

## LOW DOSE OF COPPER IS MORE EFFECTIVE ON INTESTINAL MORPHOLOGY IN WEANED PIGS

*(Baixa dose de cobre é mais eficaz na morfologia intestinal de leitões desmamados)*

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*Editora de Seção:* Juliana Sperotto Brum

*Editora:* Julia Arantes Galvão

**ABSTRACT** - The present experiment was carried out to determine the relation between pharmacological levels of copper (Cu) (125 and 200 mg/kg) and source (copper sulfate (CuSO<sub>4</sub>) and tribasic copper chloride (TBCC)) on growth performance, diarrhea incidence and intestinal morphology of piglets after weaning. At 21 days of age, 96 weaned piglets were sorted by body weight (BW, 6.14 ± 0.269 kg) and randomly assigned to 4 treatments according to a block design of a 2 x 2 factorial arrangement: level (125 and 200 mg/kg) and source (CuSO<sub>4</sub> or TBCC). Growth performance was evaluated by measuring BW, average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR) from 21 to 63 days of age. Fecal score was evaluated daily and at 63 days of age tissue samples were collected from the duodenum, jejunum and ileum for intestinal morphology analysis. At this moment, it was evaluated villus height (VH), crypt depth (CD) and villus height to crypt depth ratio (VH:CD ratio). There was no difference between level and source (P>0.05) for any growth performance. Piglets fed 200 mg/kg TBCC had the lowest diarrhea frequency (P<0.05) in overall nursery period than the other treatments during the whole experimental period. Supplementation with 125 mg/kg increased VH (P<0.05) and VH:CD ratio (P<0.05) in the duodenum when compared to 200 mg/kg, regardless of the source. Based on our results, we can conclude that 125 mg/kg of Cu improve the VH and VH:CD ratio in the duodenum and may show growth performance results similar to 200 mg/kg of Cu.

**Key words:** Copper sulfate; pharmacological level; tribasic copper chloride; weaning.

**RESUMO** - O presente experimento foi realizado para determinar a relação entre os níveis farmacológicos de cobre (Cu) (125 e 200 mg/kg) e a fonte (sulfato de cobre

(CuSO<sub>4</sub>) e cloreto de cobre tribásico (TBCC)) sobre o desempenho, a incidência de diarreia e morfometria intestinal dos leitões após o desmame. Aos 21 dias de idade, 96 leitões desmamados foram separados pelo peso corporal (PC, 6,14 ± 0,269 kg) e distribuídos aleatoriamente em 4 tratamentos, segundo delineamento em blocos e arranjo fatorial 2 x 2: nível (125 e 200 mg/kg) e fonte (CuSO<sub>4</sub> ou TBCC). O desempenho foi avaliado através da mensuração do peso corporal, consumo de ração médio diário (CRMD), ganho de peso médio diário (GPMD) e conversão alimentar (CA) dos 21 aos 63 dias de idade. A consistência fecal foi avaliada diariamente e, aos 63 dias de idade, foram coletadas amostras de tecido do duodeno, jejuno e íleo para análise da morfometria intestinal. Neste momento, foram avaliadas a altura da vilosidade (AV), profundidade da cripta (PC) e relação altura da vilosidade:profundidade da cripta (AV:PC). Não houve diferença entre nível e fonte (P>0,05) para o desempenho. Os leitões alimentados com 200 mg/kg de TBCC apresentaram menor incidência de diarreia (P<0,05) do que os outros tratamentos durante todo o período experimental. A suplementação com 125 mg/kg aumentou o AV (P<0,05) e a relação AV:PC (P<0,05) no duodeno, quando comparado a 200 mg/kg, independentemente da fonte. Com base em nossos resultados, podemos concluir que os leitões suplementados com 125 mg/kg de Cu tiveram melhor AV e relação AV:PC no duodeno e demonstram resultados de desempenho semelhantes ao 200 mg/kg de Cu.

