

## Effects of Compound Probiotics and Fiber Oligosaccharides on Growth Performance, Fecal Microbiota, and Serum Parameters in Weaned Piglets (Postprint)

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### Abstract

This experiment aimed to investigate the effects of compound probiotics and fiber oligosaccharides on growth performance, fecal microorganisms, and serum indices in weaned piglets. A total of 120 weaned piglets with an initial average body weight of  $(9.72 \pm 2.25)$  kg were randomly divided into 4 groups, with 3 replicates per group and 10 piglets per replicate. The piglets were fed a basal diet (control group), basal diet + 0.30% compound probiotics (Group A), basal diet + 0.08% fiber oligosaccharides (Group B), or basal diet + 0.30% compound probiotics + 0.08% fiber oligosaccharides (Group C). The experiment consisted of a 4-day preliminary period followed by a 35-day formal experimental period, which was divided into two phases: Phase 1 (days 1-14) and Phase 2 (days 15-35). The results showed: 1) Except for the feed-to-gain ratio (F/G) of piglets in Group C being significantly lower than that of the control group during days 15-35 ( $P < 0.05$ ), there were no significant differences in average daily feed intake (ADFI), average daily gain (ADG), or feed-to-gain ratio among groups at other stages ( $P > 0.05$ ). The diarrhea rate (DR) of piglets in Groups A and C was significantly lower than that of the control group during days 1-14 ( $P < 0.05$ ). 2) On days 14 and 35, there were no significant differences in *Bacillus* count, *Lactobacillus* count, or *Escherichia coli* count in feces among groups ( $P > 0.05$ ). 3) There were also no significant differences in any serum indices among groups ( $P > 0.05$ ). In conclusion, under the conditions of this experiment, supplementation with compound probiotics and fiber oligosaccharides improved the diarrhea status of weaned piglets during days 1-14 post-weaning and enhanced feed utilization efficiency during days 15-35 post-weaning.

## Full Text

# Effects of Compound Probiotics and Cello-Oligosaccharide on Growth Performance, Fecal Microflora and Serum Parameters of Weaned Piglets

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**Abstract:** This study investigated the effects of dietary compound probiotics and cello-oligosaccharide on growth performance, fecal microflora, and serum parameters in weaned piglets. One hundred twenty weaned piglets with an initial average body weight of (9.72±\$2.25) kg were randomly allocated into 4 groups with 3 replicates per group and 10 piglets per replicate. The four dietary treatments were: basal diet (control group), basal diet + 0.30% compound probiotics (Group A), basal diet + 0.08% cello-oligosaccharide (Group B), and basal diet + 0.30% compound probiotics + 0.08% cello-oligosaccharide (Group C). The experiment consisted of a 4-day pre-test period followed by a 35-day formal test period, which was divided into Phase 1 (days 1-14) and Phase 2 (days 15-35). The results showed that (1) no significant differences were observed in average daily feed intake (ADFI), average daily gain (ADG), or feed-to-gain ratio (F/G) among groups during any experimental period (P>0.05), except that the F/G in Group C was significantly lower than that in the control group during days 15-35 (P<0.05). The diarrhea rate in Groups A and C was significantly lower than that in the control group during days 1-14 (P<0.05). (2) No significant differences were found in fecal Bacillus, Lactobacillus, or E. coli counts among groups on days 14 and 35 (P>0.05). (3) No significant differences were observed in any serum parameters among groups (P>0.05). In conclusion, under the conditions of this experiment, dietary supplementation with compound probiotics and cello-oligosaccharide improved diarrhea status during days 1-14 post-weaning and enhanced feed utilization efficiency during days 15-35 post-weaning in weaned piglets.

