Effects of Oyster Polysaccharide-Zinc Complex on Growth Performance and Intestinal Health in Lipopolysaccharide-Challenged Weaned Piglets (Postprint)

Authors: Ma Maotao, Lin Shaowei, YIN Guangwen, Huang Zhijian

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Abstract

This experiment aimed to investigate the effects of oyster polysaccharide-zinc complex on growth performance, serum antioxidant capacity in lipopolysaccharide-challenged weaned piglets, as well as anti-inflammatory cytokine content and disaccharidase activity in jejunal mosa. Thirty piglets at (28$±1)daysofagewereselectedandrandomlydividedintoblankcontrolgroup(Group), stresscontrolgroup(Gro
doseoysterpolysaccharide−zinccomplexgroup(Group), medium−doseoysterpolysaccharide−
zinccomplexgroup(Group), andhigh−doseoysterpolysaccharide−zinccomplexgroup(Group)accordingtosim
zinccomplexinthebasaldiet, respectively.Aftera30−dayfeedingtrial, pigsinGroups , , , and wereintraperito
injectiontodetermineserumantioxidantcapacity; jejunalmucosawascollected, andinterleukin(IL)−
2, IL − 10, andtumornecrosisfactor− $ (TNF-α) contents and disaccharidase activity were detected by enzyme-linked immunosorbent assay. The results showed: 1) Compared with Group I and Group , Groups and had significantly higher average daily gain (P<0.05), while Groups , , and showed a decreasing trend in feed-to-gain ratio (P>0.05). 2) Compared with Group I, Groups , , and exhibited significantly increased serum total superoxide dismutase (P<0.05); compared with Group , Groups , , and showed significantly elevated serum glutathione peroxidase activity (P<0.05) and significantly reduced serum malondialdehyde content (P<0.05). 3) Compared with Group I, Groups , , and had significantly increased IL-2 and IL-10 contents in jejunal mucosa (P<0.05), among which Group also showed significantly higher IL-10 content than Group (P<0.05); compared with Group , Groups and exhibited significantly decreased TNF-α content in jejunal mucosa (P<0.05). 4) Compared with Group , Groups , , and all demonstrated significantly enhanced activities of jejunal sucrase, maltase, and lactase (P<0.05). These results indicate that dietary supplementation with oyster polysaccharide-zinc complex can effectively improve serum antioxidant capacity and immune

tolerance capacity, enhance disaccharidase activity in jejunal mucosa, and significantly alleviate immune stress, with the optimal effect observed at the dietary addition level of 3,000 mg/kg oyster polysaccharide-zinc complex.

Full Text

Effects of Oyster Polysaccharide-Zinc Complex on Growth Performance and Intestinal Health of Weaned Piglets Challenged with Lipopolysaccharide

MA Maotao, LIN Shaowei, YIN Guangwen, HUANG Zhijian*
(Engineering Laboratory of Animal Pharmaceuticals, College of Animal Science, Fujian Agriculture and Forestry University, Fuzhou 350002, China)

Abstract

This study was conducted to investigate the effects of oyster polysaccharide-zinc complex (Zn-OPS) on growth performance, serum antioxidant capacity, and the content of anti-inflammatory cytokines and disaccharidase activity in jejunal mucosa of weaned piglets challenged with lipopolysaccharide (LPS). Thirty piglets at (28$±1)daysofagewererandomlyallocatedintofivegroupsaccordingtosimilarbodyweight : blankcontrol(Group), stresscontrol(Group), low−doseZn−OPS(Group), medium− doseZn−OPS(Group), andhigh−doseZn−OPS(Group), withsixreplicatespergroupandonepigperreplicate.C OPS, respectively.Aftera30−dayfeedingtrial, pigletsinGroups , , , and wereintraperitoneallyinjectedwith1 injectiontodetermineserumantioxidantcapacity.Jejunalmucosawasharvestedtomeasureinterleukin(IL)− 2, IL − 10, andtumornecrosisfactor− $ (TNF-α) contents and disaccharidase activity via enzyme-linked immunosorbent assay.

