Zinc Oxide: Development of novel products for weaned piglets

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Abstract

Diarrhea in piglets is one of the main causes of animal death before and after weaning; In recent decades, zinc oxide has been used in high doses to control this disease. These doses are considered to be of concern for the pollutant potential of animal waste through soil and groundwater pollution. New technologies such as nanotechnology, new matrices such as biopolymers and encapsulation are suggestions that appear as possible innovations that can minimize the challenges imposed by piglet weaning. This review aims to collect and analyze information on novel zinc oxide products developed with innovative technologies.

Introduction

The challenges of weaning piglets

Modern pig industry is one of the most developed and technological sectors of agribusiness, however the mortality of live-born piglets is still a major challenge, with no significant advances in recent decades. Early weaning of piglets is the most critical stage of production, the first weeks are particularly stressful and immunosuppressive for animals and usually accompanied by delayed growth, weight loss, diarrhea and mortality (Lallès, 2007; Lima, 2009; Campbell *et al*., 2013; Sutherland *et al*., 2014). The physical separation from the sows and transition to a solid and complex diet, associated to an immature digestive tract, resulting in intestinal structural damage characterized by lower intestinal villi height, greater crypt depth and decreased intestinal enzymatic activity. Further the action of some pathogens, such as *Escherichia coli* and rotavirus (Pluske *et al*., 1997, Boudry *et al*., 2004, Esquerra *et al*., 2011). *E. coli* is the most important factor for post-weaning diarrhea, also known as colibacillosis (Fairbrother *et al*., 2005, Heo, *et al*., 2013).

The gastrointestinal disturbances cause large economic losses in the pig industry, the mortality among piglets in the EU is approximately 17% and a significant part of these losses may be associated with infections through mucosal surfaces (Lallès, 2007)**.** In this context, many studies have been developed in search of products that contribute to minimize the challenges imposed by piglet weaning. This review aims to collect and analyze information on novel zinc oxide (ZnO) products developed with innovative technologies such as nanoparticles, encapsulation and use of biopolymers.

Methodology

This is a literature review of a systematic study of ZnO products for weaned piglets in worldwide.

Research Strategies

Online scientific articles available at the Pubmed, Scielo, Science Direct, Google scholar and Periodicals/CAPES databases, between 2015 and 2018, were selected. The evaluation of the selected articles was divided into three stages. As an initial screening, in addition to the term Zinc Oxide or ZnO, studies regarding nanotechnology and biopolymers, conducted in animal nutrition.

After this initial screening, a second stage was performed based on the selected articles describing the experiments performed with ZnO on weaned piglets. Finally, a third step comprised the separation of results from *in vitro* and *in vivo* studies.

Check the stages of bibliographic research (identification, selection, eligibility and inclusion of data) for literature review in Fig.1.

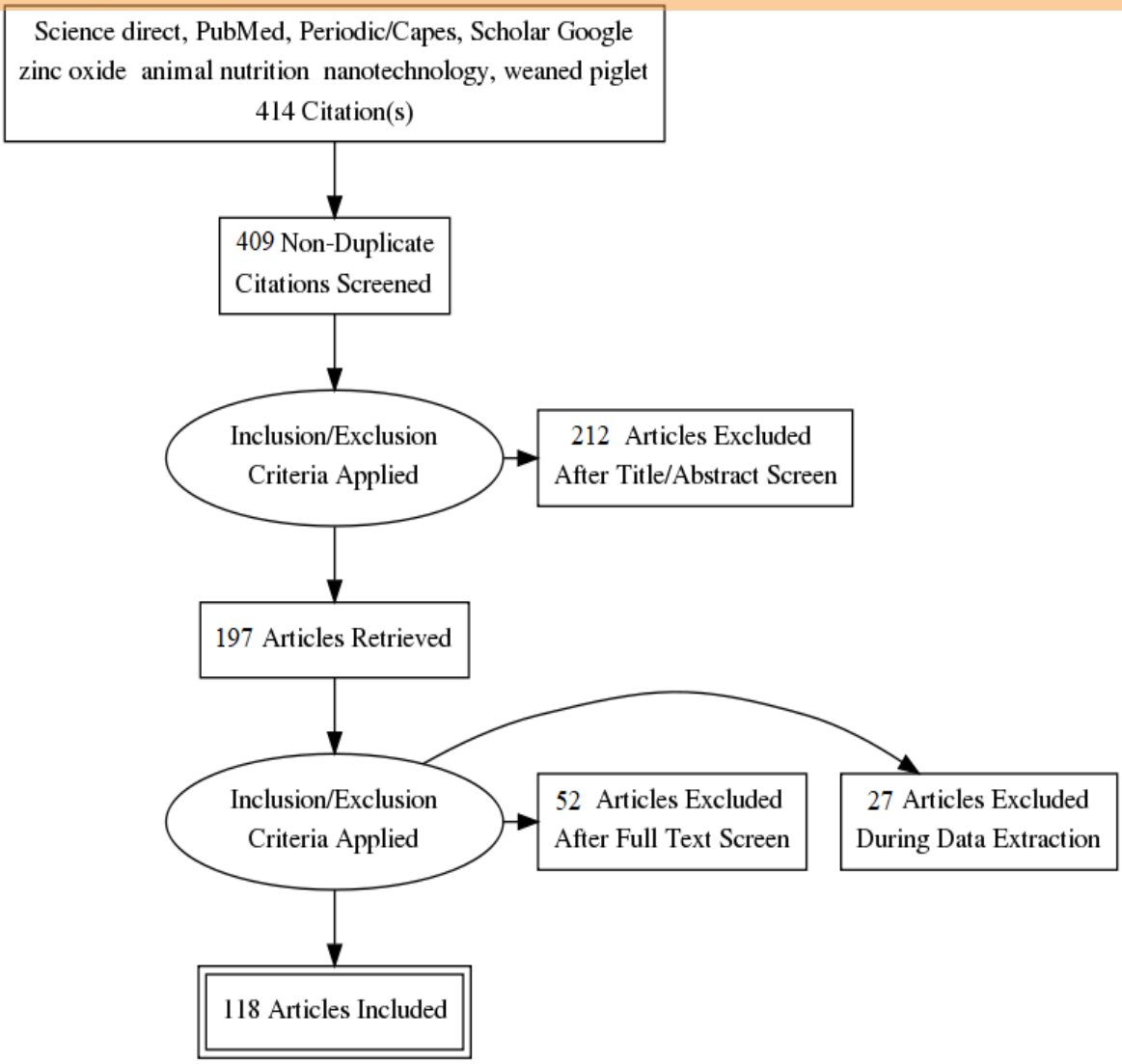

Figure 1: Stages of bibliographic research (identification, selection, eligibility and inclusion of data) for literature review.

ZnO as an additive to improve animal performance

For decades, pharmacological doses of ZnO, up to 2,000 mg ZnO/kg, have been applied to combat post-weaning diarrhea and to improve animal performance (Poulsen, 1995; Hollis *et al.*, 2005; Pettigrew, 2006). Li *et al.* (2006) have observed that *in vitro* intestinal epithelial regeneration capacity increases in the presence of exogenous ZnO and that there is an increase in the level of intestinal insulin-like growth factor 1 (IGF-1 gene) in piglets fed with high concentrations of ZnO (Li *et al.*, 2006). The IGF-1 gene is a hormone that regulates cell

growth and may contribute to intestinal tissue repair. Studies indicate that the ZnO promoter effect occurs mainly in the first two weeks after weaning (Shelton *et al*., 2009), in the same period in which piglet intestinal adaptation is practically complete. Different studies have shown that the inclusion of ZnO has effects on the increase of the gene expression of antimicrobial peptides in the small intestine, with a positive action on the stability and diversity of the microbiota, besides the reduction of the electrolyte secretion from enterocytes. This association of events results in a bactericidal action (Pluske *et al*., 2007; Zhang & Guo, 2009).