**Keywords:** compound probiotics; cello-oligosaccharide; weaned piglets; growth performance; fecal microflora; serum parameters

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## Introduction

Weaned piglets are susceptible to weaning stress due to their underdeveloped physiological systems and changes in environmental factors. While antibiotics effectively treat early weaning syndrome caused by stress, excessive antibiotic

use enhances bacterial resistance in the gut, disrupts the original intestinal microflora balance, and can ultimately cause severe diarrhea or even death. Therefore, seeking environmentally friendly additives represents an effective approach to mitigating the negative effects of antibiotics. Currently, microecological preparations are widely used in piglet diets to improve intestinal microecological balance and alleviate early weaning stress. Probiotics are live microbial preparations and their metabolites that can improve host intestinal flora structure and enhance host health status [?]. Numerous studies have investigated the effects of single or compound probiotics on weaned piglet growth, but results remain inconsistent. Huang et al. [?] found that dietary compound probiotics significantly improved growth performance, nutrient digestibility, and immune function in weaned piglets. However, De Moraes et al. [?] reported that dietary probiotics had no significant effect on weaned piglet growth performance. Dong et al. [?] also observed no significant effects of compound probiotics on weaned piglet growth performance. These discrepancies may be related to differences in probiotic strains and dosages, dietary composition, weaning age, and environmental conditions. Therefore, further investigation of compound probiotics containing *Lactobacillus plantarum*, *Bacillus licheniformis*, and *Bacillus subtilis* on weaned piglet growth performance is warranted.

Cello-oligosaccharide is an oligosaccharide composed of 2–10 glucose molecules linked by  $\beta$ -1,4 glycosidic bonds, representing a product of cellulose degradation [?]. The  $\beta$ -1,4 glycosidic bonds in cello-oligosaccharide structure can serve as carbon sources for beneficial intestinal bacteria (such as *Bifidobacterium* and *Lactobacillus*), while *E. coli* and *Eubacterium* cannot utilize them or have extremely low metabolic utilization rates. Therefore, dietary cello-oligosaccharide can stimulate *Bifidobacterium* proliferation in the large intestine, increase lactic acid concentration, reduce colonic pH, and improve piglet growth performance. Otsuka et al. [?] confirmed that dietary cello-oligosaccharide supplementation improved growth performance in weaned piglets. Current research on probiotics and cello-oligosaccharide effects on piglet growth performance has primarily focused on individual supplementation, with few studies investigating their combined effects. Therefore, this experiment was conducted to evaluate the effects of dietary compound probiotics and cello-oligosaccharide on growth performance, fecal microflora, and serum parameters in weaned piglets, providing theoretical basis and practical reference for application of environmentally friendly additives in weaned piglet production.

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## 1.1 Experimental Materials

The compound probiotics used in this experiment contained *Lactobacillus plantarum* ( $1 \times 10^8$  CFU/g), *Bacillus licheniformis* ( $1 \times 10^7$  CFU/g), and *Bacillus subtilis* ( $1 \times 10^7$  CFU/g), provided by the Feed Research Institute of Chinese Academy of Agricultural Sciences. These strains were isolated from soil near a pig farm in Daxing District, Beijing. In vitro analysis demonstrated

that all strains could inhibit the growth of Gram-positive and Gram-negative pathogens, resist low pH and bile salts, and grow in simulated artificial gastrointestinal environments, indicating their probiotic potential.

The cello-oligosaccharide used in this experiment (purity 98%) was also provided by the Feed Research Institute of Chinese Academy of Agricultural Sciences, with cellobiose, cellotriose, and cellotetraose accounting for approximately 75% of total sugars. Cello-oligosaccharide was prepared from alfalfa hay through mixed acid degradation with  $\beta$ -glucanase addition. Since animal gastrointestinal tracts lack digestive enzymes to degrade  $\beta$ -1,4 glycosidic bonds, cello-oligosaccharide passes directly into the large intestine to be utilized by *Bifidobacterium*, exerts intestinal regulatory effects, and can be used as a dietary additive.

## 1.2 Experimental Design

This experiment employed a single-factor completely randomized design. One hundred twenty “Landrace  $\times$  Large White” crossbred weaned piglets at  $(32 \pm 4)$  days of age with an initial average body weight of  $(9.72 \pm 2.25)$  kg were randomly divided into 4 groups with 3 replicates per group and 10 piglets per replicate. The four dietary treatments were: basal diet (control group, CON), basal diet + 0.30% compound probiotics (Group A), basal diet + 0.08% cello-oligosaccharide (Group B), and basal diet + 0.30% compound probiotics + 0.08% cello-oligosaccharide (Group C). The experiment included a 4-day pre-test period followed by a 35-day formal test period, which was divided into Phase 1 (days 1-14) and Phase 2 (days 15-35).

