Effects of Chitosan Oligosaccharide on Growth Performance, Antioxidant Capacity, and Jejunal Nutrient Digestion and Transport Capacity in Oxidatively Stressed Piglets: Postprint

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Abstract

This study aimed to investigate the effects of dietary chitosan oligosaccharide (COS) supplementation on growth performance, antioxidant capacity, and jejunal nutrient digestion and transport capacity in piglets under normal feeding conditions and oxidative stress. Twenty-four healthy Duroc × Landrace × Yorkshire weaned piglets at 24 days of age with an average body weight of (7.34±0.09) kg were randomly allocated to four groups according to a 2×2 factorial design: control group, COS group, diquat group, and COS+diquat group, with 6 replicates per group and 1 piglet per replicate. The experimental period lasted 28 days. The dietary COS supplementation level was 50 mg/kg, which was provided throughout the entire trial. On day 22 of the experiment, a single intraperitoneal injection of diquat at 10 mg/kg body weight was administered, while piglets not receiving diquat injection were given an equal volume of physiological saline. Digestion trials were conducted using the internal indicator method on days 18–21 of the experiment. On the morning of day 22, blood was collected from the anterior vena cava of fasted piglets prior to diquat treatment. On the morning of day 29, blood was collected from the anterior vena cava of piglets before slaughter, and jejunal mucosa samples were collected for subsequent analysis. The results showed that: 1) Before diquat injection, dietary COS supplementation had no significant effect on average daily gain (ADG) and average daily feed intake (ADFI) of piglets ($P>0.05$), but tended to decrease the feed-to-gain ratio (F/G) ($P=0.09$); significantly increased the apparent digestibility of dietary dry matter, organic matter, crude protein, ether extract, energy, crude ash, calcium, and phosphorus ($P<0.05$); and significantly elevated plasma superoxide dismutase (SOD) activity and total antioxidant capacity (T-AOC) ($P<0.05$). 2) Diquat injection highly significantly decreased ADG and

ADFI of piglets (P<0.01) and highly significantly increased F/G (P<0.01); dietary COS supplementation significantly inhibited the diquat-induced decrease in ADG (P<0.05). 3) Diquat injection highly significantly decreased plasma catalase (CAT) activity (P<0.01) and significantly decreased the activities of jejunal mucosal lactase, sucrase, and maltase, as well as the mRNA expression levels of glucose transporter 2 (GLUT2) and sodium/glucose cotransporter 1 (SGLT1) (P<0.05); dietary COS supplementation significantly increased plasma SOD activity and T-AOC (P<0.05) and significantly alleviated the reduction in jejunal mucosal disaccharidase activities and the downregulation of GLUT2 and SGLT1 mRNA expression in oxidative stress piglets (P<0.05). In conclusion, under normal feeding conditions, dietary supplementation with 50 mg/kg COS significantly improved nutrient digestibility and antioxidant capacity in piglets and tended to decrease F/G; under oxidative stress conditions, COS could alleviate diquat-induced oxidative stress by improving antioxidant capacity, enhance jejunal nutrient digestion and transport capacity in stressed piglets, and mitigate the growth reduction caused by oxidative stress.

Full Text

Abstract

This study was conducted to investigate the effects of dietary chitooligosaccharide (COS) supplementation on growth performance, antioxidant capacity, and jejunal nutrient digestion and transport capacity in piglets under normal feeding conditions and oxidative stress. Twenty-four healthy 24-day-old weaned "Duroc × Landrace × Yorkshire" piglets with an average body weight of (7.34±0.09) kg were randomly allocated to four groups following a 2×2 factorial design: control, COS, diquat, and COS+diquat, with six replicates per group and one piglet per replicate. The experimental period lasted 28 days. The COS supplementation level was 50 mg/kg throughout the entire trial. On day 22, piglets received a single intraperitoneal injection of diquat at 10 mg/kg body weight, while non-challenged piglets received an equivalent volume of saline. A digestion trial using the endogenous indicator method was conducted from days 18 to 21. On day 22, blood samples were collected from the anterior vena cava after overnight fasting, followed by diquat administration. On day 29, blood samples were again collected before slaughter, and jejunal mucosal samples were obtained for analysis.

