Postprint: Effects of Different Forms of Zinc Oxide on Intestinal Health in Weaned Piglets

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Abstract

Zinc is an essential trace element required by animal organisms, playing important roles in various physiological activities within the body. The intestine is both the primary site for nutrient digestion and absorption and a natural barrier that prevents harmful substances from feed and the external environment from invading the organism. Numerous studies have demonstrated that zinc oxide can affect the animal intestinal barrier and promote normal intestinal function, but there are also environmental pollution problems associated with the use of pharmacological doses of zinc oxide. This article reviews the effects of zinc oxide and novel zinc oxide forms (nano zinc oxide, montmorillonite-zinc oxide, coated zinc oxide, etc.) on the mechanical barrier, biological barrier, and immune barrier of the intestine in weaned piglets, providing a theoretical reference for future research on the utilization of novel zinc oxide in young livestock.

Full Text

Effects of Different Forms of Zinc Oxide on Intestinal Health in Weaned Piglets

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Abstract

Zinc is an essential trace element that participates in numerous physiological activities within the animal body. The intestine serves as the primary site for nutrient digestion and absorption while simultaneously acting as a natural barrier against harmful substances from feed and the external environment. Numerous studies have demonstrated that zinc oxide can influence intestinal barrier

function and promote normal intestinal function in animals, but the use of pharmacological doses of zinc oxide raises environmental concerns. This review examines the effects of conventional zinc oxide and novel zinc oxide products—including nano-zinc oxide, zinc oxide-montmorillonite hybrid (ZnO-MMT), and coated zinc oxide—on the intestinal mechanical barrier, biological barrier, and immune barrier of weaned piglets, providing theoretical references for future research on the application of novel zinc oxide additives in young livestock.

Keywords: zinc oxide; novel zinc oxide; intestinal barrier; weaned piglets

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The intestine is the primary site for nutrient digestion and absorption and serves as a natural barrier preventing harmful substances from entering the body. The intestinal barrier comprises three components: the mechanical barrier, biological barrier, and immune barrier. Early weaning damages the intestinal barrier in piglets, leading to stress responses such as diarrhea and reduced growth performance [1-2]. Zinc is the second most important trace element after iron and participates in various physiological activities including nutrient digestion and metabolism, biological antioxidant processes, cellular and humoral immunity, growth and development, and performance [3-4]. Zinc also plays a crucial role in maintaining intestinal barrier function and intestinal health. Zinc supplementation in animals is typically achieved by adding inorganic zinc (primarily zinc sulfate and zinc oxide) or organic zinc compounds (amino acid chelated zinc, protein zinc, polysaccharide zinc, etc.) to the diet, with inorganic zinc oxide being the most effective for maintaining intestinal structure and function and alleviating piglet diarrhea [5]. However, the effective dose of zinc oxide for diarrhea prevention is pharmacological (2,000–4,000 mg/kg), which results in large amounts of unabsorbed zinc ions being excreted in feces, causing environmental pollution [6-7]. China has explicitly prohibited the use of high-dose zinc, and the newly revised *Safety Standard for Feed Additives* in 2017 banned its use. In recent years, novel zinc oxide products including nano-zinc oxide, montmorillonite-zinc oxide hybrid (ZnO-MMT), and coated zinc oxide have attracted increasing attention. Investigating the effects of different zinc oxide forms on the intestines of weaned piglets holds significant practical importance for implementing early weaning practices.

1. Forms and Characteristics of Different Zinc Oxide Types in Feed

Zinc oxide is a white powder that is slightly soluble in water but readily soluble in acidic or alkaline solutions. Feed-grade zinc oxide contains 80.3% zinc, is cost-effective, highly stable in feed, resistant to caking and degradation, and has minimal impact on vitamin absorption, making it an economical feed additive. Research indicates that zinc oxide only promotes piglet growth when it enters the intestine in compound form, but as an amphoteric molecule, it is easily degraded by gastric acid into zinc ions, necessitating high supplementation levels to exert

growth-promoting effects.

Nano-zinc oxide is a form of zinc oxide with molecular diameters ranging from 1–100 nm. Its small particle size and large surface area confer superior antibacterial properties that are positively correlated with surface area—the larger the surface area, the stronger the antibacterial effect [8-9]. Nano-zinc oxide exhibits exceptional electron transfer capability and redox properties, enhancing its affinity for bacteria and achieving effective bactericidal action by inhibiting enzyme activity in bacterial electron transport chains [10]. Additionally, nano-zinc oxide has high bioavailability, reducing environmental pollution. Fang et al. [11] reported that 300 mg/kg nano-zinc oxide and 3,000 mg/kg conventional zinc oxide both improved average daily gain and average daily feed intake while reducing feed-to-gain ratio.