The results showed: (1) Compared with Groups and , average daily gain (ADG) in Groups and was significantly increased (P<0.05), while feed-to-gain ratio in Groups , , and showed a decreasing trend (P>0.05). (2) Serum total superoxide dismutase (T-SOD) activity in Groups , , and was significantly higher than in Group (P<0.05). Compared with Group , serum glutathione peroxidase (GSH-Px) activity in Groups , , and was significantly increased (P<0.05), while serum malondialdehyde (MDA) content was significantly decreased (P<0.05). (3) Jejunal mucosal IL-2 and IL-10 contents in Groups , , and were significantly higher than in Group (P<0.05), with IL-10 content in Group also significantly higher than in Group (P<0.05). Compared with Group , TNF-α content in Groups and was significantly reduced (P<0.05). (4) Sucrase, maltase, and lactase activities in Groups , , and were all significantly higher than in Group (P<0.05). In conclusion, dietary Zn-OPS supplementation effectively improved serum antioxidant capacity and immune tolerance, enhanced jejunal mucosal disaccharidase activity, and alleviated immune stress in weaned piglets, with 3,000 mg/kg Zn-OPS showing the optimal effect.

Keywords: oyster polysaccharide-zinc complex; weaned piglets; growth performance; antioxidant capacity; inflammatory cytokines; disaccharidase

Introduction

Polysaccharides exhibit hypolipidemic, antioxidant, immunomodulatory, antimutagenic, antiviral, and antitumor activities without toxic side effects on normal cells, attracting increasing research attention. Using polysaccharides and other immunomodulatory agents as immune enhancers to improve animal immunity has become an important direction in livestock disease prevention. Previous research by Li demonstrated that oyster polysaccharides (OPS) possess antitumor, anti-oxidative aging, antibacterial, antiviral, and immune-enhancing functions.

Zinc is an essential trace element for all animals, present in nearly all tissues and organs. At least 300 enzymes in the body contain zinc, performing diverse biological functions critical for animal growth, immunity, metabolism, and reproduction. Sun reported that zinc regulates intestinal epithelial cell immune function in weaned piglets, while Yue found that dietary zinc supplementation improves growth performance, enhances antioxidant capacity, and boosts intestinal mucosal immune function to protect gut health. Modern pharmacological studies indicate that complexes formed between active natural compounds and metal ions exhibit enhanced pharmacological activity. Zhao' s comparative study on the antitumor effects and in vitro antioxidant activity of Flammulina velutipes polysaccharide before and after zinc chelation showed improved antioxidant activity after chelation.

Lipopolysaccharide (LPS) triggers systemic inflammation. During Gram-negative bacterial infection, bacteria or LPS enter the bloodstream, activate various inflammatory cells, and induce release of inflammatory mediators, causing inflammatory responses while generating numerous free radicals that promote oxidative processes. LPS is currently the most effective immunostimulant for establishing immune stress models, possessing pyrogenic, antitumor, and infection resistance-enhancing properties.

Previous studies by Luo and Chen demonstrated that OPS promotes growth performance and alleviates intestinal health issues in immunologically stressed piglets. Building upon this work, our study chelated OPS with the trace element zinc under specific reaction conditions to form Zn-OPS. We investigated its effects on growth performance and its impact on serum antioxidant capacity, intestinal mucosal anti-inflammatory cytokine content, and disaccharidase activity in LPS-challenged weaned piglets, aiming to determine whether dietary Zn-OPS alleviates immune stress and supports intestinal health, thereby providing a theoretical basis for Zn-OPS application in pig production.