The results showed: (1) Prior to diquat injection, dietary COS supplementation had no significant effect on average daily gain (ADG) or average daily feed intake (ADFI) (P>0.05), but tended to decrease feed-to-gain ratio (F/G) (P=0.09). COS significantly increased the apparent digestibility of dietary dry matter, organic matter, crude protein, ether extract, energy, ash, calcium, and phosphorus (P<0.05), and markedly elevated plasma superoxide dismutase (SOD) activity and total antioxidant capacity (T-AOC) (P<0.05). (2) Diquat injection dramatically reduced ADG and ADFI (P<0.01) and increased

F/G (P<0.01). Dietary COS significantly mitigated the diquat-induced decline in ADG (P<0.05). (3) Diquat injection markedly decreased plasma catalase (CAT) activity (P<0.01) and significantly reduced jejunal mucosal lactase, sucrase, and maltase activities, as well as mRNA expression of glucose transporter 2 (GLUT2) and sodium/glucose transporter 1 (SGLT1) (P<0.05). Dietary COS significantly increased plasma SOD activity and T-AOC (P<0.05) and alleviated the reduction in disaccharidase activities and the downregulation of GLUT2 and SGLT1 mRNA expression in oxidative stress piglets (P<0.05). In conclusion, under normal feeding conditions, 50 mg/kg dietary COS significantly improved nutrient digestibility and antioxidant capacity while tending to decrease F/G. Under oxidative stress conditions, COS alleviated diquat-induced oxidative stress by enhancing antioxidant capacity, improved jejunal nutrient digestion and transport, and mitigated the growth depression caused by oxidative stress.

Keywords: chitooligosaccharides; weaned piglets; antioxidant; jejunum mucosa; nutrient digestion and transport

Introduction

In swine production, numerous factors can trigger excessive free radical production in pigs, including mycotoxin contamination, high temperature, viral infections, and weaning. When these radicals accumulate without timely elimination, the balance of the oxidation-antioxidant defense system is disrupted, leading to oxidative stress. This condition compromises animal immunity, reduces production performance, deteriorates product quality, and may even cause death, resulting in significant economic losses. Consequently, identifying functional products that effectively protect piglets from oxidative stress has become a research priority in piglet nutrition. Chitooligosaccharides (COS) are oligosaccharides derived from the deacetylation and degradation of chitin, consisting of 2–10 glucosamine units linked by -1,4-glycosidic bonds. Research has demonstrated that COS possess multiple biological activities and, compared to chitosan, offer advantages including low molecular weight, low viscosity, high water solubility, and no toxic side effects. In vitro studies have confirmed that COS can reduce cellular oxidative stress, while in vivo experiments have shown that dietary COS supplementation enhances animal immunity and performance, reduces harmful bacteria in piglet intestines, and decreases diarrhea incidence. In broilers, COS supplementation significantly improves antioxidant capacity. However, the protective effects of COS against oxidative stress in piglets remain unclear. Therefore, this study was designed to investigate the effects of dietary COS supplementation on growth performance, antioxidant function, and jejunal nutrient digestion and transport capacity in piglets under oxidative stress induced by a single intraperitoneal injection of diquat (10 mg/kg body weight), providing an experimental basis for the rational application of COS in piglet diets.

Materials and Methods

Experimental Materials

Chitooligosaccharides were provided by Beijing Zhongtaihe Biotechnology Co., Ltd., under the product name "Oligosaccharide-COS (II)" with an effective content of 10% and maltodextrin as the carrier. Diquat was purchased from Sigma-Aldrich (Shanghai) and dissolved in sterile saline to a concentration of 10 mg/mL for use.

Experimental Design and Animals

A 2×2 factorial design was employed, with two main factors: intraperitoneal diquat injection (0 or 10 mg/kg body weight) and dietary COS supplementation (0 or 50 mg/kg), resulting in four treatment groups: control, COS, diquat, and COS+diquat. Twenty-four healthy 24-day-old weaned "Duroc × Landrace × Yorkshire" piglets with an average body weight of (7.34±0.09) kg were randomly assigned to four groups based on similar body weight, with six replicates per group and one pig per replicate. The trial lasted 28 days. COS supplementation began on day 1 and continued throughout the experiment. Intraperitoneal diquat injection was administered on day 22, while non-challenged piglets received an equivalent volume of saline. All piglets were housed individually in cages.

Experimental Diets

The basal diet was formulated according to NRC (2012) nutrient requirements for 7–11 kg and 11–25 kg piglets. The composition and nutrient levels are presented in . Experimental diets were prepared by adding the designated amount of COS to the basal diet.

