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Zinc oxide in phytase-low phosphorus diets impairs performance of weanling pigs

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Abstract
The simultaneous utilisation of zinc oxide (ZnO) at a pharmacological dose of 3000ppm and of low phosphorus phytase supplemented diets for piglets was studied. One hundred and twelve piglets were housed in 28 pens during 5 weeks. Four experimental treatments were used, consisting of: a positive control diet with normal P content and with ZnO (T1); a low P diet supplemented with phytase and ZnO (T2); a low P phytase supplemented diet without ZnO (T3); and a low P diet with phytase and ZnO but without the mineral and vitamin premix (T4). There were significant differences between treatments for feed intake (437, 274, 443 and 274 g/d; P<0.001), weight gain (344, 175, 346 and 198 g/d; P<0.001) and feed conversion ratio (1.28, 1.58, 1.28 and 1.40 kg/kg; P<0.001). For all the parameters T1 and T3 were significantly better than T2 and T4. The low P diets resulted in lower serum P concentrations at day 28 and this was further reduced in combination with ZnO (8.0, 3.1, 5.1 and 3.1 mg/100ml; P<0.001). It can be concluded that the use of pharmacological doses of ZnO in low P diets is dangerous for the P homeostasis and that it may affect performance. Although phytase is useful to reduce the dietary P concentration, attention must be paid to possible interactions with other nutrients present in the feed.

Introduction

Plant-based diets fed to livestock generally contain substantial quantities of P in form of phytic acid. The digestion and utilization of dietary Phytate-P by monogastric animals requires its hydrolysis by phytases. Even though some plants contain an intrinsic phytase activity that may contribute to the dietary phytate-P utilization, the results observed in the field with growing pigs are very variable (Kemme et al., 1998). In recent years, the dietary supplementation with microbial phytase has become a common practice to improve phytate-P digestibility, to reduce the need for the dietary supply of inorganic P, and consequently, to reduce the excretion of P in slurry. In weanling pigs, phytase supplementation increased P retention by 50% and decreased fecal P by 42% (Lei et al., 1993). However, intrinsic or microbial phytase does not hydrolyse dietary phytate completely and a substantial portion of phytate-P remains undigested even at high levels of phytase in the diet. The mineral content of the diet is known to influence the phytate-P digestibility. For example increasing dietary Ca levels has been shown to decrease phytate-P digestibility in pigs (Sandberg et al., 1993), to reduce plasma P concentration and apparent P absorption (Lantzsch et al., 1995) and to impair performance (Lei et al., 1993). On the other hand, phytic acid can readily form chelates with divalent minerals, such as Ca, Fe, Zn and Mg (Morris, 1986) and reduce mineral bioavailability.

In the United States, zinc is routinely fed at pharmacological doses (1.5 to 3.0 $g.kg^{-1}$) to newly weaned piglets for the possible alleviation or prevention of diarrhea (Poulsen, 1995) and for growth promotion (Hahn and Baker, 1993; Case and Carlson, 2002). However, zinc utilization, as feed additive in EU countries, is limited to 150 $mg.kg^{-1}$ (Comission Regulation Nº1334/2003) to reduce harmful effects caused by Zn excretion on the environment. Even so, some EU countries have adopted the strategy to temporarily authorize the pharmacological utilization of Zn to prevent piglet diarrhea during the first two-weeks after weaning.

In vitro data has shown that Zn is a potent inhibitor of phytase-P hydrolysis by phytases, which was due in part to Zn binding causing a conformational change in the phytate moiety, thereby rendering it less accessible to phytase (Maenz et al., 1999). Additionally, it appeared that one Zn ion might bridge two phytate molecules over time and the complex formed is quite stable and precipitate out of solution at low molar ratios (Champagne and Fisher, 1990). If the same

reactions occurred in vivo, the efficacy of microbial phytase may be negatively affected in diets supplemented with pharmacological doses of zinc.
Due to the increasing use of microbial phytases in diets for swine and the risk of their interactions with minerals, our objective was to study the use of phytase alone or in combination with a pharmacological dose of zinc oxide (ZnO) on piglet growth performance after weaning.

Material and Methods

Four experimental treatments corresponding to 4 different diets were used in the experiment (table 1): treatment 1 corresponded to a control diet, containing normal inorganic P supplementation and a pharmacological dose of ZnO (3000ppm); treatment 2 corresponded to an alternative diet containing a very low level of inorganic P, supplemented with microbial phytase and ZnO; treatment 3 was similar to T2 but without supplementation of ZnO; and finally, treatment 4 was similar to T2 but without the supplementation of the vitamin-mineral premix. Diets were formulated to contain 4.3 $g.kg^{-1}$ of digestible P in diet T1 (without phytase supplementation), and 2.8 $g.kg^{-1}$ for phytase supplemented diets. All diets contained 13.8 $MJ.kg^{-1}$ ME and 12.4 $g.kg^{-1}$ of digestible lysine and were medicated. Feed was pelleted at low temperature to avoid the destruction of the microbial phytase and offered *ad libitum* during all the experimental period.
One hundred and twelve Duroc piglets, weaned at two weeks of age according to the medicated early weaning principle and weighing 4.56 ± 0.69 kg bodyweight (BW) were used. Piglets were allocated by groups of 4 animals in 7 blocks according to their BW and penned in a nursery room. The experiment lasted 33 days during which the room temperature was set to gradually decrease from 32 to 24ºC. Piglets were controlled for growth performance (ADWG), feed intake (ADFI), and feed conversion ratio (FCR) at days 0, 14 and 33 after weaning. Eight piglets at the start, and 28 at the end (7 per treatment) were bled and their Ca and P serum concentrations were analysed. Data were analysed as a randomised complete block design using the GLM procedure of SAS? .

