

## Effects of Attapulgite Nano-Zinc Oxide on Growth Performance, Organ Indices, and Blood Biochemical Parameters in Weaned Piglets (Postprint)

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### Abstract

This study aimed to investigate the effects of dietary supplementation with attapulgite-loaded nano zinc oxide (attapulgite nano zinc oxide) on growth performance, organ indices, and blood biochemical parameters in weaned piglets. A total of 210 healthy Duroc × Landrace × Yorkshire weaned piglets at 21 days of age with similar body weight [(6.30±0.51) kg] were selected and randomly divided into 7 groups, with 6 replicates per group and 5 piglets per replicate. The control group (CON group) was fed the basal diet, the antibiotic group (ANT group) was fed the basal diet + 100 g/kg 50% olaquinox + 150 g/kg 15% chlortetracycline + 50 g/kg 10% colistin sulfate, the zinc oxide group (ZO group) was fed the basal diet + 3,000 mg/kg zinc oxide, the nano zinc oxide group (NZO group) was fed the basal diet + 800 mg/kg nano zinc oxide, the low attapulgite nano zinc oxide group (LA-ZO group) was fed the basal diet + 700 mg/kg attapulgite nano zinc oxide, the medium attapulgite nano zinc oxide group (MA-ZO group) was fed the basal diet + 1,000 mg/kg attapulgite nano zinc oxide, and the high attapulgite nano zinc oxide group (HA-ZO group) was fed the basal diet + 1,300 mg/kg attapulgite nano zinc oxide. The experiment consisted of a 3-day pre-trial period and a 9-day formal trial period. The results showed that: 1) Compared with the CON group, dietary supplementation with attapulgite nano zinc oxide significantly increased the average daily feed intake and average daily gain of weaned piglets ( $P < 0.05$ ). 2) The diarrhea rate in the LA-ZO group was significantly lower than that in the CON and ANT groups ( $P < 0.05$ ), and the diarrhea index in the LA-ZO group was significantly lower than that in the CON, ANT, and NZO groups ( $P < 0.05$ ). 3) The pancreatic index in the LA-ZO and MA-ZO groups was significantly higher than that in the NZO group ( $P < 0.05$ ). 4) Compared with the CON group, the MA-ZO group

exhibited significantly decreased blood total cholesterol (TC) and triglyceride (TG) contents ( $P < 0.05$ ), and significantly increased blood high-density lipoprotein (HDL) content ( $P < 0.05$ ), which was also significantly higher than that in the ANT and NZO groups ( $P < 0.05$ ); the blood HDL content in the HA-ZO group was significantly higher than that in the CON and ANT groups ( $P < 0.05$ ). The results demonstrated that dietary supplementation with attapulgite nano zinc oxide in weaned piglets could improve growth performance, reduce diarrhea rate, and enhance piglet development, while decreasing blood TG and TC contents, increasing blood HDL content, strengthening lipid metabolism and pancreatic organ development, and could replace the use of antibiotics and high-level zinc.

## Full Text

### Effects of Attapulgite Nano Zinc Oxide on Growth Performance, Organ Indices and Blood Biochemical Indices of Weaned Piglets

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## Abstract

This experiment was conducted to investigate the effects of dietary attapulgite nano zinc oxide on growth performance, organ indices, and blood biochemical indices of weaned piglets. A total of 210 healthy Duroc × Landrace × Yorkshire piglets at 21 days of age with similar body weight [(6.30±0.51) kg] were randomly allocated into 7 groups with 6 replicates per group and 5 pigs per replicate. The control group (CON) was fed a basal diet, the antibiotic group (ANT) received the basal diet supplemented with 100 mg/kg 50% olaquinox + 150 mg/kg 15% chlortetracycline + 50 mg/kg 10% colistin sulfate, the zinc oxide group (ZO) received the basal diet + 3,000 mg/kg zinc oxide, the nano zinc oxide group (NZO) received the basal diet + 800 mg/kg nano zinc oxide, the low attapulgite nano zinc oxide group (LA-ZO) received the basal diet + 700 mg/kg attapulgite nano zinc oxide, the medium attapulgite nano zinc oxide group (MA-ZO) received the basal diet + 1,000 mg/kg attapulgite nano zinc oxide, and the high attapulgite nano zinc oxide group (HA-ZO) received the basal diet + 1,300 mg/kg attapulgite nano zinc oxide. The experiment included a 3-day preliminary period followed by a 9-day formal experimental period. The results showed: 1) Compared with the CON group, dietary attapulgite nano zinc oxide significantly increased average daily feed intake and average daily gain of

