# Effects of Chlorogenic Acid on Growth Performance, Serum Immunoglobulin, Intestinal Mucosal Morphology, and Digestive and Absorptive Capacity in Piglets: Postprint

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## Abstract

This experiment aimed to investigate the effects of chlorogenic acid (CA) on growth performance, serum immunoglobulins, intestinal mucosal morphology, and digestion and absorption capacity in piglets. Twenty-four healthy three-way cross weaned piglets (Duroc × Landrace × Yorkshire) with similar initial body weight [(9.45±0.20) kg] were randomly divided into 4 groups: 1) control group, basal diet; 2) CA250 group, basal diet + 250 mg/kg CA; 3) CA500 group, basal diet + 500 mg/kg CA; 4) CA1000 group, basal diet + 1,000 mg/kg CA. Each group had 6 replicates, with 1 pig per replicate. The experimental period was 14 days. The results showed: 1) There were no significant differences in average daily gain (ADG) and average daily feed intake (ADFI) among all groups ($P>0.05$), and the feed-to-gain ratio (F/G) in the CA1000 group was significantly lower than that in the control group ($P<0.05$). 2) There were no significant differences in serum IgA and IgM contents among all groups ($P>0.05$), and serum IgG content in the CA1000 group was significantly higher than that in the control and CA250 groups ($P<0.05$). 3) Villus height and villus height-to-crypt depth (V/C) ratio in the duodenum of the CA1000 group were significantly higher than those in the control group ($P<0.05$), and crypt depth in the duodenum of all treatment groups was significantly lower than that in the control group ($P<0.05$); villus height and villus width in the jejunum of the CA1000 group were significantly higher than those in the control group ($P<0.05$); there were no significant differences in villus height, villus width, crypt depth, and V/C ratio in the ileum among all groups ($P>0.05$). 4) Sucrase activity in the duodenum of all treatment groups increased compared with the control group, but without significant difference ($P>0.05$), while maltase activity was significantly higher than that in the control group ($P<0.05$); there was no significant

difference in sucrase activity in the jejunum among all groups (P>0.05), and maltase activity in the jejunum of treatment groups increased compared with the control group, but without significant difference (P>0.05); there was no significant difference in sucrase activity in the ileum among all groups (P>0.05), and maltase activity in the ileum of the CA1000 group was significantly higher than that in the control group (P<0.05). 5) There were no significant differences in relative expression levels of sodium-glucose cotransporter 1 (SGLT1) and glucose transporter 2 (GLUT2) genes in the duodenum among all groups (P>0.05); there was also no significant difference in relative expression level of SGLT1 gene in the jejunum among all groups (P>0.05), while relative expression level of GLUT2 gene in the jejunum of the CA1000 group was significantly higher than that in the control group (P<0.05); relative expression levels of SGLT1 gene in the ileum of treatment groups all increased compared with the control group, and that in the CA1000 group was significantly higher than in the control group (P<0.05), but there were no significant differences in relative expression level of GLUT2 gene in the ileum among all groups (P>0.05). In summary, the optimal supplementation level of CA in weaned piglet diets is 1,000 mg/kg, and supplementation at this dose can significantly reduce F/G, enhance immune function, improve small intestinal morphology and digestion/absorption capacity, thereby improving growth performance of weaned piglets.

## Full Text

# Effects of Chlorogenic Acid on Growth Performance, Serum Immunoglobulins, Intestinal Mucosa Morphology, and Digestive and Absorptive Capacity of Piglets

