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Research Article

Bone Mineral Density and Weaning Weight of Piglets from First-Parity Sows Fed Zinc during Gestation and Lactation

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Abstract

This study aimed to evaluate the effects of zinc provided to sows and their progeny on reproductive parameters, litter and nursery piglet's performance, zinc (Zn) concentration in plasma, colostrum and milk, bone densitometry, fecal score and intestinal morphometry in piglets. A total of 18 first-parity sows and their hundred and eighty weaned piglets at 21-d-old, were distributed according to a randomized block experimental design in a 3 x 3 factorial arrangement into nine treatments, consisting of the supplementation of 100 mg Zn/kg of three different sources (zinc-glycine (ZnGly), zinc amino acid complex (ZnAA) or zinc oxide (ZnO) in the maternal (gestation and lactation) and in the nursery diets. Data were analyzed by MIXED procedures of SAS, and means were compared by the test of Tukey-Kramer. Significance was assessed at $P \leq 0.05$ and trends were discussed at $P > 0.05 \leq 0.1$. Pregnant sows fed ZnO had greater total feed intake than those fed organic Zn, and lower body weight loss ($P < 0.05$). Also, piglets from sows fed ZnO had greater ADG ($P = 0.05$) compared with other treatments. Colostrum and milk Zn concentration were not affected by diet ($P > 0.05$). Plasma Zn concentration at 21d of lactation in the ZnAA sows were higher than those fed with ZnGly, but it was similar to ZnO ($P < 0.05$). At 21-d-old, ZnO sows had heavier piglets ($P < 0.05$) compared to ZnGly sows, but were similar to ZnAA sows. Also, the piglets from sows fed ZnAA had higher ($P < 0.05$) BMD than those from ZnO sows, but it was similar to sows fed ZnGly the ZnO + ZnGly piglets weaned showed 28.2% of incidence of pasty feces than those in the ZnGly + ZnAA (18.2%) and ZnGly + ZnGly (16.4%) groups, but it similar to the other groups. At 70-d-old, the lowest villus height ($P < 0.05$) was measured in the duodenum of ZnAA + ZnGly piglets compared to other treatments. The larger CD was observed when the piglets received ZnO and ZnGly ($P < 0.05$), regardless Zn source from sow. These data suggest that sows fed diets supplemented with ZnO during gestation may be able to increase total feed intake of sow and piglets weaning weight and reduce body weight loss sow. However, in the nursery pig there seems to be very little difference among zinc source on intestinal morphometry, performance and zinc plasma concentration.

Keywords: Colostrum; Diarrhea; Immunity; Nursery; Organic Trace Minerals

Introduction

Pig production has become increasingly efficient due to advances in genetic breeding, nutrition, the use of reproductive biotechnologies, among other factors. This improvement made the hyperprolific swine females be able to produce a high number of live-born piglets, and little is known about the mineral requirements of reproductive females during gestation and lactation periods.

Zinc is involved in the metabolism of proteins, carbohydrates, nucleotides and vitamin A, and it is required for calcium deposition in the bones, for immune system development, and the synthesis of hormones, insulin and adrenal corticosteroids [1]. Besides, it acts as a cofactor in several enzyme systems, and it is also a component of several enzymes [2], that are essential for cellular functions, and the deficiency of zinc iron and copper is a serious problem for infants [3,4].

The results of some researcher have shown contradictory effects, it is yet not clear whether the different Zn sources provided for sows during pregnant and lactating could help in the development from newborn piglets until wean and, whether would be interesting an additional in the diet of nursery piglets.

The delivery of an adequate supply of trace elements from the pregnant sow to the fetus is fundamental for growth and subsequently for the survival of the postnatal pig [5]. The minerals in sow milk are especially necessary for breastfeeding newborns, who can only get minerals from milk [6]. Zn accumulates in the liver of fetus from the last trimester of gestation, which functions as the primary source of nutrients for piglet [7].

Depending on its dose and chemical form, Zn can act as a nutrient, antioxidant or may even become toxic. The nutritional requirement of inorganic Zn for reproductive females is 119.7 mg/kg and organic 53.9 mg/kg [8]. Differences may occur between organic and inorganic Zn source in terms of their bioavailability [9]. Also, nursery piglets the requirement is 80 to 100 mg/kg [10,11] and 123 mg/kg [8]. Other researcher reported that a Zn concentration between 50 and 60 mg/kg feed would meet the requirements in cereal-based diets for young pigs [12]. In corn, soybean, whey-based diets 75 mg Zn per kg feed were considered to be sufficient [13].

Among the inorganic sources, ZnO is the most commonly used in animal nutrition. However, due to its low bioavailability and to the possible interactions, the inclusion of inorganic Zn concentration is usually higher than those recommended, which can result in environmental pollution [14].

