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Anim. Res. 51 (2002) 315–326

DOI: 10.1051/animres:2002028

Original article

Bioavailability of two sources of zinc in weanling pigs

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(Received 13 March 2002; accepted 19 September 2002)

Abstract — Thirty-two pigs, weaned at 28 days of age with an average body weight of 9 kg, were used in an experiment devoted to the assessment of the bioavailability of an organic Zn source (ZnOrg) compared to Zn sulfate. Pigs were fed a basal diet containing 28 mg·kg^{-1} of Zn supplemented with $ZnSO_4$ or ZnOrg to provide 0, 10, 20 and 30 mg·kg^{-1} of supplemental Zn. In order to reduce the amount and the variability of their Zn stores, pigs were fed the basal diet for a 7-day adjustment period preceding a 19-day experimental period. Growth performance and bone bending moment were not affected by the level nor the source of zinc. On the contrary, plasma zinc concentration and alkaline phosphatase activity ($P < 0.001$), bone zinc concentration ($P < 0.001$), liver and empty body zinc concentrations ($P < 0.01$) as well as the amount of Zn retained estimated both by the balance technique (BT) ($P < 0.001$) and by the comparative slaughter technique (CST) ($P < 0.01$) increased linearly in response to supplemental Zn. However, the two Zn sources exhibited a similar bioavailability. Twenty-seven percent of Zn intake was retained when measured by means of the balance technique, regardless of the dietary level or zinc source. Zn retention was 54% lower when measured by means of the CST as compared to the BT.

pig / zinc / bioavailability / source / indicator

Résumé — Biodisponibilité de deux sources de zinc chez le porcelet en post-sevrage. Trente-deux porcs, sevrés à 28 jours et d'un poids moyen de 9 kg, ont été utilisés dans un essai de 19 jours ayant pour objectif la détermination de la biodisponibilité d'une source organique de zinc (ZnOrg) par rapport au sulfate de zinc. Les porcs ont été nourris avec un aliment de base contenant 28 mg·kg^{-1} de zinc additionné de 0, 10, 20 ou 30 mg·kg^{-1} de zinc sous forme $ZnSO_4$ ou ZnOrg. Avant la période expérimentale, les porcs ont été nourris avec l'aliment de base pendant une période d'ajustement de 7 jours afin de réduire le niveau et la variabilité de leurs réserves de zinc. Les performances

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de croissance et le moment de flexion de l'os n'ont été affectés ni par le niveau ni par la source de zinc supplémentaire. Au contraire, la teneur en zinc et l'activité de la phosphatase alcaline du plasma ($P < 0,001$), les teneurs en zinc osseuse ($P < 0,001$), hépatique et corporelle ($P < 0,01$) ainsi que la quantité de zinc retenu estimée par la technique du bilan ($P < 0,001$) et celle des abattages comparés ($P < 0,01$) augmentaient linéairement avec l'addition du zinc. Cependant, aucune différence entre les sources n'a été détectée. Le coefficient de rétention de zinc, estimé par la technique du bilan, était de 27 % en moyenne, indépendamment du niveau ou de la source de zinc supplémentaire. Estimée par la technique des abattages comparés, la rétention de Zn était de 54 % inférieure à la valeur obtenue selon la technique du bilan.

porc / zinc / biodisponibilité / source / indicateur

1. INTRODUCTION

Zinc is an essential trace element which is involved in many metabolic functions. Zinc is a cofactor of more than 300 metalloenzymes and is required for at least one enzyme in all six enzyme classes [6]. Zinc deficiency in pigs is associated with parakeratosis, degraded growth performance (decreased food intake and weight gain) and diarrhea, particularly in young animals. Zinc requirements decrease from 100 to 50 $mg \cdot kg^{-1}$ diet when pig body weight increases from 5 to 50 kg and remain steady thereafter [19]. Such a dietary supply allows an optimal growth but also the maximization of zinc concentration in some tissues [36]. However, zinc contained in most plant feedstuffs is poorly available to chicks [20] and pigs [21], because it forms insoluble complexes with some organic ligands, mainly phytates. Therefore, the diets must be supplemented with zinc. In practice, because large safety margins are applied or because of their growth factor effect, zinc and other trace elements like copper are often oversupplied in pig diets and are highly concentrated in pig manure. Consequently, excessive zinc and copper accumulation in soils occurs in areas of intensive animal production, which may cause phytotoxicity of cultivated plants [8, 15, 27]. Because of this risk of environmental pollution, European regulation should move to a drastic reduction of maximal trace element concentrations authorized in pig diets. Zinc supplementation of pig diets can be achieved by means of different sources. Zinc sulfate ($ZnSO_4$) and zinc oxide (ZnO) are commonly used in pig feeding. Organic (complexes or chelates) zinc sources have been suggested to exhibit a higher bioavailability in broilers than inorganic sources [33]. An enhanced bioavailability of organic Zn could allow the reduction of zinc supply in pig diets and, in turn, would contribute to environmental protection. A zinc-methionine complex exhibited a higher bioavailability (206%) than $ZnSO_4$ in chicks fed corn-soybean meal diets [35]. In contrast, studies with pigs have failed to demonstrate differences in zinc bioavailability between organic and inorganic sources [7, 10, 34, 36].

