

## Synthesis and antibacterial activity of a ZnO-fibre complex

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**Abstract:** In this experiment, a ZnO-fibre complex was prepared using the hydrothermal methods of "water solubility," "coupling agent," and "high temperature and high pressure". Binding rate, antibacterial activity, microstructure, and the infrared spectrum were measured using biomimetic digestion, bacterial proliferation tests, and ultra-fine electron microscopes. At first, ZnO-fibre complexes were prepared with different ratios of material and water. They were divided into five groups with ratios of 1:0, 1:4, 1:6, 1:8, and 1:10, respectively. The ZnO-fibre complexes were prepared with different coupling agents on the basis of experiment 1. They were divided into four groups. The ratio for material and water in the control group was 1:0, and in the treatment group, was 1:4. Treatment groups 2 and 3 had 10% guar gum or 10% bamboo fibre polymer composites (BFP) added on the basis of group 1. A ZnO-fibre complex was successfully prepared by adding 10% BFP at a ratio of material:water of 1:4, at a high temperature of 120 °C and a high pressure of 0.3 MPa for 20 min. The ZnO-binding rate reached 99.05%. The zinc oxide may bind to the carbonyl group of bamboo powder and adhere to the surface of and gaps in the bamboo fibre. The growth inhibition rate of *Escherichia coli*, *Salmonella*, and *Staphylococcus aureus* was double that of the common ZnO additive and Zn concentration. It is expected to be used as a slow-release ZnO additive.

**Keywords:** bacterial proliferation, binding rate of ZnO, hydrothermal method, infrared spectrum, zinc oxide

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## Introduction

Zinc oxide (ZnO) at pharmacological doses has been used to control diarrhoea of suckling piglets in the pig industry for decades. At present, zinc oxide or basic zinc chloride up to 1600 ppm (calculated by zinc element) is allowed to be added to piglet feed for 1 to 2 weeks after weaning according to the safety use standards for feed additives in China. However, massive use of ZnO may increase Zinc emissions, resulting in environmental pollution and damage to immune function (Komatsu et al., 2020). Studies on piglets infected with porcine epidemic diarrhoea have found that ZnO can inhibit bacterial proliferation and improve the anti-inflammatory response and damage caused to intestinal villi by viruses, thereby improving growth performance (Zhang et al., 2021). ZnO used in the first 10 days

after weaning can substantially improve the average daily feed intake, average daily gain, and feed conversion rate of weaned piglets, and reduce the incidence of diarrhoea; the use of three probiotics instead of ZnO failed to improve intestinal health and growth performance (Satessa *et al.*, 2020). Therefore, some researchers have replaced conventional ZnO with porous ZnO (Peng *et al.*, 2020) and nano-ZnO (Pei *et al.*, 2019) in order to reduce the amount of ZnO and environmental pollution.

In recent years, some new ZnO composites have shown good antibacterial properties and slow-release effects, which provides potential for further low-dose ZnO in weaned piglets' diets. Nano ZnO was used to prepare a preservative wrap for pork cryopreservation, and it was found that nano ZnO coating improved meat quality in the process of preservation by increasing the rupture of microbial cell membranes (Suo *et al.*, 2017). Using mushroom carboxymethyl chitosan as a natural polymer stabilizer, nano-ZnO composites were prepared using ultrasound, which showed synergistic antibacterial properties against gram-positive bacteria (*Staphylococcus aureus*) (Rao *et al.*, 2020). Nano-ZnO composites prepared with chitosan/alginate can effectively protect ZnO in simulated gastric juice, and the concentration of Zn<sup>2+</sup> is six times higher than that of conventional ZnO. The newly synthesized nano-ZnO composite can inhibit the proliferation of *E. coli* and *S. aureus* (Barreto *et al.*, 2017). Nano-sized ZnO prepared by hot melt extrusion of graft copolymer material and ZnO was applied to weaned piglets, which showed better zinc digestibility compared with conventional ZnO, helping to reduce zinc emission pollution (Oh *et al.*, 2020). These studies showed that the composite ZnO materials can be prepared by special technology using nano-ZnO, copolymers, and stabilizers as materials.

Bamboo powder is mainly composed of cellulose, hemicellulose, lignin, and protein, of which the cellulose accounts for the largest proportion. However, bamboo powder contains more than 75% insoluble dietary fibre (IDF), so it is necessary to use modification methods to increase the soluble components of dietary fibre to bring the physiological function of bamboo fibre into full play. Studies have found that the content of soluble dietary fibre (SDF) increases with the smaller particle size of bamboo powder dietary fibre prepared by ultra-fine grinding (Donadelli *et al.*, 2019). It has been shown that ultrasonic treatment improves the extraction efficiencies of hemicellulose and phenolic lignin compounds 2.6-fold from bamboo bast fibre powder, and the results of input energy and radical formation correlated with the calculated values of the anti-nodal point (Wang *et al.*, 2018). A GO (graphene oxide)/ZnO/Cu<sub>2</sub>O antibacterial coating was successfully sprayed on the ultrafine glass fibres using room temperature hydrothermal synthesis and air spraying techniques (Li *et al.*, 2022). Hybrid nanomaterials based on zinc oxide were synthesized with different silane coupling agents, and the sizes of the ZnO nanoparticles are changed as function of the silane precursor used in synthesis (Purcar *et al.*, 2017).

Our previous study found that micronized bamboo powder (MBP) at less than 100 µm processed by an impact mill could contribute to reducing harmful bacteria and improving growth performance of weaned piglets (Dai *et al.*, 2021) and broilers (Dai *et al.*, 2022). It has been shown that SDF is mostly fermented before reaching the colon, whereas IDF is mostly fermented in the colon of pigs (Jaworski & Stein, 2017). Therefore, it is assumed that if ZnO is coupled with bamboo fibre, it can be prevented from being digested in the stomach of piglets and can enter the hindgut to play a role. In the current experiment, a type of ZnO-fibre complex was prepared using a hydrothermal coupling reaction with ultrafine ZnO as a copolymer and MBP as a carrier. Its antibacterial properties were studied in order to develop a new type of sustained-release, alternative ZnO material to reduce the use of conventional zinc oxide feed additives.

## Material and methods

Ultramicar ZnO: 95% feed-grade ZnO was crushed using an impact mill (ZJ-C100, Sichuan Zhongjin Powder Equipment Co., Ltd). The particle size distribution of ZnO was determined using a laser particle size distribution analyser (BT-9300ST, Dandong Bettersize Instrument Co., Ltd, China). A particle size  $D_{90}$  value of 12.50  $\mu\text{m}$  corresponds to 90% of the cumulative particle size distribution.

Micronized bamboo powder (MBP): bamboo poles of *Phyllostachys pubescens* (Sichuan Province, China) was collected after growing for 5–6 years and processed into MBP as per a previously reported method (Dai *et al.*, 2022). The particle size value of  $D_{90}$  for MBP was 55.47  $\mu\text{m}$ .

Bamboo fibre-polymer composites (BFP) were provided by the Sichuan Provincial Key Laboratory of Bamboo Diseases, Insects, and Resources Development. The total dietary fibre content was 78.2%, soluble dietary fibre content was 19.2%, insoluble dietary fibre content was 59.0%, and water absorption expansion volume was 215–438 mL/g.