In contrast, organic trace minerals showed higher absorption by the animal, as they are absorbed through the pathways of organic molecules to which they are associated [15,16].

Aim of the Study

Thus, the present study aimed to investigate the effects of zinc provided to sows and their progeny on reproductive parameters, litter and nursery piglets' performance, zinc concentration in plasma, colostrum and milk, bone densitometry, fecal score and intestinal morphometry in piglets.

Materials and Methods

The trial was performed in the Swine Research Laboratory of the School of Veterinary Medicine and Animal Science, University of Sao Paulo. The basal diets were based on corn and soybean meal. No zinc was supplemented in the basal diets. The ingredient and nutritional composition of the basal gestation, lactation and nursery diets is shown in table 1.

All the animals were supplemented with 100 mg/kg of one of three Zn sources (ZnGly, ZnAA or ZnO). The ZnGly (B-Traxim® 2C Zn, Pancosma, Geneva, Switzerland) contained 26% of Zn chelated with glycine, the Zn amino acid complex (Availa® Zn, Zinpro Corporation, Eden Prairie, MN, USA) contained 10% of Zn chelated with methionine, and the ZnO product (*Óxido* de zinco CR®, Sul *Óxidos*, Forquilhinha, Brazil) contained 80% Zn.

Ingredients1	Gestation	Lactation	Nursery		
		Pre-starter	Starter 1	Starter 2	
Corn	76	60	36.8	47.8	59.2
Soybean meal (46% CP)	20	28.4	20.5	25.0	35.0
Sugar	-	4	-	-	-
Soybean Oil	-	3.6	-	-	-
Pregnancy premix[1]	4	-			
Lactation premix[2]	-	4	-	-	-
Pre-starter premix[3]	-	-	40.0	-	-
Starter 1 premix[4]	-	-	-	25.0	-
Starter 2 premix[5]	-	-	-	-	2.5
Energy premix[6]	-	-	2.5	2.0	0.3
Flavor[7]	-	-	0.2	0.2	3.0
Calculated composition					
ME, MJ/kg	13.39	14.15	14.28	14.10	13.71
CP (%)	14.69	18.14	20.84	20.07	21.42
Crude fiber (%)	2.98	3.08	2.39	2.66	3.40
Ash (%)	5.53	5.74	5.40	5.18	5.92
Calcium (%)	0.78	0.78	0.72	0.61	0.82
Total phosphorus	0.53	0.53	0.54	0.56	0.57
Digestible lysine (%)	0.65	1	1.34	1.27	1.05
Total Lactose (%)	-	-	10.00	5.00	0
Zn (mg/kg)[8]	100	100	100	100	100

Table 1: Composition of gestation, lactation and nursery diets (%,as-fed basis).

[1]Pregnancy feed unit supplies (per kg of product) folic acid 36 mg, pantothenic acid 325 mg, biotin 2.5 mg, calcium 170 g, cobalt 4 mg, copper 375 mg, choline 12,000 mg, iron 625 mg, phosphorus 50 g, phytase 12,500 FTU, iodine 35 mg, manganese 1,000 mg, niacin 487.5 mg, selenium 11.16 mg, sodium 50 g, vitamin A 375,000 IU, vitamin B1 50 mg, vitamin B12 620 mcg, vitamin B2 96 mg, vitamin B6 50 mg, vitamin D3 93,750 IU, vitamin E 825 IU, vitamin K3 82.5 mg.

[2]Lactation feed unit supplies (per kg of product) folic acid 36 mg, pantothenic acid 325 mg, biotin 2.92 mg, calcium 175 g, cobalt 4 mg, copper 450 mg, choline 12,000 mg, iron 625 mg, phosphorus 45 g, phytase 12,500 FTU, iodine 35 mg, manganese 1,000 mg, niacin 487.5 mg, selenium 11.16 mg, sodium 45 g, vitamin A 375,000 IU, vitamin B1 50 mg, vitamin B12 620 µg, vitamin B2 96 mg, vitamin B6 50 mg, vitamin D3 93,750 IU, vitamin E 825 IU, vitamin K3 82.5 mg.

[3]Pre-starter feed unit supplies (per kg of product) folic acid 2.25 mg; fumaric acid 20 g; pantothenic acid 78 mg; biotin 1.13 mg; calcium 16 g; copper 500 mg; choline 2,900 mg; iron 225 mg; phosphorus 8,000 mg; phytase 1,250 FTU, iodine 2 mg; lysine 18 g; manganese 75 mg; methionine 7,000 mg; niacin 112 mg; selenium 1.35 mg; sodium 7,500 mg; threonine 10.20 g; tryptophan 2,300 mg; valine 9,000 mg; Vit. A 30,000 IU; Vit. B1 11 mg; Vit. B12 112 µg; Vit. B2 22 mg; Vit. B6 13 mg; Vit. D3 8,000 IU; Vit. E 335 IU; Vit. K 25 mg.