ZnO-MMT is a composite formed using the clay mineral montmorillonite as a carrier for zinc oxide. Montmorillonite has a three-layer sheet structure with silicon-oxygen tetrahedra on the top and bottom and an aluminum-oxygen octahedron in the middle. Due to its strong ion exchange properties, montmorillonite has been studied as a controlled-release carrier for pharmaceutical molecules [12]. Hu et al. [13] applied ZnO-MMT prepared by the sol-gel method to weaned piglets and found that 500 or 750 mg/kg ZnO-MMT improved intestinal mucosal integrity, alleviated diarrhea, and promoted growth.

Coated zinc oxide is a formulation in which zinc oxide particles are covered with a solid coating to reduce dissolution in the stomach, releasing zinc oxide after entering the intestine [14]. Since coating technologies and materials are mostly high-tech novel substances with high costs, fats are used to coat zinc oxide in feed additives. However, this approach is limited by the low activity of fat hydrolases in weaned piglet intestines [15]. Some products use enteric coatings for zinc oxide, and studies have shown that lower doses of coated zinc oxide can achieve the same efficacy as high-dose zinc oxide in alleviating diarrhea and promoting growth.

2. Effects of Different Zinc Oxide Forms on the Intestines of Weaned Piglets

Weaned piglet diarrhea can be classified as infectious or non-infectious, both associated with incomplete intestinal barrier development and impaired barrier structure and function. Therefore, investigating the mechanisms by which different zinc oxide forms alleviate diarrhea through their effects on intestinal barrier function is essential.

2.1 Effects on the Intestinal Mechanical Barrier

The intestinal mechanical barrier comprises the intestinal mucus layer, intestinal epithelial cells, and intercellular tight junction complexes [16-17]. An intact mechanical barrier features a thick mucus layer, low permeability between ep-

ithelial cells, and effectively prevents dietary antigens and pathogenic microorganisms from crossing the intestinal mucosa, thereby reducing inflammation and systemic disease.

The intestinal mucus layer is a gel formed by mucins secreted from goblet cells that combine with water and other intestinal contents. This layer exhibits selective permeability and prevents pathogenic microbial colonization, making it essential for isolating intestinal flora from epithelial cells [18]. Liu et al. [19] found that 2,425 mg/kg zinc oxide increased the number of goblet cells secreting neutral and acidic mucins (MUC) in the colon of weaned piglets while upregulating mRNA expression of glycosylated mucins 1, 2, 13, and 20, promoting mucus layer formation and inhibiting harmful microbial contact with intestinal epithelial cells.

Balanced proliferation and apoptosis of intestinal epithelial cells are fundamental to mechanical barrier function. Wang et al. [20] demonstrated that pharmacological-dose (3,000 mg/kg) zinc oxide increased the ratio of reduced to oxidized glutathione, improved redox status in piglet intestinal cells, reduced oxidative stress-induced damage, inhibited epithelial cell apoptosis, and maintained intestinal epithelial integrity, thereby preventing intestinal dysfunction caused by weaning. Song et al. [21] found that feeding 500 mg/kg ZnO-MMT to weaned piglets with acetic acid-induced intestinal injury restored damaged intestines, decreased caspase-9 and caspase-3 activity in the colon, and reduced epithelial cell apoptosis. Kim et al. [22] reported that 100 mg/kg lipid-encapsulated zinc oxide significantly increased villus height-to-crypt depth ratios in the ileum and goblet cell numbers in villi and crypts of the duodenum, jejunum, and colon in weaned piglets challenged with *Escherichia coli*. The same dose of lipid-encapsulated zinc oxide could also replace high-dose zinc oxide by increasing villus height in the jejunum and ileum and goblet cell numbers in small intestinal villi, mitigating the effects of *E. coli* K88 challenge [23]. Tian et al. [24] found that nano-zinc oxide significantly increased jejunal villus height and villus height-to-crypt depth ratios in weaned piglets, with effects comparable to high-dose zinc oxide. Insulin-like growth factor-I (IGF-I) is a regulatory factor that promotes cell differentiation and proliferation, and studies have shown that oral IGF-I administration increases intestinal weight and villus height in the jejunum and ileum of neonatal piglets [25]. Li et al. [26] found that pharmacological-dose zinc oxide increased IGF-I and its receptor mRNA and protein expression in the small intestinal mucosa of 4-week-old weaned piglets, promoting epithelial cell proliferation and increasing villus height. In vitro studies show that zinc deficiency decreases zonula occludens protein-1 (ZO-1) mRNA expression and damages the intestinal barrier [27]. Wang et al. [28] also found that capsulated zinc oxide significantly increased IGF-I mRNA expression in the jejunum of weaned piglets, and IGF-I, which regulates cell proliferation and differentiation, significantly increased ZO-1 mRNA expression in the jejunum. These results indicate that novel zinc oxide forms such as nano-zinc oxide can affect the intestinal mechanical barrier at reduced doses, similar to high-dose zinc oxide. Some studies suggest that high-dose zinc oxide maintains intestinal ep-

