antibiotics.

Full Text

Effects of *Lactobacillus plantarum* and NSP Compound Enzyme on Growth Performance, Fecal Microbial Flora and Serum Parameters of Weaned Piglets

CHAI Jianmin[1], WEI Ronggui[2]*, LIU Xifeng[3], LIU Jinlan , CUI Kai[1], DIAO Qiyu[1], ZHANG Naifeng[1]*

[1]Key Laboratory of Feed Biotechnology, Ministry of Agriculture, Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, China
[2]Beijing Animal Husbandry and Veterinary Station, Beijing 100107, China
[3]Beijing Daxing District Animal Health Administration, Beijing 102600, China
 Livestock and Poultry Seed-Breeding Extension Center of Beijing Fangshan District, Beijing 102444, China

Corresponding authors: WEI Ronggui, Senior Engineer, E-mail: wrgyf@126.com; ZHANG Naifeng, Associate Professor, E-mail: zhangnaifeng@caas.cn

Abstract

This experiment was conducted to investigate the effects of dietary supplementation with *Lactobacillus plantarum* and non-starch polysaccharide (NSP) compound enzyme on growth performance, fecal microbial flora, and serum parameters of weaned piglets, with the aim of developing antibiotic alternatives. Using a single-factor completely randomized design, 192 healthy 28-day-old weaned piglets with an average body weight of (7.15±0.36) kg were randomly allocated to 4 groups, each consisting of 6 replicates with 8 piglets per replicate. The control group (CT) received a basal diet supplemented with 40 mg/kg bacitracin zinc, while the experimental groups received the basal diet supplemented with 1 g/kg *L. plantarum* (PT group), 1 g/kg NSP compound enzyme (ZT group), or 1 g/kg *L. plantarum* + 1 g/kg NSP compound enzyme (PZT group). The experimental period lasted 35 days. The results showed: (1) No significant differences were observed among groups in final body weight, average daily gain, average daily feed intake, or feed-to-gain ratio ($P>0.05$). (2) No significant differences were found in fecal lactobacilli count, *E. coli* count, or lactobacilli/*E. coli* ratio among groups ($P>0.05$). However, compared with the control group, the PZT group showed a trend toward increased fecal lactobacilli count and lactobacilli/*E. coli* ratio (0.05 $P<0.10$) and a trend toward decreased *E. coli* count (0.05 $P<0.10$). (3) No significant differences were detected in serum biochemical or immune parameters among groups ($P>0.05$). These findings indicate that dietary supplementation with *L. plantarum* and NSP compound enzyme can promote intestinal microbial balance and benefit piglet health, thereby achieving the objective of antibiotic substitution.

Keywords: piglets; probiotics; enzyme preparations; antibiotics; growth performance; serum parameters

Introduction

Early weaning in piglets is associated with decreased immune antibody levels and cellular immunity, facilitating pathogen proliferation in the intestine and compromising healthy growth. In practice, antibiotics are commonly used to treat and prevent piglet diseases, thereby alleviating stress and promoting growth. However, due to antibiotic residues and bacterial resistance development, antibiotics can disrupt intestinal microbial balance, negatively affecting animal growth and potentially accumulating in humans with adverse health effects. Consequently, identifying antibiotic alternatives has become critically important.

Probiotics represent one of the most promising antibiotic alternatives, demonstrating positive effects on growth performance and disease prevention in pigs [1]. *Lactobacillus plantarum*, a probiotic bacterium, can improve intestinal microbial balance, reduce disease incidence, and enhance animal growth performance [2-3]. Previous studies have shown that feeding *L. plantarum* can improve growth performance and immune function in weaned piglets [4-5]. Dong et al. [6] demonstrated that *L. plantarum* complex preparations did not affect growth performance but enhanced immune function in weaned piglets. Enzyme preparations can improve digestibility of corn-soybean meal diets by increasing digestive enzyme activity, promoting lactobacilli proliferation, degrading feed cell walls, and breaking down non-starch polysaccharides and antinutritional factors [7]. Tang [8] reported that combined supplementation of probiotics and enzyme preparations (including amylase, protease, and NSP enzymes) significantly improved daily gain and reduced diarrhea rates in nursery piglets.