This study was conducted to compare the bioavailability of zinc from an organic Zn source with that of $ZnSO_4$, on the basis of a wide range of response criteria in order to evaluate the zinc status of the pigs. The sources were compared with regards to growth performance, plasma zinc concentration and alkaline phosphatase (AP) activity, bone zinc concentration and bending moment, liver and body zinc concentration and zinc retention. For the assessment of Zn retention, both the collection of urine and feces (balance technique) and the comparative

slaughter technique were performed and the two methods were compared.

2. MATERIALS AND METHODS

2.1. Experimental diets

A basal diet, analyzed to contain 28 mg·kg^{-1} Zn, was formulated to meet or exceed all nutrient requirements [12] for pigs between 5 and 20 kg, except zinc (Tab. I). The six other experimental diets were obtained by supplementing the basal diet with 10, 20 and 30 mg·kg^{-1} Zn as either sulfate ($ZnSO_4$, $7H_2O$) or as an organic source (ZnOrg). ZnOrg was a zinc-methionine complex with a molar ratio of 2 methionines for 1 zinc, coated by an algal matrix. The feedstuffs were ground in a hammer mill fitted with a 2.5 mm screen prior to their incorporation in the meal. During processing, the temperature did not exceed 50 °C. The diets were offered to the pigs in a pelleted form.

2.2. Animals and experimental procedures

The experiment was conducted under the guidelines of the French Ministry of Agriculture for Animal Research. Thirty-two castrated male crossbred (Piétrain, Large White × Landrace) piglets, weaned at 28 ±2 days of age, were housed in collective pens and allowed ad libitum access to the basal diet for a 7-day adjustment period. This adjustment period was aimed at reducing the variability of their zinc stores. Four blocks of 8 piglets each were then constituted on the basis of body weight. In each block, 7 piglets were randomly assigned to one of the seven diets and 1 piglet was used as the control and slaughtered at the beginning of the experimental period.

Thereafter, the pigs were individually housed in stainless steel metabolism pens for the subsequent 19-day experimental period. During this period, the daily allowance of feed was adjusted to 4.5% of the body weight and offered in three equal meals. The amount of feed intake was individually recorded daily. Pigs had free access to demineralized drinking water, free of detectable zinc. The room temperature was maintained at 25 ±1 °C. The pigs were individually weighed at the beginning and at the end of the experimental period.

During the last 8 days of the experiment, the feces and urine of each piglet were quantitatively collected daily and stored at 0 °C. At the end of the collection period, the feces of each animal were ground in a blender. Three sub-samples were taken: two were oven-dried (102 °C for 48 hours) for dry matter determination, and the other one was freeze-dried prior to analysis.

Urine was blended after being acidified with 10% sulfuric acid, and a representative sample was filtered prior to analysis.

Blood samples of each pig were drawn by punction of the vena jugularis by means of a 10 mL heparinized vacutainer® both at the initiation and termination of the experimental period, after an overnight fast. Plasma was obtained by centrifugation (1360 × *g*, 10 min, 4 °C) and the supernatant was frozen at –20 °C prior to analysis.