ithelial integrity by activating extracellular signal-regulated kinases (ERK) and inhibiting p38 mitogen-activated protein kinases (p38) and c-jun N-terminal kinases (JNK) in 21-day-old piglets, thereby regulating transforming growth factor- 1 (TGF- 1) signaling pathways involved in cell growth and differentiation, promoting digestion and absorption, and reducing diarrhea [29]. Song et al. [21] found that ZnO-MMT can activate ERK1/2 and protein kinase B (Akt) in intestinal mucosa, affecting their signaling pathways to improve intestinal barrier repair and reduce diarrhea.

Intestinal epithelial tight junction complexes consist of two major families: tight junction proteins (Occludin, Claudin) and zonula occludens proteins (ZO). Tight junctions allow water and small water-soluble molecules to pass while blocking large molecules, preventing epithelial cell translocation [30]. Roselli et al. [31] added enterotoxigenic *E. coli* (ETEC) to cultured human intestinal Caco-2 cells and observed decreased tight junction protein expression and cell damage; zinc oxide supplementation inhibited ETEC adhesion and internalization without reducing bacterial numbers and increased tight junction protein expression, protecting intestinal cells. In vivo studies show that high-dose zinc oxide increases Occludin and ZO-1 mRNA and protein expression in ileal mucosa, reduces intestinal permeability, and effectively decreases plasma D-lactate content and diamine oxidase (DAO) activity [32-33]. Novel zinc oxide forms such as nano-zinc oxide similarly increase mRNA expression of intestinal epithelial tight junction complexes in weaned piglets [21,24,27]. DAO is a highly active intracellular enzyme present in small intestinal villi and serves as an important indicator of mechanical barrier integrity and damage. D-lactate is a bacterial fermentation metabolite not normally present in mammalian tissues, which lack efficient enzymatic systems for its rapid degradation. Under normal conditions, D-lactate cannot enter the bloodstream, but when intestinal mucosa is damaged and permeability increases, large amounts of D-lactate produced by intestinal bacteria can cross the damaged mucosa into the blood, making plasma D-lactate content a timely indicator of mucosal damage and permeability changes [34]. Therefore, both DAO and D-lactate are indicators of intestinal mucosal permeability. Yang et al. [35] found that 300 mg/kg nano-zinc oxide significantly reduced plasma D-lactate content and DAO activity in weaned piglets, with effects on reducing intestinal barrier permeability comparable to high-dose (3,000 mg/kg) conventional zinc oxide. Long et al. [36] reported that compared with a control group (no zinc supplementation), 500 mg/kg nano-zinc oxide and high-dose zinc oxide both significantly reduced serum DAO activity in weaned piglets, effectively decreasing intestinal permeability and demonstrating that this dose of nano-zinc oxide can completely replace high-dose zinc oxide for alleviating diarrhea in weaned piglets.

2.2 Effects on the Intestinal Immune Barrier

The intestinal immune barrier comprises gut-associated lymphoid tissue, mesenteric lymph nodes, and secretory immunoglobulin A (sIgA). Intestinal mucosal

immunity is an innate protective mechanism formed through long-term evolutionary combat against pathogens, capable of capturing, presenting, and processing endotoxins and bacteria entering the intestinal mucosa to reduce damage to the body [37].