[4]Starter 1 feed unit supplies (per kg of product) Folic acid 3 mg; fumaric acid 24 g; pantothenic acid 100 mg; biotin 1.23 mg; calcium 23.50 g; copper 800 mg; choline 3,600 mg; iron 360 mg; phosphorus 8,000 mg; phytase 2,000 FTU, iodine 2 mg; lysine 18 g; manganese 75 mg; methionine 7,000 mg; niacin 112 mg; selenium 1.35 mg; sodium 7,500 mg; threonine 10.20 g; tryptophan 2,300 mg; valine 9,000 mg; Vit. A 30,000 IU; Vit. B1 11 mg; Vit. B12 112 µg; Vit. B2 22 mg; Vit. B6 13 mg; Vit. D3 8,000 IU; Vit. E 335 IU; Vit. K 25 mg.

[5]Starter 2 feed unit supplies (per kg of product) Folic acid 12 mg; pantothenic acid 320 mg; biotin 2 mg; calcium 135 g; copper 2,500 mg; choline 4,000 mg; phosphorus 3,900 mg; phytase 10,000 FTU, iodine 20 mg; lysine 34 g; manganese 1,400 mg; methionine 1,200 mg; niacin 600 mg; selenium 6 mg; sodium 3,560 mg; threonine 17 g; Vit. A 240,000 IU; Vit. B1 40 mg; Vit. B12 400 µg; Vit. B2 120 mg; Vit. B6 60 mg; Vit. D3 40,000 IU; Vit. E 900 IU; Vit. K 60 mg.

[6]Energy feed unit supplies (per kg of product) Beta-mananase 2,003.4 u; calcium 5,000 mg; phosphorus 3,000; lysine 8,400 mg; methionine 2,500 mg.

[7]Flavor feed unit supplies (per kg of product) Neoesperidine 1,200 mg; Saccharin sodic 58,2 g; vanilla aroma 25 g.

[8]Zinc100 mg de Zn/kg of diet in the ZnGly product (B-Traxim® 2C Zn, Pancosma, Geneva, Switzerland) contained 26% of Zn chelated with glycine, the ZnAA product (Availa® Zn, Zinpro, Minnesota, USA) contained 10% of Zn chelated with methionine, and the ZnO product (Óxido de zinco CR®, Sul Óxidos, Forquilhinha, Brazil) contained 80% Zn.

Gestation and lactation

Eighteen commercial hybrid gilts (C23, Agroceres PIC, Rio Claro, Brazil) were individually penned and used in a complete randomized design experiment. Six gilts per treatment received one of three Zn sources (ZnGly, ZnAA and ZnO).

After AI and during the first week of pregnancy, gilts of groups were fed twice a day, totaling 1.5 kg/d of a diet containing 14.7% CP and 13.39 MJ/kg of ME. From 7 to 80 d of pregnancy, the amount of feed was adjusted according to body condition score (BCS), it was divided into 5 classes: 1 = very thin, 2 = thin, 3 = normal, 4 = fat, and 5 = very fat. Females that showed BCS equal to or less than 2 received more feed to reach score 3.

From 80 d of pregnancy to farrowing, the females received 3.3 kg/d gestation diet. At 3 d before farrowing, gilts were fed 1.5 kg/d of their treatment feed and an additional 0.5 kg/d of wheat bran. At the first 24 h after farrowing, sows were fed 1.5 kg/sow of lactation feed containing 18.14% CP and 14.15 MJ/kg of ME, and this amount was gradually increased until 120 h. Afterward, sows were fed lactation diets until weaning to approximate *ad libitum* intake. Uneaten feed was removed and weighed daily before the morning feeding.

At the moment of AI, gilts were in fifth estrus, average body weight and back fat (136.93 ± 6.63 kg and 11.62 ± 1.70 mm), making up a homogenous sample. Gilts were checked for standing estrus by placing an intact boar into the pen for 15 min/d. Gilts were inseminated at intervals of 12 hours until the end of estrus with pooled semen.

Approximately 35d after AI, pregnancy was detected using a real-time ultrasound (Scanner 100, 5 MHz transducer, Pie Medical, Maastricht, the Netherlands). At approximately 107d of pregnancy, gilts were moved into individual crates in rooms with 8 farrowing crates. Routine procedures (teeth clipping, tail docking, ear notch-

ing, and iron injection) were conducted 2d after farrowing, and no creep feed was offered. Within 36 hours of farrowing, attempts were made via cross-fostering within treatment to adjust each litter to approximately 12 piglets per sow. The pigs were weaned at an average age of 21-d and all piglets were weaned on the same day regardless of the farrowing date.