Guar gum, yeast extract (LP0021 YEAST EXTRACT), tryptone (LP0042 TRYPTONE), agar powder, and sodium chloride (analytically pure) were all procured from Chengdu Cologne Chemicals Co., Ltd. *Escherichia coli*, *S. aureus* and *Salmonella* strains were obtained from the strain preservation room of the Industrial Technology Innovation Research Institute of Beijing, Vica Biotechnology Co., Ltd, China.

Preparation of ZnO-fibre complex with different material-water ratios: The experiment was divided into five groups, with three replicates per group. The ratio of mixed base material and distilled water in Groups A, B, C, D, and E was 1:0, 1:4, 1:6, 1:8, and 1:10, respectively. The preparation steps were: (1) MBP and ultramicro ZnO were mixed evenly according to the mass ratio of 9:1 to make the basic material; (2) distilled water was added to 10 g of mixed basic material according to the experimental setup of the material-water quality ratio, then mixed and stirred evenly; (3) the mixed reactants were subjected to a high temperature of 120 °C and high pressure of 0.3 MPa for 20 min; and (4) the material was dried at 105 °C to a constant weight, and crushed through an 80-mesh sieve. The samples were stored in an air dryer for testing.

Preparation of ZnO-fibre complex with different coupling agents: On the basis of the above preparation process, the optimum ratio of material to water was found to be 1:4. The experiment was divided into four groups with three replicates each. The base material was made by mixing MBP and ultramicro ZnO according to the mass ratio of 9:1, and the trial was conducted with different coupling agents and different ratios of base material and distilled water. The treatment schemes of each experimental group are shown in Table 1. The preparation step was the same as the above step in the preparation of the ZnO-fibre complexes with different material-water ratios.

**Table 1** Preparation of ZnO-fibre complexes with different coupling agents

Treatment	CON	Group 1	Group 2	Group 3
<b>Coupling agent</b>	/	/	guar gum added to the base material at a mass ratio of 9:1	bamboo fibre polymer material added to the base material according to a mass ratio of 9:1
<b>The ratio of material to water</b>	1: 0	1: 4	1: 4	1: 4

The determination of binding rate: The ZnO-fibre complex (1 g) prepared in the above test was

added to 16 mL buffer (pH 2) in a 50 mL flask, and then put into a triangular flask shaker (37 °C, 180 rpm) for 4 h according to the *in vitro* transfer model (Auch *et al.*, 2019). After filtration, filtrate and filtrate residue were collected. The filter residue was dried at 105 °C to a constant weight, and the content of zinc in the filter residue and filtrate was determined using flame atomic absorption spectrometry.

$$\text{ZnO binding rate (\%)} = \frac{\text{total zinc of filter residue}}{\text{total zinc of filter residue} + \text{total zinc of filtrate}} \times 100$$

The determination of bacterial proliferation: *E. coli*, *Salmonella*, and *S. aureus* were activated, and a single colony was inoculated into LB medium and shaken overnight at 37 °C and 180 rpm. The activated strains were inoculated in LB medium at  $1 \times 10^5$  CFU/mL, and then incubated at 37 °C and 180 rpm shaker until the OD<sub>610nm</sub> value was close to 1.00. Then, 100 mL of the bacterial solution was added to the ZnO-fibre complex prepared in the above experiment, and the activated strains were incubated at 37 °C and 180 rpm. The OD<sub>610nm</sub> value was measured by spectrophotometer every two hours for three consecutive times. The experiment was divided into six groups with three replicates in each group (Table 2). The zinc concentration in CON1 and CON2 was twice and five times higher than that in TRE1, TRE2 and TRE3, respectively.

$$\text{Proliferation rate (\%)} = \frac{\text{OD}_{610\text{nm}} \text{ test} - \text{OD}_{610\text{nm}} \text{ start}}{\text{OD}_{610\text{nm}} \text{ start}} \times 100$$

**Table 2** Experimental design of bacterial proliferation test for ZnO-fibre complex

Group	Treatment
TRE1	0.1% ZnO-fibre complexes prepared in Group 1
TRE2	0.1% ZnO-fibre complexes prepared in Group 2
TRE3	0.1% ZnO-fibre complexes prepared in Group 3
CON1	0.1% ultrafine ZnO
CON2	0.02% ultrafine ZnO
CON	blank control

Electron microscopy: A small amount of dried sample was prepared using ion sputtering (GVC-2000, Beijing Zhongke Instrument Co., Ltd, China) to improve the resolution of the picture. MBP (CON) and three ZnO-fibre complexes (TRE1, TRE2 and TRE3) prepared in experiment 1.3.2 were observed using 17KV accelerated voltage electron microscopy (KYKY-FM6900LV, Beijing Zhongke Instrument Co., Ltd, China) at 13.3KX magnification.

Infrared spectrum observation: The infrared spectra of the ZnO loaded with MBP were determined using the KBr tableting method. The infrared characteristic absorption spectra of MBP, ultramicro ZnO, the mixture of the MBP and ultramicro ZnO (mass ratio 9:1), and the complex of ZnO-fibre were determined using the KBr method with a scanning wave number of 100–4000 cm<sup>-1</sup> (PK-6000 FT-IR infrared spectrometer, Mattson Company, USA). The variation in the stretching vibration peak of ZnO-fibre complex was analysed by comparing the stretching vibration peak of MBP and ultramicro ZnO.

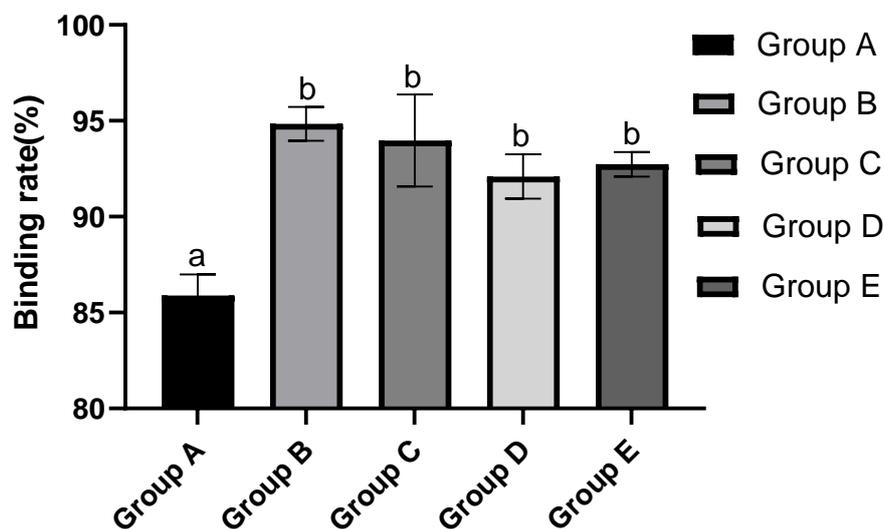
Data processing and statistical analysis: SPSS 22.0 was applied for statistical analysis. The differences in ZnO binding rate and bacterial proliferation rate among groups were compared using one-way analysis of variance (ANOVA), followed by the least significant difference test (LSD). Statistically significant differences were determined between groups (treatment group and control group)

by independent sample *t*-test.  $P < 0.05$  indicated that the difference was significant, and  $P < 0.10$  indicated a tendency of difference.