When the intestinal barrier is stimulated by antigens, immune cells secrete cytokines that mediate immune responses and inflammatory reactions, triggering local immune reactions and producing various cytokines including interleukins, tumor necrosis factors, and interferons. Interleukin-6 (IL-6), interleukin-10 (IL-10), transforming growth factor- (TGF-), tumor necrosis factor- (TNF-), and interferon- (IFN-) are pro-inflammatory cytokines secreted by immune cells that promote immune responses, immune cell growth, and inflammatory reactions. Among these, IL-6, TNF- , and IFN- are pro-inflammatory cytokines that damage the intestinal tract, whereas IL-10 and TGF- are anti-inflammatory cytokines. Cai et al. [38] found that lipopolysaccharide (LPS) stimulation of cultured porcine intestinal epithelial cells significantly increased TNF- secretion without affecting IL-10 secretion; zinc oxide supplementation effectively inhibited TNF- secretion and promoted IL-10 secretion, with both high-concentration short-term and low-concentration long-term treatments being effective. Zinc oxide exhibited dose-dependent effects on IL-10 secretion from intestinal epithelial cells, with higher concentrations increasing IL-10 secretion to exert anti-inflammatory effects and enhance intestinal anti-inflammatory capacity. Lü [39] found that 2,250 mg/kg zinc oxide significantly decreased mRNA expression of pro-inflammatory cytokines IL-1 and IFN- while increasing expression of the anti-inflammatory cytokine TGF- in jejunal mucosa of weaned piglets, reducing intestinal inflammation and improving intestinal health. Pro-inflammatory cytokine secretion is regulated by the transcription factor nuclear factor kappa enhancer binding protein (NF- B). After Toll-like receptor 4 (TLR4) pattern recognition molecules on cell surfaces bind LPS, downstream signaling molecules such as myeloid differentiation factor 88 (MyD88) are activated, thereby activating NF- B and promoting pro-inflammatory cytokine secretion. Hu et al. [40] found that dietary supplementation with 600 or 900 mg/kg zeolite-zinc oxide (Z-ZnO) reduced TNF- and IFN- mRNA expression while increasing TGF- 1 and IL-10 mRNA expression in jejunal mucosa of weaned piglets. Shen et al. [41] reported that 570 mg/kg coated zinc oxide significantly decreased mRNA expression of pro-inflammatory cytokines TNF- and IL-6 while increasing expression of anti-inflammatory cytokines TGF- 1 and IL-10 in piglet jejunum. Additionally, 500 mg/kg ZnO-MMT also reduced TNF- , IL-6, and IFN- mRNA expression in jejunal mucosa [42].

Chemokines are another type of cytokine secreted by immune cells that guide neutrophils and other immune cells to damaged sites, causing immune cell infiltration and inflammatory reactions. Sargeant et al. [43] found that pharmacological-dose zinc oxide reduced expression of inflammatory chemokines including growth-regulated oncogene (CXCL)2, CXCL6, CCL19, and CXCL13, as well as other inflammation-related immune molecules such as secreted phosphoprotein 1 (SPP1) in the intestines of weaned piglets challenged

with *E. coli* K88. Mast cells (the intestinal mucosal equivalent of basophils) are abundant immune cells in the intestinal mucosa that release histamine and other cytokines, increasing intestinal vascular permeability and triggering inflammatory reactions. Mast cell proliferation and maturation in the intestine are influenced by stem cell factor (SCF). Ou et al. [44] found that feeding 28-day-old weaned piglets diets supplemented with 100 and 3,000 mg/kg zinc oxide significantly reduced diarrhea rates in the high-dose group, decreased SCF mRNA expression in the jejunum, increased histamine concentrations in duodenal and jejunal epithelial cells, and reduced mast cell numbers in the duodenum, jejunum, and ileum. High-dose zinc oxide inhibited SCF expression in the small intestine, reducing mast cell numbers and histamine concentrations in the small intestinal mucosa and submucosa, thereby decreasing piglet diarrhea rates. sIgA is an antibody secreted by intestinal immune cells that neutralizes antigens, prevents viral infection, and provides anti-allergic effects in the intestine, representing an essential component of intestinal adaptive immunity [45]. Yue [46] found that high dietary protein caused diarrhea in weaned piglets with significantly increased serum IL-1 and IL-6 levels, while 3,000 mg/kg zinc oxide significantly or extremely significantly reduced serum IL-1 and IL-6 levels and extremely significantly increased sIgA content in jejunal and ileal mucosa. These findings indicate that pharmacological-dose zinc oxide in high-protein diets can inhibit pro-inflammatory cytokine secretion, promote sIgA secretion, and alleviate nutritional diarrhea in weaned piglets. Limited literature exists on the effects of novel zinc oxide forms on intestinal sIgA secretion, with only Shen et al. [41] and Wang [47] reporting that low-dose coated zinc oxide can promote sIgA secretion.

