

## Effects of Compound Microecological Preparation on Growth Performance, Diarrhea Rate, Immune Function, and Gut Microbiota in Weaned Piglets (Postprint)

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### Abstract

This experiment aimed to investigate the effects of compound microecological preparations on growth performance, diarrhea rate, immune performance, and intestinal microflora of weaned piglets. Ninety-six Laiwu black pig weaned piglets aged ( $30\pm 2$ ) days with an average body weight of ( $8.15\pm 0.05$ ) kg were selected and randomly divided into 4 groups, with 6 replicates per group (half male and half female), and 4 piglets per replicate. Group I was the control group, fed a basal diet without any probiotics or antibiotics; Group II was the compound microecological preparation group, with 1.0‰ peptide bacteriocin added to the basal diet; Group III was the antibiotic group, with 500 mg/kg colistin sulfate added to the basal diet; Group IV was the combined peptide bacteriocin and antibiotic group, with 1.0‰ peptide bacteriocin, 200 mg/kg olaquinox, and 110 mg/kg kitasamycin added to the basal diet. The pre-trial period was 3 days, and the formal trial period was 28 days. The results showed that: 1) The average daily gain of Group II was significantly increased by 13.16%, 15.77%, and 10.66% compared with Groups I, III, and IV, respectively ( $P<0.05$ ), and the F/G was significantly decreased by 8.54%, 12.10%, and 6.50%, respectively ( $P<0.05$ ). Compared with Group I, Groups II, III, and IV all significantly reduced the diarrhea rate of piglets ( $P<0.05$ ). 2) Compared with Groups I and III, the liver index and spleen index of Group II were increased by 12.13%, 24.76% and 14.60%, 12.45%, respectively, and the serum immunoglobulin G and immunoglobulin A contents were increased by 49.73%, 37.50% and 61.05%, 46.67%, respectively, with all differences being significant ( $P<0.05$ ). 3) Compared with Groups I and III, Group II significantly decreased the pH of gastric and duodenal contents ( $P<0.05$ ), and significantly increased the number of *Lactobacillus* in the cecum and colon ( $P<0.05$ ). It can be concluded that under the conditions of this experiment, dietary supplementation

with 1.0‰ compound microecological preparation peptide bacteriocin could improve the growth performance of weaned piglets, reduce diarrhea rate, promote the development of immune organs, increase serum immunoglobulin content, enhance piglet immunity, decrease gastric and duodenal content pH, and improve intestinal microflora balance.

## Full Text

### Effects of Compound Probiotics on Growth Performance, Diarrhea Rate, Immune Performance and Intestinal Microflora of Weaned Piglets

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#### Abstract

This trial was conducted to evaluate the effects of compound probiotics on growth performance, diarrhea rate, immune performance, and intestinal microflora of weaned piglets. A total of 96 Laiwu black piglets at (30±2) days of age with an average body weight of (8.15±0.05) kg were randomly allocated into 4 groups with 6 replicates per group (half male and half female) and 4 piglets per replicate. Group I served as the control, receiving a basal diet without any probiotics or antibiotics. Group II received the basal diet supplemented with 1.0‰ antibacterial probiotics (peptide probiotics). Group III received the basal diet supplemented with 500 mg/kg colistin sulphate. Group IV received the basal diet supplemented with 1.0‰ antibacterial probiotics, 200 mg/kg olaquinox, and 110 mg/kg kitasamycin. The pre-trial period lasted 3 days, followed by a 28-day experimental period. The results showed: 1) Compared with groups I, III, and IV, group II exhibited significantly higher average daily gain (ADG) by 13.16%, 15.77%, and 10.66%, respectively ( $P<0.05$ ), and significantly lower feed-to-gain ratio (F/G) by 8.54%, 12.10%, and 6.50%, respectively ( $P<0.05$ ). Groups II, III, and IV all showed significantly reduced diarrhea rates compared with group I ( $P<0.05$ ). 2) Group II demonstrated significantly increased liver index and spleen index by 12.13% and 24.76% compared with group I, and by 14.60% and 12.45% compared with group III ( $P<0.05$ ), respectively. Serum immunoglobulin G (IgG) and immunoglobulin A (IgA) contents in group II were significantly elevated by 49.73% and 37.50% compared with group I, and by 61.05% and 46.67% compared with group III ( $P<0.05$ ), respectively. 3) Group II showed significantly decreased pH in stomach and duodenal contents ( $P<0.05$ ) and significantly increased Lactobacillus counts in cecum and colon ( $P<0.05$ ) compared with groups I and III. In conclusion, under the conditions of this experiment, dietary supplementation with 1.0‰ compound antibacterial probiotics (peptide probiotics) improved growth performance, reduced diarrhea rate, promoted immune organ development, increased serum immunoglobulin

contents, enhanced immunity, decreased pH in stomach and duodenal contents, and improved intestinal microflora balance in weaned piglets.

