

## Effects of Compound Microecological Preparation on Growth Performance, Serum Biochemical and Immune Indices, and Fecal Volatile Fatty Acid Content in Weaned Piglets (Postprint)

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

### Abstract

This experiment aimed to investigate the effects of compound microecological preparation on growth performance, serum biochemical and immune indices, and fecal volatile fatty acid content in weaned piglets. A total of 240 weaned piglets ( $(10.0 \pm 0.3)$  kg), with half males and half females, were randomly divided into 4 groups, with 6 replicates per group and 10 piglets per replicate. Group I (control group) was fed a basal diet (containing 1% kitasamycin and 2% colistin), while Groups II, III, and IV were supplemented with 50, 100, and 150 mL of compound microecological preparation per piglet per day, respectively, based on the basal diet.

The trial consisted of a 5-day pre-trial period and a 28-day formal trial period. On days 1 and 28 of the experiment, blood samples were collected from the anterior vena cava of piglets to determine serum biochemical and immune indices, and rectal contents were collected to measure fecal volatile fatty acid content.

The results showed: 1) Compared with the control group, Group II exhibited significant improvements in weaned piglets, with average daily gain increasing by 23.53% ( $P < 0.05$ ), feed-to-gain ratio decreasing by 15.86% ( $P < 0.05$ ), and diarrhea rate decreasing by 47.16% ( $P > 0.05$ ). 2) On day 1 of the experiment, there were no significant differences in serum biochemical and immune indices among groups ( $P > 0.05$ ), while fecal volatile fatty acid content in the treatment groups was higher than that in the control group. 3) On day 28 of the experiment, Group II showed higher activities of alkaline phosphatase and aspartate aminotransferase in serum compared with the control group ( $P > 0.05$ ), as well as higher contents of glucose, total protein, albumin, and globulin in serum ( $P > 0.05$ ), while serum urea nitrogen content decreased by 18.27% compared with

the control group ( $P > 0.05$ ). Compared with the control group, Group II exhibited increased fecal acetate, propionate, and butyrate contents by 21.03% ( $P > 0.05$ ), 41.08% ( $P > 0.05$ ), and 77.84% ( $P < 0.05$ ), respectively.

In conclusion, dietary supplementation of 50 mL of compound microecological preparation per piglet per day can improve growth performance, reduce the diarrhea rate, enhance immune function, and increase fecal volatile fatty acid content in weaned piglets.

## Full Text

### Effects of Compound Probiotics on Growth Performance, Serum Biochemical and Immune Indices, and Volatile Fatty Acid Contents in Feces of Weaned Piglets

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## Abstract

This experiment was conducted to investigate the effects of compound probiotics on growth performance, serum biochemical and immune indices, and volatile fatty acid contents in feces of weaned piglets. Two hundred and forty weaned piglets (half male and half female) with an initial body weight of  $(10.0 \pm 0.3)$  kg were randomly allocated into four groups with six replicates per group and ten pigs per replicate. Pigs in group I (control group) were fed a basal diet containing 1% kitasamycin and 2% colistin, while groups II, III, and IV were fed the basal diet supplemented with 50, 100, and 150 mL of compound probiotics per piglet daily, respectively. The experiment lasted for 28 days following a 5-day pre-trial period. On days 1 and 28 of the experiment, blood samples were collected via anterior vena cava puncture to determine serum biochemical and immune indices, and rectal contents were collected to measure volatile fatty acid concentrations in feces. The results showed that: (1) Compared with the control group, group II exhibited a significant 23.53% increase in average daily gain ( $P < 0.05$ ), a significant 15.86% decrease in feed-to-gain ratio ( $P < 0.05$ ), and a 47.16% reduction in diarrhea rate ( $P > 0.05$ ). (2) On day 1, no significant differences were observed in serum biochemical and immune indices among groups ( $P > 0.05$ ), while volatile fatty acid contents in feces were higher in the treatment groups than in the control group. (3) On day 28, serum alkaline phosphatase and aspartate aminotransferase activities, as well as glucose, total protein, albumin, and globulin concentrations in group II were higher than those in the control group ( $P > 0.05$ ), whereas serum urea nitrogen content decreased by 18.27% compared with the control group ( $P > 0.05$ ). Additionally, compared with the control group, group II showed increases of 21.03% ( $P > 0.05$ ), 41.08% ( $P > 0.05$ ),

