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Effects of a potentiated zinc oxide on growth performance, incidence of diarrhea, mineral excretion, and bone breaking strength of nursery pigs

Efeito de uma fonte potencializada de oxido de zinco no crescimento, incidência de diarreia, excreção mineral, e resistência óssea de leitões em fase de creche

Efectos de un óxido de zinc potenciado sobre el rendimiento del crecimiento, la incidencia de diarrea, la excreción de minerales y la resistencia a la rotura ósea de cerdos de destete

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Abstract

The objective of the present study was to evaluate the effects of a potentiated (PZnO) and conventional source of zinc oxide (ZnO) on growth performance, bone breaking strength, incidence of diarrhea, and fecal excretion of phosphorus (P), zinc (Zn), and copper (Cu) of nursery pigs. A total of 84 pigs weaned at 21 d of age (6.1 ± 0.9 kg) were allotted based on a completely randomized block design. The nutritional program was divided in four dietary phases with four dietary treatments: [NC negative control: no ZnO and regular P levels (0.440; 0.420; 0.400; 0.380%); PC positive control: inclusion of ZnO (3,000, 3,000, 2,200, 1,000 ppm) and regular P levels (0.440; 0.420; 0.400; 0.380%); ZnO + PZnO: association between ZnO (1,000, 1,000, 500, 0 ppm) and PZnO (500, 500, 500, 500 ppm) and 10% low P levels (0.396; 0.379; 0.360; 0.340%); PZnO: inclusion of the PZnO (500, 500, 250, 250 ppm) and 10% low P levels (0.396; 0.379; 0.360; 0.340%)]. Data were analyzed using the GLM procedure of SAS 9.4. Pigs fed diets

supplemented with PZnO increased overall ADFI ($P < 0.05$) when compared with treatment with ZnO. Pigs fed diets supplemented with PZnO reduced ($P < 0.05$) Zn excretion when compared to treatment with ZnO. The treatments with PZnO reduced ($P < 0.05$) P excretion during phase 3 when compared with treatment with ZnO. In conclusion, the use of PZnO supplemented at lower levels than the conventional source of ZnO, can be a potential alternative to reduce the environmental impact of the swine production systems by optimizing the P utilization, reducing Zn excretion, and still maintaining an optimal feed intake.
Keywords: Bone health; Fecal excretion; Newly weaned pigs; Zinc oxide.

Resumo

O objetivo do presente estudo foi avaliar os efeitos de uma fonte potencializada (PZnO) e convencional de óxido de zinco (ZnO) no desempenho de crescimento, resistência à fratura óssea, incidência de diarreia e excreção fecal de fósforo (P), zinco (Zn) e cobre (Cu) de leitões. Um total de 84 leitões desmamados aos 21 dias de idade (6,1 ± 0,9 kg) foram distribuídos com base em um delineamento de blocos inteiramente casualizado. O programa nutricional foi dividido em quatro fases com quatro tratamentos: [controle negativo NC: sem inclusão de ZnO e níveis regulares de P (0,440; 0,420; 0,400; 0,380%); Controle positivo de PC: inclusão de ZnO (3,000, 3,000, 2,200, 1,000 ppm) e níveis regulares de P (0,440; 0,420; 0,400; 0,380%); ZnO + PZnO: associação entre ZnO (1,000, 1,000, 500, 0 ppm) e PZnO (500, 500, 500, 500 ppm) e 10% níveis baixos de P (0,396; 0,379; 0,360; 0,340%); PZnO: inclusão do PZnO (500, 500, 250, 250 ppm) e 10% de níveis baixos de P (0,396; 0,379; 0,360; 0,340%)]. conteúdo mineral. Os dados foram analisados usando o procedimento GLM do SAS 9.4. Leitões alimentados com dietas suplementadas com PZnO aumentaram o consumo durante o período experimental (P < 0,05) quando comparados com o tratamento com ZnO. Leitões alimentados com dietas suplementadas com PZnO reduziram (P < 0,05) a excreção de Zn quando comparados ao tratamento com ZnO. Os tratamentos com PZnO reduziram (P < 0,05) a excreção de P durante fase 3 quando comparados com o tratamento com ZnO. Em conclusão, o uso de PZnO suplementado em níveis inferiores à fonte convencional de ZnO, pode ser uma alternativa potencial para reduzir o impacto ambiental dos sistemas de produção de suínos, otimizando a utilização de P, reduzindo a excreção de Zn e ainda mantendo um consumo de ração ideal.
Palavras-chave: Saúde óssea; Excreção fecal; Leitões desmamados; Óxido de zinco

Resumen

El objetivo del presente estudio fue evaluar los efectos de una fuente potenciada (PZnO) y convencional de óxido de zinc (ZnO) sobre el rendimiento del crecimiento, la resistencia a la rotura ósea, la incidencia de diarrea y la excreción fecal de fósforo (P), zinc (Zn), y cobre (Cu) de lechones de destete. Un total de 84 cerdos destetados a los 21 días de edad (6,1 ± 0,9 kg) se asignaron en base a un diseño de bloques completamente al azar. El programa nutricional se dividió en cuatro fases dietéticas con cuatro tratamientos dietéticos: [Control negativo NC: sin inclusión de ZnO y niveles regulares de P (0,440; 0,420; 0,400; 0,380%); Control positivo PC: inclusión de ZnO (3000, 3000, 2200, 1000 ppm) y niveles regulares de P (0,440; 0,420; 0,400; 0,380%); ZnO + PZnO: asociación entre ZnO (1000, 1000, 500, 0 ppm) y PZnO (500, 500, 500, 500 ppm) y 10% niveles bajos de P (0,396; 0,379; 0,360; 0,340%); PZnO: inclusión de PZnO (500, 500, 250, 250 ppm) y 10 % de niveles bajos de P (0,396; 0,379; 0,360; 0,340 %)]. Los datos se analizaron utilizando el procedimiento GLM de SAS 9.4. Los cerdos alimentados con dietas suplementadas con PZnO aumentaron el ADFI general (P < 0,05) en comparación con el tratamiento con ZnO. Los cerdos alimentados con dietas suplementadas con PZnO redujeron (P < 0,05) la excreción de Zn en comparación con el tratamiento con ZnO. Los tratamientos con PZnO redujeron (P < 0,05) la excreción de P durante fase 3 en comparación con el tratamiento con ZnO. En conclusión, el uso de PZnO suplementado en niveles más bajos que la fuente convencional de ZnO puede ser una alternativa potencial para reducir el impacto ambiental de los sistemas de producción porcina al optimizar la utilización de P, reducir la excreción de Zn y aún así mantener un consumo de alimento óptimo.
Palabras clave: La salud ósea; Excreción fecal; Cerdos recién destetados; Óxido de zinc.