**Keywords:** antibacterial probiotics; weaned piglets; antibiotics; growth performance; diarrhea rate; immune performance; intestinal microflora

## Introduction

Weaning is one of the most critical stages in pig production. Early weaning can improve sow reproductive efficiency and reduce feeding costs [1]. However, the intestinal immune system of piglets generally matures around 4-7 weeks of age. Consequently, early-weaned piglets have low levels of self-produced immune antibodies and cellular immunity, and the cessation of maternal antibody supply results in significant immunosuppression [2]. In modern pig production systems, forced early weaning causes psychological, dietary, and environmental stress, often leading to weaning stress syndrome. This adversely affects piglet growth performance and intestinal health, resulting in intestinal microflora imbalance, reduced feed intake, slow growth, diarrhea, and even death, causing substantial economic losses [3-4]. Therefore, ensuring smooth weaning is crucial for improving pig production efficiency.

Feed antibiotics exhibit significant disease-resistant, anti-stress, and growth-promoting effects, and are commonly used in practice to treat and prevent piglet diseases, thereby alleviating stress and promoting growth [5]. However, with increasing global economic integration, China's animal husbandry standards and regulations must align with those of developed countries, including the "feed antibiotic ban." As living standards improve, public concern for food safety has intensified, particularly regarding antibiotic residues in animal products. Social pressure will inevitably drive the implementation of "feed antibiotic bans." In current Chinese livestock production, antibiotic abuse and misuse have significantly impacted the industry, with excessive antibiotic use leading to enhanced bacterial resistance in piglet intestines, disruption of normal microflora balance, and ultimately severe diarrhea or even death [6]. Therefore, developing green and environmentally friendly feed additives represents an effective solution to the negative impacts of antibiotics.

Probiotics are among the best alternatives to antibiotics. They are live microbial preparations and their metabolites that can improve host intestinal microflora structure and enhance host health status [7]. Microecological preparations have been widely applied in weaned piglet diets to improve intestinal microflora balance and alleviate early weaning stress. Numerous recent studies have investigated the effects of dietary compound probiotics on weaned piglet growth, diarrhea, immune performance, and intestinal microflora. Liu et al. [8] found that dietary compound probiotics significantly increased ADG and decreased F/G in weaned piglets. Li et al. [9] reported that adding 600 mg/kg probiotic preparation to weaned piglet diets could replace antibiotic feed additives. Zhao et al. [10] observed that feeding weaned piglets with liquid compound

probiotics containing *Lactobacillus*, yeast, *Bacillus*, and *Geotrichum candidum* significantly reduced diarrhea rate. Lei et al. [11] found that adding 1,000 mg/kg compound probiotics to weaned piglet diets increased ADG by 13.52% compared with 200 mg/kg oxytetracycline and by 14.49% compared with 300 mg/kg furazolidone. Xiao et al. [12] demonstrated that replacing antibiotics with compound probiotics in pre- and post-weaning piglet diets produced disease-resistant and growth-promoting effects, with superior diarrhea reduction compared with feed antibiotics. Therefore, this experiment aimed to investigate the effects of the company's product peptide probiotics (antibacterial probiotics) partially or completely replacing dietary antibiotics on weaned piglets, measuring its impacts on growth performance, diarrhea rate, immune performance, and intestinal microflora to provide theoretical and practical basis for peptide probiotic application as an antibiotic substitute.

## Materials and Methods

### 1.1 Experimental Materials Compound microecological preparation

—**peptide probiotics:** Composed of *Lactobacillus plantarum*, *Pediococcus pentosaceus*, *Bacillus subtilis*, *Clostridium butyricum*, yeast and its culture, *Aspergillus* and its culture, antimicrobial peptide CEC-38, and citric acid, with total viable bacteria count  $5.0 \times 10^8$  CFU/g.

**Feed antibiotics:** Commercially available colistin sulphate, olaquinox, and kitasamycin, with colistin sulphate purity at 10%, and both olaquinox and kitasamycin purity at 50%.