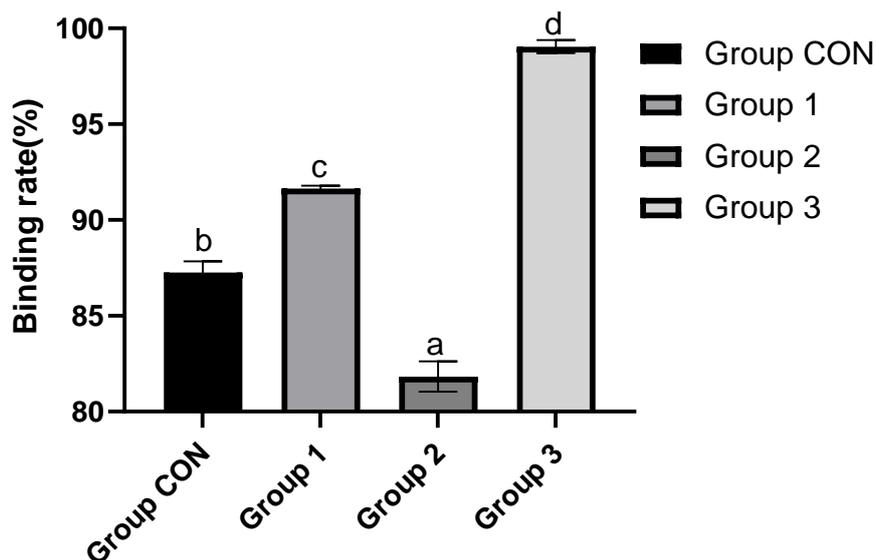
## Results and Discussion

The ZnO binding rates of the ZnO-fibre complexes prepared with different material-water ratios were determined. The ZnO binding rates of ZnO-fibre complexes in group B, C, D, and E were higher than those in Group A ( $P < 0.05$ ), while there was no difference among groups B, C, D, and E ( $P > 0.05$ ) (Figure 1). The results indicated that the ultramicro ZnO and MBP were more easily combined under hydration, but further increasing the proportion of water had no obvious effect on the combination. Considering the binding rate and the cost of subsequent drying, the ZnO-fibre complex can be prepared with the ratio of material to water of 1:4.

The ZnO binding rate was determined using the ZnO-fibre complexes prepared with different coupling agents (Figure 2). The ZnO binding rate of ZnO-fibre complexes in experimental group 3 was the highest (up to 99.05%) and was higher than that in control group, experimental group 1, and group 2 ( $P < 0.05$ ), while that in experimental group 1 was higher than that in control group 1 ( $P < 0.05$ ), and that in experimental group 2 was lower than that in control group 1 ( $P < 0.05$ ). The above results confirm that ultramicro ZnO and MBP are more easily combined under hydration. Bamboo fibre-polymer composite as coupling agent can further improve the combination rate of ultramicro ZnO and MBP, while guar gum will reduce their combination efficiency.



**Figure 1** Effect of different ratios of material to water on the binding rate of ZnO-fibre complex  
 Note: Columns with different small letter superscripts indicate a significant difference ( $P < 0.05$ )



**Figure 2** Effect of different coupling agents on the binding rate of ZnO-fibre complex

Note: Columns with different small letter superscripts indicate a significant difference ( $P < 0.05$ )

Synthesis of coordination compounds by processing plays a vital role in coordination chemistry research. At present, the main preparation methods of zinc oxide nanoparticles include the chemical precipitation method (Raja *et al.*, 2014), sol-gel hydrothermal method (Chalanger *et al.*, 2021), solvothermal method (Bai *et al.*, 2015), and thermal decomposition method (Zhu *et al.*, 2022). Due to the differences and particularity of chemical structures in different complexes, some new synthesis methods should be explored and studied according to the properties of substrates. ZnO possesses good convergent and bacteriostatic properties and has been widely used in the synthesis of new ZnO composite materials in biological antibacterial fields. Hot-melt extruded ZnO was produced with soluplus (grafted copolymer) and ZnO and extruded using a twin-screw hot-melt extruder (Oh *et al.*, 2020). A commercial silane coupling agent was used to improve the dispersion of zinc oxide nanoparticles in thin polyacrylonitrile fibres, and maintained excellent antibacterial activity (Aamer *et al.*, 2021). Electroplating and electrochemical anodization method were successfully used for the synthesis of zinc oxide nanostructures on carbon fibre surfaces (Aykaç & Akkaş, 2022).

According to the physical and chemical characteristics of the carrier material, the synthesis process can be further optimized to improve the loading rate. A new composite antibacterial material ZnO/Cu<sup>2+</sup>-Chitosan/Montmorillonite (ZCCM) was prepared with montmorillonite as carrier, Zn(Ac)<sub>2</sub>·2H<sub>2</sub>O, Cu(NO<sub>3</sub>)<sub>2</sub>·3H<sub>2</sub>O, and chitosan as raw materials, and displayed excellent antibacterial activity which was higher than ZnO-Montmorillonite, Cu<sup>2+</sup>-Montmorillonite, or ZnO/Cu<sup>2+</sup>-Montmorillonite (Ma *et al.*, 2021). Furthermore, soluble corn starch could be used to stabilize ZnO-nanoparticles (NPs) onto the surface of cotton fabrics as entrapped species and improved the adhesion properties of the cotton fibres towards ZnO-NPs (El-Nahhal *et al.*, 2020). According to the above literature, reaction parameters, carrier characteristics, and coupling agents have important effects on the binding rate of zinc oxide and the carrier.

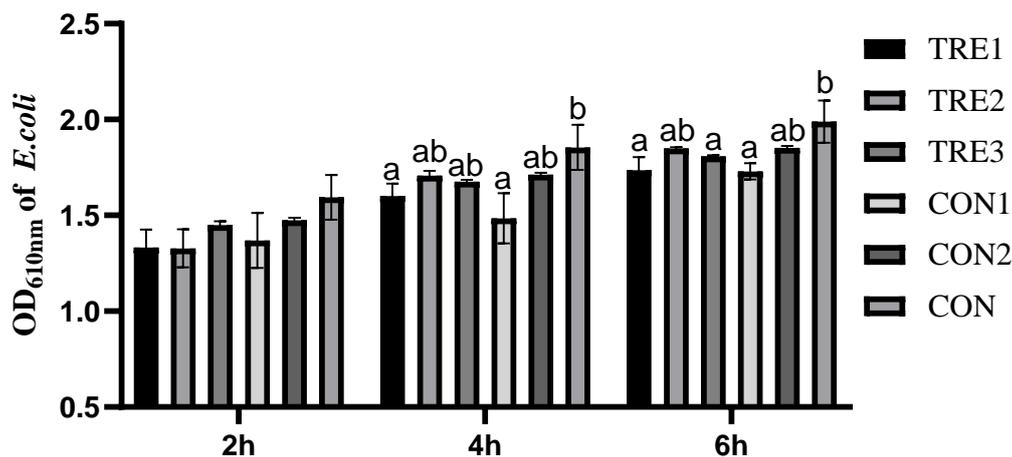
According to the characteristics of bamboo fibre rich in carbonyl structures and zinc oxide containing divalent ionic bonds, a ZnO-fibre complex was prepared using "water dissolution," "high temperature and high pressure," and "coupling agent" methods. By optimizing the ratio of material to

water and coupling agent, a ZnO complex with a high binding rate of 99% was obtained. Compared with the synthetic method mentioned above, this method has the advantages of simple operation, high loading rate, and no other chemical residues, achieving the aim of developing a highly-efficient ZnO-fibre complex as a substitute for ZnO additive in feed.