Antibacterial activity of ZnO

The antibacterial activity of ZnO is scientifically grounded; however, the mechanisms by which it modifies the gastrointestinal microbiota are not well elucidated. Roselli *et al*. (2003) suggested that ZnO does not inhibit directly enteropathogenic *E. coli* growth, but rather the ability of the microorganism to bind to intestinal cells. Concerning the efficacy of ZnO in gram-positive and gram-negative bacteria, there are controversy results in the literature. Vahjen *et al*. (2011) verified a greater susceptibility of gram-positive bacteria to ZnO, an increase in the diversity of the species present in the intestine, besides the increase of Enterobacteriaceae that would compete with enteropathogenic *E. coli* controlling its growth indirectly. While Applerot (2009), Trandafilovi´c *et al*. (2012) and Barreto *et al*. (2017) found that gram-positive bacteria, such as *Staphylococcus aureus*, are more resistant to the action of ZnO when compared to gram-negative, *E. coli*. The difference in antibacterial activity against both microorganisms may be related to the different chemical and structural compositions of cell membranes, particularly in the cell wall, in addition to the material used (Jones *et al*., 2008; Shantikumar *et al*., 2008; Xie *et al*., 2011). According to Ann and Mahmoud (2014), *S. aureus* tends to develop defenses against oxidative stress producing enzymes, such as

superoxide dismutase, catalase and thioredoxin reductase. Superoxide dismutase can convert O_2 of H_2O_2, catalase can convert H_2O_2 to H_2O and O_2, and thioredoxin reductase can protect the cell against toxic oxygen species (Ann and Mahmoud, 2014; Raghupathi *et al*, 2011; Ballal and Manna, 2010). In this way, *S. aureus* can construct an effective "shield" to reduce the toxicity of ZnO samples. On the other hand, some ZnO particles may attach to the surface of the bacterial membrane, and this mechanical damage could also be considered another method of antimicrobial inhibition (Ann and Mahmoud, 2014).

High doses of ZnO altered intestinal microbial diversity in the ileum and colon of weaned piglets and had comparable effects to antibiotics, especially affecting the non-predominant microbiota population in ileum. Understanding the effects of high ZnO on intestinal bacterial communities may provide information on the future application of the alternative strategy for the treatment of diarrhea in piglets (Yu *et al.*, 2017).

Limitation associated with using ZnO as a performance enhancer

ZnO is currently effective in improving performance and intestinal health in weaned piglets at doses ranging from 2,000 to 4,000 mg Zn/kg of feed, which are considered to be of concern for the pollutant potential of animal waste. These values are much higher than those found in the *in vitro* tests, where minimum inhibitory concentrations (MIC) and bactericidal (MBC) concentrations are often lower for different bacteria. In the literature there are MIC and MBC results between 260 and 500 ppm for different strains of *E. coli* (Liedtke and Vahjen, 2012; Barreto *et al*., 2017). Studies indicate that the efficacy of ZnO for piglets is not related to their absorption but to their action on the intestinal lumen, which consequently causes a large part of the ingested Zn to be excreted via feces (Poulsen, 1995; Rincker *et al*., 2005). Piglets supplemented with high levels of ZnO excrete between 60% and 80% of the amount ingested, the higher the excretion being the higher the levels of Zn in the diet (Carlson *et al*., 2004).

The high level of Zn^{2+} excreted in feces is an environmental concern. Although Zn is present in a relatively small amount, there is interest about the possible accumulation of this metal in the environment (Gräber *et al*., 2005). Zn is a necessary nutrient for the maintenance and growth of plant tissues, but high concentrations in the soil can cause phytotoxicity (Zhang *et al*., 2012). Concern over the contamination of water by metals is even greater in artesian well production systems or in farms close to rivers and lakes due to the low tolerance of various species of fish to Zn toxicity (Gräber *et al*., 2005; Zhang *et al*., 2012). In addition, fecal Zn is also a potential environmental inducer of bacterial resistance (Hölzel *et al*., 2012; Bednorz *et al*., 2013; Yazdankhah *et al*., 2014). In view of this, European legislation limits a maximum of 150 mg Zn kg in pig diets (European Communities, 2003), dose well below the pharmacological levels of ZnO reported as growth promoters for these animals.

ZnO Nanoparticles (ZnO Nano)

Nanoparticulate ZnO is one of the most researched oxides due to its differentiated physical, chemical and biological properties. Its low cost, versatility and availability make it highly suitable for numerous industrial applications (Giraldi, *et al*., 2012). According to the literature, ZnO nanoparticles can be synthesized by different techniques, such as hydrothermal (Suwanboon *et al*., 2013), sol-gel (Muneer *et al*., 2013), ultrasound (Khorsand *et al*., 2013) of precipitation (Chang *et al*., 2008), among others. Nanoparticles are particles smaller than 100 nanometers (nm), in this way, ZnO nano has smaller particle size, larger number of particles per unit mass and greater specific surface area compared to conventional ZnO microparticles (Raghupathi *et al*., 2011, Xie *et al*., 2011), characteristics that make them more reactive in chemical and biological systems. In addition, nanoparticles more easily cross biological barriers such as the intestine (Buzea *et al*., 2007).

Research on ZnO nanoparticles (Jones *et al*., 2008, Xie *et al*., 2011) indicates that the

surface effects of the molecule may also be responsible for its antibacterial action. The interaction of the nanoparticles with the microorganisms, damaging the integrity of bacterial cells (Zhang *et al*., 2008) and the formation of reactive oxygen species (ROS) (Jalal *et al*., 2010) are considered the main mechanisms. Jones *et al*. (2008) and Barreto *et al*. (2017) suggest that ZnO nanoparticles have significant antibacterial effects on *S. aureus*.

The antibacterial effect of ZnO nanoparticles was investigated in *Campylobacter jejuni* for inhibition and inactivation of cell growth. The results demonstrated that *C. jejuni* was extremely sensitive to the treatment with ZnO nanoparticles and that the action of the nanoparticles was bactericidal rather than bacteriostatic. According to the authors, the data indicate that the antibacterial mechanism of the ZnO nanoparticles occurs due to the rupture of the cell membrane and the cellular oxidative stress (Xie *et al*., 2011). Nanoparticles of ZnO *in vitro* exhibited strong antibacterial activity against *E. coli* in studies by Wang *et al*. (2012) and Barreto *et al*. (2017), who believe that nanoparticles can damage the membrane and cause lysis of bacterial cells. Studies have shown that ZnO nanoparticles may be potentially antibacterial for the treatment of diseases caused by *E. coli*.

Minor particles of ZnO with larger contact surface with the gastrointestinal medium seem to favor the effectiveness of ZnO as a growth promoter. In the literature, there are few studies investigating the effects of ZnO nanoparticles on the zootechnical indexes of animals, especially on swine. Nanoparticles of ZnO have been reported to increase growth performance, improve power utility and provide benefits in weaned piglets (Yang and Sun, 2006), a study with piglets fed basal diets supplemented with 200, 400, 600 mg/kg of ZnO nano or 3,000 mg/kg Zn showed encouraging average daily gain (Hongfu, 2008). On the other hand, Li *et al*. (2016) and Milani *et al*. (2017) did not find concrete results of the effectiveness of ZnO nanoparticles in piglets, but Milani *et al*. (2017) verified that ZnO nanoparticles promoted a reduction in Zn excretion in feces of animals and, consequently, the environment.

Lina *et al*. (2009) in their study indicated that ZnO nano improved the performance and production of broiler chickens at 42 days of feeding at the 40 mg/kg in the diet. The supplementation of 20 to 60 mg of Zn/kg in the diet in the form of nanoparticulate ZnO improved performance in broilers without presenting toxic or harmful effects when compared to conventional ZnO (Ahmadi *et al*., 2014; Zhao *et al*., 2014). These authors observed higher weight gain and better feed conversion in animals supplemented with ZnO nano compared to conventional ZnO (Ahmadi *et al*., 2013; Zhao *et al*., 2014). According to Tsai *et al*. (2016), ZnO nanoparticles for dietary supplementation of laying hens can increase Zn retention, carbonic anhydrase enzyme activity, growth hormone and serum Zn level, and egg shell thickness, proving that this nanometric oxide may increase Zn uptake in the intestine and have positive effects when compared to ZnO conventionally used in laying diets.

Buentello *et al.* (2009) in their study reported that there are differences in the growth rate in response to different dietary sources of Zn and different chemical substances Zn forms showed differential bioavailability in fish. According to Tawfik *et al*. (2017) supplementation of ZnO nanoparticles with fish feed may possibly improve the growth rates of these animals, such as weight gain, specific growth rates and growth hormone in blood. This could be better than conventional ZnO, so that it could be used in fish farms and aquaculture with their low concentrations and this could improve the economy of aquiculture.

