UNIVERSIDADE FEDERAL DE LAVRAS

VITOR HUGO CARDOSO MOITA

PERFORMANCE AND MINERAL FECAL EXCRETION OF POST WEANED PIGLETS ARE INFLUENCED BY DIFFERENT SOURCES OF ZINC OXIDE

LAVRAS-MG

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Dissertação apresentada a Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Nutrição e Produção de Não Ruminantes para obtenção do título de Mestre

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Aprovada em 12 de julho de 2019.

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LAVRAS

2019

À minha família, em especial aos meus pais Antonio Marcos e Nilce, por toda dedicação e apoio incondicional ao longo desta etapa.

Dedico

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RESUMO GERAL

A adição do óxido de zinco (ZnO) nas dietas pós-desmame é uma prática já difundida, com o objetivo de melhorar o desempenho e reduzir a diarreia pós desmame. O grande entrave para o uso do ZnO é o seu potencial poluidor do zinco (Zn), já que a maior parte da quantidade ingerida é excretada nas fezes. O presente estudo teve como objetivo avaliar os efeitos da suplementação em dietas pós desmame de uma fonte convencional de ZnO e/ou uma fonte potencializada de óxido de zinco (PZnO) e diferentes níveis de fósforo (P) sobre o desempenho produtivo, incidência de diarreia, excreção mineral e resistência óssea. Um total de 84 leitões desmamados aos 21 dias foram distribuídos em um delineamento em blocos casualizado e dividido em 4 tratamentos (T1 controle negativo: sem inclusão de ZnO e níveis regulares de P (0.440%; 0.420%; 0.400%; 0.380%); T2 controle positivo: inclusão de ZnO e níveis regulares de P (0.440%; 0.420%; 0.400%; 0.380%); T3: associação entre ZnO e PZnO e redução de 10% nos níveis de P (0.396%; 0.379%; 0.360%; 0.340%); T4 inclusão do PZnO e redução de 10% nos níveis de P (0.396%; 0.379%; 0.360%; 0.340%) e 7 repetições com 3 leitões por baia. O programa nutricional foi dividido em 4 fases (pré-inicial 1: 21 a 28 dias; pré-inicial II: 28 a 35 dias; inicial I: 35 a 49 dias; e inicial II: 49 a 63 dias). Quando houve diferença estatística pelo teste F ($P < 0.05$), adotou-se o teste de Tukey para comparar as médias. A variável de incidência de diarreia foi analisada aplicando-se o modelo linear generalizado GENMOD (SAS INSTITUTE INC., 2011). Avaliando o período do dia 1 ao dia 14, o T2 melhorou o ganho de peso diário (GPD) ($P = 0.0128$) e o peso corporal (PC) ($P = 0.0085$) ao dia 14. No período do dia 1 ao dia 28, o T2 otimizou o GPD ($P = 0.0208$), consumo de ração diário (CRD) ($P = 0.0099$) e consequentemente maior PC ($P = 0,001$) ao dia 28. Durante o período do dia 1 ao dia 42, os suínos alimentados com dieta T4 apresentaram o maior CRD ($P = 0.032$). Em relação aos dados de resistência óssea, não houve quaisquer diferenças significativas entre os tratamentos ($P > 0.05$). Avaliando os dados de incidência de diarreia, o T2 ($P < 0.05$) reduziu a incidência de diarreia em todos os períodos. O T4 reduziu a excreção fecal de Zn e P durante o período de 7 a 28 dias ($P < 0.05$). Portanto, a inclusão de PZnO nas dietas pós desmame reduziu a excreção fecal de Zn e P. Além disso, o uso de ZnO e PZnO proporcionaram os mesmos resultados de desempenho no período entre o dia 1 e o dia 42 após o desmame. Sendo assim, o PZnO apresenta potencial para reduzir a alta inclusão da fonte convencional de ZnO e também reduzir a poluição ambiental derivada da excreção mineral advinda dos sistemas pecuários, principalmente os de produção de suínos.

Palavras-chave: Creche, Diarreia, Fósforo, Nutrição, Resistência óssea.

ABSTRACT

The supplementation of zinc oxide (ZnO) on post weaning diets is a widespread practice, with the aim of improving the growth performance and decreasing the incidence of diarrhea. The great barrier to use ZnO in piglet's diets is due to the zinc-polluting potential, since most of the amount ingested is excreted, causing environmental pollution. The study aimed to evaluate the impact of the supplementation on post weaning diets of the conventional source of zinc oxide (ZnO) and/or a potentiated source of zinc oxide (PZnO) and different phosphorus (P) levels on productive performance, incidence of diarrhea , zinc (Zn) and copper (Cu) fecal excretion and bone resistance. A total of 84 piglets weaned at 21 days of age were allocated in a randomized block design with four treatments [T1 negative control: no inclusion of ZnO and regular P levels (0.440%; 0.420%; 0.400%; 0.380%); T2 positive control: inclusion of ZnO and regular P levels (0.440%; 0.420%; 0.400%; 0.380%); T3: association between ZnO and PZnO and 10% low P levels (0.396%; 0.379%; 0.360%; 0.340%); T4 inclusion of the PZnO and 10% low P levels (0.396%; 0.379%; 0.360%; 0.340%)], with seven repetitions and three piglets per pen. The nutritional program was divided into four dietary phases (pre-initial 1: 21 to 28 days; pre-initial II: 28 to 35 days; initial I: 35 to 49 days; and initial II: 49 to 63 days). When there was statistical difference by the F test ($P < 0.05$), the Tukey test was adopted to compare the means. The variable of incidence of diarrhea was analyzed by applying the generalized linear model in the GENMOD procedure (SAS INSTITUTE INC., 2011). Evaluating the period from day 1 to day 14, treatment T2 improved the average daily gain (ADG) ($P = 0.0128$) and BW ($P = 0.0085$) at day 14. From the period of day 1 to day 28, pigs fed with T2 diets showed higher ADG ($P = 0.0208$), ADFI (average daily feed intake) ($P = 0.0099$) and, consequently, higher Birth Weight ($P = 0.001$) at day 28. During the period from day 1 to day 42, pigs fed with PZnO diet performed the highest ADFI ($P = 0.032$). Regarding the bone resistance results, there was no significant differences between the treatments ($P > 0.05$). When evaluating overall data of diarrhea, T2 ($P < 0.05$) reduced the incidence of diarrhea during all periods. T4 reduced Zn and P fecal excretion during the period from 7 to 28 days ($P < 0.05$). Therefore, the inclusion of PZnO reduced the Zn and P fecal excretion. Furthermore, the use of ZnO and PZnO provide the same performance results of piglets on the period from day 1 to day 42 after weaning. Thus, PZnO represents a great potential to reduce the high dietary doses of the conventional source of ZnO and also to reduce the environmental pollution derived by the mineral excretion from livestock systems, especially swine production systems.

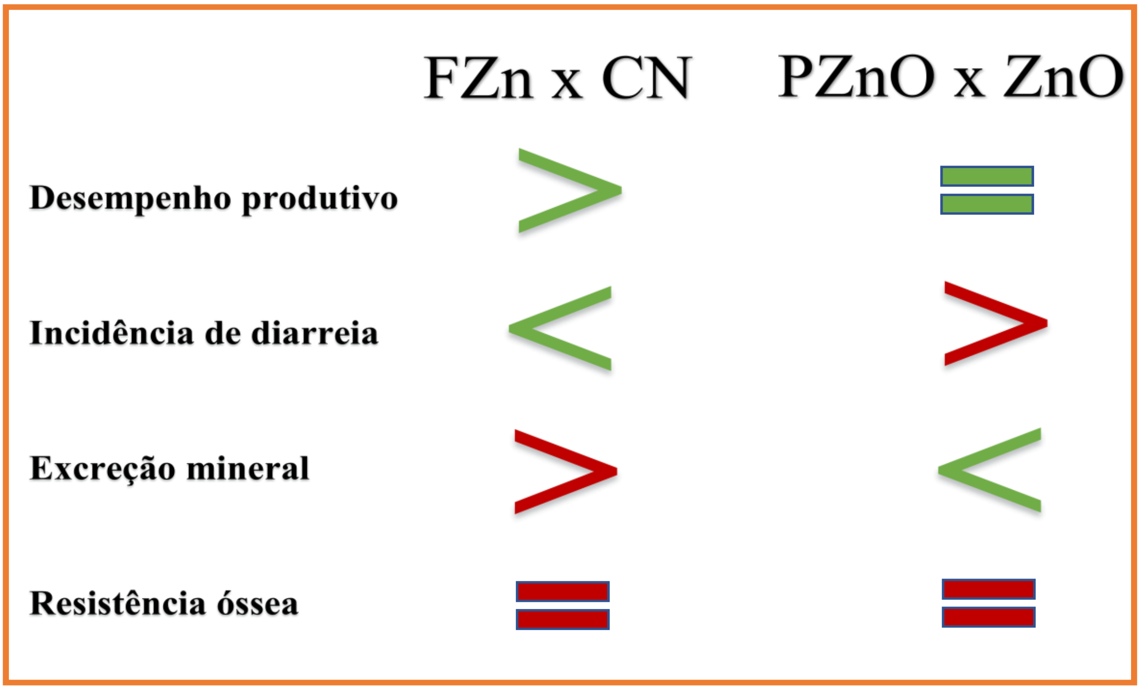
Keywords: Bone resistance, Diarrhea, Phosphorus, Nursery, Nutrition.

Performance and mineral fecal excretion of post-weaned piglets are influenced by different sources of zinc oxide

Elaborado por **Vitor Hugo Cardoso Moita** e orientado por **Márvio Lobão Teixeira de Abreu**

A adição do óxido de zinco (ZnO) nas dietas pós-desmame de leitões é uma prática já difundida, com o objetivo de melhorar o desempenho e reduzir a diarreia pós desmame. O grande entrave para o uso do ZnO é o seu potencial poluidor do zinco (Zn), já que a maior parte da quantidade ingerida é excretada nas fezes. Desta forma, durante 42 dias foram avaliados os efeitos da suplementação em dietas de leitões recém desmamados de uma fonte convencional de ZnO e/ou uma fonte potencializada e de menor granulometria de óxido de zinco (PZnO) e diferentes níveis de fósforo (P) sobre o desempenho produtivo, incidência de diarreia, excreção mineral e resistência óssea. Um grupo de 84 leitões foram desmamados aos 21 dias de idade e divididos em quatro tratamentos (Controle negativo (CN): sem inclusão de ZnO e níveis regulares de P; Controle positivo: inclusão de ZnO e níveis regulares de P; T3: associação entre ZnO e PZnO e redução de 10% nos níveis de P; T4 inclusão do PZnO e redução de 10% nos níveis de P. Em geral, ficou demonstrado que o PZnO e ZnO proporcionaram o mesmo desempenho produtivo dos animais. ZnO resultou em menor incidência de diarreia. E PZnO resultou em menor excreção de minerais nas fezes dos animais. Portanto, PZnO apresenta potencial de substituição do ZnO em dietas para leitões no período pós desmame, necessitando avaliar o impacto econômico dessa substituição.