Polysaccharidases promote degradation of antinutritional factors in diets, and the resulting oligosaccharides can serve as prebiotics to stimulate probiotic proliferation. However, most research has focused on single polysaccharidase supplementation, with few studies investigating compound enzymes. Additionally, while both probiotics and enzyme preparations can independently promote piglet growth, improve digestibility, and enhance immunity to achieve antibiotic-free production, most reports on their combined use have been conducted in broilers rather than weaned piglets. Moreover, previous studies have primarily examined single-additive effects, with limited research on combined probiotic-enzyme supplementation.

Therefore, this experiment was designed to investigate the effects of a combined probiotic-enzyme preparation on growth performance, fecal microbial flora, and serum parameters in weaned piglets, providing a theoretical basis for replacing antibiotics with enzyme preparations and probiotics in piglet diets.

1. Materials and Methods

1.1. Experimental Materials *Lactobacillus plantarum*: Viable count 2×10 CFU/g, provided by the Feed Research Institute, Chinese Academy of Agricultural Sciences.

NSP compound enzyme: Provided by Beijing Zhongnongxing Feed Technology Co., Ltd., primarily containing -glucanase (activity 8,000 U/g), -xylanase (activity 70,000 U/g), -mannanase (activity 150,000 U/g), and protease (activity 1,500 U/g).

1.2. Experimental Design A single-factor completely randomized design was employed. One hundred ninety-two healthy 28-day-old weaned piglets with an average body weight of (7.15±0.36) kg were randomly divided into 4 groups, each comprising 6 replicates with 8 piglets per replicate. The groups were: control (CT) group receiving basal diet + antibiotics (40 mg/kg bacitracin zinc); *L. plantarum* (PT) group receiving basal diet + 1 g/kg *L. plantarum*; NSP compound enzyme (ZT) group receiving basal diet + 1 g/kg NSP compound enzyme; and *L. plantarum* + NSP compound enzyme (PZT) group receiving basal diet + 1 g/kg *L. plantarum* + 1 g/kg NSP compound enzyme. The trial was conducted during the nursery period and lasted 35 days.

1.3. Experimental Diets and Management The corn-soybean meal basal diet was formulated according to the apparent ileal digestible amino acid pattern for piglets recommended by NRC [9] (1988). The composition and nutrient levels of the basal diet are presented in Table 1 .

The experiment was conducted at Fangshan Breeding Pig Farm in Beijing. Piglets were housed in enclosed pens with slatted floors. Feed was provided in mash form, water was supplied ad libitum via nipple drinkers, and piglets had free access to feed and water. Immunization and deworming procedures followed the farm' s standard management protocols.

Table 1. Composition and nutrient levels of the basal diet (DM basis), %

Ingredients	Content
Corn	[value]
Soybean meal	[value]
Whey powder	[value]
Extruded soybean	[value]
Fish meal	[value]
CaHPO	[value]
Limestone	[value]

Ingredients	Content
NaCl	[value]
L-Lys · HCl	[value]
DL-Met	[value]
Premix[1])	[value]
Total	[value]

Nutrient levels[2]) - DE/(MJ/kg): [value] - CP: [value] - Ca: [value] - TP: [value] - Lys: [value] - Met: [value]

[1]) Premix provided the following per kg of diet: VA 5,512 IU, VD 640 IU, VK 2.2 IU, VE 20 IU, VB 1.5 mg, VB 5.5 mg, VB 2.2 mg, VB 27.6 g, D-pantothenic acid 14.8 mg, nicotinic acid 30.3 mg, biotin 0.05 mg, choline 500 mg, Cu 50 mg, Fe 100 mg, Mn 10 mg, Zn 50 mg, I 0.85 mg, Se 0.25 mg.
[2]) Nutrient levels were measured values.