There are several researches focuses on investigating the toxicity of ZnO nanoparticles in mices. It is known that doses above 500 and 1000 mg Zn/kg body weight cause small toxic effects with elevated plasma Zn concentration, accumulation in kidneys, liver and lungs, nephrotoxicity, respiratory tract inflammation and oxidative stress in cell membranes (Wang *et al*., 2008; Yan *et al*., 2012; Chung *et al*., 2013; Hong *et al*., 2014; Roy, *et al*., 2015).

In recent years, the contribution of several researchers has increased in the investigation of the antibacterial effect of ZnO nano, of the involved mechanisms, of safety,

and how its use can enable the reduction of the total amount of Zn needed to optimize the results and, consequently, the polluting effect of the waste generated. Free ZnO nanoparticles, structured or encapsulated in different matrices, appear as possible technological alternatives in the use of ZnO as an additive for swine farming.

Biopolymers

Polyelectrolytes and Complexes Polyelectrolytes (PEC)

According to Canevarolo (2006), polymer is a word of greek origin composed of the radical poly (many) and mere (unit of repetition). Polymers are macromolecules composed of tens of thousands of repeating units and joined by covalent bonds. The monomers can be of different types and present themselves in several combinations, they are the ones that determine the length of the molecule and its molar mass (Canevarolo, 2006).

A polyelectrolyte is a polymer in which some groups of monomers along the chain have ionic and/or ionizable groups (Hess *et al*., 2006). In polar solution, the groups generally ionize and acquire positive charge (polyactions), negative (polyanions) or both (polyphoton). This feature gives polyelectrolytes the ability to interact strongly with macromolecules and charged surfaces with opposing charges. Generally, they are quite hydrophilic, sensitive to pH variation and the amount and types of electrolytes present in the solution (Lyklema, 2005).

The polyelectrolytes stand out for the ability to form interpolymer complexes, called Polyelectrolyte Complexes (PECs) (Schatz *et al*., 2004). The formation of PECs occurs spontaneously by the simple mixing of oppositely charged polyelectrolytes with release of counterions (Fig. 2).

The main force that leads to the formation of the PECs is the entropy gain caused by the release of the low molar mass counterions. However, hydrogen bonds, hydrophobic interactions and van der Waals forces, or the combination of these interactions, are important

in the formation of complexes (Dautzenberg, 2001).

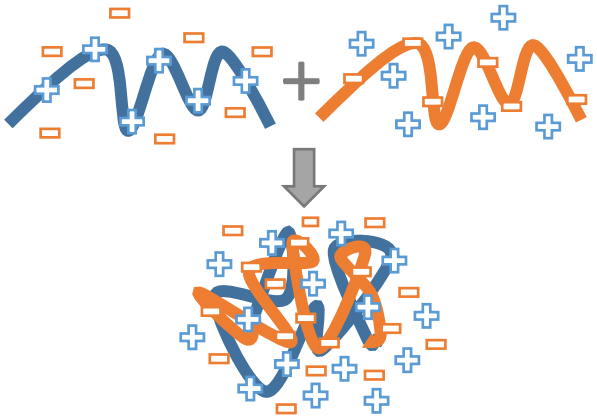

Figure 2: Schematic representation of the formation of the PECs.

Because of their properties, the PECs have attracted interest for biotechnological, food and pharmaceutical applications such as protein immobilizers and drug carriers (Dautzenberg, 2001). The use of polymers in the synthesis of ZnO has been proposed by some authors as an interesting strategy to control the growth and agglomeration of nanoparticles. The chitosan (Murugadoss, Chattopadhyay, 2008, Shih, *et al*, 2009), the starch (Bozani'c *et al*, 2011; Raveendran *et al* 2003) and alginate (Gutowska *et al*., 2001; Chang *et al*., 2011) are particularly interesting as matrix polymers because their chains have a large number of hydroxyl groups that form complexes with the metal ions, for example Zn.

In addition to nanoparticle growth controllers, natural biopolymers are examples of materials that allow the development of capsules resistant to the upper gastrointestinal tract and release the encapsulated material into the intestinal environment, as verified by different authors (Braccini & Pérez, 2001; Chen & Subirade, 2005; Liu *et al*., 2007; Jones & McClements, 2010; Patel *et al*., 2011; Barreto *et al*., 2017). In this sense, biopolymers are interesting as coatings or complexing materials with ZnO.

Alginate

Sodium alginate is a sodium salt of alginic acid, a water-soluble anionic polymer. Alginic acid is a natural polysaccharide, extracted from brown seaweed, formed by β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues bound by binding (1 → 4), composition and sequence varied (Fig. 3). The ratio between these two units influences the physical properties of this biopolymer (Lawrie *et al*., 2007). The carboxylic acid groups on these units assign negative charges to the alginate, making it capable of interacting electrostatically with the positively charged molecules to form gels. The gels formed due to the dimeric association of the G-G blocks in egg-box are induced by multivalent cation.

The alginate can be easily cross-linked with bivalent cations such as Ca^{2+}, Sr^{2+}, Zn^{2+} or Ba^{2+}, among which Ca^{2+} is the most investigated (Luo & Wang, 2014). Alginate is characterized by being biocompatible, hydrophilic, biodegradable under normal physiological conditions and chemically stable at pH values between 5.0 and 10.0 (Sankalia *et al*., 2007; Saether *et al*., 2008; Aelenei *et al*., 2009). Alginate is one of the most widely used biopolymers as a matrix for drug release, being abundant, easily manipulable, low cost and interesting physicochemical properties (Liew, 2006).

Several authors have demonstrated the antimicrobial activity of different alginate compositions and ZnO nanoparticles (Trandafilovi´c *et al*., 2012; Trandafilovi´c *et al*., 2014; Chopra *et al*., 2015; Cordero-Arias *et al*., 2015; Karbowniczek *et al*., 2017).

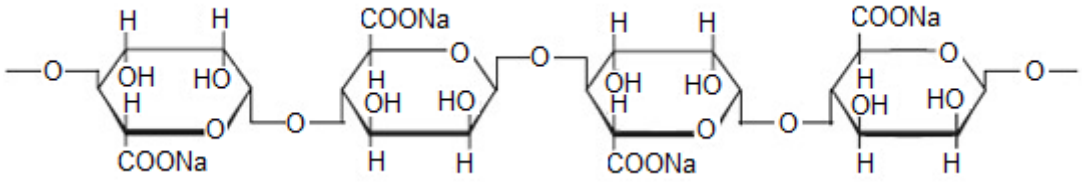

Figure 3: Sodium alginate chemical structure.

Chitosan

Chitosan is a biopolymer composed of repeating units of β-(1,4)-2-deoxy-2-amino-D-glucose and is obtained from chitin by deacetylation in alkaline medium (Fig. 4) (Sharma, 2009; Shukla, 2013). In turn, chitin is a polysaccharide constituted by repetitive constitutional units of β-(1,4)-2-deoxy-2-acetylamino-D-glucose (N-acetylglucosamine), abundantly found in nature.

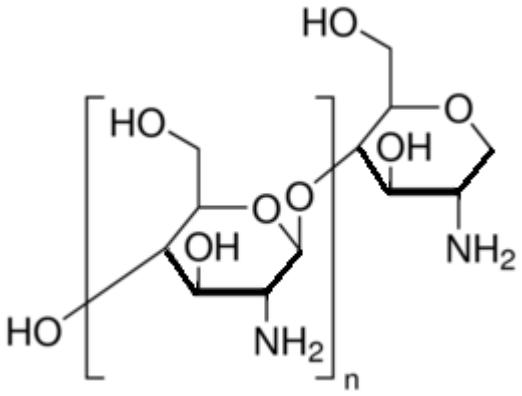

Figure 4: Chemical structure chitosan molecule.

The degree of deacetylation of chitosan ranges from 70 to 95% and the molar mass between 10-1000 kDa (Hamman, 2010). Chitosan is a weak base with pKa values between 5.9-6.6 (Kumar *et al*., 2004; Park *et al*., 2010), and above this value its solubility is limited. The amino groups present along the polymer chain act as cationic polyelectrolytes at pH <6.5 (George & Abraham, 2006). Therefore, chitosan is soluble in dilute weak acid solutions, such as acetic acid (Laranjeira & Fávere, 2009). Thus, the cationic character of chitosan, acquired in acid solution, by protonation of the amino groups, allows the electrostatic interaction with specific polyanions and, consequently, the formation of intra- and intermolecular crosslinks. However, as the pH is adjusted above 6.5, the amino groups become deprotonated and the chitosan loses its charges, becoming insoluble (Dash *et al*., 2011).