Figura 1 - Fontes de zinco em dietas para leitões na creche: desempenho, incidência de diarreia, excreção mineral e resistência óssea.



Dissertação de mestrado em Zootecnia na UFLA, defendida em 12 de julho, 2019.

LIST OF TABLES

LIST OF FIGURES

SUMMARY

FIRST PART

1 INTRODUCTION

The weaning process is a stressful event in swine's life and can compromise the piglet gut health, which can lead to growth performance decrease. To achieve high production indexes in the swine industry, piglets are commonly weaned earlier than their organism have established a stable microbial population and the immune system activity. The stress caused at weaning will further disrupts the gut microbial ecosystem, increasing susceptibility to post-weaning diarrhea.

The strong social, environmental and nutritional changes related to weaning are factors that cause great stress and may frequently cause the manifestation of factors, which can reduce or paralyze growth rates during the post-weaning period, mainly due to diarrhea. All of that can lead to a reduction in the secretion of some enzymes, which, consequently, affects the digestive and absorptive capacity of the nutrients in the small intestine, besides important changes in their morphology. The poorly digested food can be used as substrate for the growth of microorganisms, causing post-weaning diarrhea. For this reason, feed additives have been widely used in swine farming as growth promoters in order to minimize the incidence of post weaning diarrhea.

The use of antibiotics growth promoters (AGP's) tends to smooth these disturbances by decreasing the incidence of post weaning diseases. The exaggerated and indiscriminate use of AGP's has increased selective pressure for anti-microbial resistant bacteria. Zinc oxide (ZnO) has been widely used for diarrhea control and as an AGP's for post weaning diets. Among the benefits, it can prevent pathogenic bacteria adhesion and also provide improvements in the immune system action and in the growth performance. Considering the severe restriction or total ban on the use of AGP's in swine production, different types of sources of ZnO have been suggested as an alternative.

The development of antibiotic resistance in several important pathogenic bacterial species becomes the most pressing issue in public health, requiring safe antibiotic alternatives for piglets. Although ZnO is widely used to decrease the incidence of diarrhea and to improve the growth performance of piglets, more attention ought to be paid on the excretion caused by the high doses of the conventional source of ZnO. Due to its low absorption, nearly 76% of ZnO is excreted as zinc in the feces (BUFF et al., 2005).

In the last decades, nanotechnology has been developing fast, providing a new dimension for the exploration of new substitutes for dietary pharmacological concentrations of the conventional source of ZnO on post weaning diets. Owing to the decreased particle size, increased surface area and high chemical stability, zinc oxide nanoparticles (NZnO) possess a high potential for use in the swine industry. The potentiated zinc oxide (PZnO) was obtained from a commercial company (Animine Company, 335 Chemin Du Noyer, Sillingy, 74330, France). A patented manufacturing technology is at the origin of the PZnO. The following physicochemical properties are modified: specific particle size and shape, with increased specific surface area, which drastically increases the surface of contact. The high porosity of the powder amplifies the antibacterial activity of PZnO.

Based on this previous information, we hypothesized that the potentiated zinc oxide might be a potential substitute for the conventional source of ZnO to improve growth performance, decreasing diarrhea incidence and fecal zinc excretion. Therefore, this study was conducted to evaluate effects of PZnO on growth performance, as well as copper, phosphorus and zinc fecal excretion, incidence of diarrhea and bone resistance of post weaning piglets. The results of the present study might provide insights on the application of PZnO as an additive to replace or decrease the practiced high doses of the conventional source of ZnO.

2 LITERATURE REVIEW

2.1 Antibiotics restriction

One of the most important aspects of animal husbandry research is to improve the production and quality of their products (meat, milk and egg) for human consumption. Pork is the most consumed meat in the world. Nevertheless, it can be a source of pathogens such as *Salmonella* and *Escherichia coli* (KORSAK et al., 2003). Therefore, it becomes crucial to the animal producers to identify the best solutions to reduce the incidence of *Salmonella* and *E. coli* infection from pig meat. In the past, antibiotics were included at subtherapeutic levels, acting as growth promoters and reducing the pathogen load.

Nevertheless, there is a concern of the global population towards the use of antibiotics as growth promoters (AGP's) removal from animal production systems, due to health and environmental issues that are increasing the bacterial strain resistance against many antibiotics. Increases in the incidence of human infections from antibiotic-resistant bacteria have been directly related to the overuse of antibiotics required to therapeutics in animal production (LIU et al., 2018). Langlois et al. (1988) stated that complete removal of antibiotics from animal production has decreased the resistance of lactose-fermenting fecal coliform bacteria. Thus, a tremendous pressure on the livestock industry to develop alternatives against pathogens has been installed (CHEN et al., 2006).

However, by eliminating AGP's from diets, diseases may be increased, and growth performance reduced. Nevertheless, to avoid the negative effects of removing AGP's from the production systems, modifications and adaptations in management protocol and nutritional strategies are required (KIL & STEIN, 2010). The focus of these changes is to improve the prevention of pathogenic bacteria colonize the intestinal system. This can be accomplished via feed additives, which can prevent the pathogens bacteria from compromising the animals` health.

Considering the severe restriction or total ban on the use of AGP's in swine production, different alternatives, such as zinc oxide, have been suggested as a viable solution.

2.2 Post weaning challenges

Weaning is defined as the separation of the piglets from the sow after the lactation period and represents one of the most critical and determinant moments of the piglet's life. Throughout their lives, piglets are exposed to a lot of stressful factors, such as removal from the sow, dietary changes, adapting to a new environment among others. These factors may negatively affect the immune system response, which may lead to commitment on the growth performance and animal's health (POHL et al., 2017).

One of the main factors involved on the decrease of the piglet's performance on the first two weeks after weaning is the low daily feed intake related to the alterations in the diets (JAYARAMAN & NYACHOTI, 2017). The transition from the sow milk to a cereal-based solid diet with low digestibility and palatability results on a decrease on the daily feed intake. According to the same authors, the low feed intake associated with the other weaning stressful factors can compromise the health and the nutrient absorption of the post weaning piglets. Furthermore, many stress factors associated with the weaning period, such as removal from the sow, dietary changes, adapting to a new environment and histological and morphological changes in the small intestine, may negatively affect the immune system response and may lead to decreased piglet`s performance (HEO et al., 2013).

The diet transition on post weaning period represents a big challenge for nursery piglets due to anti-nutritional compounds in some feedstuffs. β-conglycinin, glycinin, lectin are some examples of allergic proteins that can cause irritations and inflammations in the mucosa of the small intestine. Ordinary feed formulation does not apply the ideal protein concept, but crude proteins antinutritional factors are causing nitrogenous fermentation in the large intestine (WANG et al., 2017). These can cause proliferation of pathogenic proteolytic bacteria, which acts against benefic lactogenic bacteria impacting in the microbiota balance. Immune response in the small intestine and microbial unbalance cause intestinal diseases, affecting impaired growth performance and the average daily feed intake of the animals. According to Liu et al. (2018), high incidence of inflammation can cause increases in the oxidative stress, which in turn, disturbs the integrity of enterocytes and compromise intestinal permeability. Intestinal leaking provides pathogenic infection and, consequently, intestinal disorders. Therefore, post-weaning piglets with affected intestinal health present reduced ability for nutrient digestion and absorption.

Post-weaning diarrhea is an economically important disease and one of the most common causes that can lead to morbidity and mortality in swine production by affecting piglets during their first weeks on nursery facilities (BAUERFEIND et al., 2016). Post-weaning diarrhea is usually associated with proliferation of enterotoxigenic *E. coli* (LUPPI et al., 2016). This pathotype is characterized by the production of enterotoxins, which are essential for the disease development (RHOUMA et al., 2017). Roselli et al. (2003) suggested that the reduction of *E. coli* adhesion by ZnO action could be due to the binding of this metal to bacterial fimbriae, which interferes with *E. coli* recognition by the specific receptors on intestinal cells, since it has been shown that the fimbriae of *E. coli* are able to exclusively bind the oxide form of zinc.

During post-weaning diarrhea, there is a greater amount of mast cells present in the mucosa and submucosa of the intestine. Mast cells can suppress this inflammation produces and releases histamine, prostaglandins and tumor necrosis factors. Biomarkers have the capacity to increase the permeability of the intestinal epithelium, which can aggravate the diarrhea by increasing the endotoxin translocation enterocytes damaging.

It is well documented that the occurrence of diarrhea is closed related to the increase in intestinal permeability (HUANG et al., 2015; FAN et al., 2017). Zhang and Guo (2009) reported that supplemental pharmacological level of zinc oxide reduced intestinal permeability by enhancing the expression and production of tight junction proteins (occludin and zonula occludens protein-1). Thus, the alleviated post-weaning diarrhea by conventional zinc oxide can be attributed to the decrease in intestinal permeability.

These events also mark the approximately 50% decrease in the secretion of disaccharidase enzymes as lactase, sucrase and isomaltase over the first five days after weaning, causing the animal to poor digestion and absorption of certain nutrients such as carbohydrates, amino acids and electrolytes at the small intestine (LALLÈS et al., 2004). The association of all these changes leads to an appearance of osmotic diarrhea. Thus, with the incidence of diarrhea, the animals` growth performance and health are remarkably affected and subsequently increasing the morbidity and mortality rates of the herd, causing high economic losses (THOMSON & FRIENDSHIP, 2019).