At 4 h, the OD<sub>610nm</sub> value of *E. coli* in the TRE1 and CON1 groups was lower than in the CON group ( $P < 0.05$ ), and the OD<sub>610nm</sub> value of *E. coli* in the TRE2, TRE3, and CON2 groups was lower than that in the CON group, but did not reach a significant level ( $P > 0.05$ ) (Figure 3). At 6 h, the OD<sub>610nm</sub> value of *E. coli* in the TRE1, TRE3, and CON1 groups was lower than that in the CON group ( $P < 0.05$ ), and the OD<sub>610nm</sub> value of *E. coli* in the TRE2, TRE3, and CON2 groups was lower than that in the CON group ( $P > 0.05$ ).

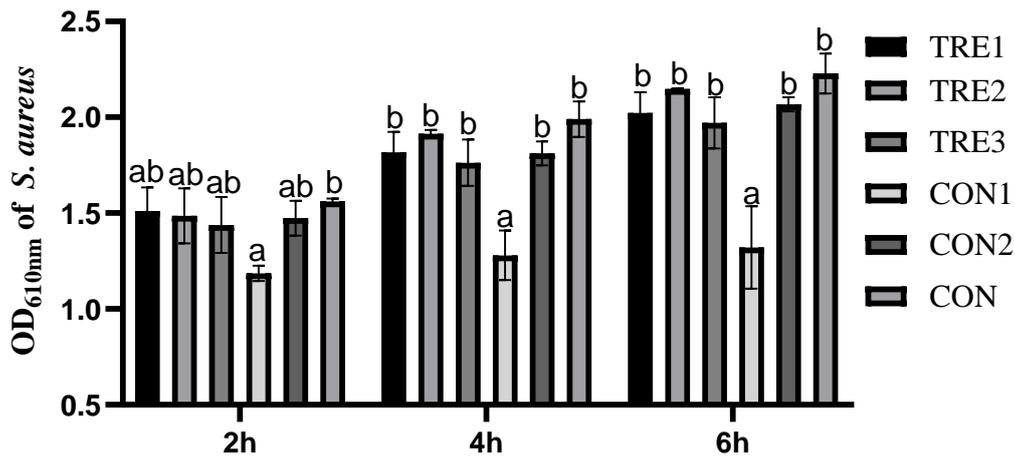
At 2 h, 4 h, and 6 h, the OD<sub>610nm</sub> values of *S. aureus* in the CON1 group were lower than those in the CON group ( $P < 0.05$ ), and the OD<sub>610nm</sub> values of *S. aureus* in TRE1, TRE2, TRE3, and CON2 groups were all lower than those in the CON group, but did not reach a significant level ( $P > 0.05$ ) (Figure 4). Among the experimental groups, the value of TRE3 group was the lowest.

At 2 h and 4 h, OD<sub>610nm</sub> values of *Salmonella* in the TRE3 and CON1 groups were lower than those in the CON group ( $P < 0.05$ ), and OD<sub>610nm</sub> values of *Salmonella* in the TRE1, TRE2, and CON2 groups were lower than those in CON group, but did not reach a significant level ( $P > 0.05$ ) (Figure 5). At 6 h, the OD<sub>610nm</sub> values of *Salmonella* in the TRE1, TRE2, TRE3, CON1, and CON2 groups were lower than those in the CON group ( $P < 0.05$ ), but there was no significant difference among the TRE1, TRE2, TRE3, CON1, and CON2 groups ( $P > 0.05$ ). At 2 h, 4 h and 6 h, the OD<sub>610nm</sub> value of *Salmonella* in TRE3 group was the lowest, and at 4 h, the OD<sub>610nm</sub> value of *Salmonella* in TRE3 group was lower than that in the TRE1 group ( $P < 0.05$ ). Comprehensive analysis of the above results indicates that the ZnO-fibre complex prepared by TRE3 had a strong inhibitory effect on *E. coli*, *S. aureus*, and *Salmonella*.



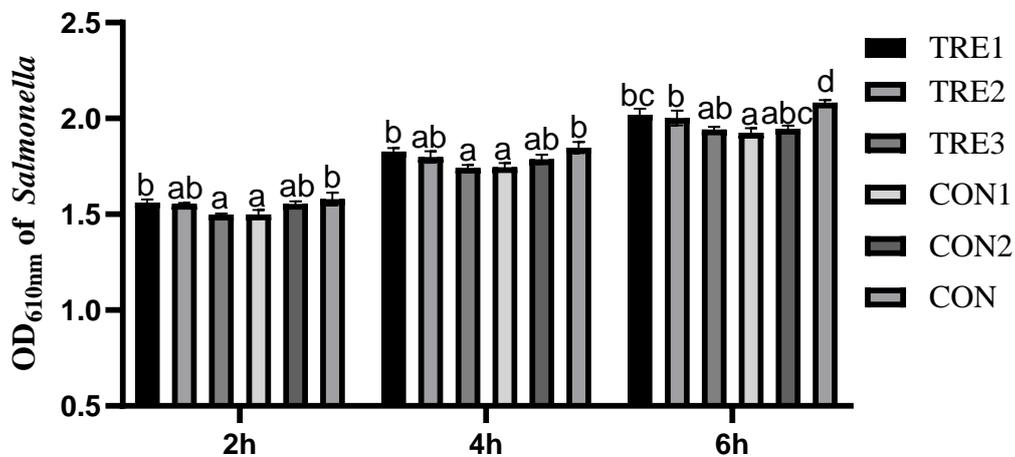
**Figure 3** Effect of ZnO-fibre complex on the proliferation of *E. coli*

Note: Columns with different small letter superscripts indicate a significant difference ( $P < 0.05$ )



**Figure 4** Effect of ZnO-fibre complex on the proliferation of *S. aureus*

Note: Columns with different small letter superscripts indicate a significant difference ( $P < 0.05$ )



**Figure 5** Effect of ZnO-fibre complex on the proliferation of *Salmonella*

Note: Columns with different small letter superscripts indicate a significant difference ( $P < 0.05$ )

New antibacterial composites involving ZnO have become a research hotspot due to its excellent antibacterial, thermal, and chemical stability. The newly synthesized nano-ZnO is a kind of inorganic, antibacterial agent with good antibacterial properties, which shows remarkable inhibitory effects on many bacteria. Studies have found that nano-ZnO can achieve this antibacterial effect by interacting with bacteria through surface contact or zinc ion dissolution (Mirzaei & Darroudi, 2017). Compared with ordinary ZnO, the specific surface area of nano-ZnO increases and its antibacterial performance is enhanced (Luo *et al.*, 2013).

