Effects of Dietary Colistin Supplementation on Growth Performance and Serum Biochemical Parameters in Weaned Piglets (Postprint)

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Abstract

This experiment aimed to investigate the application effects of enterobacterin peptide in weaned piglet production. A total of 144 healthy "Duroc × Landrace × Large White" crossbred weaned piglets at 28 days of age with an average body weight of (7.35±0.21) kg were selected and randomly divided into 6 groups, with 4 replicates per group and 6 piglets per replicate (half male and half female). Piglets in the control group were fed a basal diet, while those in the experimental groups were fed experimental diets supplemented with colistin sulfate (30 g/t), cecropin antimicrobial peptide (300 g/t), and enterobacterin peptide (300, 400, and 500 g/t) based on the basal diet. The experimental period lasted 28 days and was divided into two phases: the early phase (days 1–14) and the late phase (days 15–28). The results showed: 1) During the early phase, late phase, and entire experimental period, the average daily gain (ADG) of the 400 and 500 g/t enterobacterin peptide groups was significantly higher than that of the control group ($P<0.05$), and the feed-to-gain ratio was significantly lower than that of the control group ($P<0.05$). On days 7, 14, and 28 of the experiment, the diarrhea rate of each experimental group was extremely significantly lower than that of the control group ($P<0.01$), with the 500 g/t enterobacterin peptide group being the lowest. 2) On day 14 of the experiment, the serum urea nitrogen (UN) content of the 500 g/t enterobacterin peptide group was significantly lower than that of the control group and the 30 g/t colistin sulfate group ($P<0.05$); on day 28, the serum UN content of the 400 and 500 g/t enterobacterin peptide groups was significantly lower than that of the control group ($P<0.05$), and the 500 g/t enterobacterin peptide group was also significantly lower than the 30 g/t colistin sulfate group ($P<0.05$). On days 14 and 28 of the experiment, the serum globulin (GLB) content of the 400 and 500 g/t enterobacterin peptide groups was significantly higher than that of the control group ($P<0.05$). 3) On days 14 and 28 of the experiment, the serum immunoglobulin G (IgG) content

of the 500 g/t enterobacterin peptide group was 17.27% and 16.20% higher than that of the control group, respectively (P<0.05). On day 14 of the experiment, the serum complement 4 (C4) content of each antimicrobial peptide group was significantly higher than that of the control group (P<0.05) and the 30 g/t colistin sulfate group (P<0.05). 4) On days 14 and 28 of the experiment, the serum malondialdehyde (MDA) content of each antimicrobial peptide group was extremely significantly lower than that of the control group (P<0.01) and the 30 g/t colistin sulfate group (P<0.01). On day 28 of the experiment, the serum superoxide dismutase (SOD) activity and total antioxidant capacity (T-AOC) of the 500 g/t enterobacterin peptide group were significantly higher than those of the control group and the 30 g/t colistin sulfate group (P<0.05). In conclusion, enterobacterin peptide is superior to cecropin antimicrobial peptide and the antibiotic colistin sulfate in improving the growth performance, immune performance, and antioxidant capacity of weaned piglets, and the appropriate supplementation level of enterobacterin peptide in the diet is 500 g/t.

Full Text

Effects of Enterobacitracin Supplementation on Performance and Serum Biochemical Indices of Weaning Piglets

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Abstract

This experiment was conducted to investigate the application effects of enterobacitracin in weaning piglet production. A total of 144 healthy "Duroc × Landrace × Large White" crossbred weaning piglets at 28 days of age with an average body weight of (7.35±0.21) kg were randomly allocated to six groups, each consisting of four replicates with six piglets per replicate (half male and half female). Piglets in the control group were fed a basal diet, while those in the experimental groups were fed the basal diet supplemented with colistin sulfate (30 g/t), cecropin (300 g/t), or enterobacitracin (300, 400, and 500 g/t). The 28-day trial period was divided into two phases: days 1–14 (early period)