1. Introduction

The weaning process includes a myriad of environmental and physiological changes that can result in negative impacts on the intestinal health and subsequent performance of nursery pigs (Campbell et al., 2013; Moeser et al., 2017). Antibiotic growth promoters (AGP) have been widely used as growth and health promoters by reducing the post-weaning diarrhea and promoting growth (Heo et al., 2013). However, recent increases in of human infections from antibiotic-resistant bacteria has been hypothesized to be associated with imprudent and overuse of AGP in livestock production systems (Martin et al., 2015; Liu et al., 2018). Considering the restrictions or total ban of the use of AGP around the world, zinc oxide (ZnO) have been suggested as an alternative to antibiotics as growth promoters due to its antimicrobial effects preventing the post-weaning diarrhea (de Lange et al., 2010). On the other hand, ZnO has been supplemented in nursery diets at very high levels (above

1,500 ppm) (Zhang et al., 2007), which can increase zinc excretion in the feces leading to environmental issues, because around 80% of the amount ingested ZnO is excreted in the feces (Buff et al., 2005). Even though the use of ZnO is still allowed in some countries, such as Brazil, the European Food Safety Authority (EFSA) announced the ban of the pharmacological use of ZnO in animal feeds from June of 2022 (Article 35 of directive 2001/82/EC). Thus, a potentiated source of zinc oxide (PZnO) has emerged as a potential alternative to reduce the negative effects associated with the high supplemental doses of ZnO associated with environmental issues. The PZnO contains different physicochemical properties and a high porosity that will increase the surface area resulting in a greater absorption and lower excretion in feces. Long et al. (2017), evaluated the effects of PZnO compared to the conventional source of ZnO and observed positive effects related to intestinal permeability and growth performance of nursery pigs. Therefore, it was hypothesized that PZnO supplemented at lower doses can maintain an optimal growth performance of nursery pigs by reducing the incidence of diarrhea and mineral fecal excretion. The objective of the present study was to evaluate the effects of a potentiated and conventional source of ZnO on growth performance, bone breaking strength, incidence of diarrhea, and fecal excretion of P, Zn, and Cu of nursery pigs.

2. Methodology

The experimental protocol was approved by the Institutional Animal Welfare and Ethics/Protection committee from the Universidade Federal de Minas Gerais (UFMG) – Campus Montes Claros (CEUA), MG, Brazil, under the protocol nº 296/2017.

Animals, Experimental Procedure, and Sample Collection

The study was conducted in the nursery facilities of the swine production unit at the Universidade Federal de Minas Gerais (UFMG) – Campus Montes Claros (CEUA), MG, Brazil. A total of eighty-four nursery pigs (42 barrows and 42 gilts) weaned at 21 d of age (6.1 ± 0.9 kg) were obtained from a commercial pig herd (TN70® x Traxx®). The pigs were allocated to four dietary treatments with seven replicates each and three pigs per pen based on a randomized complete block design using the initial body weight and sex as the blocks. The dietary treatments were formulated and are described as follows: NC negative control: no inclusion of ZnO and regular P levels (0.440, 0.420, 0.400, 0.380%); PC positive control: inclusion of ZnO (3,000, 3,000, 2,200, 1,000 ppm) and regular P levels (0.440, 0.420, 0.400, 0.380%); ZnO + PZnO: association between ZnO (1,000, 1,000, 500, 0 ppm) and PZnO (500, 500, 500, 500 ppm) and 10% low P levels (0.396; 0.379; 0.360; 0.340%); PZnO inclusion of the PZnO (500, 500, 250, 250 ppm) and 10% low P levels (0.396, 0.379, 0.360, 0.340%). The ZnO and PZnO sources were added volumetrically with the substitution of corn.

The nutritional program was divided into four dietary phases: pre-starter 1 (P1): d 1 to d 7; pre-starter II (P2): d 8 to d 14; starter I (P3): d 15 to d 28; and starter II (P4): d 28 to d 42. Diets were formulated to meet the nutrient requirements proposed by Rostagno et al. (2017). The nutrient composition of the experimental diets including the inclusion of P and Zn are shown from Table 1 to Table 4. The PZnO was obtained from a patented manufacturing technology (Animine, 10 rue Leon Rey-Grange, 74960 Annecy, France).

Table 1 - Experimental diets used on phase 1.

Feedstuffs, %	**Phase 1 - Pre-starter I**			
	NC	**PC**	**ZnO + PZnO**	**PZnO**
Corn yellow dent, 7,9% CP	24.22	23.92	24.16	24.26
Soybean meal, 46% CP	16.00	16.00	16.00	16.00
Soybean protein concentrate	8.00	8.00	8.00	8.00
Pre-cooked corn	10.35	10.35	10.35	10.35
Blood plasma	5.00	5.00	5.00	5.00
Bakery meal	5.00	5.00	5.00	5.00
Soybean oil	3.55	3.55	3.55	3.55
Sugar	3.50	3.50	3.50	3.50
Whey permeate	21.00	21.00	21.00	21.00
Dicalcium phosphate	1.045	1.045	0.724	0.724
Limestone	0.453	0.453	0.659	0.659
Salt	0.400	0.400	0.400	0.400
L-Lysine HCl	0.424	0.424	0.424	0.424
DL-Methionine	0.220	0.220	0.220	0.220
L-Threonine	0.197	0.197	0.197	0.197
L-Tryptophan	0.065	0.065	0.065	0.065
L-Valine	0.110	0.110	0.110	0.110
Micromineral premix[1]	0.100	0.100	0.100	0.100
Vitamin premix[2]	0.050	0.050	0.050	0.050
Phytase[3]	0.005	0.005	0.005	0.005
Organic acids blend[4]	0.200	0.200	0.200	0.200
Toxin binder[5]	0.100	0.100	0.100	0.100
Sweetener	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
Calculated values				
Metabolizable Energy, kcal/kg	3,500	3,500	3,500	3,500
Crude Protein, %	22.22	22.22	22.22	22.22
Total Calcium, %	0.70	0.70	0.70	0.70
STTD P[6], %	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 AA[7], %				
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
Analyzed values				
Zinc, mg/kg	148.5	2,304.9	1,226.2	475.3
Total 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), Niacin (120,000 mg/kg), Pantothenic 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 1,000 FTU 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]Standardized total tract digestibility of phosphorus.
[7]Standardized ileal digestible amino acids.
Source: Authors.

