



## Two different forms and levels of $\text{CuSO}_4$ in piglet feeding: liver, plasma and faeces copper status

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**ABSTRACT.** A 35-d experiment involving 150 crossbred pigs weaned at  $26 \pm 2$  d of age was conducted to evaluate the effect of different forms of  $\text{CuSO}_4$  on the copper content of liver, plasma and faeces. Piglets were randomly allocated to five dietary groups with increasing supplementation of protected and unprotected Cu in the diet as follows: 0 ppm (CON), 75 ppm Cu unprotected ( $\text{Cu } 75_{\text{UNP}}$ ), 75 ppm protected ( $\text{Cu } 75_{\text{P}}$ ), 150 ppm unprotected ( $\text{Cu } 150_{\text{UNP}}$ ) and 150 ppm protected ( $\text{Cu } 150_{\text{P}}$ ). After 18 days of the experimental trial, 30 piglets were sacrificed to determine liver and plasma copper status. Faecal samples were taken for determination of copper content at the end of the whole experimental trial on the 35<sup>th</sup> day. The highest liver Cu concentration ( $P < 0.001$ ) was found in  $\text{Cu } 150_{\text{UNP}}$  compared with all other groups. The plasma Cu concentration showed a significant ( $P < 0.05$ ) increase in the  $\text{Cu } 150_{\text{P}}$  vs CON group. Groups Cu 150, either in the protected or unprotected form, showed a higher content in faeces compared with the CON and Cu 75 groups. The protection did not negatively influence bioavailability at Cu 75 ppm integration.

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### Introduction

Pigs require copper (Cu) at concentrations no greater than 5 to 6 ppm in their diet (NRC, 2012). Due to the elevated daily gain and feed efficiency arising from the inclusion of large amounts of dietary copper (100–200–250 ppm) (Coffey et al., 1994; Cromwell et al., 1989, 1998) it is common practice to use high concentrations of dietary Cu in pig production, up to the limit imposed by the European Union (Commission Regulation (EC) No. 1334/2003). Adequate amounts of Cu and Zn are generally added to pig diets *via* a premix. Cu has some antibacterial properties that may explain its

growth-promoting effect, but there is a lack of scientific evidence to explain its exact mode of action (Jacela et al., 2010). Other researchers have reported that copper enhances growth through a systemic, rather than antimicrobial, effect in the intestinal tract (Zhou et al., 1994). The idea that supplemental copper may be acting systemically is supported by the wide variety of biological systemic functions of copper. If a systemic effect is the more likely mode of action, then nutritionists and the feed industry need to develop ways to improve the efficacy of delivering copper into the circulation.

On the other hand, limiting the pharmacological inclusion of Cu in the diet is an approach that

some countries have enforced, even setting a maximum allowable total mineral concentration in pig feed. The majority of dietary Cu is excreted in the manure, thus contributing to the accumulation of this element in soil and surface waters (Bikker et al., 2012). A factor that may affect micromineral requirements is the form of the mineral added to the diet (Martin et al., 2011).

The present study was designed to determine the copper content in liver, plasma and faeces in piglets fed with two different forms of copper and its levels in the post-weaning period. Moreover, we hypothesized that copper in a protected form may reduce the quantity of active principle administered.

## Material and methods

### Animals, dietary treatments and experimental procedures

All procedures involving animals were in accordance with the European Community guidelines (n. 86/609/CEE) and approved by the Italian Ministry of Health (L. n. 116/92).

At weaning, 150 female piglets (Landrace × Large White;  $8.4 \pm 1.08$  kg of body weight (BW), 26 days old) were randomly allocated to five experimental dietary groups (3 pens per diet with 10 piglets per pen) according to initial BW. The five dietary treatments consisted of a basal no-Cu-supplemented diet (CON) with increasing supplementation of unprotected (UNP) and protected (P) copper. The latter consisted of copper sulphate that was microencapsulated in a protective matrix of hydrogenated vegetable lipids using spray cooling technology (Sintal Zootechnica, Isola Vicentina, Vicenza, Italy). The different concentrations of copper supplemented as  $\text{CuSO}_4$  in the two-phase feeding regimes from d 0–d 18 and d 18–d 35 were as follows: 0 ppm (CON), 75 ppm Cu unprotected ( $\text{Cu } 75_{\text{UNP}}$ ), 75 ppm protected ( $\text{Cu } 75_{\text{P}}$ ), 150 ppm unprotected ( $\text{Cu } 150_{\text{UNP}}$ ) and 150 ppm protected ( $\text{Cu } 150_{\text{P}}$ ). None of the diets was medicated. The innate calculated content of the control diet was 10 mg for the first phase (Sauvant et al., 2004).