and days 15–28 (late period). The results showed: (1) During the early period, late period, and overall experimental period, the average daily gain (ADG) of piglets in the 400 and 500 g/t enterobacitracin groups was significantly higher than that of the control group ($P<0.05$), while their feed-to-gain ratio (F/G) was significantly lower ($P<0.05$). On days 7, 14, and 28, the diarrhea rate of all experimental groups was extremely significantly lower than that of the control group ($P<0.01$), with the 500 g/t enterobacitracin group showing the lowest rate. (2) On day 14, the serum urea nitrogen (UN) content in the 500 g/t enterobacitracin group was significantly lower than that in both the control group and the 30 g/t colistin sulfate group ($P<0.05$). On day 28, the serum UN content in the 400 and 500 g/t enterobacitracin groups was significantly lower than that in the control group ($P<0.05$), and the 500 g/t group was also significantly lower than the 30 g/t colistin sulfate group ($P<0.05$). On both days 14 and 28, the serum globulin (GLB) content in the 400 and 500 g/t enterobacitracin groups was significantly higher than that in the control group ($P<0.05$). (3) On days 14 and 28, the serum immunoglobulin G (IgG) content in the 500 g/t enterobacitracin group was 17.27% and 16.20% higher than that in the control group, respectively ($P<0.05$). On day 14, the serum complement 4 (C4) content in all antimicrobial peptide groups was significantly higher than that in both the control group and the 30 g/t colistin sulfate group ($P<0.05$). (4) On days 14 and 28, the serum malondialdehyde (MDA) content in all antimicrobial peptide groups was extremely significantly lower than that in both the control group and the 30 g/t colistin sulfate group ($P<0.01$). On day 28, the serum superoxide dismutase (SOD) activity and total antioxidant capacity (T-AOC) in the 500 g/t enterobacitracin group were significantly higher than those in both the control group and the 30 g/t colistin sulfate group ($P<0.05$). In conclusion, enterobacitracin is superior to cecropin and the antibiotic colistin sulfate in improving the performance, immune function, and antioxidant capacity of weaning piglets, with an optimal dietary supplementation level of 500 g/t.

Keywords: enterobacitracin; weaning piglets; performance; immune function; antioxidant capacity

Introduction

Dietary antibiotic supplementation has been proven effective in alleviating weaning stress and promoting growth in piglets, as confirmed by numerous domestic and international studies. However, long-term antibiotic use has led to increasing drug resistance, posing growing problems for human health. Consequently, many countries have issued bans on antibiotic use. According to Announcement No. 2428 by China' s Ministry of Agriculture, colistin sulfate was prohibited as a growth promoter in feed additives starting November 1, 2016. Globally, the rise of resistant bacteria and the ban on antibiotic growth promoters have made the search for alternatives imperative [1].

Antimicrobial peptides are small peptide substances widely found in natural organisms. They exhibit stable structures, broad antimicrobial spectra, heat resistance, and low potential for inducing resistance in target bacterial strains. These peptides play crucial roles in innate immune systems and hold promising applications in medicine, animal husbandry, food processing, and agriculture [2]. Due to their low resistance potential, antimicrobial peptides are considered ideal antibiotic substitutes [1-3]. Cecropin exhibits broad-spectrum bactericidal activity with low resistance development, showing wide application prospects in animals, plants, and microorganisms. However, research on antimicrobial peptides faces several challenges, including insensitivity of some bacteria, difficulties in large-scale production, susceptibility to protease degradation in vivo, and limited toxicological and pharmacological studies [4].

Enterobacitracin is an antimicrobial peptide produced efficiently in *Lactobacillus plantarum* using protein engineering technology. It possesses strong antibacterial activity and low resistance potential, featuring a lasso-like structure that provides exceptional stability. It exhibits potent bactericidal and inhibitory effects against *Escherichia coli*, *Salmonella*, and *Shigella*, with no hemolytic activity, making it significant for human and animal resistance against pathogenic bacteria. Enterobacitracin has been applied in livestock and poultry production, with numerous trials demonstrating its ability to improve animal performance [5]. As research on antimicrobial peptides has progressed, increasing numbers of bacteria have been found to develop resistance to them [2]. Currently, studies on bacterial resistance to antimicrobial peptides remain at the laboratory stage, while extensive clinical trials have shown that resistance induced by antimicrobial peptides is significantly milder than that caused by conventional antibiotics [6]. Decades of intensive research have revealed that different antimicrobial peptides exhibit significantly different antibacterial mechanisms, and no universal mechanism has been identified that can generalize all antimicrobial peptides [2,7]. Research on antimicrobial peptides as feed additives for livestock and poultry remains in its early stages.

Numerous reports have documented the application of antimicrobial peptides as antibiotic alternatives in weaning piglets, but studies on enterobacitracin—a novel antimicrobial peptide—remain limited. Therefore, this experiment used weaning piglets as subjects to investigate the effects of dietary enterobacitracin supplementation at different levels on performance and serum biochemical indices, aiming to identify optimal supplementation levels and provide scientific basis for its rational use.

Materials and Methods

1.1 Experimental Materials

The antibiotic used in this experiment was colistin sulfate, purchased commercially. Antimicrobial peptides, including cecropin and enterobacitracin, were

provided by Beijing Zhongnong Yingtai Company.

1.2 Experimental Design

One hundred forty-four 28-day-old "Duroc × Landrace × Large White"crossbred weaning piglets with consistent genetic background, good health status, and an average body weight of (7.35±0.21) kg were selected as experimental animals. Using a single-factor experimental design, the piglets were randomly divided into six groups, each comprising four replicates with six piglets per replicate (half male and half female). Replicates were balanced for body weight (P>0.05). The trial included a 7-day pre-test period followed by a 28-day formal test period. To better validate the feeding effects of enterobacitracin, comparisons were made by adding colistin sulfate and cecropin to the basal diet. Details of group allocation and supplementation levels are presented in Table 1 .

