Effects of Nano-Zinc Oxide on Growth Performance, Serum Immune and Biochemical Indices in Weaned Piglets: Postprint

Authors: Wang Bin, Liu Lujie, Zhu Jia, Zou Junbiao, Leng Dongbi, Wang Minqi

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Abstract

This experiment was conducted to investigate the effects of dietary supplementation with different doses of nano zinc oxide on growth performance, serum immune and biochemical indices in weaned piglets. One hundred and fifty 28-day-old "Duroc × Landrace × Yorkshire" three-way crossbred weaned piglets with a body weight of $(9.37\$\pm0.48)kgwererandomlydividedinto5groups, with3replicatespergroupand10pigsperreplic$ $1)Comparedwiththecontrolgroup, dietarysupplementationwithnanozincoxidesignificantlyincreasedtheaver$ $0.05), andsignificantlydecreasedthefeed-to-gainratioanddiarrhearate(P <$ $0.05), withthe300and450mg/kgdosegroupsshowingbettereffects, comparabletoconventionalzincoxide; 2)Com$ $6, andtumornecrosisfactor-$ $ contents (P<0.05), and significantly decreased serum urea nitrogen content (P<0.05). In conclusion, dietary supplementation with 300 or 450 mg/kg nano zinc oxide can improve the immune function and growth performance of weaned piglets, reduce the diarrhea rate, and has potential application value as a replacement for conventional zinc oxide.

Full Text

Effects of Zinc Oxide Nanoparticles on Growth Performance, Serum Immune and Biochemical Indices of Weaned Piglets

WANG Bin[1], LIU Lujie[1], ZHU Jia[1], ZOU Junbiao[2], LENG Dongbi[2], WANG Minqi[1]* [1]College of Animal Science, Zhejiang University, Hangzhou 310058, China
[2]Jiangxi Innovating Science & Technology Co., Ltd., Nanchang 330115, China

Abstract

This study was conducted to investigate the effects of dietary supplementation with different doses of zinc oxide nanoparticles on growth performance, serum immune and biochemical indices of weaned piglets. A total of 150 crossbred piglets (Duroc × Landrace × Yorkshire) at 28 days of age with an average initial body weight of (9.37$±0.48)$kgwererandomlyallocatedintofivegroupswiththreereplicatespergroupandtenpigle zincgroupreceivedthebasaldietsupplementedwith3,000mg/kgofconventionalzincoxide.Theexperimentalperi 1)Comparedwiththecontrolgroup,dietaryzincoxidenanoparticlessignificantlyincreasedaveragedailyfeedint 0.05),andsignificantlydecreasedfeed − to − gainratioanddiarrhearate($P <$ 0.05),withthe300and450mg/kgdosegroupsshowingoptimaleffectscomparabletoconventionalzincoxide.2)Die 6,andtumornecrosisfactor− $ contents (P<0.05), and significantly decreased serum urea nitrogen content (P<0.05). These findings indicate that supplementation with 300 or 450 mg/kg zinc oxide nanoparticles can enhance immune function, improve growth performance, and reduce diarrhea rate in weaned piglets, suggesting potential application value as a substitute for conventional zinc oxide.

Keywords: zinc oxide nanoparticles; weaned piglets; growth performance; cytokines; immune indices; serum biochemical indices

Introduction

Zinc is an essential mineral element for animals. Although present in small quantities in the body, it plays a crucial regulatory role in growth and development, immune function, and intestinal health. Early weaning of piglets is an important method for improving sow utilization efficiency in large-scale pig production, but the associated weaning stress leads to decreased feed intake, growth retardation, increased diarrhea rates, and substantially reduced production performance. Numerous studies have confirmed that dietary supplementation with high doses of zinc oxide can reduce diarrhea rates in weaned piglets and alleviate early weaning stress, thereby improving production performance. Consequently, high-dose zinc oxide has been widely used to treat and prevent diarrhea in weaned piglets. However, its low absorption efficiency and high excretion in feces have become increasingly problematic, causing not only significant resource waste but also environmental pollution. Therefore, research on new zinc sources with high absorption efficiency has become a hot topic in animal nutrition. Nanotechnology development provides new ideas for finding novel zinc sources. Zinc oxide nanoparticles are one of the most mature and earliest commercially applied nanomaterials. As a new zinc source, zinc oxide nanoparticles possess excellent characteristics including small particle size, large specific surface area, and high bioavailability. Various preparation methods exist for zinc oxide nanoparticles, primarily categorized into physical and chemical methods, including solid-phase reaction, gas-phase reaction, direct precipitation, precipitation transformation, sol-gel, and microemulsion methods. Different preparation methods yield zinc