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### Abstract

This study investigated the effects of chlorogenic acid (CA) on growth performance, serum immunoglobulins, intestinal mucosa morphology, and digestive and absorptive capacity of piglets. Twenty-four healthy Duroc × Landrace × Yorkshire weaned piglets with similar initial body weight [(9.45±0.20) kg] were randomly allocated to four groups: 1) control group, fed a basal diet; 2) CA250 group, fed the basal diet supplemented with 250 mg/kg CA; 3) CA500 group, fed the basal diet supplemented with 500 mg/kg CA; and 4) CA1000 group, fed the basal diet supplemented with 1,000 mg/kg CA. Each group comprised 6 replicates with 1 piglet per replicate. The experiment lasted for 14 days. The results showed: 1) No significant differences in average daily gain (ADG) or average

daily feed intake (ADFI) were observed among groups (P>0.05), while the feed-to-gain ratio (F/G) in the CA1000 group was significantly lower than that in the control group (P<0.05). 2) Serum IgA and IgM contents did not differ significantly among groups (P>0.05), but serum IgG content in the CA1000 group was significantly higher than in the control and CA250 groups (P<0.05). 3) Duodenal villus height and villus height/crypt depth (V/C) ratio in the CA1000 group were significantly higher than in the control group (P<0.05), while duodenal crypt depth in all treatment groups was significantly lower than in the control group (P<0.05). Jejunal villus height and width in the CA1000 group were significantly higher than in the control group (P<0.05). No significant differences in ileal villus height, villus width, crypt depth, or V/C ratio were found among groups (P>0.05). 4) Duodenal sucrase activity increased in treatment groups but without significant difference from the control (P>0.05), whereas duodenal maltase activity was significantly higher in treatment groups (P<0.05). Jejunal sucrase activity did not differ significantly among groups (P>0.05), and jejunal maltase activity increased in treatment groups but without significant difference (P>0.05). Ileal sucrase activity showed no significant differences among groups (P>0.05), but ileal maltase activity in the CA1000 group was significantly higher than in the control group (P<0.05). 5) No significant differences were observed in relative expression of sodium-glucose cotransporter 1 (SGLT1) and glucose transporter 2 (GLUT2) genes in the duodenum among groups (P>0.05). Jejunal SGLT1 expression did not differ significantly among groups (P>0.05), but jejunal GLUT2 expression in the CA1000 group was significantly higher than in the control group (P<0.05). Ileal SGLT1 expression increased in treatment groups, with the CA1000 group showing significantly higher expression than the control group (P<0.05), while no significant differences in ileal GLUT2 expression were found among groups (P>0.05). In conclusion, the appropriate supplementation level of CA in weaned piglet diets is 1,000 mg/kg. This dosage significantly reduces F/G, enhances immune function, improves intestinal morphology, and promotes digestive and absorptive capacity, thereby improving growth performance of weaned piglets.

**Keywords:** chlorogenic acid; piglet; growth performance; serum immunoglobulin; intestinal morphology; digestive and absorptive capacity

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## Introduction

The nursery phase from weaning to 70 days of age is a critical period for piglets, characterized by immature digestive function, low digestive enzyme activity, short gastric emptying time, defective immune systems, and poor thermoregulatory capacity. Additionally, piglets experience abrupt dietary changes from easily digestible liquid sow milk to solid feed, causing morphological and physiological alterations in intestinal tissue such as villus atrophy, crypt hyperpla-

sia, and increased intestinal mucosal permeability. These changes lead to decreased digestive and absorptive capacity, compromised immune function, and ultimately result in diarrhea and reduced growth performance. Previous studies have shown that dietary antibiotics, high copper, and high zinc can promote piglet growth and alleviate weaning stress. However, the widespread use of these additives has caused increasingly prominent problems including environmental pollution, residues, and antimicrobial resistance. Consequently, there is growing demand for eliminating antibiotics and restricting high copper and zinc usage in feed, while research and development of novel green feed additives has attracted considerable attention.