Table 1 Experimental Design

Items	Control Group	30 g/t Colistin Sulfate Group	300 g/t Cecropin Group	300 g/t Enterobacitracin Group	400 g/t Enterobacitracin Group	500 g/t Enterobacitracin Group
Colistin Sulfate (g/t)	0	30	0	0	0	0
Cecropin (g/t)	0	0	300	0	0	0
Enterobacitracin (g/t)	0	0	0	300	400	500

1.3 Basal Diet

The basal diet was formulated according to NRC (1998) standards. Its composition and nutrient levels are shown in Table 2 .

Table 2 Composition and Nutrient Levels of the Basal Diet (Air-Dry Basis)

Items	Content
Ingredients	
Corn	52.00
Rice bran meal	5.00
Broken rice	10.00
High protein soybean meal	15.00
Extruded soybean meal	5.00

Items	Content
Fermented soybean meal	3.00
Imported fish meal	2.50
Whey powder	4.00
Glucose	1.00
Sucrose	1.00
Soybean oil	1.00
Limestone	0.80
Ca(H PO) ·H O	0.80
NaCl	0.30
Premix[1]	0.50
Lysine	0.30
Methionine	0.10
Threonine	0.10
Choline chloride	0.10
Total	**100.00**
Nutrient Levels[2]	
Digestible Energy (MJ/kg)	14.23
Crude Protein (%)	20.50
Crude Fat (%)	4.50
Crude Fiber (%)	3.50
Crude Ash (%)	5.80
Lysine (%)	1.35
Methionine (%)	0.40
Methionine + Cysteine (%)	0.70
Available Phosphorus (%)	0.45

[1]The premix provided the following per kg of diet: Fe 80 mg, Cu 12 mg, Zn 75 mg, Mn 10 mg, I 0.35 mg, Se 0.30 mg, VA 1,500 IU, VD 3,300 IU, VE 60 IU, VK 6.0 mg, riboflavin 6.2 mg, nicotinic acid 60 mg, D-pantothenic acid 18.0 mg, VB 0.03 mg, biotin 0.46 mg.
[2]Nutrient levels were calculated values.

1.4 Animal Management

Piglets were housed in nursery pens with room temperature maintained at 25–28°C and humidity at 55–65%. Powdered feed was provided three times daily with ad libitum access to feed and water. Other routine management procedures followed standard farm protocols. Feed intake, diarrhea incidence, and mental status were observed and recorded daily.

1.5 Measurements

1.5.1 Performance Metrics Piglets were weighed after fasting on days 1, 14, and 28. Daily feed intake per group was recorded, and diarrhea incidence was

documented on days 7, 14, and 28. Average daily gain (ADG), average daily feed intake (ADFI), feed-to-gain ratio (F/G), and diarrhea rate were calculated.

1.5.2 Serum Biochemical Indices On days 14 and 28, after fasting and weighing, one piglet near the average body weight was selected from each replicate per group. Blood samples (10 mL) were collected via anterior vena cava puncture, centrifuged to obtain serum, and stored at -20°C for analysis. Serum urea nitrogen (UN), total protein (TP), albumin (ALB), and globulin (GLB) were analyzed using a Shenzhen Mindray BS-420 automatic biochemical analyzer with reagent kits from Zhongsheng Beikong Co., Ltd. Serum immunoglobulin G (IgG), complement 4 (C4), malondialdehyde (MDA) content, glutathione peroxidase (GSH-Px) activity, superoxide dismutase (SOD) activity, and total antioxidant capacity (T-AOC) were analyzed using a Beijing Songshang A6 semi-automatic biochemical analyzer with reagent kits from Beijing Huaying Biotechnology Research Institute.

1.6 Statistical Analysis

Experimental data were analyzed using one-way ANOVA in SPSS 17.0 software. Duncan' s multiple comparison test was used for post-hoc analysis. Differences were considered significant at $P<0.05$ and extremely significant at $P<0.01$. Results are expressed as "mean ± standard deviation."

Results

2.1 Effects of Enterobacitracin Supplementation on Growth Performance of Weaning Piglets

As shown in Table 3 , during the early period (days 1-14), ADG in all enterobacitracin groups was significantly higher than that in the control group ($P<0.05$), while ADG in the 30 g/t colistin sulfate and 300 g/t cecropin groups showed no significant change ($P>0.05$). During the late period (days 15-28) and the overall experimental period (days 1-28), ADG in the 400 and 500 g/t enterobacitracin groups was significantly higher than that in the control group ($P<0.05$), with no significant differences among other groups ($P>0.05$).

During both the early and late periods, ADFI did not differ significantly among groups ($P>0.05$). Over the entire experimental period, ADFI in all enterobacitracin groups was higher than that in the control group, but the difference was not significant ($P>0.05$).

During the early and late periods, F/G did not differ significantly among groups ($P>0.05$). However, over the entire experimental period, although all experimental groups showed reduced F/G compared with the control group, only the 400 and 500 g/t enterobacitracin groups showed significant reductions ($P<0.05$), decreasing by 7.61% and 8.15%, respectively.