The animals were housed in an environmentally controlled nursery and given *ad libitum* access to water and meal diet (Table 1).

Individual body weights and feed intake (FI) were recorded on days 0, 18 and 35. Based on these data, average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (feed intake:weight gain, FCR) were calculated. After 18 days of experimental trial, 30 piglets (six piglets/treatment, 2 from each repetition) were randomly

**Table 1.** Composition of diets,  $\text{g} \cdot \text{kg}^{-1}$  as-fed basis)

Indices	Phase 1 8–15 kg BW	Phase 2 15–30 kg BW
Ingredients		
steam-rolled maize	280	1
maize, yellow	150	200
barley	150	200
wheat middlings	80	80
dried whey	50	20
soya protein concentrate	40	44
soyabean meal, 48	60	80
fish meal, 70	28	–
rice protein meal, 65	24	20
dextrose	25	10
wheat bran	30	80
soya oil	30	30
vitamin-mineral premix <sup>1</sup>	35	35
dicalcium phosphate	10	14
L-lysine-HCl	5	4
preservative <sup>2</sup>	3	3
Calculated chemical composition <sup>3</sup> , $\text{g} \cdot \text{kg}^{-1}$		
crude protein	206.1	195.4
ether extract	83.3	75.6
crude fibre	30.9	39.9
ash	61.3	65.9
lysine	13.1	11.9
methionine + cysteine	7.9	7.2
threonine	8.5	7.7
tryptophan	2.6	2.4

<sup>1</sup>provided per kg of complete diet, g: Ca – 2.8, P – 0.14, Na – 1.33; vitamins, IU: A – 16000, D<sub>3</sub> – 2000, E – 175; mg: K (menadione sodium bisulphite) – 3.8, B<sub>1</sub> – 4.9, B<sub>2</sub> – 9.8, calcium D-pantothenate – 40, niacin – 57.8, B<sub>12</sub> – 0.09, B<sub>6</sub> – 7.7, folic acid – 3.4, biotin – 0.33, choline chloride – 1000.0, Zn (ZnO) – 100.0, Cu ( $\text{CuSO}_4$ ) – 0, 75, 150 mg for control and treated groups; Mn (MnO) – 108.0, Fe ( $\text{FeCO}_3$ ), 270.0, I (KI) – 3.85, Co ( $\text{CoSO}_4$ ) – 1.40, Se ( $\text{Na}_2\text{SeO}_3$ ) – 0.49. Premix containing calcium formate, *Saccharomyces cerevisiae*, sodium chloride, barley, butyric acid, dl-tryptophan, dl-methionine, l-treonine

<sup>2</sup> composition per kg of complete feed, g: formic acid – 0.3, lactic acid – 1.1, colloidal silica carrier 1.6

<sup>3</sup> calculated based on Sauvant et al. (INRA, 2004)

selected and sacrificed. The slaughter procedure included stunning with a bolt gun followed by exsanguination. At slaughtering, blood samples were obtained from the anterior vena cava into 10 ml heparinized (143 units of sodium heparin per tube) vacutainers (Venoject, Terumo Europe N.V., Leuven, Belgium). Livers were dissected, weighed and collected from each animal for further determination of Cu concentration. The blood was centrifuged (10 min, 2100 g), and plasma was isolated and stored at  $-20^\circ\text{C}$  until Cu measurements. Performance data of the remaining animals were registered until the 35<sup>th</sup> day after the start of the trial.

Faecal samples were taken directly from the floor of each barn at the end of the experimental trial after 35 days of dietary Cu ingestion. At least 10

faecal sub-samples were taken from different areas of the unit floor. Sub-samples of each pen/treatment were then pooled and thoroughly mixed to provide one representative sample/treatment of approximately 0.5 kg for analysis.

### Faeces, plasma and liver copper analysis

Approximately 0.5 g faeces were mineralized in a Milestone 1200 (FKV, Bergamo, Italy) microwave system using 3 ml nitric acid (65% SPA grade, Romil, Cambridge, UK). The samples were diluted to 50 ml by addition of bi-distilled water. Plasma samples (1 ml) were mineralized in the same microwave system using 1 ml nitric acid (65% SPA grade). The samples were then diluted to 20 ml using bi-distilled water. Cu determinations were carried out using an inductively coupled plasma atomic emission spectrophotometer Ultima 2 (Horiba Jobin Yvon, Milan, Italy).