## 1.3 Experimental Diets and Management

The basal diet was formulated according to the pig farm’s practical formulation. The composition and nutrient levels of the basal diet are presented in Table 1. All experimental piglets were housed in the same building, with each replicate in the same pen. Piglets were allowed free access to feed and water. Room temperature was maintained at approximately 25°C with good ventilation. Manure was cleaned once daily, and pens were disinfected 2-3 times weekly. Immunization and deworming were conducted according to routine farm management practices.

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

Items	Phase 1 (1 to 14 days)	Phase 2 (15 to 35 days)
<b>Ingredients</b>		
Corn	58.00	65.00
Soybean meal	20.00	24.00
Whey powder	10.00	5.00
Fish meal	5.00	2.00

Items	Phase 1 (1 to 14 days)	Phase 2 (15 to 35 days)
Soybean oil	2.50	1.50
Limestone	0.80	0.90
Premix <sup>1)</sup>	3.00	1.00
CaHPO <sub>4</sub>	0.40	0.35
NaCl	0.20	0.20
Lys	0.10	0.05
Calcium propionate	0.05	0.05
<b>Total</b>	<b>100.00</b>	<b>100.00</b>
<b>Nutrient levels<sup>2)</sup></b>		
DM	89.00	88.50
DE (MJ/kg)	14.50	14.00
CP	20.00	18.50
EE	5.50	4.50
Ash	5.50	5.00
Ca	0.85	0.75
TP	0.65	0.60
Lys	1.25	1.10

<sup>1)</sup> Premix provided the following per kg of diet: VA 13,000 IU, VD 4,500 IU, VE 60 IU, VK<sub>3</sub> 2.5 IU, VB<sub>1</sub> 3.5 mg, VB<sub>2</sub> 5.5 mg, VB<sub>6</sub> 3.3 mg, VB<sub>12</sub> 27.6 g, D-pantothenic acid 14.6 mg, nicotinic acid 30 mg, biotin 25 g, choline 500 mg, Cu 30 mg, Fe 110 mg, Mn 30 mg, Zn 110 mg, I 0.65 mg, Se 0.3 mg.

<sup>2)</sup> DE was a calculated value, while the others were measured values.

#### 1.4 Measurement Indices and Methods

**1.4.1 Growth Performance** Body weight (BW) was measured on a per-pen basis on the morning of days 1, 14, and 35 of the formal test period for calculation of average daily gain (ADG). Feed allowance was recorded daily on a per-pen basis, and residual feed was weighed before feeding the next morning for calculation of average daily feed intake (ADFI). Feed-to-gain ratio (F/G) was then calculated from ADFI and ADG. The calculation formulas were:

- $ADG = \text{total pen weight gain} / (\text{number of piglets in pen} \times \text{number of test days})$
- $ADFI = \text{total pen feed intake} / (\text{number of piglets in pen} \times \text{number of test days})$
- $F/G = ADFI / ADG$

**1.4.2 Diarrhea Rate** Diarrhea status of all experimental piglets was observed and recorded daily. Each day a piglet experienced diarrhea was recorded as one diarrhea incident. Diarrhea rate (DR) was calculated for each group after the experiment:

Diarrhea rate (%) = [total diarrhea incidents / (total number of piglets × total test days)] × 100

**1.4.3 Rectal Fecal Microbial Counts** Fecal microflora counts were determined using selective culture medium plate counting methods [?]. On the morning of days 14 and 35, one piglet per pen was randomly selected to provide fresh fecal samples, which were transported to the laboratory in a low-temperature incubator for microbial culture and counting. Nutrient agar plates, MRS plates, and MacConkey agar plates were prepared in advance. All operations were performed in a sterile hood. One gram of fecal sample was weighed and mixed with 9 mL of sterile physiological saline to prepare a 1:10 sample dilution ( $10^{-1}$  dilution), which was vortexed for 3-5 minutes. Then, 1 mL of this dilution was accurately transferred to a tube containing 9 mL of sterile physiological saline, vortexed for 1-2 minutes to prepare a  $10^{-2}$  dilution. This process was repeated sequentially to prepare  $10^{-3}$  through  $10^{-8}$  dilutions.