On days 7, 14, and 28, the diarrhea rate of all experimental groups was extremely significantly lower than that of the control group (P<0.01), with the 500 g/t enterobacitracin group showing the lowest rate. On day 7, the diarrhea rate of the 500 g/t enterobacitracin group was 10.88%, which was 30.21% (P<0.01), 17.14% (P<0.01), and 14.40% (P<0.01) lower than that of the control, 30 g/t colistin sulfate, and 300 g/t cecropin groups, respectively. On day 14, the diarrhea rate of the 500 g/t enterobacitracin group was 4.71%, which was 54.23% (P<0.01), 43.53% (P<0.01), and 18.65% (P<0.05) lower than that of the control, 30 g/t colistin sulfate, and 300 g/t cecropin groups, respectively. On day 28, the diarrhea rate of the 500 g/t enterobacitracin group was extremely significantly lower than all other groups (P<0.01). Additionally, the diarrhea rates of the 300 and 400 g/t enterobacitracin groups were significantly lower than that of the 30 g/t colistin sulfate group (P<0.05), with no significant difference from the 300 g/t cecropin group (P>0.05).

Table 3 Effects of Enterobacitracin Supplementation on Growth Performance of Weaning Piglets

Items	Control Group	30 g/t Colistin Sulfate Group	300 g/t Cecropin Group	300 g/t Enterobacitracin Group	400 g/t Enterobacitracin Group	500 g/t Enterobacitracin Group
ADG (g)						
Days 1-14	239.17±28.20	247.00±18.43	261.23±15.26	266.14±14.27	274.21±17.18	276.06±15.03
Days 15-28	437.18±48.24	448.29±18.15	447.23±19.26	465.31±18.47	468.29±19.50	471.38±17.93
Days 1-28	338.17±38.23	347.30±18.29	354.23±17.18	365.73±16.35	371.25±18.33	373.73±16.45
ADFI (g)						
Days 1-14	438.99±43.91	439.81±14.87	454.83±11.75	450.74±12.50	457.46±15.96	454.45±12.06
Days 15-28	807.56±79.67	798.78±12.77	793.96±14.68	814.29±12.61	811.41±12.49	808.18±13.72
Days 1-28	623.27±61.90	618.95±13.55	624.40±13.23	632.52±12.55	634.44±14.23	631.32±12.70
F/G (%)						

Items	Control Group	30 g/t Colistin Sulfate Group	300 g/t Cecropin Group	300 g/t Enterobacitracin Group	400 g/t Enterobacitracin Group	500 g/t Enterobacitracin Group
Days 1-14	1.84±0.0	1.79±0.10	1.75±0.14	1.70±0.13	1.68±0.16	1.65±0.12
Days 15-28	1.85±0.0	1.80±0.05	1.78±0.11	1.75±0.10	1.74±0.10	1.72±0.08
Days 1-28	1.84±0.0	1.79±0.06	1.76±0.07	1.73±0.09	1.70±0.05	1.69±0.09
Diarrhea Rate (%)						
Day 7	15.59±1.2	12.13±0.21	12.71±0.42	12.21±0.93	11.77±0.31	10.88±0.16
Day 14	10.29±0.9	8.84±0.52	5.79±0.54	5.75±0.32	5.33±0.41	4.71±0.34
Day 28	7.33±0.7	6.17±0.56	1.59±0.10	1.37±0.09	1.21±0.08	0.00±0.00

In the same row, values with different capital letter superscripts indicate extremely significant differences (P<0.01), different small letter superscripts indicate significant differences (P<0.05), and same small letters or no letters indicate no significant difference (P>0.05). The same notation applies to subsequent tables.

2.2 Effects of Enterobacitracin Supplementation on Serum Protein Synthesis Indices

As shown in Table 4 , on day 14, serum UN content in the 30 g/t colistin sulfate, 300 g/t cecropin, and 300 and 400 g/t enterobacitracin groups did not differ significantly from the control group (P>0.05), but the 500 g/t enterobacitracin group showed significantly lower serum UN content than both the control and 30 g/t colistin sulfate groups (P<0.05). On day 28, serum UN content in the 30 g/t colistin sulfate, 300 g/t cecropin, and 300 g/t enterobacitracin groups did not differ significantly from the control group (P>0.05), but the 400 and 500 g/t enterobacitracin groups were significantly lower than the control group (P<0.05), with the 500 g/t group also being significantly lower than the 30 g/t colistin sulfate group (P<0.05). Overall, enterobacitracin demonstrated superior effects in reducing serum UN content compared with colistin sulfate and cecropin, with the 500 g/t enterobacitracin group showing the best results.

On days 14 and 28, serum TP and ALB contents did not differ significantly among groups (P>0.05), though all experimental groups showed varying degrees of increase compared with the control group, with the 500 g/t enterobacitracin group showing the greatest increase.