Chitosan presents, among other properties, biocompatibility, biodegradability, antimicrobial activity and bioadhesiveness due to its polycationic nature. The number of studies investigating chitosan as carrier and nanocarrier has increased significantly in the last decades (Gan *et al*., 2005; Boddohi *et al*., 2009; Hamman, 2010; Dash *et al*., 2011; Luo & Wang, 2014). According to Laranjeira and Fávere (2009), the rate of drug release from chitosan matrices is affected by the pH change. *In vitro* studies, simulating the gastrointestinal tract, revealed that the dissolution profiles of these systems depend on the type of polymer matrix and the pH of the simulated fluid (Laranjeira & Fávere, 2009).

Different methodologies can be used for the preparation of nano and microparticles containing chitosan, which involve one or two types of association between macromolecules (Tavares *et al*., 2012). The formation of covalent crosslinking that requires a crosslinking agent, such as glutaraldehyde, genipine and sodium tripolyphosphate (TPP) (Banerjee, *et al*., 2002; Liu & Huang, 2008; Ocak, 2012) and the physical interaction, which involves methods such as spray-drying, ionic gelling, reverse microemulsion, solvent evaporation/diffusion, polyelectrolyte complexation and coacervation/precipitation (Harris *et al*., 2011; Lee *et al*., 2010; Mukhopadhyay *et al.* 2012).

In the literature, it is possible to verify that the complexation of chitosan with polyanions such as pectin, collagen, xanthan gum, cellulose and sodium alginate has been shown to be efficient in the development of formulations for drug delivery (Davidenko *et al*., 2009; Plapied *et al*., 2011). Alginate is often used in the formation of complexes with chitosan, in addition to the complex formed remain biodegradable and biocompatible, it becomes mechanically strong at low pH values where chitosan is soluble. While chitosan is easily degraded by lysozymes, the chitosan-alginate complex has shown greater strength due to the strong interaction between the polymer chains (Hamman, 2010). Several results have been found on the use of chitosan and sodium alginate in the formation of micro and

nanoparticles by means of ionic interaction (Sarmento *et al*., 2006; Cafaggi *et al*., 2007; Sankalia *et al*., 2007; Li *et al*., 2008; Aelenei *et al*., 2009; Lertsutthiwong *et al*., 2009; Barreto *et al*., 2017).

Studies of the antibacterial activity of the nano complexed or chitosan coated ZnO presented positive results regarding the control of gram positive and gram negative bacteria (Bhadra *et al*., 2011; Malini *et al*., 2015; Vaseeharan *et al*., 2015). Investigating Zn nanoparticles encapsulated in biocompatible chitosan polymer, Bhadra *et al*. (2011) found that the chitosan encapsulation helped to restrict the growth of the ZnO nanomaterial and that the chitosan coated ZnO compound was bound to the external cell membrane of the bacteria through the chitosan-NH_2. This increased the permeability of the cell membrane and resulted in cell cytoplasm to leak the entire cell leading to destruction. The inhibition zone study confirmed the highest antibacterial effect of chitosan-capped ZnO nanoparticles compared to unencapsulated ZnO nanomaterial as well as chitosan against *E. coli* (Bhadra *et al*., 2011). In another study, the antibacterial activity of membranes synthesized with chitosan/ZnO nanoparticles was investigated in *Klebsiella planticola* and *Bacillus substilis*. In all cases, the gram negative *K. planticola* was inhibited in a greater proportion compared to gram positive *Bacillus substilis* and according to the authors, the chitosan/ZnO nanocomposites appeared to be very promising in relation to their antibacterial property (Malini *et al*., 2015). Similarly, antibacterial activity of chitosan-ZnO composite demonstrated the effective growth control as gram negative *Vibrio parahaemolyticus* as gram positive *Bacillus lechiniformis* bacteria isolated from aquatic environments (Vaseeharan *et al*., 2015).

In the literature, the application of complexes of ZnO and biopolymers in the animal nutrition is not observed. In an *in vitro* study, Barreto *et al*. (2017) synthesized ZnO nanoparticles complexed with chitosan and alginate polyelectrolytes with and without TPP. The authors obtained compounds that showed a pronounced antibacterial effect against *E. coli*

and *S. aureus*, in addition to a release profile of the Zn^{2+} ions suitable for delivery to the simulated enteric piglet environment, indicating that the polyelectrolyte complexes can efficiently protect ZnO in the piglets' gastric environment (Barreto *et al*, 2017).

Encapsulated ZnO

The encapsulation of ZnO in different matrices has emerged as one of the alternatives to protect the ZnO in the stomach and to allow the maximization of its release in the small intestine.

In piglets, the addition of coated and microencapsulated zinc containing 100 g of ZnO/kg of finished product, showed results equal to or greater than those of conventional ZnO. Microencapsulated Zn is designed to provide ions (Zn^{2+}) in the ideal fraction of the gastrointestinal tract resulting in maximum efficiency of control and treatment of post-weaning diarrhea (Moran, 2007). Shen *et al*. (2014) found that a low concentration of coated ZnO alleviated diarrhea in piglets and promoted intestinal development by protecting the intestinal mucosa barrier from damage, stimulating the mucosal immune system and regulating the intestinal microbiota, as well as high concentration of ZnO. In addition, compared to a high concentration of ZnO, a low concentration of coated ZnO prevented excessive accumulation of Zn in animals and reduced the Zn concentration of excreted feces in order to achieve the goal of saving the Zn source and reducing the environmental pollution of Zn (Shen *et al*., 2014). However, Park *et al*. (2014) investigated the effects of lipid encapsulated ZnO supplementation at a physiological level and the result was superior to that of conventional ZnO at the same level in the weight gain of weaned piglets, but their effects on suppression of diarrhea were lower than that of conventional ZnO. The results found by Kim *et al*. (2015) indicated that dietary supplementation of 72 ppm of Zn as ZnO encapsulated in lipids was as effective as that of 2,000 to 2,500 ppm Zn supplied as

conventional ZnO or antibiotics to improve growth performance, goblet cell density in the intestine, as well as reduction of diarrhea in challenged piglets with a low dose of *E. coli* (K88). The authors suggest that studies be performed under production conditions to determine the effects of lipid-coated ZnO over conventional ZnO in weaned piglets.

Table 1: A brief overview of ZnO products and data found in vivo and in vitro assays.

References	Products	Results
Jones *et al*. (2008)	ZnO nano (50 - 70 nm)	Antibacterial activity: *Staphylococcus aureus*
Xie *et al*. (2011)	ZnO nano (50 nm)	Antibacterial activity: *Campylobacter jejuni*
Zhang *et al*. (2008)	ZnO nano (90-100 nm)	Antibacterial activity: *Escherichia coli*
Jalal *et al*. (2010)	ZnO nano (37-41 nm)	Antibacterial activity: *Escherichia coli*
Barreto *et al*. (2017)	ZnO nano (17 nm)	Antibacterial activity: *Escherichia coli and Staphylococcus aureus*
Wang *et al*. (2012)	ZnO nano (55-95 nm)	Antibacterial activity: *Escherichia coli*
Yang and Sun (2006)	ZnO nano (ND)	*In vivo*: Increase growth performance, improve power utility and provide benefits in weaned piglets.