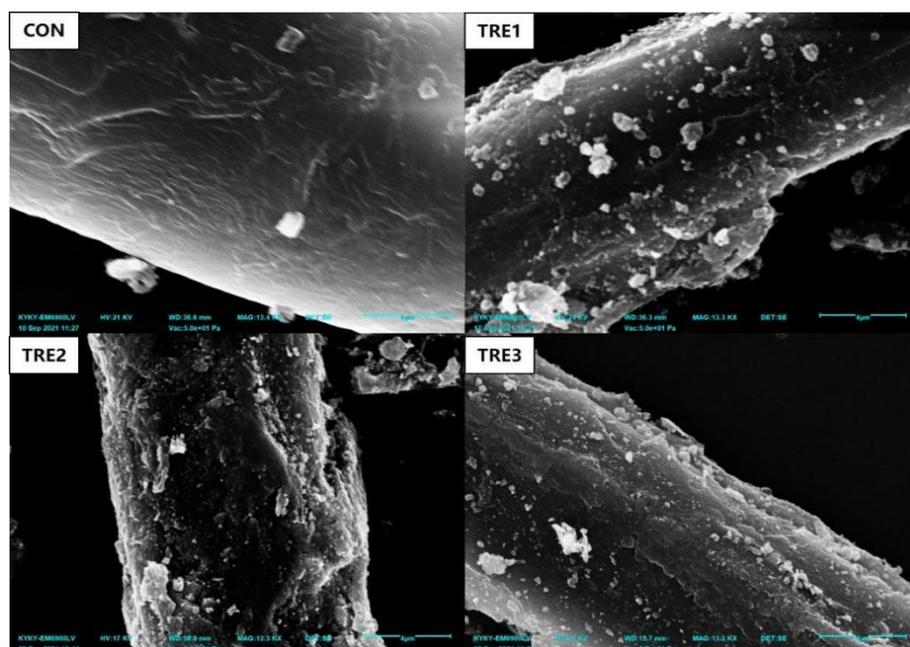
At present, pharmacological doses of ZnO can increase animal feed intake (Walk *et al.*, 2015), increase anti-inflammatory cytokines and reduce pro-inflammatory cytokines (Peng *et al.*, 2020), and promote restoration of intestinal barrier function and development (Zhu *et al.*, 2017), and is widely used for diarrhoea control (Sun *et al.*, 2019) and growth promotion in weaned piglets (Wang *et al.*, 2018). However, pharmacological doses of ZnO can also cause environmental pollution and bacterial

resistance to antibiotics (Bednorz *et al.*, 2013). In the current study, the modified ZnO could reduce the amount of ZnO and environmental emission reduction in actual production on the premise of maintaining the growth performance of piglets. In order to improve the efficiency of ZnO, impact grinding was used to obtain ultramicro ZnO. The  $D_{90}$  value was 12.50  $\mu\text{m}$ , close to the quasi-nanometer level.

The addition of nano-ZnO to a copolymer of 3-hydroxybutyrate and 3-hydroxyvalerate had a significant inhibitory effect on *E. coli*. (Li *et al.*, 2012; Song *et al.*, 2020), which may be related to the release of reactive oxygen species (ROS) in nano-ZnO (Banoee *et al.*, 2010). Studies have found that UV irradiation of nano-ZnO promote the generation of ROS, thereby improving the antibacterial ability of nano-ZnO against *E. coli* (Li *et al.*, 2012; Sirelkhatim *et al.*, 2015). However, more evidence has shown that the accumulation of Zn ions released by nano-ZnO is positively correlated with the inhibitory effect on *E. coli* (Zhang *et al.*, 2018; Song *et al.*, 2020). Studies have also found that nano-ZnO has better antibacterial effect on *E. coli* than *S. aureus* (Díez-Pascual & Díez-Vicente, 2014). The results of the current study showed that the 6-h growth rates of *E. coli*, *S. aureus*, and *Salmonella* were 94.44%, 83.16%, and 88.64%, respectively, after the addition of a ZnO-fibre complex prepared using the third process, which may be related to the different characteristics of the bamboo fibre carrier.

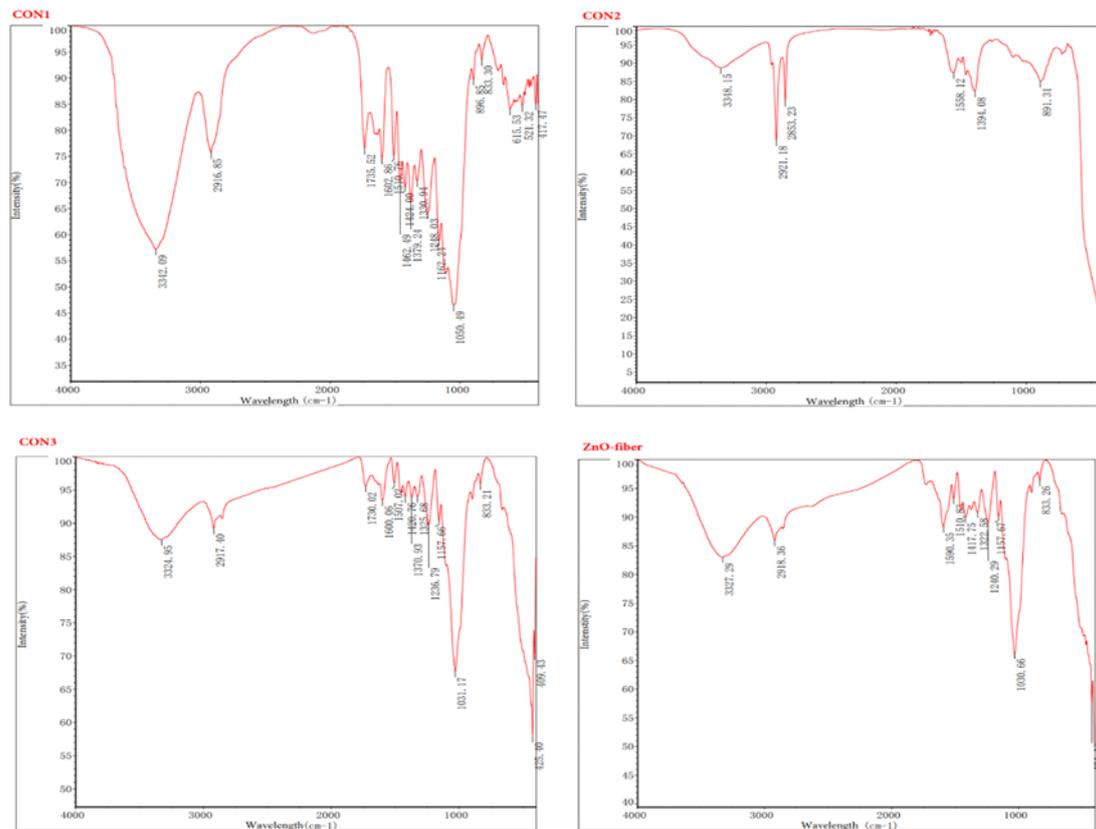
The bacteriostatic activity of the complex is mainly determined by the carrier, and the efficiency of bacteriostatic activity can also be increased by improving the adsorbability and cytocompatibility of the carrier. This experiment showed that the 6-h growth rates of *E. coli*, *S. aureus*, and *Salmonella* after the addition of ZnO-fibre complex prepared using the third process were lower than that of the control group. The effect is comparable to that of the double-dose ZnO treatment, which may be related to the improved adsorbability and compatibility of the MBP polymer material. In this study, the ZnO-fibre complex prepared using "water-soluble," "coupling agents," "high temperature and high pressure" methods can achieve strong antibacterial effects, which is expected to be provide an alternative additive of zinc oxide to achieve zinc emission reduction and contribute to 'green' and healthy pig breeding.