On days 14 and 28, serum GLB content in the 400 and 500 g/t enterobacitracin groups was significantly higher than that in the control group (P<0.05), with no significant differences among other groups (P>0.05). The 500 g/t enterobacitracin group exhibited the highest serum GLB content.

Table 4 Effects of Enterobacitracin Supplementation on Serum Protein Synthesis Indices of Weaning Piglets

Items	Control Group	30 g/t Colistin Sulfate Group	300 g/t Cecropin Group	300 g/t Enterobacitracin Group	400 g/t Enterobacitracin Group	500 g/t Enterobacitracin Group
UN (mmol/L)						
Day 14	4.36±0.14	4.32±0.21	4.16±0.29	4.15±0.35	3.97±0.24	3.87±0.28
Day 28	3.35±0.35	3.29±0.26	3.02±0.27	2.93±0.23	2.87±0.24	2.66±0.24
TP (g/L)						
Day 14	60.81±3.63	63.71±4.35	64.21±4.78	64.96±4.46	65.17±4.71	66.49±4.95
Day 28	63.49±3.65	65.24±4.03	66.01±4.05	66.61±4.32	68.32±4.57	69.21±4.23
ALB (g/L)						
Day 14	39.10±1.39	39.90±2.07	39.80±2.33	40.41±2.07	39.59±2.32	40.66±2.41
Day 28	40.31±2.44	40.87±2.40	41.10±2.52	41.32±2.75	42.40±2.85	42.87±2.52
GLB (g/L)						
Day 14	21.71±2.22	22.81±2.29	24.41±2.45	24.55±2.39	25.58±2.39	25.84±2.55
Day 28	23.18±1.25	24.37±1.63	24.91±1.54	25.29±1.57	25.92±1.72	26.34±1.71

2.3 Effects of Enterobacitracin Supplementation on Serum Immune Indices

As shown in Table 5 , on days 14 and 28, serum IgG content in the 500 g/t enterobacitracin group was 17.27% and 16.20% higher than that in the control group, respectively (P<0.05), with no significant differences among other groups (P>0.05).

On day 14, serum C4 content in all antimicrobial peptide groups was significantly higher than that in both the control group and the 30 g/t colistin sulfate group (P<0.05), though no significant differences were observed among the antimicrobial peptide groups themselves (P>0.05). On day 28, serum C4 content did not differ significantly among groups (P>0.05), with the 500 g/t enterobacitracin group showing the highest value.

Table 5 Effects of Enterobacitracin Supplementation on Serum Immune Indices of Weaning Piglets

Items	Control Group	30 g/t Colistin Sulfate Group	300 g/t Cecropin Group	300 g/t Enterobacitracin Group	400 g/t Enterobacitracin Group	500 g/t Enterobacitracin Group
IgG						
Day 14	4.17±0.38	4.23±0.36	4.38±0.40	4.37±0.39	4.60±0.43	4.89±0.45
Day 28	4.63±0.35	4.81±0.42	5.17±0.45	5.19±0.46	5.31±0.47	5.38±0.48
C4						
Day 14	0.082±0.006	0.093±0.006	0.095±0.007	0.095±0.008	0.095±0.007	0.099±0.010
Day 28	0.145±0.004	0.147±0.004	0.149±0.004	0.151±0.005	0.153±0.013	0.160±0.021

2.4 Effects of Enterobacitracin Supplementation on Serum Antioxidant Indices

As shown in Table 6 , on days 14 and 28, serum GSH-Px activity did not differ significantly among groups (P>0.05), though the 500 g/t enterobacitracin group showed the highest activity at 570.00 and 602.00 U/mL, respectively.

On days 14 and 28, serum MDA content in all experimental groups was lower than that in the control group. Specifically, all antimicrobial peptide groups showed extremely significantly lower MDA content than both the control group and the 30 g/t colistin sulfate group (P<0.01), with no significant differences among the antimicrobial peptide groups themselves (P>0.05). The 500 g/t enterobacitracin group exhibited the lowest serum MDA content.

On day 14, serum SOD activity did not differ significantly among groups (P>0.05). On day 28, compared with the control group, the 30 g/t colistin sulfate and 300 g/t cecropin groups showed no significant improvement in serum SOD activity (P>0.05), while all enterobacitracin groups showed significant increases (P<0.05). Furthermore, the 500 g/t enterobacitracin group was significantly higher than the 30 g/t colistin sulfate group (P<0.05).

On day 14, serum T-AOC did not differ significantly among groups (P>0.05). On day 28, serum T-AOC in the 500 g/t enterobacitracin group was 19.45% (P<0.05), 17.38% (P<0.05), and 8.15% (P>0.05) higher than that in the control, 30 g/t colistin sulfate, and 300 g/t cecropin groups, respectively. No significant differences in serum T-AOC were observed among antimicrobial peptide groups (P>0.05).