**Palavras-chave** - Cloreto de cobre tribásico; desmame; nível farmacológico; sulfato de cobre.

## INTRODUCTION

Weaning is a critical event that directly related to piglet's productivity and subsequently, profitability for the producer. As a result of this stressful event, changes may occur in the post-weaning period about gastrointestinal physiology, microbiology, and immunology. Therefore, the post-weaning period is often characterized by a high incidence of diarrhea and depression in piglet's growth performance (Heo et al., 2013).

The use of copper (Cu) in diet can help animals face new challenges, especially in the antibiotic ban, as growth promoters. The supplementation of Cu at pharmacological levels (100 to 250 mg/kg) post-weaning has been associated with improving growth performance, feed intake and efficiency of feed utilization in weanling pigs (Cromwell et al., 1998; Hill et al., 2000; Armstrong et al., 2004; Shelton et al., 2011; Ma et al., 2015; Bikker et al., 2016; Carpenter et al., 2019, Lin et al., 2020). However, Cu as copper sulfate (CuSO<sub>4</sub>) is poorly absorbed, around 15-30% of the ingested in young animals, and 5-10% in adult animals (Gattás; Barbosa, 2004) and thereby it is necessary to know a different

kind of sources in order to reduce the environmental impact generated by this management.

The most used source of Cu is copper sulfate (CuSO<sub>4</sub>). Nevertheless, it has been reported that tribasic copper chloride (TBCC) to be equally effective as CuSO<sub>4</sub> for improving the performance of weanling pigs (Cromwell *et al.*, 1998; Fry *et al.*, 2012). Tribasic copper chloride is a less reactive form of Cu and is a potential alternative to CuSO<sub>4</sub> (Kim and Kil, 2015). Due to its lower hygroscopicity and solubility in water and the crystalline structure of the TBCC, it dissolves slowly in the digestive tract, leading to greater efficacy (Olukosi *et al.*, 2018). Furthermore, the TBCC-complex possesses a stronger bonding that can be responsible for reduced reactivity with other components of the diet and improve bioavailability (Cemin *et al.*, 2017). Also, tribasic copper chloride is a possible solution for reducing Cu excretion in the environment.

Thus, this study aimed to evaluate the effects of association between the pharmacological levels (125 or 200 mg/kg) and source (CuSO<sub>4</sub> or TBCC) of Cu on performance, diarrhea incidence, and intestinal morphology piglets after weaning.

## **MATERIAL AND METHODS**

The procedures and experimental use of animals were approved by the Ethic Committee on Animal Use (CEUA) with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), protocol number 6255070515.

Ninety-six commercial crossbred [(Large White × Landrace) × Duroc] weanling piglets of 21 days of age were used for this trial. The animals were distributed into four treatments based on BW, according to a block design of a 2 × 2 factorial arrangement: level (125 or 200 mg/kg) and source (CuSO<sub>4</sub> or TBCC). The piglets received one of four treatments: 125 mg/kg of CuSO<sub>4</sub>, 125 mg/kg of TBCC, 200 mg/kg of CuSO<sub>4</sub> and 200 mg/kg of TBCC.

It was prepared a basal diet based on Rostagno *et al.* (2017) recommendations with the mineral's inclusion according to the treatments. The animals were housed in two climate-controlled rooms for nursery pigs, with 12 pens of 1.2 m<sup>2</sup>/4 piglets. Each treatment consisted of 6 replicate pens with 4 animals each (2 males and 2 females).

Water and feed were supplied *ad libitum* and feed was offered according to the period of the rearing cycle, described below: pre-starter I period, from 21 to 28 days of age; pre-starter II period, from 28 to 35 days of age; starter I period, from 35 to 49 days of age, and starter 2 period, from 49 to 63 days of age (Table 1). Zinc oxide (ZnO) was supplemented at 2,500 mg/kg to all treatments during the pre-starter periods I and II.

The piglets' performance was evaluated by measuring BW at 21, 28, 35, 49 and 63 days of age. The average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated for each interval.

Visual analysis of the feces on the pen's floor was carried out every day, with scores ranging from 1 to 3 for each pen: 1 = solid feces (normal); 2 = feces softer than normal (pasty); and 3 = liquid feces (severe diarrhea) according to Pascoal et al. (2012) with modifications. Subsequently, it was calculated the diarrhea incidence, considering diarrhea the sum of scores 2 and 3.