2.3 Nutritional strategies for intestinal health and performance on nursery phase

The feed cost represents about 70% of the total cost in a swine production system (PORTES et al., 2017). It means that every nutritional strategy must be evaluated and discussed based on the objectives of the producer and the estimated budget. Nowadays, there are a lot of consolidated strategies applied to smooth the post weaning challenges and the post-weaning diarrhea. According to the Official Journal of the European Union (2003), feed additives are defined as substances, micro-organisms or preparations, other than feed material and premix, which are included on the water or/on the diets in order to perform important functions such as, positive effects on the ingredients characteristics, attend the nutritional requirements, improve the performance and animal welfare and also increase digestibility of feeding stuffs. Therefore, a feed additive must not perform any adverse effect on animal health or the environment, without any performance decrease. Among the feed additives currently used on swine production, plant extracts, acidifiers and ZnO are already consolidated and has registered positive effects.

Acidifiers, for example, are often used as alternatives to AGP's, due to their ability to stimulate a favorable intestinal environment for healthy microorganisms, which might result in greater nutrient digestibility, increased growth performance, and significant reduction on the incidence of diarrhea.

Plant extracts have potential biological functions, such as antiviral, antimicrobial, antioxidant and anti-inflammatory effects (Liu et al., 2013a, b; 2014). Based on previous studies, plant extracts may improve animal health through several mechanisms, such as direct suppression of the proliferation of pathogens, alteration of gut microbial populations and enhancement of immune functions (LIU et al., 2018).

According to Raquipo et al. (2018), the inclusion of zinc oxide on post weaning diets improves growth performance and reduces the incidence of diarrhea. It possesses an antimicrobial function, mainly acting on *Escherichia coli*, which is one of the main issues on nursery phase. Likewise, Hedemann et al. (2006) suggested that the supplementation with high levels of zinc has a marked effect on the physiology of the gastrointestinal tract taking into account the enzymatic activity, since this supplementation leads to greater production of enzymes in the pancreatic tissue such as amylase, carboxypeptidase A, chymotrypsin, trypsin and lipase, which may lead to better digestion of the food. Considering the severe restriction or total ban on the use of AGP's in swine production, different alternatives, such as zinc oxide, have been suggested as a viable solution.

2.4 Zinc functions and mechanisms

Zinc (Zn) plays crucial and determinant roles and functions throughout swine's life. However, the absorption and bioavailability of this mineral can be affected depending of the nutrition, phase of life of the animal, environmental conditions, etc. According to Tacnet et al. (1993), the optimal pH for Zn absorption and for re-absorption by saturate or peptide carriers is near 7. It has also been shown that the ileum has the greatest capacity for Zn absorption among the three segments of the small intestine (duodenum, jejunum and ileum). When absorbed into intestinal cells, Zn is bound to high molecular weight proteins or metallothionein (ANTONSON et al., 1979).

One of the proposed functions of Zn is to play a role of signaling hormones that regulate growth, such as insulin, insulin-like growth factor-1 (IGF-1), insulin-like growth factor-2 (IGF-2) and growth hormone (GH). Yin et al. (2009) found that piglets fed with ZnO showed hepatic and duodenal high IGF-1 mRNA expression. The composition of the diet and the nutritional status are the major influences on circulating IGF-1 levels. Moreover, if the intake of protein or energy is reduced, it can decrease circulating IGF-1 levels. The liver is the responsible to produce the IGF-1 and growth hormone secretions signal the production from the hypophysis (HAASE & MARET, 2003). It was shown that zinc deficiency might lead to a reduction of IGF-1 levels, regardless of energy intake (COSSACK, 1991).

Insulin is an important signaling factor that acts as a feedback mechanism and regulatory factor in many physiology and metabolism aspects. This hormone is produced by the β-cells of the pancreas and is highly sensitive to the composition of the diet (BROWNING et al., 1998). Additionally, insulin can directly activate the mTOR pathway by activation of insulin receptor substrate 1 (IRS-1) (RUVINKY & MEYUHAS, 2006).

One of the main entry points for pathogens into animal's system is the digestive tract, which determines the intestine as a major physical barrier that prevents pathogens bacteria from damaging the mucosa (MANI et al., 2012). Subsequently, the commitment of this structure that acts as a physical barrier can allow the passage of pathogens into the system and thereby activate the immune response of the organism.

Zinc will also play a key role in immune function, being a potent mediator of host resistance to an infection (SHANKAR & PRASAD, 1998). Immune response can be characterized into two categories, innate and adaptive response. Innate immunity is the first biological defense mechanism against infections. Zinc deficiency has been documented to create several problems associated with innate immunity (IBS & RINK, 2003).

Thus, Zn plays important and crucial functions in the whole animal physiology and metabolism. It contributes to increase growth performance by acting as a regulatory factor in the synthesis of GH, IGF-1 and has also been shown to directly regulate mTOR pathway. Immune response is also altered by zinc and in the absence of Zn, in which the immune function can be drastically impacted.

2.5 Zinc oxide on nursery diets

Zinc oxide (ZnO) usage in swine production has been practiced worldwide to improve growth performance, reduce the incidence of diarrhea and as alternatives to AGP's. Zinc oxide is included on post weaning diets to regulate gut microbiota by suppressing pathogenic bacterial load in the small intestine. It helps smoothing post-weaning diarrhea caused by *Escherichia coli*. However, there is a concern regarding its palatability, piglets tend to prefer diets without the ZnO inclusion (REYNOLDS et al., 2010).

According to NRC (2012), the basal levels of Zn for piglets are in the range of 70 to 100 ppm. On the other hand, higher dietary doses of ZnO, such as 2000 - 3000 ppm, are generally used to promote growth performance, reduce intestinal permeability and/or decrease incidence of diarrhea in post weaning piglets (WANG et al., 2017).

The benefits of the inclusion of ZnO on post weaning diets are already known and consolidated. The use of ZnO promotes a greater growth performance, a remarkable decrease on the incidence of diarrhea and a reduction on pathogenic microorganisms, such as *E. coli* (POULSEN, 1995; HILL et al., 2001; CHO et al., 2015; GRILLI et al., 2015; SONG et al., 2015; MILANI et al., 2017; BAI et al., 2019).

Furthermore, several studies reported an increased proportion of *E. coli* resistant to tetracycline and sulfonamides on post weaning piglets fed with high dietary doses of ZnO (VAHJEN et al., 2015). This may explain why antimicrobial resistance persists even in the absence of antimicrobial exposure to any drug. Moreover, the use of high dietary levels of ZnO has led to heavy metal contamination in the soil, raising environmental concerns (HOLMAN & CHÉNIER, 2015).

2.6 Zinc oxide on mineral excretion

Even though all the benefits provided using high doses of Zn as ZnO, there is a serious concern about their impacts on the environment. In post-weaning diets supplemented with high levels of ZnO, about 80% Zn will be excreted as Zn on piglets' feces (Buff et al., 2005). Studies performed by Case & Carlson (2002) and Carlson et al. (2004) showed that the supplementation of ZnO on levels higher than 500 ppm generates an excess of Zn excreted in feces. According to Jensen et al., (2018), feces are the largest way of Zn excretion. It can become toxic to microorganisms and plants due to its soil accumulation, especially in intensive pig production areas (GRÄBER et al., 2005). Moreover, fecal Zn is also a possible environmental inducer of bacterial resistance (HÖLZEL et al., 2012; BEDNORZ et al., 2013; YAZDANKHAH et al., 2014).

However, a several minerals, including Zn, have their bioavailability affected by anti-nutritional factors, being phytate the most determinant regarding to mineral availability (Lopez et al., 2002). Phytate is the main storage form for P in plants (Selle et al., 2009) but is also considered an anti-nutritional factor for humans and animals, as it has the capacity to chelate nutritionally important cations such as Cu^{+2}, Zn^{+2}, Mg^{+2}, Fe^{+2} and Ca^{+2} (Selle et al., 2009).

The proposed interaction between the minerals Zn and Cu happen in the presence of excess of Zn, and will reduce the copper absorption (BREWER et al., 1990). The proposed mechanism of this interaction was based on the increase of the synthesis of a protein named metallothionein, which has the function to form chelates with free minerals and then to perform an antioxidant function in the organism.

Copper is a microelement that can be used as a growth promoter when added at high levels in poultry and swine feeds. The use of copper as growth promoters for these animals has been documented with effects like those of antibiotics growth promoters (GÁTTAS & BARBOSA, et al., 2004). In monogastric animals, around 10% of the ingested Cu in young animals is absorbed, and the duodenum is the main site of absorption (McDOWEL, 2003). The improvements on growth performance when high dietary levels of copper are supplemented are similar in magnitude when compared with AGP's added on the diets (CROMWELL, 2001). Therefore, it appears that the growth-promoting properties of high dietary levels of copper are in addition to it antimicrobial effect.

2.7 Zinc oxide on bone resistance

Bone resistance is a parameter that has gained relevance due to recent advances in genetic improvement, which is leading to a higher slaughter weight. Considering the genetic growth potential of the actual animals and the tendency to keep slaughtering heavier pigs, the bone structure must be sufficiently good to support this upcoming practice.

Phosphorus (P) is one of the most important minerals used for animal production playing innumerous roles in the organism. Moreover, its absence can lead to decreases in the animal performance and unbalance metabolism and other vital functions (SUTLLE, 2010). Approximately 70% of the total P is bound to phytate, and, therefore, it has low digestibility by pigs, due to his anti-nutritional characteristics (JEONG et al., 2015). Thus, it is known as an inhibitor of important enzymes and that the digestibility as well as the availability of nutrients will be affected due to the formation of insoluble and indigestible complexes (COWIESON et al., 2016).

The phytase enzyme activity was nearly 30% reduced when dietary ZnO was added on post weaning diets (AUGSPURGER et al., 2004). The authors found that Zn exerts its effect on P availability and phytase activity through making a complex with the phytate molecule and preventing access for phytase action. Phytate preferentially precipitates with Zn, creating an insoluble complex, and will reduce phytase activity through of promoting Zn-phytate complexes (CHAMPAGNE et al., 1990).

A post-weaning diet that is deficient in digestible P may have some restrictions on the growth performance and potentially will lead to skeletal disease (BÜHLER et al., 2010). Therefore, there is a consensus that phytase enzyme can improve bone mineralization and animal performance, as well as digestibility of phosphorus and other nutrients.

2.8 Alternative sources of zinc oxide

Recent developments in nanotechnology provide new dimensions for researches on substitutes of antibiotics and high dietary ZnO. Nanoparticles exhibit great potentials for many areas. As an alternative to ordinary therapies, the nanoparticles products show various advantages, such as better drug absorption, and improved bioavailability (WANG et al., 2018). Zinc oxide nanoparticles are the most widely used nanoparticles due to their high surface area, especially for their high chemical stability and easy synthesis. The particle size and shape are important characteristics of nanoparticles, which are closely associated with their properties (GAJJAR et al., 2009).