Sows body weight was measured at 45, 65, 106 days of gestation, 1d after farrowing and 21 days of lactation. Back fat (BF) was measured by ultrasound (5 MHz transducer) at P2 position in the similar days of body weight. The blood of six sows per treatment was collected from the jugular vein at 15d before AI, 45, 65, 106 days of gestation, and 21 days of lactation for the analysis of plasma Zn concentration. Also, it was evaluated Zn concentration in colostrum (day of farrowing) and milk (21 days of lactation) by atomic absorption spectrophotometry [17].

Nursery

One hundred and eighty piglets were supplemented same Zn sources in diet during nursery period, compound a 3 × 3 treatment factorial design, totaling nine treatments with five replicates (pens) of four pigs (two males and two females) each.

The nursery basal diets (pre-starter, from 21 to 35 days; starter 1, from 36 to 49 days, and starter 2, from 50 to 70 days of age) were formulated according to the nutritional recommendations of the [11].

Piglets were weighed at 21, 35, 49 and 70 days of age. Average daily feed intake, Average daily gain, and feed gain ratio (g feed intake/g weight gain) were calculated for the periods of 21 to 35 days, 36 to 49 days and 50 to 70 days of age.

Tissue and bone collection and analyses

The blood of five piglets per treatment was collected from the cranial vena cava, at 21 and 70 days of age for the analysis of plasma Zn concentration by atomic absorption spectrophotometry [17]. Piglets were vaccinated at 28 and 42 days of age against *Streptococcus suis* according to the manufacturer's recommendations. Blood collection was performed at 42 and 70 days of age, for the measurement of vaccine antibodies using ELISA. The blood collected both for Zn serum and antibody measurements was centrifuged at 3.000 rpm for 15 min, and the serum samples were stored at -20°C in a freezer until analyses.

Four piglets per treatment were euthanized by electronarcosis, followed by bleeding, at 21 and 70 days of age, and their right metacarpal bone was collected for bone densitometry analyses. Bone mineral content (BMC) and bone mineral density (BMD) were determined using a dual-emission X-ray densitometer (DXA) (model DPX-1 ALPHA, GE Healthcare Lunar®, United Kingdom) coupled with software specially designed for small animals. Bone densitometric readings were performed by scanning and storing the radiographic images in a microcomputer to obtain BMC and BMD values [18].

The duodenum of the same piglets euthanized for bone densitometry analyses was collected to measure villus height, crypt depth, and villus height: crypt depth ratio. The methodology proposed by Pekas [19] was applied. Each collected fragment was washed and fixed in Bouin's solution. After 24 hours, samples were washed in ethyl alcohol at 70°C and afterward dehydrated in graded alcohol series. After dehydration, samples were trimmed, cleared in benzol, embedded in paraffin, and sectioned for slide assemble. Slides were stained with Harris' hematoxylin eosin. Villus height and crypt depth were measured under light microscopy using the image analyzer system at 230x magnitude.

Visual analysis of the feces was carried out daily, with scores ranging from 1 to 3 for each animal: 1 = solid feces (normal); 2 = feces softer than normal (pasty); and 3 =liquid feces (severe diarrhea).

Statistical analysis

Data collected during gestation and lactation were analyzed by ANOVA appropriate for a completely randomized design using the GLM procedures of SAS [20]. The individual sow was the experimental unit for all data. Data collected during the nursery phases were analyzed using the MIXED procedure of SAS [20] for a randomized block design. The pen of pigs served as the experimental unit for ADFI, FGR, and fecal score. For individual body weight, ADG, Zn serum concentration, bone densitometry, antibody levels and intestinal morphometry, the piglet was considered as experimental unit. Means were compared by the test of Tukey-Kramer. Significance was assessed at $P \leq 0.05$ and trends were discussed at $P > 0.05 \leq 0.1$. All results were expressed as means.

Results

Gestation and lactation

There was no effect of diet on sows' performance ($P > 0.05$). Pregnant sows fed ZnO had greater total feed intake than those fed organic Zn ($P < 0.05$), 259.96 ± 4.16, 253.86 ± 3.53 and 249.26 ± 2.33 kg, ZnO, ZnAA and ZnGly, respectively. Sows ZnAA

fed had higher body weight loss than those fed ZnO (0.46 ± 0.14, 0.24 ± 0.23 kg/d) (P < 0.05), but it was similar to ZnGly (0.44 ± 0.09 kg/d).

Litter size and body weight at birth were similar among sows supplemented with organic and inorganic zinc, although there was a tendency (P = 0.066) towards higher body weight at weaning in piglets from sows fed ZnO (6.45 kg), almost the equivalent of 1 kg more, compared with organic Zn sows, regardless source (5.63 and 5.47 kg, ZnAA and ZnGly, respectively. Also, piglets from sows fed ZnO had greater ADG (P = 0.05) than those fed organic Zn (0.24 vs 0.21 and 0.20 kg/day, ZnO vs ZnAA and ZnGly, respectively).