Chlorogenic acid (CA) is a phenolic acid derived from various plants, synthesized as a secondary metabolite through the shikimic acid pathway via condensation of caffeic acid and quinic acid during aerobic respiration. Current research reports indicate that CA possesses extensive biological activities, including antibacterial, anti-inflammatory, antiviral, and antioxidant properties. Liu et al. found that dietary CA supplementation enhanced antioxidant capacity in weaned piglets. Liu et al. reported that adding CA to feed in commercial pig farms significantly reduced feed-to-gain ratio and diarrhea frequency while lowering cost per kilogram of weight gain. These findings suggest that CA supplementation benefits weaned piglet growth and improves economic returns. However, research on CA application in weaned piglets remains limited, and its effects are not fully elucidated. Therefore, this study aimed to investigate the effects of different CA supplementation levels on growth performance, serum immunoglobulin content, and intestinal mucosal morphology and absorptive capacity in weaned piglets, evaluating its potential to promote intestinal health, enhance immune function, and improve growth performance, thereby providing a scientific basis for CA application in pig production.

### 1.1 Experimental Materials

Experimental animals were healthy Duroc × Landrace × Yorkshire crossbred castrated male piglets purchased from Sichuan Tieqi Lishi Industrial Co., Ltd. The CA used in the experiment was a synthetic product (purity 99.5%) provided by Sichuan Junzheng Biological Feed Co., Ltd.

### 1.2 Experimental Design and Diets

The experiment was conducted at the research base of the Institute of Animal Nutrition, Sichuan Agricultural University. Twenty-four piglets with average body weight of (9.45±0.20) kg were selected and randomly divided into 4 groups according to the principle of consistent body weight: control group (fed basal diet), CA250 group (basal diet + 250 mg/kg CA), CA500 group (basal diet + 500 mg/kg CA), and CA1000 group (basal diet + 1,000 mg/kg CA). Each group had 6 replicates with 1 piglet per replicate, housed individually. The experimental period lasted 14 days. Piglets were fed at 08:00, 12:00, 16:00, and 20:00 daily (4 times per day) with free access to feed and water. The basal diet

was formulated according to NRC (2012) nutrient requirements for pigs. The composition and nutrient levels of the basal diet are shown in Table 1 .

**Table 1** Composition and nutrient levels of the basal diet (air-dry basis)

| Items | Content |
|---|---|
| **Ingredients** | |
| Corn | |
| Extruded corn | |
| Soybean meal | |
| Extruded soybean | |
| Soy protein concentrate | |
| Fish meal | |
| Whey powder | |
| Soybean oil | |
| Glucose | |
| NaCl | |
| Limestone | |
| CaHPO4 | |
| Choline chloride | |
| L-Lys · HCl | |
| DL-Met | |
| Thr | |
| Trp | |
| Vitamin premix[1] | |
| Mineral premix[1] | |
| **Total** | |
| **Nutrient levels[2]** | |
| DE/(MJ/kg) | |
| CP | |
| TP | |
| AP | |
| Total Lys | |
| Total Met | |
| Total Thr | |
| Total Met+Cys | |
| Total Trp | |

[1]The premix provided the following per kg of diet: VA 6,000 IU, VD3 400 IU, VE 10 IU, VK3 2.0 mg, VB1 0.80 mg, VB2 6.4 mg, VB6 2.4 mg, VB12 12 g, folic acid 0.20 mg, nicotinic acid 14 mg, D-pantothenic acid 10 mg, Fe (as ferrous sulfate) 120 mg, Cu (as copper sulfate) 6 mg, Mn (as manganese sulfate) 40 mg, Zn (as zinc sulfate) 100 mg, I (as potassium sulfate) 0.30 mg, Se (as sodium sulfate) 0.30 mg.

[2]DE was a calculated value, while the others were measured values.

### 1.3 Experimental Procedures

**1.3.1 Growth Performance** On the morning of day 1, piglets were weighed after overnight fasting. Daily feed intake was recorded throughout the experimental period, including feed offered, residual feed, and wasted feed. On the morning of day 14, piglets were weighed again after overnight fasting. Average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G) were calculated.

**1.3.2 Serum Immunoglobulin Content** On day 14, 10 mL of blood was collected from the anterior vena cava after overnight fasting. After standing for 30 minutes, serum was separated by centrifugation at 3,500 r/min for 10 minutes and stored at -20°C for determination of serum immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM) contents using ELISA kits from Beijing Chenglin Biotechnology Co., Ltd., following the manufacturer' s instructions strictly.