and 77.84% ( $P < 0.05$ ) in acetic acid, propionic acid, and butyric acid contents in feces, respectively. These findings indicate that dietary supplementation with 50 mL of compound probiotics per piglet daily can improve growth performance, reduce diarrhea rate, enhance immune function, and increase volatile fatty acid contents in feces of weaned piglets.

**Keywords:** compound probiotics; weaned piglets; growth performance; serum biochemical index; volatile fatty acid

## Introduction

Intensive modern farming practices readily disrupt the intestinal microbial ecosystem in animals, leading to digestive disorders, impaired growth, and increased production costs. Antibiotic supplementation can influence initial microbial colonization in the host intestine, thereby promoting growth. Although antibiotics as feed additives can improve economic returns, their extensive use is associated with numerous adverse effects, including antibiotic residues in animal products that pose potential health risks to humans and may increase microbial resistance to antibiotics. Research has demonstrated that probiotics can replace antibiotics in piglet production by improving feed intake, growth performance, and immune function while promoting beneficial intestinal bacteria and inhibiting pathogenic microorganisms. Therefore, this study investigated the effects of different supplementation levels of compound probiotics on growth performance, serum biochemical and immune indices, and volatile fatty acid contents in feces of weaned piglets, aiming to provide a foundation for developing safe, efficient, and environmentally friendly compound probiotics for widespread application in animal production.

## Materials and Methods

### Experimental Materials

The compound probiotics used in this study were provided by Guangzhou Baiaofei Biotechnology Co., Ltd. as a mixture of lactic acid bacteria, yeast, and their culture medium in light yellow powder form. The powder was added to water heated to 24°C in a fermentation tank and anaerobically fermented at constant temperature for 18 hours. The fermented compound probiotics had a pH of 3.59, with viable counts of  $1 \times 10^8$  CFU/mL for lactic acid bacteria and  $8 \times 10^8$  CFU/mL for yeast. Each batch of fermented compound probiotics was used within 2 days, and anaerobic conditions were maintained throughout fermentation and use. The experimental diets were formulated according to NRC (2012) standards, with basal diet composition and nutrient levels shown in Table 1.

## Experimental Design

Two hundred and forty weaned piglets (Duroc × Landrace) with an average body weight of  $(10.0 \pm 0.3)$  kg (half male and half female) were randomly divided into four groups with six replicates per group and ten piglets per replicate. Group I (control) was fed the basal diet containing 1% kitasamycin and 2% colistin, while groups II, III, and IV were fed the basal diet supplemented with 50, 100, and 150 mL of compound probiotics fermentation liquid per piglet daily, respectively. The experiment was conducted at Tianzhong Breeding Base in Luyi County, Henan Province, and lasted for 28 days following a 5-day pre-trial period.

## Feeding Management

The on-farm trial was conducted strictly according to conventional pig farm management protocols, including required vaccination and deworming procedures. All groups were fed mixed diets at 07:00, 10:00, 13:30, and 15:30 daily.

## Measurement Indicators

**Growth Performance** On days 1 and 28 of the experimental period, piglets were weighed after fasting to calculate average daily gain. Daily feed intake was recorded for each group, and total feed consumption was calculated at the end of the experiment to determine average daily feed intake and feed-to-gain ratio. Fecal consistency was observed daily at 08:00, and the number of diarrheic piglets was recorded to calculate diarrhea rate using the formula: Diarrhea rate (%) =  $100 \times (\text{number of diarrheic piglets}) / (\text{total number of piglets} \times \text{experimental days})$ .