Feeding Management

The experiment was conducted at the research base of the Institute of Animal Nutrition, Sichuan Agricultural University. Room temperature was maintained at approximately 26°C. Piglets were fed four times daily (08:00, 12:00, 16:00, 20:00) with feed provided ad libitum (slight excess remaining after satiety). Water was freely available. Pens were cleaned daily, with regular ventilation and periodic disinfection.

Sample Collection

Fecal Samples From days 18 to 21, approximately 150 g of feces were collected daily from each replicate. Ten percent sulfuric acid (10% of fecal weight) and two drops of toluene were added, mixed thoroughly in sample bags, and stored at 4°C. The four-day collections from each replicate were pooled, mixed thoroughly, dried at 65°C to constant weight, ground to pass through a 40-mesh sieve, and stored at -20°C for analysis.

Plasma Samples After 12 hours of fasting, blood samples (10 mL) were collected from the anterior vena cava on days 22 and 29 into heparinized tubes, allowed to stand for 30 minutes, then centrifuged at 3,500 rpm for 15 minutes to separate plasma. Plasma was aliquoted and stored at -20°C for analysis.

Jejunal Mucosa On day 29, after weighing and blood collection, all piglets were anesthetized. The abdomen was opened to isolate the jejunum, and a 10 cm segment of intact mid-jejunum was excised. The segment was rinsed with chilled saline, blotted dry with filter paper, opened longitudinally on ice, and the mucosa was gently scraped with a clean glass slide. Mucosal samples were placed in cryovials, snap-frozen in liquid nitrogen, and stored at -80°C for analysis.

Measurement Indicators

Growth Performance Daily feed intake was recorded accurately for each replicate. Body weight was measured on days 1, 22, and 29 after overnight fasting. Average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G) were calculated for days 1–21 and 22–28.

Apparent Nutrient Digestibility Dry matter (DM), organic matter (OM), ash, crude protein (CP), calcium (Ca), phosphorus (P), ether extract (EE), and energy in diets and feces were determined according to Zhang Liying' s methods. Acid-insoluble ash (AIA) content was measured using the combustion method according to GB/T 23743–2009. Nutrient digestibility was calculated as:

$$\text{Nutrient digestibility } (\%) = 100 - (A1 \times F2) / (A2 \times F1) \times 100$$

where F1 is the nutrient content in diet (%), F2 is the nutrient content in feces (%), A1 is the AIA content in diet (%), and A2 is the AIA content in feces (%).

Plasma Redox Status Indicators Plasma total antioxidant capacity (T-AOC), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) activities, and malondialdehyde (MDA) content were determined using colorimetric assay kits purchased from Nanjing Jiancheng Bioengineering Institute, following the manufacturer' s instructions.

Jejunal Mucosal Disaccharidase Activities Activities of lactase, sucrase, and maltase in jejunal mucosa were determined using kits from Nanjing Jiancheng Bioengineering Institute according to Cao et al.' s method, following the manufacturer' s instructions.

mRNA Expression of Nutrient Transporters in Jejunal Mucosa Real-time quantitative PCR was used to determine the relative mRNA expression levels of glucose transporter 2 (GLUT2), sodium/glucose transporter 1 (SGLT1), basic amino acid transporter 1 (SLC7A1), neutral amino acid

transporter (NAAT), and basic amino acid transporter 7 (SLC7A7) in jejunal mucosa. Total RNA extraction and quality assessment were performed according to Chen et al.' s method. cDNA synthesis was carried out using a Prime Script™ reagent kit (TaKaRa, Japan) following the manufacturer' s protocol. Target gene CDS sequences were obtained from NCBI, and primers were designed using Primer Premier 5.0 software. Primer specificity was verified by BLAST analysis in NCBI, and selected primers were synthesized by BGI Tech Solutions Co., Ltd. Primer sequences are listed in . Real-time quantitative PCR was performed using an ABI-7900 system with a 10 L reaction mixture containing 5 L SYBR Premix Ex Taq™ II (TaKaRa, Japan), 0.4 L each of forward and reverse primers, 1 L cDNA, and 3.2 L ddH2O. PCR conditions were: 95°C for 30 s; 40 cycles of 95°C for 5 s and 60°C for 30 s; and 95°C for 15 s. -actin served as the internal reference gene, and relative expression was calculated using the 2-ΔΔCt method.