**1.3.3 Intestinal Mucosal Morphology** After slaughter at the end of the experiment, approximately 1 cm segments from the middle of duodenum, jejunum, and ileum were immediately collected and fixed in 4% paraformaldehyde. Paraffin-embedded tissue blocks were prepared routinely, and 5 m thick sections were stained with hematoxylin-eosin and mounted. Villus height and width and crypt depth were measured in 10 randomly selected fields under an optical microscope, and villus height/crypt depth (V/C) ratio was calculated.

**1.3.4 Intestinal Mucosal Disaccharidase Activity** After slaughter, duodenal, jejunal, and ileal segments were immediately collected. The intestinal contents were removed, and the segments were rinsed with physiological saline and blotted dry with filter paper. Mucosal tissue was scraped with a glass slide, aliquoted into cryovials, and temporarily stored at -80°C. Appropriate amounts of mucosal tissue were weighed and placed in a homogenizer, and cold physiological saline was added at a 1:9 ratio (tissue weight to saline volume) to prepare mucosal homogenates under ice bath conditions. The homogenates were transferred to centrifuge tubes and centrifuged at 4°C, 3,500 r/min for 15 minutes. The supernatant was aliquoted into 0.5 mL tubes and stored at -20°C for determination of sucrase and maltase activities using kits from Nanjing Jiancheng Bioengineering Institute, following the manufacturer' s instructions strictly.

**1.3.5 Intestinal Glucose Transporter Gene Expression** Real-time quantitative PCR was used to detect expression of sodium-glucose cotransporter 1 (SGLT1) and glucose transporter 2 (GLUT2) genes. The kit was purchased from Tiangen Biotech Co., Ltd. The main procedures were as follows: Total RNA was extracted from duodenal, jejunal, and ileal mucosal tissues using Trizol reagent. RNA concentration and purity were determined using a nucleic acid detector measuring absorbance at OD260, OD280, and OD320. cDNA synthesis was performed according to the kit instructions. Primer sequences for the two target

genes and the reference gene -actin are detailed in Table 2 . SYBR Green real-time quantitative PCR was performed according to the kit instructions. PCR conditions were: 95°C for 1 min; 45 cycles of 95°C for 5 s and annealing temperature for 35 s according to Table 2. Relative expression of the two target genes was calculated using the 2-ΔΔCt method.

**Table 2** Primer sequences for quantitative real-time PCR

| Genes | Primer sequences (5' –3' ) | Product size/bp |
|---|---|---|
| Sodium-glucose cotransporter 1 (SGLT1) | F: TGTATTTGAGGCCAGTGTCA R: GGGCACCACAACTCTTAAA | |
| Glucose transporter 2 (GLUT2) | F: TGGAATCAGCCAACCTGTTT R: ACAAGTCCCACCACATGA | |
| -actin | F: TCTGGCACCACACCTTCT R: TGATCTGGGTCATCTTCTCAC | |

### 1.4 Statistical Analysis

Experimental data were analyzed using SPSS 19.0 software with one-way ANOVA. Duncan' s multiple comparison test was used for post-hoc analysis. Differences were considered significant at $P<0.05$. Results are expressed as mean ± standard deviation.

## Results

### 2.1 Effects of CA on Growth Performance of Piglets

As shown in Table 3 , no significant differences were observed in ADG or ADFI among groups ($P>0.05$), although ADG and ADFI in the CA1000 group were slightly higher than in the control group. The F/G in CA250 and CA500 groups did not differ significantly from the control group ($P>0.05$), but F/G in the CA1000 group was significantly lower than in the control group ($P<0.05$).

