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Effects of reducing copper and zinc supplementation on the performance and mineral status of fattening pigs

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ABSTRACT

Pig manure with high copper (**Cu**) and zinc (**Zn**) concentration is applied to the soil, and these trace minerals can accumulate in the topsoil and decrease its fertility. Thus, adjusting concentrations of Cu and Zn in pig diets below current maximum allowance can prevent this risk. Reduction of dietary concentrations of Cu and Zn reduces their faecal excretion since only a small portion is retained in the pig's body. The aim of this study was to evaluate the effects of reducing concentration of dietary Cu and Zn or withdrawing their supplementation on the performance and mineral status of fattening pigs. Four dietary treatments were compared: a basal diet (**WS**; withdraw supplementation), with no Cu or Zn supplementation (5 and 29 mg/kg of native Cu and Zn, respectively); intermediate concentration ($\mathbf{O_{INT}}$), supplemented with Cu and Zn oxides to obtain mean dietary concentration of 7.4 and 47.5 mg/kg of Cu and Zn, respectively; and two diets supplemented with oxides ($\mathbf{O_{REG}}$) or sulphates ($\mathbf{S_{REG}}$) at concentration similar to European Union limits (i.e. 25 and 120 mg/kg of total Cu and Zn, respectively), as commonly used on commercial farms. Ninety-six pigs (24.3 ± 3.3 kg BW) were each assigned to one of the four treatments and reared in individual pens for 14 weeks (up to 110.3 ± 8.9 kg BW). Animal performances were measured, and samples of plasma (on day 1 and day 41 of experimentation and at slaughter), bones and the liver (at slaughter) were collected from all pigs. Faecal samples were collected from all pigs every 3 weeks to determine the Cu and Zn excretion. Over the entire experiment, neither the concentration nor the source of Cu and Zn influenced feed intake, BW or the feed conversion ratio. Plasma Cu and Zn concentrations were not influenced by the treatment but increased as the age of the pigs increased. Liver Cu concentration increased ($P < 0.05$) as dietary concentrations increased (O_{REG}> WS). Neither the concentration nor the source of Cu and Zn influenced bone Cu and Zn concentration or physical bone parameters. However, S_{REG} had a higher maximum load until bone breaking ($P < 0.05$) than $O_{REG.}$ As expected, faecal excretion of Cu and Zn decreased ($P < 0.01$) as dietary concentration decreased. Dietary Cu and Zn can be reduced without decreasing the performance or mineral status of pigs, and these results should be validated on commercial farms that have more challenging health conditions.
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Implications

Copper and zinc are essential nutrients for pigs and are thus added to their diets. However, more than 90% of ingested copper and zinc is excreted in manure, which is commonly spread on agricultural soil, where they can accumulate and cause negative environmental impacts. Reducing their concentrations in pig diets can decrease the amounts excreted. Under the conditions of this study, it was shown that dietary copper and zinc can be reduced without decreasing the performance or mineral status of pigs.

Introduction

Copper (**Cu**) and zinc (**Zn**) are added to pig diets to meet their nutritional requirements, which are estimated for fattening pigs at 5–6 mg and 50 mg of Cu and Zn per kg of feed, respectively (National Research Council, 2012). To forestall antagonistic effects

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with elements of the feed ration (such as calcium or phytate) and considering that exact requirements are not well established, Cu and Zn are supplemented in pig feed (Suttle, 2010). In general, more than 90% of ingested Cu and Zn are excreted in pig manure (Jondreville et al., 2004; Dourmad et al., 2015), and pig manure can contain up to 1 000 and 2 000 mg of Cu and Zn per kg DM, respectively (Dourmad et al., 2002; Jondreville et al., 2002; Marcato, 2007). Direct field application of stored manure is a common management practice for pig waste, but it results in the accumulation of Cu and Zn in the soil (Gross et al., 2021), which may negatively impact soil microbial activity (McGrath, 1981; López Alonso et al., 2000) and, in the medium term, reduce crop yields. Several strategies can reduce the environmental impacts of Cu and Zn in pig farming systems, including reducing their concentrations in feed to reduce the amounts excreted. For the past several years, European Union (**EU**) regulations (Commission implementing regulation (EU) (2016/1095 and 2018/1039)) have imposed significant limits on concentrations of Cu and Zn in pig feed (currently 25 and 120 mg/kg of as-fed Cu and Zn, respectively, in fattening pig diets), but this regulation may further reduce maximum authorised dietary levels. Several studies have shown the potential to reduce Cu and Zn in pig feed without compromising animal performance (Peter et al., 2001; Ma et al., 2018; Ding et al., 2021), especially in piglets. Further research is required, as few studies have been conducted on growing and finishing pigs and tested such low dietary levels of Cu and Zn as in the present study or supplementation withdrawal.

The objective of this study was to evaluate the influence of reducing Cu and Zn concentrations in feed below current EU limits on the growth performance and Cu and Zn status of fattening pigs, as well as Cu and Zn excretion in faeces. Our hypothesis was that pig performance and Cu and Zn status can be maintained by feeding Cu and Zn at very low concentrations close to the estimated requirements (National Research Council, 2012). We analysed an oxide source of Cu and Zn that has been few studied in fattening pigs before. We compared this source to a reference sulphate source that contained the same dietary levels of Cu and Zn.

Material and methods

The study was conducted from January to May 2022 at the Pig Physiology and Phenotyping Experimental Facility (https://doi.org/10.15454/1.5573932732039927E12) of the French National Research Institute for Agriculture, Food and the Environment located in Saint-Gilles, France. Ethical approval according to French legislation on experimental animal care was approved by the Ethics Committee on Animal Experimentation in Rennes, France (authorisation for living animals no. 2021041318055831).

