Application of ZnO-Functionalised-Sepiolite in weaning piglet diets

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ABSTRACT

The objective was to evaluate the effect of ZnO-Functionalised-sepiolite (ZnO-Sepiolite) to fulfil Zn requirements and health status of weaning piglets. Pre-starter Basal Diet (BD, corn-soybean based, from weaning till 14 days on trial) was calculated to provide 27 mg Zn/kg feed from raw materials and had no added ZnO and no antibiotics or organic acids. Treatments during pre-starter period were: 1) BD + 90% of NRC Zn requirements completed with ZnO (ZnO90); 2) BD + 90% of NRC Zn requirements completed with ZnO-Sepiolite (ZnOS90); 3) BD + 3000 mg ZnO /kg of diet (ZnO3000); 4) BD + 150 mg added Zn/kg diet from ZnO-Sepiolite (ZnOS150). The starter feed (corn-soybean based, from 14 till 31 days on trial) was common for all piglets, and met 90% NRC Zn requirements by adding ZnO. Diarrhea affected more than 50% of the animals of ZnO90, ZnOS90 and ZnOS150, and 33% of the ZnO3000 animals. Animals from ZnOS90 tended ($P<0.10$) to improve Gain to Feed ratio (G:F) compared to animals from ZnO90 (0.830 kg/kg vs. 0.811 kg/kg for G:F). Performance of animals from ZnO3000 was not significantly different from the other treatments, and numerically similar to animals from ZnOS90. The inclusion of ZnO at 3000 mg/kg of feed in the pre-starter period numerically decreased P in serum at the end of this period,

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with no effect on Ca level; normal levels were restored after 2 weeks of feeding the same levels of Zn than other animals. Animals fed ZnO-Sepiolite diets had numerically higher serum Ca than ZnO90 and ZnO3000 at 12 days and higher than ZnO90 at 28 days. Serum Zn levels were significantly higher for ZnO3000 than the other treatments.

INTRODUCTION

Zinc oxide is fed at pharmacological levels to weaning piglets to decrease diarrhea incidence and increase performance (Hahn and Baker, 1993; Hill *et al.*, 2001). Zinc has been described to keep the integrity of the intestinal mucosa at weaning, favouring villi development and nutrient absorption. Li *et al.* (2006) detected increased villi height when ZnO was supplemented at 3000 mg Zn/kg. ZnO also modifies the intestinal microflora. Weaning piglets fed 2500 mg/kg ZnO kept the stability of the intestinal microflora and the diversity of coliforms for the first two weeks post weaning (Katouli *et al.*, 1999) increasing resistance to pathogen colonization (Owusu-Asiedu *et al.*, 2003). According to Højberg *et al.* (2005) the influence of ZnO at 2500 mg Zn/kg on the intestinal microflora could be similar to the mechanism of action of antibiotic growth promoters, suppressing gram + commensal bacteria and consequently reducing the fermentation of digestible nutrients in the proximal part of the gastrointestinal tract rendering more energy for the host.

However, the increasing concern about zinc content of slurry led to search for alternatives. Organic sources, like Zn methioninate or Zn polysaccharide products have been studied with variable efficacy in piglets (Hollis *et al.*, 2005; Buff *et al.*, 2005).

Sepiolite ($\text{Mg}_8\text{Si}_{12}\text{O}_{30}(\text{OH})_4(\text{H}_2\text{O})_4 \cdot 8\text{H}_2\text{O}$) is a clay mineral of the phyllosilicates group with a characteristic chain-like structure that confers capillary arrangement and special physical-chemical properties such as high porosity and surface area. Furthermore