**Table 3** Effects of CA on growth performance of piglets

| Items | Control | CA250 | CA500 | CA1000 |
|---|---|---|---|---|
| ADG (kg) | 0.41±0.01 | 0.44±0.06 | 0.45±0.01 | 0.47±0.01 |
| ADFI (kg) | 0.67±0.07 | 0.69±0.08 | 0.68±0.02 | 0.70±0.04 |
| F/G | 1.58±0.04 | 1.53±0.03 | 1.53±0.01 | 1.44±0.02 |

In the same row, values with no letter or the same small letter superscripts mean no significant difference (P>0.05), while different small letter superscripts mean significant difference (P<0.05), and different capital letter superscripts mean extremely significant difference (P<0.01). The same as below.

### 2.2 Effects of CA on Serum Immunoglobulin Content of Piglets

As shown in Table 4 , no significant differences in serum IgA content were observed among groups (P>0.05), although IgA in the CA1000 group was slightly higher than in the control group. Serum IgG content in CA250 and CA500 groups did not differ significantly from the control group (P>0.05), but IgG content in the CA1000 group was significantly higher than in the control and CA250 groups (P<0.05). No significant differences in serum IgM content were found among groups (P>0.05).

**Table 4** Effects of CA on serum immunoglobulin content of piglets

| Items | Control | CA250 | CA500 | CA1000 |
|---|---|---|---|---|
| IgA | 0.81±0.07 | 0.81±0.06 | 0.83±0.04 | 0.86±0.06 |
| IgG | 2.46±0.26 | 2.41±0.20 | 2.49±0.40 | 2.75±0.53 |
| IgM | 0.21±0.04 | 0.22±0.04 | 0.17±0.03 | 0.18±0.03 |

### 2.3 Effects of CA on Intestinal Mucosa Morphology of Piglets

As shown in Table 5 , in the duodenum, villus height increased in all treatment groups compared with the control, with CA1000 group being significantly higher (P<0.05). Villus width showed an increasing trend in treatment groups (P>0.05). Crypt depth in treatment groups was significantly lower than in the control group (P<0.05). The V/C ratio increased in treatment groups, with CA500 and CA1000 groups being significantly higher than the control (P<0.05), and the difference between these two groups was also significant (P<0.05). In the jejunum, villus height and width in the CA1000 group were significantly higher than in the control group (P<0.05). No significant differences in jejunal crypt depth or V/C ratio were observed among groups (P>0.05). In the ileum, no significant differences in villus height, villus width, crypt depth, or V/C ratio were found among groups (P>0.05).

**Table 5** Effects of CA on intestinal mucosa morphology of piglets

| Items | Control | CA250 | CA500 | CA1000 |
|---|---|---|---|---|
| **Duodenum** | | | | |
| Villus height ( m) | 308.46±14.87 | 317.56±12.67 | 337.93±21.65 | 433.54±12.99 |
| Villus width ( m) | 112.09±4.47 | 111.89±5.83 | 122.51±4.22 | 125.32±5.16 |

| Items | Control | CA250 | CA500 | CA1000 |
|---|---|---|---|---|
| Crypt depth ( m) | 217.33±4.24 | 191.37±7.23 | 182.89±6.80 | 174.76±6.04 |
| V/C | 1.42±0.07 | 1.66±0.04 | 1.84±0.06 | 2.49±0.14 |
| **Jejunum** | | | | |
| Villus height ( m) | 212.47±13.44 | 219.62±13.82 | 229.65±7.10 | 246.46±2.37 |
| Villus width ( m) | 109.17±3.41 | 107.63±1.14 | 112.26±3.36 | 117.82±0.85 |
| Crypt depth ( m) | 177.11±6.21 | 185.04±5.74 | 174.94±9.12 | 172.09±12.84 |
| V/C | 1.29±0.11 | 1.20±0.11 | 1.33±0.09 | 1.46±0.13 |
| **Ileum** | | | | |
| Villus height ( m) | 234.04±4.05 | 232.91±8.91 | 234.45±11.73 | 246.92±9.88 |
| Villus width ( m) | 95.71±4.00 | 97.38±5.40 | 99.33±3.94 | 102.93±5.15 |
| Crypt depth ( m) | 133.96±1.50 | 133.66±6.15 | 133.69±4.71 | 133.78±2.94 |
| V/C | 1.74±0.04 | 1.76±0.15 | 1.77±0.14 | 1.86±0.10 |