Table 1. Composition and chemical analysis of experimental diets ($g.kg^{-1}$, as fed).

Treatments	**T1**	**T2**	**T3**	**T4**
Phosphorus level	**normal**	**low**	**low**	**low**
Phytase	**-**	**+**	**+**	**+**
Zinc	**+**	**+**	**-**	**+**
Vit-min premix	**+**	**+**	**+**	**-**
Ingredients				
Cereals	547.0	477.9	477.9	477.9
Cereal by-products	70.0	150.0	150.0	150.0
Soya by-products	210.0	175.0	175.0	175.0
Other protein meals	10.0	34.6	34.6	34.6
Sweet whey	80.0	80.0	80.0	80.0
Lard	23.5	26.0	26.0	26.0
Synthetic aminoacids (Lys, Met, Trp, Trh)	12.3	10.9	10.9	10.9
Acidifiers (Ca formiate, Citric Ac.)	3.0	23.0	23.0	23.0
Other (Salt, sepiolite, sweetener)	92.9	9.9	10.2	10.3
Dicalcium phosphate	22.1	2.8	2.8	2.8
Calcium carbonate	6.1	3.7	3.7	3.7
Vit-min premix	**4.0**	**4.0**	**4.0**	**-**
Microbial phytase	**-**	**0.1**	**0.1**	**0.1**
Zinc oxyde 80%	**3.0**	**3.0**	**-**	**3.0**
Chemical analysis				
Energy ($MJ\ ME.kg^{-1}$)	13.78	13.82	13.82	13.82
Crude protein	184.0	183.3	185.2	186.1
Lysine	14.5	14.6	14.1	14.0
Calcium	10.25	7.45	7.10	7.15
Phosphorus	7.45	4.25	4.20	4.30
Zinc	2.47	2.75	0.27	2.55
Phytase activity ($IU.kg^{-1}$)[a]	122	403	539	403

[a] After pelleting; the corresponded values of mash feed were: 125, 434, 541 and 433, respectively.

Results and Discussion

Feed analysis showed that gross energy, crude protein and lysine contents were similar between diets. As expected, the Ca and P contents of diet T1 were higher than that in the low P diets, and these were relatively identical between them. Furthermore, Zn concentration ranged between 2.5 - 2.7 $g.kg^{-1}$ in diets T1, T2 and T4, and was 10-times lower in diet T3. The activity of the endogenous phytase observed in diet T1 was 122 $IU.kg^{-1}$ and it was lower than that of the diets with added microbial phytase. The values of phytase activity before and after pelleting are practically identical, indicating that the microbial phytase was not damaged during pelleting (at low temperature).

Despite an identical bodyweight at the start of the experiment, piglet growth performance (ADWG) after weaning was significantly affected by the experimental treatments (table 2). During the first 14-d period, the piglets of treatment T3 grew faster than all the other treatments, and those of T1grew faster than those of T2 and T4 (P<0.001). Feed intake (ADFI) and feed conversion ratio (FCR) were also affected in a similar way, with T3 and T1 being superiors than T2 and T4. So, piglets of T3 achieved an intermediary bodyweight slightly higher than that of treatment T1. Compared to T2 and T4, the piglets of T3 were practically 1 kg heavier. During the second 19-d period, ADWG and ADFI were similar for T3 and T1 and these practically doubled that of treatments T2 and T4. Weight gain of T4 piglets was also higher than that of treatment T2. As a consequence FCR of T2 was higher than that of all the other treatments. The performance during the whole experimental period clearly showed that piglets on treatments T3 and T1 grew faster and ate more feed than those on treatments T4 and T2. The FCR for T3 and T1 were better than for T4, and that of piglets on treatment T2 was the worst. The final bodyweight was similar for T3 and T1 and those were higher than that for T4 and T2. It is really surprising, that such an important difference in bodyweight (5kg) could be achieved in such a short period of time.

Table 2. Effects of P level, phytase and ZnO on the performance and on the Ca and P concentration in blood serum of weanling pigs.