Experimental diets

Four dietary treatments were formulated and compared. All diets were based on the same basal grower and basal finisher diets (Table 1), which is a meal without any supplementation and that is commonly used in the experimental unit. A premix (the same for all dietary treatments) without any Cu and Zn supplementation was used and a first mix of the premix with Cu and Zn supplementation products (copper sulphate ($CuSO_4$) and zinc sulphate ($ZnSO_4$) containing 25 and 35% of Cu and Zn, respectively, and oxides Cu_2O and ZnO (CoRouge® and Hizox®, respectively; Animine, France) containing 75% of Cu and Zn, respectively) was proceed to obtain eight different premixes corresponding to the four dietary treatments and the two fattening phases (growing phase and finishing phase). Then, the premix was added to the basal diet with different mixing steps (mix of the premix with a portion of the basal diet, then mix of the mixture with another portion of the basal diet etc...) in order to obtain homogenous mixing. Table 2 gives the amount of supplementation used for each treatment per kg feed. The first treatment (**WS**=withdraw supplementation) contained no Cu or Zn supplementation (4.8 and 5.1 mg total Cu/kg feed and 29.0 and 29.6 mg total Zn/kg feed for growers and finishers, respectively). The second, intermediate treatment (**O_{INT}**) was supplemented with Cu and Zn oxides (Cu_2O and ZnO; CoRouge® and Hizox®, respectively; Animine, France) to obtain dietary concentrations of 7.8 and 7.1 mg Cu/kg feed and 44.2 and 50.2 mg Zn/kg feed for growers and finishers, respectively. Formulation of this O_{INT} diet was based on a simulation of Cu and Zn flows through a feed–animal–excretion–treatment chain to obtain the maximum Cu and Zn concentrations allowed in fertilisers for organic farming (Ecolabel, 100 mg Cu and 300 mg Zn per kg DM), which are lower than those allowed for conventional farming (300 mg Cu and 800 mg Zn per kg DM (Regulation (EU) 2019/1009)) (Gourlez et al., 2023). The remaining diets were supplemented with oxides (**O_{REG}**) or sulphates (**S_{REG}**) to obtain concentrations close to EU limits, which are commonly used on commercial farms in the EU. The mineral concentrations in the diets for each phase were analysed (Table 2). The diets were fed to pigs as pellets.

Table 1
Ingredients and composition of the basal pig diet for the grower and finisher phases (as-fed basis).

Item	Grower	Finisher
Ingredients, g/kg		
Wheat	319.1	338.2
Maize	150.0	150.0
Barley	250.0	250.0
Wheat bran	50.0	80.0
Rapeseed	70.0	90.0
Soya bean cake	115.0	55.0
Plant oil	10.0	10.0
Calcium carbonate	12.0	8.6
Monocalcium phosphate	1.9	0.0
Salt	4.5	4.0
Lysine	7.4	6.2
PM-Valine	0.6	0.0
DL-Methionine	0.7	0.3
L-Threonine	1.7	1.2
Tryptophan	1.1	0.5
Acidifier	1.0	1.0
Vitamin–trace mineral mix[1]	5.0	5.0
Calculated composition		
DM, g/kg	877.6	876.7
Crude fat, g/kg	29.9	30.5
NDF, g/kg	151.7	164.2
ADF, g/kg	53.1	56.8
Net Energy (NE), Kcal	2 304	2 303
Digestible Energy (DE), MJ	3 208	3 171
Phytase, FTU[2]	850	500
Analysed composition		
DM, g/kg	883.4	888.0
Ash, g/kg	44.8	44.2
CP, g/kg	127.0	115.6
P, g/kg	4.2	3.9
Ca, g/kg	6.8	5.8
K, g/kg	7.4	6.7
Mg, g/kg	1.6	1.6
Na, g/kg	1.9	1.5
Fe, mg/kg	164.2	141.2

Abbreviations: FTU=phytase unit.
[1] Premix without Cu or Zn supplementation Composition of the premix (per kg of diet): vitamin A, 5 000 IU; vitamin D, 1 000 IU; vitamin E, 20 mg; vitamin B1, 2 mg; vitamin B2, 4 mg; calcium pantothenate, 10.0 mg; niacin, 15 mg; vitamin B12, 0.02 mg; vitamin B6, 1 mg; vitamin K3, 4.35 mg; folic acid, 1 mg; biotin, 0.2 mg; choline chloride, 667 mg; iron (sulphate), 80 mg; iodine (calcium), 0.2 mg; and selenium (selenite), 0.15 mg.
[2] Phytase source = phytase G5000.

Table 2
Quantity of added product and copper and zinc total concentrations of the experimental pig diets.

Phase and element	Treatment[1]			
	WS	O_{INT}	O_{REG}	S_{REG}
Amount of added product[2]				
Grower				
Cu, mg/kg feed	0	6.3	19.6	58.8
Zn, mg/kg feed	0	25.2	105	225
Finisher				
Cu, mg/kg feed	0	5.6	25.6	76.8
Zn, mg/kg feed	0	31.2	111	238
Total concentrations (analysed)				
Grower				
Cu, mg/kg	4.8	7.8	18.2	18.3
Zn, mg/kg	29.0	44.2	102	95.9
Finisher				
Cu, mg/kg	5.1	7.1	22.0	22.5
Zn, mg/kg	29.6	50.2	105	101

[1] WS=Withdraw supplementation of Cu and Zn; O_{INT}=intermediate concentration supplemented with Cu and Zn oxides; O_{REG}=concentrations close to the maximum regulatory concentration authorised by the European Union (EU) and supplemented with Cu and Zn oxides; S_{REG}=concentrations close to the maximum regulatory concentration authorised by the EU and supplemented with Cu and Zn sulphates.
[2] For O_{INT} and O_{REG} treatments, products are added as Cu_2O and ZnO, containing 75 % Cu and Zn; for S_{REG} treatment, products are added as $CuSO_4$ and $ZnSO_4$ containing 25 % Cu and 35 % Zn.

Animals and experimental procedures

The experiment was conducted in two replicates. A total of 96 crossbred pigs (Piétrain, Landrace × Large White; about 70 days of age at 24.3 ± 3.3 kg BW, corresponding to day 1 of the experiment) were each assigned to one of the four treatments in a randomised complete block design by sex and litter origin and reared in individual pens for 14 weeks (until slaughter, day 90 and day 97of the experiment for the first and second replicate, respectively, at 110.3 ± 8.9 kg BW). Each experimental group had an equal number of females and entire males (n = 12 per treatment per sex). Pens (2.65 × 0.85 m) were separated by steel rods and had solid concrete floors. Manure in the pen was scraped out every morning. The pigs were fed according to a feeding plan based on their metabolic BW (180 g/kg $BW^{0.6}$ per day, corresponding to a diet close to the animal voluntary feed intake). All pigs in a given replicate changed feed at the same time: they were fed the grower diets from 24.3 ± 3.3 kg to 57.2 ± 6.2 kg (day 1 to day 41 of the experiment) and then fed the finisher diets until slaughter (until day 90 (replicate 1) or 97 (replicate 97) of the experiment). Feed was distributed once per day during the growing phase and twice per day during the finishing phase. Feed refusals were weighed every week. Pigs were weighed every two weeks to adjust the amount of feed fed daily. The room temperature was maintained at 20–22 °C. At the end of the experiment and after overnight fasting, the experimental pigs were slaughtered by electro-immobilisation and exsanguination.