weaned piglets ( $P < 0.05$ ). 2) The diarrhea rate in the LA-ZO group was significantly lower than that in the CON and ANT groups ( $P < 0.05$ ), and the diarrhea index in the LA-ZO group was significantly lower than that in the CON, ANT, and NZO groups ( $P < 0.05$ ). 3) The pancreas index in the LA-ZO and MA-ZO groups was significantly higher than that in the NZO group ( $P < 0.05$ ). 4) Compared with the CON group, the MA-ZO group exhibited significantly decreased blood total cholesterol (TC) and triglyceride (TG) contents ( $P < 0.05$ ) and significantly increased blood high-density lipoprotein (HDL) content ( $P < 0.05$ ), which was also significantly higher than that in the ANT and NZO groups ( $P < 0.05$ ). The blood HDL content in the HA-ZO group was significantly higher than that in the CON and ANT groups ( $P < 0.05$ ). These findings indicate that dietary supplementation with attapulgite nano zinc oxide can improve growth performance, reduce diarrhea rate, promote growth and development, decrease blood TG and TC contents, increase blood HDL content, enhance lipid metabolism and pancreatic organ development, and thus may serve as a viable substitute for antibiotics and high-dose zinc in weaned piglets.

**Keywords:** attapulgite nano zinc oxide; weaned piglets; growth performance; organ index; blood biochemical index

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Weaning imposes significant stress on piglets due to their underdeveloped intestinal tracts and immature immune systems, often leading to weaning stress syndrome characterized by intestinal flora disturbance and diarrhea, which severely impairs growth performance [1]. Antibiotics and high-dose zinc have been widely used in production to mitigate these adverse effects [2]. However, research has revealed numerous drawbacks, including antibiotic residues, bacterial resistance, and environmental pollution from zinc excretion due to its low digestibility [3]. Consequently, the European Union, the Netherlands, the United States, and other countries have restricted zinc supplementation levels in piglet feed. Therefore, exploring rational zinc utilization methods and identifying effective antibiotic alternatives to alleviate weaning stress is imperative. Nano zinc oxide represents a promising alternative to conventional zinc oxide, demonstrating superior efficacy [4]. With particle sizes of 1-100 nm, nano zinc oxide exhibits high specific surface area and absorption rate, and can modulate immune function and reproductive performance [5]. Studies have confirmed that dietary supplementation with 200-1,000 mg/kg nano zinc oxide promotes growth in livestock and poultry, improves intestinal mucosal morphology, reduces diarrhea rate, and stimulates cellular, humoral, and non-specific immune functions [6-8]. Zhao et al. [9] reported that 60 mg/kg nano zinc oxide improved later-stage daily weight gain in broilers, while Long et al. [10] found that 500 mg/kg nano zinc oxide in weaned piglets significantly increased daily gain and feed intake while reducing diarrhea rate, with effects comparable to 3,000 mg/kg conventional zinc oxide. Nevertheless, high-dose nano zinc oxide may exert toxic side effects [11].