sepiolite has been used to improve nutrient utilization in pigs (Parisini *et al.*, 1999) and decrease the incidence and severity of diarrhea in weaning piglets (Sardi *et al.*, 2004). Zinc oxide can be deposited onto sepiolite's surface; the resulting compound (functionalized sepiolite) has novel properties as antibacterial activities (Jones *et al.*, 2008). The effect of ZnO functionalized with sepiolite on piglets' performance and health has not been studied yet. Sepiolite was functionalized with ZnO by a controlled precipitation method (Pecharromán *et al.*, 2006; Esteban-Cubillo *et al.*, 2008) to obtain a surface effect of ZnO by its homogeneous dispersion onto sepiolite. The hypotheses were that ZnO-Functionalized-sepiolite (ZnO-Sepiolite) could fulfill the Zn nutrient requirements of piglets at weaning at lower Zn levels than ZnO, and could also exert the same health benefits than pharmacological levels of ZnO at much lower Zn level.

MATERIAL AND METHODS

Animals and housing

The experiments followed the EU principals for animal care and experimentation, and procedures used were approved by the Ethical Committee for Animal Care and Experimentation. The trial was performed with 144 entire male piglets (*Duroc x Landrace*), weaned at 21 days (6.1 kg average Body Weight -BW).

Experimental design

The experiment was set as completely randomized design, blocked by piglets' initial BW. Each treatment was assigned to 6 pens with 6 piglets each. Feeds were corn-soybean based and formulated according to animal requirements except for Zn requirements, in two periods (pre-starter -Basal Diet, BD- from weaning till 14 days on trial, 3375 kcal/kg ME; 1.50% lysine; and starter from 14 till 31 days on trial, 3300 kcal/kg ME; 1.25% lysine); no antibiotics or organic acids were included in the feeds. Treatments during pre-starter period were: 1) BD + 90% of NRC Zn

requirements completed with ZnO (ZnO90); 2) BD + 90% of NRC Zn requirements completed with ZnO-Sepiolite (ZnOS90); 3) BD + 3000 mg ZnO /kg of diet (ZnO3000); 4) BD + 150 mg added Zn/kg diet from ZnO-Sepiolite (ZnOS150). The starter feed was common for all piglets, and met 90% NRC Zn requirements by adding ZnO.

Pigs were weighed at the start of the trial (weaning), on day 14 and 31. Average daily gain (ADG), average daily feed intake (ADFI) and gain to feed ratio (G:F) were calculated for each period and for the overall experiment. Appearance of diarrhea was individually controlled and recorded, animals were not medicated against diarrhea. On day 12 and 28, individual blood samples were collected from the anterior vena cava of two pigs per pen (12 pigs per treatment) for atomic absorption spectrophotometry analysis of Ca, P and Zn serum content.

Statistical Analysis

Data were analyzed according the design of the experiment. One pen was considered the experimental unit for performance variables while one pig was considered the experimental unit for serum Ca, P and Zn content. Analysis was performed using the following procedures of SAS System for Windows V9.1.3 (SAS, 2003): GLM for performance variables, NPAR1WAY for number of dead animals or animals with diarrhea, MIXED for serum analysis. Level of significance was set at $P=0.05$.

RESULTS AND DISCUSSION

Results of performance and diarrhea appearance are presented in Table 1. During the pre-starter period diarrhea affected approximately half of the animals of Treatments ZnO90, ZnOS90 and ZnOS150, and less than one third of the animals of ZnO3000. Similar results were obtained by Hahn and Baker (1993) and Hill *et al.* (2001). However all the animals gained weight during this period and no animal had to be taken

off trial due to diarrhoea. Animals from ZnOS90 significantly improved G:F compared to animals from ZnO90 (0.825 vs. 0.742); ADG was also improved by 6%, although differences did not reach significance. Animals from ZnO3000 significantly improved G:F compared to animals from ZnO90, and ADG was numerically improved by 17%. Incidence of diarrhea was lower in the starter than in the pre-starter period for all treatments except for ZnO3000 which presented the same incidence in both periods. In the starter period animals from ZnO3000 had similar ADG to animals from ZnO90, but G:F was significantly impaired compared to ZnO90 and ZnOS150 (0.818 vs. 0.847 vs. 0.847).