Data and sample collection

Diet samples were taken every week, and average samples of grower and finisher diets were pooled at the end of the experiment. The two samples were ground in a blender (1 mm) and stored at 4 °C until analysis. Faecal samples were collected every 3 weeks to determine the Cu and Zn excretion (weeks 4, 7, 10 and 13). Faecal samples were spot samples and were taken by rectal sampling and manual stimulation from all pigs. Sampling time did not exceed 5 min, in compliance with the ethical rules of experimentation and one sample was taken per pig per timepoint. The faeces were freeze-dried, ground in a blender and stored at 4 °C until analysis. A blood sample was collected from each pig by puncturing the jugular vein using 10-ml heparinised vacutainer tubes at the beginning of the experiment (day 1), at the grower-finisher transition (day 41) and at slaughter (day 90 or 97). Plasma was obtained via centrifugation (2 000 × g, 10 min, 4 °C), and the supernatant was frozen at −20 °C until analysis.

After slaughter, the liver and front feet were immediately removed. The liver was weighed, and the entire right paramedian lobe was sampled, freeze-dried and ground and stored at 4 °C until analysis. The right foot was autoclaved at 120 °C for 20 min to facilitate the removal of muscle and connective tissue from the extracted metacarpals III and IV. Bones were then frozen at −20 °C before fat was extracted from the intact bone in anhydrous ethyl ether (24 h). After the remaining ether evaporated, the fat-free bone was dried at 103 °C for 16 h (Revy et al., 2005). Samples were then incinerated at 550 °C for 18 h in a muffle furnace to determine the ash content. Samples were then ground and stored at 4 °C until analyses. No heat treatment was applied to the left foot, from which the two metacarpals (III and IV) were removed and dissected free of soft tissues. The bones of the left foot were then stored at −20 °C until 24 h before analysis, when they were transferred to 4 °C (Revy et al., 2005; Schlegel and Gutzwiller (2020)). Bone mineral content and bone mineral density of the left metacarpals III and IV were measured using dual-energy X-ray absorptiometry (GE Healthcare iDXA, GE Medical Systems, Glattbrugg, Switzerland). The calibration of the device was checked and passed by scanning a calibration phantom according to the manufacturer's instructions. The acquisition mode used in the enCORE software (version 18) was "Small Animal – Small" (100 kV, 0.188 mA). Images were processed to place the bones on the right arm region of interest for each metacarpal. The bones' maximum load until breaking was then measured using a three-point bending test (Zwick Roell, Ulm, Germany). Bones were held by two supports spaced 45 mm apart and were broken by a wedge lowered on the centre of the bone at a speed of 10 mm/min, with a maximum pressure of 2 500 N. The force was measured by a pressure-sensitive cell, and peaks of maximum force and the maximum work required to reach this maximum force were recorded.

Chemical analyses of samples

For feed, bone and faecal samples, DM concentrations were quantified by heating the samples at 103 °C for 24 h, and ash content and organic matter were subsequently determined after incineration at 550 °C for 6 h (Commission regulation (EC) No 152/2009). The Dumas method was used to determine the nitrogen content of the feed using a rapid nitrogen analyser (Elementar, Lyon, France) according to the Association of Official Analytical Chemists (AOAC, 1990). Mineral analyses were performed on samples of diets (ground sample), blood plasma, metacarpals III and IV of the right foot (ash from samples), liver (lyophilised and ground sample) and faeces (lyophilised and ground sample). Phosphorus, calcium, magnésium, potassium, sodium, iron, Zn, Cu and manganese were analysed using an inductively coupled plasma optical emission spectrometer (ICP-OES, Agilent 5110, Coutaboeuf, France), according to NF-EN 15621 (for feed, bone and liver) and NF-EN 16174 (for faeces). All analyses were performed in duplicate at INRAE laboratories.

Validation of methods used

Intra-assay CV of nitrogen content of the feed determined with the Dumas method was 0.44%; inter-assay CV was 0.43%. Intra-assay CV of Cu concentration determined with inductively coupled plasma optical emission spectrometer (ICP-OES) in diets, plasma,

liver, bones and faeces was 4.2,2.1, 2.5, 4.8 and 3.8%, respectively; inter-assay CV was 3.2, 1.4, 2.3, 5.4 and 9.0%, respectively. Intra-assay CV of Zn concentration determined with inductively coupled plasma optical emission spectrometer (ICP-OES) in diets, blood, plasma, liver and faeces was 2.4, 2.2, 1.5, 1.3 and 4.1%, respectively; inter-assay CV was 1.6, 1.5, 1.4, 1.2 and 9.2%, respectively.

Calculations and statistical analyses

Average daily gain (**ADG**), average daily feed intake (**ADFI**) and feed conversion ratio (**FCR**) were calculated for each pig over the growing phase, finishing phase and the entire experiment based on pig BW, the amount of feed distributed and the amount of feed refused. Total excretion of Cu and Zn (g) was calculated for the entire experiment based on digestibility of the DM measured in each diet in another experiment using the same experiment diets (Gourlez et al., 2024: 85.2 and 84.7% for WS, 84.7 and 85.8% for O_{INT}, 84.0 and 84.8% for O_{REG} and 83.3 and 85.3% for S_{REG} for growers and finishers, respectively), feed intake and faecal Cu and Zn concentrations.

Statistical analyses were performed using R software (version 4.1.2, R Core Team, 2021). Block effects of the experimental design were analysed and were not significant. Linear mixed-effects models were used with the "lm" function of the "nlme" package. Data on animal performance, liver concentrations and bones were analysed using the following mixed model:

$$Y_{ijk} = \mu + Treatment_i + Sex_j + Replicate_k + Treatment_i \times Sex_j + Treatment_i \times Replicate_k + Sex_j \times Replicate_k + e_{ijk}$$

with Y_{ijk} the analysed variable; μ the overall mean; $Treatment_i$ the fixed effect of the dietary treatment; Sex_j the fixed effect of the sex; $Replicate_k$ the fixed effect of the replicate; $Treatment_i \times Sex_j$, $Treatment_i \times Replicate_k$ and $Sex_j \times Replicate_k$ are the two-way interaction of each effects and e_{ijk} the residual error term.