Cu determinations in liver samples were carried out by inductively coupled plasma optical emission spectrometry as described by Lopez et al. (2007). Samples were homogenized, mineralized and diluted according to the MP-1289-R4/06 method. All analyses were performed in duplicate. Liver Cu is expressed on a tissue DM basis. The accuracy of the methods was tested by analysing certified human plasma (SRM 1950) and bovine liver (SRM 1577c) from the National Institute of Standards and Technology (Canada).

### Statistical analysis

Performance data were performed using one-way ANOVA with dietary treatment as a fixed effect. Blood and liver Cu contents were analysed in a completely randomised design with an individual pig as the experimental unit. Faeces Cu content was analysed by ANOVA. Differences among means were determined using a Student-Newman-Keuls test. Significance was declared at  $P < 0.05$  and levels of 5%–10% ( $0.05 < P < 0.10$ ) were considered a trend. All analyses were performed using SPSS software (SPSS/PC Statistics 18.0, 2009).

## Results

Throughout the study there were no mortalities and no pigs needed to be removed for poor performance or health reasons.

Over the entire experimental period (1–35 d), growth of the piglets did not show any significant difference among the treatments (Table 2). Pigs fed Cu<sub>150</sub> in both forms of Cu showed numerically higher ADG than the other groups. At 18 days, piglets fed the highest level of Cu in the unprotected form tended to improve ( $P = 0.07$ ) FCR.

Table 3 summarizes the results in the liver and plasma at d 18. Concerning the liver, neither its absolute weight nor that related to BW were influenced by the dietary treatments. Liver Cu was not increased in pigs fed 75 ppm Cu or 150 ppm as Cu 150<sub>p</sub>. The highest Cu concentration in the liver was found in the Cu 150<sub>UNP</sub> group ( $P < 0.001$ ).

**Table 2.** Growth performance of piglets in response to dietary copper inclusion

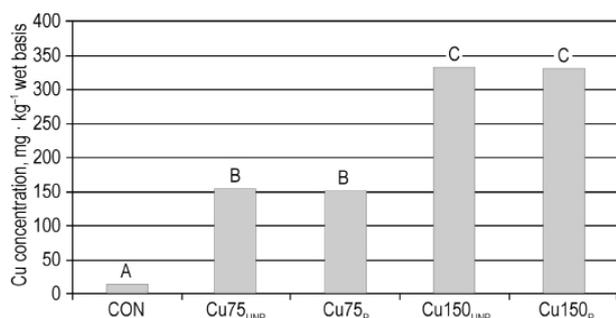
Indices	Experimental diets					SEM	P-value
	CON	75 <sub>UNP</sub>	75 <sub>p</sub>	150 <sub>UNP</sub>	150 <sub>p</sub>		
Body weight, kg							
0 d <sup>1</sup>	8.44	8.48	8.43	8.43	8.42	0.09	1.00
18 <sup>1</sup> d	12.26	12.47	12.37	12.55	12.08	0.15	0.89
35 <sup>2</sup> d	19.43	19.64	19.50	20.40	20.09	0.34	0.81
ADG, g · d <sup>-1</sup>							
0 d–18 d <sup>1</sup>	212	221	219	229	203	6.05	0.66
18 d–35 d <sup>2</sup>	420	437	418	453	464	12.77	0.74
0 d–35 d <sup>2</sup>	314	320	316	344	331	8.68	0.80
ADFI, g · d <sup>-1</sup>							
0 d–18 d	554	457	456	374	446	20.57	0.22
18 d–35 d	633	743	807	814	690	30.78	0.31
0 d–35 d	596	600	620	594	554	15.55	0.81
FCR, kg · kg <sup>-1</sup>							
0 d–18 d	2.61 <sup>a</sup>	2.07 <sup>ab</sup>	2.08 <sup>ab</sup>	1.63 <sup>b</sup>	2.20 <sup>ab</sup>	0.113	0.07
18 d–35 d	1.51	1.70	1.93	1.80	1.49	0.67	0.20
0 d–35 d	1.89	1.87	1.96	1.72	1.68	0.05	0.42

CON = 0; 75<sub>p</sub> = 75 ppm as copper protected; 75<sub>UNP</sub> = 75 ppm as copper unprotected; 150<sub>p</sub> = 150 ppm as copper protected; 150<sub>UNP</sub> = 150 ppm as copper unprotected; <sup>1</sup>n = 30; <sup>2</sup>n = 24; SEM – standard error of the mean; ADG – average daily gain; ADFI – average daily feed intake; FCR – feed conversion ratio

**Table 3.** Effect of dietary copper on liver weight and copper concentration (mean ± SE) in liver (DM basis) and plasma of pigs after 18 d treatment