Attapulgite, a silicate-based clay mineral [theoretical formula:  $\text{Si}_2\text{Mg}_2\text{O}_5(\text{OH}) \cdot 4\text{H}_2\text{O}$ ], is non-toxic, tasteless, non-irritating, and inexpensive. Its porous struc-

ture, large specific surface area, and cation exchange capacity enable effective adsorption of  $Pb^{2+}$ ,  $Cu^{2+}$ , and antibiotics [12-15]. Furthermore, clay minerals can serve as controlled-release carriers for bioactive molecules, drugs, and nutrients [16-18], leading to its approval as a feed additive since 2011 [19]. Research demonstrated that 2,000 mg/kg attapulgite in weaned piglets significantly increased average daily feed intake, improved feed conversion ratio, and enhanced intestinal health [20], while Tang et al. [21] reported that 1,800 mg/kg attapulgite increased average daily gain and reduced diarrhea. However, excessive attapulgite supplementation may negatively affect piglet growth [21-23]. Previous studies have focused on high-dose attapulgite, with limited evidence on low-dose effects, and inconsistent results regarding 500-1,000 mg/kg nano zinc oxide supplementation. Therefore, this study integrated low-dose attapulgite with varying levels of nano zinc oxide to form attapulgite nano zinc oxide, aiming to determine the optimal supplementation level for early-stage weaned piglets and evaluate its potential as a novel green feed additive to replace antibiotics and high-dose zinc.

## 1 Materials and Methods

### 1.1 Experimental Materials

The zinc oxide (white powder), nano zinc oxide (white powder, purity 99%, average particle size 45 nm), and attapulgite nano zinc oxide (purity 85%, composed of 80% nano zinc oxide and 20% attapulgite, with the nano zinc oxide having the same physicochemical properties as described above and the attapulgite consisting primarily of silicate with a double-chain structure) were provided by the College of Chemical Engineering, Yangzhou University. The 50% olaquinox, 15% chlortetracycline, and 10% colistin sulfate were supplied by the Yangzhou University Feed Mill. The experimental piglets (21-day-old Duroc  $\times$  Landrace  $\times$  Yorkshire) were provided by Taicang Jinzhu Agricultural Development Co., Ltd., Suzhou.

The colistin sulfate used was produced in September 2016. According to the Ministry of Agriculture Announcement No. 2428, its use was permitted until April 30, 2017, making the experimental period compliant with regulations. These materials were used exclusively for research purposes to explore antibiotic alternatives.

### 1.2 Experimental Design

A total of 210 healthy 21-day-old Duroc  $\times$  Landrace  $\times$  Yorkshire weaned piglets with similar body weight [(6.30 $\pm$ 0.51) kg] were randomly assigned to 7 groups: control (CON, basal diet), antibiotic (ANT, basal diet + 100 mg/kg 50% olaquinox + 150 mg/kg 15% chlortetracycline + 50 mg/kg 10% colistin sulfate), zinc oxide (ZO, basal diet + 3,000 mg/kg zinc oxide), nano zinc oxide (NZO, basal diet + 800 mg/kg nano zinc oxide), low attapulgite nano zinc oxide (LA-ZO, basal diet + 700 mg/kg attapulgite nano zinc oxide), medium

attapulgitic nano zinc oxide (MA-ZO, basal diet + 1,000 mg/kg attapulgitic nano zinc oxide), and high attapulgitic nano zinc oxide (HA-ZO, basal diet + 1,300 mg/kg attapulgitic nano zinc oxide). Each group comprised 6 replicates with 5 pigs per replicate. The experiment was conducted at Taicang Jinzhu Pig Farm in Jiangsu Province in April 2017, with a 3-day preliminary period and a 9-day formal experimental period.

### 1.3 Feeding Management and Experimental Diets

The pig farm operated at commercial scale with complete facilities and comprehensive disease prevention and management protocols. All experimental piglets were housed in the same building and fed powdered diets ad libitum (four times daily at 06:30, 10:30, 14:30, and 18:30, with slight excess in troughs) with free access to water. Pens were cleaned daily to maintain hygiene, with natural ventilation. Routine management, disinfection, and disease prevention followed standard farm procedures. The basal diet was formulated according to NRC (2012) nutrient requirements for piglets and practical production conditions, with composition and nutrient levels shown in Table 1.