On day 63, six piglets per treatment, 3 males and 3 females, were euthanized by electronarcosis and subsequent exsanguination to collect samples from the duodenum, jejunum and ileum. Tissue samples, with a length of 3 cm were collected and gently washed using NaCl solution 9%, pinned on cork and fixed in a 10% formalin solution for 24 hours for histological examination. Formalin-fixed samples were embedded in paraffin, sectioned at a 5  $\mu$ m thickness, and stained with hematoxylin and eosin for histological examination.

Characteristics determined during examination were villus height (VH), crypt depth (CD) and villus height: crypt depth ratio (VH:CD). Samples were examined using a Nikon Eclipse Ni-U 80i<sup>®</sup> light microscope (Nikon Instruments Inc., Melville, NY) coupled to a Nikon DigitalSight DS RI1<sup>®</sup> camera (Nikon Instruments Inc., Melville, NY). The parameters were determined using NIS Elements AR 4 13.01<sup>®</sup> software (Nikon Instruments Inc., Melville, NY). To perform the analysis, it was used at 10x magnification (Pekas, 1986).

Data were analyzed using the MIXED procedure of SAS software (Statistical Analysis System, version 9.3), according to a block design containing treatments as fixed effect and body weight as random effect. Each pen was considered as one experimental unit. Effect of treatment and time were evaluated using PDIFF. Data on the frequency of diarrhea was evaluated by the chi-square method. Differences were considered significant when  $P < 0.05$  and marginally significant when  $P < 0.10$  and  $P \geq 0.05$ . All results were expressed as mean values and standard deviations.

The statistical model used to test the effect of treatments and blocks was

$Y_{ijk} = \mu + B_j + L_i + S_k + \varepsilon_{ijk}$ , in which  $Y_{ijk}$  is the quantitative response variable;  $\mu$  is the overall mean;  $L$  is the effect of level (" $i$ " = 125 and 200 mg/kg),  $S$  is the effect of source (" $k$ " = CuSO<sub>4</sub> and TBCC);  $B$  is the effect of block (" $j$ " = BW); and  $\varepsilon$  is the random error.

**Table 1.** Composition of experimental diets (as-fed basis) provided to the weaned piglets (from 21 to 63 days of age).<sup>1,2,3</sup>

Ingredients, g/kg	Pre-starter I	Pre-starter II	Starter I	Starter II
Ground corn	486.00	525.00	617.90	663.30
Soybean meal	190.00	250.00	300.00	300.00
Milk whey	186.30	124.60	-	-
Whole milk powder	20.00	25.20	-	-
Degummed soybean oil	22.90	8.40	11.80	7.00
Spray-dried plasm	42.00	15.00	-	-
Sugar	20.00	20.00	20.00	-
Limestone	-	6.10	4.10	8.70
Fish meal	-	-	22.00	-
Dicalcium phosphate	15.70	8.60	6.90	6.20
Salt	-	4.00	5.70	4.70
Choline chloride	-	-	-	0.80
L-Lysine HCL	4.20	3.00	2.80	2.00
DL-Methionine	3.60	2.50	1.90	1.20
L-Threonine	2.90	1.50	1.20	0.60
L-Tryptophan	0.90	0.30	0.20	0.05
L-Valine	0.40	0.70	-	-
Vitamin and mineral premix <sup>4</sup>	5.00	5.00	5.00	5.00
Calculated composition, g/kg				
ME, kcal/kg	3450.00	3400.00	3380.00	3350.00
Crude protein	184.10	190.30	198.20	198.50
Calcium	5.50	6.20	6.00	6.00
Available phosphorus	5.70	4.90	4.40	3.70
SID lysine	12.70	11.70	11.20	10.50
SID met + cys	6.40	6.20	6.10	6.10
SID threonine	7.00	6.50	6.30	6.20
SID Tryptophan	2.10	1.80	1.80	1.80
Sodium	3.60	3.60	2.60	2.00

SID - standardized ileal digestible.

<sup>1</sup>CuSO<sub>4</sub> was added to the basal diets at the expense of ground corn at 0.5 and 0.8 g/kg of diet to achieve the dietary concentrations of 125 and 200 mg/kg Cu, respectively, for each period (as-fed basis).