### 2.4 Effects of CA on Intestinal Mucosal Disaccharidase Activity of Piglets

As shown in Table 6 , in the duodenum, sucrase activity increased in treatment groups compared with the control but without significant difference (P>0.05). Maltase activity in treatment groups was significantly higher than in the control group (P<0.05), with a significant difference between CA250 and CA1000 groups (P<0.05). In the jejunum, no significant differences in sucrase activity were observed among groups (P>0.05). Maltase activity in treatment groups increased compared with the control but without significant difference (P>0.05). In the ileum, no significant differences in sucrase activity were found among groups (P>0.05). Maltase activity in CA500 group was significantly higher than in CA250 group (P<0.05), and maltase activity in CA1000 group was significantly higher than in the control and CA250 groups (P<0.05).

**Table 6** Effects of CA on intestinal mucosal disaccharidase activity of piglets (U/g prot)

| Items | Control | CA250 | CA500 | CA1000 |
|---|---|---|---|---|
| **Duodenum** | | | | |
| Sucrase | 27.53±3.31 | 36.94±4.21 | 39.52±3.77 | 39.11±3.92 |
| Maltase | 71.58±2.84 | 110.61±2.97 | 135.14±9.34 | 143.25±12.81 |
| **Jejunum** | | | | |

| Items | Control | CA250 | CA500 | CA1000 |
|---|---|---|---|---|
| Sucrase | 243.73±9.38 | 244.61±37.60 | 241.00±22.85 | 239.26±36.82 |
| Maltase | 270.35±24.45 | 271.82±36.46 | 284.56±16.65 | 302.54±50.54 |
| **Ileum** | | | | |
| Sucrase | 90.70±0.86 | 80.80±8.76 | 87.10±12.80 | 88.49±4.98 |
| Maltase | 115.27±13.62 | 103.04±3.68 | 147.89±15.23 | 177.93±13.07 |

### 2.5 Effects of CA on Intestinal Glucose Transporter Gene Expression of Piglets

As shown in Table 7 , no significant differences in relative expression of SGLT1 and GLUT2 genes in the duodenum were observed among groups (P>0.05). Jejunal SGLT1 expression did not differ significantly among groups (P>0.05). However, jejunal GLUT2 expression differed among groups, with CA250 group showing significantly lower expression than the control (P<0.05) and CA1000 group showing significantly higher expression than the control (P<0.05). Ileal SGLT1 expression increased in treatment groups compared with the control, with CA1000 group being significantly higher (P<0.05). No significant differences in ileal GLUT2 expression were found among groups (P>0.05).

**Table 7** Effects of CA on expression of intestinal mucosa glucose transporter genes of piglets

| Items | Control | CA250 | CA500 | CA1000 |
|---|---|---|---|---|
| **Duodenum** | | | | |
| SGLT1 | 1.00±0.17 | 1.10±0.24 | 1.13±0.09 | 1.16±0.09 |
| GLUT2 | 1.00±0.09 | 1.14±0.25 | 1.02±0.06 | 0.84±0.10 |
| **Jejunum** | | | | |
| SGLT1 | 1.00±0.06 | 0.99±0.05 | 1.06±0.20 | 1.16±0.02 |
| GLUT2 | 1.00±0.12 | 0.53±0.03 | 0.74±0.06 | 1.28±0.10 |
| **Ileum** | | | | |
| SGLT1 | 1.00±0.04 | 1.24±0.16 | 1.23±0.19 | 1.45±0.04 |
| GLUT2 | 1.00±0.14 | 1.01±0.22 | 0.97±0.12 | 0.73±0.11 |