PZnO is obtained by a patented manufacturing technology. The following physicochemical properties are modified: specific particle size and shape with increased (10 to 15 times higher than conventional sources) specific surface area, which drastically increases the surface of contact. Several studies were performed to evaluate the applications and functions of PZnO. The authors stated that due to their particle size, PZnO could be highly absorbed that other sources, being able to express their characteristics. They also registered a high antimicrobial effect and an equivalent performance compared with the conventional source of ZnO (LONG et al., 2017; WANG et al., 2017; LEI et al., 2018; WANG et al., 2018; BAI et al., 2019).

3 GENERAL CONSIDERATIONS

The weaning process is a critical transition phase for the piglets, being characterized by several physical and physiological changes that can compromise the health and growth performance of the post weaning piglets. Nutritional and farm managements become the major challenges on this phase, in order to minimize stressors and ensuring adequate productive conditions throughout the nursery phase and until the slaughter.

In the last decades, different additives have been developed focusing on reducing or smoothing the post weaning challenges. Moreover, ingredient processing, such as nanotechnology process, has been perfecting fast and providing a new dimension for the exploration of new substitutes for the high dietary levels of the conventional source of ZnO in post weaning piglets. Based on this information, the alternative sources of ZnO have a great potential for improving growth performance, decreasing incidence of diarrhea and decreasing mineral fecal excretion. Furthermore, these alternative sources can be included at low inclusion, might leading to economics benefits.

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SECOND PART

Performance and mineral fecal excretion of post-weaned piglets are influenced by different sources of zinc oxide

ABSTRACT

The study aimed to evaluate the impact of the supplementation of the conventional source of zinc oxide (ZnO) and/or a potentiated source of zinc oxide (PZnO) and different phosphorus (P) levels in weaned piglets' diets on productive performance, incidence of diarrhea P, zinc (Zn) and copper (Cu) fecal excretion and bone resistance. A total of 84 piglets weaned at 21 days of age (6.13±0.92 kg), were allocated in a randomized block design with four treatments [T1 negative control: no inclusion of ZnO and regular P levels (0.440%; 0.420%; 0.400%; 0.380%); T2 positive control: inclusion of ZnO and regular P levels (0.440%; 0.420%; 0.400%; 0.380%); T3: association between ZnO and PZnO and 10% low P levels (0.396%; 0.379%; 0.360%; 0.340%); T4 inclusion of the PZnO and 10% low P levels (0.396%; 0.379%; 0.360%; 0.340%)], with seven repetitions and three piglets per pen. The nutritional program was divided into four dietary phases (pre-starter 1: 21 to 28 days of age; pre-starter II: 28 to 35 days of age; starter I: 35 to 49 days of age; and starter II: 49 to 63 days of age). When there was a statistical difference by the F test (P <0.05), the Tukey test was adopted to compare the means. The variable of incidence of diarrhea was analyzed by applying the generalized linear model in the GENMOD procedure (SAS INSTITUTE INC., 2011). During the period from day 1 to day 7, treatments did not influence the performance of the piglets (P > 0.05). When evaluating the period from day 1 to day 14, treatment T2 improved the average daily gain (ADG) (P = 0.0128) and BW (P = 0.0085) at day 14. From the period of day 1 to day 28, pigs fed with T2 diets showed higher ADG (P = 0.0208), ADFI (P = 0.0099) and, consequently, higher BW (P = 0.001) at day 28. Overall, pigs fed with PZnO diet performed the highest ADFI (P = 0.032). By evaluating overall data of diarrhea, it was found that T2 (P < 0.05) reduced the incidence of diarrhea during all studied periods. Treatment T4 reduced Zn and P fecal excretion during the period from 7 to 28 days (P < 0.05). Therefore, the inclusion of PZnO reduced the Zn and P fecal excretion. Moreover, the use of ZnO and PZnO provide the same performance results of piglets on the period from day 1 to day 42 after weaning.

Keywords: Bone resistance. Diarrhea. Phosphorus. Nursery. Nutrition.

1 INTRODUCTION

The weaning process provides some physiological changes and consequences for the animals, mainly due to some factors that occur during this period, such as withdrawal of the pig from contact with the mother, substitution of breast milk for solid foods, fights in nursery pens, sanitary and management challenge, and inadequate temperature.

These factors, associated with weaning, lead to a reduction in the secretion of some enzymes, which consequently affects the digestive and absorptive capacity of the nutrients in the small intestine, besides important changes in their morphology. For this reasons, antibiotics growth promoters (AGP) have been widely used in swine production as growth promoters in order to minimize the incidence of diarrhea after weaning and to improve the performance of the piglets.

However, increases in the incidence of human infections from antibiotic-resistant bacteria have been hypothesized to be directly related to the overuse of antibiotics required for human medical prophylactics and therapeutics in the feeding programs for animal production, especially swine (JENSEN et al., 2018). Considering the severe restriction or total ban on the use of AGP in swine production, ZnO have been suggested as an alternative to antibiotics as growth promoters. The issue regarding the high doses of ZnO in piglet's diets is the environmental pollution, since approximately 80% of the amount ingested is excreted in the feces (BUFF et al., 2005). Moreover, new forms of zinc oxide processing were developed in order to improve the efficiency of use in increasingly smaller doses.

In the last few years, nanotechnology has been developing fast, providing a new dimension for the exploration of new substitutes for high dietary doses of the conventional source of ZnO in post weaning diets. A patented manufacturing technology is at the origin of the potentiated source of zinc oxide (PZnO). It exhibits high antibacterial activity and due to his particle size, PZnO have higher bioavailability than the conventional source of ZnO, being added in small amount on post weaning diets.

Thus, the objective of the present study was to evaluate the impact of the supplementation of the conventional source of ZnO and/or a potentiated source of zinc oxide and different phosphorus levels in post weaning diets on productive performance, incidence of diarrhea, phosphorus, zinc and copper excretion and bone resistance.

2 OBJECTIVE

The present study aims to evaluate the impact of the piglets' post weaning diets supplemented with the conventional source of zinc oxide and/or a potentiated source of zinc oxide (PZnO) and different phosphorus on productive performance, incidence of diarrhea, phosphorus, zinc and copper excretion and bone resistance.

3 MATERIALS AND METHODS

All methods involving animal handling were performed in accordance with the regulations approved by the Institutional Animal Welfare and Ethics/Protection committee from the Federal University of Minas Gerais – Campus Montes Claros (UFMG – CETEA), Brazil, under the protocol nº 296/2017.

3.1 Animals, diets and experimental Design

The study was performed between October and December 2018 and it was conducted in the nursery facilities of the swine production farm of the Federal University of Minas Gerais – Campus Montes Claros. A total of 84 piglets were obtained from a commercial pig herd (TN 70® Topigs Norsvin x TN Traxx® Topigs Norsvin) and weaned at 21 days of age, presenting an initial body weight of 6.13 ± 0.92 kg. The pigs were allocated in a randomized block design using the initial body weight as the main criteria for the formation of the blocks, and divided among four treatments and seven repetitions per treatment, with 3 piglets per pen. The following experimental treatments were used: T1 negative control: no inclusion of ZnO and regular P levels (0.440%; 0.420%; 0.400%; 0.380%); T2 positive control: inclusion of ZnO and regular P levels (0.440%; 0.420%; 0.400%; 0.380%); T3: association between ZnO and PZnO and 10% low P levels (0.396%; 0.379%; 0.360%; 0.340%); T4 inclusion of the PZnO and 10% low P levels (0.396%; 0.379%; 0.360%; 0.340%). The zinc sources were added volumetrically in substitution of the corn. The nutrient compositions of the experimental diets are shown from Table 1 to Table 4, as well as the obtained phosphorus and zinc values from laboratory analysis, which are similar to those of the calculated composition of the diets.

PZnO was obtained from a commercial company (Animine Company, 335 Chemin Du Noyer, Sillingy, 74330, France). A patented manufacturing technology is at the origin of the potentiated source of zinc oxide. The following physicochemical properties are modified:

specific particle size and shape with increased (10 to 15 times higher than conventional sources) specific surface area, which drastically increases the surface of contact. The high porosity of the powder amplifies the antibacterial activity of PZnO.

The nutritional program was divided into four dietary phases (pre-starter 1 (P1): 21 to 28 days of age; pre-starter II (P2): 28 to 35 days of age; starter I (P3): 35 to 49 days of age; and starter II (P4): 49 to 63 days of age. The applied diets were isoenergetic, isoproteic, isoaminoacidic and formulated to meet the requirements of this animal category according to Rostagno et al. (2017), except for the phosphorus levels. The piglets were housed in groups of three animals per pen into the nursery facilities with access to feed and water ad libitum.

Table 1 - Experimental diets used on phase 1 (21 to 28 days of age).