Hongfu (2008)	ZnO nano (ND)	*In vivo*: Diarrhoea incidence reduced in piglets
Milani *et al*. (2017)	ZnO nano (31-108 nm)	*In vivo*: Weaning diarrhea control
Trandafilovi´c *et al*. (2012)	ZnO nano + Alginate (3.9 - 6.8 nm)	Antibacterial activity: *Staphylococcus aureus* and *Escherichia coli*
Trandafilovi´c *et al*. (2014)	ZnO nano + Alginate (100 nm)	Antibacterial activity: *Staphylococcus aureus* and *Escherichia coli*
Chopra *et al*. (2015)	ZnO nano + Alginate (75-80 nm)	Antibacterial activity: *Pseudomonas aeruginosa*
		High encapsulation efficiency
Cordero-Arias *et al*. (2015)	ZnO nano + Alginate (20-60 nm)	Antibacterial activity: *Escherichia coli*
Karbowniczek *et al*. (2017)	ZnO nano (40,8 nm) + Alginate + Chitosan	Antibacterial activity: *Salmonella enteric* and *Staphylococcus aureus*
Bhadra *et al*. (2011)	ZnO nano (80 nm) + Chitosan	Antibacterial activity: *Escherichia coli*

Malini et al. (2015)	ZnO nano + Chitosan	Antibacterial activity: *Klebsiella planticola* and *Bacillus substilis*
Vaseeharan *et al*. (2015)	ZnO nano (30 - 60 nm) + Chitosan	Antibacterial activity: *Vibrio parahaemolyticus* and *Bacillus lechiniformis*
Barreto *et al*. (2017)	ZnO nano (17 nm) + Chitosan + Alginate	Antibacterial activity: Escherichia coli and Staphylococcus aureus
		In vitro: Optimum *in vitro* release profile of Zn^{2+} in simulated enteric fluids assays.
Moran (2007)	Microencapsulated ZnO: Lipid matrix	In vivo: Maximum efficiency of control and treatment of post-weaning diarrhea
Shen *et al*. (2014)	Microencapsulated ZnO: Lipid matrix	*In vivo*: Reduced diarrhoea and reduced the Zn concentration of excreted faeces
Park *et al*. (2014	Microencapsulated ZnO: Lipid matrix	*In vivo*: Weight gain of weaned piglets
Kim *et al*. (2015)	Microencapsulated ZnO: Lipid matrix	*In vivo*: Reduced plasma Zn concentration and faecal Zn excretion levels

ConsiderationsZnO is an important antimicrobial used to combat diarrhea common to weaned piglets, its antimicrobial activity has been extensively researched and confirmed for gram negative and gram positive bacteria. Elevated doses of ZnO have been shown to be effective in the field; however, much is lost with animal waste, contaminating soils and

groundwater. In the last two decades, the interest has been increased by alternatives that protect ZnO in the gastric environment and allow the maximization of its release in the enteric environment. Novel knowledge such as nanotechnology and biopolymers as release matrices arise bringing new perspectives to animal nutrition and to the challenges inherent in weaning piglets.

However, scientific research on novel ZnO products, such as ZnO nanoparticles, complexes ZnO/biopolymers, or ZnO encapsulated in animal nutrition, especially in pigs, is still scarce. Further *in vitro* and *in vivo* studies are recommended for a better understanding of the effect of ZnO nanoparticles, their location and mechanism of absorption, and the risk of intoxication in animals. Adequate levels of ZnO in the diet should be focused in order to minimize piglet mortality, confer better animal performance and economic benefits in a safe way.

References

1. Aelenei, N.; Popa, M. I.; Novac, O.; Lisa, G.; Balaita, L. 2009. Tannic acid incorporation in chitosan-based microparticles and in vitro controlled release. Journal of Material Science: Materia Medica, 20:1095–1102.
2. Ahmadi, F., Ebrahimmezhad, Y., Sis, N.M., Ghalehkandi, J.G., 2013. The effects of zinc oxide nanoparticles on performance, digestive organs and serum lipid concentrations in broiler chickens during starter period. Int. J. Biosci. 3: 23-29.
3. Ahmadi, F., Ebrahimnezjad, Y., Ghalehkandi, J.G., Sis, N.M., 2014. The Effect of Dietary Zinc Oxide Nanoparticles on the Antioxidant State and Serum Enzymes Activity in Broiler Chickens During Starter. In: International Conference on Biological, Civil and Environmental Engineering, 2014, Dubai, pp. 26-28.
4. Ann, L.C., Mahmud, S., Bakhori, S.K.M., Sirelkhatim, A., Mohamad, D., Hasan, H.,

et al. 2014. Antibacterial responses of zinc oxide structures against *Staphylococcus aureus*, Pseudomonas aeruginosa and Streptococcus pyogenes. Ceram. Int., 40: 2993-3001.

5. Applerot, G. A., Lipovsky, R. Dror, N. Perkas, Y. Nitzan, R. Lubart, A. Gedanken, 2009. Enhanced antibacterial activity of nanocrystalline ZnO due to increased ROS-mediated cell injury, Advanced Functional Materials, 19:842–852.
6. Ballal, A., A.C. Manna. 2010. Control of thioredoxinreductase gene (trxB) transcription by SarA in Staphylococcus aureus. Journal of Bacteriology. 192:336–345.
7. Banerjee, T.; Mitra, S.; Singh, A. K.; Sharma, R. K.; Maitra, A. 2002. Preparation, characterization and biodistribution of ultrafine chitosan nanoparticles. International Journal of Pharmaceutics, 243:93-105.
8. Barreto, M.S.R., Andrade, C.T., da Silva, L.C.R.P., Cabral, L.M., Paschoalin, V.M.F., Del Aguila, E.M. 2017. *In vitro* physiological and antibacterial characterization of ZnO nanoparticle composites in simulated porcine gastric and enteric fluids. BMC Veterinary Research, 13:181-191.
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. International Journal of Medical Microbiology, Jena, 303(6/7): 396-403.
10. Bhadra, P., Mitra, M.K., Das, G.C., Dey, R., Mukherjee, S. 2011. Interaction of chitosan capped ZnO nanorods with *Escherichia coli*. Materials Science and Engineering C, 31: 929–937.
11. Braccini, I; Pérez, S. 2001. Molecular basis of Ca2+-induced gelation in alginates and

pectins: the egg-box model revisited. Biomacromolecules, 2:1089-1096.

12. Boddohi, S., Moore, N., Patrick A. 2009. Johnson and Matt J. Kipper. Polysaccharide-based polyelectrolyte complex nanoparticles from chitosan, heparin, and hyaluronan. Biomacromolecules. 10:1402–1409.
13. Boudry, G., Péron V., Le Huërou-Luron I, Lallès, J.P., Sève, B. 2004. Weaning induces both transient and long-lasting modifications of absorptive, secretory, and barrier properties of piglet intestine. J. Nutr. 134(9):2256-62.
14. Bozani'c, D. K. B. 2011. Silver nanoparticles encapsulated in glycogen biopolymer: Morphology, optical and antimicrobial properties. Carbohydrate Polymers, 83: 883–890.
15. Buentello, J.A., Goff, J.B., Gatlin, D.M. 2009. Dietary Zinc Requirement of Hybrid Striped Bass, Morone chrysops×Morone saxatilis, and Bioavailability of Two Chemically Different Zinc Compounds, Journal of the World Aquaculture Society, 40(5): 687–694.
16. Buzea, C.; Blandino, I.I.P.; Robbie, K. 2007. Nanomaterials and nanoparticles: Sources and toxicity. Biointerphases, New York, 2(4): MR17-MR172.
17. Cafaggi, S.; Russo, E.; Stefani, R.; Leardi, R.; Caviglioli, G.; Parodi, B.; Bignardi, A.; De Totero, B.; Aiello, C.; Viale, M. 2007. Preparation and evaluation of nanoparticles made of chitosan or N-trimethyl chitosan and a cisplatin–alginate complex. Journal of Controlled Release, 121:110–123.
18. Campbell, J.M, Crenshaw, J.D., Polo, J., 2013. The biological stress of early weaned piglets. J. Anim. Sci. Biotechnol. 4: 1-4.
19. Canevarolo, V. S. 2006. Ciência dos Polímeros, 2. ed. São Paulo, SP: Artliber Editora. 280p.
20. Carlson, M. S., C. A. Boren, C. Wu, C. E. Huntington, D. W. Bollinger, and T. L.

Veum. 2004. Evaluation of various inclusion rates of organic zinc either as a polysaccharide or proteinate complex on the growth performance, plasma, and excretion of nursery pigs. J. Anim. Sci. 82:1359.–1366.