Data Processing and Statistical Analysis

All data were analyzed using SPSS 17.0 statistical software. Growth performance, nutrient digestibility, and serum indices before stress were analyzed using t-tests. Post-stress growth performance, serum, and jejunal indices were analyzed using two-way ANOVA with interaction, with diquat (with/without), dietary COS (with/without), and their interaction as main effects, followed by Duncan' s multiple comparison test. Results are expressed as means and pooled standard errors. $P<0.01$ was considered highly significant, $P<0.05$ significant, and 0.05 $P<0.10$ indicated a trend.

Results

Effects of COS and Oxidative Stress on Piglet Growth Performance

As shown in , prior to diquat injection (days 1–21), dietary COS supplementation had no significant effect on ADG or ADFI ($P>0.05$) but tended to reduce F/G ($P=0.09$). Diquat injection dramatically decreased ADG and ADFI ($P<0.01$) and increased F/G ($P<0.01$) during days 22–28. Dietary COS significantly alleviated the diquat-induced reduction in ADG ($P<0.05$) and increased ADFI by 7.69% while decreasing F/G by 13.69%, though these differences were not statistically significant ($P>0.05$).

Effects of COS on Nutrient Digestibility Before Oxidative Stress

As presented in , dietary COS significantly increased the apparent digestibility of CP, energy, ash, and P ($P<0.01$) and elevated the apparent digestibility of OM, DM, EE, and Ca ($P<0.05$) in piglets before oxidative stress induction.

Effects of COS and Oxidative Stress on Plasma Antioxidant Capacity

As shown in , before diquat injection, dietary COS markedly increased plasma SOD activity ($P<0.01$) and T-AOC ($P<0.05$) but had no significant effect on plasma CAT and GSH-Px activities or MDA content ($P>0.05$). Diquat injection significantly decreased plasma CAT activity ($P<0.01$). Dietary COS significantly increased plasma SOD activity and T-AOC ($P<0.05$) and tended to elevate plasma GSH-Px activity ($P=0.07$) in oxidative stress piglets.

Effects of COS and Oxidative Stress on Jejunal Disaccharidase Activities and Nutrient Transporter mRNA Expression

As shown in , diquat injection significantly reduced jejunal mucosal sucrase, lactase, and maltase activities ($P<0.05$). Dietary COS significantly improved these disaccharidase activities ($P<0.05$). Compared with the diquat group, the diquat+COS group showed 24.09% ($P>0.05$), 30.66% ($P<0.05$), and 20.16% ($P>0.05$) increases in lactase, sucrase, and maltase activities, respectively. No significant interaction between dietary COS and diquat injection was observed for disaccharidase activities ($P>0.05$).

As presented in , diquat injection significantly downregulated mRNA expression of GLUT2 and SGLT1 ($P<0.05$) but had no significant effect on SLC7A7, NAAT, and SLC7A1 mRNA expression ($P>0.05$). Dietary COS significantly prevented the diquat-induced downregulation of GLUT2 and SGLT1 mRNA expression ($P<0.05$). No significant interaction was found between dietary COS and diquat injection for nutrient transporter mRNA expression ($P>0.05$).

Discussion

The effects of dietary COS supplementation on piglet growth performance under normal feeding conditions have been extensively reported. Chen et al. found that COS significantly improved ADG and ADFI in piglets, with 0.5% supplementation being superior to 0.25%. Yang et al. also observed that dietary COS at 0.04% and 0.06% increased ADG and feed conversion ratio in weaned piglets. However, Han et al. reported that 0.3% and 0.4% dietary COS had no significant effect on ADG but improved feed conversion. The current study found that 50 mg/kg COS tended to reduce F/G in piglets under normal conditions. These inconsistent results may be attributed to differences in piglet physiological stages and COS products used. COS are oligosaccharides composed of 2–10 glucosamine units with varying component ratios and acetylation degrees, leading to different biological effects. Numerous studies have demonstrated that dietary COS improves feed conversion efficiency, possibly by significantly enhancing nutrient digestibility. Liu et al. reported that 200 mg/kg COS markedly improved the absorption of total energy, DM, CP, EE, Ca, and P in 16-day-old weaned piglets. Walsh et al. found that among different molecular weight COS fractions supplemented at 250 mg/kg, the 5–10 kDa fraction most effectively improved apparent digestibility of DM, OM, and CP. The present study also revealed that

50 mg/kg COS significantly or highly significantly improved apparent digestibility of CP, energy, and OM, possibly due to enhanced antioxidant capacity and improved intestinal barrier function. Under normal conditions, 50 mg/kg COS significantly increased plasma SOD activity and T-AOC, consistent with Long et al.' s findings that 30 mg/kg COS elevated blood antioxidant enzyme activities and reduced MDA content in late-gestation sows and newborn piglets. Additionally, COS has been shown to improve intestinal morphology by increasing villus density and height, reducing crypt depth, and enlarging absorptive surface area to promote nutrient digestion and absorption.