Slaughter was achieved by electro-immobilization and exsanguination. The liver was immediately removed, weighed, ground in a blender and freeze-dried. The two metacarpals III were extracted from the feet of the front legs, weighed and individually frozen at –20 °C. The following operations were implemented at slaughter for 3 blocks only. Total blood was collected, weighed and a sample was taken. Digesta in the gastrointestinal tract were removed, the tract was rinsed with water and the bladder was emptied. The empty body, except the whole blood, the two metacarpals III and the liver, was weighed, stored at –20 °C and subsequently ground, minced using a 1 mm grid and homogenized. A sample of this compartment was weighed and stored at

Table I. Composition and analytical characterization of the basal diet (as fed basis).

Ingredients	**g·kg^{-1}**
Barley two-rowed	397.22
Low lactose dried whey	210.00
Isolated soybean protein	100.00
Milk powder	80.00
Wheat	70.00
Cornstarch	60.00
Fish meal	40.00
Soybean meal 50	20.00
Monocalcium phosphate	9.90
Calcium carbonate	5.18
L-Threonine	1.10
L-Lysine HCl	0.80
D-L Methionine	0.80
Mineral and vitamin premix [1]	5.00
Analytical characteristics	
Dry matter (DM) [2]	904
Crude protein (CP) [2]	199
Ash [2]	60.5
Crude fiber (CF) [2]	20.7
Fat [2]	49.0
Starch [2]	305
Phosphorus [2]	8.02
Digestible phosphorus [2, 4]	5.13
Calcium	9.49
Phytic phosphorus [2]	0.80
	mg·kg^{-1}
Magnesium	1497
Iron	215
Zinc[3]	28.0
Copper	22.3
Manganese	53.9
	MJ·kg^{-1}
DE[4]	14.5
NE[5]	10.2

[1] Zn-free mineral and vitamin premix – provided per kilogram of diet: (mg) Fe: 100; Cu: 20; Mn: 40; Co: 2; I: 1; Se: 0.3; Vit K3 (menadione): 2; Vit B1 (thiamine): 2; Vit B2 (riboflavine): 10; Vit B3 (PP, niacin): 30; Vit B5 (pantothenic acid): 15; Vit B6 (pyridoxine): 10; Vit B8 (biotin, H): 0.2; Vit B9 (folic acid): 2; Vit B12 (cyanocobalamin): 0.05; choline: 800; Vit C (ascorbic acid): 100; (UI) Vit A: 15000; Vit D3: 3000; Vit E (DL α-tocopherol acetate): 40.

[2] Analyses: DM: drying to constant weight at 103 °C, CP: Dumas method, Ash: muffle furnace (550 °C, 8 h), CF: Weende method, fat: without hydrolysis prior to analysis, starch: Ewers method, phytic P: chromatography after acid extraction, P: Vanadate colorimetric method.

[3] The analyzed Zn content of the diets was 40, 46, 56, 38, 46 and 53 mg·kg^{-1} for the 10, 20, 30 mg·kg^{-1} of Zn from $ZnSO_4$, and 10, 20, 30 mg·kg^{-1} from ZnOrg, respectively.

[4] Digestible energy and P calculated by measuring the energy and P balance of the pigs fed the control diet.

[5] Net energy calculated from Noblet et al. [17]: NE = 0.703 DE + 0.066 fat + 0.020 starch – 0.041 CP – 0.041 CF.

–20 °C. The empty body sample and the blood sample were separately freeze-dried and ground in a blender. The lyophilized empty body sample and blood sample were combined in the same relative proportion present at slaughter according to their DM contents.

2.3. Analysis

Plasma AP activity was measured using the Sigma procedure (Sigma 245, St Louis, MO, USA) by means of the Cobas Mira apparatus (Hoffman-LaRoche, Nutley, NJ). The results are expressed in $U \cdot L^{-1}$. One unit is defined as the amount of enzyme required to produce 1 μmole of p-nitrophenol in 1 minute at 37 °C.

Analysis of zinc in blood plasma, bone, liver and blood combined with the empty body, in urine and the feces and the analysis of calcium, magnesium, zinc, iron, copper and manganese in the diets were performed by flame atomic absorption spectrophotometry (SpectrAA 220 FS, Varian, Springvale, Australia).