Materials and Methods

1.1 Experimental Materials

Weaned piglets were provided by a pig farm in Nanping, Fujian Province. Zn-OPS was prepared in our laboratory (zinc content determined as 4.6% by flame atomic absorption spectrometry). LPS (E. coli serotype O55:B5) was purchased from Sigma. ELISA kits were from Cusabio. A microplate reader (Thermo Labsystems) was used. Catalase (CAT) activity assay kit, malondialdehyde (MDA) content assay kit, total superoxide dismutase (T-SOD) activity assay kit, glutathione peroxidase (GSH-Px) activity assay kit, and total protein content assay kit were all purchased from Nanjing Jiancheng Bioengineering Institute.

1.2 Establishment of Weaned Piglet Immune Stress Model

Thirty Duroc × Landrace × Large Yorkshire crossbred barrows at (28$±$1) days of age were randomly divided into five groups according to body weight and litter: blank control (Group), immune stress control (Group), low-dose Zn-OPS (Group), medium-dose Zn-OPS (Group), and high-dose Zn-OPS (Group), with six replicates per group and one pig per replicate. Groups and received basal diets (composition and nutrient levels shown in Table 1), while Groups , , and received basal diets supplemented with 1,000, 3,000, and 5,000 mg/kg Zn-OPS, respectively. The trial consisted of a 3-day pre-feeding period followed by a 30-day formal experimental period. After the feeding trial and 12 hours of fasting, pigs in Groups , , , and were intraperitoneally injected with 100 g/kg BW LPS, while Group received an equivalent volume of saline.

1.3 Sample Collection and Measurements

Blood was collected from the anterior vena cava and centrifuged at 3,500 rpm for 10 minutes to separate serum for antioxidant capacity analysis. Pigs were euthanized by jugular exsanguination. The jejunum was removed from the mesentery and sampled on ice. A 20-cm segment from the middle jejunum was opened and gently rinsed in 0.9% saline to remove intestinal contents. After blotting on filter paper, 10 g of jejunal mucosa was scraped with a knife blade, placed in sterile cryovials, and immediately stored on ice. The mucosa was homogenized with 10 mL distilled water, centrifuged at 3,500 rpm for 10 minutes, and the supernatant was collected for determination of IL-2, IL-10, TNF-α contents and disaccharidase activity.

1.4 Measurement Indicators

1.4.1 Growth Performance Piglets were weighed individually at the beginning and end of the trial after 12 hours of fasting. Daily feed intake was recorded to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G).

1.4.2 Serum Antioxidant Capacity CAT and GSH-Px activities were measured by colorimetric assay, MDA content by thiobarbituric acid method, and T-SOD activity by xanthine oxidase method. Assay kits were purchased from Nanjing Jiancheng Bioengineering Institute. Measurements were performed using a PERSEE-TU1810 UV spectrophotometer (Beijing Purkinje General Instrument Co., Ltd.) according to kit instructions.

1.4.3 Jejunal Mucosal Anti-inflammatory Cytokine Content IL-2, IL-10, and TNF-α contents were determined by ELISA using kits from Cusabio and a TECAN-F50 microplate reader, following the manufacturer' s protocols.

1.4.4 Jejunal Disaccharidase Activity Maltase, lactase, and sucrase activities were measured according to kit instructions (Nanjing Jiancheng Bioengineering Institute) using a PERSEE-TU1810 UV spectrophotometer.

1.5 Statistical Analysis

Data were analyzed by one-way ANOVA using SPSS 17.0 software. Duncan' s multiple range test was used for pairwise comparisons. Differences were considered significant at P<0.05. Results are expressed as "mean ± standard deviation."

Results

2.1 Effects of Zn-OPS on Growth Performance of Weaned Piglets

As shown in Table 2 , ADG in Groups and was significantly higher than in Groups and (P<0.05), with Group showing an 18.3% increase compared to Group (P<0.05). ADFI in Groups , , and was significantly higher than in Groups and (P<0.05). Feed-to-gain ratio in Groups and was lower than in Groups , , and , though the difference was not significant (P>0.05).

