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Zinc availability and digestive zinc solubility in piglets and broilers fed diets varying in their phytate contents, phytase activity and supplemented zinc source

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The study was conducted to evaluate the effects of dietary zinc addition (0 or 15 mg/kg of Zn as inorganic or organic zinc) to three maize–soybean meal basal diets varying in their native Zn, phytic P contents and phytase activity (expressed in kg of feed: P− with 25 mg Zn and 1.3 g phytic P, P+ with 38 mg Zn and 2.3 g phytic P or P+/ENZ being P+ including 500 units (FTU) of microbial phytase per kg) in two monogastric species (piglets, broilers). Measured parameters were growth performance, zinc status (plasma, and bone zinc) and soluble zinc in digesta (stomach, gizzard and intestine). The nine experimental diets were fed for 20 days either to weaned piglets (six replicates per treatment) or to 1-day-old broilers (10 replicates per treatment). Animal performance was not affected by dietary treatments ($P > 0.05$) except that all P− diets improved body weight gain and feed conversion ratio in piglets ($P < 0.05$). Piglets fed P− diets had a better Zn status than those fed P+ diets ($P < 0.05$). In both species, Zn status was improved with supplemental Zn ($P < 0.05$), irrespective of Zn source. Phytase supplementation improved piglet Zn status to a higher extent than adding dietary Zn, whereas in broilers, phytase was less efficient than supplemental Zn. Digestive Zn concentrations reflected the quantity of ingested Zn. Soluble Zn (mg/kg dry matter) and Zn solubility (% of total Zn content) were highest in gizzard contents, which also presented lower pH values than stomach or intestines. The intestinal Zn solubility was higher in piglet fed organic Zn than those fed inorganic Zn ($P < 0.01$). Phytase increased soluble Zn in piglet stomach ($P < 0.001$) and intestine ($P = 0.1$), but not in broiler gizzard and intestinal contents. These results demonstrate (i) that dietary zinc was used more efficiently by broilers than by piglets, most probably due to the lower gizzard pH and its related higher zinc solubility; (ii) that zinc supplementation, irrespective of zinc source, was successful in improving animal's zinc status; and (iii) suggest that supplemented Zn availability was independent from the diet formulation. Finally, the present data confirm that phytase was efficient in increasing digestive soluble Zn and improving zinc status in piglets. However, the magnitude of these effects was lower in broilers probably due to the naturally higher Zn availability in poultry than in swine.

Keywords: zinc, phytate, piglet, broiler, availability

Implications

The study conducted in parallel on two species (weaned piglets and broilers) allow a better understanding in the use of dietary zinc originating from native or supplemented sources. To optimize feed formulations on Zn, this study confirms that both Zn sources were equivalent in their efficacy to provide available Zn. This, as supplemental Zn would not react with native phytate molecules. This study also illustrates, especially in pigs, the efficacy of phytase to improve the availability of dietary native Zn. The known antagonism between dietary phytate and Zn should therefore be considered only for native Zn.

Introduction

Zinc is an essential trace mineral for swine and poultry, but also a toxic metal for the soil and environment when supplied in excess. Natural Zn concentrations in feedstuffs are generally lower than the daily Zn requirement for weaning piglets or broilers leading to the necessity of dietary Zn supplementation. Because of the high variation in dietary, Zn and Zn antagonists (calcium, phytate, fibers

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and so on), Zn supplementation is generally supplied with high safety margins for Zn; even when not supplied for pharmacological purposes in piglets. High safety margins in Zn lead to unbalanced manure composition relative to nitrogen (Mohanna and Nys, 1998) and to environmental concerns in areas with concentrated livestock production (Dourmad and Jondreville, 2008; Römkens *et al*., 2008). Along with new legal limitations on dietary Zn supply (e.g. European Community, 2003), there is a need for new approaches to maximize the efficiency of Zn use to optimize productivity and minimize environmental Zn load.

Zinc is supplemented either in inorganic (oxides, sulfates and so on) or organic forms (non-ionic chemical bond between the Zn atom and the ligand, generally an amino acid). Inorganic salts are ionized in liquid environments and are suspected to react with other feed components. Zn in particularly, forms insoluble complexes with phytate in the digestive tract leading to reduced Zn availability for monogastrics (O'Dell and Savage, 1960; Davies and Nightingale, 1975; Windisch and Kirchgessner, 1999). Calcium reinforces this reduced Zn availability by stabilizing phytates (Oberleas *et al*., 1966). According to Dintzis *et al*. (1995) and Susaki *et al*. (1999), these antagonists alter Zn availability by depressing its solubility in the digestive tract. Demonstrations of increased Zn availability when feeding organic Zn sources compared to Zn sulfate in the presence of phytate were reported by Power *et al*. (1994) and Schlegel and Windisch (2006) in rats. According to these authors, organic Zn sources have a reduced reaction to dietary Zn antagonists, such as phytate, and present a solubility in the digestive tract favorable for an improved Zn absorbability. Increased Zn availability in broilers using organic Zn sources has also been reported by Wedekind *et al*. (1992) but remains unconfirmed in piglets by the same research group (Wedekind *et al*., 1994). Referring to these studies, the authors suggested that the higher Ca content in the broiler diets, a potential Zn antagonist, may explain the increased availability of the organic source in broilers compared with piglets (Wedekind *et al*., 1994). However, physicochemical conditions and transit time in the digestive tract of both species may also contribute to different Zn solubility in the digestive tract, and, in turn, to different Zn availability in piglets and broilers.