## Discussion

### 3.1 Effects of CA on Growth Performance of Piglets

Current research reports indicate that dietary CA supplementation effectively improves growth performance in aquatic animals. Zhang et al. demonstrated that 200 mg/kg CA significantly promoted growth and increased weight gain rate in Jian carp. Li et al. found that grass carp fed diets containing 0.04% CA showed 16.4% higher weight gain rate and 13.1% lower feed coefficient. Wen et al. reported that 200-400 mg/kg CA improved growth performance in Chinese

soft-shelled turtles. However, some studies have reported no beneficial effects on growth performance. For example, Wang et al. showed that 100-400 mg/kg CA had no significant effect on growth performance of Pacific white shrimp. Research on CA effects in weaned piglets has been reported but results remain inconclusive. Liu et al. found that 300 mg/kg CA fed for 4 weeks had no significant effect on ADG, ADFI, or feed conversion rate. Lai' s recent study demonstrated that CA supplementation significantly increased ADG and ADFI while reducing F/G in weaned piglets. Our results showed that although CA had no significant effect on ADG and ADFI, it significantly reduced F/G. Liu et al. also reported similar results from a large-scale commercial pig farm study (140 pigs per group), where CA supplementation had no significant effect on ADG or ADFI but significantly reduced F/G, consistent with our findings. The mechanism by which CA promotes animal growth remains unclear but may involve: 1) CA' s adrenalin-like effects that enhance central nervous system excitability and intestinal contractility, promoting nutrient absorption; 2) CA' s antibacterial, antiviral, and anti-inflammatory properties that help animals resist pathogenic invasion and maintain healthy growth; and 3) CA's antioxidant and free radical scavenging activities that reduce oxidative stress and maintain normal oxidative balance, thereby promoting healthy animal growth.

### 3.2 Effects of CA on Serum Immunoglobulin Content of Piglets

CA has been confirmed to possess multiple biological activities, including anti-inflammatory and immunity-enhancing effects. Zhang et al. reported that CA significantly enhanced specific immune function in mice, improving both cellular and humoral immunity. Additionally, CA can enhance non-specific immune function. Zhang et al. found that dietary CA improved leukocyte phagocytic capacity and serum lysozyme activity in Jian carp, enhancing non-specific immunity. Li et al. also reported that CA improved lysozyme and other non-specific immune indices in grass carp. However, limited information is available regarding CA effects on immunoglobulins, particularly in piglets. Gong et al. demonstrated that CA increased immunoglobulin E (IgE) and IgG contents in mice and promoted interleukin-4 (IL-4) production in mesenteric lymph nodes. Lin et al.' s meta-analysis indicated that CA significantly increased serum IgG content and IgG1 antibody-forming cell numbers. However, Wang et al. reported that dietary CA had no significant effect on serum IgG content in rex rabbits, possibly related to animal species and CA dosage. Our results showed that CA supplementation had no significant effect on serum IgA or IgM contents but significantly increased serum IgG content at high dosage. IgG is the most abundant immunoglobulin in serum and the primary antibody mediating humoral immunity, playing crucial roles in antibacterial and antiviral functions. The increased IgG content in our study indicates that dietary CA can enhance immune function in piglets. The mechanism may involve: directly promoting development of immune organs such as thymus and spleen; activating calcineurin-mediated signaling pathways to enhance phagocytic function of macrophages; and promoting IL-4 synthesis and secretion, which facilitates B lymphocyte proliferation and

differentiation, enhances immunoglobulin production, and promotes CD4+ T lymphocyte differentiation into Th2 cells, thereby strengthening humoral and cellular immunity.