Colostrum and milk Zn concentration were not affected by diet (P > 0.05). Plasma Zn concentration at 21 days of lactation in the sows fed ZnAA (1.34 mg/L) were higher than those fed with ZnGly (1.00 mg/L), but it was similar to ZnO (1.22 mg/L, P < 0.05).

Nursery

There was no interaction between maternal (gestation and lactation) and nursery diets for any of the analyzed parameters, except for fecal score. The piglets in the ZnO + ZnGly group presented higher incidence of pasty feces (28.2%) than those in the ZnGly + ZnAA (18.2%) and ZnGly + ZnGly (16.4%) groups, whereas the other groups presented intermediate values (Figure 1). Higher incidence of normal feces (P < 0.05) was detected during the periods of 36 to 49 days (76.98%) and of 50 to 70 days (71.76%) compared with the period of 21 to 35 days (60%).

The sow's fed ZnO had heavier piglets at 21-d-old (P < 0.05) compared to females fed ZnGly, but were similar to ZnAA sows. At 49 days of age, there was a tendency (P = 0.068) towards higher body weight in the pigs from sow fed ZnO compared with ZnAA and ZnGly sows. No effects of Zn sources supplemented in maternal or nursery diets (P > 0.05) were observed on ADG, ADFI and FGR piglets between 21 and 70 days of age (Table 3). Although, there was a trend (P = 0.073) towards greater ADG in pigs from sow fed ZnO related to other treatments, in the period of 50 to 70 days. Besides, Also, there was a tendency (P = 0.071) towards better FGR in pigs who received ZnAA in diet compared with ZnGly and ZnO.

Plasma Zn concentration and antibody levels of 70-d-old piglets were not affected by diets (P > 0.05). At 21 days of age, piglets from sows fed ZnAA (0.12 g/cm^2) had higher (P < 0.05) BMD than those from females receiving ZnO (0.08 g/cm^2), but it was similar to sows fed ZnGly (0.09 g/cm^2). However, at 70 days, there were no difference among treatments (P > 0.05, Table 2). Also, BMC at 21 and 70 days of age were not affected by Zn diet (P > 0.05, Table 2).

The piglets from sows fed different Zn sources had similar duodenal morphometry measured in 21-d-old piglets. However, at 70 days of age, the lowest villus height (P <0.05) was measured in the duodenum of piglets of the ZnAA + ZnGly group compared to other treatments. Concerning CD, regardless of the source of zinc provided for sows, the larger CD were observed when the piglets received ZnO and ZnGly in the diets (P < 0.05). No villus height to crypt depth ratio differences were observed among treatments (P > 0.05). The results are presented in table 2.

Item	Treatment									SEM	P-value		
M	ZnO			ZnAA			ZnGly						
N	ZnO	ZnAA	ZnGly	ZnO	ZnAA	ZnGly	ZnO	ZnAA	ZnGly		M	N	M*N
Zn plasma concentration (mg/L)													
70	1.54	1.47	1.42	1.34	1.32	1.45	1.55	1.48	1.50	0.029	0.137	0.735	0.729
Bone densitometry													
BMC	1.98	1.65	1.86	1.84	1.72	1.72	2.01	1.58	1.65	0.055	0.813	0.115	0.865
BMD	0.25	0.23	0.24	0.26	0.24	0.23	0.28	0.26	0.23	0.006	0.599	0.178	0.722
Duodenal Morphometry (μm)													
VH	544.63^{a}	566.49^{a}	579.23^{a}	577.12^{a}	573.46^{a}	521.94^{b}	550.60^{a}	592.77^{a}	579.36^{a}	4.079	0.283	0.146	0.003
CD	326.00^{ab}	288.73^{b}	363.72^{a}	377.08^{a}	327.77^{b}	342.60^{ab}	349.34^{b}	318.96^{b}	408.71^{a}	3.981	0.003	<.0001	0.002
VH:CD	1.82	2.23	1.71	1.66	1.90	1.63	1.73	2.16	1.55	6.110	0.278	0.266	0.242

Table 2: Zinc plasma concentration, bone densitometry and intestinal morphometry parameters measured on 70-d-old piglets.

M - maternal diet; N- nursery diet, M*N - interaction between maternal and nursery diet Zn supplementation sources; BMC - bone mineral content (g); BMD - bone mineral density (g/cm^2); VH - villus height; CD - crypt depth; VH:CD - villus height to crypt depth ratio.

Means followed by different superscripts in the same row are statistically different by the test of Tukey-Kramer (p < 0.05).