Piglets frequently experience various stressors during their life cycle that generate excessive reactive oxygen species (ROS), including high temperature, inflammation, weaning, and high metabolic burden. When ROS cannot be effectively eliminated by the redox system, oxidative stress occurs, compromising piglet health and performance. Therefore, developing functional products to protect piglets from oxidative stress is crucial. Previous studies have demonstrated that diquat injection reduced ADFI and ADG by 29.74% and 40.57%, respectively, while increasing F/G by 45.35%. The current study found that diquat injection dramatically decreased ADG and ADFI and increased F/G during days 22–28, while dietary COS significantly mitigated the ADG reduction and decreased F/G by 13.69%, indicating that COS effectively alleviates growth depression caused by oxidative stress.

The protective effects of COS on oxidative stress piglets may be mediated by enhanced antioxidant function, preservation of intestinal digestive enzyme activities, and protection of nutrient transporters. Sun et al. demonstrated in vitro that COS effectively scavenged superoxide anions, comparable to vitamin C and SOD. Other studies have shown that COS free radical scavenging capacity increases with concentration. The present study found that diquat injection dramatically decreased plasma CAT activity, while dietary COS significantly improved plasma SOD activity and T-AOC and tended to increase GSH-Px activity in oxidative stress piglets. This suggests that dietary COS enhances plasma antioxidant enzyme activity and free radical scavenging capacity during oxidative stress, thereby alleviating oxidative damage. Additionally, diquat injection causes abrupt feed intake reduction, vomiting, and diarrhea, making the intestine the first organ to suffer ischemia and the last to recover. During reperfusion, explosive oxygen consumption generates massive superoxide radicals, causing intestinal damage and functional impairment. Small intestinal disaccharidases are key enzymes for disaccharide hydrolysis, and their activities directly reflect carbohydrate digestion capacity. Diquat injection significantly reduced jejunal mucosal disaccharidase activities, possibly related to the sharp decrease in feed intake. Nutrient absorption depends on various carrier transport systems in epithelial cell brush borders and basolateral membranes. Glucose absorption is primarily mediated by the sodium/glucose cotransporter (SGLT) family and glucose transporter (GLUT) family, with SGLT1 and GLUT2 being important members distributed in intestinal epithelial cells. Amino acid absorption requires amino acid transporters classified as neutral, basic, and acidic. Li found

that oxidative stress significantly reduced SGLT1 and GLUT2 mRNA expression in piglet intestinal mucosa, while Yin et al. observed that diquat-induced oxidative stress had no significant effect on mRNA expression of amino acid transporters SLC7A1, NAAT, and SLC7A7. The current study demonstrated that diquat injection significantly downregulated GLUT2 and SGLT1 mRNA expression without affecting SLC7A1, NAAT, and SLC7A7 expression. COS supplementation effectively alleviated intestinal structural and functional damage. Xu et al. reported that COS increased jejunal amylase activity in weaned piglets in a dose-dependent manner. Xiao et al. found that 30 mg/kg COS in diets of piglets challenged with enterotoxigenic *E. coli* significantly repaired jejunal morphology by increasing villus height and reducing crypt depth. The present study also revealed that 50 mg/kg COS significantly increased disaccharidase activities and GLUT2 and SGLT1 mRNA expression in oxidative stress piglets without affecting amino acid transporter expression. Collectively, COS protects stressed piglets by enhancing antioxidant capacity, reducing ROS generation and/or increasing ROS scavenging, and maintaining intestinal structural and functional integrity, thereby alleviating the detrimental effects of stress on growth performance.

Conclusion

1. Under normal feeding conditions, dietary COS supplementation significantly improved nutrient digestibility and antioxidant capacity in piglets while tending to reduce feed-to-gain ratio.
2. Under oxidative stress conditions, COS alleviated diquat-induced oxidative stress by enhancing antioxidant capacity, improved jejunal nutrient digestion and transport capacity, and mitigated the growth depression caused by oxidative stress.

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