Indices	CON	Cu 75 <sub>UNP</sub>	Cu 75 <sub>p</sub>	Cu 150 <sub>UNP</sub>	Cu 150 <sub>p</sub>	P-value
BW at slaughter, kg	12.18	12.31	12.31	12.06	11.73	0.809
Liver weight, g	253 ± 11.76	256 ± 16.52	275 ± 12.65	273 ± 9.21	254 ± 9.91	0.523
Liver, % BW	2.07	2.07	2.23	2.26	2.16	0.447
Copper, mg liver	21.24 <sup>A</sup> ± 3.70	15.81 <sup>A</sup> ± 1.93	23.34 <sup>A</sup> ± 4.57	48.50 <sup>B</sup> ± 6.52	32.06 <sup>A</sup> ± 4.53	<0.001
Cu in plasma, mg · l <sup>-1</sup>	1.34 ± 0.05 <sup>a</sup>	1.47 ± 0.07 <sup>ab</sup>	1.38 ± 0.08 <sup>ab</sup>	1.52 ± 0.01 <sup>ab</sup>	1.65 ± 0.07 <sup>b</sup>	0.045

BW – body weight; <sup>A,B,C</sup> means with different superscripts differ significantly ( $P < 0.001$ ); <sup>a,b,c</sup> means with different superscripts differ significantly ( $P < 0.05$ );



**Figure 1.** Concentration of copper in faeces of pigs in response to dietary copper integration (0 = CON; 75<sub>p</sub> = 75 ppm as copper protected; 75<sub>UNP</sub> = 75 ppm as copper unprotected; 150<sub>p</sub> = 150 ppm as copper protected; 150<sub>UNP</sub> = 150 ppm as copper unprotected) at the end of experimental period; bars with different letters differ significantly at  $P < 0.001$

Pigs fed Cu at increased levels had numerically higher plasma Cu concentrations compared with the control. Only the Cu 150<sub>p</sub> group showed a significant ( $P < 0.05$ ) increase vs the CON group.

The Cu concentrations in piglet faeces (Figure 1) show that the level of Cu supplementation influenced the concentration of Cu ( $P < 0.001$ ) at the end of the experimental period. In particular, groups receiving Cu 150, either in the protected or unprotected form, showed a higher content in faeces compared with those in the CON and Cu 75 groups, attaining levels 20-fold higher than those of the CON group. The Cu 75 groups showed an intermediate value.

## Discussion

Most pig producers include pharmacological levels of Cu in the post-weaning diet to enhance growth and feed conversion (Hill et al., 2001). In the literature, the effects of dietary copper on growth performance are controversial. Cromwell et al. (1998) reported that 200 ppm Cu from Cu chloride or sulphate improved ADG in piglets from 7.9 to 17.7 kg BW. Armstrong et al. (2004) showed that lower dietary Cu concentrations (125 ppm from either Cu citrate or CuSO<sub>4</sub>) were as effective as 250 ppm of Cu from CuSO<sub>4</sub> at stimulating the growth of weaning pigs over a 45-d nursery period. Zhao et al. (2007) found that 200 ppm Cu from a commercial Cu proteinate complex is an effective growth promoter in pig the first 10 days of the post-weaning period, but has no effect if considered during the overall post-weaning period (35 days) of dietary treatment. Smith et al. (1997) found that 28-d growth performance of weaning pig fed 250 ppm Cu from CuSO<sub>4</sub> on a commercial farm did not differ from that of controls.

The present study was not, however, focused on growth performance of piglets since a low number of replicates was used; therefore a definitive conclu-

sion on the obtained results could be not appropriate. We speculate in accordance with Stansbury et al. (1990) that a response in performance from the addition of Cu should not be expected when animals are healthy or housed in a clean environment.

The main objective of this study was to determine the copper status in liver, plasma and faeces of piglets fed with two different forms of copper and levels in the post-weaning period.