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

*Note: The premix provided per kilogram of diet: VA 6,000 IU, VD 3,400 IU, VE 30 mg, VK 2 mg, VB 3.5 mg, VB 5.5 mg, VB 3.5 mg, VB 25.0 g, biotin 0.05 mg, folic acid 0.3 mg, D-pantothenic acid 20 mg, niacin 20 mg, choline chloride 500 mg, Fe (as ferrous sulfate) 110 mg, Zn (as zinc sulfate) 100 mg, Cu (as copper sulfate) 20 mg, Mn (as manganese sulfate) 40 mg, Se (as sodium selenite) 0.30 mg, I (as potassium iodide) 0.40 mg. Nutrient levels were calculated values.*

### 1.4 Measurement Indices and Methods

**1.4.1 Growth Performance** Feed intake was recorded by replicate throughout the experiment. Piglets were weighed at 08:00 on day 1 and the final day after overnight fasting to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G).

**1.4.2 Diarrhea Rate and Diarrhea Index** Fecal consistency was scored daily at 07:00 using the method of Castillo et al. [24]: 0 = formed feces or pellets; 1 = soft but formed feces; 2 = thick, unformed, no water separation; 3 = liquid, unformed, water separation. Diarrhea was defined as a score 2. Diarrhea rate and index were calculated as:

Diarrhea rate (%) =  $100 \times (\text{total diarrhea incidents}) / (\text{experimental days} \times \text{number of pigs per group})$

Diarrhea index =  $100 \times (\text{total diarrhea scores}) / (\text{number of pigs per group})$

**1.4.3 Organ Indices** At the end of the experiment, piglets were slaughtered and the heart, liver, spleen, lungs, kidneys, thymus, and pancreas were col-

lected. After blotting excess blood with absorbent paper, organs were immediately weighed to calculate organ indices:

$$\text{Organ index (g/kg)} = \text{organ wet weight (g)} / \text{live body weight (kg)}$$

**1.4.4 Blood Biochemical Indices** At 08:00 on the final day, one piglet with body weight 接近 the replicate average was randomly selected from each replicate for blood collection (3 mL) from the anterior vena cava into anticoagulant tubes. Blood alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) activities, and total protein (TP), albumin (ALB), globulin (GLB), triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) contents were determined, and albumin-to-globulin ratio (A/G) was calculated.

## 1.5 Statistical Analysis

Data were initially processed using Excel 2010, then subjected to one-way ANOVA using SPSS 20.0 software. Duncan's multiple range test was used for inter-group comparisons, with  $P < 0.05$  considered statistically significant.

## 2 Results

### 2.1 Effects of Attapulgit Nano Zinc Oxide on Growth Performance of Weaned Piglets

As shown in Table 2, dietary supplementation with antibiotics, zinc oxide, nano zinc oxide, and attapulgit nano zinc oxide had no significant effects on final body weight or F/G ( $P > 0.05$ ). Compared with the CON group, attapulgit nano zinc oxide supplementation significantly increased ADFI and ADG ( $P < 0.05$ ). Additionally, ADFI in the HA-ZO group was 34.78% higher than that in the ANT group ( $P < 0.05$ ).

**Table 2** Effects of attapulgit nano zinc oxide on growth performance of weaned piglets

*In the same row, values with different letter superscripts indicate significant difference ( $P < 0.05$ ), while the same or no letter superscripts indicate no significant difference ( $P > 0.05$ ). The same applies below.*

### 2.2 Effects of Attapulgit Nano Zinc Oxide on Diarrhea of Weaned Piglets

Table 3 shows that the diarrhea rate in the LA-ZO and HA-ZO groups was significantly lower than that in the CON group ( $P < 0.05$ ), with the LA-ZO group also significantly lower than the ANT group ( $P < 0.05$ ). All attapulgit nano zinc oxide groups exhibited significantly reduced diarrhea index compared with the CON group ( $P < 0.05$ ), and the LA-ZO group had a significantly lower diarrhea index than the ANT and NZO groups ( $P < 0.05$ ).