The aim of this study was to investigate the digestive zinc solubility and zinc availability in piglets and broilers fed diets varying in their phytate contents, phytase activity and zinc source supplementation. The experimental diets were identical for piglets and broilers to allow direct species comparison. The nine experimental diets were therefore formulated to meet the nutrient requirements, except Zn, of the most demanding species.

Material and methods

Experimental diets

Three iso-energetic, iso-nitrogenous and iso-Ca/P maize–soybean meal basal diets (P−; P+; P+/ENZ) were formulated to be differentiated in their phytic P concentration and phytase activity and to meet or exceed all nutrient requirements of weaned piglets and broiler starters, except for Zn (Institut National de la Recherche Agronomique, 1989; Institut National de la Recherche Agronomique-Association Française de Zootechnie, 2004). Nutrients, except Zn, were therefore adjusted to meet requirements of the most demanding specie. To achieve two different concentrations of phytic P, plant feedstuffs (soybean meal, heated wheat bran and isolated soybean protein) present in the basal diet high in phytic P (P+) were replaced with fish meal, casein, corn starch and cellulose in the basal diet low in phytic P (P−). The third basal diet (P+/ENZ) was made by incorporating 500 units (FTU) of microbial phytase per kg (phytase produced by *Aspergillus niger*, 6970 FTU/g; Natuphos®, BASF, Ludwigshafen, Germany) into basal diet P+. Wheat bran was heated (twice 2 min at 900 W in microwave oven) to eliminate any intrinsic phytase activity. Frapin (1996) indicated that this heating treatment denatures plant phytase to a major extent. No basal diet was supplemented with Zn and their premix was Zn-free. The ingredient and chemical composition of the three basal diets P−, P+ and P+/ENZ are presented in Table 1. Nine experimental diets were formulated: each basal diet was supplemented with 0 or 15 mg Zn/kg issued from zinc sulfate heptahydrate (ZnSulf, 326 g Zn/kg feed grade) or 15 mg Zn/kg from zinc glycine chelate (ZnGly, 214 g Zn/kg; B-TRAXIM®, Pancosma, Geneva, Switzerland) according to the defined terms in EU legislation (European Community, 2006). All experimental diets were pelleted (maximum 62°C, 2.5 mm). Piglets and broilers were fed the same experimental diets with the exception of anticoccidiostats (500 mg/kg), which were incorporated before pelletting into the experimental diets fed to broilers.

Animals and experimental procedures

Piglet experiment. One hundred and eight piglets (Pietrain × (Landrace × Large White); female : castrate 1 : 1) were weaned and placed by pairs into plastic/inox pens and fed, *ad libitum*, the unsupplemented experimental diet P+ for a 6-day adaptation period. Following the adaptation period, 54 piglets were allocated to six blocks on the basis of gender (three blocks of female and three of castrates), BW and litter. The 54 selected piglets were 27 ± 1-day old and weighed 8.8 ± 1.1 kg at weaning. The nine piglets from each block were placed in individual plastic/inox pens and randomly allocated to one of the nine experimental diets for 20 days. Diets were fed restrictively (3.5% of BW) divided in three daily and equal meals. Water was available *ad libitum*. Before the first and the last experimental days, a night of fasting was introduced. On the last experimental day (day 21), piglets were fed two-thirds of their previous daily consumption until 2 h before anesthesia (electric shock), which was followed by bleeding. The two climatically controlled rooms were set at a constant temperature (25°C ± 1°C).

Broiler experiment. Two hundred and twenty, 1-day-old broilers (Ross) were placed in 96 plastified cages, each containing a plastified feeder and a plastic water nipple.

Table 1 *Composition of basal diets P− and P+ and P+/ENZ (as fed basis)*

	P−	P+	P+/ENZ
Ingredients (g/kg)[a]			
Maize	593.0	593.0	593.0
Wheat bran, heated[b]	0.0	112.5	112.5
Soybean meal, 48% crude protein	0.0	39.5	39.5
Soybean protein, isolated	0.0	123.5	123.5
Casein	106.5	30.0	30.0
Maize starch	159.2	0.0	0.0
Fishmeal	73.0	0.0	0.0
Sunflower oil	6.0	42.0	42.0
Cellulose	18.0	0.0	0.0
Monodicalcium phosphate	14.2	24.5	24.5
Calcium carbonate	18.3	18.5	18.5
NaCl	2.0	3.0	3.0
L-Lysine HCl	1.1	4.0	4.0
L-Threonine	1.3	1.6	1.6
DL-Methionine	1.7	2.2	2.2
L-Tryptophan	0.7	0.6	0.6
Phytase (FTU/kg)[c]	0	0	500
Vitamin and mineral premix[d] (Zn-free)	5.0	5.0	5.0
Nutrient composition			
Dry matter (DM, g/kg)[e]	884	896	896
Crude protein (CP, N × 6.25, g/kg)[e]	213	233	233
Crude fiber (CF, g/kg)[f]	30.2	29.9	29.9
Ash (g/kg)[e]	45.5	60.5	60.5
Net energy pig (MJ/kg)[f]	10.8	10.6	10.6
Metabolizable energy broiler (ME, MJ/kg)[f]	13.4	13.3	13.3
Ca (g/kg)[e]	10.3	14.0	14.0
P (g/kg)[e]	6.6	10.4	10.4
Phytic P (g/kg)[e]	1.3	2.3	2.3
Phytase activity (FTU/kg)[e]	27	201	688
Zn (mg/kg)[eg]	25	38	38
Phytic acid/Zn (mol/mol)	18	21	21
Phytic acid × Ca/Zn (mol/kg DM)	5.3	8.3	8.3