Ingredients (%)	**Phase 1 - Pre-starter I**			
	T1	**T2**	**T3**	**T4**
Corn (7.86%)	24.228	23.928	24.166	24.266
Soybean meal (46%)	16.000	16.000	16.000	16.000
Soybean protein concentrate	8.000	8.000	8.000	8.000
Pre-Cooked corn	10.350	10.350	10.350	10.350
Swine plasma	5.000	5.000	5.000	5.000
Biscuit meal	5.000	5.000	5.000	5.000
Soybean oil	3.550	3.550	3.550	3.550
Sugar	3.500	3.500	3.500	3.500
Milk whey	21.000	21.000	21.000	21.000
Dicalcium fosfate	1.045	1.045	0.724	0.724
Calcium carbonate	0.453	0.453	0.659	0.659
Sodium cloride	0.400	0.400	0.400	0.400
Ultracid® (Organic Acid blend)[4]	0.200	0.200	0.200	0.200
L-Lysina HCl (78%)	0.424	0.424	0.424	0.424
DL-Methionine (98%)	0.220	0.220	0.220	0.220
L-Threonine (98%)	0.197	0.197	0.197	0.197
L-Triptophane (98%)	0.065	0.065	0.065	0.065
L-Valine (98%)	0.110	0.110	0.110	0.110
Premix Micromineral SALUS®[1]	0.100	0.100	0.100	0.100
Premix Vitamin Crescimento OVN®[2]	0.050	0.050	0.050	0.050
Phytase Ronozyme Hyphos®[3]	0.005	0.005	0.005	0.005
Toxi-nil® (Mycotoxin binder)[5]	0.100	0.100	0.100	0.100
Powersweet®	0.030	0.030	0.030	0.030
Zinc Oxide 80%	0.000	0.300	0.100	0.000
Potentiated Zinc Oxide	0.000	0.000	0.050	0.050
TOTAL	**100.00**	**100.00**	**100.00**	**100.00**
Nutritional specifications				
Metabolizable Energy, (kcal/kg)	3500	3500	3500	3500

Crude Protein, (%)	22.22	22.22	22.22	22.22
Total Calcium, (%)	0.70	0.70	0.70	0.70
Digestible Phosphorus, (%)	0.440	0.440	0.396	0.396
Lactose, (%)	14.7	14.7	14.7	14.7
Sodium, (%)	0.25	0.25	0.25	0.25
SID[6] AAS (%)				
Lysine	1.50	1.50	1.50	1.50
Methionine + Cysteine	0.84	0.84	0.84	0.84
Threonine	1.00	1.00	1.00	1.00
Valine	1.06	1.06	1.06	1.06
Tryptophan	0.31	0.31	0.31	0.31

Laboratory analysis report	**T1**	**T2**	**T3**	**T4**
Zinc, (mg/kg)	148.47	2.304,91	1.226,24	475.31
Phosphorus, (%)	0.61	0.61	0.57	0.56

[1] Inorganic manganese (50,000 mg/kg), Inorganic zinc (97,000 mg/kg), Inorganic iron (100,001 mg/kg), Inorganic copper (13,000 mg/kg), Total iodine (1,000 mg/kg).; [2] Vitamin A (225,00000 UI/kg), Vitamin D3 (380,0000 UI/kg), Vitamin E (200,000 UI/kg), Vitamin K (10,000 mg/kg), Biotin (1,000 mg/kg), Folic acid (9,000 mg/kg), Nyacin (120,000 mg/kg), Pantotenic acid (60,000 mg/kg), Vitamin B2 (20,000 mg/kg), Vitamin B1 (8,000 mg/kg), Vitamin B6 (12,000 mg/kg), and Vitamin B12 (100,000 mcg/kg).; [3] Phytase inclusion equivalent to 1000 FYT and 0.120% available phosphorus.; [4] Mixture of organic acids: Formic Acid / Acetic Acid / Propionic Acid / Lactic Acid / Citric acid / Carrier & anticaking.; [5] Toxin binder composed by Fermentation extracts of *Saccharomyces cerevisiae*, citric acid, lactic acid, phosphoric acid, and propylene glycol.; [6] SID – Standardized ileal digestibility

Table 2 - Experimental diets used on phase 2 (28 to 35 days of age).

Ingredients (%)	**Phase 2 - Pre-starter II**			
	T1	**T2**	**T3**	**T4**
Corn (7.86%)	32.189	31.889	32.142	32.242
Soybean meal (46%)	19.000	19.000	19.000	19.000
Soybean protein concentrate	5.000	5.000	5.000	5.000
Pre-Cooked corn	10.000	10.000	10.000	10.000
Swine plasma	3.000	3.000	3.000	3.000
Biscuit meal	5.000	5.000	5.000	5.000
Soybean oil	3.350	3.350	3.350	3.350
Sugar	3.500	3.500	3.500	3.500
Milk whey	15.000	15.000	15.000	15.000
Dicalcium fosfate	1.187	1.187	0.885	0.885
Calcium carbonate	0.538	0.538	0.737	0.737
Sodium cloride	0.400	0.400	0.400	0.400
Ultracid® (Organic Acid blend)[4]	0.200	0.200	0.200	0.200
L-Lysina HCl (78%)	0.576	0.576	0.576	0.576
DL-Methionine (98%)	0.247	0.247	0.247	0.247
L-Threonine (98%)	0.273	0.273	0.273	0.273
L-Triptophane (98%)	0.080	0.080	0.080	0.080
L-Valine (98%)	0.175	0.175	0.175	0.175

Premix Micromineral SALUS®[1]	0.100	0.100	0.100	0.100
Premix Vitamin Crescimento OVN®[2]	0.050	0.050	0.050	0.050
Phytase Ronozyme Hyphos®[3]	0.005	0.005	0.005	0.005
Toxi-nil® (Mycotoxin binder)[5]	0.100	0.100	0.100	0.100
Powersweet®	0.030	0.030	0.030	0.030
Zinc Oxide 80%	0.000	0.300	0.100	0.000
Potentiated Zinc Oxide	0.000	0.000	0.050	0.050
TOTAL	**100.00**	**100.00**	**100.00**	**100.00**
Nutritional specifications				
Metabolizable Energy, (kcal/kg)	3450	3450	3450	3450
Crude Protein, (%)	20.34	20.34	20.34	20.34
Total Calcium, (%)	0.720	0.720	0.720	0.720
Digestible Phosphorus, (%)	0.420	0.420	0.379	0.379
Lactose, (%)	10.5	10.5	10.5	10.5
Sodium, (%)	0.25	0.25	0.25	0.25
SID[6] AAS (%)				
Lysine	1.45	1.45	1.45	1.45
Methionine + Cysteine	0.81	0.81	0.81	0.81
Threonine	0.97	0.97	0.97	0.97
Valine	1.02	1.02	1.02	1.02
Tryptophan	0.29	0.29	0.29	0.29
Laboratory analysis report	**T1**	**T2**	**T3**	**T4**
Zinc, (mg/kg)	265.77	2.555.63	1.260.39	556.12
Phosphorus, (%)	0.61	0.60	0.53	0.52

[1] Inorganic manganese (50,000 mg/kg), Inorganic zinc (97,000 mg/kg), Inorganic iron (100,001 mg/kg), Inorganic copper (13,000 mg/kg), Total iodine (1,000 mg/kg).; [2] Vitamin A (225,00000 UI/kg), Vitamin D3 (380,0000 UI/kg), Vitamin E (200,000 UI/kg), Vitamin K (10,000 mg/kg), Biotin (1,000 mg/kg), Folic acid (9,000 mg/kg), Nyacin (120,000 mg/kg), Pantotenic acid (60,000 mg/kg), Vitamin B2 (20,000 mg/kg), Vitamin B1 (8,000 mg/kg), Vitamin B6 (12,000 mg/kg), and Vitamin B12 (100,000 mcg/kg).; [3] Phytase inclusion equivalent to 1000 FYT and 0.120% available phosphorus.; [4] Mixture of organic acids: Formic Acid / Acetic Acid / Propionic Acid / Lactic Acid / Citric acid / Carrier & anticaking.; [5] Toxin binder composed by Fermentation extracts of *Saccharomyces cerevisiae*, citric acid, lactic acid, phosphoric acid, and propylene glycol.; [6] SID – Standardized ileal digestibility

Table 3 - Experimental diets used on phase 3 (35 to 49 days of age).

Ingredients (%)	**Phase 3 - Starter I**			
	T1	**T2**	**T3**	**T4**
Corn (7.86%)	46.884	46.663	46.936	46.960
Soybean meal (46%)	25.000	25.000	25.000	25.000
Soybean protein concentrate	3.000	3.000	3.000	3.000
Pre-Cooked corn	7.000	7.000	7.000	7.000
Biscuit meal	5.000	5.000	5.000	5.000
Soybean oil	3.700	3.700	3.700	3.700
Sugar	2.000	2.000	2.000	2.000
Milk whey	3.000	3.000	3.000	3.000

Dicalcium fosfate	1.556	1.563	1.269	1.269
Calcium carbonate	0.520	0.514	0.705	0.706
Sodium cloride	0.400	0.400	0.400	0.400
Ultracid® (Organic Acid blend)[4]	0.200	0.200	0.200	0.200
L-Lysina HCl (78%)	0.625	0.625	0.625	0.625
DL-Methionine (98%)	0.245	0.245	0.245	0.245
L-Threonine (98%)	0.304	0.304	0.304	0.304
L-Triptophane (98%)	0.090	0.090	0.090	0.090
L-Valine (98%)	0.191	0.191	0.191	0.191
Premix Micromineral SALUS®[1]	0.100	0.100	0.100	0.100
Premix Vitamin Crescimento OVN®[2]	0.050	0.050	0.050	0.050
Phytase Ronozyme Hyphos®[3]	0.005	0.005	0.005	0.005
Toxi-nil® (Mycotoxin binder)[5]	0.100	0.100	0.100	0.100
Powersweet®	0.030	0.030	0.030	0.030
Zinc Oxide 80%	0.000	0.220	0.000	0.000
Potentiated Zinc Oxide	0.000	0.000	0.050	0.025
TOTAL	**100.00**	**100.00**	**100.00**	**100.00**
Nutritional specifications				
Metabolizable Energy, (kcal/kg)	3400	3400	3400	3400
Crude Protein, (%)	19.21	19.21	19.21	19.21
Total Calcium, (%)	0.720	0.720	0.720	0.720
Digestible Phosphorus, (%)	0.400	0.400	0.360	0.360
Lactose, (%)	2.1	2.1	2.1	2.1
Sodium, (%)	0.25	0.25	0.25	0.25
SID[6] AAS (%)				
Lysine	1.35	1.35	1.35	1.35
Methionine + Cysteine	0.77	0.77	0.77	0.77
Threonine	0.91	0.91	0.91	0.91
Valine	0.94	0.94	0.94	0.94
Tryptophan	0.27	0.27	0.27	0.27

Laboratory analysis report	**T1**	**T2**	**T3**	**T4**
Zinc (mg/kg)	170.66	1.766.67	485.90	208.67
Phosphorus (%)	0.60	0.60	0.54	0.55

[1] Inorganic manganese (50,000 mg/kg), Inorganic zinc (97,000 mg/kg), Inorganic iron (100,001 mg/kg), Inorganic copper (13,000 mg/kg), Total iodine (1,000 mg/kg).; [2] Vitamin A (225,00000 UI/kg), Vitamin D3 (380,0000 UI/kg), Vitamin E (200,000 UI/kg), Vitamin K (10,000 mg/kg), Biotin (1,000 mg/kg), Folic acid (9,000 mg/kg), Nyacin (120,000 mg/kg), Pantotenic acid (60,000 mg/kg), Vitamin B2 (20,000 mg/kg), Vitamin B1 (8,000 mg/kg), Vitamin B6 (12,000 mg/kg), and Vitamin B12 (100,000 mcg/kg).; [3] Phytase inclusion equivalent to 1000 FYT and 0.120% available phosphorus.; [4] Mixture of organic acids: Formic Acid / Acetic Acid / Propionic Acid / Lactic Acid / Citric acid / Carrier & anticaking.; [5] Toxin binder composed by Fermentation extracts of *Saccharomyces cerevisiae*, citric acid, lactic acid, phosphoric acid, and propylene glycol.; [6] SID – Standardized ileal digestibility

Table 4 - Experimental diets used on phase 4 (49 to 63 days of age).