Fecal dilutions of  $10^{-2}$  to  $10^{-4}$  were plated on nutrient broth solid medium to count *Bacillus*;  $10^{-3}$  to  $10^{-5}$  dilutions were plated on MacConkey medium to count *E. coli*; and  $10^{-6}$  to  $10^{-8}$  dilutions were plated on MRS solid medium to count *Lactobacillus*. *E. coli* and *Bacillus* were cultured aerobically at 37°C for 20 hours, while *Lactobacillus* was cultured aerobically at 37°C for 36 hours. Two parallel plates were prepared for each dilution, and the average of two counting results was taken. Fecal microbial counts were expressed as lg(CFU/g) (logarithm of colony-forming units per gram of feces) [?].

**1.4.4 Serum Biochemical and Immune Indices** On day 35, one piglet per pen was randomly selected for blood collection (10 mL) from the anterior vena cava. Blood samples were centrifuged at 3,000 rpm for 20 minutes to separate serum, which was stored at -20°C for subsequent analysis. Serum total protein (TP), albumin (ALB), and globulin (GLB) contents, as well as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities were measured. TP content was determined by the biuret method, ALB by the bromocresol green method, SOD activity by the pyrogallol autoxidation method, and GSH-Px activity by chemical colorimetry. All measurements were performed using an automatic biochemical analyzer. GLB content was calculated as: GLB = TP - ALB.

Serum immune indices included immunoglobulin G (IgG), immunoglobulin A (IgA), and immunoglobulin M (IgM) contents, which were measured by enzyme-linked immunosorbent assay (ELISA) using a microplate reader. ELISA kits were provided by Beijing Huaying Biotechnology Research Institute, and operations were performed according to kit instructions.

## 1.5 Data Processing

Experimental data were initially processed using Excel software. Statistical analysis was performed using one-way ANOVA and Duncan's multiple comparison

test with SAS 9.1 software. Differences were considered significant at  $P < 0.05$ .

## 2.1 Effects of Compound Probiotics and Cello-Oligosaccharide on Growth Performance of Weaned Piglets

As shown in Table 2, no significant differences were observed in initial average body weight among groups ( $P > 0.05$ ). During days 1-14, no significant differences were found in ADFI, ADG, or F/G among groups ( $P > 0.05$ ). During days 15-35, the F/G in Group C was significantly lower than that in the control group ( $P < 0.05$ ), while ADFI and ADG were 11.92% and 34.31% higher than the control group, respectively.

During the entire experimental period (days 1-35), no significant differences were observed in ADFI, ADG, or F/G among groups ( $P > 0.05$ ). However, compared with the control group, Group C showed 4.38% higher ADFI, 13.76% higher ADG, and 9.09% lower F/G.

**Table 2** Effects of dietary compound probiotics and cello-oligosaccharide on growth performance of weaned piglets

Items	Groups		P-value
	CON	A	
Initial average body weight (kg)	9.72	9.71	
<b>Days 1-14</b>			
ADFI (g)	395.67	396.33	
ADG (g)	203.33	203.81	
F/G	1.95	1.95	
<b>Days 15-35</b>			
ADFI (g)	672.00	695.00	
ADG (g)	344.29	376.67	
F/G	1.95	1.85	
<b>Days 1-35</b>			
ADFI (g)	558.57	579.43	
ADG (g)	286.43	303.33	
F/G	1.95	1.91	

Values in the same row with different superscripts differ significantly ( $P < 0.05$ ).

## 2.2 Effects of Compound Probiotics and Cello-Oligosaccharide on Fecal Microflora of Weaned Piglets

Table 3 shows that no significant differences were observed in fecal *Bacillus*, *Lactobacillus*, or *E. coli* counts among groups on days 14 and 35 ( $P > 0.05$ ). On day 14, *Bacillus* counts in Groups A, B, and C were 0.19%, 4.59%, and 13.77%

higher than the control group, respectively, while *E. coli* counts were 12.96%, 13.29%, and 15.78% lower than the control group, respectively. On day 35, *Bacillus* counts in Groups A and B were 6.95% and 13.68% higher than the control group, respectively, while *E. coli* counts in Groups A, B, and C were 13.05%, 14.20%, and 16.89% lower than the control group, respectively.