**Table 3** Effects of attapulgite nano zinc oxide on diarrhea of weaned piglets

### **2.3 Effects of Attapulgite Nano Zinc Oxide on Organ Indices of Weaned Piglets**

Table 4 indicates that dietary attapulgite nano zinc oxide supplementation had no significant effects on heart, liver, spleen, lung, kidney, or thymus indices compared with the CON group ( $P>0.05$ ). Heart index tended to increase with attapulgite nano zinc oxide dosage compared with the ANT and NZO groups ( $P>0.05$ ). The pancreas index in the LA-ZO and MA-ZO groups was significantly higher than that in the NZO group ( $P<0.05$ ).

**Table 4** Effect of attapulgite nano zinc oxide on organ indices of weaned piglets

### **2.4 Effects of Attapulgite Nano Zinc Oxide on Blood Biochemical Indices of Weaned Piglets**

Table 5 demonstrates that attapulgite nano zinc oxide supplementation had no significant effects on blood ALP and LDH activities or TP, ALB, GLB, and LDL contents, nor on A/G ratio ( $P>0.05$ ). The TG content in the LA-ZO and MA-ZO groups was significantly lower than that in the CON group ( $P<0.05$ ). The TC content in the MA-ZO group was 23.04% lower than that in the CON group ( $P<0.05$ ) and 25.94% lower than that in the ZO group ( $P<0.05$ ). The HDL content in the LA-ZO group showed no significant difference from the CON, ANT, and NZO groups ( $P>0.05$ ) but was significantly lower than that in the ZO group ( $P<0.05$ ). The HDL content in the MA-ZO group was 54.55% higher than that in the CON and ANT groups ( $P<0.05$ ), while the HA-ZO group exhibited 16.36% higher HDL content than the CON and ANT groups ( $P<0.05$ ).

**Table 5** Effects of attapulgite nano zinc oxide on blood biochemical indices of weaned piglets

## **3 Discussion**

### **3.1 Effects of Attapulgite Nano Zinc Oxide on Growth Performance of Weaned Piglets**

Early weaning compromises intestinal development and nutrient absorption efficiency in piglets, resulting in high diarrhea incidence and impaired growth. Feed intake and feed conversion efficiency are primary determinants of growth rate during this critical period [25-26]. Previous studies have shown that both attapulgite and nano zinc oxide can alleviate weaning stress and improve growth performance. Wang et al. [27] reported that 1,200 mg/kg nano zinc oxide improved intestinal barrier function and reduced diarrhea compared with a colistin sulfate-zinc oxide mixture, while decreasing zinc accumulation in tissues and excreta, indicating enhanced absorption and utilization that achieves high-dose conventional zinc oxide effects with lower supplementation. Long et al. [10] demon-

strated that 500 mg/kg nano zinc oxide significantly increased ADG, ADFI, and feed conversion efficiency, with effects comparable to 3,000 mg/kg conventional zinc oxide. Meng [28] found that 3,000 mg/kg attapulgite enhanced intestinal digestive enzyme activity, promoted carbohydrate conversion, and accelerated nutrient digestion, thereby increasing ADG and ADFI. Our results suggest that reduced attapulgite dosage can achieve similar outcomes, though the specific efficacy of low-dose attapulgite requires further investigation. This study evaluated attapulgite nano zinc oxide, a combination of low-dose attapulgite and nano zinc oxide particles. Compared with the CON group, attapulgite nano zinc oxide significantly increased ADG and ADFI, with the LA-ZO and MA-ZO groups showing effects comparable to the ZO group. The LA-ZO group exhibited superior growth performance to the ANT group and numerically better results than the NZO group, with the lowest F/G, indicating that reduced zinc and attapulgite dosages can maintain efficacy. However, feed conversion efficiency decreased with increasing attapulgite nano zinc oxide dosage. Attapulgite's viscosity, rheological properties, and ion exchange capacity enable formation of colloidal films that prolong intestinal retention time and improve villus growth [18,20,28-29]. We hypothesize that attapulgite forms a protective colloidal film on the intestinal mucosa, reducing damage and harmful substance contact while gradually releasing nano zinc oxide particles for absorption. Ion exchange between attapulgite and digestive tract ions may promote digestive enzyme release and accelerate feed conversion, thereby improving growth performance. However, excessive attapulgite nano zinc oxide coverage on the mucosal surface may hinder nutrient absorption and reduce feed conversion efficiency, though not significantly. Additionally, nano zinc oxide can enhance proliferation of Lgr5 and Bmil stem cells in the ileum, promoting intestinal epithelial renewal and improving nutrient absorption [30-31]. Thus, the synergistic action of attapulgite and nano zinc oxide on the intestine promotes piglet growth and development.