[a]Diets for broilers contained 0.5 g coccidiostats/kg.
[b]Heated twice for 2 min at 900 W in a microwave oven.
[c]Microbial phytase produced by *Aspergillus niger* (6970 FTU/g; Natuphos®, BASF, Ludwigshafen, Germany).
[d]Vitamins supplied per kg of diet: 15 000 IU vitamin A, 3000 IU vitamin D3, 40 IU vitamin E (DL α-tocopherolacetate), 2 mg vitamin K3 (menadion), 2 mg vitamin B1 (thiamine), 10 mg vitamin B2 (riboflavin), 30 mg vitamin B3 (PP, niacin), 15 mg vitamin B5 (panthotenic acid), 10 mg vitamin B6 (pyridoxine), 0.2 mg vitamin B8 (biotin, H), 2 mg vitamin B9 (folic acid), 0.05 mg vitamin B12 (cyanocobalamin), 100 mg vitamin C (ascorbic acid), 800 mg cholin, 30 mg Fe ($FeSO_4$), 20 mg Cu ($CuSO_4$), 40 mg Mn (MnO), 2 mg Co ($CoSO_4$), 1 mg I ($Ca(IO_3)_2$), 0.3 mg Se (Na_2SeO_3).
[e]Analyzed as described under the section Material and Methods.
[f]Calculated using Institut National de la Recherche Agronomique – Association Française de Zootechnie (2004).
[g]Analyzed Zn concentration in P−, P+ and P+/ENZ supplemented with Zn as sulfate was 41, 54 and 54 mg/kg, respectively; analyzed Zn concentration in P−, P+ and P+/ENZ supplemented with Zn as glycinate was 42, 52 and 53 mg/kg, respectively.

Broilers were fed a standard commercially available starter diet during the first 2 days following hatching. On the second day, 180 broilers having the nearest BW to the mean were selected and allocated by BW to 10 blocks of nine cages. Each cage contained two animals, and one from each block was randomly allocated to one of the nine experimental diets for 20 days. Feed and water was available *ad libitum*. To stimulate intake before the end of the experience, all experimental diets were removed for 5.5 h and reintroduced for the last 2.5 h before lethal injection of pentobarbital. Broilers were housed in one climatically controlled room. The temperature was progressively decreased from 33°C to 26°C. The light was permanently on during the first 2 days following hatching, then on for 23 h per day.

Data and sample collection and analysis

A sample from each treatment diet was taken and frozen (−20°C). A sample of 10 ml of drinking water was taken (daily in the piglet unit and weekly in the broiler unit) and analyzed for Zn content. Feed intake (FI) was recorded for the individual pig on a daily basis and for each broilers cage on a weekly basis. Piglets and broilers were individually weighed at the start and end of the experimental period. Blood samples were collected from each piglet at the jugular vein with heparinized 10-ml tubes on weighing days. Blood samples from each broiler were collected with heparinized 7.5-ml tubes from the alary vein before lethal injection. Blood samples were centrifuged (3000 × **g**, 10 min, 4°C) and plasma was frozen (−20°C). At slaughter, the right metacarpal IV from the piglets and the right tibia from the broilers were collected, autoclaved at 120°C for 20 min, cleaned from any soft tissues and were frozen (−20°C). Within 30 min following piglet slaughter, stomacal and intestinal (length of 70 cm from the pylori) contents were collected separately, lyophilized and frozen (−20°C) after their pH was measured. Within 10 min after broiler slaughter, the same process was conducted for the gizzard and the complete small intestine.

Basal diets P− and P+ were analyzed for crude protein (CP; N × 6.25), phytic P, P and Ca contents. Basal diets P−, P+ and P+/ENZ were analyzed for phytase activity. Zn was analyzed in all experimental diets, water samples, bones, plasma and digesta. Soluble Zn was analyzed in digesta. Alkaline phosphatase (AP) activity was measured in plasma. All analyses were performed in duplicate. Dry matter (DM) was measured by drying at 103°C until constant weight. Nitrogen was determined by the Kjeldahl method according to the French standard AFNOR (NFV 18-100) using a Kjelfoss apparatus (A/S N Foss Electric, Denmark). Phytic P was determined by ion-pair HPLC (Column C_{18}, Hypersyl C 18-5 μm 200 × 2 mm, Interchim, Montluçon, France) after acidic extraction and anionic exchange purification according to the method developed by Sandberg and Adherinne (1986) and modified by Lehrfeld (1989). Phytase activity was measured colorimetrically after incubation in a sodium phytate solution (Engelen *et al.*, 1994). One phytase activity unit (FTU) is the amount of enzyme that liberates 1 μmol of inorganic P from 5.1 mmol/l solution of sodium phytate per minute at pH 5.5 and 37°C. Plasmatic AP activity was determined using the procedure from Biomérieux (Biomérieux 63609, Marcy l'Etoile, France) on Cobas Mira apparatus (Hoffman-La Roche, Nutley, NJ, USA).