2.2 Effects of Zn-OPS on Serum Antioxidant Capacity of Immunologically Stressed Piglets

Table 3 shows that serum CAT activity in Group was significantly higher than in Groups , , , and (P<0.05), with no significant differences among the latter four groups (P>0.05). Serum GSH-Px activity in Group was significantly lower than in Groups , , , and (P<0.05), with no significant differences among these four groups (P>0.05). Serum MDA content in Groups , , , and was significantly reduced by 11.6%, 5.9%, 7.3%, and 5.4%, respectively, compared to Group (P<0.05), with no significant differences among Groups , , and (P>0.05). Serum T-SOD activity in Groups , , and increased by 0.9% (P>0.05), 2.5% (P<0.05), and 0.9% (P>0.05), respectively, compared to Group , with no significant differences among the Zn-OPS groups (P>0.05).

2.3 Effects of Zn-OPS on Intestinal Mucosal Anti-inflammatory Cytokine Content

As shown in Table 4 , IL-2 and IL-10 contents in Groups , , , and were significantly higher than in Group (P<0.05). Specifically, IL-2 content in Group increased by 1.3% compared to Group (P>0.05), while IL-10 content in Groups , , and increased by 14.5% (P>0.05), 27.1% (P<0.05), and 20.3% (P>0.05), respectively, compared to Group . No significant differences in IL-2 and IL-10 contents were observed among Zn-OPS groups (P>0.05). TNF-α content in Group was significantly higher than in Groups , , and (P<0.05), while Groups , , and showed reductions of 23.5% (P<0.05), 35.3% (P<0.05), and 5.9% (P>0.05), respectively, compared to Group .

2.4 Effects of Zn-OPS on Jejunal Disaccharidase Activity in Immunologically Stressed Piglets

Table 5 shows that lactase, maltase, and sucrase activities in Groups , , , and were all significantly higher than in Group (P<0.05). Lactase activity in Group was significantly lower than in Groups , , and (P<0.05). Maltase activity in Group was significantly higher than in Zn-OPS-supplemented groups (P<0.05), while no significant differences in maltase or sucrase activities were observed among Zn-OPS groups (P>0.05).

Discussion

3.1 Effects of Zn-OPS on Growth Performance of Weaned Piglets

Numerous studies have shown that dietary polysaccharide supplementation improves ADG and promotes healthy growth in piglets. Chen reported that OPS supplementation increased ADG and improved growth performance. Song demonstrated that dietary Hedysarum polybotrys polysaccharide significantly enhanced piglet growth performance. Zinc is an essential trace element that increases feed intake, enhances intestinal digestive capacity, improves feed utilization, and promotes piglet growth. Yue' s research indicated that high dietary zinc improved growth performance, antioxidant capacity, and intestinal mucosal immune function in piglets.

Our results showed that Groups , , and exhibited improved ADFI, ADG, and feed-to-gain ratio compared to Groups and , with Group showing the most pronounced effects. This suggests that Zn-OPS supplementation increases feed intake, improves feed efficiency, and enhances growth performance in weaned piglets. The underlying mechanism may involve enhanced intestinal immune function, thereby improving appetite and reducing diarrhea incidence. Cai' s study on Pseudostellaria heterophylla polysaccharide in weaned piglets similarly demonstrated reduced diarrhea rates and improved growth performance.

3.2 Effects of Zn-OPS on Serum Antioxidant Capacity in Immunologically Stressed Piglets

LPS challenge alters cellular membrane oxidase systems and microsomal metabolic processes, releasing numerous free radicals that strongly oxidize cell membranes, proteins, and nucleic acids, causing substantial damage. The body maintains free radical balance through enzymatic (T-SOD, GSH-Px, CAT) and non-enzymatic antioxidant systems. T-SOD scavenges superoxide anions, CAT reduces hydrogen peroxide concentrations, and GSH-Px inhibits free radical generation by clearing hydroxides and lipid oxides. MDA, a product of lipid peroxidation, serves as an important indicator of oxidative cellular damage.