Compared with the control MBP, tiny, white particles increased on the surface of the bamboo fibre loaded with ZnO prepared using TRE1, TRE2, and TRE3, indicating that the ultramicro-ZnO can adhere to the surface of MBP through the action of "water solubility" and "high temperature and pressure" (Figure 6). Compared with TRE1, the surface cracks of bamboo fibres were reduced in TRE2 and TRE3 when supplemented with the coupling agent, which may be caused by the filling effect of coupling agent. There were fewer tiny, white particles on the surface of TRE2 than on TRE3, which also confirmed that the ZnO binding rate of TRE2 was lower than that of TRE3. Using scanning electron microscope ultrastructure, it is observed that bamboo fibre polymer material is more suitable as a coupling agent than guar gum for the binding reaction between ultramicro ZnO and MBP.



**Figure 6** Scanning electron microscopy images of the ZnO-fibre complex

Infrared spectroscopy was used to study the molecular vibration and polar bonds between different atoms (Figure 7). The infrared spectra of ZnO-fibre complex prepared in experimental group 3 were similar to those of MBP (CON1) and the mixture of MBP and ultramicro ZnO (CON3), but differed greatly from those of ultramicro ZnO (CON2). The stretching vibration peak of the hydroxyl group in bamboo powder is at  $3342.09\text{cm}^{-1}$ , which moves to  $3327.29\text{cm}^{-1}$  after combining with ZnO and  $3324.95\text{cm}^{-1}$  after mixing with ZnO. This phenomenon may be caused by an induction effect or dipole field effect, which can lead to a change in electron cloud density of the hydroxyl group. The stretching vibration peak of carbonyl group appeared at  $1735.52\text{cm}^{-1}$  in bamboo powder, the peak disappeared after the binding reaction with ZnO, and finally moved to  $1730.02\text{cm}^{-1}$  after mixing with ZnO. This indicates that the ultramicro ZnO has been loaded on bamboo fibres, and the carbonyl group of bamboo powder may be involved in the binding reaction with ZnO.



**Figure 7** Fourier transform infrared spectroscopy spectra for the ZnO-fibre complex. micronized bamboo powder, MBP (CON1); ultramicro ZnO (CON2); mixture of MBP and ultramicro ZnO (CON3)

Studies have found that the surface of the ZnO quantum dots has hydroxyl and amino functional groups using infrared spectrum analysis in order to explore the functional groups on the surface of ZnO quantum dots in the nano-ZnO complex (Naseri *et al.*, 2017). When the content of the hydroxyl group is high, the hydrolysis process will be affected by 3-aminopropyl trimethoxysilane (PTES) into silicon dioxide. The content of functional groups on the surface of ZnO quantum dots is small, and the hydroxyl and amino functional groups are hydrophilic groups, which helps to enhance the stability of ZnO quantum dots in the aqueous dispersion system (Patra *et al.*, 2009; Lores-Padín *et al.*, 2020). The polar bonds and molecular vibrations was studied between different atoms of the ZnO-fibre complex using infrared spectroscopy. The results showed that the carbonyl group of bamboo fibre may participate in the binding reaction with ZnO, which is helpful in improving the stability of the complex.

In order to improve the loading rate of the complex, some scholars have reported that alkali treatment can effectively remove some hemicellulose, lignin, wax, and other substances on the fibre surface or even inside the fibre (Roy *et al.*, 2012), expose the matrix fibre, increase the fibre surface area as well as surface roughness (Zhou *et al.*, 2016), and enhance mechanical properties of the monofilament (Goda *et al.*, 2006), thermal stability (Mohd Edeerozey *et al.*, 2018), and fracture toughness (Zhang *et al.*, 2018). In the current experiment, ultramicroscopic electron microscopy was used to determine that the loading rate of ZnO increased in the treatment group with high temperature and high pressure, which may be related to the decrease in fibre crystallinity and increase in fibre surface void. On this basis, the loading rate can be further increased using a coupling agent treatment.

The results indicate that guar gum tended to reduce the load of ZnO, and bamboo fibre polymer material tended to enhance the load. This indicates that the choice of coupling agent plays a very vital role in the preparation of ZnO-fibre complexes. The experimental synthesis method can effectively increase the load of zinc oxide, and has the advantages of safety, high efficiency, and no pollution.

## Conclusions

It was concluded that the best synthesis method of a ZnO-fibre complex was treatment with high temperature and high pressure at a ratio 9:1 for MBP to ultramicro ZnO and 10% bamboo fibre polymer composites, and that the best ratio was 9:1 for material to water. The binding rate of the ZnO-fibre complex reached 99.05%. The carbonyl group of the bamboo fibre is involved in the binding reaction with zinc oxide, and was mainly attached to the surface and pores of the bamboo fibre. *In vitro* bacterial proliferation showed that the ZnO-fibre complex had better inhibitory performance against *E. coli.*, *Salmonella*, and *S. aureus*, and the effect was nearly twice that of the ZnO control group at the same zinc concentration.

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## Authors' contributions

Fawen Dai initiated the idea, finished the experimental design. Fawen Dai, Xia Huang, Mujia Shi, and Fei Zhao conducted the laboratory index determination. Tao Lin and Fawen Dai prepared the initial manuscript in English. Yaojun Yang and Xiang Nong made the final revision. All authors read and approved the final manuscript.

## Conflicts of interest declaration

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work. All results were strictly in accordance with the experimental data.

## References

- Aamer, H., Kim, S.B., Oh, J.M., Park, H. & Jo, Y.M. 2021. ZnO-impregnated polyacrylonitrile nanofibre filters against various phases of air pollutants. *Nanomaterials (Basel)*. 11(9), 2313. doi 10.3390/nano11092313
- Auch, C., Jede, C., Harms, M., Wagner, C. & Mäder, K. 2020. Impact of amorphization and GI physiology on supersaturation and precipitation of poorly soluble weakly basic drugs using a small-scale *in vitro* transfer model. *Int J Pharm.* 574, 118917. doi 10.1016/j.ijpharm.2019.118917
- Aykaç, A. & Akkaş, E.Ö. 2022. Synthesis, characterization, and antibacterial properties of ZnO nanostructures functionalized flexible carbon fibres. *Recent Pat Nanotechnol.* doi 10.2174/1872210516666220414103629
- Bai, X., Li, L., Liu, H., Tan, L., Liu, T. & Meng, X. 2015. Solvothermal synthesis of ZnO nanoparticles and anti-infection application *in vivo*. *ACS Appl Mater Interfaces*. 7(2), 1308-1317. doi 10.1021/am507532p
- Banoee, M., Seif, S., Nazari, Z.E., Jafari-Fesharaki, P., Shahverdi, H.R., Moballegh, A., Moghaddam, K.M. & Shahverdi, A.R. 2010. ZnO nanoparticles enhanced antibacterial activity of ciprofloxacin against *Staphylococcus aureus* and *Escherichia coli*. *J Biomed Mater Res B Appl Biomater.* 93(2), 557-561. doi 10.1002/jbm.b.31615