### 3.3 Effects of CA on Intestinal Mucosal Morphology of Piglets

The fundamental function of the small intestine is digestion and absorption of nutrients, and its capacity is closely related to villus height, crypt depth, and V/C ratio of the intestinal mucosal structure. Montagne et al. reported that increased villus length, shallow crypt depth, or increased V/C ratio generally indicates enhanced digestive and absorptive function, while the opposite represents reduced function. To date, research on CA effects on intestinal mucosal morphology is extremely limited. Ruan et al. found that CA supplementation in SD rat diets for 15 days post-weaning resulted in significantly higher jejunal villus height, significantly lower crypt depth in jejunum and ileum, and significantly higher V/C ratios compared with controls, suggesting that CA improves intestinal mucosal morphology in weaned rats. In our study, feeding weaned piglets diets supplemented with three different CA levels for 14 days increased villus height and width and V/C ratio while decreasing crypt depth in duodenal and jejunal mucosa compared with controls, with more pronounced changes at higher CA supplementation levels. This suggests that CA supplementation benefits intestinal mucosal morphology in weaned piglets. Furthermore, increased villus height and width inevitably enhance effective absorptive area and promote absorptive function, consistent with Ruan et al.' s findings in weaned rats. The mechanism underlying CA' s beneficial effects on mucosal morphology remains unclear but may involve: 1) CA' s antioxidant activity reducing oxidative stress and preventing oxidative damage to maintain mucosal integrity; 2) CA' s ability to increase tight junction protein expression and reduce intestinal permeability; and 3) CA' s capacity to maintain diversity of intestinal microbial communities and protect microecological balance, which also benefits mucosal growth.

### 3.4 Effects of CA on Intestinal Digestive and Absorptive Capacity of Piglets

Digestion and absorption of nutrients, including carbohydrates, is fundamental to animal life activities. Digestible carbohydrates in animal diets mainly include polysaccharides, oligosaccharides, disaccharides, and monosaccharides. Polysaccharides and oligosaccharides must be converted to disaccharides by digestive enzymes, then hydrolyzed to monosaccharides such as glucose by intestinal disaccharidases before absorption. Therefore, intestinal maltase and sucrase are key digestive enzymes for final carbohydrate digestion and are considered markers of mature intestinal function. No previous reports have addressed CA effects on intestinal disaccharidases. In our study, CA supplementation increased duodenal sucrase activity (though not significantly) and significantly increased duodenal maltase activity compared with controls. Jejunal maltase activity also increased, and ileal maltase activity was significantly higher in the CA1000 group. These

results indicate that CA can promote intestinal disaccharidase activity in piglets, facilitating digestion of carbohydrate nutrients into monosaccharides.

Glucose constitutes the majority of monosaccharides, and its absorption and transport in the small intestine are accomplished primarily by two types of transmembrane glucose transporters located on intestinal epithelial cells—SGLT1 and GLUT2. SGLT1, coupled with the sodium pump, actively transports glucose against concentration gradients from intestinal lumen into epithelial cells in an energy-dependent manner. Additionally, SGLT1 can regulate GLUT2-mediated glucose transport by activating protein kinase C-dependent signaling pathways. GLUT2 passively transports glucose from intestinal epithelial cells into blood along concentration gradients without energy consumption. Therefore, SGLT1 and GLUT2 play crucial roles in glucose absorption. Research on CA effects on SGLT1 and GLUT2 is extremely limited, with only scattered reports in high-fat fed animals. Liang et al. and Peng et al. reported that CA significantly increased GLUT4 mRNA expression in skeletal muscle of high-fat emulsion-fed mice and SD rats. Peng et al. showed that CA decreased SGLT1 mRNA and protein expression in high-fat fed rats, with no significant effect on duodenal, ileal, or colonic SGLT1, but increased GLUT2 mRNA expression in duodenum, jejunum, and ileum. Our results demonstrated that high-dose CA significantly increased GLUT2 expression in jejunum and SGLT1 expression in ileum, suggesting that high-dose CA supplementation promotes glucose absorption and transport in the small intestine. The mechanism by which CA increases glucose transporter expression remains unclear but may be related to CA' s promotion of intestinal villus growth, as Dong et al. found that expression of intestinal glucose transporters is positively correlated with villus height. Other mechanisms may exist and require further investigation.

## Conclusion

Based on comprehensive evaluation of all indices under our experimental conditions, the appropriate supplementation level of CA in weaned piglet diets is 1,000 mg/kg. This dosage significantly reduces F/G, enhances immune function, improves small intestinal morphology, and promotes digestive and absorptive capacity, thereby improving growth performance of weaned piglets.

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