### 3.2 Effects of Attapulgite Nano Zinc Oxide on Diarrhea of Weaned Piglets

Post-weaning piglets have immature defense systems and poor resistance to environmental stressors, making them susceptible to intestinal flora disturbance and pathogen colonization, particularly *E. coli*, leading to diarrhea [32]. Attapulgite has demonstrated efficacy in treating diarrhea in humans and ruminants [33-35], but its application in piglets remains limited. Nano zinc oxide has been widely used in weaned piglets with good results in reducing diarrhea frequency [25-26]. Hu et al. [1] found that 500 and 750 mg/kg montmorillonite-zinc oxide mixtures (25% zinc content) significantly reduced diarrhea index in 4-14 day-old weaned piglets, with 500 mg/kg showing effects equivalent to 2,000 mg/kg zinc oxide, by improving intestinal mucosal integrity and digestive enzyme activity while reducing continuous solid feed irritation. Han [31] reported that both 2,000 mg/kg conventional zinc oxide and 500 mg/kg nano zinc oxide significantly reduced diarrhea incidence, with no difference between groups, primarily because nano zinc oxide decreased intestinal permeability and improved villus

height and crypt depth in the ileum and colon. Our results indicate that all three attapulgite nano zinc oxide levels effectively reduced diarrhea rate and index, with 700 mg/kg supplementation achieving the lowest values—superior to 3,000 mg/kg zinc oxide and 800 mg/kg nano zinc oxide, and significantly better than the ANT group. The 140 mg/kg attapulgite component, combined with nano zinc oxide, may achieve equivalent anti-diarrheal effects to 200 mg/kg attapulgite alone, while 1,000 mg/kg supplementation produced diarrhea index similar to 3,000 mg/kg zinc oxide, consistent with Hu et al. [1]. Therefore, 700 mg/kg attapulgite nano zinc oxide demonstrates optimal anti-diarrheal efficacy. The mechanism may involve nano zinc oxide increasing expression of tight junction proteins Occludin and Claudin, repairing the intestinal physical barrier, and eliminating Gram-negative bacteria and heat-resistant spores to facilitate beneficial flora colonization [36]. Attapulgite's adsorptive properties can bind mycotoxins, protecting the intestinal barrier. This synergistic action maintains intestinal integrity and flora stability, reducing diarrhea [37]. During the experiment, the CON and ANT groups primarily exhibited mild diarrhea and soft feces, resulting in higher diarrhea rates, though antibiotics improved feed utilization, yielding lower F/G than the CON group. High-dose zinc oxide alleviated diarrhea but may have impaired nutrient absorption and conversion, reducing feed conversion efficiency.