Ingredients (%)	Phase 4 - Starter II			
	T1	**T2**	**T3**	**T4**
Corn (7.86%)	58.255	58.155	58.305	58.330
Soybean meal (46%)	28.000	28.000	28.000	28.000
Biscuit meal	5.000	5.000	5.000	5.000
Soybean oil	4.500	4.500	4.500	4.500
Dicalcium fosfate	1.520	1.520	1.233	1.233
Calcium carbonate	0.593	0.593	0.780	0.780
Sodium cloride	0.400	0.400	0.400	0.400
Ultracid® (Organic Acid blend)[4]	0.200	0.200	0.200	0.200
L-Lysina HCl (78%)	0.574	0.574	0.574	0.574
DL-Methionine (98%)	0.186	0.186	0.186	0.186
L-Threonine (98%)	0.275	0.275	0.275	0.275
L-Triptophane (98%)	0.067	0.067	0.067	0.067
L-Valine (98%)	0.145	0.145	0.145	0.145
Premix Micromineral SALUS®[1]	0.100	0.100	0.100	0.100
Premix Vitamin Crescimento OVN®[2]	0.050	0.050	0.050	0.050
Phytase Ronozyme Hyphos®[3]	0.005	0.005	0.005	0.005
Toxi-nil® (Mycotoxin binder)[5]	0.100	0.100	0.100	0.100
Powersweet®	0.030	0.030	0.030	0.030
Zinc Oxide 80%	0.000	0.100	0.000	0.000
Potentiated Zinc Oxide	0.000	0.000	0.050	0.025
TOTAL	**100.00**	**100.00**	**100.00**	**100.00**
Nutritional specifications				
Metabolizable Energy, (kcal/kg)	3399	3399	3399	3399
Crude Protein, (%)	18.32	18.32	18.32	18.32
Total Ca, (%)	0.720	0.720	0.720	0.720
Digestible P, (%)	0.380	0.380	0.340	0.340
Lactose, (%)	0	0	0	0
Sodium, (%)	0.25	0.25	0.25	0.25
SID [6] AAS, (%)				
Lysine	1.25	1.25	1.25	1.25
Methionine + Cysteine	0.71	0.71	0.71	0.71
Threonine	0.84	0.84	0.84	0.84
Valine	0.87	0.87	0.87	0.87
Tryptophan	0.24	0.24	0.24	0.24
Laboratory analysis report	**T1**	**T2**	**T3**	**T4**
Zinc, (mg/kg)	126.7	844.34	487.06	303.14
Phosphorus, (%)	0.59	0.54	0.55	0.58

[1] Inorganic manganese (50,000 mg/kg), Inorganic zinc (97,000 mg/kg), Inorganic iron (100,001 mg/kg), Inorganic copper (13,000 mg/kg), Total iodine (1,000 mg/kg).; [2] Vitamin A (225,00000 UI/kg), Vitamin D3 (380,0000 UI/kg), Vitamin E (200,000 UI/kg), Vitamin K (10,000 mg/kg), Biotin (1,000 mg/kg), Folic acid (9,000 mg/kg), Nyacin (120,000 mg/kg),

Pantotenic acid (60,000 mg/kg), Vitamin B2 (20,000 mg/kg), Vitamin B1 (8,000 mg/kg), Vitamin B6 (12,000 mg/kg), and Vitamin B12 (100,000 mcg/kg).; [3] Phytase inclusion equivalent to 1000 FYT and 0.120% available phosphorus.; [4] Mixture of organic acids: Formic Acid / Acetic Acid / Propionic Acid / Lactic Acid / Citric acid / Carrier & anticaking.; [5] Toxin binder composed by Fermentation extracts of *Saccharomyces cerevisiae*, citric acid, lactic acid, phosphoric acid, and propylene glycol.; [6] SID – Standardized ileal digestibility

3.2 Experimental procedures

Every morning, feed refusals were collected when available, and fresh feed was immediately distributed once per day between 6:30 am and 7:30 am. Feed intake was determined as the difference between feed allowance and the refusals collected on the next morning. Feed samples (1 sample per treatment and per phase) were analyzed for zinc and phosphorus contents (AOAC, 1990). The variations in ambient temperature (maximum and minimum), relative humidity (RH) and photoperiod were following closely the outdoor conditions. Ambient temperature and RH was continuously recorded (one measurement every day at 5:00 pm) in the barns, using an infrared thermometer placed inside the facilities (Didai Tecnologia Ltda., Campinas, Brazil). Pigs were individually weighed at the beginning and at the end of each period of the experiment. All the piglets received one shot of Draxxin® Zoetis (0.15 mL per animal) to control respiratory diseases. For each growing stage, the average daily gain, average daily feed intake and gain: feed ratios were calculated.

During the experiment, piglets that presented high intense diarrhea were medicated with an intramuscular injection of Tylan® and Quinotril® during three consecutive days at a dosage of 2.0 mL/piglet. The diarrhea score of each piglet was assessed daily, according to the method proposed by Casey et al. (2007). The incidence of diarrhea was observed by a visual inspection of the consistency of fecal material on the floor of each pen on a scale of 1–5: whereas, 1, no diarrhea hard and dry consistency; 2, no diarrhea soft and humid consistency considered as normal; 3, no diarrhea soft, humid and pasty consistency; 4, pasty diarrhea; and 5, liquid diarrhea. Diarrhea scores were taken from all repetitions in each treatment group on a daily basis early in the morning (7:00 am) and in the afternoon (5:00 pm). The presence of diarrhea was recorded when at least a piglet of the pen developed pasty or watery fecal consistency (scores 4 to 5). The diarrhea incidence (%) was calculated as the sum of daily diarrheal piglet observations over the period and then divided by the number of piglet days in the period, and the quotient multiplied by 100 (CASEY et al., 2007).

The fecal sampling was followed a standard protocol: three non-consecutive periods of collection throughout each phase of approximately 40g of feces from every pen of each treatment. Then they were sampled and pooled and then, marked and frozen in a temperature of -72 ºC in a Consul® Freezer. Afterwards, samples from Phase 2 (28 to 35 days) and Phase 3 (35 to 49 days) were thawed at room temperature, oven-dried at 55 °C for three days and grinded to be analyzed about the excretion of P, Zn and Cu using the technique proposed by Detmann et al. (2012).

On day 42 of the experiment, one pig with BW closest to the repetition average weight was chosen from 24 experimental pens and euthanized, totaling 24 pigs. The slaughter was performed through stunning by electronarcosis (>300 V, 1.25 A, for 6 s) followed by exsanguination. The slaughter was performed in a commercial slaughterhouse in the city of Januária (Minas Gerais, Brazil) accompanied by a registered veterinarian. The front foot of the right side of each carcass was removed and frozen (-20 °C) for subsequent determination of bone characteristics. The paws were placed in boiling water in an aluminum container to smooth the skin and flesh around the bones to facilitate the removal of the third metacarpal. Subsequently, the third metacarpus samples were taken to a forced-ventilation oven at 55 °C for 24 hours. The bone bending moment was determined by the Universal Testing Machine WDW 20E (Jinan Liangong Testing Technology®, Jiangsu, China) in the “Wood Resistance Laboratory” of the Forest Engineering Department – UFLA (Universidade Federal de Lavras). The test was performed in a climatic chamber with temperature controlled at 17 °C and relative humidity of 65%. A force was applied at the midpoint of each bone at a constant velocity of 2 mm/min with a span length of 2.0 cm. The analysis ended with the deformation and subsequent rupture of the bone. The load (“kgf”) was recorded by the equipment.

3.3 Calculations and statistical analyses

The data were submitted to the statistical software SAS INSTITUTE INC, 2011. The Shapiro-Wilk test was used to analyze the normality of the data and when they did not present this distribution the transformation was performed using PROCRANK (SAS INSTITUTE INC, 2011). The initial weight of the piglets was considered as a covariate in the model. All variables were submitted to analysis of variance. When there was a statistical difference by the F test ($P < 0.05$), the Tukey test was adopted to compare the means. For the variable of incidence of diarrhea, the influence of each treatment on the occurrence of diarrhea was analyzed by applying the generalized linear model in the GENMOD procedure (SAS INSTITUTE INC, 2011).

4 RESULTS AND DISCUSSION

4.1 Environmental indexes

Average minimum and maximum temperatures and relative humidity level measured during the entire experimental period were disposed on Table 5. These environmental parameters indicate that the pigs were kept in a comfortable thermoneutral environment, so that the environment did not represent an influencing factor in the test results.

Table 5 - Average minimum and maximum temperature and relative humidity during the experimental periods.

Indexes	**Values**			
	1-7 days	**7-14 days**	**14-28 days**	**28-42 days**
Minimum Temperature (ºC)	23.7	22.9	23.7	23.7
Maximum Temperature (ºC)	31.6	33.9	32.1	31.7
Relative Humidity (%)	59.8	59%	61%	60%

4.2 Performance and incidence of diarrhea

During the period from day 1 to day 7, treatments did not influence the performance (Table 6) of the piglets ($P > 0.05$). This result contrasts with studies conducted by Wang et al. (2018) and Lei & Kim (2018), who observed that the inclusion of zinc oxide on post weaning diets showed an improvement in the performance and a decrease in the incidence of diarrhea during the firsts weeks after weaning.

Due to nutritional, psychological and environmental stressors, weaning is often associated with diarrhea (KIM et al., 2012). However, the incidence of diarrhea (Table 7) during the first week after weaning was not different between the treatments ($P > 0.05$). This finding contradicts the information presented in the study of Shen et al. (2014), which showed that groups of piglets fed with diets without the inclusion of ZnO showed worse performance and high incidence of diarrhea during the first weeks after weaning and subsequently during the entire trial.

Table 6 - Effects of dietary levels of conventional zinc oxide and/or the use of PZnO on the performance of piglets during nursery phase.