1.4. Measurements and Methods 1.4.1. Growth Performance

Piglets were weighed before morning feeding at the beginning and end of the experiment on a per-replicate basis to calculate average daily gain (ADG). Daily feed provision and residual feed were recorded for each replicate to calculate average daily feed intake (ADFI). Feed-to-gain ratio (F/G) was then calculated based on ADG and ADFI.

1.4.2. Rectal Fecal Microbial Counts

Fecal microbial counts were determined using plate count methods. On the final day of the experiment, one piglet was randomly selected from each replicate before weighing, and rectal fecal samples were collected using sterile spoons, refrigerated, and immediately transported to the laboratory for analysis. In a sterile workstation, 1.0 g of fecal sample was mixed with 9 mL of sterile physiological saline to prepare a 1:10 dilution (10^{1} dilution), vortexed for 3-5 min, and then 1 mL of this dilution was transferred to 9 mL of sterile physiological saline, vortexed for 1-2 min to prepare a 10^{2} dilution. Serial dilutions were continued to 10 . Fecal dilutions of 10 to 10 were plated on MRS solid medium to enumerate lactobacilli, while 10^{3} to 10 dilutions were plated on MacConkey medium to enumerate *E. coli*. *E. coli* were incubated aerobically at 37°C for 24 h, and lactobacilli for 36 h. Each dilution was plated in triplicate, and the average count was calculated. Microbial counts were expressed as lg(CFU/g) (logarithm of colony-forming units per gram of feces) [6].

1.4.3. Serum Biochemical and Immune Parameters

On the final day, one piglet per replicate was randomly selected, and 10 mL of blood was collected from the anterior vena cava. Serum was separated by centrifugation at 3,000 r/min for 20 min and stored at -20°C. Serum total protein

(TP), albumin (ALB), globulin (GLB), urea nitrogen (UN), glucose (GLU), total cholesterol (TC), and non-esterified fatty acid (NEFA) concentrations were measured using an automatic biochemical analyzer (Hitachi 7600, Japan).

Serum immunoglobulin G (IgG), insulin-like growth factor I (IGF-I), insulin (INS), and growth hormone (GH) concentrations were determined using porcine immunoglobulin enzyme-linked immunosorbent assay kits provided by Nanjing Jiancheng Bioengineering Institute, following the manufacturer' s instructions.

1.5. Data Analysis Experimental data were organized using Excel 2007 and analyzed using one-way ANOVA in SAS 9.2 statistical software. Duncan' s multiple range test was used for post-hoc comparisons when significant differences were detected. $P<0.05$ was considered statistically significant, and 0.05 $P<0.10$ was considered a trend toward improvement or reduction.

---

2. Results

2.1. Effects of *L. plantarum* and NSP Compound Enzyme on Growth Performance of Weaned Piglets As shown in Table 2 , no significant differences were observed in initial body weight among groups ($P>0.05$), confirming the validity of the experimental design. No significant differences were found in final body weight, average daily gain, average daily feed intake, or feed-to-gain ratio among groups ($P>0.05$).

Table 2. Effects of *Lactobacillus plantarum* and NSP compound enzyme on growth performance of weaned piglets

Items	Groups	P-value
Initial body weight/kg	[values]	>0.05
Final body weight/kg	[values]	>0.05
ADG/g	[values]	>0.05
ADFI/g	[values]	>0.05
F/G	[values]	>0.05

2.2. Effects of *L. plantarum* and NSP Compound Enzyme on Fecal Microbial Flora of Weaned Piglets As shown in Table 3 , no significant differences were observed in fecal lactobacilli count, *E. coli* count, or lactobacilli/*E. coli* ratio among groups ($P>0.05$). However, compared with the control group, the PZT group exhibited a trend toward increased fecal lactobacilli count and lactobacilli/*E. coli* ratio (0.05 $P<0.10$) and a trend toward decreased *E. coli* count (0.05 $P<0.10$).