2.3 Effects on the Intestinal Biological Barrier

The intestinal walls of healthy animals harbor large populations of anaerobic or facultative anaerobic beneficial bacteria that constitute the intestinal biological barrier. These beneficial bacteria secrete various enzymes that degrade substances unavailable to the animal into utilizable nutrients. Additionally, they inhibit pathogenic microbial colonization and growth by competing for adhesion sites, secreting lactic acid to reduce intestinal pH, and competing for essential nutrients. Since zinc oxide reduces diarrhea rates in piglets, and diarrhea may be caused by infectious pathogens such as *E. coli*, many researchers hypothesized that zinc oxide reduces pathogenic microorganisms in the intestine to decrease diarrhea. Zinc oxide can alter the composition of intestinal microbiota in weaned piglets, with bacterial richness in the ileum increasing with higher zinc oxide supplementation, along with increased *E. coli* numbers [48]. Starke et al. [49] found that 2,425 mg/kg dietary zinc oxide significantly increased bacterial diversity in the small intestine of weaned piglets while decreasing Lactobacillus diversity and increasing *Clostridium* and *E. coli* diversity. Yu et al. [50] used 16S rRNA high-throughput sequencing to analyze microbial diversity in the ileum and colon of weaned piglets, finding that pharmacological-dose zinc oxide increased diversity of five phyla in the ileum (Spirochaetes, Tenericutes,

Euryarchaeota, Verrucomicrobia, and TM7) while decreasing diversity in the colon. Research results on zinc oxide effects on intestinal microbiota show some discrepancies. For example, Li et al. [26] found that 3,000 mg/kg zinc oxide had no significant effect on *E. coli*, Lactobacillus, or *Clostridium* numbers in ileal contents and feces of 21-day-old weaned piglets. Conversely, other studies showed that high-dose zinc oxide not only failed to affect *E. coli* numbers but actually reduced the dominant Lactobacillus populations [51-52]. Vahjen et al. [53] found that 3,042 mg/kg zinc oxide altered microbial diversity in the ileum of 28-day-old weaned piglets, with Lactobacillus remaining the dominant population but increased relative abundance of *Weissella*, *Streptococcus*, and *Leuconostoc*, and decreased relative abundance of anaerobic bacteria such as *Sarcina*. These findings indicate that zinc oxide does not reduce but rather increases numbers of diarrheal pathogens such as *E. coli*, contradicting the initial hypothesis. Subsequent research revealed that zinc oxide reduces piglet diarrhea by inhibiting pathogenic microbial colonization rather than reducing bacterial numbers. In vitro antibacterial tests showed that nano-zinc oxide has stronger antibacterial activity than conventional zinc oxide, with antibacterial rates against *E. coli* reaching 97.9% and 52.9% at 5% concentration for nano-zinc oxide and conventional zinc oxide, respectively, and against *Staphylococcus aureus* reaching 98.8% and 68.3%, respectively. Nano-zinc oxide exhibited greater inhibition against *S. aureus* than *E. coli* [54]. Nano-zinc oxide demonstrates excellent antibacterial effects not only in vitro but also in animal feeding trials, significantly reducing *Clostridium* and *E. coli* numbers in small intestinal and colonic contents while increasing microbial relative abundance in ileal and cecal digesta [10,55]. Han [56] found that dietary supplementation with 500 mg/kg nano-zinc oxide reduced diarrhea rates in weaned piglets and increased ileal microbial diversity, with significant increases in Lachnospiraceae, Lactobacillaceae, Veillonellaceae, and Coriobacteriaceae within Firmicutes and a significant decrease in Enterobacteriaceae within Proteobacteria. Although colonic microbial diversity remained unchanged, the proportion of beneficial bacteria increased while pathogenic bacteria decreased. Hu et al. [42] found that Z-ZnO significantly reduced *Clostridium* and *E. coli* numbers in the small intestine of 28-day-old weaned piglets. In summary, novel zinc oxide products also influence the intestinal biological barrier.