**Serum Biochemical and Immune Indices** On days 1 and 28 of the experimental period, five mL of blood was collected via anterior vena cava puncture from six piglets of similar body weight randomly selected from each group at 08:00. Blood was placed in procoagulant vacuum tubes, left on an inclined plane for 0.5 hours, then centrifuged at 3,000 rpm for 15 minutes. The supernatant was collected and stored at  $-20^{\circ}\text{C}$ . Serum samples were sent to Heilongjiang Provincial People's Hospital for determination of alkaline phosphatase (ALP) and aspartate aminotransferase (AST) activities, as well as glucose (GLU), urea nitrogen (UN), total protein (TP), albumin (ALB), and globulin (GLB) concentrations using a Hitachi 7020 automatic biochemical analyzer.

**Determination of Volatile Fatty Acid Content in Feces** On days 1 and 28 at 15:00, rectal contents were aseptically and anaerobically collected from three randomly selected piglets per group and placed in 50 mL sterile screw-cap tubes, which were immediately stored at  $-20^{\circ}\text{C}$ . Approximately 2 g of feces was transferred to a centrifuge tube, mixed with double-distilled water, and centrifuged at 10,000 rpm for 15 minutes at  $4^{\circ}\text{C}$ . The supernatant was collected and aliquoted into two EP tubes as volatile fatty acid samples. For analysis, 1.5

mL of fecal supernatant was mixed with 0.2 mL of 8.2% metaphosphoric acid, incubated in a water bath for 30 minutes, then centrifuged at 10,000 rpm for 10 minutes at 4°C. The supernatant was analyzed for acetic acid, propionic acid, and butyric acid contents using a GC-2010 gas chromatograph.

### Statistical Analysis

Data were analyzed using SPSS 22.0 software via one-way ANOVA, with LSD test for multiple comparisons. Differences were considered significant at  $P < 0.05$ . Results are expressed as mean  $\pm$  standard error.

## Results

### Effects of Compound Probiotics on Growth Performance of Weaned Piglets

As shown in Table 2, group II had the highest average daily gain, while group I had the lowest. Average daily gain in groups II, III, and IV was significantly higher than in group I ( $P < 0.05$ ), with increases of 23.53%, 20.59%, and 14.71%, respectively. No significant differences were observed in average daily feed intake among the four groups ( $P > 0.05$ ). Feed-to-gain ratio differed significantly among groups ( $P < 0.05$ ), with group I showing a significantly higher ratio than the other groups ( $P < 0.05$ ). Compared with group I, group II exhibited a 15.86% reduction in feed-to-gain ratio ( $P < 0.05$ ). Although diarrhea rate did not differ significantly among the four groups ( $P > 0.05$ ), group II had the lowest rate, showing a 47.16% reduction compared with group I ( $P > 0.05$ ).

### Effects of Compound Probiotics on Serum Biochemical and Immune Indices of Weaned Piglets

As presented in Table 3, on day 1, no significant differences were detected in serum alkaline phosphatase and aspartate aminotransferase activities, urea nitrogen and glucose concentrations, or total protein, albumin, globulin, and albumin/globulin ratio among the four groups ( $P > 0.05$ ). On day 28, serum alkaline phosphatase activity did not differ significantly among groups ( $P > 0.05$ ), though group III showed the highest activity. Similarly, no significant differences were observed in serum aspartate aminotransferase activity among groups ( $P > 0.05$ ). Serum urea nitrogen and glucose concentrations did not differ significantly among groups ( $P > 0.05$ ), but group II showed an 18.27% reduction in urea nitrogen content compared with group I ( $P > 0.05$ ). No significant differences were found in serum total protein, albumin, globulin, or albumin/globulin ratio among groups ( $P > 0.05$ ).

## Effects of Compound Probiotics on Volatile Fatty Acid Contents in Feces of Weaned Piglets

As shown in Table 4, on day 1, significant differences were observed in fecal acetic acid content among the four groups ( $P < 0.05$ ), with groups II, III, and IV showing increases of 63.41%, 61.76%, and 76.63% compared with group I ( $P < 0.05$ ). Fecal propionic acid content also differed significantly among groups ( $P < 0.05$ ), with groups II, III, and IV showing increases of 88.86%, 28.20%, and 35.21% compared with group I ( $P < 0.05$ ). Although fecal butyric acid content did not differ significantly among the four groups ( $P > 0.05$ ), groups II, III, and IV all showed increased levels compared with group I.