Our results showed significantly increased serum MDA content and T-SOD activity and elevated CAT activity in Groups , , , and compared to Group , consistent with Ben-Shaul' s findings, indicating that LPS injection induced immune stress by increasing serum free radicals. The significant reduction in serum GSH-Px activity in Group compared to Group likely resulted from acute immune stress following LPS injection, leading to increased free radical levels. Zn-OPS supplementation significantly increased serum T-SOD and GSH-Px activities and decreased MDA content compared to Group . Numerous studies have confirmed that dietary zinc and polysaccharides enhance antioxidant capacity. Leng reported that dietary aspartate increased mucosal antioxidant enzyme activity and significantly reduced MDA content in jejunum and ileum. Song demonstrated that Hedysarum polybotrys polysaccharide enhanced antioxidant capacity in immunologically stressed piglets. Our findings indicate that Zn-OPS supplementation activates the enzymatic free radical scavenging system and improves serum antioxidant capacity.

3.3 Effects of Zn-OPS on Intestinal Mucosal Anti-inflammatory Cytokine Content

LPS, the major component of Gram-negative bacterial outer membranes, stimulates production of IL-2, IL-10, TNF-α, and other cytokines upon entering the body through the gastrointestinal tract, thereby activating immune cells and enhancing specific and non-specific immune responses. IL-2, secreted by T lymphocytes, is a bidirectional immunomodulatory cytokine that enhances immune responses and mediates immune tolerance. Han demonstrated that dietary polysaccharide supplementation increased IL-2 levels in weaned piglets. IL-10 is a pleiotropic cytokine with both immunosuppressive and immunostimulatory functions, while TNF-α is a multifunctional cytokine primarily mediating immunity and inflammation.

Our results showed significantly increased IL-2, IL-10, and TNF-α contents in intestinal mucosa of Groups and Zn-OPS groups compared to Group , consistent with Zhu and Xu, confirming that LPS challenge induced immune stress in weaned piglets. Compared to Group , Zn-OPS groups showed increased IL-2 and IL-10 contents and significantly decreased TNF-α content. While Luo and Zhou reported increased serum TNF-α levels, our observed decrease may be attributed to: (1) Zn-OPS enhancing immune capacity and tolerance, thereby increasing IL-2 and IL-10 while decreasing TNF-α, consistent with Shi's findings on oxymatrine effects in psoriasis mouse models; and (2) the zinc component in Zn-OPS improving intestinal immune function, suggesting Zn-OPS protects the intestinal tract by modulating the immune system in immunologically stressed piglets.

3.4 Effects of Zn-OPS on Jejunal Disaccharidase Activity in Immunologically Stressed Piglets

The small intestine is the primary site for food digestion. Since disaccharides cannot be directly absorbed by intestinal mucosa, disaccharidase activity is crucial for carbohydrate absorption, with maltase, sucrase, and lactase being the most important enzymes, particularly in the jejunum where activity is highest. Weaning causes a decline in small intestinal disaccharidase activity in piglets.

Our results showed that jejunal disaccharidase activities in Groups , , , and were significantly lower than in Group , confirming that LPS reduces disaccharidase activity. However, Groups , , and showed significantly higher disaccharidase activities than Group . Chen reported that LPS injection decreased small intestinal disaccharidase activity in weaned piglets, while dietary OPS significantly increased it. Xu showed that trace zinc enhanced sucrase activity but inhibited maltase activity to some extent. Guo demonstrated that Cu^{2+}/ZnO-montmorillonite significantly increased jejunal mucosal disaccharidase activity. Our findings indicate that Zn-OPS enhances jejunal disaccharidase activity and effectively alleviates LPS-induced immune stress in weaned piglets.

Conclusion

Dietary Zn-OPS supplementation improves growth performance, serum antioxidant capacity, and immune tolerance while significantly enhancing jejunal disaccharidase activity in weaned piglets, thereby alleviating immune stress. The optimal supplementation level is 3,000 mg/kg.

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