21. Chang, C.C., Ping L; Chun, H.L. 2008. Synthesis and characterization of nano-sized ZnO powders by direct precipitation method. Chemical Engineering Journal 144:509–513.
22. Chang, P.R., Yua, J., Ma, X., Anderson, D.P. 2011. Polysaccharides as stabilizers for the synthesis of magnetic nanoparticles. Carbohydrate Polymers, 83: 640–644.
23. Chen, L.; Subirade, M. 2005. Chitosan/ [beta]-lactoglobulin core-shell nanoparticles as nutraceutical carriers. Biomaterials, v. 26, n. 30, p. 6041-6053.
24. Chopra, M., Bernela, M., Kaur, P., Manuja, A., Kumar, B., Thakur, R. 2015. Alginate/gum acacia bipolymeric nanohydrogels — Promising carrier for Zinc oxide nanoparticles. International Journal of Biological Macromolecules 72: 827–833.
25. Chung, H.E.; Yu, J.; Baek, M.; Lee, J.A.; Kim, M.S.; Kim, S.H.; Maeng, E.H.; Lee, J.K.; Jeong, J.; Choi, S.J. 2013. Toxicokinetics of zinc oxide nanoparticles in rats. Journal of Physics: Conference Series, Bristol, 429: 12037-12045.
26. Cordero-Arias, L., Cabanas-Polo, S., Goudouri, O.M., Misra, S.K., Gilabert, J., Valsami-Jones, E., Sanchez, E., Virtanen, S., Boccaccini, A.R. 2015. Electrophoretic deposition of ZnO/alginate and ZnO-bioactive glass/alginate composite coatings for antimicrobial applications, Materials Science & Engineering C, 55:137– 144.
27. Dash, M., F. Chiellini, R. M. Ottenbriteb, E. Chiellini. 2011. Chitosan—A versatile semisynthetic polymer in biomedical applications. Progress in Polymer Science, 36:981–1014.
28. Dautzenberg, H. 2001. Em: RADEVA. T. Physical chemistry of polyelectrolytes: surfactant science series, v 99, New York: Marcel Dekker, 2001. 743.

29. Davidenko, N.; Blanco, M.D.; Peniche, C.; Bechera´N, L.; GuerrerO, S.; Teijo´N, J.M. 2009. Effects of Different Parameters on the Characteristics of Chitosan–Poly(acrylic acid) nanoparticles obtained by the method of coacervation. Journal of Applied Polymer Science, 111:2362–2371.

30. Esquerra R.G., Zhao J., Harrell R. 2011. Zinco e cobre como promotores de crescimento para suínos. Anais: IV Simpósio Brasil Sul de Suinocultura, Chapecó, SC – Brasil.

31. European Communities. Official Journal of the European Union. Commission regulation (EC) n° 1334/2003 of 25 July 2003. Amending the conditions for authorization of a number of additives in feeding stuffs belonging to the group of trace elements, 2003. 6 p.

32. Fairbrother, J. M., Nadeau, E. & Gyles, C. L. 2005. Escherichia coli in postweaning diarrhea in pigs: an update on bacterial types, pathogenesis, and prevention strategies. Anim Health Res Rev 6:17–39.

33. Gräber, I.; Hansen, J.F.; Olesen, S.E.; Petersen, J.; Ostergaard, H.S.; Krogh, L. 2005. Accumulation of Copper and Zinc in Danish Agricultural Soils in Intensive Pig Production Areas. Geografisk Tidsskrift - Danish Journal of Geography, Kjøbenhavn, 105(2):15-22.

34. Gan, Q., T. Wang, C. Cochrane, P. McCarron. 2005. Modulation of surface charge, particle size and morphological properties of chitosan–TPP nanoparticles intended for gene delivery. Colloids and Surfaces B: Biointerfaces. 44:65–73.

35. George, M., T. E. Abraham. 2006. Polyionic hydrocolloids for the intestinal delivery of protein drugs: alginate and chitosan – a review. Journal of Controlled Release, 114:1-14.

36. Giraldi, T. R., Santos, G. V. F., de Mendonca, V. R., Ribeiro, C., Weber, I.T. 2012.

Effect of synthesis parameters on the structural characteristics and photocatalytic activity of ZnO. Materials Chemistry and Physics, 136:505–511.

37. Gutowska, A., Jeong, B., Jasionowski, M. 2001. Injectable gels for tissue engineering. The Anatomical Record, 263:342–3499.
38. Hamman, J. H. 2010. Chitosan based polyelectrolyte complexes as potential carrier materials in drug delivery systems. Marine Drugs, 8:1305-1322.
39. Harris, R.; Lecumberri, E.; Mateos-Aparicio, I.; Mengibar, M.; Heras, A. 2011. Chitosan nanoparticles and microspheres for the encapsulation of natural antioxidants extracted from Ilex paraguariensis. Carbohydrate Polymers, 84:803-806.
40. Hess, M.; Jones, R. G; Kahovec, J.; Kitayama, T.; Kratochvíl, P.; Kubisa, P.; Mormann, W., Stepto, R.F.T., Tabak, D., Vohlídal, J., Wilks, E.S. 2006. Terminology of polymers containing ionizable or ionic groups and of polymers containing ions (IUPAC Recommendations 2006). Pure and Applied Chemistry, 78(11):2067-2074.
41. Heo, J. M., F. O. Opapeju, J. R. Pluske, J. C. Kim, D. J. Hampson, C. M. Nyachoti. 2013. Gastrointestinal health and function in weaned pigs: a review of feeding strategies to control post-weaning diarrhea without using in-feed antimicrobial compounds. Journal of Animal Physiology and Animal Nutrition, 97:207–237.
42. Hollis, G.R.; Carter, S.D.; Cline, T.R. Crenshaw, T.D., Cromwell, G.L., Hill, G.M., Kim, S.W., Lewis, A.J., Mahan, D.C., Miller, P.S., Stein, H.H., Veum, T.L 2005. Effects of replacing pharmacological levels of dietary zinc oxide with copper dietary levels of various organic zinc sources for weanling pigs. Journal of Animal Science, 83(9): 2123-2129.
43. Hölzel, C.S.; Müller, C.; Harms, K.S.; Mikolajewski, S.; Schäfer, S.; Schwaiger, K.; Bauer, J. 2012. Heavy metals in liquid pig manure in light of bacterial antimicrobial resistance. Environmental Research, New York, 113:21-27.

546 44. Hong, J.-S.; Park, M.-K.; Kim, M.-S.; Lim, J.-H.; Park, G.-J.; Maeng, E.-H.; Shin, J.-
547 H.; Kim, M.-K.; Jeong, J.; Park, J.-A.; Kim, J.-C.; Shin, H.-C. 2014. Prenatal
548 development toxicity study of zinc oxide nanoparticles in rats. International Journal of
549 Nanomedicine, Auckland, 9(2): 159-171.
550 45. Hongfu, Y.B.Z. 2008. Effects of Nano-ZnO on growth performance and diarrhea rate
551 in weaning piglets, China Feed, 1:08.
552 46. Jalal, R., Goharshadi, E.K., Abareshi, M., Moosavi, M., Yousefi, A., Nancarrow, P.
553 2010. ZnO nanofluids: green synthesis, characterization, and antibacterial activity.
554 Materials Chemistry and Physics, 121:198–201.
555 47. Jones, N., Ray, B., Koodali, T.R., Adhar, C.M. 2008. Antibacterial activity of ZnO
556 nanoparticle suspensions on a broad spectrum of microorganisms. FEMS FEMS
557 Microbiology Letters, 279:71-76.
558 48. Jones, O. G.; McClements, D. J. 2010. Functional Biopolymer Particles: Design,
559 Fabrication, and Applications. Comprehensive Reviews in Food Science and Food
560 Safety, 9(4):374-397.
561 49. Karbowniczek, J., Cordero-Arias, L., Virtanen, S., Misra, S.K., Valsami-Jones, E.,
562 Rutkowski, B., Górecki, K., Bała, P., Czyrska-Filemonowicz, A., Boccaccini, A.R.
563 2017. Electrophoretic deposition of organic/inorganic composite coatings containing
564 ZnO nanoparticles exhibiting antibacterial properties. Materials Science &
565 Engineering C, doi: 10.1016/j.msec.2017.03.180.
566 50. Khorsand Zak, A; Majid, W.H; Wang, H.Z; Yousefi, R; Moradi Golsheikh, A; Ren,
567 Z.F. 2013. Sonochemical synthesis of hierarchical ZnO nanostructures, Ultrasonics
568 Sonochemistry, 20:395–400.
569 51. Kim, S.J., Chang, H.K., Park, B.C., Lee, C.Y., Han, J.H. 2015. Effects of a lipid-
570 encapsulated zinc oxide dietary supplement, on growth parameters and intestinal

morphology in weanling pigs artificially infected with enterotoxigenic Escherichia coli. Journal of Animal Science and Technology, 57:4.