Table 2 - Experimental diets used on phase 2.

Feedstuffs, %	Phase 2 - Pre-starter II			
	NC	PC	ZnO + PZnO	PZnO
Corn yellow dent, 7,9% CP	32.18	31.88	32.14	32.24
Soybean meal, 46% CP	19.00	19.00	19.00	19.00
Soybean protein concentrate	5.00	5.00	5.00	5.00
Pre-cooked corn	10.00	10.00	10.00	10.00
Blood plasma	3.00	3.00	3.00	3.00
Bakery meal	5.00	5.00	5.00	5.00
Soybean oil	3.35	3.35	3.35	3.35
Sugar	3.50	3.50	3.50	3.50
Whey permeate	15.00	15.00	15.00	15.00
Dicalcium phosphate	1.187	1.187	0.885	0.885
Limestone	0.538	0.538	0.737	0.737
Salt	0.400	0.400	0.400	0.400
L-Lysine HCl	0.576	0.576	0.576	0.576
DL-Methionine	0.247	0.247	0.247	0.247
L-Threonine	0.273	0.273	0.273	0.273
L-Tryptophan	0.080	0.080	0.080	0.080
L-Valine	0.175	0.175	0.175	0.175
Micromineral premix[1]	0.100	0.100	0.100	0.100
Vitamin premix[2]	0.050	0.050	0.050	0.050
Phytase[3]	0.005	0.005	0.005	0.005
Organic acids blend[4]	0.200	0.200	0.200	0.200
Toxin binder[5]	0.100	0.100	0.100	0.100
Sweetener	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
Calculated values				
Metabolizable Energy, kcal/kg	3,450	3,450	3,450	3,450
Crude Protein, %	20.34	20.34	20.34	20.34
Total Calcium, %	0.720	0.720	0.720	0.720
STTD P[6], %	0.420	0.420	0.379	0.379
Lactose, %	10.50	10.50	10.50	10.50
Sodium, %	0.25	0.25	0.25	0.25
SID AA[7], %				
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
Analyzed values				
Zinc, mg/kg	265.8	2,555.6	1,260.4	556.1
Total 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), Niacin (120,000 mg/kg), Pantothenic 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 1,000 FTU 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]Standardized total tract digestibility of phosphorus.
[7]Standardized ileal digestible amino acids.
Source: Authors.

Table 3 - Experimental diets used on phase 3.

Feedstuffs, %	Phase 3 – Starter I			
	NC	**PC**	**ZnO + PZnO**	**PZnO**
Corn yellow dent, 7,9% CP	46.88	46.66	46.93	46.96
Soybean meal, 46% CP	25.00	25.00	25.00	25.00
Soybean protein concentrate	3.00	3.00	3.00	3.00
Pre-cooked corn	7.00	7.00	7.00	7.00
Bakery meal	5.00	5.00	5.00	5.00
Soybean oil	3.70	3.70	3.70	3.70
Sugar	2.00	2.00	2.00	2.00
Whey permeate	3.00	3.00	3.00	3.00
Dicalcium phosphate	1.556	1.563	1.269	1.269
Limestone	0.520	0.514	0.705	0.706
Salt	0.400	0.400	0.400	0.400
L-Lysine HCl	0.625	0.625	0.625	0.625
DL-Methionine	0.245	0.245	0.245	0.245
L-Threonine	0.304	0.304	0.304	0.304
L-Tryptophan	0.090	0.090	0.090	0.090
L-Valine	0.191	0.191	0.191	0.191
Micromineral premix[1]	0.100	0.100	0.100	0.100
Vitamin premix[2]	0.050	0.050	0.050	0.050
Phytase[3]	0.005	0.005	0.005	0.005
Organic acids blend[4]	0.200	0.200	0.200	0.200
Toxin binder[5]	0.100	0.100	0.100	0.100
Sweetener	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
Calculated values				
Metabolizable Energy, kcal/kg	3,400	3,400	3,400	3,400
Crude Protein, %	19.21	19.21	19.21	19.21
Total Calcium, %	0.720	0.720	0.720	0.720
STTD P[6], %	0.400	0.400	0.360	0.360
Lactose, %	2.10	2.10	2.10	2.10
Sodium, %	0.25	0.25	0.25	0.25
SID AA[7], %				
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
Analyzed values				
Zinc, mg/kg	170.66	1,766.67	485.90	208.67
Total 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), Niacin (120,000 mg/kg), Pantothenic 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 1,000 FTU 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]Standardized total tract digestibility of phosphorus.
[7]Standardized ileal digestible amino acids.
Source: Authors.

Table 4 - Experimental diets used on phase 4.

Feedstuffs, %	**Phase 4 – Starter II**			
	NC	**PC**	**ZnO + PZnO**	**PZnO**
Corn yellow dent, 7,9% CP	58.25	58.15	58.30	58.33
Soybean meal, 46% CP	28.00	28.00	28.00	28.00
Bakery meal	5.00	5.00	5.00	5.00
Soybean oil	4.50	4.50	4.50	4.50
Dicalcium phosphate	1.520	1.520	1.233	1.233
Limestone	0.593	0.593	0.780	0.780
Salt	0.400	0.400	0.400	0.400
L-Lysine HCl	0.574	0.574	0.574	0.574
DL-Methionine	0.186	0.186	0.186	0.186
L-Threonine	0.275	0.275	0.275	0.275
L-Tryptophan	0.067	0.067	0.067	0.067
L-Valine	0.145	0.145	0.145	0.145
Micromineral premix[1]	0.100	0.100	0.100	0.100
Vitamin premix[2]	0.050	0.050	0.050	0.050
Phytase[3]	0.005	0.005	0.005	0.005
Organic acids blend[4]	0.200	0.200	0.200	0.200
Toxin binder[5]	0.100	0.100	0.100	0.100
Sweetener	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
Calculated values				
Metabolizable Energy, kcal/kg	3,399	3,399	3,399	3,399
Crude Protein, %	18.32	18.32	18.32	18.32
Total Calcium, %	0.720	0.720	0.720	0.720
STTD P[6], %	0.380	0.380	0.340	0.340
Lactose, %	0	0	0	0
Sodium, %	0.25	0.25	0.25	0.25
SID AA[7], %				
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
Analyzed values				
Zinc, mg/kg	126.70	844.34	487.06	303.14
Total 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), Niacin (120,000 mg/kg), Pantothenic 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 1,000 FTU 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]Standardized total tract digestibility of phosphorus.
[7]Standardized ileal digestible amino acids.
Source: Authors.