Prior to mineral analysis, plasma samples were mixed with 20% TCA in a 1:1 volume ratio and centrifuged at $1020 \times g$ for 15 min. One milliliter of supernatant was collected and diluted with 4 mL of 0.6 N HCl. The metacarpal III from the right foot was autoclaved at 120 °C for 20 min to facilitate removal of the muscle and connective tissue from the bone. It was longitudinally sectioned and fat was extracted by means of a 48 h-treatment with anhydrous ethyl ether. After the remaining ether was evaporated, the fat-free bone was dried at 103 °C overnight and ashed at 550 °C for 12 h in a muffle furnace. Ash samples were ground into a fine powder. Samples of the diets, lyophilized feces, liver and blood combined with the empty body were ashed at 550 °C for 8 h in a muffle furnace. Ash samples were wet-ashed with 70% HNO_3 and 30% H_2O_2 on a sand heater until dry. The samples were next diluted in 0.4 N HNO_3 prior to mineral analysis.

The bone bending moment was determined on the metacarpal III from the left foot with an INSTRON testing machine (Model 5543, INSTRON S.A., Buc, France) according to the formula bending moment = $F \times L/4$, where F is the force applied on the midpoint of the shaft until breaking and L the length between the 2 fulcrum points that the bone rested on, and was expressed as N·m [25].

2.4. Calculations and statistical analysis

The Zn content of the pig was calculated from the Zn content of the blood and the empty body together and the Zn content of the metacarpal and liver were analyzed separately. The retained Zn, estimated by the comparative slaughter technique, was calculated as the difference between the body Zn of the experimental pigs at the end of the experiment and that of the control ones slaughtered at the beginning of the experiment.

All the data were analyzed using the GLM procedure of SAS [30] statistical software with a model appropriate for a randomized complete block design and using the individual pig as the experimental unit for all the response criteria of the study.

Zinc bioavailability of the organic source was determined by means of a multiple linear regression and the slope ratio method [13]. First, three assumptions were sequentially tested for validity of the model: the response is linear for each zinc source, the intercepts for the 2 lines are equal (common intercept) and the response to the zero level is equal to the common intercept value. After these assumptions were checked, the regression of the response criteria to the amount of supplemental zinc ingested was fitted for each of the 2 zinc sources. The model included the block. For plasma zinc concentration and AP activity, the initial value was used as a covariate. The

relative bioavailability value of ZnOrg relative to $ZnSO_4$ was defined as the ratio between the slopes of the fitted regression lines. The differences were considered significant when $P < 0.05$.

3. RESULTS

Two pigs were removed from the data set because of abnormal feed intake which was not associated with the dietary treatments. The analyzed Zn concentration of the diets fitted well with the expected values (Tab. I).

3.1. Growth performance

Growth performance, average daily feed intake (ADFI), average daily gain (ADG) and feed/gain of the pigs were not affected by the zinc level nor by the source (Tab. II) and reached on average 579, 453 $g \cdot d^{-1}$ and 1.28, respectively.

3.2 Plasma zinc

Plasma zinc concentration increased linearly ($P < 0.001$) with supplemental zinc intake (Tab. II). The addition of 10, 20 and 30 $mg \cdot kg^{-1}$ of zinc, in comparison with no supplementation increased plasma zinc concentration by 52, 125 and 164%, respectively. Although a quadratic effect of supplemental Zn intake was detected ($P < 0.05$), it explained only 6% of the total variance in addition to the 83% explained by the linear model. The source of supplemental Zn did not influence plasma Zn concentration.

3.3. Plasma alkaline phosphatase activity

Initial plasma AP activity was higher than final plasma AP activity irrespective of the diet (Tab. II). Plasma AP activity increased linearly ($P < 0.001$) and quadratically ($P < 0.01$) with supplemental zinc intake. This quadratic effect explained 12% of the variance in addition to the 76% explained by the linear model. The addition of 10, 20 and 30 $mg \cdot kg^{-1}$ of zinc, in comparison with no Zn supplementation increased plasma AP activity by 153, 300 and 339%, respectively. The two sources did not differ for this criterion.

3.4. Bone Zn concentration and bending moment

The results are presented in Table II. Bone zinc concentration, expressed as either $mg \cdot kg^{-1}$ FFDM (fat free dry matter) or $mg \cdot kg^{-1}$ ash, increased linearly ($P < 0.001$) with supplemental zinc intake. The results were similar for pigs fed diets containing either $ZnSO_4$ or ZnOrg. The intercepts for the 2 lines were equal but the measured response for the basal diet tended to be higher ($P < 0.10$) than the common intercept value of the lines. Indeed, bone zinc concentration was not increased by the addition of 10 $mg \cdot kg^{-1}$ of Zn to the basal diet, but was increased by 33 and 46% when 20 and 30 $mg \cdot kg^{-1}$ of Zn were added to the basal diet, respectively. The bone bending moment did not differ significantly between the treatments.