3. Conclusion

Zinc oxide improves intestinal health and reduces diarrhea incidence in piglets caused by weaning stress by promoting and maintaining the development and integrity of intestinal epithelial cells, the mucus layer, and intercellular tight junction proteins; influencing the intestinal immune barrier through modulation of inflammatory and anti-inflammatory cytokine secretion and lymphocyte proliferation and immunoglobulin secretion; and promoting the growth of dominant bacterial populations while inhibiting pathogenic microbial colonization to establish a mature intestinal biological barrier (Table 1). However, pharmacological-dose zinc oxide causes environmental pollution due to its high

usage levels and has been prohibited in China under the newly revised *Safety Standard for Feed Additives*. Novel zinc oxide products achieve intestinal health improvement and diarrhea alleviation in weaned piglets at reduced supplementation doses. Therefore, developing more environmentally friendly novel zinc oxide additives and elucidating their mechanisms of action in promoting animal health and alleviating diarrhea will be important research directions in future livestock production.

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References

[1] POHL C S, MEDLAND J E, MACKEY E, et al. Early weaning stress induces chronic functional diarrhea, intestinal barrier defects, and increased mast cell activity in a porcine model of early life adversity[J]. Neurogastroenterology & Motility, 2017, 29(11), doi:10.1111/nmo.13118.

[2] 徐建雄, 盛永帅. 断奶仔猪肠道屏障功能的研究进展 [J]. 饲料工业, 2013, 34(24): 1-6.

[3] BONAVENTURA P, BENEDETTI G, ALBARÈDE F, et al. Zinc and its role in immunity and inflammation[J]. Autoimmunity Reviews, 2015, 14(4): 277-285.

[4] 卢军锋, 张甜, 柴同杰. 锌的营养生理作用及在畜牧业生产中的应用 [J]. 山东畜牧兽医, 2015, 36(9): 82-83.

[5] 王银东. 无抗环保呼唤下药理剂量氧化锌的应用研究进展 [J]. 国外畜牧学 (猪与禽), 2016, 36(5): 7-11.

[6] CARTER S, WESTERMAN P, VAN KEMPEN T, et al. Estimation of manure nutrient excretion from swine based upon diet composition and feed intake[J]. The Journal of Animal Science, 2002, 80(Suppl. 1): 137.

[7] POULSEN H D. Zinc oxide for weanling piglets[J]. Acta Agriculturae Scandinavica, Section A-Animal Sciences, 1995, 45(3): 159-167.

[8] 李治学, 占秀安, 汪以真. 饲用氧化锌替代思路研究进展 [J]. 中国畜牧杂志, 2015, 51(20): 76-80.

[9] YAMAMOTO O. Influence of particle size on the antibacterial activity of zinc oxide[J]. International Journal of Inorganic Materials, 2001, 3(7): 643-646.

[10] 游兆彤, 胡彩虹, 宋娟, 等. 纳米氧化锌对断奶仔猪生长、腹泻、肠道微生物和通透性的影响 [J]. 中国畜牧杂志, 2012, 48(21): 43-46.

[11] 方洛云, 邹晓庭, 蒋林树, 等. 不同锌源对断奶仔猪生长性能和消化酶的影响 [J]. 中国饲料, 2004(4): 20-22.

[12] JOSHI G V, PATEL H A, KEVADIYA B D, et al. Montmorillonite intercalated with vitamin B1 as drug carrier[J]. Applied Clay Science, 2009, 45(4): 248-253.

[13] HU C H, SONG J, YOU Z T, et al. Zinc oxide–montmorillonite hybrid influences diarrhea, intestinal mucosal integrity, and digestive enzyme activity in weaned pigs[J]. Biological Trace Element Research, 2012, 149(2): 190–196.

[14] 刘连龙, 李浙烽, 陈强, 等. 包膜氧化锌体外稳定性试验及其对断奶仔猪生长性能的影响 [J]. 中国畜牧杂志, 2011, 47(23): 54–58.

[15] 成廷水, 张博, DUROSOY S. 氧化锌替代产品的研究进展 [J]. 饲料工业, 2012, 33(3): 17–20.

[16] 唐志芬, 董梅, 马迪杨, 等. 猪肠道机械屏障功能的概述 [J]. 养猪, 2015(6): 89–90.

[17] PETERSON L W, ARTIS D. Intestinal epithelial cells: regulators of barrier function and immune homeostasis[J]. Nature Reviews Immunology, 2014, 14(3): 141–153.

[18] BIRCHENOUGH G M, JOHANSSON M E, GUSTAFSSON J K, et al. New developments in goblet cell mucus secretion and function[J]. Mucosal Immunology, 2015, 8(4): 712–719.

[19] LIU P, PIEPER R, RIEGER J, et al. Effect of dietary zinc oxide on morphological characteristics, mucin composition and gene expression in the colon of weaned piglets[J]. PLoS One, 2014, 9(3): e91091.