Data on plasma and faecal concentrations were analysed using the following mixed model:

$$Y_{ijkl} = \mu + Treatment_i + Sex_j + Time_k + Treatment_i \times Sex_j + Treatment_i \times Time_k + Sex_j \times Time_k\, Pig_l + e_{ijkl}$$

with Y_{ijkl} the analysed variable; μ the overall mean; $Treatment_i$ the fixed effect of the dietary treatment; Sex_j the fixed effect of the sex; $Time_k$ the fixed effect of the day of sampling; $Treatment_i \times Sex_j$, $Treatment_i \times Time_k$ and $Sex_j \times Time_k$ are the two-way interaction of each effects; Pig_l the random effect of the pig and e_{ijkl} the residual error term. Differences were considered significant at $P < 0.05$ and considered trends for P of 0.05–0.10. For each model built, every hypothesis was validated (independence of the residuals, normality of the distribution of the residuals, homogeneity of the residuals). Pairwise comparisons were tested according to a Tukey test.

Results

For results, least squares mean are shown with SEs from the statistical model.

Growth performance and carcass characteristics

Four pigs (one from WS, two from O_{REG} and one from S_{REG}) were removed from the study due to death (2) or abnormal feed intake and ADG (one with an ADG of 217 g/day at the dietary transition compared to an average of 802 g/day for other pigs of the experiment and one with an ADG of 631 g/day at the end of the experiment compared to an average of 1 022 g/day for other pigs of the experiment). Growth performances are presented in Table 3. Over the entire experiment, neither the concentration nor the source of Cu and Zn in the feed influenced ADFI, BW, ADG or FCR ($P > 0.05$) for females. Carcass characteristics were similar among the treatments ($P > 0.05$).

Over the entire experiment, males had higher ADG and lower FCR than females did ($P < 0.05$). During the different phases studied (Growing phase, finishing phase and overall phase), the ADG of males was influenced by the treatment but that of females was not (treatment × sex, $P < 0.01$). During growing phase, male fed with WS had a better ADG and FCR than those fed with O_{REG} and during finishing phase, male fed with WS had a better ADG and final BW (at slaughter) than those fed with other three treatments. Females had higher carcass yield and lean meat percentage than males ($P < 0.01$).

Plasma zinc and copper

Reducing Cu and Zn concentrations in the feed did not significantly influence plasma Cu or Zn concentration ($P > 0.10$, Fig. 1) at day 41 or at slaughter. Plasma Cu and Zn concentrations did not differ significantly between the oxide and sulphate sources. The mean plasma concentration of Cu increased from day 41 to slaughter ($P < 0.01$) (from 1.69 to 1.86 mg/L for the average of the four treatments) but did not differ between day 1 and day 41 ($P > 0.10$). The mean plasma concentration of Zn increased from day 1 to slaughter (e.g. 0.80–1.09 mg/L, for the average of the four treatments).

The plasma concentration of Zn was not influenced by the sex of the pig ($P = 0.320$). In contrast, females had higher plasma concentration of Cu ($P < 0.05$) than males over the entire experiment (1.82 vs 1.70 mg/L, respectively).

Bone copper and zinc concentrations and physical parameters

Neither the concentration nor the source of Cu and Zn influenced the DM, ash or Cu and Zn concentrations, or the bone mineral content or bone mineral density, of metacarpals III or IV ($P > 0.10$) (Table 4). However, the maximum load until breaking of metacarpal IV was higher ($P < 0.05$) for S_{REG} than for O_{REG}. No significant effects on the other physical parameters (i.e. elasticity and maximum work) were observed. The Zn concentration, bone mineral density of metacarpal III and metacarpal IV, and elasticity and maximum work of bones of females were higher ($P < 0.01$) than those of males.

Liver copper and zinc concentrations

Neither the concentration nor the source of Cu and Zn influenced liver weight or Zn concentration (Table 5). Liver Cu concentration increased ($P < 0.05$) as the dietary Cu concentration increased. In fact, liver Cu concentration was 26% higher for pigs fed the O_{REG} diet compared to the WS diet, while that of pigs fed the S_{REG} diet did not differ from that of pigs fed the WS diet. Males had a heavier liver and higher Cu concentration in the liver than females did ($P < 0.01$).

Faecal copper and zinc concentrations

Faecal Cu and Zn concentrations in DM decreased significantly ($P < 0.01$) as dietary Cu and Zn concentrations decreased (Fig. 2), and the source of supplementation had no influence. For pigs fed with diet WS and O_{INT}, no difference was shown with the age of animal for Cu and Zn concentrations in faeces ($P > 0.05$). For pigs fed with O_{REG}, Cu concentrations in faeces increased significantly

Table 3
Influence of dietary copper and zinc concentrations on growth performance and carcass characteristics in growing-finishing pigs.