3.5. Liver zinc

Liver zinc concentration increased linearly ($P < 0.01$) with supplemental zinc intake (Tab. II). Similarly to bone zinc concentration, it was not modified by the addition of 10 $mg \cdot kg^{-1}$ of Zn to the basal diet. The addition of 20 and 30 $mg \cdot kg^{-1}$ increased the response by 15 and 39%, respectively. The Zn source did not influence the liver Zn concentration.

3.6. Empty body zinc concentration and zinc retained estimated by the comparative slaughter technique

The empty body zinc content increased linearly ($P < 0.01$) with supplemental zinc intake (Tab. III) but did not differ between

Table II. Effect of dietary zinc level and source on growth performance, plasma zinc concentration and alkaline phosphatase activity, bone and liver zinc concentrations.

Source		$ZnSO_4$			ZnOrg			RSD[2]	P[2]		
Zinc level ($mg \cdot kg^{-1}$)	0	10	20	30	10	20	30		Linear	Quadratic	Source
No. of pigs	3	4	4	4	3	4	4				
$mg \cdot d^{-1}$ Zn intake[1]	16.8	23.1	26.1	32.6	22.1	25.9	30.8				
Supplemental Zn intake[1]	0.0	6.6	9.9	15.9	5.6	9.7	14.2				
Initial weight (kg)	9.25	9.23	8.87	9.22	8.92	8.72	9.08				
Final weight (kg)	17.8	18.2	17.5	17.8	17.3	16.8	17.8	0.8	0.60	0.08	0.36
ADFI ($g \cdot d^{-1}$)[1, 3]	590	580	568	585	581	567	581	14	0.97	0.24	0.63
ADG ($g \cdot d^{-1}$)[3]	463	474	455	449	444	427	460	28	0.81	0.12	0.38
Feed/gain	1.27	1.22	1.25	1.31	1.31	1.33	1.27	0.08	0.70	0.31	0.45
Plasma Zn ($mg \cdot L^{-1}$)[5, 7]	0.263	0.384	0.634	0.695	0.413	0.550	0.695	0.076	<0.001	<0.05	0.62
AP activity ($U \cdot L^{-1}$)[6, 7]	37	92	164	157	95	132	168	24	<0.001	<0.01	0.76
Bone Zn ($mg \cdot kg^{-1}$ FFDM)[4, 8]	66	66	91	103	58	83	89	13	<0.001	0.29	0.21
Zn ($mg \cdot kg^{-1}$ ash)	122	122	173	192	107	150	170	21	<0.001	0.26	0.17
Bending moment (N·m)	1.39	1.34	1.55	1.50	1.44	1.32	1.44	0.21	0.53	0.20	0.44
Liver Zn ($mg \cdot kg^{-1}$ DM)[4, 8]	112	116	131	156	105	127	157	13	<0.01	0.98	0.85

[1]For the whole 19-day experimental period, for a 91% DM diet.
[2] RSD: residual standard deviation; *P*: probability.
[3] ADFI: average daily feed intake; ADG: average daily gain.
[4] FFDM: fat-free dry matter; DM: dry matter.
[5] Adjusted means for an initial plasma Zn concentration of 0.598 $mg \cdot L^{-1}$.
[6] Adjusted means for an initial alkaline phosphatase activity of 223 $U \cdot L^{-1}$.
[7] Contrast: basal diet vs. diets supplemented with 10 ppm Zn, $P < 0.05$.
[8] Contrast: basal diet vs. diets supplemented with 10 ppm Zn, $P > 0.10$.

the Zn sources ($P > 0.10$). Retained zinc ($mg \cdot d^{-1}$ or $mg \cdot kg^{-1}$ BWG) increased linearly ($P < 0.01$) with supplemental zinc intake, regardless of the source. The addition of 10, 20 and 30 $mg \cdot kg^{-1}$ of zinc increased the amount of zinc retained ($mg \cdot d^{-1}$) by 27, 70 and 100%, respectively.