Item	Treatment									SEM	P-value		
M	ZnO			ZnAA			ZnGly						
N	ZnO	ZnAA	ZnGly	ZnO	ZnAA	ZnGly	ZnO	ZnAA	ZnGly		M	N	M*N
BW (kg)													
21	6.53	6.57	6.50	6.34	6.29	6.29	5.72	5.69	5.65	0.110	0.007	0.979	1.000
35	9.57	9.52	9.36	9.14	9.23	9.10	8.93	8.48	8.54	0.139	0.299	0.396	0.966
49	17.13	17.05	17.26	16.85	16.54	16.45	16.30	15.85	15.69	0.220	0.068	0.824	0.978
70	33.23	31.98	31.98	32.15	32.00	31.74	32.09	30.48	30.63	0.338	0.111	0.841	0.989
ADG (Kg/d)													
21-35	0.22	0.21	0.20	0.20	0.21	0.20	0.23	0.20	0.21	0.006	0.479	0.664	0.635
36-49	0.54	0.54	0.56	0.55	0.52	0.53	0.53	0.53	0.51	0.009	0.452	0.805	0.823
50-70	0.77	0.71	0.70	0.73	0.74	0.71	0.75	0.70	0.71	0.008	0.898	0.073	0.701
21-70	0.54	0.52	0.52	0.53	0.52	0.52	0.54	0.50	0.51	0.006	0.779	0.225	0.929
ADFI (Kg/d)													
21-35	0.25	0.24	0.24	0.21	0.23	0.23	0.22	0.23	0.21	0.005	0.751	0.489	0.921
36-49	0.74	0.74	0.78	0.78	0.75	0.75	0.74	0.69	0.71	0.012	0.099	0.307	0.822
50-70	1.30	1.15	1.22	1.27	1.22	1.21	1.22	1.14	1.25	0.018	0.448	0.107	0.670
21-70	0.76	0.71	0.75	0.75	0.73	0.73	0.73	0.68	0.72	0.011	0.192	0.084	0.968
FGR													
21-35	1.15	1.15	1.19	1.09	1.12	1.17	0.98	1.21	1.06	0.031	0.286	0.680	0.463
36-49	1.44	1.38	1.46	1.42	1.43	1.43	1.41	1.31	1.40	0.010	0.175	0.130	0.750
50-70	1.70	1.61	1.75	1.74	1.65	1.72	1.63	1.64	1.77	0.021	0.729	0.071	0.188
21-70	1.55	1.48	1.57	1.57	1.52	1.60	1.52	1.48	1.57	0.016	0.378	0.170	0.760

Table 3: Growth performance of nursery pigs supplemented Zn sources.

M - maternal diet; N- nursery diet, M * L - interaction between maternal and nursery diet Zn supplementation sources; BW – body weight; ADG – average daily gain; ADFI – daily feed intake; FGR - feed gain ratio.

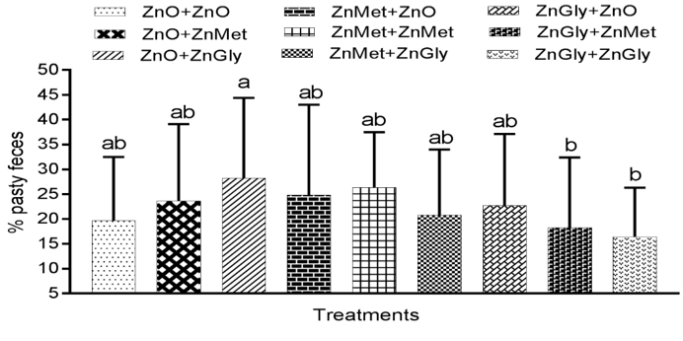

Figure 1: Effect of the supplementation of Zn sources in the maternal and nursery diets on the incidence of pasty feces.

Different superscripts indicate statistical differences by the test of Tukey-Kramer ($p < 0.05$).

Discussion

Gestation and lactation

Zinc is a component of many metalloenzymes, including DNA and RNA synthases and transferases, and many digestive enzymes, and also is associated with the hormone insulin [21]. Thus, the nutrition of sow during gestation and lactation is vital because it should provide the necessary nutrients to support the growth and development of the fetus and piglets [4]. The minerals in sow milk are especially necessary for breastfeeding newborns, who can only get minerals from milk [6].

Current recommendations for Zn in diets for sows during gestation and lactation are 100 mg/kg feed and take into account some safety margins due to possible interactions of Zn with phytate [22], calcium or other factors [11] (NRC, 2012). Besides, differences in bioavailability may occur between organic and inorganic Zn source [9].

We observed no effect of Zn provided to sow diet on sow weight, back fat. Our data agree with those of other researchers [4,23]. Although there was an increase in ADFI during gestation and lower body weight loss for sows fed ZnO, these results were dissimilar to those reported by Payne., *et al*. [23]. We have no explanations for this result. The sows were randomly allocated in treatments, and the sow was similar body weight and back fat before of AI, so it is not clear how ZnO could have increased the ADFI gestation, we believe that this higher feed intake could have helped in reduced body weight loss.