	Male				Female					*P*-value[1]		
Item	WS	O_{INT}	O_{REG}	S_{REG}	WS	O_{INT}	O_{REG}	S_{REG}	RMSE	T	S	T×S
Number of pigs	12	12	11	12	12	11	11	11				
Grower phase (day 0 –41)												
Duration, day	41	41	41	41	41	41	41	41				
Initial BW, kg	24.4	23.6	23.4	23.0	24.1	25.1	25.8	25.4	3.0	0.98	0.04	0.48
Final BW, kg	59.7	57.4	54.8	54.9	56.4	56.8	59.8	58.0	5.5	0.85	0.40	0.10
ADFI, kg/day	1.69	1.65	1.61	1.60	1.68	1.69	1.75	1.72	0.12	0.91	< 0.01	0.23
ADG, g/day	861[a]	824[ab]	764[b]	778[ab]	788	775	830	794	78	0.48	0.59	0.04
FCR, kg/kg	1.96[a]	2.01[ab]	2.12[b]	2.09[ab]	2.15	2.21	2.12	2.16	0.12	0.26	< 0.01	0.05
Finisher phase (day 41 – slaughter)												
Duration, day	51.5	51.5	51.8	51.5	52.4	52.5	52.8	52.2				
Final BW, kg	118.4[a]	112.5[ab]	108.5[b]	108.5[b]	107.0	106.3	112.6	108.2	7.2	0.33	0.05	< 0.01
ADFI, kg/day	2.81	2.73	2.69	2.67	2.73	2.71	2.80	2.75	0.12	0.47	0.48	0.06
ADG, g/day	1 141[a]	1 071[b]	1 044[b]	1 041[b]	964	946	1 002	964	57	0.04	< 0.01	< 0.01
FCR, kg/kg	2.47	2.56	2.58	2.57	2.83	2.88	2.80	2.86	0.13	0.36	< 0.01	0.36
Overall phase (day 0 – slaughter)												
Duration, day	92.5	92.5	92.8	92.5	93.4	93.5	93.8	93.2				
ADFI, kg/day	2.31	2.25	2.21	2.20	2.27	2.27	2.34	2.30	0.12	0.67	0.07	0.09
ADG, g/day	1 017[a]	962[ab]	920[b]	925[b]	887	871	927	891	55	0.08	< 0.01	< 0.01
FCR, kg/kg	2.28[a]	2.35[ab]	2.41[b]	2.38[b]	2.56	2.61	2.52	2.58	0.09	0.11	< 0.01	0.02
Carcass characteristics												
Carcass weight, kg	93.7[a]	88.8[ab]	86.6[ab]	85.3[b]	86.5	85.7	90.8	88.1	5.9	0.30	0.54	0.01
Carcass yield, %	76.2	76.0	77.0	75.7	78.0	77.9	78.3	78.4	1.1	0.29	< 0.01	0.29
Lean meat percentage (LMP), %	62.1	61.8	62.4	62.2	62.8	62.5	63.6	63.1	1.2	0.21	< 0.01	0.89

Abbreviations: ADFI=average daily feed intake; ADG=average daily gain; FCR=feed conversion ratio; WS=Withdraw supplementation of Cu and Zn; O_{INT}=intermediate concentration supplemented with Cu and Zn oxides; O_{REG}=concentrations close to the maximum regulatory concentration authorised by the European Union (EU) and supplemented with Cu and Zn oxides; S_{REG}=concentrations close to the maximum regulatory concentration authorised by the EU and supplemented with Cu and Zn sulphates.
[a-b]Values within a row with different superscripts differ significantly at $P<0.05$ (Tukey test).
[1] T=effect of treatment; S=effect of sex; T×S=interaction treatment × sex.

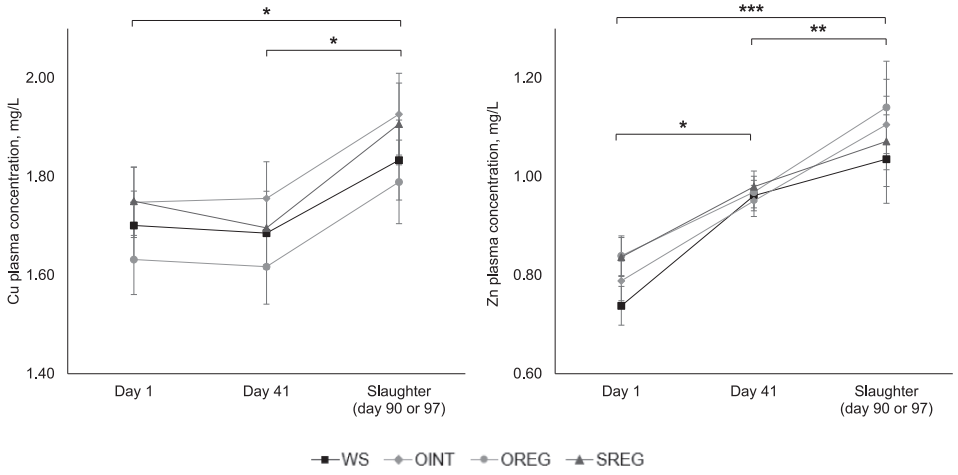

Fig. 1. Cu (left) and Zn (right) plasma concentrations in pigs fed different dietary concentrations and sources of Cu and Zn at different days of sampling. WS=Withdraw supplementation of Cu and Zn; O_{INT}=intermediate concentration supplemented with Cu and Zn oxides; O_{REG}=concentrations close to the maximum regulatory concentration authorised by the European Union (EU) and supplemented with Cu and Zn oxides; S_{REG}=concentrations close to the maximum regulatory concentration authorised by the EU and supplemented with Cu and Zn sulphates; treatment had no significant effect ($P > 0.10$) on plasma Cu or Zn; sex had no significant effect ($P > 0.10$) on plasma Zn but had a significant effect on plasma Cu ($P < 0.05$) with a higher plasma Cu concentration for females compared to males (1.8 vs 1.7 mg Cu /L); time (days of sampling) had a significant effect ($P < 0.05$) on plasma Cu and Zn. SDs correspond to the SE of the mean from the statistical model. *,**,*** Values with different asterisks differ significantly at $P < 0.05$ (Tukey test).

between weeks 7 and 10 of sampling ($P < 0.05$) and stayed constant between weeks 10 and 13 of sampling and Zn concentrations in faeces differed significantly ($P < 0.05$) between week 4 of sampling and week 13. For pigs fed with $S_{REG,}$ Cu concentrations in faeces increased significantly between weeks 7 and 10 of sampling ($P < 0.05$) and remained constant between weeks 10 and 13 of sampling no difference between weeks of sampling were shown for Zn concentrations ($P > 0.05$).

Likewise, total Cu and Zn excretion (calculated based on digestibility of the DM measured in each diet in another experiment using the same experiment diets, Gourlez et al., 2024) decreased significantly ($P < 0.05$) as dietary concentrations decreased, but the source had no influence. Over the entire experiment, total faecal excretion were 1.11 ± 0.05 g Cu and 6.5 ± 0.8 g Zn per pig for pigs fed WS, 1.81 ± 0.08 g Cu and 10.4 ± 1.1 g Zn per pig for pigs fed O_{INT}, 4.47 ± 0.17 g Cu and 21.3 ± 1.8 g Zn per pig for

Table 4
Bone ash and mineral concentrations, and physical bone parameters of fattening pigs as a function of dietary copper and zinc concentrations, supplement source and sex.