Samples were treated as follows before mineral analyses: 1.0 ml of plasma, mixed with 0.5 ml HCl 3N and 0.5 ml of 40% trichloroacetic acid, was centrifuged at 3000 × **g** for 15 min. The supernatant was collected and diluted in 3 ml of deionized water. Each bone was sectioned longitudinally, dried overnight at 103°C, weighed and then ashed at 550°C for 12 h in a muffle furnace. Ash was weighed and ground. Samples of diets and lyophilized digesta were ashed at 550°C for 8 h in a muffle furnace. Bone, feed and digesta ashes were solubilized using nitric acid 16 N and hydrogen peroxide 30% on a digestion block until dry and nitric acid 0.4 N for dilution before analysis. To determine the soluble Zn content in the digesta, samples were rehydrated (0.3 g lyophilized digesta for 10 ml deionized water) and stirred constantly at 38°C during 2 h. Supernatants were clarified by ultracentrifugation (18000 × **g**, 1 h, 20°C) and separated by filtration (45 μm). The filtrated supernatant was acidified with one drop of HNO_3 16 N before mineral analysis. Calcium and Zn were analyzed by flame atomic absorption spectrophotometry (SpectrAA 220 FS; Varian, Springvale, Australia). Phosphorus was analyzed by the Vanadate colorimetric method on a Cobas Mira apparatus (Hoffman-La Roche).

Statistical analysis

Data were submitted to an ANOVA followed by a comparison of means (NCSS, 2001). The individual pig and the cage of two birds were considered as experimental units. Data were analyzed as a 3 × 3 factorial design. The model included block, basal diet (P−, P+, P+/ENZ), supplemental Zn (0, ZnSulf, ZnGly) and basal diet × supplemental Zn. The values obtained before feeding the experimental diets to piglets were used as covariates for final plasma Zn and AP activity values. The feed consumed during the 2 h before slaughter was used as covariate for piglet digestive tract data analysis. Differences were considered significant when $P < 0.05$ and trends were noted at $P < 0.1$.

Results

Diets

The analyzed and calculated nutrient values of the three basal diets were consistent with the expected values (Table 1). The analyzed Zn and phytic P contents were higher in basal diet P+ and P+/ENZ than in basal diet P−. The analyzed phytase activity in basal diet P+ reached 200 FTU/kg, probably due to an incomplete denaturation of plant phytase by heat treatment of wheat bran. The increased phytase activity (487 FTU) in basal diet P+/ENZ, compared to basal diet P+ was consistent with the expected phytase inclusion. Analyzed Zn concentration in Zn supplemented experimental diets were according to theoretical values. Zn concentration in drinking water did not exceed values of 0.03 and 0.46 mg/l in piglet and broiler units, respectively.

Animal performance

Three non-zinc supplemented piglets (two from P− and one from P+ diets) had to be removed from the study due to low FI. Broilers fed P− diets performed in reduced intake (−44% compared to P+ and P+/ENZ) and increased cases of morbidity, particularly leg weakness (30% *v.* 5% in P+ and P+/ENZ). Such low feed intake associated with depressed growth and increased morbidity was not representative of expected broiler performance fed. This situation was most probably due to the hardness of the P-pellets, which is not related to the experimental variable. Broiler data from P-treatments were therefore removed.

Results relative to piglets are presented in Table 2. Piglet performance was affected by the basal diet ($P < 0.05$), but not by supplemental Zn ($P > 0.1$). The restricted FI was 404 ± 16 g/day, irrespective of the experimental treatment, but body weight gain (BWG) and feed conversion ratio (FCR) were improved by 8.7% ($P < 0.05$) and 7.9% ($P < 0.05$), respectively, when P− diets were fed compared to P+ or P+/ENZ diets. No interaction was measured between basal diet and supplemental Zn ($P > 0.1$).

Results relative to broilers are presented in Table 3. Mean broiler performance was 56 ± 4 g/day FI, 43 ± 3 g/day BWG and 1.32 ± 0.06 FCR and was neither affected by the basal diet, supplemental Zn nor by their interaction ($P > 0.1$).

Zinc status: plasma and bone characteristics

In piglets, plasma Zn concentrations and AP activity remained similar, while bone Zn level was reduced ($P < 0.001$) by 24% when feeding P+ diets compared to P− diets. Supply of phytase to P+ diets increased plasma Zn, AP activity and bone Zn ($P < 0.001$) by 104%, 157% and 105%, respectively. Dietary Zn supplementation (15 mg/kg) increased plasma AP activity by 49% ($P < 0.01$) and bone Zn by 17% ($P < 0.05$), irrespective of Zn source. Plasma Zn was increased by 15% ($P < 0.05$) with ZnSulf and by 12% ($P > 0.05$) with ZnGly.

In broilers, dietary Zn supplementation increased ($P < 0.001$) plasma Zn by 19%, irrespective of Zn source. The use of phytase increased plasma Zn (+25%) only in the experimental diet without supplemental Zn. The dietary addition of ZnSulf or ZnGly increased plasma Zn by 32% and 37%, respectively, when no phytase was fed (interaction basal diet × Zn, $P < 0.05$). Plasma AP activity was not influenced by dietary treatments ($P > 0.1$). Dietary Zn supplementation increased ($P < 0.001$) bone Zn by 42%, irrespective of Zn source and phytase increased it by 11% ($P < 0.001$). Bone Zn was increased by 29% when adding phytase to non-Zn supplemented diets or by 58% when adding Zn to P+ diet or by 27% (ZnSulf) and 30% (ZnGly) when adding Zn to P+/ENZ diet (interaction basal diet × Zn, $P < 0.01$).

Digestive pH, Zn and soluble Zn contents

In piglets, mean pH values of the stomacal and intestinal contents were 5.1 ± 0.2 and 5.4 ± 0.2, respectively, and were independent of basal diet and supplemental Zn ($P > 0.1$). Zn concentration in the stomach closely reflected Zn concentration in diets. P+ and P+/ENZ diets, containing 13 mg/kg more Zn than P− diet also led to there being 11 to 14 mg/kg DM more Zn in the stomach ($P < 0.001$).