Table 6 Effects of Enterobacitracin Supplementation on Serum Antioxidant Indices of Weaning Piglets

Items	Control Group	30 g/t Colistin Sulfate Group	300 g/t Cecropin Group	300 g/t Enterobacitracin Group	400 g/t Enterobacitracin Group	500 g/t Enterobacitracin Group
GSH-Px (U/mL)						
Day 14	512.31±37.90	527.00±49.91	510.55±50.48	542.83±29.43	558.32±53.11	570.00±55.99
Day 28	529.00±48.25	548.50±42.25	580.00±48.59	598.00±55.02	602.00±53.89	602.00±53.89
MDA (nmol/mL)						
Day 14	5.09±0.46	4.80±0.33	4.11±0.37	4.12±0.34	4.00±0.38	3.72±0.32
Day 28	3.71±0.32	3.99±0.28	3.09±0.24	2.95±0.23	2.80±0.21	2.80±0.21
SOD (U/mL)						
Day 14	34.57±3.39	39.88±2.95	36.37±3.24	36.97±3.50	38.42±3.38	39.55±3.53
Day 28	41.33±3.42	42.01±2.18	44.13±3.31	44.53±1.41	45.63±1.43	45.63±1.43
T-AOC (U/mL)						
Day 14	6.11±0.33	6.09±0.52	6.46±0.52	6.46±0.50	6.68±0.58	6.87±0.60

Items	Control Group	30 g/t Colistin Sulfate Group	300 g/t Cecropin Group	300 g/t Enterobacitracin Group	400 g/t Enterobacitracin Group	500 g/t Enterobacitracin Group
Day 28	6.22±0.47	7.33±0.58	6.87±0.67	6.89±0.61	7.22±0.70	7.43±0.90

Discussion

3.1.1 Effects of Enterobacitracin Supplementation on Growth Performance of Weaning Piglets

Colistin sulfate is a basic polypeptide antibiotic primarily used to prevent and treat diarrhea caused by pathogenic bacteria, with additional growth-promoting effects. Li et al. [8] reported that low-dose colistin sulfate alone could not achieve ideal growth-promoting effects, which aligns with our findings that 30 g/t colistin sulfate improved ADG and reduced F/G in weaning piglets, but without significant effects. Huan et al. [9] found that combined supplementation of 20 g/t colistin sulfate and 40 g/t zinc bacitracin improved ADG in weaning piglets, but the effect was not significant. However, Wang et al. [10] reported that dietary supplementation with 20 g/t colistin sulfate significantly improved ADG and reduced F/G in piglets. Zhang et al. [11] found that 400 g/t colistin sulfate improved ADG and significantly reduced F/G in weaning piglets. The inconsistent significance of these results may be related to the source, dosage, and application method of colistin sulfate, as well as the basal diet composition.

Our results demonstrate that antimicrobial peptides can improve ADG and reduce F/G in weaning piglets, indicating that antimicrobial peptides enable piglets to better utilize dietary nutrients. Antimicrobial peptides primarily enhance digestive and absorptive functions by eliminating harmful intestinal bacteria, regulating intestinal flora balance, increasing the villus height-to-crypt depth ratio, and improving small intestinal mucosal morphology, thereby enhancing growth performance [12]. Previous studies have shown that dietary antimicrobial peptide supplementation can improve performance in weaning piglets [13-18] and broilers [19-20]. Zhang et al. [21] reported that enterobacitracin improved ADG and reduced F/G in weaning piglets. Sun et al. [22] demonstrated that enterobacitracin improved performance in laying hens. These findings corroborate our results. Regarding antimicrobial peptide efficacy, our study shows that enterobacitracin outperforms cecropin. This superiority may be attributed to two factors: first, antimicrobial peptides are susceptible to protease degradation in vivo, and linear polypeptides synthesized using amino acid methods lack biological activity, with structural alterations leading to reduced or eliminated antibacterial capacity. Enterobacitracin' s lasso structure provides exceptional stability, enabling resistance to acid, alkali, pepsin, and

trypsin while maintaining high activity in vivo. Second, weaning piglets are particularly vulnerable to Gram-negative bacteria during the early period, and enterobacitracin exhibits potent bactericidal effects against Gram-negative bacteria, whereas cecropin primarily targets Gram-positive bacteria such as *Staphylococcus*. This specificity gives enterobacitracin unique advantages in preventing weaning piglet diarrhea and consequently promotes better growth. Additionally, Zhang et al. [23] reported that dietary antimicrobial peptide supplementation could achieve growth effects comparable to colistin sulfate, similar to our finding that 300 g/t enterobacitracin and 300 g/t cecropin produced effects similar to 30 g/t colistin sulfate.

3.1.2 Effects of Enterobacitracin Supplementation on Diarrhea Rate of Weaning Piglets

Antibiotics exert antibacterial effects by controlling bacterial metabolic pathways (e.g., DNA, protein, and cell wall synthesis) [24]. Our results indicate that 30 g/t colistin sulfate can extremely significantly reduce diarrhea rates in piglets, consistent with the findings of Chen [25]. However, colistin sulfate was less effective than enterobacitracin, which aligns with the results of Zhang et al. [21].