	Treatment[1]				Sex[2]			*P*-value[3]		
Item	WS	O_{INT}	O_{REG}	S_{REG}	F	M	RMSE	T	S	T×S
Number of pigs	24	23	22	23	45	47				
DM, g/kg	617	628	634	634	642	614	40	0.50	< 0.01	< 0.01
Ash, g/kg DM	561	562	534	550	555	549	41	0.20	0.57	0.55
Cu										
Concentration, mg/kg DM	0.329	0.322	0.328	0.307	0.308	0.334	0.072	0.65	0.12	0.88
Concentration, mg/kg ash	0.587	0.572	0.619	0.561	0.557	0.611	0.133	0.52	0.07	0.73
Zn										
Concentration, mg/kg DM	91.2	92.4	91.6	94.6	95.8	89.2	10.8	0.81	< 0.01	0.93
Concentration, mg/kg ash	163	165	172	172	173	162	15	0.14	< 0.01	0.99
Metacarpal III										
Bone mineral content, g	4.77	4.96	5.02	4.72	5.04	4.70	0.93	0.77	0.09	0.26
Bone mineral density, g/cm^2	0.357	0.375	0.372	0.359	0.383	0.349	0.056	0.71	< 0.01	0.54
Metacarpal IV										
Elasticity, N/mm	313	300	277	311	326	276	71	0.34	< 0.01	0.23
Maximum load, N	1 173 ^{ab}	1 131 ^{ab}	1 066 ^{a}	1 231 ^{b}	1 156	1 147	174	0.03	0.68	0.36
Maximum work (Wmax), J	5.60	5.35	5.42	5.18	5.10	5.67	0.99	0.67	0.02	0.42
Bone mineral content, g	4.63	4.81	4.66	4.56	4.79	4.55	0.83	0.81	0.18	0.06
Bone mineral density, g/cm^2	0.362	0.379	0.367	0.364	0.384	0.352	0.053	0.73	< 0.01	0.40

[a-b]Values within a row with different superscripts differ significantly at $P<0.05$.
[1] WS=Withdraw supplementation of Cu and Zn; O_{INT}=intermediate concentration supplemented with Cu and Zn oxides; O_{REG}=concentrations close to the maximum regulatory concentration authorised by the European Union (EU) and supplemented with Cu and Zn oxides; S_{REG}=concentrations close to the maximum regulatory concentration authorised by the EU and supplemented with Cu and Zn sulphates.
[2] F=female; M=male.
[3] T=effect of treatment; S=effect of sex; T×S=interaction treatment × sex.

Table 5
Liver weight and copper and zinc concentrations as a function of dietary copper and zinc concentrations, supplement source and sex for fattening pigs.

	Treatment[1]				Sex[2]			*P*-value[3]		
Item	WS	O_{INT}	O_{REG}	S_{REG}	F	M	RMSE	T	S	T×S
Number of pigs	24	23	22	23	45	47				
Liver weight, kg	2.07	2.04	1.95	1.98	1.91	2.11	0.21	0.257	< 0.01	0.630
Cu, mg/kg DM	29.9 ^{a}	32.5 ^{ab}	40.3^{b}	31.5 ^{ab}	29.5	37.2	12.2	0.04	< 0.01	0.980
Zn, mg/kg DM	189	208	210	202	208	196	41	0.341	0.164	0.845

[a-b]Values within a row with different superscripts differ significantly at $P < 0.05$ (Tukey test).
[1] WS=Withdraw supplementation of Cu and Zn; O_{INT}=intermediate concentration supplemented with Cu and Zn oxides; O_{REG}=concentrations close to the maximum regulatory concentration authorised by the European Union (EU) and supplemented with Cu and Zn oxides; S_{REG}=concentrations close to the maximum regulatory concentration authorised by the EU and supplemented with Cu and Zn sulphates.
[2] F=female; M=male.
[3] T=Influence of treatment; S=Influence of sex; T×S=interaction treatment × sex.

pigs fed O_{REG}, and 4.18 ± 0.19 g Cu and 21.7 ± 1.9 g Zn per pig for pigs fed S_{REG}.

Discussion

Effects of dietary copper and zinc concentrations and their source on growth performance

Over the entire experiment, performances of males fed WS (i.e. ADFI, ADG, FCR and final BW) were numerically (but not significantly) higher than those of pigs fed the other treatments (O_{INT}, O_{REG} and S_{REG}). This is not the case for females. ADG differed significantly between dietary treatments during the finishing phase and tended to do so over the entire experiment. This difference appears especially for males and not for females. We hypothesise this difference may be due to the hormonal status of the pigs and this result requires further study about sex effect. In comparison, Ding et al. (2021) observed no significant difference in animal performance when reducing Cu and Zn concentration in diets compared with regulation levels but noted that groups of pigs (25–60 kg BW) fed 4 mg/kg Cu also had higher ADG than those fed 20 mg/kg Cu (numerical difference, 665 vs 641 g per day, respectively). In the study of Ding et al. (2021), there is no indication if they whether or not added phytase to the pig diet, it may have an influence on Zn absorption.

Results of the present study indicate that supplementing concentrations of Cu and Zn lower than the EU maximum limits, or even withdrawal supplementation, maintains growth performance and carcass quality. Only a few studies have evaluated the effects of such low dietary Cu and Zn concentrations on fattening pigs. Similar results have been observed after removing or reducing Cu and Zn supplementation in grower–finisher diets (Peter et al., 2001; Ma et al., 2018; Ding et al., 2021). In the present study, the dietary Cu concentration was close to the National Research Council (2012) requirement in the WS diet and exceeded it in the other diets. In comparison, dietary Zn concentration was less than the requirement of the National Research Council (2012) in the WS diet (29 vs 50 mg/kg feed) but met or exceeded the requirement in the other diets. Adding phytase to the diet increases the bioavailability of native Zn, which according to Schlegel (2010) is equivalent to 40 and 29 mg of Zn added as sulphate (based on the equivalence equation of Jondreville et al., 2005: Zn [mg] = 59.3–58*EXP(−0.00127 * FTU)) in the present study's grower and finisher