- Barreto, M.S.R., Andrade, C.T., da Silva, L.C.R.P., Cabral, L.M., Flosi Paschoalin, V.M. & Del Aguila, E.M. 2017. *In vitro* physiological and antibacterial characterization of ZnO nanoparticle composites in simulated porcine gastric and enteric fluids. *BMC Vet Res.* 13(1),181. doi 10.1186/s12917-017-1101-9
- Bednorz, C., Oelgeschläger, K., Kinnemann, B., Hartmann, S., Neumann, K., Pieper, R., Bethe, A., Semmler, T., Tedin, K., Schierack, P., Wieler, L.H. & Guenther, S. 2013. The broader context of antibiotic resistance: Zinc feed supplementation of piglets increases the proportion of multi-resistant *Escherichia coli in vivo*. *Int J Med Microbiol.* 303(6-7), 396-403. doi 10.1016/j.ijmm.2013.06.004
- Chalangar, E., Nur, O., Willander, M., Gustafsson, A. & Pettersson, H. 2021. Synthesis of vertically aligned ZnO nanorods using sol-gel seeding and colloidal lithography patterning. *Nanoscale Res Lett.* 16(1), 46. doi 10.1186/s11671-021-03500-7
- Dai, F.W., Lin, T., Cheng, L.M., Wang, J., Zuo, J.J. & Feng, D.Y. 2022. Effects of micronized bamboo powder on growth performance, serum biochemical indexes, cecal chyme microflora, and metabolism of broilers aged 1–22 days. *Trop Anim Health Prod.* 54(3), 166. doi 10.1007/s11250-022-03172-0
- Dai, F.W., Lin, T., Su, B.Y., Yao, H.M., Gu, D.C. & Yang, Y.J. 2021. Effects of feeding bamboo powder on growth performance, serum biochemical indexes and fecal microorganism of weaned piglets. *Chinese Journal of Animal Nutrition (in Chinese).* 33(12), 6709-6720
- Díez-Pascual, A.M. & Díez-Vicente, A.L. 2014. ZnO-reinforced poly(3-hydroxybutyrate-co-3-hydroxyvalerate) bionanocomposites with antimicrobial function for food packaging. *ACS Appl Mater Interfaces.* 6(12), 9822-9834. doi 10.1021/am502261e
- Donadelli, R.A., Stone, D.A., Aldrich, C.G. & Beyer, R.S. 2019. Effect of fibre source and particle size on chick performance and nutrient utilization. *Poult Sci.* 98(11),5820-5830. doi 10.3382/ps/pez382
- El-Nahhal, I.M., Salem, J., Anbar, R., Kodeh, F.S. & Elmanama, A. 2020. Preparation and antimicrobial activity of ZnO-NPs coated cotton/starch and their functionalized ZnO-Ag/cotton and Zn(II) curcumin/cotton materials. *Sci Rep.* 10(1), 5410. doi 10.1038/s41598-020-61306-6
- Goda, K., Sreekala, M., Gomes, A., Kaji, T. & Ohgi, J. 2006. Improvement of plant based natural fibres for toughening green composites—Effect of load application during mercerization of ramie fibres. *Compos Part A-Appl S.* 2006, 37(12): 2213-2220. doi 10.1016/j.compositesa.2005.12.014
- Jaworski, N.W. & Stein, H.H. 2017. Disappearance of nutrients and energy in the stomach and small intestine, cecum, and colon of pigs fed corn-soybean meal diets containing distillers dried grains with solubles, wheat middlings, or soybean hulls. *J Anim Sci.* 95(2), 727-739. doi 10.2527/jas.2016.0752
- Komatsu, T., Sugie, K., Inukai, N., Eguchi, O., Oyamada, T., Sawada, H., Yamanaka, N. & Shibahara, T. 2020. Chronic pancreatitis in farmed pigs fed excessive zinc oxide. *J Vet Diagn Invest.* 32(5), 689-694. doi 10.1177/1040638720944368
- Li, M., Chen, Z., Yang, L., Li, J., Xu, J., Chen, C., Wu, Q., Yang, M. & Liu, T. 2022. Antibacterial Activity and Mechanism of GO/Cu<sub>2</sub>O/ZnO coating on ultrafine glass fibre. *nanomaterials (Basel).* 12(11), 1857. doi 10.3390/nano12111857
- Li, Y., Zhang, W., Niu, J. & Chen Y. 2012. Mechanism of photogenerated reactive oxygen species and correlation with the antibacterial properties of engineered metal-oxide nanoparticles. *ACS Nano.* 6(6), 5164-5173. doi 10.1021/nn300934k
- Lores-Padín, A., Menero-Valdés, P., Fernández, B. & Pereiro, R. 2020. Nanoparticles as labels of specific-recognition reactions for the determination of biomolecules by inductively coupled plasma-mass spectrometry. *Anal Chim Acta.* 1128, 251-268. doi 10.1016/j.aca.2020.07.008