Parameters	**Treatments**				**CV%**	***P*-value**
	T1	**T2**	**T3**	**T4**		
	1-7 days					
Initial BW, kg	6.182	6.052	6.172	6.123	14.80	0.8144
BW – d 7, kg	7.337	7.380	7.313	7.109	12.68	0.4295
ADG, kg	0.171	0.179	0.146	0.139	32.25	0.2555
ADFI, kg	0.219	0.230	0.218	0.216	20.05	0.8349
F:C	1.40	1.34	1.38	1.49	19.29	0.7780
	1-14 days					
BW – d 14, kg	8.893^{b}	9.740^{a}	9.00^{b}	8.88^{b}	13.77	0.0085
ADG, kg	0.198^{b}	0.256^{a}	0.205^{ab}	0.196^{b}	21.80	0.0128
ADFI, kg	0.273	0.303	0.276	0.271	12.49	0.1956
F:C	1.42	1.24	1.30	1.39	13.68	0.1843
	1-28 days					
BW – d 28, kg	14.931^{b}	16.199^{a}	14.987^{b}	14.801^{b}	11.51	0.0031
ADG, kg	0.315^{ab}	0.357^{a}	0.317^{ab}	0.309^{b}	11.57	0.0208
ADFI, kg	0.446^{b}	0.504^{a}	0.447^{b}	0.445^{b}	11.12	0.0099
F:C	1.42	1.41	1.41	1.45	4.18	0.7149
	1-42 days					
BW – d 42, kg	24.149	25.728	24.762	24.354	8.36	0.0997
ADG, kg	0.430	0.465	0.435	0.433	7.87	0.0620
ADFI, kg	0.674^{ab}	0.634^{b}	0.716^{a}	0.735^{a}	7.74	0.0032
F:C	1.57	1.55	1.60	1.59	4.28	0.4196

BW – body weight; ADFI – average daily feed intake; ADWG – average daily gain; F:C – feed conversion ratio; T1 – Negative control; T2 – Positive control (ZnO); T3 – association zinc oxide and PZnO; T4 – PznO; CV – variation coefficient.
^{a,b,c} Within a row with different letters differ by Tukey test (P < 0.05).

The results of the first week after weaning may lead us to reflect about the composition and nutrient availability of each diet in the first phase. The first phase diets contain important quantities of milk by-products and lactose-rich ingredients that may have stimulated growth conditions in the immediate post-weaning period (O'SHEA et al., 2013). Several studies have demonstrated the benefits of high dietary lactose inclusion on post weaning in diets for piglet's performance, nutrient digestibility and homeostasis of intestinal microbiota (O'DOHERTY et al., 2004; PIERCE et al., 2005; O'DOHERTY et al., 2010). Lactose is instantly fermented by the weaned piglet to lactic acid due to the presence of *Lactobacillus*. The resultant lower pH in the stomach increases gastric proteolysis and nutrient digestibility (PARTRIDGE, 1993). Moreover, it is important to consider that the piglets received solid diets during their lactation, and this practice associated with sow milk, might lead to a faster adaptation on nursery. According to Rhouma et al. (2017), providing solid diets to piglets during lactation may reflect on the post weaning performance due to a previous adaptation of the organism of the animal.

Table 7 - Effects of dietary levels of zinc oxide and/or the use of PZnO on the incidence of diarrhea (%) based on the fecal score of piglets during nursery phase.

Period	Treatments				
	T1	**T2**	**T3**	**T4**	***P*-value**
1-7 days, %	11.2	13.3	8.2	16.3	0.350
7-14 days, %	35.7^{a}	14.2^{b}	24.4^{a}	28.5^{a}	0.005
14-28 days, %	50.5^{a}	17.3^{c}	29.5^{b}	37.2^{b}	<0.001
28-42 days, %	39.7^{a}	18.8^{b}	27.5^{ab}	39.7^{a}	<0.001

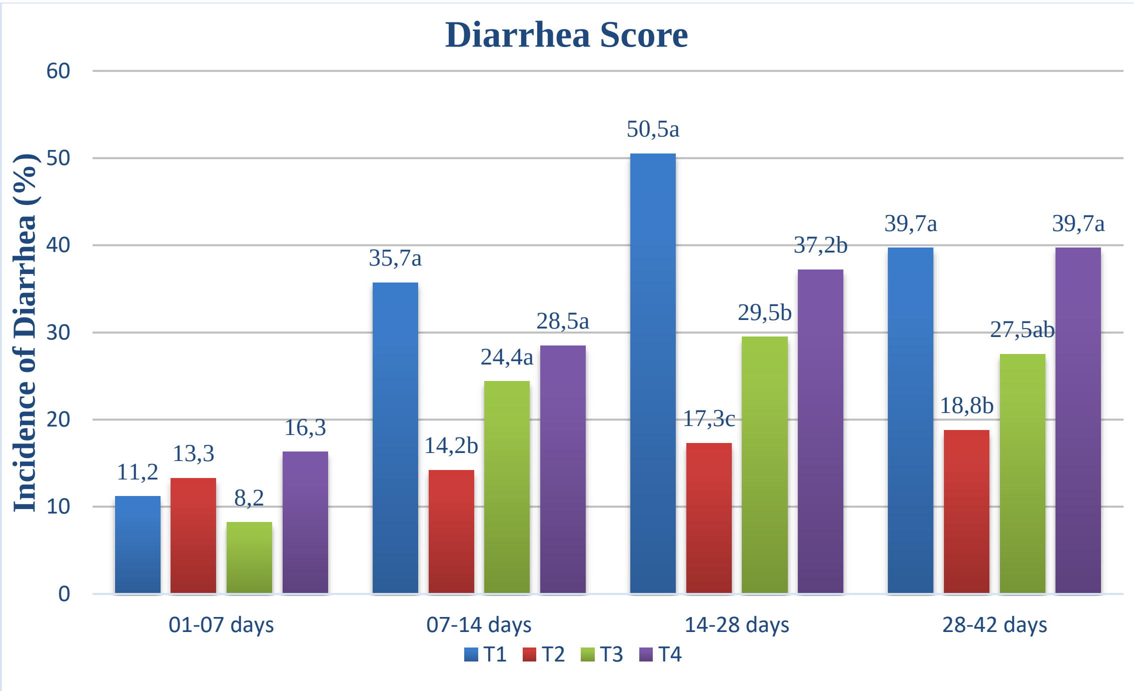
T1 – Negative control; T2 – Positive control (ZnO); T3 – Association zinc oxide and PZnO; T4 – PZnO
[a,b,c] Within a row with different letters differ by Tukey test ($P < 0.05$).

When evaluating the period from day 1 to day 14, the diets containing the conventional zinc oxide improved the ADG (P = 0.0128) and, consequently, the body weight (BW) (P = 0.0085) at fourteen days. It also presented a significant effect by decreasing the incidence of diarrhea from day 7 to day 14 of the trial (Figure 2). The performance and diarrhea results from the present study supports the findings of Cho et al. (2015) and Song et al. (2015), showing that feeding zinc oxide can reduce the post-weaning diarrhea and improve the performance of the piglets. Likewise, Raquipo et al. (2017) performed a study evaluating the supplementation of different sources of zinc oxide. The authors reported a significant effect of the conventional source of zinc oxide by improving the ADG of the piglets during the first weeks after weaning when compared with the group without inclusion zinc oxide in the diets.

Treatments containing the conventional zinc oxide also influenced the performance of the piglets during the period from day 1 to day 28. Pigs fed with treatment 2 (T2 - ZnO) diets showed a greater ADG (P = 0.0208), ADFI (P = 0.0099) and consequently a higher BW (P = 0.0031) at twenty-eight days when compared to other treatments. The supplementation of conventional ZnO, in agreement with the study conducted by Heo et al. (2013), significantly reduced the incidence of diarrhea and improved the performance in newly weaned piglets. Thus, ZnO exerts an antibacterial activity and provides reduction on the incidence diarrhea and improvement of gut health (GRILLI et al., 2015). Moreover, Long et al. (2017) tested the same commercial source of PZnO for post weaned piglets and the authors observed that PZnO significantly increased growth performance compared to the conventional source of zinc oxide.

Regarding to the diarrhea results during the period from day 14 to day 28, there was a positive effect of PZnO ($P < 0.001$), by reducing the incidence of diarrhea when compared with treatment 1 (T1 - negative control). However, the conventional source of ZnO ($P < 0.001$) presented the highest reduction on the incidence of diarrhea. The diarrhea results agreed with the findings of the study performed by Bai et al. (2019), when the authors found that dietary supplementation of an alternative source of zinc oxide decreased the diarrhea. However, the effect was not as strong as the conventional source of zinc oxide. Thus, PZnO remarkably becomes a promising alternative for the reduction of the high doses of conventional zinc oxide mostly practiced on post weaning diets.

Figure 2 - Effects of dietary levels of zinc oxide and/or the use of PZnO on the incidence of diarrhea (%) based on the fecal score of piglets during nursery phase.



From day 1 to day 42, ZnO negatively influenced the feed intake of the piglets. The greater values of feed intake were observed on the piglets fed with diets containing PZnO ($P = 0.0032$). Likewise, Milani et al. (2017) performed a study about an alternative source of zinc oxide compared with conventional one. The authors concluded that the dietary supplementation of this alternative source of zinc oxide (nanoparticle size zinc oxide) improved the immunity and growth performance of piglets. Nevertheless, the results regarding ADG and F:C showed no significant difference ($P > 0.05$). In addition, the conventional source of ZnO did not perform an effect on growth performance of weanling pigs. On the other hand, it performed a significantly effect on post-weaning diarrhea. Regarding PZnO, there was a high anti-inflammatory capacity against microorganisms, and they attributed this effect to the porosity and the particle size of the PZnO. The antibacterial activity of ZnO is due to the generation of hydrogen peroxide. Therefore, it is assumed that increasing the surface area of ZnO particle (decreasing particle size) can elevate the efficient of the hydrogen peroxide generation, as well as antibacterial activity (LONG et al., 2017; SIRELKHATIM et al., 2015 and ZHANG et al., 2007).

Improvements in the manufacturing process with past years, such as envelope and nanotechnology, may feature advantages for the efficiency of absorbing nutrients and additives. Nanoparticles decrease the particle size of ingredients, which can then more readily cross gut barriers and have better absorption and permeability rates (BUZEA et al., 2017; CHO et al., 2013). Therefore, reducing minerals to nanoscale may lead to an improvement on the utilization and absorption.