oxide nanoparticles with varying performance and production costs. Our research group previously prepared non-agglomerated zinc oxide nanoparticles with both water and oil dispersibility by coupling zinc oxide formation with esterification of acetate and ethanol under solvothermal conditions using zinc acetate and ethanol as raw materials. This study investigates the effects of zinc oxide nanoparticles on growth performance, serum immune and biochemical indices of weaned piglets compared with conventional zinc oxide to explore the potential for substitution and provide a scientific basis for their application in weaned piglets.

1.1 Experimental Materials

Conventional zinc oxide was purchased commercially (feed grade). Zinc oxide nanoparticles were provided by Jiangxi Innovating Science & Technology Co., Ltd., with an average particle size of 71.61 nm, polymer dispersity index (PDI) of 0.097, Zeta potential of +31.1 mV, and specific surface area of 21.041 m^2/g.

1.2 Experimental Design

The experiment utilized 150 crossbred weaned piglets at 28 days of age with an average body weight of (9.37$±$0.48) kg, randomly divided into five groups with three replicates per group and ten piglets per replicate (half male and half female). The experimental period lasted 21 days. The five groups were fed: 1) basal diet (control group, containing 100 mg/kg zinc), 2) basal diet + 150 mg/kg zinc oxide nanoparticles, 3) basal diet + 300 mg/kg zinc oxide nanoparticles, 4) basal diet + 450 mg/kg zinc oxide nanoparticles, and 5) basal diet + 3,000 mg/kg conventional zinc oxide. The basal diet was formulated as a powdered complete feed according to NRC (2012) nutrient requirements for weaned piglets. The composition and nutrient levels of the basal diet are shown in Table 1 .

1.3 Feeding Management

Piglets were group-housed in pens with ad libitum access to feed and water. Routine immunization procedures and standard management practices were followed. The pig house was disinfected before the experiment. The trial included a 7-day pre-period followed by a 21-day formal experimental period. Daily feed consumption and diarrhea conditions were recorded throughout the trial. At the end of the experiment, piglets were weighed after a 24-hour fasting period. Average daily feed intake (ADFI), average daily gain (ADG), feed-to-gain ratio (F/G), and diarrhea rate were calculated.

Diarrhea rate (%) = 100 × (number of piglets with diarrhea × days of diarrhea) / (total number of piglets × formal experimental days).

1.4 Sample Collection and Preservation

At the end of the feeding trial, six piglets with similar body weight (half male and half female) were selected from each group, totaling 30 piglets. On the final day at 08:00, blood samples were collected from the anterior vena cava of all 30 piglets and centrifuged at 3,000 r/min for 15 minutes. The serum supernatant was transferred to Eppendorf tubes and stored at -70°C. Feed was withheld from 20:00 the day before blood collection, resulting in a 12-hour fasting period.

1.5.1 Immune Index Determination Serum immunoglobulin G (IgG), immunoglobulin M (IgM), immunoglobulin A (IgA), complement 3 (C3), and complement 4 (C4) contents were determined using immunoturbidimetry with kits purchased from Nanjing Jiancheng Bioengineering Institute and analyzed using an OLYMPUS AU400 automatic biochemical analyzer.

1.5.2 Serum Cytokines Serum interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) contents were determined using enzyme-linked immunosorbent assay (ELISA) with kits purchased from Shanghai Yanhui Biotechnology Co., Ltd.

1.5.3 Serum Biochemical Indices Serum total protein (TP), glucose (GLU), urea nitrogen (UN), total cholesterol (TCHO), triglycerides (TG), high-density lipoprotein (HDL), calcium (Ca), and phosphorus (P) contents, as well as glutamic-pyruvic transaminase (GPT), glutamic-oxaloacetic transaminase (GOT), and alkaline phosphatase (AKP) activities were determined using an OLYMPUS AU400 automatic biochemical analyzer with kits purchased from Nanjing Jiancheng Bioengineering Institute.