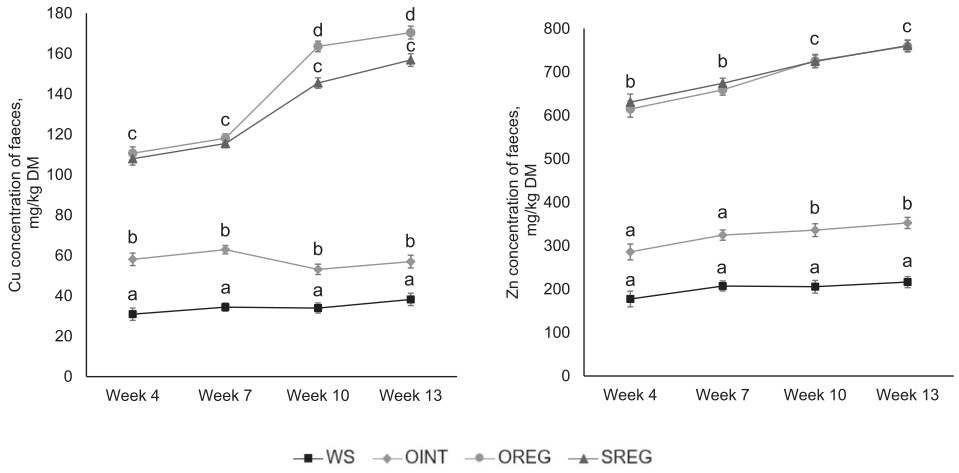

Fig. 2. Cu (left) and Zn (right) concentrations in faeces of pigs fed different dietary concentrations of Cu and Zn at different weeks of sampling. WS=Withdraw supplementation of Cu and Zn; O_{INT}=intermediate concentration supplemented with Cu and Zn oxides; O_{REG}=concentrations close to the maximum regulatory concentration authorised by the European Union (EU) and supplemented with Cu and Zn oxides; S_{REG}=concentrations close to the maximum regulatory concentration authorised by the EU and supplemented with Cu and Zn sulphates; time (weeks of sampling) had a significant effect ($P < 0.05$) on Cu and Zn in faeces: no difference of the age on Cu concentration in faeces for WS and O_{INT} but Cu concentration differs significantly between week 1 and 2 and week 10 and 13 ($P < 0.05$) for pigs fed with O_{REG} and S_{REG}; no difference of the age on Zn concentration in faeces for WS, O_{INT} and S_{REG} but Zn concentration differs significantly between week 1 and week 13 ($P < 0.05$) for pigs fed with O_{REG}. SDs correspond to the SE of the mean from the statistical model. $^{a-c}$ For each timepoint of sampling, values with different letters differ significantly at $P < 0.05$ (Tukey test).

diets, respectively (corresponding to 850 and 500 FTU, respectively). Given this equivalency, all treatments met the pigs' Zn requirement.

Nevertheless, this practice requires adequate knowledge of basal diet concentrations before applying it to pigs. In fact, the native Cu and Zn concentration may change according to the ingredients (cereals) used in the basal diet and may be lower than the basal levels used in the present study. Moreover, it is important to know if the diet is a phytase–enriched diet or not, influencing Zn absorption. As pigs in this study were housed under good sanitary conditions without competition for feed, these results must be evaluated on commercial farms that have more challenging health conditions. In fact, the strict requirements estimated by the National Research Council (2012) are estimated in good sanitary situations. A sanitary constraint may involve more stress or impact microbiota, leading to possible diarrhoea and a higher requirement in Cu and Zn to maintain the health of pigs. Furthermore, it is important to notice that all pig farms do not have the same level of sanitary conditions.

Effects of dietary copper and zinc concentrations and their source on mineral status

Cu and Zn requirements are evaluated through an empiric approach consisting of experimentally testing the effects of gradually increasing dietary Cu and Zn concentrations in order to evaluate the response of several zootechnical or physiological parameters. Statistical processing of the results using a linear plateau model is then generally used to describe the response and define the breakpoint requirement. (Brugger et al., 2022). A parameter is considered characteristic of the animal's Cu or Zn status when this specific response is obtained (Kirchgessner, 1993; Schlegel et al., 2013, Brugger et al., 2022). For Cu, the parameter used to evaluate its requirement is its concentration in the liver (Brugger et al., 2022). For Zn, the most representative parameter usually used is its concentration in bone or plasma. According to the literature, the plasma Zn of piglets increases as Zn in the diet increases and reaches a stable homeostasis when dietary Zn exceeds the requirement (Kirchgessner, 1993; Gourlez et al., 2022), unless pharmacological supplements are provided, which increases plasma Zn greatly. The results obtained in the present study indicate that feeding lower concentrations of Cu and Zn maintained the Cu and Zn status of fattening pigs, even when feeding the lowest concentrations of Cu and Zn (WS treatment), corresponding to native Cu and Zn.

The plasma Cu and Zn concentrations observed in the present study agreed with those in the literature (i.e. 1.71 mg Cu and 0.81 mg Zn per L at day 1, 1.86 mg Cu and 1.09 mg Zn per L at slaughter). Plasma Cu and Zn concentrations lay within the normal physiological range (1.3–3.0 mg Cu and 0.7–1.5 mg Zn per L; Puls, 1994). In a meta-analysis, Bikker et al. (2012) observed plasma concentrations of 1.65 mg Cu and 0.74 mg Zn per L for growing pigs fed a maximum dietary Cu supplement of 20 mg/kg, a maximum dietary Zn supplement of 100 mg/kg and 500 phytase units. Nevertheless, plasma Zn increased over time as animal BW increased. Okwonko et al. (1979) observed an increase in plasma Cu in piglets from 2 to 10 weeks of age.

Cu and Zn concentrations in bones measured in the present study agreed with those in the literature for similar concentrations of dietary Cu and Zn (Hernández et al., 2008; Schlegel and Gutzwiller, 2020). The physical parameters of bone (i.e. maximum load until breaking, maximum work to reach this maximum load and elasticity) were lower in the present study than those in the literature. Schlegel and Gutzwiller (2020) measured the same parameters of the same bones of growing-finishing pigs and observed mean bone mineral content and bone mineral density twice as high as those in the present study. They also observed higher maximum loads until bone breaking (1 580–1 766 N) than those in the present study (1 066–1 231 N). One reason for the differences in bone mineralisation between these two studies may be that pigs in the present study were housed in individual pens, whereas Schlegel and Gutzwiller (2020) housed their pigs in a single large pen on deep straw litter. We thus hypothesise that physical activity promotes mineralisation and strengthens the physical properties of bone, as shown in the study of Weiler et al., 2006. In the present study, the dietary treatment did not influence the physical parameters of bones, except for the maximum load until breaking, which was higher for pigs fed S_{REG} than for those fed O_{REG}. This result was unexpected because no other differences were shown on the bones. In fact, it would have been possible to imagine that maximum load until breaking could have been related to bone mineral density or bone ash content. The literature

shows there is a good correlation between bone mineralisation and bone bending moment (Crenshaw et al., 1981). Thus, this result requires further study.