**Table 3** Effects of dietary compound probiotics and cello-oligosaccharides on fecal microflora of weaned piglets (lg CFU/g)

Items	Groups		P-value
	CON	A	
<b>Day 14</b>			
<i>Bacillus</i>	5.26	5.27	
<i>Lactobacillus</i>	7.08	7.15	
<i>E. coli</i>	6.48	5.64	
<b>Day 35</b>			
<i>Bacillus</i>	5.90	6.31	
<i>Lactobacillus</i>	7.48	7.49	
<i>E. coli</i>	6.36	5.53	

### 2.3 Effects of Compound Probiotics and Cello-Oligosaccharide on Diarrhea Rate of Weaned Piglets

As shown in Table 4, during days 1-14, the diarrhea rate in Groups A and C was significantly lower than that in the control group ( $P < 0.05$ ), with reductions of 73.88% and 67.28%, respectively. Group B showed a 40.90% reduction compared with the control group, but the difference was not significant ( $P > 0.05$ ). During days 15-35, Groups A and C showed 49.62% and 44.36% reductions in diarrhea rate compared with the control group, respectively, but these differences were not significant ( $P > 0.05$ ). During the entire experimental period (days 1-35), Groups A, B, and C showed 71.88%, 13.89%, and 62.91% reductions in diarrhea rate compared with the control group, respectively, but these differences were not significant ( $P > 0.05$ ).

Figure 1 [Figure 1: see original paper] illustrates that the diarrhea rate in the control group was higher than that in all treatment groups, with Group A showing the lowest rate. Diarrhea rates in all groups increased to varying degrees during days 1-7 post-weaning, showed a stable decreasing trend during days 7-19, and rarely occurred after day 19.

**Table 4** Effects of dietary compound probiotics and cello-oligosaccharides on diarrhea rate of weaned piglets (%)

Items	Groups		P-value
	CON	A	

Items	Groups	P-value
Days 1-14	19.56	5.11
Days 15-35	3.17	1.60
Days 1-35	9.12	2.57

**Figure 1** Diarrhea rate of weaned piglets in different groups

## 2.4 Effects of Compound Probiotics and Cello-Oligosaccharide on Serum Parameters of Weaned Piglets

Table 5 shows that no significant differences were observed in serum biochemical indices among groups ( $P>0.05$ ). Compared with the control group, Groups A, B, and C showed 1.48%, 8.37%, and 0.17% higher serum TP content, respectively; 7.07%, 16.72%, and 2.59% higher ALB content, respectively; and 11.26%, 16.90%, and 2.82% higher ALB/GLB ratio, respectively. Groups B and C showed 15.93% and 2.29% higher SOD activity, respectively, while Group C showed 15.36% higher GSH-Px activity compared with the control group.

**Table 5** Effects of dietary compound probiotics and cello-oligosaccharides on serum biochemical parameters of weaned piglets

Items	Groups	P-value
	CON	A
TP (g/L)	52.98	53.76
ALB (g/L)	28.86	30.90
GLB (g/L)	24.12	22.86
ALB/GLB	1.20	1.33
SOD (U/mL)	179.76	182.31
GSH-Px (U/mL)	742.86	748.57

Table 6 shows that no significant differences were observed in serum immune indices among groups ( $P>0.05$ ). Groups A and C showed 4.00% and 9.39% higher serum IgM content, respectively, while Groups A and B showed 0.46% and 0.50% higher IgG content, respectively, compared with the control group.