4.3 Mineral excretion

The supplementation of PZnO reduced zinc fecal excretion ($P < 0.0001$) when compared with the other treatments from day 7 to day 42 (Table 8). Furthermore, from the period of day 14 to day 28, the same source reduced zinc ($P < 0.0001$) and phosphorus ($P = 0.0011$) fecal excretion. Furthermore, the treatment containing only the conventional source of zinc oxide performed a decrease in copper fecal excretion on the period from day 7 to day 14 ($P = 0.0033$), from day 14 until day 28 ($P = 0.0003$) and from day 28 to day 42 ($P = 0.0403$), when compared with the other treatments.

Table 8 - Effects of dietary levels of zinc oxide and/or the use of PZnO on the phosphorus, zinc and copper fecal excretion of piglets during nursery the period from 7 to 14 days and the period from 14 to 28 days.

Minerals	Treatments				CV%	*P*-value
	T1	T2	T3	T4		
7 to 14 days						
Cu (mg/kg)	140.42[a]	112.11[b]	122.83[ab]	124.49[ab]	11.84	0.0033
Zn (mg/kg)	12.883[d]	89.683[a]	60.931[b]	34.978[c]	60.81	<0.0001

P (%)	1.511	1.401	1.413	1.373	8.26	0.1956
14 to 28 days						
Cu (mg/kg)	96.781^{a}	80.488^{b}	98.606^{a}	92.911^{a}	10.30	0.0003
Zn (mg/kg)	7.710^{c}	75.510^{a}	33.686^{b}	13.160^{c}	82.09	<0.0001
P (%)	1.266^{ab}	1.348^{a}	1.190^{bc}	1.098^{c}	9.95	0.0011
28 to 42 days						
Cu (mg/kg)	211.90^{a}	170.95^{b}	208.84^{ab}	196.12^{ab}	14.14	0.0403
Zn (mg/kg)	2.261^{d}	11.421^{a}	7.424^{b}	4.319^{c}	58.08	<0.0001
P (%)	1.311	1.103	1.200	1.236	16.99	0.3922

T1 – Negative control; T2 – Positive control (ZnO); T3 – Association zinc oxide and PZnO; T4 – PznO; CV – variation coefficient; Cu – copper; Zn – zinc; P – phosphorus.

$^{a, b, c, d}$ Within a row with different letters differ by Tukey test (P < 0.05).

In the last few years, numerous studies regarding the environmental pollution caused by an animal's productions systems deject have been performed, aiming to evaluate the impacts and to understand the components of the contamination. They found high zinc concentrations and other heavy metals in the soils of pig production areas. Zn is an essential mineral (micronutrient) for the most of living organisms including husbandry, mainly swine. It plays a vital role in the organism such as being component of metalloenzymes such as DNA and RNA synthetases, transferases and many digestive enzymes (JENSEN et al., 2018).

Furthermore, Zn plays essential roles on the animal's metabolism (HEO et al., 2013). Although the inclusion of ZnO is widely used to decrease the incidence of diarrhea and improve the growth performance in piglets, more attention should be paid to the excretion caused by the high doses of the conventional source of ZnO. Due to its low absorption, around 80% of the ZnO is excreted as Zn in the feces (BUFF et al., 2005). Therefore, concerns related to environmental pollution and adverse effects on environmental microbial diversity caused by fecal Zn excretion have increased. Zinc can become toxic to microorganisms and plants due to its soil accumulation, especially in intensive pig production areas (GRÄBER et al., 2005). Moreover, fecal Zn is also a possible environmental inducer of bacterial resistance (HÖLZEL et al., 2012; BEDNORZ et al., 2013; YAZDANKHAH et al., 2014).

As observed in the present study, the decreased Zn fecal excretion in both evaluated periods indicated that PZnO can be an alternative to the conventional source of ZnO on post weaning diets, being able to be beneficial to reduce the environmental Zn contamination. The results are in concordance with Upadhaya et al. (2018), who showed that using lower doses of an alternative source of ZnO reduced the Zn fecal excretion when compared with the conventional source of ZnO. PZnO presents strong bioactivity at low concentrations due to their high surface area to volume ratio and unique chemical and physical properties (RAI et al., 2009). However, according to Bai et al. (2019), no alternative source of ZnO effectively suppressed the incidence of diarrhea compared with the conventional source of ZnO.

Regarding the levels of Cu in fecal excretion, Cu is a microelement that can be used as a growth promoter when added at high levels in poultry and pork feeds. The use of Cu as a growth promoter for these animals has been documented with effects similar to those of the antibiotics growth promoters (GÁTTAS & BARBOSA, et al., 2004).

In monogastric animals, about 15-30% of Cu ingested in young animals and 5-10% in adult animals are absorbed, and the duodenum is the main site of absorption (McDOWEL, 2003). However, a new issue that has been raised in the scientific environment is the concern about the environmental pollution, due to the excess of Cu present in animal feces, fed with high dietary levels of this mineral. Copper, when is eliminated in animal excretion may contaminate soil and water sources increasing the concentration of this mineral.

The interaction between the mineral zinc and copper has been documented in the last few years, where the excess of zinc reduces the copper absorption (BREWER et al., 1990). The proposed mechanism of this interaction was based on the increase of the synthesis of a protein named metallothionein, which has the function to form chelates with free minerals and then to exercise an antioxidant function in the organism. This protein has high affinity for minerals such as cadmium and copper, and less for the zinc (COUSINS, 1994).

Thereby, because of this high affinity for copper by the metallothionein, Cu stays stuck inside the enterocytes, prevented to get in the bloodstream and between two or three days it will be excreted. However, in the results of the present study, it was noticed a significant reduction on Cu fecal excretion in the diets of high ZnO supplementation (T2). It contradicts the interaction proposed by Brewer et al. (1990), because it was expected a reduction on Cu fecal excretion as while as we reduce the inclusion of ZnO or when we try a different source of ZnO.

Phosphorus is one of the most important minerals used for animal production, mainly swine, and it plays numerous roles in the organism. Moreover, its deficiency can lead to decreases in the animal performance and unbalance metabolism and other vital functions (SUTLLE, 2010). Approximately 70% of the total P in these plant ingredients is bound to phytate, and, therefore, has low digestibility by pigs (JEONG et al., 2015). Phytic acid or phytate is the primary storage form of P in most organic feedstuff. Phytate is a powerful chelator of proteins, amino acids, starch and cations in monogastric diets.

Nevertheless, P is known as an inhibitor of important enzymes such as β-amylase, trypsin, pepsin and that the digestibility as well as the availability of nutrients will be affected due to the formation of insoluble and undigestible complexes (COWIESON et al., 2016).

Therefore, inorganic P sources, such as dicalcium phosphate and monocalcium phosphate, must be added to diets for pigs to meet the requirements of digestible P. Thus, the inclusion of the *phytase* enzyme improves phosphorus digestibility originated by phytate and reduces P excretion in diets of post-weaned piglets and growing-finishing pigs (ROJAS et al., 2013; SHE et al., 2015). It is important that P supplementation is optimized during the nursery phase in order to provide bone mineral reserves for the growing-finishing phases (VARLEY et al., 2011).

The *phytase* enzyme efficacy was reduced approximately 30% when dietary ZnO was added in corn–soybean meal–based post weaning diets (AUGSPURGER et al., 2004). The authors stated that Zn exerts its effect on P availability and *phytase* efficacy through binding with the phytate molecule and preventing access for *phytase* hydrolysis. Phytate preferentially precipitates with Zn, creating an insoluble complex, and will reduce *phytase* efficacy through of promoting Zn-phytate complexes, which are inaccessible to *phytase* (CHAMPAGNE et al., 1990).

In the present study, we hypothesized that the improvement in the P utilization by increasing the *phytase* efficacy with the utilization of small doses of a PZnO with high absorption capacity, due to his “nanoparticle” size. However, it is not clear that PZnO improved the *phytase* efficacy. It was observed a less P (P = 0.0011) fecal excretion in the treatments containing PZnO and this can lead to an increase in the *phytase* efficacy. Nevertheless, it is important to evualate more parameters to draw a proper conclusion.

4.4 Bone resistance

Data relative to bone resistance are presented in Table 9. There were no significant differences (P > 0.05) between treatments on the bone resistance, length and weight of the third metacarpus of piglets.

Table 9 - Effects of dietary levels of zinc oxide and/or the use of PZnO on the bone resistance, length and weight of the third metacarpus of piglets during nursery phase.

Parameters	Treatments				CV%	*P*-value
	T1	T2	T3	T4		
Lenght, (cm)	3.026	3.017	2.955	2.949	4.91	0.7314
Weight, (g)	5.835	5.817	5.578	5.670	13.49	0.9097

Resistance, (kgf)	45.700	43.327	48.577	48.305	18.76	0.7317

T1 – Negative control; T2 – Positive control (ZnO); T3 – association zinc oxide and PZnO; T4 – PznO; CV – variation coefficient.

The literature indicates that *phytase* is an effective hydrolyzer of the ester linkage of phytate with phosphorus. In addition, it affects the concentration of phosphorus in feces and phosphorus digestibility (MAISON, LIU & STEIN, 2015; SHE et al., 2015; SOTAK PEPER et al., 2016). A diet deficient in digestible P may have some restrictions on the growth performance of weanling pigs, and potentially will lead to skeletal disease (BÜHLER et al., 2010).

Overall, there is a consensus that *phytase* can improve bone mineralization and animal performance, as well as digestibility of phosphorus and other nutrients (SHE et al., 2015). However, even observing a reduction on P fecal excretion on T4 (PZnO), possibly caused by an improvement on *phytase* efficacy due to a better utilization of the new source of ZnO or because of the 10% phosphorus reduction on the T3 and T4, no differences in the bone resistance parameters were observed.

Bone resistance has gained relevance due to recent advances in genetic improvement, which lead to higher slaughter weight. Considering the genetic growth potential of the actual animals and the tendency to keep slaughtering pigs in a high weight, the bone structure must be sufficiently good to support this upcoming practice.

5 CONCLUSION

The inclusion of the conventional source of zinc oxide improves the piglet performance during nursery phase and reduces the incidence of diarrhea. The diets containing PZnO reduces zinc and phosphorus fecal excretion during the period from day 7 to day 42. The use of the conventional source of ZnO compared with the PZnO provides the same performance results of piglets in nursery phase analyzing the period from day 1 to day 42 after weaning.

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