### **3.3 Effects of Attapulgite Nano Zinc Oxide on Organ Indices of Weaned Piglets**

Visceral organs are essential components that maintain normal growth and development while resisting environmental changes and pathogen invasion. Organ indices reflect organ growth, metabolism, function, and health status, thereby indicating animal performance and physiological condition. As a vital gastrointestinal component, the pancreas secretes endogenous digestive enzymes that influence nutrient absorption and utilization [38]. Healthy organ development is crucial for stress resistance and affects piglet growth and nutrient absorption. Our results show that compared with 800 mg/kg nano zinc oxide, 700 and 1,000 mg/kg attapulgite nano zinc oxide significantly increased pancreatic weight, slightly exceeding the CON group, though the pancreas index decreased with increasing dosage. Previous research indicates that high-dose nano zinc oxide can be toxic to mouse organs, impairing development [12]. We speculate that elevated zinc levels may inhibit activity of stress-related enzymes or stem cell differentiation in the pancreas, exacerbating oxidative stress and negatively affecting organ growth, while attapulgite may mitigate this damage—though the exact mechanism requires further study. Heart index tended to increase with attapulgite nano zinc oxide dosage, exceeding the ANT group but not differing significantly from the CON group, suggesting no significant effect on heart development or other organ growth.

### 3.4 Effects of Attapulgit Nano Zinc Oxide on Blood Biochemical Indices of Weaned Piglets

Blood is a critical component of the internal environment, and changes in biochemical parameters indirectly reflect alterations in organ function and metabolism. Healthy metabolism promotes nutrient absorption and enzyme release, maintaining nutritional balance, improving growth performance, and enhancing production efficiency. Triglycerides (TG), derived from fat breakdown or carbohydrate conversion, provide energy; serum TG levels reflect fat catabolism and body fat changes. Decreased TG indicates enhanced fat breakdown or conversion, with appropriate levels beneficial for homeostasis [39]. Total cholesterol (TC) is a lipid component and precursor for steroid hormone synthesis, reflecting lipid absorption and metabolism. Meng [28] reported that 3,000 mg/kg attapulgit had no significant effect on blood TG in weaned piglets, while Xu et al. [41] found that 40 mg/kg nano zinc oxide significantly reduced serum TC in broilers after 28 days. Our results with 1,000 mg/kg attapulgit nano zinc oxide align with the latter regarding TC reduction, differing from the former regarding TG, possibly because attapulgit alone does not significantly affect blood TG and TC, while nano zinc oxide modulates their synthesis and conversion, appropriately reducing levels to promote fat catabolism and lipid conversion/absorption, thereby improving energy utilization and growth performance. High-density lipoprotein (HDL) plays a crucial transport role, delivering cholesterol to adrenal glands and other tissues for metabolism and transporting it from peripheral tissues to the liver for biliary excretion, alleviating atherosclerosis [42]. Wang et al. [43] found that 150 and 300 mg/kg nano zinc oxide increased serum HDL in weaned piglets without significant difference from high-zinc groups, suggesting enhanced crude protein absorption and growth. Our results indicate that 1,000 mg/kg attapulgit nano zinc oxide significantly increased blood HDL content, surpassing the ANT group but not differing from the ZO group, demonstrating that attapulgit nano zinc oxide can enhance HDL synthesis, accelerate cholesterol conversion and inter-tissue transport, and promote nutrient metabolism and absorption, thereby improving animal performance.

## 4 Conclusion

1. Dietary attapulgit nano zinc oxide supplementation significantly increased ADG and ADFI while reducing diarrhea rate in weaned piglets, thereby improving growth performance. However, F/G increased with supplementation level.
2. Supplementation with 1,000 mg/kg attapulgit nano zinc oxide significantly decreased blood TG and TC contents and significantly increased blood HDL content, promoting fat catabolism, accelerating cholesterol conversion, and enhancing lipid metabolism.
3. Based on comprehensive results, 700 mg/kg attapulgit nano zinc oxide

can replace antibiotics, 800 mg/kg nano zinc oxide, and 3,000 mg/kg conventional zinc oxide for improving growth performance and reducing diarrhea in weaned piglets, enabling low-dose zinc application and improving production efficiency. Therefore, the recommended optimal supplementation level is 700 mg/kg.

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