1.6 Data Processing and Analysis

All experimental data are expressed as mean ± standard deviation. One-way ANOVA was performed using SPSS 20.0 statistical software. Duncan' s multiple comparison test was used for inter-group difference significance testing, with $P<0.05$ considered statistically significant and $P>0.05$ considered not significant.

Results

2.1 Growth Performance and Diarrhea Rate

As shown in Table 2 , compared with the control group, dietary supplementation with 300 and 450 mg/kg zinc oxide nanoparticles significantly increased ADG and ADFI ($P<0.05$) and significantly decreased F/G ($P<0.05$) in weaned piglets. The 150 mg/kg zinc oxide nanoparticles significantly increased ADG and ADFI ($P<0.05$) but had no significant effect on F/G ($P>0.05$). The 3,000 mg/kg conventional zinc oxide significantly increased ADG and ADFI ($P<0.05$) and

significantly decreased F/G (P<0.05), with effects comparable to the 300 and 450 mg/kg zinc oxide nanoparticles.

Dietary supplementation with different doses of zinc oxide nanoparticles showed varying anti-diarrheal effects, with 300 and 450 mg/kg doses being optimal. Compared with the control group, diarrhea rates decreased by 28.95% (P>0.05), 57.24% (P<0.05), and 79.14% (P<0.05) in the 150, 300, and 450 mg/kg zinc oxide nanoparticle groups, respectively, and by 58.09% (P<0.05) in the 3,000 mg/kg conventional zinc oxide group.

2.2 Serum Immune Indices

As shown in Table 3 , dietary supplementation with different doses of zinc oxide nanoparticles had varying effects on immune performance of weaned piglets. Compared with the control group, serum IgA content increased by 16.67% (P>0.05), 28.43% (P<0.05), and 32.35% (P<0.05) in the 150, 300, and 450 mg/kg zinc oxide nanoparticle groups, respectively, and by 20.59% (P<0.05) in the 3,000 mg/kg conventional zinc oxide group. Different doses of zinc oxide nanoparticles and 3,000 mg/kg conventional zinc oxide significantly decreased serum IgM content (P<0.05) but had no significant effect on serum C3, C4, and IgG contents (P>0.05).

2.3 Serum Cytokine Contents

As shown in Table 4 , dietary supplementation with different doses of zinc oxide nanoparticles had varying effects on serum IL-6 and TNF-α contents in weaned piglets. Compared with the control group, serum IL-6 content increased by 9.72% (P<0.05), 11.95% (P<0.05), and 18.27% (P<0.05) in the 150, 300, and 450 mg/kg zinc oxide nanoparticle groups, respectively, and by 22.47% (P<0.05) in the 3,000 mg/kg conventional zinc oxide group. Different doses of zinc oxide nanoparticles and 3,000 mg/kg conventional zinc oxide significantly increased serum TNF-α content (P<0.05), with increases of 27.47%, 29.41%, 32.35%, and 36.43% in the 150, 300, and 450 mg/kg zinc oxide nanoparticle groups and the 3,000 mg/kg conventional zinc oxide group, respectively.

2.4 Serum Biochemical Indices

As shown in Table 5 , compared with the control group, dietary zinc oxide nanoparticles significantly increased serum AKP activity (P<0.05) and significantly decreased serum GOT activity and UN content (P<0.05). Serum GPT activity increased by 8.03% (P>0.05), 15.68% (P>0.05), and 18.34% (P<0.05) in the 150, 300, and 450 mg/kg zinc oxide nanoparticle groups, respectively, while AKP activity increased by 55.29% (P<0.05), 92.55% (P<0.05), and 80.74% (P<0.05), respectively. Serum GOT activity decreased by 36.87% (P<0.05), 24.59% (P<0.05), and 14.19% (P<0.05), respectively, and UN content decreased by 23.53% (P<0.05), 39.80% (P<0.05), and 24.31% (P<0.05), respectively. The 3,000 mg/kg conventional zinc oxide group showed increases of 31.36% (P<0.05)

and 62.30% (P<0.05) in serum GPT and AKP activities, respectively, and decreases of 13.14% (P>0.05) and 23.92% (P<0.05) in serum GOT activity and UN content, respectively. No significant differences were observed among groups in serum TP, TCHO, TG, HDL, GLU, Ca, and P contents (P>0.05).