Table 3. Effects of *Lactobacillus plantarum* and NSP compound enzyme on fecal microbial flora of weaned piglets

Items	Groups	P-value
Lactobacilli	[values]	>0.05
E. coli	[values]	>0.05
Lactobacilli/*E. coli*	[values]	>0.05

2.3. Effects of *L. plantarum* and NSP Compound Enzyme on Serum Parameters of Weaned Piglets As shown in Table 4 , no significant differences were observed in serum biochemical or immune parameters among groups (P>0.05).

Table 4. Effects of *Lactobacillus plantarum* and NSP compound enzyme on serum parameters of weaned piglets

Items	Groups	P-value
TP/(g/L)	[values]	>0.05
ALB/(g/L)	[values]	>0.05
GLB/(g/L)	[values]	>0.05
ALB/GLB	[values]	>0.05
UN/(mmol/L)	[values]	>0.05
GLU/(mmol/L)	[values]	>0.05
TC/(mmol/L)	[values]	>0.05
NEFA/(mmol/L)	[values]	>0.05
IgG/(g/L)	[values]	>0.05
IGF-I/(ng/mL)	[values]	>0.05
INS/(IU/mL)	[values]	>0.05
GH/(ng/mL)	[values]	>0.05

3. Discussion

3.1. Effects of Dietary *L. plantarum* and NSP Compound Enzyme on Growth Performance of Weaned Piglets During the weaning period, piglets typically experience reduced appetite, decreased nutrient intake, lowered intestinal digestive enzyme activity, reduced feed efficiency, and diarrhea due to various stress factors including dietary changes, environmental shifts, and management practices. The primary underlying cause is disruption of intestinal microecological balance. In this experiment, the control group received bacitracin zinc, a complex of bacitracin (a polypeptide antibiotic produced by *Bacillus licheniformis*) and zinc, which exhibits excellent stability and strong bactericidal activity against Gram-positive bacteria, improving feed utilization and promoting growth while potentially causing intestinal dysbiosis and enhanced bacterial resistance. Dietary probiotic supplementation can regulate intestinal microecological balance, promote nutrient digestion and absorption, and alleviate weaning stress [10-11]. Enzyme supplementation in weaned piglet diets can compensate for decreased endogenous enzyme activity during this period, facilitating nutrient digestion and absorption, particularly by reducing the adverse effects of antinutritional factors such as non-starch polysaccharides, thereby enhancing disease resistance and promoting growth [8].

Using an antibiotic-supplemented group as a positive control, this study found no significant differences in body weight, average daily gain, average daily feed intake, or feed-to-gain ratio among groups, indicating that dietary *L. plantarum* and NSP compound enzyme supplementation can achieve antibiotic substitution. Dong et al. [6] reported that supplementing weaned piglet diets (35-70 days of age) with *L. plantarum* complex preparations yielded no significant differences in body weight, average daily gain, average daily feed intake, or feed-to-gain ratio compared with antibiotic supplementation. Ma [12] demonstrated that enzyme supplementation (xylanase and -mannanase) produced no significant differences in average daily gain or feed conversion ratio compared with antibiotic supplementation. These findings align with the effects of individual *L. plantarum* or NSP compound enzyme supplementation observed in this study. However, the combined supplementation of *L. plantarum* and NSP compound enzyme also showed no significant differences in growth performance compared with antibiotic supplementation. This may be attributed to the fact that the combined group received the same supplementation levels as the individual additive groups, suggesting that the dosage and ratio of *L. plantarum* and NSP compound enzyme may not have been optimal, resulting in limited synergistic effects and suboptimal probiotic efficacy. For probiotics, sufficient viable cell counts are crucial for efficacy. Yu et al. [4] demonstrated that supplementing weaned piglet diets with 5.8×10 CFU/g of *Lactobacillus fermentum* produced optimal effects, with higher or lower concentrations being less effective. Zhang et al. [2] confirmed that the optimal supplementation level of *L. plantarum* in growing pig diets was 1×10 CFU/kg. Similarly, Zhou et al. [13] showed that dietary -glucanase supplementation at 0.1 g/kg did not affect piglet growth performance, while Ma [11] reported that 0.1% enzyme supplementation in corn-soybean meal diets produced optimal results. Therefore, further research is needed to determine the optimal supplementation levels and ratios of *L. plantarum* and NSP compound enzyme.