For the whole trial period diarrhea affected more than half of the animals of Treatments ZnO90, ZnOS90 and ZnOS150, while only about one third of the animals from ZnO3000. Animals from ZnOS90 numerically improved G:F compared to animals from ZnO90 (0.830 vs. 0.811). Animals from ZnO3000 numerically improved G:F and ADG compared to animals from ZnO90 (by 2% and 6% respectively). Performance of animals from ZnOS150 was not significantly different from the other treatments, and numerically close to animals from ZnOS90.

The inclusion of ZnO at 3000 mg/kg of feed in the pre-starter period numerically decreased the Phosphorus in serum at the end of this period, with no effect on Ca level, and significantly increased the ratio Ca:P (Table 2). This effect has been shown in previous trials and in some cases might imply severe P deficiencies and impaired performance as found by Lizardo *et al.* (2004). Levels of P were restored after 2 weeks of feeding the same levels of Zn than other animals. Serum calcium was numerically higher at 28 days for ZnOS90 compared to ZnO90 animals; however serum Ca level was intermediate for ZnOS150 animals. Zinc levels were significantly higher for ZnO3000 than the other treatments, both at 12 and 28 days; differences were more

extreme at 12 days though. Zinc was restricted in feed to 90% of NRC requirement for a theoretical ADFI of 500 g in the pre-starter period, but actual ADFI was much lower (308 g as global average) and consequently actual Zn ingestion was approximately 40% of NRC requirement. However no differences in serum Zn were detected among ZnO90, ZnOS90 and ZnOS150 animals. Levels of Zn in serum were in accordance with results obtained by Hill *et al.* (2001) with animals fed pharmacological and 118% NRC requirement levels.

Feeding 90% NRC Zn requirements as ZnO-Functionalised Sepiolite instead of ZnO significantly improved G:F in the pre-starter period, and numerically improved G:F in the overall experiment. Addition of ZnO at 3000 mg/kg improved ADG in the pre-starter period, but impaired G:F in the starter period compared to ZnOS150. Therefore these results might suggest that animals' Zn requirement was already met in the conditions of the experiment (actually 40% NRC requirement), and the objective to compare Zn efficacy between ZnO90 and ZnOS90 could not be assessed; further research is warranted on ZnO-Functionalised-Sepiolite to assess its effect on a longer term period.

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Table 1. Summary results of performance variables (n=6 replicates (pens) with 6 piglets each per treatment)¹

	BW ² (kg)	ADG ² (g)	ADFI ² (kg/kg)	G:F ²	diarrhea ³
Pre-starter period (from weaning till 14 days)					
ZnO90	9.41 ±0.443	235 ±14.5	315 ±14.8	0.742 ±0.0140 ^B	19
ZnOS90	9.61 ±0.594	249 ±25.3	299 ±23.2	0.825 ±0.0288 ^A	23
ZnO3000	9.98 ±0.366	274 ±11.3	337 ±13.7	0.814 ±0.0170 ^A	10
ZnOS150	9.25 ±0.567	222 ±24.9	282 ±25.5	0.780 ±0.0250 ^{AB}	18
P value	0.203	0.207	0.137	0.067	χ^2 *
Starter period (from 14 to 31 days)					
ZnO90	16.56 ±0.714	419 ±16.2	496 ±23.3	0.847 ±0.0083 ^A	6
ZnOS90	16.19 ±1.043	387 ±28.2	466 ±37.2	0.834 ±0.0081 ^{AB}	10
ZnO3000	17.15 ±0.432	421 ±11.0	515 ±12.6	0.818 ±0.0094 ^B	10
ZnOS150	16.28 ±1.051	411 ±28.3	488 ±39.7	0.847 ±0.0116 ^A	13
P value	0.547	0.476	0.516	0.084	χ^2 0.326
Whole trial					
ZnO90	16.56 ±0.714	336 ±15.1	414 ±19.4	0.811 ±0.0037	20
ZnOS90	16.19 ±1.043	325 ±26.3	391 ±30.3	0.830 ±0.0066	25
ZnO3000	17.15 ±0.432	355 ±5.5	435 ±9.4	0.817 ±0.0078	14
ZnOS150	16.28 ±1.051	326 ±26.3	395 ±33.1	0.826 ±0.0096	22
P value	0.547	0.519	0.363	0.176	χ^2 0.064