Discussion

3.1 Effects of Zinc Oxide Nanoparticles on Growth Performance and Diarrhea Rate of Weaned Piglets

The results of this study indicate that dietary supplementation with zinc oxide nanoparticles can increase ADG and ADFI while decreasing F/G in weaned piglets, with the most significant effects observed in the 300 and 450 mg/kg groups, comparable to the effects of 3,000 mg/kg conventional zinc oxide. These findings suggest the potential for zinc oxide nanoparticles to replace conventional zinc oxide. These results are consistent with most previous studies. Hu et al. found that adding 300 mg/kg zinc oxide nanoparticles to weaned piglet diets significantly increased ADG by 7.26% compared with the control group, with effects equivalent to the 3,000 mg/kg high-zinc group. Fang et al. reported that 300 mg/kg zinc oxide nanoparticles significantly increased ADG and decreased F/G in piglets, demonstrating potential to replace high-zinc diets (3,000 mg/kg zinc oxide). Feng et al. showed that supplementation with 3,000 mg/kg conventional zinc oxide and 600 mg/kg zinc oxide nanoparticles significantly reduced diarrhea rates to 5.4% and 2.4%, respectively, compared with 48.9% in the unsupplemented group. The comparable growth-promoting effects of low-dose zinc oxide nanoparticles and high-dose conventional zinc oxide may be attributed to two factors: first, the small particle size of zinc oxide nanoparticles increases contact area and duration with the intestinal wall, substantially improving absorption efficiency; second, zinc is a component of gustin, which plays an important role in the structure, function, and metabolism of oral mucosal epithelial cells, affecting the morphology and function of taste pores in tongue papillae and enhancing taste sensitivity, thereby increasing feed intake through improved appetite.

This study also demonstrates that dietary supplementation with 300 and 450 mg/kg zinc oxide nanoparticles provides good anti-diarrheal effects comparable to 3,000 mg/kg conventional zinc oxide. The possible mechanisms include: 1) zinc oxide nanoparticles have large specific surface area and mismatched intermolecular bond states, providing strong inhibitory capacity against pathogenic microorganisms in the intestinal tract of weaned piglets; 2) improved adhesion to biological membranes and extended interaction time with intestinal mucosa may more effectively enhance expression of tight junction proteins between intestinal epithelial cells, reduce intestinal permeability, and protect intestinal barrier function; and 3) high biological activity of zinc oxide nanoparticles enables oxidation-reduction reactions with numerous organic substances, allowing reactions with antigenic substances in the diet to reduce allergic reactions and decrease diarrhea incidence.

3.2 Effects of Zinc Oxide Nanoparticles on Serum Immune Indices of Weaned Piglets

Immunoglobulins are important functional immune proteins in livestock and poultry, mainly including IgG, IgA, and IgM. Under normal conditions, serum contents of IgG, IgA, and IgM are positively correlated with animal immunity. IgG is the most abundant immunoglobulin in blood, accounting for approximately 85% and playing a major role in antibody-mediated humoral immunity. IgA can be divided into serum-type and secretory-type, with serum-type IgA comprising about 85% of total IgA and regulating phagocytosis. IgM is the primary initial immune antibody, with content second only to IgA in animals. Complement C3 and C4 assist, supplement, and enhance immune activity of antibodies and phagocytes, playing a synergistic role in anti-infection defense. This study found that dietary zinc oxide nanoparticles significantly increased serum IgA content and enhanced immunity but decreased IgM content, with specific mechanisms requiring further investigation. Fang et al. found that 300 mg/kg zinc oxide nanoparticles and 3,000 mg/kg conventional zinc oxide increased serum IgA content in piglets but had no significant effect on serum IgG and IgM contents. However, Yu et al. reported that different doses of zinc oxide nanoparticles increased serum IgG content in weaned piglets and inferred that the anti-diarrheal and growth-promoting effects of zinc oxide nanoparticles were related to increased IgG levels. The inconsistent results among studies may be attributed to: 1) differences in immune performance of experimental animals, and 2) differences between the novel zinc oxide nanoparticles prepared in this study and zinc sources used in other experiments.