**Table 6** Effects of dietary compound probiotics and cello-oligosaccharides on serum immune parameters of weaned piglets

Items	Groups	P-value
	CON	A
IgM (g/mL)	156.00	162.25
IgG (g/mL)	1,301.33	1,307.33
IgA (g/mL)	292.00	292.00

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Items	Groups	P-value
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### 3.1 Effects of Compound Probiotics and Cello-Oligosaccharide on Growth Performance of Weaned Piglets

Weaning stress disrupts gastrointestinal microflora balance in piglets, ultimately inhibiting their growth and development. Probiotics are biological preparations containing live bacteria and their metabolites that can effectively alleviate the decline in immune function caused by early weaning stress by promoting colonization of beneficial intestinal bacteria and optimizing intestinal microflora [?]. The  $\beta$ -1,4 glycosidic bonds in cello-oligosaccharide structure can serve as carbon sources for beneficial intestinal bacteria, which ferment cello-oligosaccharide to produce short-chain fatty acids, reduce intestinal pH, inhibit growth and reproduction of harmful and pathogenic bacteria, and ultimately improve pig growth performance [?].

Current literature reports inconsistent results regarding the effects of compound probiotics and cello-oligosaccharide on weaned piglet growth performance. Wang et al. [?] found that dietary supplementation with probiotics and oligosaccharides increased ADG, feed utilization, and feed intake by 14.17%, 8.88%, and 4.04%, respectively, while reducing diarrhea rate by 42.56% in weaned piglets. However, Shi et al. [?] reported that dietary *Enterococcus faecium* supplementation had no significant effects on ADFI, ADG, or F/G in weaned piglets during days 1-14. Yu et al. [?] also found no significant effects of dietary probiotics on ADG or F/G in weaned piglets. Jiao et al. [?] reported that dietary cello-oligosaccharide supplementation did not significantly affect growth performance in weaned piglets. Days 1-14 post-weaning represent a critical period when piglets gradually adapt to weaning and when intestinal microflora is most unstable, making this the optimal period for probiotic application [?]. In the current experiment, no significant differences were observed in ADFI, ADG, or F/G among groups during days 1-14, which contradicts the findings of Wang et al. [?] and Estienne et al. [?]. These discrepancies may be attributed to reduced feed and water intake caused by weaning stress, leading to decreased small intestinal absorption function and inhibited growth performance [?]. Alternatively, the dosages of compound probiotics and cello-oligosaccharide may have been insufficient to exhibit probiotic effects during days 1-14 post-weaning. Additionally, differences in weaning age and management practices may have contributed to inconsistent results [?]. During days 15-35, no significant differences were observed in ADFI or ADG among groups, but Group C showed 11.92% higher ADFI and 34.31% higher ADG compared with the control group, with significantly lower F/G. Fang et al. [?] reported that combined supplementation of probiotics and oligosaccharides in weaned piglet diets produced more pronounced growth-promoting effects than

individual supplementation. These results suggest that piglet gastrointestinal tracts gradually adapted to compound probiotics and cello-oligosaccharide supplementation during days 15–35, with both individual and combined supplementation promoting growth, but the combined supplementation showing better efficacy and synergistic complementary effects.

### 3.2 Effects of Compound Probiotics and Cello-Oligosaccharide on Fecal Microflora and Diarrhea Rate of Weaned Piglets

During the suckling period, piglet intestinal microflora maintains dynamic equilibrium, but weaning stress reduces *Lactobacillus* counts and increases *E. coli* counts [?]. Bon et al. [?] reported that dietary supplementation with *Saccharomyces cerevisiae* and *Pediococcus acidilactici* significantly reduced fecal *E. coli* counts, consistent with findings by Hou [?] and Mallo et al. [?]. Additionally, oligosaccharides can promote proliferation of beneficial intestinal bacteria and improve the intestinal microenvironment [?]. Yang [?] reported that fructooligosaccharide, isomaltooligosaccharide, and mannanoligosaccharide significantly promoted proliferation of *Bifidobacterium* and *Lactobacillus acidophilus* while significantly inhibiting growth of *Escherichia coli* and *Staphylococcus* in piglet intestines. The current results showed that *Bacillus* counts in Groups A, B, and C were higher than those in the control group (except Group C on day 35), while *E. coli* counts were lower than those in the control group, but these differences were not significant. These findings indicate that probiotic and cello-oligosaccharide supplementation did not significantly alter fecal *Bacillus*, *Lactobacillus*, or *E. coli* counts, which is inconsistent with previous studies. The discrepancies may be related to probiotic species, bacterial counts, and interactions between cello-oligosaccharide and probiotics.