The litter size and pig birth weight also were not affected by Zn. These results support previous studies reporting no effect on litter size and pig birth weight of sows fed diets supplemented with 100 mg/kg ZnAA or ZnSO4 during gestation and lactation [23], 120 mg/kg of ZnO or 250 mg/kg ZnAA provided for sows during the last trimester of gestation [4].

On the other hand, we observed that the pig weaning weight tended to be higher sows fed ZnO, as well as ADG during lactation was higher in the piglets from sows fed ZnO. Our results were dissimilar to those observed by other authors [4,23-25] which it no found differences. Also, disagree with Grela., *et al*. [26], once these researchers reported significant differences in the body weight of piglets on days 21, with the addition of microbial phytase in 500 FTU/kg diet of sows.

Numerically, the total pigs born and born alive were higher sows fed ZnO. Similarly, Payne., *et al*., [23] observed no effect of an additional 100 mg/kg Zn from ZnAA or ZnSO4 on litter performance, and it the same result was reported by Caine., *et al*. [4], with supplementation of 250 mg/kg ZnAA. In contrast, Grela., *et al*. [26] reported a markedly higher number of liveborn with the addition of microbial phytase in 500 FTU/kg diet of sows, showing that the use of phytase increase the digestibility of some nutrients and can improve the growing pigs.

The plasma Zn concentration during gestation, colostrum, and milk was not affected by Zn, however, at 21 days of lactation higher concentrations plasma were observed in sows fed ZnO (1.22 mg/kg) and ZnAA (1.34 mg/kg). In the current study, the highest Zn concentration in colostrum was observed 17.45 mg/kg ZnGly, following by 13.15 mg/kg ZnO and 12.14 mg/kg ZnAA. These results were similar to other studies that also obtained high Zn concentration of 15.7 mg/kg [27], 16.8 mg/kg without phytase and 24.2 mg/kg with microbial phytase [26], 15.8 mg/kg in primiparous, but in sows of 8 parity was observed concentration of 18.2 mg/kg [25] (Davin., *et al*. 2015) and 16.18 mg/kg [28].

Our results for the Zn concentration in milk at 21-d-old were lower than in the colostrum (8.29, 9.91 and 9.99 mg/kg ZnGly, ZnO and ZnAA, respectively). Results similar also were observed by other researchers 6.14 mg/kg [27], 15.7 mg/kg without phytase [26], 6.22 mg/kg [25], 5 mg/kg [28], however with the supplementation of microbial phytase, this concentration was higher than in the colostrum 27.9 mg/kg Zn [26].

These our results suggest that sows fed ZnO made more efficient use of nutrient than sows fed the other sources. Zinc concentration in milk is higher than in serum suggesting that the mammary gland provides active transport and regulation mechanisms for some trace elements [25,29].

Besides, Davin., *et al*. [25] reported that the concentration of zinc in sow milk was not influenced by the concentration of Zn in sow feed. It would be difficult to increase the concentration of Zn in the sow milk by feeding sows a high Zn diet. However, the organic sources of Zn due to raising bioavailability could be better utilized by sows, without the need to supply high concentration in the diet.

Nursery

The dietary supplementation of different Zn in the maternal and nursery diets did not influence piglet performance. These results agree with some authors [23,30,31] who did not verify any effect of the supplementation of organic Zn sources (100 to 120 mg/kg) in the maternal diet on the performance of nursery piglets.

On the other hand, Case and Carlson [32] observed better performances in nursery piglets fed 500 mg/kg of a Zn-polysaccharide complex compared with ZnO (150 mg/kg) and a ZnAA (500 mg/kg). The performance improvement obtained with the supply of organic Zn sources seems to related to Zn plasma concentration higher than 2.5 mg/L [33] which is higher than that considered normal for young pigs, of 0.5-1.5 mg/L, according to Kaneko [34]. The raise Zn plasma concentration above this threshold was obtained with dietary Zn concentration higher than 1000 mg/kg [35]; however, only 100 mg/kg were fed in the present study and the Zn plasma concentration was of 1.28 mg/L at 21-d-old and 1.45 mg/L at 70-d-old, which is considered normal for young pigs.