3.3 Effects of Zinc Oxide Nanoparticles on Serum Cytokine Contents of Weaned Piglets

Cytokines are small molecular proteins secreted by immune cells or certain tissue cells that have immunomodulatory functions. They can specifically bind to cell receptors, promote proliferation and differentiation of immune cells, enhance anti-infection capacity, and stimulate or inhibit secretion of other cytokines, thereby regulating immune function. However, excessive secretion of these cytokines can negatively affect animals by reducing nutrient utilization efficiency. Therefore, serum cytokine contents reflect immune function and health status to some extent. IL-6 is a multifunctional cytokine produced by various cells that plays key roles in immune regulation, stress response, hematopoietic stem cell differentiation, and defense mechanisms. IL-6 can stimulate activated B lymphocytes to secrete immunoglobulins, promoting thymocyte and T lymphocyte proliferation and inducing T lymphocytes to secrete interleukin-2 (IL-2). TNF-α is an autocrine activation factor produced by macrophages that not only stimulates macrophages, endothelial cells, and epithelial cells to secrete cytokines such as IL-2, interleukin-8 (IL-8), and granulocyte-macrophage colony-stimulating factor (GM-CSF), but also induces tumor cell apoptosis and promotes angiogenesis and wound healing. This study demonstrates that dietary zinc oxide nanoparti-

cles significantly increased serum IL-6 and TNF-α contents, enhancing immune performance in weaned piglets. Li et al. showed that zinc significantly increased TNF-α content in normal mouse serum in a dose-dependent manner, but IL-6 levels were inhibited with increasing zinc doses, indicating that appropriate zinc concentrations can promote immune function while high doses may reduce immunity and cause functional disorders. Li et al. also reported that 1,800 mg/kg coated zinc oxide significantly increased serum IL-6 content and enhanced immunity in weaned piglets.

3.4 Effects of Zinc Oxide Nanoparticles on Serum Biochemical Indices of Weaned Piglets

Changes in serum biochemical indices reflect alterations in tissue cell permeability and metabolism to some extent. GPT and GOT are intracellular enzymes that normally remain at low activity levels in serum due to cell membrane barrier functions. However, when cells are damaged by various factors (such as acute stress), increased cell membrane permeability accelerates their release into the blood. Elevated GPT and GOT activities indicate compromised animal health and can reflect liver function status, serving as important indicators of hepatocyte damage. GPT and GOT also play important roles in amino acid metabolism, and within normal physiological ranges, their activities in blood are positively correlated with pig ADG. The present results show that 450 mg/kg zinc oxide nanoparticles increased serum GPT activity to levels similar to the normal physiological range reported by Zhu et al., indicating no detrimental effects on liver function. AKP primarily originates from liver and bone, promotes bone calcification, and enhances deposition of Ca and P. Zinc is an essential metal ion for AKP synthesis, so its activity is easily affected by zinc status in animals, making AKP activity a reflection of zinc utilization. Serum UN is an important indicator of protein metabolism and dietary amino acid balance. Decreased serum UN content indicates increased nitrogen deposition and improved dietary protein utilization, while increased content suggests enhanced protein catabolism and reduced nitrogen deposition. This study found that dietary zinc oxide nanoparticles significantly increased serum AKP activity, significantly decreased serum UN content, but had no significant effect on serum TP, TCHO, TG, HDL, GLU, Ca, and P contents. These results indicate that the novel zinc oxide nanoparticles can improve dietary protein utilization, promote bone calcification, and consequently enhance growth in weaned piglets.

Conclusion

1. Zinc oxide nanoparticles can improve growth performance and reduce diarrhea rate in piglets, with optimal effects at 300 and 450 mg/kg doses, demonstrating potential to replace conventional zinc oxide.
2. Zinc oxide nanoparticles can increase serum IgA, IL-6, and TNF-α contents, decrease UN content, and improve dietary protein utilization.

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