Table 2 *Effect of basal diet and zinc supplementation on growth performance, digesta, plasma and bone characteristics in piglets (main factors)*

	Basal diet				Supplemented zinc				
Main factors	P−	P+	P+/ENZ	*P*-value[1]	0	ZnSulf	ZnGly	*P*-value[1]	r.m.s.e.[1]
Performance									
Initial BW (kg)	10.4	10.2	10.6		10.5	10.4	10.2		
Final BW (kg)	17.4[a]	16.6[b]	17.1[ab]	*	17.0	17.2	17.0	ns	0.8
Feed intake (g/day)	403	401	408		398	407	408		
Zn intake (mg/day)	15.3[c]	19.7[b]	20.5[a]	***	13.9y	20.9x	20.6x	***	1.0
BW gain (g/day)	351[a]	320[b]	326[b]	*	322	339	337	ns	30
Feed conversion ratio	1.16[a]	1.26[b]	1.26[b]	*	1.25	1.21	1.21	ns	0.11
Stomacal characteristics[2]									
pH	5.1	5.1	5.1	ns	5.0	5.2	5.1	ns	0.2
DM (g/kg)	301	282	290	ns	284	305	284	ns	30
Zn (mg/kg DM)	41[c]	55[a]	52[b]	***	38y	56x	54x	***	3.3
Soluble Zn (mg/kg DM)	3.8[b]	4.6[b]	7.5[a]	***	3.9y	5.7x	6.2x	**	1.5
Zn solubility (%)[3]	9.6[b]	8.5[b]	14.5[a]	***	10.6	10.4	11.6	ns	3.0
Intestinal characteristics[2]									
PH	5.3	5.4	5.4	ns	5.3	5.4	5.4	ns	0.2
DM (g/kg)	165[b]	176[b]	210[a]	*	186	181	183	ns	39
Zn (mg/kg DM)	51[b]	62[a]	63[a]	*	46z	70x	61y	***	10.7
Soluble Zn (mg/kg DM)	6.4[b]	7.8[b]	10.2[a]	**	7.0	8.1	9.2	0.09	2.4
Zn solubility (%)[3]	13.5	13.0	16.2	0.10	15.5x	11.9y	15.3x	*	3.6
Plasma and bone characteristics									
Plasma Zn (mg/l)[4]	0.51[b]	0.45[b]	0.92[a]	***	0.57y	0.67x	0.64xy	0.05	0.11
Plasma alkaline phosphatase activity (U/l)[5]	230[b]	197[b]	506[a]	***	234y	366x	333x	**	114
Metacarpal ash (g/kg DM)	425[a]	389[b]	405[b]	**	399	407	413	ns	26
Metacarpal Zn (mg/kg DM)	54[b]	41[c]	84[a]	***	54y	63x	62x	*	11

DM = dry matter.
[1]r.m.s.e. = root mean square error; ***$P<0.001$; **$P<0.01$; *$P<0.05$; ns (non-significant) $P>0.10$; two-way interaction was never significant ($P>0.10$) except for stomacal Zn solubility ($P=0.07$), and therefore, only main effects are presented. For each main factor, values in the same row not followed by the same letter (a, b, c for basal diet effect; x, y, z for supplemented zinc effect) differ significantly.
[2]Adjusted means for feed intake on last experimental day of 325 g.
[3]Calculated as the ratio of total to soluble Zn concentration.
[4]Adjusted means for initial plasma Zn of 0.83 mg/l.
[5]Adjusted means for initial plasma alkaline phosphatase activity of 619 U/l.

Dietary Zn supplementation of 15 mg/kg increased stomacal Zn concentration accordingly by 17 mg/kg DM ($P<0.001$), irrespective of Zn source. Stomacal soluble Zn concentration was similar in piglets fed P− and P+ diets ($P>0.05$), but increased ($P<0.05$) by 63% when phytase was added to P+ diets. Supplemental Zn also increased ($P<0.01$) stomacal soluble Zn by 46 (ZnSulf) and 59% (ZnGly). Stomacal Zn solubility (percentage of soluble Zn to total Zn concentration) was increased by P+/ENZ diet ($P<0.001$) and by supplemental Zn in P+ and P+/ENZ diets (interaction basal diet × Zn, $P=0.07$). Intestinal Zn concentration in piglets was increased by 12 mg/kg DM with P+ and P+/ENZ diets compared to P− diet ($P<0.05$) and by 24 (ZnSulf) and 15 mg/kg (ZnGly) DM with Zn supplementation ($P<0.001$). As in the stomach, intestinal soluble Zn was not dependent on the dietary phytate content ($P>0.05$), but on the addition of phytase into P+ diets (+31%, $P<0.01$). Intestinal soluble Zn increased by 16% when ZnSulf was supplied and by 31% when ZnGly was supplied ($P=0.09$). Intestinal Zn solubility was higher in piglets fed Zn as ZnGly compared to those fed ZnSulf ($P<0.05$). Zn solubility was the highest in the intestine of piglets fed P+/ENZ, ZnGly experimental diet (18.4%).