3.7. Zn balance

The results of the Zn balance are presented in Table IV.

Fecal zinc excretion increased linearly ($P < 0.001$) with supplemental Zn intake. There was a quadratic effect ($P < 0.05$) of supplemental ingested Zn on the urinary Zn excretion. However, urinary Zn only represented 2% of the total zinc excretion which linearly increased ($P < 0.001$) with supplemental Zn intake. Similarly, the amounts of zinc absorbed and retained linearly increased ($P < 0.001$) with the amount of supplemental zinc intake. Regardless of the dietary zinc level or source, an average of 27% of the total zinc intake was retained by the pigs. Neither fecal or urinary excretion of Zn nor absorption or retention were affected by the source of Zn supplementation.

Table III. Effect of dietary zinc level and source on empty body zinc concentration and retained zinc estimated by the comparative slaughter technique.

Source		$ZnSO_4$			ZnOrg			RSD[2]	P [2]		
Zinc level (mg·kg^{-1})	0	10	20	30	10	20	30		Linear	Quadratic	Source
No. of pigs	3	3	3	3	3	3	3				
Zn EB (mg·kg^{-1} DM)[3]	41.6	45.1	47.0	49.9	41.8	47.4	49.7	3.4	<0.01	0.61	0.67
Zn retained (mg·d^{-1})[1]	2.27	3.51	4.01	4.65	2.24	3.70	4.44	0.83	<0.01	0.56	0.86
Zn retained (mg·kg^{-1} BWG)[1, 3]	5.49	7.65	9.30	10.52	5.52	8.71	10.04	1.90	<0.01	0.37	0.88

[1]For the whole 19-day experimental period, for a 91% DM diet.
[2]RSD: residual standard deviation; *P*: probability.
[3]EB: empty body; BWG: body weight gain.

Table IV. Effect of dietary zinc level and source on zinc balance.

Source		$ZnSO_4$			ZnOrg			RSD[2]	P [2]		
Zinc level (mg·kg^{-1})	0	10	20	30	10	20	30		Linear	Quadratic	Source
No. of pigs	3	4	4	4	3	4	4				
Feed intake (g·d^{-1})[1]	781	779	766	781	794	770	777	11	0.50	0.23	0.75
Zinc (mg·d^{-1}) Intake[1]	22.8	31.1	35.3	43.6	30.2	35.1	41.1				
Supplemental intake[1]	0.0	8.9	13.4	21.3	7.6	13.2	19.0				
Fecal excreted	16.7	21.7	26.2	29.9	20.4	24.7	30.1	1.6	<0.001	0.64	0.53
Urinary excreted	0.675	0.424	0.529	0.551	0.571	0.864	0.557	0.217	0.70	<0.05	0.14
Total excreted	17.4	22.2	26.8	30.4	21.0	25.6	30.6	1.6	<0.001	0.67	0.41
Apparently absorbed	6.2	9.4	9.0	13.7	9.8	10.4	11.1	1.7	<0.001	0.61	0.51
Retained	5.5	8.9	8.5	13.1	9.2	9.6	10.5	1.7	<0.001	0.58	0.40
% of intake Apparent absorption	26.9	29.9	25.5	31.5	32.4	29.7	26.9	4.6	0.72	0.70	0.70
Retention	23.9	28.6	24.1	30.2	30.5	27.3	25.6	4.7	0.52	0.67	0.57

[1] For the 8-day collection period.
[2] RSD: residual standard deviation; *P*: probability.

4. DISCUSSION

4.1. Growth performance, plasma zinc concentration and AP activity, bone and liver zinc concentration

In our study, the zinc content of the basal diet (28 mg·kg^{-1}) was appropriate for a maximum growth of piglets weighing between 9 and 18 kg. Similar results were previously reported by Hill et al. [10]. In contrast, an improved growth rate of the piglets of similar live weight was observed by Adeola et al. [1] by adding 100 mg·kg^{-1} of Zn in a corn-soybean meal diet containing 23 mg·kg^{-1} of Zn. Cheng et al. [7] observed inconsistent responses to the addition of Zn on growth performance between 2 trials when pigs fed corn-soybean meal diets containing 30.5 and 33.5 mg·kg^{-1} of Zn. As pointed out by these authors, this difference may originate from several dietary factors, mainly phytic acid and Ca