Treatments	**T1**	**T2**	**T3**	**T4**		
Phosphorus level	**normal**	**low**	**low**	**low**		
Phytase	**-**	**+**	**+**	**+**		
Zinc	**+**	**+**	**-**	**+**		
Vit-min premix	**+**	**+**	**+**	**-**	*RSD*	*P*
Performance of the 1st period						
Initial weight (kg)	4.55	4.56	4.57	4.56	0.15	NS
Weight gain ($kg.d^{-1}$)	0.180b	0.133c	0.207a	0.129c	0.041	***
Feed intake ($kg.d^{-1}$)	0.206ab	0.185b	0.230a	0.180b	0.035	*
Feed conversion ratio ($kg.kg^{-1}$)	1.17b	1.45a	1.12b	1.47a	0.19	**
Performance of the 2nd period						
Intermediary weight (kg)	7.05b	6.42c	7.47a	6.37c	0.59	***
Weight gain ($kg.d^{-1}$)	0.472a	0.209c	0.448a	0.254b	0.068	***
Feed intake ($kg.d^{-1}$)	0.607a	0.340b	0.600a	0.343b	0.044	***
Feed conversion ratio ($kg.kg^{-1}$)	1.29b	1.64a	1.34b	1.35b	0.08	***
Performance of all experiment						
Weight gain ($kg.d^{-1}$)	0.344a	0.175b	0.346a	0.198b	0.534	***
Feed intake ($kg.d^{-1}$)	0.437a	0.274b	0.443a	0.274b	0.034	***
Feed conversion ratio ($kg.kg^{-1}$)	1.28c	1.58a	1.28c	1.40b	0.068	***
Final weight (kg)	15.90a	10.32b	15.98a	11.10b	1.75	***
Blood serum concentration [a]						
Total calcium ($mg.100mL^{-1}$)	12.50b	13.96a	12.77b	11.57c	0.85	**
Inorganic phosphorus ($mg.100mL^{-1}$)	8.00a	3.10c	5.06b	3.07c	0.57	***
Ca:P ratio	1.57d	4.51a	2.57c	3.80b	0.38	***

[a] the reference values at start were 11.36 and 7.56 $mg.100mL^{-1}$ for Ca and P, respectively.

In pigs, serum Ca concentration remains relatively constant from birth until slaughter weight whereas inorganic P concentration increases from birth until 2 weeks and decreases afterwards

(Ullrey et al., 1967). Considering that piglets of T1 and T3 have normal growth it seems that Ca concentration slightly increases after weaning and is highly dependent of dietary treatments (table 2). Inorganic P concentration did not vary from weaning to the end of the experiment in case of treatment T1, it decreases slightly in T3, and dramatically in other treatments. As a consequence the Ca:P ratios varied tremendously between treatments, from 1.6 to 4.5 for T1 and T2, respectively. This ratio was of 1.5 at the start of the experiment.
Simultaneous supplementation of ZnO and replacement of inorganic-P supply in the diet by the addition of microbial phytases strongly decrease feed intake, growth performance and serum inorganic-P concentration. Withdrawal of ZnO allows piglets to ate more, grew faster and recover normal P and Ca concentration. At this point, is difficult to discriminate what is the normal serum P concentration, that of T1 (with ZnO supplementation) or that of T3 (with low inorganic-P supply). Nonetheless, Ca:P ratio of 2.6 seems very high indicating a possible sub-optimal P level in low-P diets supplemented with phytases used in the experiment. Those diets were formulated to contain 0.28 available P compared with 0.43 for the control diet. In case of phytase binding by Zn ions, phytate-P will not available, so piglets suffered a P deficiency, which could affect bone mineralisation. Phosphorus requirements for maximum rates of animal growth are generally lower than those necessary for maximum bone development. Visual scores of leg structure in growing animals may not detect differences in bone development, especially at a young age (Cera and Mahan; 1988) but P (and Ca) level have a carry-over effect from the younger to the finisher phase. Although growth performance may not always appear to be depressed in pigs fed sub-optimal levels of P, reports of animals suffering from broken bones during slaughtering process are common (Anselm, 2000). In young pigs, inadequate P supplementation can result in rickets and, by the time the clinical symptoms are observed, recovery of the animal is unlikely; culling is the most cost-effective but an expensive solution. So, it appears beneficial to increase P level instead of using phytases in diets for young pigs. The use a pharmacological dose of Zn does not seem necessary for growth promotion, even immediately after weaning; piglets of treatment without ZnO oxide supplementation grew more than controls in the first two weeks of the trial. It must be noted that feed was also medicated with antibiotics and piglets did not suffer from diarrhea. So the risk of Zn interaction with other elements of the diet and its contribution to the environmental pollution does not compensate the possible advantages of its inclusion in diets at so high dosage. In a recent study with young pigs, the use of pharmacological levels of Zn in low-P diets significantly failed to improve growth performance and bone ash (Augspurger et al., 2004). Supplementation with microbial phytase recovers growth performance but only in part comparatively to that of piglets fed a diet supplemented with an inorganic-P source. Authors suggested that pharmacological levels of Zn chelate the phytate complex, thereby decreasing its availability for hydrolysis, and the efficacy of phytase. Although the potential utilization of phytase in early-weaned piglets may be limited, this nevertheless shows that its inclusion level must be adjusted according to the possible interactions with other components of the diet.

Conclusion

It can be concluded that the use of pharmacological doses of ZnO in low P diets is dangerous for the P homeostasis and that it may affect performance. Although phytase is useful to reduce the dietary P concentration, attention must be paid to possible interactions with other nutrients present in the feed, particularly in diets for young animals.

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