Our study demonstrates that antimicrobial peptides extremely significantly reduced diarrhea rates in weaning piglets. The decreased resistance, increased diarrhea susceptibility, and reduced growth performance observed during weaning may be associated with decreased expression of antimicrobial peptides. Exogenous antimicrobial peptide supplementation represents an important approach to enhancing animal defense functions and improving autoimmunity and disease resistance [26]. Enterobacitracin inhibits pathogenic microorganisms through two primary mechanisms: interfering with bacterial mRNA and protein synthesis by inhibiting RNA polymerase activity, and altering cell membrane permeability to cause membrane rupture and bacterial death [5]. Cecropin exerts its antibacterial activity by disrupting bacterial cell membrane structure and function, altering membrane permeability through membrane penetration, causing massive leakage of bacterial contents and ultimately leading to bacterial death [27]. Studies have shown that dietary antimicrobial peptide supplementation can reduce diarrhea rates in weaning piglets [14,28-29] and broilers [19], and that enterobacitracin has the potential to replace antibiotics in preventing weaning piglet diarrhea [8,21], which corroborates our findings. This is because antimicrobial peptides can increase immunoglobulin content in piglets when intestinal toxin concentrations rise, and immunoglobulins play a critical role in protecting the intestine against microbial invasion. Regarding antimicrobial peptide efficacy, our results show that enterobacitracin outperforms cecropin, likely because piglets are more susceptible to Gram-negative bacteria during the early period, and enterobacitracin demonstrates superior efficacy against Gram-negative bacteria compared with cecropin.

3.2.1 Effects of Enterobacitracin Supplementation on Serum UN Content of Weaning Piglets

Serum UN content reflects protein metabolism and dietary amino acid balance in piglets. When piglets exhibit high protein utilization efficiency and balanced dietary amino acids, serum UN content decreases. Chen [25] reported that dietary colistin sulfate supplementation reduced serum UN content in weaning piglets. Wang [18] and Li et al. [30] both demonstrated that dietary antimicrobial peptide supplementation decreased serum UN content in weaning piglets. Our results align with these findings, showing that both antimicrobial peptides and antibiotics can reduce serum UN content to some extent, with enterobacitracin demonstrating superior effects. This is because antimicrobial peptides slow down protein degradation in piglets, increasing nitrogen retention and thereby enhancing the absorption of protein decomposition products.

3.2.2 Effects of Enterobacitracin Supplementation on Serum TP, ALB, and GLB Contents of Weaning Piglets

Serum TP comprises ALB and GLB, primarily functioning to maintain plasma osmotic pressure and acid-base balance while also providing buffering, transport, immune, and tissue repair functions [25]. ALB is utilized for tissue repair and energy provision, while GLB constitutes the main antibody proteins in the body. Increased serum GLB content reflects improved antibody levels [18].

Our study indicates that dietary antibiotic and antimicrobial peptide supplementation increased serum TP and ALB contents in weaning piglets, though not significantly, with enterobacitracin producing the greatest increases. This is because antimicrobial peptides promote protein synthesis and deposition in piglets, consistent with the findings of Wang [18], Chen [25], and Li et al. [30].

Our results also show that enterobacitracin significantly increased serum GLB content in weaning piglets, while colistin sulfate and cecropin did not produce significant effects. This aligns with the results of Wang [18] and Chen [25], who reported that colistin sulfate and cecropin were not effective in increasing serum GLB content. The effect of enterobacitracin on serum protein synthesis indices in weaning piglets has not been previously reported. The observed promotion of serum protein synthesis by enterobacitracin may be achieved through improved intestinal health and enhanced growth and immune performance.

3.3 Effects of Enterobacitracin Supplementation on Immune Function of Weaning Piglets

Serum IgG exists in monomeric form and is the most abundant immunoglobulin in serum. It is the primary antibody produced after active immunization and plays the most important role in humoral immunity [31]. Complement 4 (C4) plays a primary role during classical pathway activation. Weaning causes intestinal villus atrophy and crypt deepening in piglets, and protecting gastrointestinal mucosa can mitigate stress-induced damage. Immunoglobulins are critical for

protecting the intestine, respiratory tract, urogenital tract, mammary glands, and eyes against microbial invasion [32].

Our results demonstrate that both antimicrobial peptides and antibiotics can increase serum IgG and C4 contents in piglets to varying degrees, with the 500 g/t enterobacitracin group showing the greatest increases compared with the control group. Seng et al. [33] reported that colistin sulfate increased serum IgG and C4 contents in weaning piglets. Wu et al. [28] found that colistin sulfate increased serum IgG content in weaning piglets but was less effective than antimicrobial peptides. Kuang et al. [34] demonstrated that dietary antimicrobial peptide supplementation increased serum IgG and C4 contents in weaning piglets. Wang [18], Li et al. [30], Su et al. [35], and Yuan et al. [36] all reported that antimicrobial peptides increased serum IgG content in weaning piglets. Yao et al. [37] and Liu et al. [38] found that cecropin increased serum immunoglobulin and complement contents in chickens. These findings are similar to our results.