The pigs were housed in nursery facilities with free access to feed and water. Every morning, feed refusals were

collected, and feeders were replenished with fresh feed when necessary. The temperature (maximum and minimum) and relative humidity (RH) was recorded daily using an infrared thermometer placed inside the facilities (Didai Tecnologia Ltda., Campinas, São Paulo, Brazil). The body weight (BW) and feed intake were recorded at the end of each week to calculate the average BW, average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) as indicators of growth performance.

The fecal score was assessed daily in the morning and afternoon, and recorded as previously described by Casey et al. (2007). The fecal score was recorded based on a scale of 1–5: 1, no diarrhea, hard and dry consistency; 2, no diarrhea, soft and humid consistency, considered normal; 3, no diarrhea, humid, and soft consistency; 4, pasty diarrhea; and 5, liquid diarrhea. The presence of diarrhea was considered when at least one pig housed within the pen developed pasty or watery fecal consistency (score 4 to 5). The incidence of diarrhea was calculated as the sum of daily diarrhea observations per pig over the period and then divided by the recorded diarrhea observations over the experimental period, and with the quotient multiplied by one hundred.

The fecal sampling was performed in three non-consecutive days during phases 2 (d 8, d 11, and d 14) and 3 (d 15, d 22, and d 28) of the experimental period. Approximately 40g of feces were collected from every pen in the morning the afternoon during the selected days of each phase. Fecal samples were sampled, pooled, and labelled before being frozen at a temperature of -20ºC in a horizontal freezer. Later, fecal samples collected from phase 2 and phase 3 were thawed at room temperature, oven-dried at 55°C for 72 hours and grinded to be analyzed for P, Zn and Cu contents following the technique proposed by Instituto Adolfo Lutz (2008). On day 42 of the experiment, one pig of each pen with a BW closest to the pen average weight was euthanized. Euthanasia was performed through stunning by electronarcosis (> 300 V, 1.25 A, for 6 seconds) followed by exsanguination in a commercial slaughterhouse located in Januária (Minas Gerais, Brazil) accompanied by a registered veterinarian.

The right front foot of each carcass was removed and frozen at -20°C for subsequent assessment of bone characteristics. The pads of the foot 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. The third metacarpal samples were then placed in a forced-ventilation oven at 55°C for 24 hours. A bone bending analysis was performed using the Universal Testing Machine WDW 20E (Jinan Liangong Testing Technology, Jiangsu, China) in the "Wood Resistance Laboratory" of the Department of Forest Engineering of the Universidade Federal de Lavras (UFLA, Lavras, MG, Brazil). The test was performed in a climatic chamber with controlled temperature of 17°C with a relative humidity of 65%. 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 and later converted to newtons (N).

Calculations and Statistical Analyzes

Data were analyzed using the GLM procedure of SAS 9.4. software (SAS Inc., Cary, NC, USA). The experimental unit was the pen containing three animals. The Shapiro-Wilk test was used to analyze the normality of the data. Statistical differences were considered significant with $p < 0.05$ and tendency with $0.05 \leq p < 0.10$. The least square means procedure (PDIFF option) was used to compare means when a significant F-value was obtained. Means multiple comparisons were performed according to the PDIFF option of SAS using Tukey test for contrasts. For obtaining data's homogeneity of the variance, an adjustment using the sinarc of the square root described in the equation 1 was made.

Equation 1: $\text{Sinarc}\sqrt{X}=Y$

Where: X = data collected, Y = homogenized data.

The homogenized data was analyzed using the same procedures of the data classified as normal by the Shapiro-Wilk

test.

The statistic model used was according to the equation 2.

Equation 2: $yij=\mu+\alpha i+\beta j+\varepsilon ij$

Where: μ = general average, αi = diet effect, βj = block effect and εij = incidental residual effect of observation.

3. Results

Growth Performance and Incidence of Diarrhea

The supplementation of ZnO increased ($P < 0.05$) the BW of nursery pigs at d 14, d 28 and tended to increase ($P = 0.099$) at d 42, whereas without affecting the BW at d 7 (Table 5). The supplementation of ZnO tended to increase ($P = 0.062$) the overall ADG. Furthermore, the supplementation of ZnO increased ($P < 0.05$) the ADFI on phase 2 and on phase 3. The supplementation of PZnO and PZnO combined with ZnO increased ($P < 0.05$) the overall ADFI when compared to the treatment containing the conventional source of ZnO. There were no differences on the FCR during the overall period.

Table 5 - Growth performance of nursery pigs fed diets with dietary levels of conventional ZnO and/or of PZnO.