In broilers, mean pH values of the gizzard and intestinal contents were 4.2 ± 0.3 and 6.0 ± 0.2, respectively. Intestinal pH was slightly increased in birds fed P+/ENZ diets (+0.1 unit, $P<0.01$). As observed in the piglet stomach, Zn concentration in the broiler gizzard was close to dietary Zn content. In the gizzard, soluble Zn was not raised by the addition of phytase to P+ diets ($P>0.10$) as it was the case in the piglet stomach, but was increased by supplemental Zn ($P<0.01$) by 52% (ZnSulf) and 38% (ZnGly). Compared to the Zn concentration in piglet stomach and intestine and to the gizzard, Zn concentration in the broiler intestine was twice as high (82 ± 4 mg Zn/kg for unsupplemented Zn). Intestinal total and soluble Zn concentrations were similar ($P>0.1$) in chickens fed P+ and P+/ENZ diets, but increased with Zn supplementation by 45 ($P<0.001$) and by 43% ($P<0.01$), respectively, irrespective of Zn source. Intestinal Zn solubility was not influenced ($P>0.05$) by Zn supplementation, but was reduced in the presence of phytase ($P<0.05$). Zn solubility was highest

Table 3 *Effect of basal diet and zinc supplementation on growth performance, digesta, plasma and bone characteristics in broilers*

Basal diet	P+			P+/ENZ			Basal diet		Supplemented zinc			*P*-value[1]			r.m.s.e.[1]
Added ZnSulf (mg Zn/kg)	0	15	0	0	15	0	P+	P+/ENZ	0	ZnSulf	ZnGly	Diet	Zn	Diet × Zn	
Added ZnGly (mg Zn/kg)	0	0	15	0	0	15									
Added phytase (FTU/kg)	0	0	0	500	500	500									
Performance															
Initial BW (g)	58	58	58	58	58	58	58	58	58	58	58				
Final BW (g)	869	830	867	872	895	861	855	876	870	862	864	ns	ns	ns	65
Feed intake (g/day)	55.8	53.7	57.2	56.8	56.9	56.2	55.6	56.7	56.3	55.3	56.8	ns	ns	ns	3.6
Zn intake (mg/day)	2.14	2.88	2.95	2.32	2.93	2.96	2.65	2.71	2.19y	2.90x	2.95x	ns	***	ns	0.17
BW gain (g/day)	42.7	40.6	42.6	42.9	44.0	42.2	42.0	43.0	42.8	42.3	42.4	ns	ns	ns	3.4
Feed conversion ratio	1.32	1.32	1.35	1.33	1.29	1.34	1.33	1.32	1.32	1.31	1.34	ns	ns	ns	0.06
Gizzard characteristics															
pH	4.2	4.2	4.2	4.3	4.3	4.0	4.2	4.2	4.2	4.3	4.1	ns	ns	ns	0.3
DM (g/kg)	268r	268r	238rs	237rs	213s	285r	258	245	252	241	262	ns	ns	*	42
Zn (mg/kg DM)	37	40	45	34	54	44	40	44	35y	47x	45x	ns	0.05	ns	10
Soluble Zn (mg/kg DM)	9.5	11.5	12.4	9.0	16.6	12.9	11.1	12.8	9.2y	14.0x	12.7x	ns	**	ns	3.0
Zn solubility (%)[2]	26.5	30.8	28.9	26.8	32.5	29.1	28.7	29.5	26.6	31.7	29.0	ns	ns	ns	8.7
Intestinal characteristics															
PH	6.0	5.9	6.0	6.1	6.1	6.1	6.0[b]	6.1[a]	6.0	6.0	6.1	**	ns	ns	0.2
DM (g/kg)	207	202	209	212	211	204	206	209	210	206	207	ns	ns	ns	14
Zn (mg/kg DM)	81	117	111	82	115	131	103	110	82y	116x	121x	ns	***	0.09	15
Soluble Zn (mg/kg DM)	11.2	15.9	16.1	9.8	15.7	12.4	14.4	12.6	10.5y	15.8x	14.2x	ns	**	ns	5.4
Zn solubility (%)[2]	13.9	13.8	14.4	11.9	13.3	9.4	14.0[a]	11.6[b]	12.9	13.6	11.9	*	ns	ns	4.3
Plasma and bone characteristics															
Plasma Zn (mg/l)	0.91s	1.20r	1.25r	1.14r	1.20r	1.18r	1.12	1.17	1.02y	1.20x	1.22x	ns	***	*	0.15
Plasma alkaline phosphatase activity (U/l)	3820	3887	3663	3872	3059	2846	3790	3259	3846	3473	3255	ns	ns	ns	16
Tibia ash (g/kg DM)	500	498	488	483	492	485	492	487	492	490	486	ns	ns	ns	16
Tibia Zn (mg/kg DM)	120t	189r	190r	155s	196r	202r	166[b]	184[a]	137y	193x	196x	***	***	**	13

DM = dry matter.
[1]r.m.s.e. = root mean square error; ***$P<0.001$; **$P<0.01$; *$P<0.05$; ns (non-significant) $P>0.10$. For each factor, values in a same row not followed by the same letter (a, b, c for basal diet effect; x, y, z for supplemented zinc effect; r, s, t for interaction effect) differ significantly.
[2]Calculated as the ratio of total to soluble Zn concentration.

in the intestine of broilers fed P+, ZnGly experimental diet (14.4%).

Discussion

Animal performance and zinc status

In piglets, the increased growth and feed efficiency for the P− diet compared to P+ and P+/ENZ diets was not directly related to Zn use, as plasma and bone Zn concentrations of piglets fed P− diet were lower than those fed P+/ENZ diet. The energy value of ingredients such as casein, maize starch and fish meal in the P− diet may have been underestimated while formulating this diet and may have directly influenced the piglet growth performance. Neither supplemental Zn nor supplemental phytase had any effect on piglet growth performance. Since one of the first signs of Zn deficiency is depressed FI, animal performance may be considered as an adequate indicator for supplemental Zn availability under the condition that animals are fed *ad libitum* low Zn diets (Jongbloed *et al*., 2002). In this study, piglets could not express any potential effect on growth performance because their feeding was restricted.