- Luo, Z., Wu, Q., Xue, J. & Ding, Y. 2013. Selectively enhanced antibacterial effects and ultraviolet activation of antibiotics with ZnO nanorods against *Escherichia coli*. *J Biomed Nanotechnol.* 9(1), 69-76. doi 10.1166/jbn.2013.1472
- Ma, X., Gao, L., Ma, Y. & Zhang, X. 2021. Antibacterial activity and mechanism of ZnO/Cu<sup>2+</sup>-chitosan/montmorillonite. *J Wuhan Univ Technol Mater Sci Ed.* 36(4), 510-516. doi 10.1007/s11595-021-2438-2
- Mirzaei, H. & Darroudi, M. 2017. Zinc oxide nanoparticles: Biological synthesis and biomedical applications. *CeramInt.* 43(1), 907-914. doi 10.1016/j.ceramint.2016.10.051
- Mohd Edeerozey A.M., Akil, H.M., Azhar, A.B. & Zainal Ariffin, M.I. 2007. Chemical modification of kenaf fibres. *Mater Lett.* 61(10), 2023-2025. doi 10.1016/j.matlet.2006.08.006
- Naseri, A., Samadi, M., Mahmoodi, N.M., Pourjavadi, A., Mehdipour, H. & Moshfegh, A.Z. 2017. Tuning composition of electrospun ZnO/CuO nanofibres: Toward controllable and efficient solar photocatalytic degradation of organic pollutants. *J Phys Chem C.* 121(6), 3327-3338. doi 10.1021/acs.jpcc.6b10414
- Oh, S.M., Kim, M.J., Hosseindoust, A., Kim, K.Y., Choi, Y.H., Ham, H.B., Hwang, S.J., Lee, J.H., Cho, H.J., Kang, W.S. & Chae, B.J. 2020. Hot melt extruded-based nano zinc as an alternative to the pharmacological dose of ZnO in weanling piglets. *Asian-Australas J Anim Sci.* 33(6), 992-1001. doi 10.5713/ajas.19.0140
- Patra, M.K., Manoth, M., Singh, V.K., Siddaramana Gowd, G., Choudhry, V.S., Vadera, S.R. & Kumar, N. 2009. Synthesis of stable dispersion of ZnO quantum dots in aqueous medium showing visible emission from bluish green to yellow. *J Lumin.* 129, 320-324. doi 10.1016/j.jlumin.2008.10.014
- Pei, X., Xiao, Z., Liu, L., Wang, G., Tao, W., Wang, M., Zou, J. & Leng, D. 2019. Effects of dietary zinc oxide nanoparticles supplementation on growth performance, zinc status, intestinal morphology, microflora population, and immune response in weaned pigs. *J Sci Food Agri.* 99(3), 1366-1374. doi 10.1002/jsfa.9312
- Peng, P., Deng, D., Chen, S., Li, C., Luo, J., Romeo, A., Li, T., Tang, X. & Fang, R. 2020. The effects of dietary porous zinc oxide supplementation on growth performance, inflammatory cytokines and tight junction's gene expression in early-weaned piglets. *J Nutr Sci Vitaminol (Tokyo).* 66(4), 311-318. doi 10.3177/jnsv.66.311
- Purcar, V., Şomoghi, R., Niţu, S.G., Nicolae, C.A., Alexandrescu, E., Gîfu, I.C., Gabor, A.R., Stroescu, H., Ianchiş, R., Căprărescu, S. & Cintează, L.O. 2017. The effect of different coupling agents on nano-ZnO materials obtained via the sol-gel process. *Nanomaterials (Basel).* 7(12), 439. doi 10.3390/nano7120439
- Raja, K., Ramesh, P.S. & Geetha, D. 2014. Synthesis, structural and optical properties of ZnO and Ni-doped ZnO hexagonal nanorods by Co-precipitation method. *Spectrochim Acta A Mol Biomol Spectrosc.* 120, 19-24. doi 10.1016/j.saa.2013.09.103
- Rao, K.M., Suneetha, M., Park, G.T., Babu, A.G. & Han, S.S. 2020. Hemostatic, biocompatible, and antibacterial non-animal fungal mushroom-based carboxymethyl chitosan-ZnO nanocomposite for wound-healing applications. *Int J Biol Macromol.* 155, 71-80. doi 10.1016/j.ijbiomac.2020.03.170
- Roy, A., Chakraborty, S., Kundu, S.P., Basak, R.K., Majumder, S.B. & Adhikari, B. 2012. Improvement in mechanical properties of jute fibres through mild alkali treatment as demonstrated by utilisation of the Weibull distribution model. *Bioresour Technol.* 107, 222-228. doi 10.1016/j.biortech.2011.11.073
- Satessa, G.D., Kjeldsen, N.J., Mansouryar, M., Hansen, H.H., Bache, J.K. & Nielsen, M.O. 2020. Effects of alternative feed additives to medicinal zinc oxide on productivity, diarrhoea incidence, and gut development in weaned piglets. *Animal.* 26, 1-9. doi 10.1017/S1751731120000154

- Sirelkhatim, A., Mahmud, S., Seeni, A., Kaus, N.H.M., Ann, L.C., Bakhori, S.K.M., Hasan, H. & Mohamad, D. 2015. Review on Zinc oxide nanoparticles: Antibacterial activity and toxicity mechanism. *Nanomicro Lett.* 7(3), 219-242. doi 10.1007/s40820-015-0040-x
- Song, K., Zhang, W., Sun, C., Hu, X., Wang, J. & Yao L. 2020. Dynamic cytotoxicity of ZnO nanoparticles and bulk particles to *Escherichia coli*: A view from unfixed ZnO particle: Zn<sup>2+</sup> ratio. *Aquat Toxicol.* 220, 105407. doi 10.1016/j.aquatox.2020.105407
- Sun, Y.B., Xia, T., Wu, H., Zhang, W.J, Zhu, Y.H., Xue, J.X., He, D.T. & Zhang, L.Y. 2019. Effects of nano zinc oxide as an alternative to pharmacological dose of zinc oxide on growth performance, diarrhoea, immune responses, and intestinal microflora profile in weaned piglets. *Anim Feed Sci Tech.* 258, 114312. doi 10.1016/j.anifeedsci.2019.114312
- Suo, B., Li, H., Wang, Y., Li, Z., Pan, Z. & Ai, Z. 2017. Effects of ZnO nanoparticle-coated packaging film on pork meat quality during cold storage. *J Sci Food Agric.* 97(7), 2023-2029. doi 10.1002/jsfa.8003
- Walk, C.L., Wilcock, P. & Magowan, E. 2015. Evaluation of the effects of pharmacological zinc oxide and phosphorus source on weaned piglet growth performance, plasma minerals and mineral digestibility. *Animal.* 9(7), 1145-52. doi 10.1017/S175173111500035
- Wang, C., Tallian, C., Su, J., Vielnascher, R., Silva, C., Cavaco-Paulo, A., Guebitz, G.M. & Fu, J. 2018. Ultrasound-assisted extraction of hemicellulose and phenolic compounds from bamboo bast fibre powder. *PLoS One.* 13(6), e0197537. doi 10.1371/journal.pone.0197537
- Wang, C., Zhang, L., Ying, Z., He, J., Zhou, L., Zhang, L., Zhong, X. & Wang, T. 2018. Effects of dietary zinc oxide nanoparticles on growth, diarrhoea, mineral deposition, intestinal morphology, and barrier of weaned piglets. *Biol Trace Elem Res.* 185(2), 364-374. doi 10.1007/s12011-018-1266-5
- Zhang, Q., Wu, T., Li, S., Meng, Y., Tan, Z., Wu, M., Yi, D., Wang, L., Zhao, D. & Hou, Y. 2021. Protective effect of zinc oxide and its association with neutrophil degranulation in piglets infected with porcine epidemic diarrhoea virus. *Oxid Med Cell Longev.* 2021, 3055810. doi 10.1155/2021/3055810
- Zhang, R., Carlsson, F., Edman, M., Hummelgård, M., Jonsson, B.G., Bylund, D. & Olin, H. 2018. *Escherichia coli* bacteria develop adaptive resistance to antibacterial ZnO nanoparticles. *Adv Biosyst.* 2(5), e1800019. doi 10.1002/adbi.201800019
- Zhou, Y., Fan, M. & Chen, L. 2016. Interface and bonding mechanisms of plant fibre composites: An overview. *Compos Part B-Eng.* 101: 31-45. doi 10.1016/j.compositesb.2016.06.055
- Zhu, C., Lv, H., Chen, Z., Wang, L., Wu, X., Chen, Z., Zhang, W., Liang, R. & Jiang, Z. 2017. Dietary zinc oxide modulates antioxidant capacity, small intestine development, and jejunal gene expression in weaned piglets. *Biol Trace Elem Res.* 175(2), 331-338. doi 10.1007/s12011-016-0767-3
- Zhu, X., Wang, J., Cai, L., Wu, Y., Ji, M., Jiang, H. & Chen, J. 2022. Dissection of the antibacterial mechanism of zinc oxide nanoparticles with manipulable nanoscale morphologies. *J Hazard Mater.* 430, 128436. doi 10.1016/j.jhazmat.2022.128436