concentrations as well as from the initial Zn status of the animals and the duration of the experiment. Some biochemical indicators of Zn status are more responsive to Zn deficiency than growth performance in large domestic animals [2]. In pigs, zinc deficiency is characterized by serum and bone zinc concentrations from 0.18 to 0.25 mg·L^{-1} and 60 to 90 mg·kg^{-1} DM, respectively [28]. These values are close to the 0.263 mg·L^{-1} for plasma zinc and the 66 mg·kg^{-1} FFDM in bone we measured in pigs fed the basal diet. As previously reported with pigs and other species [11, 16, 35, 36], plasma and bone zinc concentrations were commonly used for assessing Zn requirements because the response to dietary Zn intake increases linearly and presents an inflexion point when Zn intake meets the requirement [11, 36]. These indicators were relevant for the estimation of the relative bioavailability value (RBV) of the sources only with Zn levels below the inflexion point [3, 35]. Nevertheless, some Zn sources were previously compared with dietary Zn supply above this point [7, 29, 32]. All the indicators of Zn status we investigated in this study, except growth performance and bone bending moment, responded linearly to the dietary Zn level. As previously reported by Pallauf et al. [22–24], our study confirms that the Zn requirement is above 56 mg·kg^{-1} Zn in complex diets.

However, when pigs were supplemented with 10 mg·kg^{-1} Zn, bone and liver zinc concentrations were unresponsive because Zn stores may have been completely depleted and the remaining zinc, part of tissue structure, could have not been released. The comparison for bone and liver zinc contents (mg·pig^{-1}), by a contrast analysis of the experimental pigs slaughtered at the end of the experiment, to the control pigs slaughtered at the beginning of the experiment corroborated this assumption. The bone Zn content (mean ± SD) was 0.232 ± 0.036, 0.145 ±0.012, 0.134 ±0.034, 0.184 ±0.039, 0.224 ±0.016 mg·pig^{-1} and the liver Zn content was 13.1 ±3.1, 11.2 ±0.7, 11.6 ±1.2, 13.2 ±2.1, 16.1 ±5.3 mg·pig^{-1} for the control and the experimental pigs fed 0, 10, 20 and 30 mg·kg^{-1} Zn diets, respectively. It decreased, significantly ($P < 0.01$) for bone and numerically for the liver, when the pigs were fed the 0 and 10 mg·kg^{-1} Zn diets. On the contrary, it tended to increase ($P = 0.06$) in the liver and remained unchanged in the bone for the pigs fed the 20 mg·kg^{-1} and 30 mg·kg^{-1} diets.

In contrast, other metabolic functions and/or zinc stores, like plasma zinc concentration and AP activity were responsive to the addition of 10 mg·kg^{-1} Zn in the basal diet. However, Bobilya et al. [4] with neonatal pigs and Brown et al. [5] with rats, observed that bone zinc concentration reached a plateau after plasma zinc concentration did during zinc depletion trials.

4.2. Zn retention evaluated by the balance technique and by the comparative slaughter technique

Our balance study indicates that the mean Zn retention amounted to 27% of Zn intake, regardless of the dietary zinc level or source. This result is in agreement with the coefficients of retention of 18 to 30% previously measured according to a similar method in pigs weighing between 9 and 35 kg and fed diets containing between 27 and 72 mg·kg^{-1} [1, 26]. Estimated by the comparative slaughter technique (CST), the pigs fed the diets supplemented with 30 mg·kg^{-1} of zinc retained 10.3 mg·kg^{-1} BWG. This value was slightly below the value of 12 mg·kg^{-1} BWG that can be derived from the mineral composition of pigs from weaning to 20 kg of body weight published by Mahan and Shields [14]. This slight difference is likely to originate from the difference in Zn content of the diets that met or exceeded the NRC [18] requirements for Zn in the study conducted by Mahan and Shields [14] in contrast to the current study.