Item	Treatments[1]				SEM	*P* value
	NC	PC	ZnO + PZnO	PZnO		
BW[2], kg						
d 1	6.18	6.05	6.17	6.12	0.36	0.814
d 7	7.34	7.38	7.31	7.11	0.37	0.429
d 14	8.89^{b}	9.74^{a}	9.00^{b}	8.88^{b}	0.49	0.008
d 28	14.93^{b}	16.20^{a}	14.99^{b}	14.80^{b}	0.68	0.003
d 42	24.15^{B}	25.73^{A}	24.76^{B}	24.35^{B}	0.81	0.099
ADG[3], g/d						
Phase 1	171	179	146	139	23	0.255
Phase 2	287	257	251	258	42	0.728
Phase 3	440	448	429	426	21	0.588
Phase 4	715	687	669	649	26	0.929
Overall	430^{B}	465^{A}	435^{B}	433^{B}	12	0.062
ADFI[4], g/d						
Phase 1	219	230	218	216	17	0.835
Phase 2	334^{B}	410^{A}	311^{B}	337^{B}	31	0.050
Phase 3	616^{b}	698^{a}	618^{b}	612^{b}	30	0.029
Phase 4	1,10	1,17	1,15	1,18	34	0.245
Overall	674^{ab}	634^{b}	716^{a}	735^{a}	17	0.002
FCR[5]						
Phase 1	1.40	1.34	1.38	1.49	0.11	0.778
Phase 2	1.50	1.47	1.42	1.42	0.37	0.994
Phase 3	1.62	1.57	1.51	1.54	0.12	0.951
Phase 4	1.69	1.72	1.73	1.83	0.07	0.841
Overall	1.57	1.55	1.60	1.59	0.05	0.496

Abbreviations: Standard error of the mean.
[1]NC: Negative control: no zinc oxide sources added, PC: Positive control: ZnO, ZnO + PZnO: association ZnO and PZnO, PZnO: supplementation of PZnO; [2]Body weight; [3]Average daily gain; [4]Average daily feed intake; [5]Feed conversion ratio.
a,b,c Within a row with different letters differ by Tukey test ($P < 0.05$).
A,B,C Within a row with different letters differ by Tukey test ($P < 0.05$).
Source: Authors.

The supplementation of ZnO reduced ($P < 0.05$) the incidence of diarrhea based on the fecal score during phase 2, phase 3 and phase 4 (Table 6).

Table 6 - Incidence of diarrhea based on the fecal score of nursery pigs fed diets with dietary levels of ZnO and/or of PZnO

Item	Treatments[1]				SEM	*P* value
	NC	PC	ZnO + PZnO	PZnO		
Incidence of diarrhea, %						
Phase 1	11.2	13.3	8.2	16.3	0.3	0.350
Phase 2	35.7[a]	14.2[b]	24.4[a]	28.5[a]	0.2	0.005
Phase 3	50.5[a]	17.3[c]	29.5[b]	37.2[b]	0.2	<0.001
Phase 4	39.7[a]	18.8[b]	27.5[ab]	39.7[a]	0.2	<0.001

Abbreviations: Standard error of the mean.
[1]NC: Negative control: no zinc oxide sources added, PC: Positive control: ZnO, ZnO + PZnO: association ZnO and PZnO, PZnO: supplementation of PZnO.
a,b,c Within a row with different letters differ by Tukey test ($P < 0.05$).
Source: Authors.

Mineral excretion

The supplementation of ZnO reduced ($P < 0.05$) the fecal excretion of Cu when compared with NC treatment during phase 2 and 3 (Table 7). The supplementation of ZnO increased ($P < 0.05$) the fecal excretion of Zn during phase 2 and 3. The supplementation of ZnO increased ($P < 0.05$) the fecal excretion of P during phase 3. The mineral fecal excretion results observed in the present study may be related to the nutrient composition of diets.

Table 7 - Mineral fecal excretion of copper, zinc and phosphorus of nursery pigs fed diets with dietary levels of ZnO and/or of PZnO.

Item	Treatments[1]				SEM	*P* value
	NC	PC	ZnO + PZnO	PZnO		
Phase 2						
Cu[3], mg/kg	140.42[a]	112.11[b]	122.83[ab]	124.49[ab]	4.80	0.003
Zn[4], mg/kg	12.88[d]	89.68[a]	60.93[b]	34.98[c]	4.84	<0.001
P[5], %	1.51	1.40	1.41	1.37	0.04	0.195
Phase 3						
Cu, mg/kg	96.78[a]	80.49[b]	98.61[a]	92.91[a]	2.81	0.003
Zn, mg/kg	7.71[c]	75.51[a]	33.69[b]	13.16[c]	1.47	<0.001
P, %	1.27[ab]	1.35[a]	1.19[bc]	1.10[c]	0.03	0.011

Abbreviations: Standard error of the mean.
[1]NC: Negative control: no zinc oxide sources added, PC: Positive control: ZnO, ZnO + PZnO: association ZnO and PZnO, PZnO: supplementation of PZnO.
a,b,c Within a row with different letters differ by Tukey test ($P < 0.05$).
Source: Authors.

Bone Parameters

The supplementation of ZnO and PZnO did not influence ($P > 0.10$) the bone parameters of nursery pigs during this study (Table 8).

Table 8 - Bone parameters of nursery pigs fed diets with dietary levels of ZnO and/or the use of PZnO;

Item	Treatments[1]				SEM	*P* value
	NC	PC	ZnO + PZnO	PZnO		
Length, cm	3.02	3.02	2.95	2.95	0.06	0.731
Weight, g	5.83	5.82	5.58	5.67	0.33	0.909
Breaking strength, N	448.16	424.82	476.31	473.66	36.77	0.731

Abbreviations: Standard error of the mean.
[1]NC: Negative control, PC: Positive control: ZnO, ZnO + PZnO: association ZnO and PZnO, PZnO: supplementation of PZnO.
a,b,c Within a row with different letters differ by Tukey test ($P < 0.05$).
Source: Authors.

4. Discussion

In this study, there were no differences on the growth performance of the pigs during the first week. Our results contrast with the studies conducted by Wang et al. (2018) and Lei and Kim, (2018), who observed that the inclusion of ZnO in nursery diets of pigs resulted in an enhanced growth performance and reduced incidence of diarrhea during the first week of nursery phase. Due to nutritional, psychological, and environmental stressors, weaning is often associated with the appearance of post-weaning diarrhea (Kim et al., 2012). However, in the present study there were no differences on the incidence of diarrhea during the first week.

The results from the first week may lead us to reflect firstly about the composition and nutrient availability of each diet, as well as the sanitary challenge and health conditions of the facilities where the animals are housed. Additionally, diets fed in the first weeks after weaning usually contain important milk by-products and lactose-rich ingredients that may smooth the negative effects of weaning during the first days in nursery phase and stimulate growth throughout their productive life (O'Shea et al., 2014). Several studies have demonstrated the benefits of high dietary lactose inclusion in nursery diets (O'Doherty et al., 2004; Pierce et al., 2005). The results of growth performance and the incidence of diarrhea from the present study supports the findings of Song et al. (2015) and Cho et al. (2015), where it was observed that high levels of ZnO (above 1,500 ppm) can reduce the incidence of diarrhea and improve the growth performance of nursery pigs.