Similarly, broiler growth performance was neither affected by Zn supplementation nor supply of microbial phytase. In accordance, no improvement in growth performance of broiler starters up to 21 days of age was observed by adding zinc (Mohanna and Nys, 1999a; Burrell *et al*., 2004; Jondreville *et al*., 2007) or microbial phytase (Mohanna and Nys, 1999b; Jondreville *et al*., 2007) to maize–soybean meal diets containing more than 30 mg of Zn/kg. Dietary Zn contents slightly below 40 mg Zn/kg may therefore be sufficient for optimal growth over a limited time period of 21 days. In contrary, Ao *et al*. (2007) observed improved growth performance when supplementing to broilers a maize–soybean meal diet with 500 FTU/kg phytase and/or 2 to 12 mg/kg Zn. However, it must be noted that the unsupplemented broilers had lower growth rates (29 g/day) than in this study.

The present values in piglet and broiler Zn status based on plasma Zn, AP activity and bone Zn are consistent with previous findings conducted on similar protocols within INRA laboratories (e.g. Mohanna and Nys, 1999a; Revy *et al*., 2004) as well as other institutes (Ao *et al*., 2007). The experimental diets were formulated to provide minimal native Zn and maximal supplemental Zn while keeping animals below homeostatic Zn regulation capacity. Consequently, the dietary treatments are expected to influence animal Zn status and present results indicate that this was clearly the case: piglets fed the P− diets had a better Zn status than the ones fed the P+ diets, despite the lower Zn concentration in the P− diets. As suggested by Fordyce *et al*. (1987), the (phytate × Ca)/Zn molar ratio is an accurate Zn availability indicator due to the presence of insoluble Zn–Ca–phytate complexes in the digestive tract. The relative lower Ca and phytate contents to Zn contents in the P− diet (phytate × Ca/Zn of 5.3 mol/kg DM) than P+ diet (phytate × Ca/Zn of 8.3 mol/kg DM) may therefore explain the better Zn status of animals fed P− diet. This study clearly demonstrated an improved Zn status when supplying phytase to the unsupplemented P+ diet. The supply of 500 FTU/kg phytase elicited a far greater response than 15 mg supplemental Zn in piglets, whereas in broilers, 15 mg supplemental Zn was more efficient than 500 FTU/kg phytase. This confirms the previously reported higher efficacy of microbial phytase for improved Zn availability in piglets (e.g. Pallauf *et al*., 1992; Jondreville *et al*., 2005; Revy *et al*., 2006) than in broilers (e.g. Yi *et al*., 1996; Mohanna and Nys, 1999b; Jondreville *et al*., 2007). Recently, the minimal supplementation of 500 FTU/kg phytase in maize–soybean meal diets were evaluated to an equivalent of 30 mg dietary Zn as $ZnSO_4$ in piglets (Jondreville *et al*., 2005) and below 15 mg dietary Zn as $ZnSO_4$ in broilers (Biehl *et al*., 1995; Mohanna and Nys, 1999b; Jondreville *et al*., 2007).

In broilers, supplemental Zn led to a greater plasma and bone Zn response when added to P+ diet than P+/ENZ diet. These results are consistent with Ao *et al*. (2007). According to Jondreville *et al*. (2007), the respective dietary content of 50 and 59 mg Zn/kg (maize–soybean meal-based diet; 33 mg/kg Zn as native Zn) maximized broiler plasma and bone Zn concentration. Thus, supplementation of P+/ENZ diet (38 mg/kg Zn) with 15 mg Zn could not improve plasma Zn and only slightly improve bone Zn as supply was already close to the requirement. The present findings also suggest that Zn requirements for optimal growth are lower than for maximized metabolic Zn use (plasma AP activity, plasma Zn) and bone mineralization.

In both species, regardless of the basal diet, both supplemented Zn sources (ZnSulf and ZnGly) resulted in similar Zn status improvements indicating a similar availability. Only numerical improvements using ZnGly compared with ZnSulf were observed, such as +9% and +4% in plasma and bone Zn, respectively, for piglets fed P+ diet, +4% in plasma Zn for broilers fed P+ diet and +3% in tibia Zn for broilers fed P+/ENZ diet. These findings are compatible with broiler data (Swiatkiewicz *et al*., 2001) in which a Zn amino-acid complex numerically increased bone Zn by 3% and piglet data (Revy *et al*., 2004) in which a Zn methionine even led to a small but non-significant reduction in plasma Zn and bone Zn when compared to ZnSulf. These findings are in contrast to previous research comparing the identical ZnGly source with $ZnSO_4$ resulted in improved plasma, liver and bone Zn on growing rats (Schlegel and Windisch, 2006) and plasma and liver Zn on growing steers (Spears *et al*., 2004). Therefore, this study did tnot confirm the hypothesis of improved Zn availability using organic Zn sources relative to $ZnSO_4$ with increased dietary phytate concentration or reduced dietary phytase activity. In addition, this study did not confirm previous findings by Wedekind *et al*. (1992 and 1994) which showed advantages of using organic Zn sources in piglets relative to broilers due to low dietary Ca contents.