**1.2 Experimental Design and Diet Composition** A total of 96 Laiwu black weaned piglets at  $(30 \pm 2)$  days of age with an average body weight of  $(8.15 \pm 0.05)$  kg were randomly divided into 4 groups with 6 replicates per group (half male and half female) and 4 piglets per replicate (one pen per replicate). Group I served as the control, receiving a basal diet without probiotics or antibiotics. Group II received the basal diet supplemented with 1.0‰ peptide probiotics. Group III received the basal diet supplemented with 500 mg/kg colistin sulphate. Group IV received the basal diet supplemented with 1.0‰ peptide probiotics, 200 mg/kg olaquinox, and 110 mg/kg kitasamycin. The pre-trial period lasted 3 days, followed by a 28-day experimental period.

The basal diet was formulated according to NRC (2012) standards. Its composition and nutrient levels are shown in Table 1 .

**1.3 Management** The experiment was conducted at the Ningyang Experimental Pig Farm of Shandong Baolai-Leelai Agricultural Science and Technology Co., Ltd. All piglets were housed in a closed pig house with cement floors, provided ad libitum access to feed and water. Deworming, disinfection, and immunization followed the farm's routine management procedures.

### 1.4 Measurements

**1.4.1 Growth Performance** Feed intake was recorded by pen throughout the experiment. All piglets were weighed after overnight fasting at 08:00 on days 1 and 28 to calculate ADG, average daily feed intake (ADFI), and F/G for days 1–28 using the following formulas:

$$\begin{aligned} \text{ADG} &= \text{total pen weight gain} / (\text{number of piglets per pen} \times \text{experimental days}) \\ \text{ADFI} &= \text{total pen feed intake} / (\text{number of piglets per pen} \times \text{experimental days}) \\ \text{F/G} &= \text{ADFI} / \text{ADG} \end{aligned}$$

**1.4.2 Diarrhea Rate** Feces were observed at 09:00 and 15:00 daily during the experimental period and scored according to Shi et al. [4]: 0 = strip- or pellet-shaped; 1 = soft but formed; 2 = thick, unformed, no fecal-water separation; 3 = liquid, unformed, with fecal-water separation. Diarrhea was defined as a score  $\geq 2$ . Diarrhea rate (DR) was calculated as:

$$\text{DR (\%)} = [\text{total diarrhea incidents per group} / (\text{total piglets per group} \times \text{total experimental days})] \times 100$$

**1.4.3 Immune Organ Indices** At the end of the experiment, one piglet per replicate (near average body weight) was selected from each group for slaughter. The liver and spleen were removed, connective tissue was trimmed, blood was blotted with filter paper, and wet weight was measured to calculate organ indices:

$$\text{Organ index (g/kg)} = \text{organ wet weight} / \text{live body weight}$$

**1.4.4 Gastrointestinal pH** At the end of the experiment, one piglet per replicate (near average body weight) was selected from each group for slaughter. The abdominal cavity was opened, and pH values of stomach, duodenal, jejunal, ileal, and cecal contents were measured using a PHB-2 portable pH meter.

**1.4.5 Serum Immune Indices** At the end of the experiment, one piglet per replicate (near average body weight) was selected from each group. Blood was collected from the anterior vena cava, allowed to clot for 1 h, then centrifuged at 4,000 r/min for 10 min to separate serum. Serum immunoglobulin G (IgG) and immunoglobulin A (IgA) contents were determined by enzyme-linked immunosorbent assay using kits from Beijing Yanda Biological Technology Co., Ltd., following the manufacturer's instructions.

**1.4.6 Intestinal Microflora Counts** At the end of the experiment, one piglet per replicate (near average body weight) was selected from each group for slaughter. Cecal and colonic contents were aseptically collected, homogenized in sterile physiological saline, and bacterial counts were performed using plate culture methods. Microflora counts were expressed as log<sub>10</sub> colony-forming units per gram of intestinal content (lg CFU/g). One gram of content was weighed in a sterile environment, added to 99 mL sterile physiological saline with glass

beads, and shaken. One milliliter of this suspension was transferred to a tube containing 9 mL sterile 0.9% saline, serially diluted 10-fold to  $10^{-8}$ . Lactobacillus was cultured on LBS agar at 37°C under anaerobic conditions for 48 h before colony counting. *Escherichia coli* was cultured on eosin-methylene blue agar at 37°C under aerobic conditions for 24 h before counting.

**1.5 Data Processing and Analysis** Experimental data were processed using Excel and analyzed for significance using the ANOVA procedure in SPSS 13.0 software. Multiple comparisons were performed using LSD method, with  $P < 0.05$  as the significance threshold. Results are expressed as “mean  $\pm$  standard deviation.”