The effect of enterobacitracin on serum immune indices in weaning piglets has not been previously reported. Enterobacitracin supplementation can improve immune function in weaning piglets because antimicrobial peptides are endogenous peptide substances derived from animals themselves that promote immune organ development and induce acquired immunity, constituting an important component of the natural immune defense system. Exogenous antimicrobial peptide supplementation can enhance animals' ability to resist and prevent diseases, reducing weaning stress.

3.4 Effects of Enterobacitracin Supplementation on Antioxidant Capacity of Weaning Piglets

Peroxides and free radicals produced during metabolic processes can damage tissues and cells, accelerating cellular senescence or apoptosis. Antioxidant enzymes such as SOD, GSH-Px, and reduced glutathione work synergistically to clear peroxides and free radicals, maintain cell membrane integrity, and preserve normal immune function [35]. Malondialdehyde (MDA) is a lipid peroxidation product formed when oxygen free radicals attack polyunsaturated fatty acids (PUFA) in biological membranes. It is cytotoxic and can increase cellular gaps and membrane permeability, with its content reflecting the degree of lipid peroxidation and indirectly indicating the severity of oxidative damage [39]. Weaning piglets are susceptible to various factors that induce lipid peroxidation and oxidative stress. During sustained oxidative stress, the capacity of SOD, reduced glutathione, and total hydroxyl radical inhibition decreases, demonstrating the crucial role of these enzymes in oxidative stress responses [40].

Our results show that antimicrobial peptides extremely significantly reduced serum MDA content in weaning piglets, while antibiotics did not produce significant effects. Lipid peroxidation occurs when lipids react with reactive oxygen species, initially forming lipid radicals that react with oxygen to form lipid

peroxy radicals, which further oxidize to form lipid peroxides. These peroxides attack other lipids, creating a chain reaction that damages biological membranes [40]. Xu et al. [41] reported that colistin sulfate reduced serum MDA content in weaning piglets, but the effect was not significant. Su et al. [35] demonstrated that dietary antimicrobial peptide supplementation significantly reduced serum MDA content in weaning piglets. Sun et al. [42] found that dietary antimicrobial peptide supplementation alone significantly reduced serum MDA content in broilers. These results are similar to our findings. Regarding antimicrobial peptide efficacy, our study shows that enterobacitracin outperforms cecropin, likely because enterobacitracin has unique advantages in killing Gram-negative bacteria such as *E. coli*. Therefore, it can not only protect immune cell membranes and promote IgG secretion through its own antioxidant activity but also improve piglet anti-infection levels by regulating intestinal microbial balance.

Our results also indicate that neither antimicrobial peptides nor antibiotics significantly increased serum GSH-Px activity, though enterobacitracin showed relatively better effects. Xu et al. [41] reported that colistin sulfate increased serum GSH-Px activity in weaning piglets, but the effect was not significant. Sun et al. [42] found that dietary antimicrobial peptide supplementation increased serum GSH-Px activity in broilers. These findings align with our results.

Our study demonstrates that colistin sulfate and cecropin did not significantly increase serum T-AOC in weaning piglets, while enterobacitracin produced significant effects. Xu et al. [41] reported that colistin sulfate increased serum T-AOC in weaning piglets, but the effect was not significant. Su et al. [35] showed that antimicrobial peptides significantly increased serum T-AOC in weaning piglets. These results are consistent with our findings.

Additionally, our results indicate that colistin sulfate and cecropin were less effective than enterobacitracin in increasing serum SOD activity. Xu et al. [41] found that colistin sulfate increased serum SOD activity in weaning piglets. Su et al. [35] reported that dietary antimicrobial peptide supplementation increased serum SOD activity in weaning piglets. Sun et al. [42] demonstrated that dietary antimicrobial peptide supplementation increased serum SOD activity in broilers. Dan et al. [43] showed that antimicrobial peptides effectively improved serum antioxidant capacity in weaning piglets, protected cell membrane structure and function, and enhanced resistance to weaning stress. Xiao et al. [44] reported that composite antimicrobial peptides improved antioxidant capacity in weaning piglets. These findings are similar to our results.

The effect of enterobacitracin on antioxidant capacity in weaning piglets has not been previously reported. Enterobacitracin supplementation can improve antioxidant capacity in weaning piglets, likely through mechanisms involving improved intestinal health and enhanced antioxidant performance. In this experiment, serum GSH-Px activity, SOD activity, and T-AOC in weaning piglets did not always increase simultaneously, possibly due to a balancing regulatory mechanism in the piglet antioxidant system where one mechanism may be acti-

vated while another is inhibited. The specific antioxidant regulatory mechanisms require further investigation.

Conclusion

Enterobacitracin is superior to cecropin and the antibiotic colistin sulfate in improving the performance, immune function, and antioxidant capacity of weaning piglets, with an optimal dietary supplementation level of 500 g/t.

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