¹ No antibiotics were included in the feeds. Treatments during the pre-starter period were: 1) Basal diet (BD) with 90% NRC Zn requirement completed with ZnO (ZnO90); 2) BD with 90% NRC Zn requirement completed with ZnO-Sepiolite (ZnOS90); 3) BD with ZnO added at 3000 mg/kg; (ZnO3000); and 4) BD with ZnO-Sepiolite to provide 150 mg Zn/kg (ZnOS150). A common feed was provided during the starter period that met 90% NRC Zn requirement completed with ZnO.

² BW = Body weight at the end of period; ADG = Average Daily Gain; ADFI = Average Daily Feed Intake; G:F = Gain to Feed ratio

³ Number of different animals that presented diarrhea on the scoring days during the trial (10 days from the pre-starter period; 6 days from the starter period) (Kruskal-Wallis test)

0.01 < * ≤ 0.05

^{ab} values in the same column with uncommon superscripts differ (P ≤ 0.05)

^{AB} values in the same column with uncommon superscripts differ (P ≤ 0.10)

Table 2.Summary results of Ca, P and Zn serum content (n=12 replicates (animals) per treatment)¹

	Ca (mmol/L)	P (mmol/L)	Ca:P	Zn (µg/dL)
day 12				
ZnO90	1.47 ±0.082	1.94 ±0.121	0.77 ±0.022 ^b	71.9 ±3.00 ^b
ZnOS90	1.63 ±0.132	1.96 ±0.153	0.83 ±0.029 ^b	84.4 ±7.02 ^b
ZnO3000	1.47 ±0.039	1.57 ±0.061	0.95 ±0.029 ^a	170.7 ±12.52 ^a
ZnOS150	1.65 ±0.085	2.10 ±0.126	0.79 ±0.019 ^b	81.4 ±5.18 ^b
day 28				
ZnO90	1.69 ±0.049	2.06 ±0.053	0.82 ±0.018 ^{AB}	60.4 ±3.73 ^b
ZnOS90	1.87 ±0.075	2.17 ±0.134	0.89 ±0.047 ^A	60.9 ±4.22 ^b
ZnO3000	1.82 ±0.060	2.23 ±0.061	0.82 ±0.026 ^{AB}	84.4 ±6.42 ^a
ZnOS150	1.75 ±0.077	2.17 ±0.072	0.80 ±0.014 ^B	65.3 ±3.76 ^b
Treatment P value	0.217	0.199	*	***
day P value	***	***	0.482	***
interaction P value	0.420	*	***	***

¹ No antibiotics were included in the feeds. Treatments during the pre-starter period were: 1) Basal diet (BD) with 90% NRC Zn requirement completed with ZnO (ZnO90); 2) BD with 90% NRC Zn requirement completed with ZnO-Sepiolite (ZnOS90); 3) BD with ZnO added at 3000 mg/kg; (ZnO3000); and 4) BD with ZnO-Sepiolite to provide 150 mg Zn/kg (ZnOS150). A common feed was provided during the starter period that met 90% NRC Zn requirement completed with ZnO.

0.01 < * ≤ 0.05; *** ≤ 0.001

^{ab} values in the same column within day with uncommon superscripts differ (P ≤ 0.05)

^{AB} values in the same column within day with uncommon superscripts differ (P ≤ 0.10)

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