Liver Cu concentrations in the present study lay within the range of those observed in the literature (Hernández et al., 2008; Ma et al., 2018). Pigs fed O_{REG} had significantly higher liver Cu concentration (40.3 ± 14.0 mg/kg DM) than those fed WS (29.9 ± 15.9 mg/kg DM), as also reported by Hernández et al. (2008). From data obtained under commercial conditions for a large number of pigs of different origins and ages, Hodges and Fraser (1983) observed high variability in liver Cu concentration. They concluded that Cu concentrations lower than 12 mg/kg DM could induce the risk of Cu deficiency, but that most pigs had Cu concentrations greater than 20 mg/kg DM, indicating supra-nutritional Cu intake. Considering this result from Hodges and Fraser (1983) and the liver concentration obtained in the present study, we may consider that all diets provided supra-nutritional Cu intake.

We observed no significant difference between the sources of Cu and Zn in the diet. The literature shows that the form of Cu and Zn supplemented in the diet can influence their absorption efficiency (Suttle, 2010). Nevertheless, the difference in Cu and Zn bioavailability in oxides and sulphates is very low (Schlegel et al., 2013; Brugger et al., 2022), which may explain the lack of difference between sources in the present study. Blavi et al. (2021) observed that the addition of Cu as Cu_2O with 250 mg Cu /kg feed conducted to a lower accumulation of Cu in the liver compared to the addition of Cu as $CuSO_4$ with 250 mg Cu /kg feed. We did not observe that result in the present study, but Cu and Zn concentrations were very low, it was thus complicated to observe relevant source effects at such levels. In fact, these low dietary levels of Cu and Zn may stimulate absorption mechanisms and hiding eventual source effect (Villagómez-Estrada et al., 2020). Several studies highlighted this source effect but with higher levels of Cu and Zn (Villagómez-Estrada et al., 2020).

Sex had a significant effect on plasma Cu, bone Zn and physical bone characteristics, with higher values observed for females than for entire males. Bernau et al. (2020) evaluated bone mineralisation in male pigs comparing entire boars and castrated boars. They observed significant differences in the physical bone characteristics of animals (Bone mineral content and bone mineral density) with higher values for castrated males and suggested that the result is due to the hormonal status of the pigs. Lower bone mineralisation may induce an impact on bone conformation and induce more pigs with lameness (Bernau et al., 2020). Conversely, liver Cu was higher for males than for females. Kirchgessner et al. (1994) observed no difference in carcass Cu or Zn concentrations (in lean meat, blood and internal organs) between sexes of fattening pigs fed 18 mg Cu and 113 mg Zn per kg feed, and Ruiz-Ascacibar et al. (2019) found the same results. Jondreville et al. (2004) observed higher body composition in Cu at slaughter for females than for castrated males, perhaps because the latter have a higher final BW (Jondreville et al., 2004; Ruiz-Ascacibar et al., 2019).

Effects of dietary copper and zinc concentrations and their source on faecal excretion

In the present study, faecal Cu and Zn concentrations decreased as dietary concentrations decreased. Over the entire experiment, Cu concentrations in faeces decreased by 70 and 54% for pigs fed WS and O_{INT}, respectively, compared to those fed regulatory concentrations (O_{REG} or S_{REG}), while Zn concentrations in faeces decreased by 62 and 50%, respectively. The literature provided Cu and Zn concentrations in pig manure (faeces + urine) of about 200–220 mg Cu/kg DM (Levasseur and Texier, 2001; Liu et al., 2016; Ding et al., 2021) and 1 050–1 300 mg Zn/kg DM for fattening pigs fed Cu and Zn concentrations close to EU limits, which corresponded to treatments O_{REG} and S_{REG} (Van Heugten et al., 2004; Liu et al., 2016; Ding et al., 2021). In the present study, lower average faecal Cu and Zn concentrations of the several sampling at different timepoint were observed (i.e. 128–136 mg Cu/kg DM and 725–738 mg Zn/kg DM over the entire experiment for O_{REG} and S_{REG}), perhaps due to the sampling and storage conditions of the faeces and slurry. Faecal samples in the present study were frozen until analysis, whereas samples taken after storage in a slurry pit have emitted gases, which decreases the DM content and thus increases Cu and Zn concentrations in the DM (Popovic and Jensen, 2012).

In the present study, pigs excreted most of the Cu and Zn ingested, but absorbed Zn and Cu was enough to increase plasma concentrations with time, regardless of the treatment. The source of supplemental Cu and Zn in the feed did not influence Cu and Zn excretion, as little Cu or Zn was retained. Low levels of Cu and Zn stimulate absorption mechanisms making it more effective (Villagómez-Estrada et al., 2020), which may be the reason for the absence of source effect in the present study. These results show that reducing dietary Cu and Zn had a direct effect on Cu and Zn excretion and demonstrate the relevance of unbalanced diet as an environmental issue. There is a need to better characterise the native content of Cu and Zn in the basal diet as they may be enough to fulfil animals' requirements in some phases.

Conclusion

To date, only a few studies have tested such low concentrations of Cu and Zn or their withdrawal from supplementation in the diets of fattening pigs. Our results support that pig performance and Cu and Zn status can be maintained by feeding Cu and Zn at concentrations lower than EU limits, or even by withdrawing Cu and Zn supplementation, while also reducing their concentrations in faeces. Nevertheless, this practice requires adequate knowledge of basal diet concentrations before applying it to pigs. It is, in fact, important to know if the diet is a phytase enriched diets or not, increasing the bioavailability of native Zn and thus influencing its absorption. Moreover, as pigs in this study were housed under good sanitary conditions without competition for feed, these results must be evaluated on commercial farms that have more challenging health conditions. Dietary Cu and Zn can be reduced without influencing the performance of fattening pigs, which can provide a huge environmental benefit by greatly decreasing Cu and Zn concentrations in pig manure.

Ethics approval

Ethical approval according to French legislation on experimental animal care was approved by the Ethics Committee on Animal Experimentation in Rennes, France (authorisation for living animals no. 2021041318055831).

Data and model availability statement

None of the data were deposited in an official repository. The data that support the study findings are available from the authors upon request and after authorisation by all authors.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

This study was carried out in partnership with Animine company, which supplied the oxide sources of Cu and Zn studied.

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