Furthermore, Raquipo et al. (2017) performed a study evaluating the supplementation of PZnO and a coated ZnO. The authors reported an improvement on the ADG of pigs fed with coated ZnO during the first week after weaning compared to pigs fed diets without supplementation of any source of ZnO. In the present study, pigs fed with the treatment containing ZnO experienced greater overall ADG, whereas the treatments containing PZnO showed greater overall ADFI. Our findings regarding the supplementation of conventional ZnO agrees with Heo et al. (2013), who reported a reduction on the incidence of diarrhea and enhanced the growth performance in nursery pigs. Therefore, the conventional source of ZnO exerts antibacterial activity and provides a reduction in the incidence of diarrhea and negative impacts associated with the weaning (Grilli et al., 2015).

Moreover, Long et al. (2017) evaluated the effects of PZnO in nursery diets and observed an increase on growth performance of pigs fed PZnO when compared to the diets supplemented with the conventional source of ZnO. The fecal score results presented in our study are in agreement with Bai et al. (2019), which found that the conventional source of ZnO reduced the incidence of diarrhea when compared to an alternative source of ZnO. In addition, Milani et al. (2017) performed a study evaluating the effects of alternative sources of ZnO compared to the conventional source of ZnO in nursery diets, and the authors observed that dietary supplementation of alternative sources of ZnO can improve the immune response and growth performance of nursery pigs.

Based on the physicochemical properties of PZnO, it seems to be a potential alternative for reducing the high doses of

the conventional source of ZnO that are normally supplemented in nursery diets without affecting the daily feed intake of the animals. One of the hypothesized mechanisms for PZnO efficacy is through the increase of the surface area and porosity of PZnO particles that will lead to a greater absorption of Zn molecules (Zhang et al., 2007; Sirelkhatim et al., 2015; Long et al., 2017). A greater absorption of Zn can increase the hydrogen peroxide secretion, which may result in a greater antibacterial activity (Zhang et al., 2007; Sirelkhatim et al., 2015; Long et al., 2017).

Zinc is an essential mineral for most of living organisms including pigs and can play important roles in the body, such as a been a component of metalloenzymes such as DNA and RNA synthetases, transferases and many digestive enzymes (Heo et al., 2013; Jensen et al., 2018). Although the inclusion of ZnO is widely used to decrease the incidence of diarrhea and improve growth performance in pigs, more attention should be paid to the level of Zn excreted in the manure. Around 80% of the ingested ZnO is excreted as Zn in the feces of pigs due to a low absorption of this mineral (Buff et al., 2005). As a result, increased environmental concerns have arisen regarding Zn and P footprint caused by excess levels of these minerals in the feces. Zinc can become toxic to microorganisms and plants due to its accumulation in the soil which is more commonly observed in commercial swine production systems surrounding areas (Gräber et al., 2005).

Moreover, fecal excretion of Zn is also a possible environmental inducer for bacterial resistance (Hölzel et al., 2012; Bednorz et al., 2013; Yazdankhah et al., 2014). Studies regarding environmental pollution caused by animal production systems have been performed to evaluate the impact and to better understand the contamination profile (Gräber et al., 2005; Jensen et al., 2018).

In the present study, the treatment with PZnO reduced the Zn excretion during phases 2 and 3 when compared to the treatment with ZnO. This may indicate that PZnO can be supplemented as an alternative to conventional sources of ZnO in diets for nursery pigs to reduce Zn fecal excretion, especially because PZnO can be supplemented at lower doses compared with ZnO. The results of the present study partially agrees with Upadhaya et al. (2018), where the authors concluded that using lower doses of an alternative source of ZnO can reduce the Zn fecal excretion and provide comparable results of growth performance when compared with the conventional source of ZnO supplemented at high levels (above 1,500 ppm).

The increase of Cu concentration in affected areas caused by animal manure may contaminate both soil and water sources. The interaction between Zn and Cu have been well documented in recent years, where the presence of excess Zn reduces Cu absorption (Brewer et al., 1990). In monogastric animals, around 30% of the Cu ingested by young animals and around 10% ingested by adult animals are absorbed in the duodenum, which is the main site of absorption of this mineral (McDowell, 2003). The proposed mechanism of this interaction is based on the increased synthesis of a protein called metallothionein, which acts to form chelates with free minerals and possesses an antioxidant function within the organism. This protein has high affinity for minerals like cadmium and Cu, and less for the Zn (Cousins, 1994). Due to the high affinity for Cu by metallothionein, Cu remains inside the enterocytes and prevented from entry into the bloodstream, and after two to three days after ingestion Cu will be excreted. The results from the present study showed a reduction of Cu fecal excretion in the diets supplemented with ZnO. These findings contradict the interaction proposed by Brewer et al. (1990), which states an expected reduction of Cu fecal excretion with a reduction in the inclusion of ZnO or when a different source of ZnO is supplemented within the diet. Phosphorus is one of the most important and widely supplemented minerals in swine diets and plays important roles within the organism. A P deficiency can lead to reduced animal performance and physiological functions, as well as other detrimental effects (Misiura et al., 2020).

Moreover, phytase activity can be reduced when dietary ZnO was added in corn and soybean-based diets during the nursery phase (Augspurger et al., 2004). The same authors stated that excess Zn exerts an inhibitory effect on P availability and phytase activity by binding to the phytate molecule and preventing access of phytase to catalyze the hydrolysis of this molecule (Champagne et al., 1990). In the present study, it was observed a reduction of fecal P excretion in the PZnO treatment, possibly

caused by a 10% reduction in phosphorus within the ZnO + PZnO and PZnO treatments. Based on our findings, we hypothesize that an improvement of P utilization could be attained through increasing the phytase activity due to the use small doses and high absorption capacity of PZnO. However, it is unclear that the PZnO treatment improved the phytase activity, especially because there was a reduction of P levels in the treatments containing PZnO. A diet deficient in P may have some restrictions on the growth performance of nursery pigs and could potentially lead to bone health issues (Bühler et al., 2010). However, no differences in the present study were observed on the bone parameters, which can be partially associated to an improvement of phytase effects due the PZnO properties. The conventional source of ZnO showed its benefits such as enhanced growth performance and lower incidence of diarrhea compared with PZnO. On the other hand, there is a global concern associated with high doses of ZnO and environmental impacts related with Zn and P accumulation in soils and water sources nearby swine production systems. Even though PZnO did not impact growth performance, it allowed a reduction of dietary P levels without compromising the bone breaking strength of nursery pigs.