We used two methods, BT and CST, to calculate the Zn retention, expressed as either mg·d^{-1} or mg·kg^{-1} BWG. Both measurements increased linearly with Zn intake (Tabs. III and IV) and were linearly correlated as described in Figure 1. However, Zn retention measured by CST was lower by 54 and 43% to the value obtained by BT, expressed as mg·d^{-1} and mg·kg^{-1} BWG, respectively. Two main phenomena may be involved in this difference. First, an incomplete collection of excreta, mainly feces, may have occurred with the BT. However, to explain such a difference between the two techniques, the amount of Zn excreted would have been underestimated by 16%, which seems high. Secondly, the CST lasted the whole 19-day experimental period whereas the BT was implemented during the last 8 days. Possibly, due to a progressive reduction of endogenous losses of Zn in response to Zn deficiency, the daily amount of Zn retained was increased by the end of the experimental period.

4.3. Bioavailability of Zn sources

Our study failed to demonstrate a better availability of ZnOrg compared to $ZnSO_4$. In pigs, similar observations are reported in the literature with diets containing Zn levels below the requirements [10, 34, 36] as well as with diets containing pharmacological Zn levels [9, 31]. In chicks, inconsistent results were published. In the study conducted by Mohanna and Nys [16], an organic source of Zn complexed with methionine exhibited the same availability as $ZnSO_4$, whereas Wedekind et al. [35] observed that the bioavailability of a Zn-methionine complex relative to $ZnSO_4$ increased with the presence in the diet of antagonistic factors, mainly phytate and fiber. Recently, the interaction between Zn and phytate was confirmed in chicks by Swiatkiewicz et al. [33]. They observed that the difference in bioavailability of zinc complexed with amino acids relative to $ZnSO_4$, although it remained over 100%, was reduced by the addition of microbial phytase in a high phytate and Ca diet (1.24 and 0.92%, respectively). The authors pointed out that microelements in an organic form are better protected from forming indigestible complexes with phytic acid than those in an inorganic form. Relying on their own studies [35, 36], Wedekind et al. suggested that the inconsistency in the results obtained with chicks compared to pigs may be attributed more to differences in

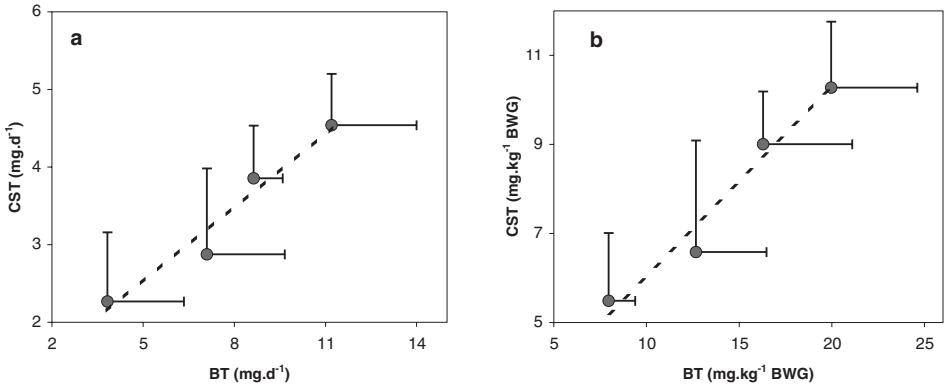

Figure 1. Regression of retained Zn estimated by the balance technique (BT) on retained Zn estimated by the comparative slaughter technique (CST); (a) retained Zn (mg·d^{-1}): Y = 0.32X + 0. 93 (R^2 = 0.95); (b) retained Zn in mg·kg^{-1} of body weight gain (BWG): Y = 0.42X + 1.88 (R^2 = 0.96).

phytate and Ca contents in diets rather than to differences in the species. Indeed their chick diets contained 0.59% phytate and 1.1% Ca, whereas the phytate and Ca contents of the pig diets were lower, 0.29 to 0.37% and 0.58 to 0.78%, respectively. Therefore, in our study, the absence of a difference between the sources, ZnOrg and $ZnSO_4$, may originate from the quite low phytate concentration of our diets (0.29%).

In conclusion, although a specific difference between pigs and poultry regarding the advantages of organic sources of zinc cannot be excluded, the effect of the presence of high amounts of phytate and Ca in pig diets on the bioavailability of ZnOrg relative to $ZnSO_4$ is worth being further investigated.

ACKNOWLEDGEMENTS

The authors wish to thank S. Hillion, F. Pontrucher, Y. Jaguelin, F. Le Gouëvec and H. Renoult for their technical assistance and C.H. Lacroix and A. Pointillart (INRA, LNSA, Jouy-en-Josas) for bone strength analysis.

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