On day 28, significant differences were observed in fecal acetic acid content among groups ( $P < 0.05$ ), with groups II, III, and IV showing increases of 21.03% ( $P > 0.05$ ), 3.19% ( $P > 0.05$ ), and 40.69% ( $P < 0.05$ ) compared with group I. Fecal propionic acid content also differed significantly among groups ( $P < 0.05$ ), with group IV showing a 107.11% increase compared with group I ( $P < 0.05$ ), while groups II and III showed numerical increases without statistical significance ( $P > 0.05$ ). Fecal butyric acid content differed significantly among groups ( $P < 0.05$ ), with groups II, III, and IV showing increases of 77.84% ( $P < 0.05$ ), 30.15% ( $P > 0.05$ ), and 76.03% ( $P < 0.05$ ) compared with group I.

## Discussion

### Effects of Compound Probiotics on Growth Performance of Weaned Piglets

Direct feeding of fermented probiotics mixed with diets is rarely reported in the literature, with most studies focusing on fermented feed ingredients or direct addition of beneficial bacteria to diets. Yue et al. reported that compound probiotics fermented soybean meal can promote intestinal development, enhance digestive enzyme activity, reduce diarrhea rate, and improve growth performance in piglets. Abe et al. demonstrated that oral administration of *Bifidobacterium* and *Lactobacillus* preparations to newborn piglets significantly increased average daily gain compared with the control group. Estienne et al. found that microbial agents can improve average daily gain in pigs. Liu et al. reported that compound probiotics fermented diets can significantly improve apparent digestibility of nutrients and growth performance in finishing pigs. Wu et al. observed that feeding compound probiotics fermented diets to finishing pigs reduced feed-to-gain ratio by 9.76% and increased weight gain by 18.03% while effectively reducing diarrhea rate in piglets. Liu et al. showed that probiotics can significantly improve average daily gain and reduce feed-to-gain ratio in weaned piglets. The present study, which used compound probiotics mixed directly with diets, yielded results consistent with previous research. Notably, comprehensive evaluation of average daily gain, average daily feed intake, feed-to-gain ratio, and diarrhea rate indicated that supplementation with 50 mL of compound probiotics fermentation liquid per piglet daily produced the op-

timal effect, significantly improving average daily gain, reducing feed-to-gain ratio, and decreasing diarrhea rate. These findings suggest that higher doses of compound probiotics do not necessarily confer better growth performance, indicating the existence of an optimal supplementation level and range.

### **Effects of Compound Probiotics on Serum Biochemical and Immune Indices of Weaned Piglets**

Serum biochemical indices provide a comprehensive reflection of metabolic function, with changes in blood biochemical parameters indicating alterations in metabolic activity. Within a certain range, higher glucose content represents stronger capacity for glucose utilization in anabolic metabolism. Glucose serves as an energy substrate for metabolism, acting as an important monosaccharide energy source that supports vital activities and provides energy for protein synthesis in animals. Additionally, serum glucose absorption can promote insulin release and facilitate protein synthesis. Serum alanine aminotransferase and aspartate aminotransferase are important markers of liver and heart function; elevated activities may indicate hepatic or cardiac damage. Tang et al. reported that serum alkaline phosphatase activity can reflect metabolic efficiency of proteins and lipids, as well as growth rate and performance. In the present study, no significant differences in serum alkaline phosphatase activity were observed among groups on day 1; however, on day 28, group II showed higher activity than group I, further confirming that compound probiotics can improve growth performance in weaned piglets. Group II exhibited the highest average daily gain and lowest feed-to-gain ratio and diarrhea rate. This outcome may be attributed to increased probiotic doses potentially causing diarrhea in weaned piglets, thereby reducing feed intake and weight gain. Meanwhile, with similar feed intake, the 100 mL supplementation level provided increased microbial numbers, which may explain the higher serum alkaline phosphatase activity observed in group III. Serum biochemical indices reflect metabolic status in weaned piglets. Serum total protein and urea nitrogen concentrations accurately indicate protein metabolism absorption and dietary volatile fatty acid balance. Lower serum urea nitrogen content suggests greater urea nitrogen deposition and more efficient conversion to body protein, thereby promoting animal growth. Gao et al. reported that supplementation with 6% probiotics fermented apple pomace can reduce serum urea nitrogen content in weaned piglets. In the present study, no significant differences in serum urea nitrogen content were observed among groups on day 1; however, on day 28, group III showed lower serum urea nitrogen content than the control group, indicating that compound probiotics can increase urea nitrogen deposition, facilitate protein synthesis, and promote piglet growth.