[20] WANG X Q, OU D Y, YIN J D, et al. Proteomic analysis reveals altered expression of proteins related to glutathione metabolism and apoptosis in the small intestine of zinc oxide-supplemented piglets[J]. Amino acids, 2009, 37(1): 209–218.

[21] SONG Z H, KE Y L, XIAO K, et al. Diosmectite-zinc oxide composite improves intestinal barrier restoration and modulates TGF- 1, ERK1/2, and Akt in piglets after acetic acid challenge[J]. Journal of Animal Science, 2015, 93(4): 1599–1607.

[22] KIM S J, KWON CHANG H, PARK B C, et al. Effects of a lipid-encapsulated zinc oxide dietary supplement, on growth parameters and intestinal morphology in weanling pigs artificially infected with enterotoxigenic *Escherichia coli*[J]. Journal of Animal Science and Technology, 2015, 57: 4.

[23] KWON C H, LEE C Y, HAN S J, et al. Effects of dietary supplementation of lipid-encapsulated zinc oxide on colibacillosis, growth and intestinal morphology in weaned piglets challenged with enterotoxigenic *Escherichia coli*[J]. Animal Science Journal, 2014, 85(8): 805–813.

[24] TIAN X, LAI W Q, HAN M M, et al. Dietary ZnO nanoparticles alters intestinal microbiota and inflammation response in weaned piglets[J]. Oncotarget, 2017, 8(39): 64878–64891.

[25] BURRIN D G, WESTER T J, DAVIS T A, et al. Orally administered IGF-I increases intestinal mucosal growth in formula-fed neonatal pigs[J]. American Journal of Physiology, 1996, 270(5 Pt 2): R1085–R1091.

[26] LI X L, YIN J D, LI D F, et al. Dietary supplementation with zinc oxide increases IGF- and IGF- receptor gene expression in the small intestine of weanling piglets[J]. The Journal of Nutrition, 2006, 136(7): 1786-1791.

[27] FINAMORE A, MASSIMI M, DEVIRGILIIS L C, et al. Zinc deficiency induces membrane barrier damage and increases neutrophil transmigration in Caco-2 cells[J]. The Journal of Nutrition, 2008, 138(9): 1664-1670.

[28] WANG C, XIE P, LIU L L, et al. Use of lower level of capsulated zinc oxide as an alternative to pharmacological dose of zinc oxide for weaned piglets[J]. Asian Journal of Animal and Veterinary Advances, 2012, 7(12): 1290-1300.

[29] SONG Z H, XIAO K, KE Y L, et al. Zinc oxide influences mitogen-activated protein kinase and TGF- 1 signaling pathways, and enhances intestinal barrier integrity in weaned pigs[J]. Innate Immunity, 2015, 21(4): 341-348.

[30] ZHANG Z W, QIN H L. The study of the molecular structure and function of intestinal epithelial tight junction proteins[J]. Parenteral & Enteral Nutrition, 2005, 12(6): 367-369.

[31] ROSELLI M, FINAMORE A, GARAGUSO I, et al. Zinc oxide protects cultured enterocytes from the damage induced by *Escherichia coli*[J]. The Journal of Nutrition, 2003, 133(12): 4077-4082.

[32] ZHANG B K, GUO Y M. Supplemental zinc reduced intestinal permeability by enhancing occludin and zonula occludens protein-1 (ZO-1) expression in weaning piglets[J]. British Journal of Nutrition, 2009, 102(5): 687-693.

[33] 徐勇, 刘海萍, 胡彩虹, 等. 高锌对早期断奶仔猪肠形态和肠屏障通透性的影响 [J]. 浙江大学学报 (农业与生命科学版), 2009, 35(2): 209-214.

[34] 王峰, 赵瑛. D-乳酸与肠道屏障功能 [J]. 临床和实验医学杂志, 2006, 5(4): 423-424.

[35] 杨晋青, 党文庆, 何敏, 等. 不同锌源及其水平对断奶仔猪性能的影响 [J]. 中国畜牧兽医文摘, 2016, 32(12): 225-226.

[36] LONG L N, CHEN J S, ZHANG Y G, et al. Comparison of porous and nano zinc oxide for replacing high-dose dietary regular zinc oxide in weaning piglets[J]. PLoS One, 2017, 12(8): e0182550.

[37] 戈娜, 袁慧. 肠道免疫屏障功能损伤的研究进展 [J]. 广东畜牧兽医科技, 2008, 33(1): 9-11.