## Results

**2.1 Effects of Compound Probiotics on Growth Performance and Diarrhea Rate** As shown in Table 2, initial body weight did not differ significantly among groups ( $P > 0.05$ ). Final body weight was highest in group II, significantly exceeding groups I, III, and IV by 5.69%, 6.79%, and 4.89%, respectively ( $P < 0.05$ ). ADFI did not differ significantly among groups ( $P > 0.05$ ). ADG was highest in group II, significantly higher than groups I, III, and IV by 13.16%, 15.77%, and 10.66%, respectively ( $P < 0.05$ ). F/G was lowest in group II, significantly lower than groups I, III, and IV by 8.54%, 12.10%, and 6.50%, respectively ( $P < 0.05$ ). Diarrhea rate was highest in group I, significantly higher than all other groups ( $P < 0.05$ ), while no significant differences were observed among groups II, III, and IV ( $P > 0.05$ ).

**2.2 Effects of Compound Probiotics on Immune Performance** As shown in Table 3, liver weight was highest in group IV, followed by group II, with no significant difference between these two groups ( $P > 0.05$ ), but both were significantly higher than groups I and III ( $P < 0.05$ ). Spleen weight did not differ significantly among groups II, III, and IV ( $P > 0.05$ ), but all were significantly higher than group I. Liver index and spleen index in group II were significantly increased by 12.13% and 24.76% compared with group I, and by 14.60% and 12.45% compared with group III ( $P < 0.05$ ), respectively, but did not differ significantly from group IV ( $P > 0.05$ ). Serum IgG and IgA contents were highest in group II, significantly higher than groups I and III ( $P < 0.05$ ), but not significantly different from group IV ( $P > 0.05$ ).

**2.3 Effects of Compound Probiotics on Gastrointestinal pH** As shown in Table 4, on day 28, group II exhibited significantly reduced pH in stomach and duodenal contents compared with group I ( $P < 0.05$ ), decreasing by 22.33% and 16.56%, respectively. However, no significant differences were observed in jejunal, ileal, and cecal contents ( $P > 0.05$ ). Group III showed no significant differences in pH values across all measured gastrointestinal segments compared with group I ( $P > 0.05$ ). Group IV had significantly lower pH in stomach and

duodenal contents compared with group I ( $P < 0.05$ ), decreasing by 21.05% and 14.33%, respectively, while jejunal, ileal, and cecal pH values were lower but not significantly different ( $P > 0.05$ ).

### 2.5 Effects of Compound Probiotics on Intestinal Microflora Counts

As shown in Table 5, no significant differences in cecal and colonic *E. coli* counts were observed among groups II, III, and IV ( $P > 0.05$ ), but all were significantly lower than group I ( $P < 0.05$ ). Group II showed significantly higher Lactobacillus counts in both cecum and colon compared with all other groups ( $P < 0.05$ ). No significant differences in cecal Lactobacillus counts were observed among groups I, III, and IV ( $P > 0.05$ ). Colonic Lactobacillus counts in group I were significantly higher than groups III and IV ( $P < 0.05$ ), with no significant difference between groups III and IV ( $P > 0.05$ ).

## Discussion

### 3.1 Effects of Compound Probiotics on Growth Performance and Diarrhea Rate

Weaning stress syndrome caused by feed changes, pathogenic, physiological, and environmental factors severely affects survival rates and economic efficiency in early-weaned piglets [13]. Researchers have added Chinese herbal medicines, acidifiers, enzymes, and microecological preparations to weaned piglet diets to alleviate weaning stress syndrome [14]. Studies have shown that probiotics can replace antibiotics in piglets, improving feed intake [15], growth performance [16], immunity [17], and promoting beneficial bacteria while inhibiting harmful bacteria. Yin et al. [18] reported that adding 0.10% and 0.05% microecological preparations (containing  $1 \times 10^8$  and  $5 \times 10^8$  CFU/g effective bacteria, respectively) to piglet diets significantly reduced diarrhea and mortality rates compared with the control group, with superior effects to antibiotics. In this experiment, the compound probiotics group significantly improved ADG and reduced F/G, with effects superior to both the antibiotic group and the combined probiotics-antibiotics group. Specifically, ADG was 15.77% higher and F/G was 12.10% lower than the antibiotic group. The compound probiotics group also significantly reduced diarrhea rate compared with the control group, though not significantly different from the antibiotic group. These results demonstrate that the compound probiotic peptide preparation alleviated post-weaning stress, improved growth performance, and achieved equivalent diarrhea control to antibiotics.