3.2. Effects of Dietary *L. plantarum* and NSP Compound Enzyme on Fecal Microbial Flora of Weaned Piglets Lactobacilli and *E. coli* are the primary indicator organisms for intestinal health, and their ratio serves as a key index for evaluating digestive tract microecological balance [14]. During weaning, decreased intestinal lactobacilli and increased *E. coli* populations are major causes of diarrhea [15-16]. Numerous studies have confirmed that probiotic supplementation in weaned piglets reduces diarrhea incidence and fecal *E. coli* counts. Dong et al. [6] and Le et al. [17] demonstrated that *L. plantarum* complex probiotic supplementation significantly reduced fecal *E. coli* counts in weaned piglets. Li [18] showed that -mannanase supplementation in corn-soybean meal diets increased intestinal lactobacilli and bifidobacteria counts while decreasing *E. coli* counts. Zhou et al. [13] reported that -glucanase supplementation reduced fecal *E. coli* counts in weaned piglets. In this study, compared with the control group, the PZT group exhibited trends toward increased fecal lactobacilli count and lactobacilli/*E. coli* ratio (0.05 $P<0.10$) and a trend toward decreased *E. coli* count (0.05 $P<0.10$), indicating that combined *L. plantarum* and NSP compound enzyme supplementation effectively improved intestinal microbial balance. Enzyme preparations facilitate degradation of nutrients into carbohydrates in the intestine, promoting lactobacilli proliferation. Lactobacilli ferment carbohydrates into organic acids, lowering intestinal pH and inhibiting *E. coli* growth and reproduction, thereby improving intestinal microflora. Combined probiotic and enzyme supplementation may fundamentally resolve intestinal microbial imbalance.

3.3. Effects of Dietary *L. plantarum* and NSP Compound Enzyme on Serum Parameters of Weaned Piglets Serum biochemical parameters reflect metabolic status and functional changes in tissues and organs, as well as nutrient deposition in animals [19]. Serum total protein, composed of albumin and globulin, is an important indicator of nutritional status. Serum globulin participates in immune responses against foreign antigens, and its concentration reflects immune status to some extent [20]. Decreased albumin/globulin ratio indicates enhanced specific immune response and improved disease resistance. In this study, no significant differences were observed in serum total protein, albumin, or globulin concentrations among groups, suggesting that *L. plantarum* and NSP compound enzyme supplementation achieved effects comparable to bacitracin zinc supplementation. Dong et al. [6] reported no significant differences in serum total protein and albumin concentrations between weaned piglets fed lactic acid bacteria complex preparations and those fed antibiotics. Li [18] demonstrated that enzyme supplementation increased serum albumin and total protein concentrations, but not significantly different from antibiotic supplementation.

Serum urea nitrogen is an important parameter reflecting protein metabolism and dietary amino acid balance, with elevated levels indicating enhanced protein catabolism [21]. Serum glucose is the direct energy source for vital activities and correlates strongly with daily gain and dietary nutrient levels [22]. Serum total cholesterol and non-esterified fatty acids reflect lipid utilization; during stress, increased fat mobilization for energy elevates these parameters. This study found no significant differences in serum biochemical parameters among groups, possibly because weaning stress had dissipated after 35 days of feeding, and nutrient digestion, absorption, and utilization in piglets receiving probiotics and NSP compound enzyme were equivalent to those receiving antibiotics. Combined with feed conversion results, this further supports that probiotic and NSP compound enzyme supplementation can substitute antibiotics. Fan [23] reported that NSP compound enzyme supplementation had no significant effect on serum total cholesterol and triglyceride concentrations in weaned piglets.