Weaned piglets are highly susceptible to intestinal microflora disturbances caused by psychological, dietary, and environmental stresses, leading to proliferation of harmful bacteria and diarrhea. The current results demonstrated that during the entire experimental period (days 1–35), Groups A, B, and C showed 71.88%, 13.89%, and 62.91% reductions in diarrhea rate compared with the control group, while differences in ADFI, ADG, and F/G among groups were relatively small. Wang et al. [?] reported that *Bacillus licheniformis* reduced diarrhea rate to some extent but did not show growth-promoting effects, similar to findings by Dong et al. [?]. These results suggest that additives such as probiotics and oligosaccharides have more pronounced effects on improving intestinal microecology and health status than on growth promotion. *Lactobacillus* and coliform bacteria are considered primary indicators of intestinal health [?]. The current experiment showed that probiotic and oligosaccharide supplementation did not significantly affect fecal microflora (*Bacillus*, *Lactobacillus*, and *E. coli*) but reduced diarrhea rate in piglets, suggesting that fecal microflora as an indicator of intestinal microbial balance and health requires further investigation.

### 3.3 Effects of Compound Probiotics and Cello-Oligosaccharide on Serum Parameters of Weaned Piglets

Blood is an important component of the internal environment in animals. Changes in nutrient metabolism, tissue organ function, and pathological conditions often cause alterations in serum biochemical indices, making blood component detection clinically significant [?]. Serum TP consists of serum ALB and GLB, and its content primarily reflects liver protein synthesis capacity. Serum ALB plays an important role in liver protein metabolism, while serum GLB increases with blood antibody levels, and its content reflects animal immune status and physiological condition to some extent [?]. The ALB/GLB ratio can measure immune status, with decreased ratios indicating increased GLB synthesis and improved immune function. SOD is an active substance that eliminates harmful substances produced during animal metabolism [?]. GSH-Px is a ubiquitous peroxide-degrading enzyme in animals that protects cell membrane structure and function from peroxide damage. The current results showed no significant differences in serum TP, ALB, GLB contents or SOD and GSH-Px activities among groups, but Groups A, B, and C showed higher ALB/GLB ratios than the control group, while Groups B and C showed higher SOD activity and Group C showed higher GSH-Px activity. These results indicate that dietary supplementation with compound probiotics and cello-oligosaccharide in weaned piglets not only helps eliminate harmful substances produced during growth but also improves immune function, consistent with other studies [?].

Serum immunoglobulins are important components of the humoral immune system [?]. IgG is the primary antibody in humoral immune responses, IgM is the antibody produced in the initial stage of immune responses, and IgA is the main antibody in mucosal immunity, playing an important role in reducing pathogens with assistance from non-specific immune defense mechanisms [?]. The current results showed no significant differences in serum IgM, IgG, or IgA contents among groups. However, You et al. [?] reported that sodium butyrate and mannanoligosaccharide promoted immune function in weaned piglets, with the best effects observed at 0.10% sodium butyrate + 0.10% mannanoligosaccharide, which is inconsistent with the current results. The discrepancy may be due to insufficient dosages of compound probiotics and cello-oligosaccharide in the current experiment to significantly affect serum immune indices. Nevertheless, Groups A and C showed 4.00% and 9.39% higher serum IgM content, respectively, while Groups A and B showed 0.46% and 0.50% higher IgG content, respectively, compared with the control group. These effects may be related to *Lactobacillus* acting as an immune adjuvant after colonizing the intestine, affecting non-specific immune responses and stimulating specific immune reactions [?].

The compound probiotics and cello-oligosaccharide used in this experiment are microecological preparations that function by establishing a healthy intestinal microflora balance system to improve animal immune function, which is a com-

plex biological process. The growth-promoting effects of microecological preparations may require time to manifest and may be more evident in older animals.

Under the conditions of this experiment, dietary supplementation with compound probiotics and cello-oligosaccharide had no significant effects on ADFI, ADG, fecal microflora counts, or serum parameters in weaned piglets, but improved diarrhea status during days 1-14 post-weaning and enhanced feed utilization efficiency during days 15-35 post-weaning.

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