### 3.2 Effects of Compound Probiotics on Immune Performance

Organ indices represent biological characteristics whose magnitude partially determines functional capacity [19-20]. The liver is the most metabolically active organ and performs important immune functions. The spleen is the largest peripheral immune organ and the primary site for immune cell production, differentiation, maturation, settlement, and immune response [21]. Numerous reports have addressed microecological preparations' effects on weaned piglet immune organs. Wang et al. [22] found that dietary *Enterococcus faecium* supplementation did

not significantly affect liver index but increased spleen weight and index. Xin et al. [23] reported that 0.75% *Bacillus* supplementation increased thymus index by 62.30% and spleen index by 12.04% in piglets. This experiment showed that on day 28, compound probiotics significantly increased liver weight, spleen weight, liver index, and spleen index compared with the control group, promoting liver and spleen development. Compared with the antibiotic group, the compound probiotics group increased liver weight, spleen weight, liver index, and spleen index by 10.27%, 8.03%, 14.60%, and 12.45%, respectively, demonstrating superior effects to colistin sulphate. The combination of 1.0‰ peptide probiotics with 200 mg/kg olaquinox and 110 mg/kg kitasamycin showed no significant differences from peptide probiotics alone in improving these indices, but was superior to colistin sulphate, indicating that peptide probiotics can be combined with these antibiotics in weaned piglet diets.

Humoral immunity represents the ability to produce antibodies and is a crucial disease resistance mechanism [24]. IgA, IgG, and IgM can specifically bind corresponding antigens and constitute the primary components of humoral immune function, with serum immunoglobulin contents reflecting immune capacity [25]. Zhang et al. [26] reported that dietary *Bacillus* supplementation significantly increased serum IgG content in piglets. This experiment demonstrated that compound probiotics increased serum IgG and IgA contents by 49.73% and 37.50% compared with the control group, and by 61.05% and 46.67% compared with the antibiotic group, with all differences being significant.

**3.3 Effects of Compound Probiotics on Gastrointestinal pH** The gastrointestinal tract is the primary site for nutrient digestion and absorption, and pH is a crucial factor affecting digestive function in weaned piglets [27]. Appropriate acidity is essential for maintaining normal physiological functions of the digestive system. Studies have shown that probiotics can increase volatile fatty acid content in piglet intestines, reduce intestinal pH [28], and inhibit pathogen proliferation [29]. Högberg et al. [30] found that increased dietary probiotics significantly elevated volatile fatty acid content while reducing pH in piglet intestines. Xin et al. [31] reported that *Bacillus* significantly reduced jejunal and cecal pH. Lactic acid bacteria produce large amounts of lactic and acetic acid, lowering intestinal pH. Citric acid, primarily used as a feed additive in weaned piglets, can reduce diet pH and buffering capacity [32], thereby increasing gastrointestinal acidity, mainly in the stomach and anterior small intestine. Risley et al. [33] found that adding 1.5% citric acid to piglet diets reduced diet pH from 6.42 to 4.92 and gastric content pH from 4.07 to 3.82. This experiment showed that 1.0‰ peptide probiotics significantly reduced stomach and duodenal content pH by 22.33% and 16.56%, respectively, compared with the control group. These results indicate that dietary compound probiotic peptide preparation can reduce gastrointestinal pH, inhibit pathogen proliferation, and maintain intestinal health in piglets.

### 3.4 Effects of Compound Probiotics on Intestinal Microflora Counts

In healthy piglets, harmful and beneficial bacteria should maintain dynamic equilibrium. Stress conditions (such as weaning) increase harmful bacteria and decrease beneficial bacteria, making microflora balance control crucial [34]. Studies have found that single or compound probiotics, particularly those based on lactic acid bacteria, can intervene and correct intestinal microecological imbalance during piglet weaning, promoting growth [35-36]. Bon et al. [37] reported that dietary *Saccharomyces cerevisiae* and *Pediococcus* significantly reduced fecal *E. coli* counts. Dong et al. [38] found that probiotic feeding significantly reduced fecal *E. coli* counts while improving growth performance and immunity in weaned piglets. This experiment demonstrated that dietary supplementation with 1.0‰ peptide probiotics significantly increased *Lactobacillus* counts in cecum and colon, effectively promoting beneficial bacteria proliferation. Compared with the control group, compound probiotics significantly reduced *E. coli* counts in cecum and colon, showing effective pathogen inhibition. No significant differences in cecal and colonic *E. coli* counts were observed between the compound probiotics and antibiotic groups, indicating that appropriate levels of peptide probiotics can achieve pathogen inhibition equivalent to antibiotics in weaned piglet diets.

### Conclusion

Under the conditions of this experiment, dietary supplementation with 1.0‰ compound microecological preparation (peptide probiotics) improved growth performance, reduced diarrhea rate, promoted immune organ development, increased serum immunoglobulin contents, enhanced immunity, decreased pH in stomach and duodenal contents, and improved intestinal microflora balance in weaned piglets.

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