Serum immunoglobulins are major components of the humoral immune system, with immunoglobulin G playing a crucial role in systemic immune responses as the primary antibody. No significant differences were observed in serum IgG concentrations among groups, indicating comparable immune function across treatments. This may be because after 35 days post-weaning, piglets had adapted to their environment and diet, with weaning stress gradually diminishing and intestinal microflora stabilizing. During weaning stress recovery, *L. plantarum* and NSP compound enzyme supplementation achieved effects equivalent to bacitracin zinc, potentially offering advantages in immune function recovery and enhancement. Kin et al. [24] found that probiotic complex supplementation in piglet diets did not affect serum immunoglobulin concentrations. Han [25] reported that enzyme supplementation increased serum IgA and IgG concentrations, but the differences were not significant.

Serum insulin-like growth factor I and growth hormone are closely associated with animal growth and weight gain [22], while serum insulin is a key hormone for lowering blood glucose. No significant differences were observed in serum IGF-I, GH, or insulin concentrations among groups. Research indicates that appropriate enzyme supplementation can increase serum IGF-I concentration, but excessive supplementation may produce the opposite effect [18]. This suggests that serum IGF-I, GH, and insulin concentrations are related to probiotic and enzyme supplementation levels, particularly requiring further investigation for combined supplementation.

Conclusions

1. Supplementation with *Lactobacillus plantarum* and NSP compound enzyme reduced fecal *E. coli* counts and increased lactobacilli counts in weaned piglets, demonstrating promotion of intestinal microecological balance and benefits for piglet health.
2. Dietary supplementation with *L. plantarum* and NSP compound enzyme achieved growth performance and serum parameter effects comparable to bacitracin zinc supplementation in weaned piglets.

References

[1] LEE J S, AWJI E G, LEE S J, et al. Effect of *Lactobacillus plantarum* CJLP243 on the growth performance and cytokine response of weaning pigs challenged with enterotoxigenic *Escherichia coli* [J]. Journal of Animal Science, 2012, 90(11): 3709–3717.

[2] ZHANG N F, WANG J, CUI K, et al. Effects of *Lactobacillus plantarum* GF103 on growth performance, nutrient digestibility and fecal microbial counts

in growing pigs [J]. Chinese Journal of Animal Nutrition, 2015, 27(6): 1853-1860.

[3] GIANG H H, VIET T Q, OGLE B, et al. Growth performance, digestibility, gut environment and health status in weaned piglets fed a diet supplemented with potentially probiotic complexes of lactic acid bacteria [J]. Livestock Science, 2010, 129(1/2/3): 95-103.

[4] YU H F, WANG A N, LI X J, et al. Effect of viable *Lactobacillus fermentum* on the growth performance, nutrient digestibility and immunity of weaned pigs [J]. Journal of Animal and Feed Sciences, 2008, 17(1): 61-69.

[5] GIANG H H, VIET T Q, OGLE B, et al. Growth performance, digestibility, gut environment and health status in weaned piglets fed a diet supplemented with a complex of lactic acid bacteria alone or in combination with *Bacillus subtilis* and *Saccharomyces boulardii* [J]. Livestock Science, 2012, 143(2/3): 132-141.

[6] DONG X L, ZHANG N F, ZHOU M, et al. Effects of compound bacterial preparation on growth performance, fecal microbes and serum parameters of weaned piglets [J]. Chinese Journal of Animal Nutrition, 2013, 25(6): 1285-1292.

[7] WALSH G A, POWER R F, HEADON D R. Enzymes in the animal-feed industry [J]. Trends in Biotechnology, 1993, 11(10): 424-430.

[8] TANG M H. Effects of combined microecological and enzyme preparations on diarrhea rate and growth performance of nursery piglets [J]. Feed Industry, 2015, 36(12): 32-35.

[9] NRC. Nutrient requirements of swine [S]. 10th ed. Washington, D.C.: National Academy Press, 1998.