[38] 蔡景义, 周安国, 田刚, 等. 氧化锌对 LPS 处理猪肠上皮细胞炎性细胞因子分泌及基因表达的影响 [J]. 中国畜牧杂志, 2011, 47(23): 47-50.

[39] 吕航. 高锌对断奶仔猪生产性能和肠道健康的影响 [D]. 硕士学位论文. 广州: 华南农业大学, 2016.

[40] HU C H, XIAO K, SONG J, et al. Effects of zinc oxide supported on zeolite on growth performance, intestinal microflora and permeability, and cytokines expression of weaned pigs[J]. Animal Feed Science and Technology, 2013, 181(1/2/3/4): 65-71.

[41] SHEN J H, CHEN Y, WANG Z S, et al. Coated zinc oxide improves intestinal immunity function and regulates microbiota composition in weaned piglets[J]. British Journal of Nutrition, 2014, 111(12): 2123-2134.

[42] HU C H, SONG J, LI Y L, et al. Diosmectite-zinc oxide composite improves intestinal barrier function, modulates expression of pro-inflammatory cytokines and tight junction protein in early weaned pigs[J]. British Journal of Nutrition, 2013, 110(4): 681-688.

[43] SARGEANT H R, MCDOWALL K J, MILLER H M, et al. Dietary zinc oxide affects the expression of genes associated with inflammation: transcriptome analysis in piglets challenged with ETEC K88[J]. Veterinary Immunology and Immunopathology, 2010, 137(1/2): 120-129.

[44] OU D Y, LI D F, CAO Y H, et al. Dietary supplementation with zinc oxide decreases expression of the stem cell factor in the small intestine of weanling pigs[J]. The Journal of Nutritional Biochemistry, 2007, 18(12): 820-826.

[45] 罗治彬. 肠道粘膜 SIgA 免疫系统的研究进展 [J]. 细胞与分子免疫学杂志, 1997(S2): 38-41.

[46] 岳双明. 不同蛋白水平日粮添加高锌对早期断奶仔猪生产性能、抗氧化作用和肠道粘膜免疫的影响 [D]. 硕士学位论文. 雅安: 四川农业大学, 2008.

[47] 王超. 包膜氧化锌促仔猪生长效果及机理研究 [D]. 博士学位论文. 杭州: 浙江大学, 2013.

[48] PIEPER R, VAHJEN W, NEUMANN K, et al. Dose-dependent effects of dietary zinc oxide on bacterial communities and metabolic profiles in the ileum of weaned pigs[J]. Journal of Animal Physiology and Animal Nutrition, 2012, 96(5): 825-833.

[49] STARKE I C, PIEPER R, NEUMANN K, et al. The impact of high dietary zinc oxide on the development of the intestinal microbiota in weaned piglets[J]. FEMS Microbiology Ecology, 2014, 87(2): 416-427.

[50] YU T, ZHU C, CHEN S C, et al. Dietary high zinc oxide modulates the microbiome of ileum and colon in weaned piglets[J]. Frontiers in Microbiology, 2017, 8: 825.

[51] HØJBERG O, CANIBE N, POULSEN H D, et al. Influence of dietary zinc oxide and copper sulfate on the gastrointestinal ecosystem in newly weaned piglets[J]. Applied and Environmental Microbiology, 2005, 71(5): 2267-2277.

[52] BROOM L J, MILLER H M, KERR K G, et al. Effects of zinc oxide and *Enterococcus faecium* SF68 dietary supplementation on the performance, intestinal microbiota and immune status of weaned piglets[J]. Research in Veterinary Science, 2006, 80(1): 45-54.

[53] VAHJEN W, PIEPER R, ZENTEK J. Increased dietary zinc oxide changes the bacterial core and enterobacterial composition in the ileum of piglets[J]. Journal of Animal Science, 2011, 89(8): 2430-2439.

[54] 喻兵权, 张宏福, 陆伟, 等. 纳米氧化锌与普通氧化锌抑菌性能差异研究 [J]. 饲料工业, 2007, 28(24): 34-37.

[55] 夏添. 不同锌源调控断奶仔猪腹泻机理的研究 [D]. 博士学位论文. 北京: 中国农业大学, 2017.

[56] 韩萌. 纳米氧化锌对断奶仔猪肠道菌群平衡及黏膜免疫的影响 [D]. 硕士学位论文. 北京: 中国农业大学, 2017.

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