52. Kumar, B.; Sandhu, K.; Kaur, I. 2004. Topical 0.25% methotrexate gel in a hydrogel base for palmoplantar psoriasis. Journal of Dermatology, 31:798-801.
53. Lallès, J., Bosi, P., Smidt, H., Stokes, C.R., 2007. Weaning-a challenge to gut physiologists. Livest. Sci. 108:82-93.
54. Laranjeira, M.C.M. & Fávere, V.T. 2009. Quitosana: biopolímero funcional com potencial industrial biomédico. Química Nova, 32:672-678.
55. Lawrie, G.; Keen, I.; Drew, B.; Chandler-Temple, A.; Rintoul, L.; Fredericks, P.; Grøndahl, L. 2007. Interactions between Alginate and Chitosan Biopolymers Characterized Using FTIR and XPS. Biomacromolecules, 8:2533-254.
56. Lee, E. J.; Khan, S. A.; Kim, Y. B.; Lim, K. H. 2010. Preparation of chitosan/carboxymethyl dextran nanoparticles by polyelectrolyte complexation. Journal of Biotechnology, 150:450-451.
57. Lertsutthiwong, P.; Rojsitthisak, P.; Nimmannit, U. 2009. Preparation of turmeric oil-loaded chitosan-alginate biopolymeric nanocapsules. Materials Science and Engineering C, 29:856–860.
58. Li, M.Z., Huang, J.T., Tsai, Y.H., Mao, S.Y., Fu, C.M., Lien, T.F., 2016. Nanosize of zinc oxide and the effects on zinc digestibility, growth performances, immune response and serum parameters of weanling piglets. Anim. Sci. J. 87:1379-1385.
59. Li, P.; Dai, Y.; Zhang, J.; Wang, A.; Wei, Q. 2008. Chitosan–Alginate Nanoparticles as a Novel Drug Delivery System for Nifedipine. International Journal of Biomedical Science, 4: 221-228.
60. Li X, Yin J, Li D, Chen X, Zang J, Zhou X. 2006. Dietary supplementation with zinc oxide increases IGF-I and IGF-I receptor gene expression in the small intestine of

596 weanling piglets. The Journal of Nutrition, 136:1786–1791.

597 61. Liedtke, J., Vahjen, W. 2012. In vitro antibacterial activity of zinc oxide on a broad
598 range of reference strains of intestinal origin. Veterinary Microbiology, 160: 251–255.

599 62. Liew, C.V.; Chan, L.W.; Ching, A.L.; Heng, P.W. 2006. Evaluation of sodium alginate
600 as drug release modifier in matrix tablets. International Journal of Pharmaceutics,
601 309:25-37.

602 63. Lima G.J.M.M., Mores N. & Sanches R.L. 2009. As diarréias nutricionais na
603 suinocultura. Acta Scientiae Veterinariae, 37:17-30.

604 64. Lina, T., Jianyang, J., Fenghua, Z., Huiying, R. and Wenli, L. 2009. Effect of nano-
605 zinc oxide on the production and dressing performance of broiler. Chinese Agricultural
606 Science Bulletin, 2: 003.

607 65. Liu, B. S. & Huang, T. B. 2008. Nanocomposites of genipin-crosslinked
608 chitosan/silver nanoparticles - structural reinforcement and antimicrobial properties.
609 Macromolecular Bioscience, 8:932.

610 66. Liu, L.; Fishman, M.; Hicks, K. 2007. Pectin in controlled drug delivery – a review.
611 Cellulose, 14(1):15-24.

612 67. Luo, Y. and Q. Wang. 2014. Recent development of chitosan-based polyelectrolyte
613 complexes with natural polysaccharides for drug delivery. International Journal of
614 Biological Macromolecules, 64:353–367.

615 68. Lyklema, J. The bottom size of colloids. 2005. Bulletin of the Polish Academy of
616 Sciences Technical Sciences, 53:317-323.

617 69. Malini, M., Thirumavalavan, M., Yang, W.Y., Lee, J.F., Annadurai, G. 2015. A
618 versatile chitosan/ZnO nanocomposite with enhanced antimicrobial properties.
619 International Journal of Biological Macromolecules 80: 121–129.

620 70. Milani, N.C., Sbardella, M. Ikeda, N.Y., Arno, A., Mascarenhas, B.C., Miyada, V.S.

2017. Dietary zinc oxide nanoparticles as growth promoter for weanling pigs, Animal Feed Science and Technology http://dx.doi.org/10.1016/j.anifeedsci.2017.03.001.

71. Moran, C. 2007. Designing a new growth promoter in the 21st century. In: J.Patterson (ed.) Australasian Pig Science Association, p. 159-169. Manipulating pig production XI, Werribee, Vic. Australia.
72. Mukhopadhyay, P.; Mishra, R.; Rana, D.; Kundu, P. 2012. Strategies for effective oral insulin delivery with modified chitosan nanoparticles: A review. Progress in Polymer Science, 37:1457–1475.
73. Muneer, M., Ba-Abbad, Abdul Amir H., Kadhum, Mohamad, A.B., Mohd S. Takriff, Kamaruzzaman Sopian. 2013. The effect of process parameters on the size of ZnO nanoparticles synthesized via the sol–gel technique. Journal of Alloys and Compounds, 550:63–70.
74. Murugadoss, A., Chattopadhyay, A. 2008. A 'green' chitosan–silver nanoparticle composite as a heterogeneous as well as micro-heterogeneous catalyst. Nanotechnology, 19: 015603 doi: 10.1088/0957-4484/19/01/015603.
75. Ocak, B. 2012. Complex coacervation of collagen hydrolysate extracted from leather solid wastes and chitosan for controlled release of lavender oil. Journal of Environmental Management, 100: 22-28
76. Park, J. H., Saravanakumar, G., Kim, K., Kwon, I. C. 2010. Targeted delivery of low molecular drugs using chitosan and its derivatives. Advanced Drug Delivery Reviews, 63:28-41.
77. Park, B.C., Jung, D.Y., Kang, S.Y., Ko, Y.H., Ha, D.M., Kwonc, C.H., Park, M.J., Hanc, J.H., Jang, I., Lee, C.Y. 2014. Effects of dietary supplementation of a zinc oxide product encapsulated with lipid on growth performance, intestinal morphology, and digestive enzyme activities in weanling pigs. Animal Feed Science and Technology,

200:112-115.

78. Patel, K.R.; Patel, M.R.; Mehta, T.J.; Patel, A.D.; Patel, N.M. 2011. Microencapsulation: Review on novel approaches. International Journal of Pharmacy and Technology, 3:894-911.

79. Pettigrew, J.E. 2006. Reduced use of antibiotic growth promoters in diets fed to weanling pigs: Dietary tools, part 1. Animal Biotechnology, 17:207–215.

80. Plapied, L.; Duhem, N.; Rieux, A.; Préat, V. 2011. Fate of polymeric nanocarriers for oral drug delivery. Current Opinion in Colloid & Interface Science, 16:228–237.

81. Pluske, J.R.; Hampson, D.J.; Williams, I.H. 1997. Factors influencing the structure and function of the small intestine in the weaned pig: a review. Livestock Production Science, Amsterdam, 51(1/3): 215-236.

82. Pluske, J.R., Hansen, C.F., Payne, H.G., Mullan, B.P., Kim, J.-C. and Hampson, D.J. 2007. Gut health in the pig. In Manipulating Pig Production XI (J.E. Paterson and J.A. Barker, eds.), pp. 147-158. Australasian Pig Science Association, Werribee, Victoria, Australia.

83. Poulsen, H.D. 1995. Zinc Oxide for Weanling Piglets. Journal Acta Agriculturae Scandinavica A, 45(3): 159-167.

84. Raghupathi, K.R., R.T. Koodali, A.C. Manna. 2011. Size-dependent bacterial growth inhibition and mechanism of antibacterial activity of zinc oxide nanoparticles, Langmuir 27:4020–4028.

85. Raveendran, P., Fu, J., Wallen, S. L. 2003. Completely green synthesis and stabilization of metal nanoparticles. Journal of the American Chemical Society, 125:13940–13941.