5. Conclusion

In conclusion, the use of PZnO supplemented at lower levels than the conventional source of ZnO, can be a potential alternative to reduce the environmental impact of the swine production systems by optimizing the P utilization, reducing Zn excretion, and still maintaining an optimal feed intake. The supplementation of PZnO allows a reduction of 10% of dietary P levels without compromising bone breaking strength or overall ADFI of nursery pigs. Considering future regulations towards a reduced use of conventional sources of ZnO, new feed additives and alternatives sources of ZnO should be developed and further investigated.

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References

Augspurger, N. R., J. D. Spencer, D. M. Webel, & D. H. Baker. (2004). Pharmacological zinc levels reduce the phosphorus-releasing efficacy of phytase in young pigs and chickens. *Journal of Animal Science*. 82:1732–1739. 10.2527/2004.8261732x.

Bai, M., H. Liu, K. Xu, C. Wen, R. Yu, J. Deng, & Y. Yin. (2019). Use of coated nano zinc oxide as an additive to improve the zinc excretion and intestinal morphology of growing pigs. *Journal of Animal Science*. 97:1772–1783. 10.1093/jas/skz065.

Bednorz, C., K. Oelgeschläger, B. Kinnemann, S. Hartmann, K. Neumann, R. Pieper, A. Bethe, T. Semmler, K. Tedin, P. Schierack, L. H. Wieler, & S. Guenther. (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 Microbiolpgy*. 303:396–403. 10.1016/j.ijmm.2013.06.004.

Brewer, G. J., V. Yuzbasiyan-Gurkan, & D. Y. Lee. (1990). Use of zinc-copper metabolic interactions in the treatment of Wilson's disease. *Journal of the American College of Nutrition*. 9:487–491. 10.1080/07315724.1990.10720405.

Buff, C. E., D. W. Bollinger, M. R. Ellersieck, W. A. Brommelsiek, & T. L. Veum. (2005). Comparison of growth performance and zinc absorption, retention, and excretion in weanling pigs fed diets supplemented with zinc-polysaccharide or zinc oxide. *Journal of Animal Science*. 83:2380–2386. 10.2527/2005.83102380x.

Bühler, K., A. Liesegang, B. Bucher, C. Wenk, & J. Broz. (2010). Influence of benzoic acid and phytase in low-phosphorus diets on bone characteristics in growing-finishing pigs. *Journal of Animal Science*. 88:3363–3371. 10.2527/jas.2009-1940.

Campbell, J. M., J. D. Crenshaw, & J. Polo. (2013). The biological stress of early weaned piglets. *Journal of Animal Science and Biotechnology*. 4:19. 10.1186/2049-1891-4-19.

Casey, P. G., G. E. Gardiner, G. Casey, B. Bradshaw, P. G. Lawlor, P. B. Lynch, F. C. Leonard, C. Stanton, R. P. Ross, G. F. Fitzgerald, & C. Hill. (2007). A five-strain probiotic combination reduces pathogen shedding and alleviates disease signs in pigs challenged with Salmonella enterica Serovar Typhimurium. *Applied Environment Microbiology*. 73:1858–1863. 10.1128/AEM.01840-06.

Champagne, E. T., M. S. Fisher, & O. Hinojosa. (1990). NMR and ESR studies of interactions among divalent cations, phytic acid, and N-acetyl-amino acids. *Journal of Inorganic Biochemistry*. 38:199–215. 10.1016/0162-0134(90)84013-F.

Cho, J. H., S. D. Upadhaya, & I. H. Kim. (2015). Effects of dietary supplementation of modified zinc oxide on growth performance, nutrient digestibility, blood profiles, fecal microbial shedding and fecal score in weanling pigs. *Animal Science Journal* 86:617–623. 10.1111/asj.12329.

Cousins, R. J. (1994). Metal Elements and Gene Expression. Annual Revison of Nutrition. 14:449–469. 10.1146/annurev.nu.14.070194.002313.

Gräber, I., J. F. Hansen, S. E. Olesen, J. Petersen, H. S. Østergaard, & L. Krogh. (2005). Accumulation of copper and zinc in Danish agricultural soils in intensive pig production areas. *Journal of Geography*. 105:15–22. 10.1080/00167223.2005.10649536.

Grilli, E., B. Tugnoli, F. Vitari, C. Domeneghini, M. Morlacchini, A. Piva, & A. Prandini. (2015). Low doses of microencapsulated zinc oxide improve performance and modulate the ileum architecture, inflammatory cytokines and tight junctions expression of weaned pigs. *Animal*. 9:1760–1768. 10.1017/S1751731115001329.

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. 10.1111/j.1439-0396.2012.01284.x.

Hölzel, C. S., C. Müller, K. S. Harms, S. Mikolajewski, S. Schäfer, K. Schwaiger, & J. Bauer. (2012). Heavy metals in liquid pig manure in light of bacterial antimicrobial resistance. *Environment Research*. 113:21–27. 10.1016/j.envres.2012.01.002.

Instituto Adolfo Lutz. (2008). *Métodos físico-químicos para análise de alimentos*. ANVISA, São Paulo, Brazil.

Jensen, J., N. C. Kyvsgaard, A. Battisti, & K. E. Baptiste. (2018). Environmental and public health related risk of veterinary zinc in pig production - Using Denmark as an example. *Environment International*. 10.1016/j.envint.2018.02.007.

Kim, J. C., C. F. Hansen, B. P. Mullan, & J. R. Pluske. (2012). Nutrition and pathology of weaner pigs: Nutritional strategies to support barrier function in the gastrointestinal tract. *Animal Feed Science and Technology*. 173:3–16. 10.1016/j.anifeedsci.2011.12.022.

de Lange, C. F. M., J. Pluske, J. Gong, & C. M. Nyachoti. (2010). Strategic use of feed ingredients and feed additives to stimulate gut health and development in young pigs. *Livestock Science*. 134:124–134. 10.1016/j.livsci.2010.06.117.