Similar Zn plasma concentration were obtained among treatments, demonstrating the absence of influence of Zn sources supplemented in the maternal and nursery diets, respectively. Metzler-Zebeli., *et al*. [30] supplemented sow lactation diets with 240 mg/kg of a ZnAA or 120 mg/kg ZnO and did not detect any differences in piglet Zn plasma concentration at birth (0.57 mg/L) or at weaning (1.05 mg/L). However, Caine., *et al*. [4] found higher Zn plasma concentration at birth and weaning (0.63 and 1.51 mg/L, respectively) in the progeny of sows fed during the last trimester of gestation with 250 mg/kg of a ZnAA compared with ZnO. Wang., *et al*. [31] reported higher Zn plasma concentration in weaned piglets supplemented with 100 mg of ZnGly (0.905 mg/L) per kg of diet compared with those not supplemented with Zn; however, the highest concentration was determined when the diet was supplemented with 3.000 mg of ZnO (1.230 mg/L) per kg of diet.

High Zn plasma concentration is commonly associated with increased synthesis of the protein metallothionein in the intestinal mucosa, which is vital for the uptake and storage of copper [35]. Although Zn plasma concentration was not different among treatments in the present study, at 21 days of age, the piglets from sows fed ZnAA showed higher bone mineral density ($P < 0.05$) compared with those fed ZnO, but not during the nursery period. Payne., *et al*. [23] reported that piglets fed 100 mg/kg ZnAA presented higher bone Zn concentration in compared with those fed 100 mg/kg ZnSO4 ($P < 0.05$), but differences in bone strength.

It is established that trace minerals (Zn, Fe, Cu, Mn, Se) are essential for the maintenance of the immune status and resistance to diseases [36]. However, the Zn sources evaluated in the current study did not influence piglet immune status, as demonstrated by the lack of differences in antibody levels among treatments. Spears., *et al*. [37] did not find any immune response differences among piglets fed 50 or 150 mg/kg of ZnSO4 or zinc proteinate. The current Zn recommendations for nursery pigs are 80 - 100 mg Zn/kg diet [10,11] and 123 mg Zn/kg diet [8]. According to van Heugten., *et al*. [38], these concentrations supply the requirements for optimal performance and immune response, and the supplementation of inorganic sources by organic ones are not able to improve these characteristics. In contrast, Caine., *et al*. [39] reported that the progeny of sows fed a ZnAA from 80 days of gestation until farrowing presented better immune function within the first 24 hours after weaning, at 14 days of age.

The incidence of fecal score 2 (pasty feces) was influenced by Zn source supplementation both in the maternal and nursery diets ($P < 0.05$). Piglets ZnO + ZnGly group showed 28.2% the incidence of soft stools. The mechanisms by which Zn controls diarrhea are still not fully elucidated. According to Carlson., *et al*. [33], the beneficial effect of pharmacological doses of Zn is due to an increase in metallothionein levels (the protein that regulates the absorption of minerals in the intestinal mucosa), protein synthesis and cell proliferation, improving intestinal health status. Besides, Zn may inhibit the active transport of succinate into Escherichia coli cells, the activity of its oxidase system and respiratory chain, or even prevent its adhesion to the intestinal mucosa [40]; however, in the present study was used in according Zn recommendations for nursery.

The duodenal morphometry of 21-d-old piglets was not influenced by Zn sources supplemented in the maternal diets. However, at 70-d-old, lower villus height was measured in the duodenum piglets of the ZnAA + ZnGly group, and lower crypt depth were measured in the ZnO + ZnAA, ZnGly + ZnAA, ZnAA + ZnAA, and ZnGly + ZnO groups. According to Nabuurs., *et al*. [41], villus height reaches the lowest values between 3 and 4 days after weaning and recover pre-weaning values between days 11 and 14 after weaning. The effects of post-weaning anorexia on digestive enzyme secretion and on the morphometry of the intestinal epithelium are multifactorial and include deprivation of luminal substrates for enterocyte growth and reduction of the expression of digestive organ growth factors, such as glucagon-like peptide 2, insulin-like growth factor I (IGF-I) [42], and cholecystokinin [43]. As a result, nutrient digestibility and absorption are impaired. Payne., *et al*. [23] reported that different dietary Zn sources (100 mg/kg $ZnSO_4$+100 mg/kg ZnAA) did not influence the duodenal morphometry of weaning piglets. Caine., *et al*. [4] also did not find any effect of the supplementation of 250 mg/kg of ZnAA sow diet on piglet duodenal villus height, crypt depth, or villus height to crypt depth ratio at weaning (14 days of age). Moreover, according to Metzler-Zebeli., *et al*. [30], the intestinal morphology of weaned piglets was not affected by the supplementation of 240 mg/kg of a ZnAA) in the diet of sows during lactation.

Conclusion

These data suggest that sows fed diets supplemented with 100 mg/kg ZnO during gestation may be able to increase total feed in-

take of sow and piglets weaning weight and reduce body weight loss sow. However, in the nursery pig there seems to be very little difference among zinc source on intestinal morphometry, performance and zinc plasma concentration.

Conflict of Interest

We certify that there is no financial interest nor conflict of interest.

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