[10] DÄNICKE S, DÖLL S. A probiotic feed additive containing spores of *Bacillus subtilis* and *B. licheniformis* does not prevent absorption and toxic effects of the Fusarium toxin deoxynivalenol in piglets [J]. Food and Chemical Toxicology, 2010, 48(1): 152-158.

[11] LÄHTEINEN T, LINDHOLM A, RINTTILÄ T, et al. Effect of *Lactobacillus brevis* ATCC 8287 as a feeding supplement on the performance and immune function of piglets [J]. Veterinary Immunology and Immunopathology, 2014, 158(1/2): 14-25.

[12] MA Y F. Effects of replacing corn with brown rice and wheat and enzyme supplementation on growth performance and digestibility in weaned piglets [D]. Master' s thesis. Beijing: Chinese Academy of Agricultural Sciences, 2002.

[13] ZHOU T X, JUNG J H, ZHANG Z F, et al. Effect of dietary -glucan on growth performance, fecal microbial shedding and immunological responses after lipopolysaccharide challenge in weaned pigs [J]. Animal Feed Science and Technology, 2013, 179(1/2/3/4): 85-92.

[14] CASTILLO M, MARTÍN-ORÚE S M, MANZANILLA E G, et al. Quantification of total bacteria, enterobacteria and lactobacilli populations in pig digesta by real-time PCR [J]. Veterinary Microbiology, 2006, 114(1/2): 165–170.

[15] FAIRBROTHER J M, NADEAU É, GYLES C L. *Escherichia coli* in postweaning diarrhea in pigs: an update on bacterial types, pathogenesis, and prevention strategies [J]. Animal Health Research Reviews, 2005, 6(1): 17–39.

[16] CASTILLO M, MARTÍN-ORÚE S M, ANGUITA M, et al. Adaptation of gut microbiota to physical structure of different types dietary fibre [J]. Livestock Science, 2007, 109(1/2/3): 149–152.

[17] LE BON M, DAVIES H E, GLYNN C, et al. Influence of probiotics on gut health in the weaned pig [J]. Livestock Science, 2010, 133(1/2/3): 179–181.

[18] LI X J. Effects of -mannanase on growth performance and its mechanism in weaned piglets [D]. PhD thesis. Shenyang: Shenyang Agricultural University, 2008.

[19] WANG J P, YOO J S, KIM H J, et al. Nutrient digestibility, blood profiles and fecal microbiota are influenced by chitooligosaccharide supplementation of growing pigs [J]. Livestock Science, 2009, 125(2/3): 298–303.

[20] LIN Q, DAI Q Z, BIN S Y, et al. Synergistic effects of probiotics and enzyme preparations on blood biochemical parameters and immune performance in yellow-feathered broilers [J]. Feed Industry, 2012, 33(14): 31–36.

[21] LIU H, JI H F, ZHANG D Y, et al. Effects of *Lactobacillus brevis* preparation on growth performance, fecal microflora and serum profile in weaned pigs [J]. Livestock Science, 2015, 178: 251–254.

[22] ZHAO X J, LI L, LUO Q L, et al. Effects of mulberry (*Morus alba* L.) leaf polysaccharides on growth performance, diarrhea, blood parameters, and gut microbiota of early-weanling pigs [J]. Livestock Science, 2015, 177: 88–94.

[23] FAN C L. Effects of -glucan and xylanase compound enzyme on growth axis hormones and its mechanism in weaned piglets [D]. PhD thesis. Hangzhou: Zhejiang University, 2008.

[24] KIM K H, INGALE S L, KIM J S, et al. Bacteriophage and probiotics both enhance the performance of growing pigs but bacteriophage are more effective [J]. Animal Feed Science and Technology, 2014, 196: 88–95.

[25] HAN G Q. Experimental study on effects of -mannanase supplementation on growth and development of piglets [D]. Master' s thesis. Beijing: China Agricultural University, 2007.

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