The concentrations of trace minerals in some target organs are often chosen for the assessment of bioavailability. According to Baker and Ammerman (1995), bioavailability is defined as the degree to which an ingested nutrient is absorbed in a form that can be metabolized by the normal animal. This definition stresses that the mineral must be available not only at the dietary level but also at the tissue level. Measurements of Cu bioavailability have traditionally focused on liver Cu concentrations measured even after a short feeding diet (Cromwell et al., 1989, 1998). The order of importance of the response criteria depends on whether the animals are fed suboptimal or adequate levels of Cu. The ranking of importance indicated that liver Cu content ranks highest in both situations (EMFEMA, 2002). Results on copper liver content in the CON group are in agreement with values found by Taranu et al. (2012) in 39-day-old piglets fed a reference diet containing 20 mg of copper (24 mg · kg<sup>-1</sup> DM vs 21.24). A concentration of 28 mg · kg<sup>-1</sup> DM was found in the livers of a control group of piglets fed without added Cu (the basal diet contained 15 mg · kg<sup>-1</sup> of Cu) (Apgar et al., 1995). Our results on the liver Cu content of the CON and Cu<sub>75</sub> groups in both forms are in agreement with those of Cromwell et al. (1998), who found no difference between the control group and pigs fed 100 ppm from CuSO<sub>4</sub> or between the control group and pigs fed 125 ppm (Cromwell et al., 1989), showing control values numerically higher than the treated groups. The higher liver Cu concentrations in pigs fed 150 mg · kg<sup>-1</sup> of Cu<sub>UNP</sub> compared with pigs fed 150 mg · kg<sup>-1</sup> of Cu<sub>p</sub> suggest that Cu from Cu<sub>UNP</sub> was absorbed more efficiently than Cu<sub>p</sub>.

In the present work, Cu<sub>150p</sub> failed to increase liver Cu content, an indication that Cu in this form is less available. Only the unprotected form at the highest dietary concentration showed a more than 2-fold increase of copper content compared with the other groups. This result could be explained by the analytical data provided by the manufacturer, indicating that less than 50% of the Cu in the protected form is soluble in water compared with a Cu solubility of >99% for Cu<sub>UNP</sub> after one hour. At 24 h, the protected Cu shows a solubility of 76%. If no responses are

obtained following Cu supplementation of the diet, it can be presumed that a difference in the solubility of the two forms of Cu in the intestine affects Cu utilization, according to the results of Omole (1980).

In the present study, the liver copper concentration in the 150<sub>UNP</sub> group increased because of dietary copper supplementation, which may indicate that the extra copper was accumulated in the liver without being transported to the circulating blood. In fact, the same group did not differ from the others in plasma concentration.

Copper concentrations in plasma are considered normal in the range of 1.3–3.0 mg · l<sup>-1</sup> (Puls, 1994); in the present work, all piglet values were within the normal range. In the present study, blood Cu levels for each treatment were not different among treatments, except for CON vs Cu150<sub>p</sub>. Shelton et al. (2011) found no dietary effects on plasma Cu levels of piglets fed with 0 or 125 ppm of sulphate Cu in the first 14 days of the experimental trial. In a trial conducted on piglets fed a diet containing CuSO<sub>4</sub> (250 ppm Cu) or Cu-proteinates (50 or 100 ppm Cu), Veum et al. (2004) found that plasma Cu concentrations of weanling pigs numerically increased with increasing dietary levels of Cu proteinates, but there were no differences in plasma Cu concentrations between the treatments. A nearly unchanged Cu concentration in the plasma over a range of Cu intakes may be the result of a homeostatic control mechanism in the animal (Turnlund et al., 1990).

In most species, the majority of the actively excreted dietary Cu is eliminated *via* the bile in the faeces. Most of the Cu excreted in the faeces is unabsorbed Cu (Underwood, 1977). In the present study, Cu concentrations in the faeces reflected their inclusion in the diet but were independent of the form; this is supported by studies with different organic sources in weanling pigs (Apgar and Kornegay 1996; Case and Carlson, 2002; Carlson et al., 2004). It is possible that the dietary concentration of Cu at the highest level used in this experiment, which was in excess of requirements, influenced the excreted fraction. Hence, regardless of the form in which they were fed, the absorption profile was similar to that observed by Dowdy (1969), who established that approximately 30% of Cu intake is absorbed.

## Conclusions

Dietary levels of 75 mg · kg<sup>-1</sup> Cu in both forms (protected and unprotected) significantly reduced the excretion of Cu *via* faeces by approximately 50% compared with a diet containing 150 mg · kg<sup>-1</sup> in the protected or unprotected form.

In respect to the form used, the protected form at the lowest supplementation level (75 mg · kg<sup>-1</sup>) did not influence bioavailability, whereas at the highest supplementation level (150 mg · kg<sup>-1</sup>) it resulted in lower availability. In this trial it has to be emphasised that only a few replicates were used to assess growth performance. To evaluate the growth promoter effect of Cu, a larger sample of animals should be used to confirm the better feed conversion ratio (–10%) and the average daily gain (+5%) found in the present study.

The decrease in Cu excretion found with the lowest inclusion would contribute to the sustainability of pig production when restrictions on nutrient pollution are increased.

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