Serum total protein content reflects protein absorption status and its relationship with humoral immunity. Compound probiotics contain abundant lactic acid bacteria and yeast, whose cellular components can effectively stimulate the intestine and act as immune adjuvants to promote immune organ development.

Serum total protein, composed of albumin and globulin, is the most abundant solid component in serum and functions to maintain normal vascular colloid osmotic pressure and pH while transporting various metabolites. Serum globulin is produced by macrophages; increased circulating antibody levels elevate serum globulin content, which can bind to foreign antigens and trigger immune responses to protect the organism from foreign substances. Globulin concentration thus reflects immune status and physiological condition to some extent, and its elevation can increase serum total protein content. Wang et al. reported that dietary *Lactobacillus* supplementation can improve non-specific immune function in juvenile Pacific white shrimp. Dong et al. demonstrated that dietary supplementation with a compound preparation of *Bacillus licheniformis*, *Bacillus subtilis*, and *Lactobacillus plantarum* can enhance immune performance. The present results are consistent with these previous findings. Compound probiotics contain abundant microorganisms that can influence intestinal microbial colonization and composition, exerting certain stimulatory effects on host digestion and immune function.

### **Effects of Compound Probiotics on Volatile Fatty Acid Contents in Feces of Weaned Piglets**

The digestive tract of piglets contains numerous microorganisms that coexist in a dynamic microecological balance system of interdependence and mutual restriction. When piglets experience stress, this intestinal microecological balance is disrupted, causing microbial dysbiosis and impaired growth performance. The digestive tract (ileum, cecum, and colon) of weaned piglets harbors abundant microorganisms capable of fermenting dietary carbohydrates to produce various volatile fatty acids (such as acetic acid, propionic acid, and butyric acid). These small molecular volatile fatty acids protect intestinal health by serving as energy substrates, inhibiting harmful bacteria, and promoting proliferation of intestinal epithelial cells and mucosal growth. The volatile fatty acid content and intestinal health status of weaned piglets can be indirectly reflected through analysis of rectal contents (feces). In the present study, volatile fatty acid contents in feces were higher in all treatment groups than in the control group, consistent with previous research. Zhang et al. reported that dietary supplementation with porcine-derived *Lactobacillus salivarius* can improve intestinal microecological balance. Hou and Mallo et al. demonstrated that dietary *Lactobacillus* supplementation in weaned piglets can significantly increase *Lactobacillus* counts and reduce *Escherichia coli* counts in the ileum, cecum, and feces. Giang et al. noted that the primary mechanism by which *Lactobacillus* inhibits pathogenic bacteria is through organic acid production, which increases intestinal organic acid content, reduces intestinal pH, and inhibits harmful bacterial growth. Gao et al. reported that supplementation with 6% probiotics fermented apple pomace can improve intestinal microbial balance in piglets. Based on the comprehensive results of this study, supplementation with 50 mL of compound probiotics per piglet daily is most appropriate, as this level can improve intestinal microbial status, nutritional status, and intestinal health in weaned piglets.

## Conclusion

Compound probiotics can improve growth performance and immune function, enhance intestinal function, reduce diarrhea rate, and increase volatile fatty acid contents in feces of weaned piglets, with optimal effects observed at a supplementation level of 50 mL per piglet daily.

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