86. Rincker, M.J.; Hill, G.M.; Link, J.E.; Meyer, A.M.; Rowntree, J.E. 2005. Effects of dietary zinc and iron supplementation on mineral excretion, body composition, and

671 mineral status of nursery pigs. Journal of Animal Science, Champaign, 83(12): 2762-
672 2774.
673 87. Roy, R.; Das, M.; Dwivedi, P.D. 2015. Toxicological mode of action of ZnO
674 nanoparticles: Impact on immune cells. Molecular Immunology, Oxford, 63(9): 184-
675 192.
676 88. Roselli, M., Finamore A., Garaguso I., Britti M.S. & Mengheri E. 2003. Zinc oxide
677 protects cultured enterocytes from the damage induced by Escherichia coli. Journal of
678 Nutrition. 133:4077-4082.
679 89. Saether, H.V.; Holme, H.K.; Maurstad, G.; Smidsrød, O.; Bjørn, T.; Stokke, H. 2008.
680 Polyelectrolyte complex formation using alginate and chitosan. Carbohydrate
681 Polymers, 74:813–821.
682 90. Sankalia, M.G.; Mashru, R.C.; Sankalia, J.M.; Sutariya, V.B. 2007. Reversed chitosan–
683 alginate polyelectrolyte complex for stability improvement of alpha-amylase:
684 Optimization and physicochemical characterization. European Journal of
685 Pharmaceutics and Biopharmaceutics, 65: 215–232.
686 91. Sarmento, B.; Ferreira, D.; Veiga, B.; Ribeiro, A. 2006. Characterization of insulin-
687 loaded alginate nanoparticles produced by ionotropic pre-gelation through DSC and
688 FTIR studies. Carbohydrate Polymers, 66:1–7.
689 92. Schatz, C., Domard, A., Viton, C., Pichot, C., Delai, T. 2004. Versatile and efficient
690 formation of colloids of biopolymer-based polyelectrolyte complexes.
691 Biomacromolecules, 5:1882-1892.
692 93. Shantikumar N., S. Abhilash, V. V. Divya, M. Deepthy, N. Seema, K Manzoor Satish.
693 2008. Antibacterial activity of zinc oxide nanoparticles. African Journal of
694 Microbiology Research, 24: 465-471.
695 94. Sharma, S., Soni, V.P., Bellare, J.R. 2009. Chitosan reinforced apatite–wollastonite

coating by electrophoretic deposition on titanium implants. Journal of Materials Science. Materials in Medicine, 20:1427–1436.

95. Shelton, N. W.; Derouchey, J. M.; Neill, C. R.; Tokach, M. D.; Dritz, S. S.; Goodband, R. D.; Nelssen, J. L. 2009. Effects of increasing feeding level during late gestation on sow and litter performance. In: SWINE DAY. Report of progress. Kansas State University, p 38-50.

96. Shen, J. H., Chen, Y., Wang, Z., Zhou, A.G., He, M., Mao, L., Zou, H., Peng, Q., Xue, B., Wang, L., Zhang, X., Wu, S., Lv, Y. 2014. Coated zinc oxide improves intestinal immunity function and regulates microbiota composition in weaned piglets. Br. J. Nutr. 111: 2123–2134.

97. Shih, C.-M., Shieh, Y.-T., e Twu, Y.-K. 2009. Preparation of gold nanopowders and nanoparticles using chitosan suspensions. Carbohydrate Polymers, 78:309–315.

98. Shukla, S. K. 2013. Chitosan-based nanomaterials: A state-of-the-art review. International Journal of Biological Macromolecules, 59:46-58.

99. Sutherland, M.A., Backus, B.L. and McGlone, J.J. 2014. Effects of Transport at Weaning on the Behavior, Physiology and Performance of Pigs. Animals 4:657-669.

100. Suwanboon, S., Amornpitoksuk, P., Bangrak, P., Randorn, C. 2013. Physical and chemical properties of multifunctional ZnO nanostructures prepared by precipitation and hydrothermal methods. Ceramics International, 40: 975-983.

101. Tawfik M.M.M, Moustafa M.M., Abumourad I.M.K., El-Meliegy EM, Refai M.K. 2017. Evaluation of Nano Zinc Oxide feed additive on tilapia Growth and Immunity, 15th International Conference on Environmental Science and Technology Rhodes, Greece, 31 August to 2 September 2017.

102. Tavares, I. S.; Caroni, C.; Dantas Neto, A.; Pereira, M. R.; Fonseca, J. 2012. Surface charging and dimensions of chitosan coacervated nanoparticles. Colloids and

721 Surfaces B: Biointerfaces, 90: 254 – 258.

722 103. Trandafilovi´c, L.V., Whiffen, R.K., Dimitrijevi´c –Brankovi´c, S., Stoiljkovi´c,
723 M., Luyt, A.S., Djokovic, V. 2014. ZnO/Ag hybrid nanocubes in alginate biopolymer:
724 Synthesis and properties. Chemical´ Engineering Journal 253: 341–349.

725 104. Trandafilovi'c, L.V.; Bo˘zani´c, D.K; Dimitrijevi´c-Brankovi´c, S.; Luyt, a.S.;
726 Djokovi´c, V. 2012. Fabrication and antibacterial properties of ZnO–alginate
727 nanocomposites. Carbohydrate Polymers, 88:263-269.

728 105. Tsai, Y.H., Mao, S.Y., Li, M.Z., Huang, J.T., Lien, T.F., 2016. Effects of
729 nanosize zinc oxide on zinc retention, eggshell quality, immune response and serum
730 parameters of aged laying hens. Anim. Feed Sci. Technol. 213:99-107.

731 106. Vahjen, W., Pieper, R., Zentek, J. 2011. Increased dietary zinc oxidechanges the
732 bacterial core and enterobacterial composition in theileum of piglets. Journal of
733 Animal Science, 89:240–243.

734 107. Vaseeharan, B., Sivakamavalli, J., Thaya, R. 2015. Synthesis and
735 characterization of chitosan-ZnO composite and its antibiofilm activity against aquatic
736 bacteria. Journal of Composite Materials, 49(2):177-184.

737 108. Wang, B., Feng, W., Wang, M., Wang, T., Gu, Y., Zhu, M., Ouyang, H., Shi, J.,
738 Zhang, F., Zhao, Y., Chai, Z., Wang, H., Wang, J. 2008. Acute toxicological impact of
739 Nano and submicro-scaled zinc oxide powder on healthy adult mice, J Nanopart Res,
740 10:263-76.

741 109. Wang, C.; Liu, L.-L.; Zhang, A.-T.; Xie, P.; Lu, J.-J.; Zou, X.-T. 2012yang.
742 African Journal of Biotechnology, Nairobi, 11(44): 10248-10254.

743 110. Yan, G.; Huang, Y.; Bu, Q.; LV, L.; Deng, P.; Zhou, J.; Wang, Y.; Yang, Y.; Liu,
744 Q.; Cen, X.; Zhao, Y. 2012. Zinc oxide nanoparticles cause nephrotoxicity and kidney
745 metabolism alterations in rats. Journal of Environmental Science and Health - Part A,

Toxic/hazardous substances & environmental engineering, New York, 47(4):577-588.

111. Yang, Z.P. and Sun, L.P. 2006. Effects of nanometre ZnO on growth performance of early weaned piglets. J. Shanxi Agric. Sci., 3: 024.

112. Yazdankhah, S.; Rudi, K.; Bernhoft, A. 2014. Zinc and copper in animal feeddevelopment of resistance and co-resistance to antimicrobial agents in bacteria of animal origin. Microbial Ecology in Health and Disease, Chichester, 25: 1-7.

113. Yu, T., Zhu, C., Chen, S., Gao, L., Lv, H., Feng, R., Zhu, Q., Xu, J., Chen, Z., Jiang, Z. (2017). Dietary High Zinc Oxide Modulates the Microbiome of Ileum and Colon in Weaned Piglets. Frontiers in Microbiology, 8, 825.

114. Xie, Y., Y. He, P. L. Irwin, T. Jin, X. Shi. 2011. Antibacterial activity and mechanism of action of zinc oxide nanoparticles against Campylobacter jejuni. Applied and Environmental Microbiology, 77: 2325–2331.

115. Zhang, L; Y. Ding, M. Povey, D. York. 2008. ZnO nanofluids – A potential antibacterial agent, Progress in Natural Science, 18:939-944.

116. Zhang, B and Guo, Y. 2009. Supplemental zinc reduced intestinal permeability by enhancing occluding and zonula occludens protein 1 (ZO-1) expression in weaning piglets. British Journal of Nutrition 102:687-693.

117. Zhang, F.S., Li, Y.X., Yang, M., Li, W. 2012. Content of heavy metals in animal feeds and manures from farms of different scales in northeast China. Int. J. Environ. Res. Pub. Health 9:2658–2668.

118. Zhao, Y.C., Shu, T.X., Xiao, Y.X., Qiu, S.X., Pan, Q.J., Tang, X.Z., 2014. Effects of dietary zinc oxide nanoparticles on growth performance and antioxidative status in broiler. Biol. Trace Elem. Res. 160, 361-367.