Lei, X. J., & I. H. Kim. (2018). Low dose of coated zinc oxide is as effective as pharmacological zinc oxide in promoting growth performance, reducing fecal scores, and improving nutrient digestibility and intestinal morphology in weaned pigs. *Animal Feed Science and Technology*. 245:117–125. 10.1016/j.anifeedsci.2018.06.011.

Liu, Y., C. D. Espinosa, J. J. Abelilla, G. A. Casas, L. V. Lagos, S. A. Lee, W. B. Kwon, J. K. Mathai, D. M. D. L. Navarro, N. W. Jaworski, & H. H. Stein. (2018). Non-antibiotic feed additives in diets for pigs: A review. *Animal Nutrition*. 10.1016/j.aninu.2018.01.007.

Long, L., J. Chen, Y. Zhang, X. Liang, H. Ni, B. Zhang, & Y. Yin. (2017). Comparison of porous and nano zinc oxide for replacing high-dose dietary regular zinc oxide in weaning piglets. Y. K. Mishra, editor. *PLoS One*. 12:e0182550. 10.1371/journal.pone.0182550.

Martin, M. J., S. E. Thottathil, & T. B. Newman. (2015). Antibiotics overuse in animal agriculture: A call to action for health care providers. *American Journal of Public Health*. 105:2409–2410. 10.2105/AJPH.2015.302870.

McDowell, L. R. (2003). Minerals in animal and human nutrition. *Elsevier Inc*. 10.1016/B978-0-444-51367-0.X5001-6.

Milani, N. C., M. Sbardella, N. Y. Ikeda, A. Arno, B. C. Mascarenhas, & V. S. Miyada. (2017). Dietary zinc oxide nanoparticles as growth promoter for weanling pigs. *Animal Feed Science and Technology*. 227:13–23. 10.1016/j.anifeedsci.2017.03.001.

Misiura, M. M., J. A. N. Filipe, C. L. Walk, & I. Kyriazakis. (2020). How do pigs deal with dietary phosphorus deficiency? *British Journal of Nutrition*. 124:256–272. 10.1017/S0007114520000975.

Moeser, A. J., C. S. Pohl, & M. Rajput. (2017). Weaning stress and gastrointestinal barrier development: Implications for lifelong gut health in pigs. *Animal Nutrition*. 3:313–321. 10.1016/j.aninu.2017.06.003.

O'Doherty, J. V., C. S. Nolan, J. J. Callan, & P. McCarthy. (2004). The interaction between lactose feed level and soya-bean meal on growth performance of weanling pigs. *Animal Science*. 78:419–427. 10.1017/S1357729800058823.

O'Shea, C. J., P. McAlpine, T. Sweeney, P. F. Varley, & J. V. O'Doherty. (2014). Effect of the interaction of seaweed extracts containing laminarin and fucoidan with zinc oxide on the growth performance, digestibility and fecal characteristics of growing piglets. *British Journal of Nutrition*. 111:798–807. 10.1017/S0007114513003280.

Pierce, K. M., J. J. Callan, P. McCarthy, & J. V. O'Doherty. (2005). Performance of weanling pigs offered low or high lactose diets supplemented with avilamycin or inulin. *Animal Science*. 80:313–318. 10.1079/ASC40900313.

Raquipo, J. M. B., F. C. C. Reyes, B. A. Moog, E. P. Angeles, S. Regaspi, Anthony Francis, & A. M. Cuizon. (2017). Performance of nursery pigs fed diets with coated or potentiated zinc oxide. *Philippine Journal of Veterinary and Animal Science*. 43:133–138.

Rostagno, H. S., L. F. T. Albino, J. L. Donzele, P. C. Gomes, R. F. de Oliveira, D. C. Lopes, A. S. Ferreira, S. L. T. Barreto, & R. Euclides. (2017). *Brazilian tables for poultry and swine: composition of feedstuffs and nutritional requirements*. 4th Edition.; UFV: Viçosa, Brazil.

Shen, J., Y. Chen, Z. Wang, A. Zhou, M. He, L. Mao, H. Zou, Q. Peng, B. Xue, L. Wang, X. Zhang, S. Wu, & Y. Lv. (2014). Coated zinc oxide improves intestinal immunity function and regulates microbiota composition in weaned piglets. *British Journal of Nutrition*. 111:2123–2134. 10.1017/S0007114514000300.

Sirelkhatim, A., S. Mahmud, A. Seeni, N. H. M. Kaus, L. C. Ann, S. K. M. Bakhori, H. Hasan, & D. Mohamad. (2015). Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. *Nano-Microbiology Letter*. 7:219–242. 10.1007/s40820-015-0040-x.

Song, Z. H., K. Xiao, Y. L. Ke, L. F. Jiao, & C. H. Hu. (2015). Zinc oxide influences mitogen-activated protein kinase and TGF-β1 signaling pathways, and enhances intestinal barrier integrity in weaned pigs. *Innate Immunology*. 21:341–348. 10.1177/1753425914536450.

Upadhaya, S. D., Y. M. Kim, K. Y. Lee, & I. H. Kim. (2018). Use of protected zinc oxide in lower doses in weaned pigs in substitution for the conventional high dose zinc oxide. *Animal Feed Science and Technology*. 240:1–10. 10.1016/j.anifeedsci.2018.03.012.

Wang, C., Ligen Zhang, Z. Ying, J. He, L. Zhou, Lili Zhang, X. Zhong, & T. Wang. (2018). Effects of dietary zinc oxide nanoparticles on growth, diarrhea, mineral deposition, intestinal morphology, and barrier of weaned piglets. *Biological Trace Elements Research.* 185:364–374. 10.1007/s12011-018-1266-5.

Yazdankhah, S., K. Rudi, & A. Bernhoft. (2014). Zinc and copper in animal feed – development of resistance and co-resistance to antimicrobial agents in bacteria of animal origin. *Microbial Ecology of Health and Disorders*. 10.3402/mehd.v25.25862.

Zhang, L., Y. Jiang, Y. Ding, M. Povey, & D. York. (2007). Investigation into the antibacterial behavior of suspensions of ZnO nanoparticles (ZnO nanofluids). *Journal Nanoparticle Research*. 9:479–489. 10.1007/s11051-006-9150-1.