Zinc in digesta and relation to zinc availability

Measurements of pH, total Zn concentrations and soluble Zn in the digestive tract (digesta) were used to evaluate their importance in relation to Zn availability. Previous

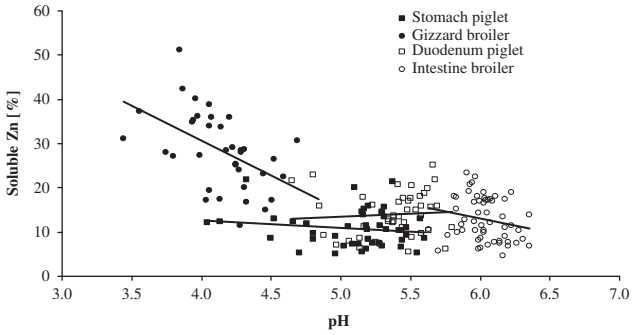

Figure 1 Effect of digesta pH on Zn solubility.

research suggested that Zn availability depends on Zn solubility in the digesta (Susaki *et al*., 1999), whereas Zn solubility may depend on various factors including digesta pH (Cao *et al*., 2000; Jongbloed *et al*., 2002), dietary Zn concentration (Dintzis *et al*., 1995; Susaki *et al*., 1999), supplemented Zn source (Cao *et al*., 2000) and interactions between dietary Zn and diet composition (Ammerman *et al*., 1995; Jongbloed *et al*., 2002).

In this study, the gastrointestinal pH increased only slightly from the piglet stomach to its intestine. This increase was more evident from the broiler gizzard to its intestine. The feeding technique before slaughter and the use of limestone as Ca source, which displays a high acid-binding capacity may have favored high pH values in the stomach and gizzard (Lawlor *et al*., 2005). Zinc concentrations in digesta reflected dietary Zn concentration, except for the broiler intestine, which may originate from the sampling technique employed and the partial dietary DM absorption and/or endogenous Zn secretions occurring in the intestine even under drastically limited Zn supply (Windisch, 2003; Schlegel and Windisch, 2006). The percentage of Zn present in a soluble form (Zn solubility) may be related to digesta pH (Figure 1) (7% to 16%, respectively) and 10% to 18% of Zn were soluble in piglet stomach and intestine, while these percentages were 26% to 32% and 9% to 14% in the broiler gizzard and intestine, respectively. Furthermore, Zn solubility was also found to be higher as a consequence of lower pH *in vitro* (Cao *et al*., 2000). The stomacal Zn solubility was low (54% to 84% on 100 kg BW pigs) compared to measures observed by Dintzis *et al*. (1995) even though pH was 4.5 and extraction methods for measuring soluble Zn were similar. The intestinal Zn solubility in piglets was, however, comparable with those of Dintzis *et al*. (1995) who reported values of 21% in the jejunum when feeding 24 mg/kg Zn, Susaki *et al*. (1999) with values of 11% in ileum when supplementing the diet with 25 mg/kg Zn, and those observed by Ashida *et al*. (2000) also showed values of 29% in small intestine when feeding 65 mg/kg Zn.

The importance of the digestive soluble Zn and Zn solubility on animal Zn status remains uncertain since several factors may have limited potential findings in this study: First, in broilers, the supply of soluble Zn near the absorption sites were not the limiting factor for plasma and bone Zn due to the fact that nutritional requirements were fulfilled. Second, the methodology implemented did not differentiate soluble Zn according to the size of the compounds to which it is associated, while Zn is more readily absorbed when associated with small compounds (Shafey *et al*., 1991). Third, the collected broiler intestinal contents may not be representative enough of digesta located close to the Zn absorption sites, probably located at the upper part of the small intestine. Nevertheless, the results from this study do present novel findings in both piglets and broilers that Zn supplementation increased the concentration of digestive Zn and soluble Zn, independently of source, resulting in increased Zn status. The absence of interaction between the basal diet and supplemental Zn on digestive soluble Zn suggests that, supplemental Zn, irrespective of its source or target specie, did not react with the dietary antagonists. This is in agreement with the response of Zn status indicators in pigs, as discussed previously. The effect of phytase on piglet Zn status was well illustrated by the positive response on the soluble Zn in stomach and intestine, proving the efficacy of phytase to hydrolyze phytic acid and to release Zn. In broilers, on the other hand, the low pH in the gizzard, the high soluble Zn contents and the absence of phytase efficiency to increase soluble Zn all indicate that phytate and Zn were already dissociated to a major extent in P+. According to Ellis *et al*. (1982), zinc–phytate complexes dissociate as soon as pH is decreased down to 4. Thus, these results suggest that the low pH in gizzard allows zinc–phytate complexes to dissociate even in the absence of phytase, whereas in the piglet stomach with higher pH, phytates must first be hydrolyzed by phytase before Zn can be released. This phenomenon would result in a physiologically higher availability of zinc in poultry than in swine, explaining the lower dietary requirements in broilers than in piglets for this trace element (40 mg/kg Zn for chickens, (Institut National de la Recherche Agronomique, 1989) and 80/100 mg/kg Zn for piglets, (National Research Council, 1998; Institut National de la Recherche Agronomique, 1989)). This phenomenon would also explain the differential efficacy of phytase for improved Zn availability in poultry and in swine.

Conclusions

The results of this study demonstrate that dietary zinc was used more efficiently by broilers than by piglets, most probably due to the lower gizzard pH and its related higher zinc solubility. The results of this study demonstrate that zinc supplementation, irrespective of zinc source, are successful in improving zinc status in both piglets and broilers, and suggest that supplemented Zn availability was independent from the diet formulation. Finally, the present data confirm that phytase was efficient in increasing digestive soluble Zn and improving zinc status in piglets. This was of a lower magnitude in broilers probably due to the naturally higher Zn availability in poultry comparedwith swine.

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