<sup>2</sup>TBCC was added to the basal diets at the expense of ground corn at 0.23 and 0.37 g/kg of diet to achieve the dietary concentrations of 125 and 200 mg/kg Cu, respectively, for each period (as-fed basis).

<sup>3</sup>The chemical composition was not determined.

<sup>4</sup> Vitamin and mineral premix (per kg of product): folic acid 3.2 mg, pantothenic acid 18 mg, biotin 0.2 mg, cobalt 0.1 mg, choline 562.5 mg, iron 125 mg, iodine 1.2 mg, manganese 48 mg, niacin 38 mg, selenium 0.5 mg, thiamine 3 mg, vitamin A 20250 IU, vitamin B12 46.5 mg, vitamin B2 7.3 mg, vitamin B6 4 mg, vitamin C 400 mg, vitamin D3 5265 IU, vitamin E 150 IU, vitamin K3 5.26 mg, zinc 2,500 mg/kg (Pre-starter 1 and 2), zinc 150 mg/kg (Starter 1 and 2).

## RESULTS

Growth performance were not affected by level or source of dietary Cu ( $P > 0.05$ , Table 2). Interaction between level and source was observed in diarrhea frequency, from 21 to 63 days of age. The lowest diarrhea frequency ( $P < 0.05$ ) was observed when the piglets received 200 mg/kg Cu as TBCC (Figure 1). The increase of concentration from 125 to 200 mg/kg Cu as TBCC led to reduction of 75% on the diarrhea frequency, and a decrease on diarrhea (75.75%) it also was verified when compared CuSO<sub>4</sub> to TBCC source.

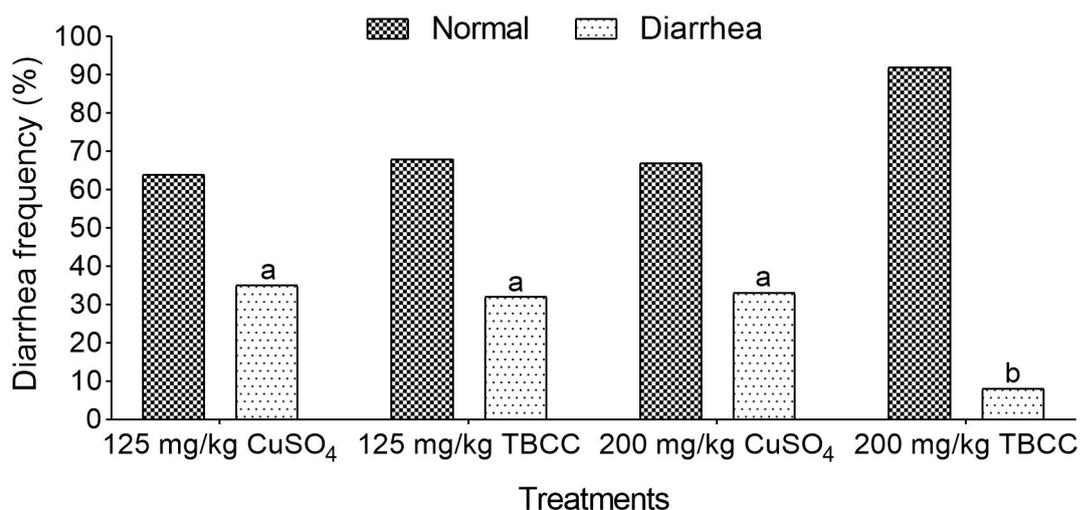
On intestinal morphology analysis, the jejunum was the only segment that was not observed differences among treatments ( $P > 0.05$ , Table 3). Piglets that received 125 mg/kg Cu had an enhance of 18.46% in VH in duodenum compared with the piglets that received 200 mg/kg ( $P < 0.05$ , Table 3). An increase of 29.17% was observed in the VH:CD ratio in duodenum of animals 125 mg/kg Cu in regarding to group that received 200 mg/kg ( $P < 0.05$ , Table 3), and also was verified marginally increase in 20.88% in this same relation in the piglets that received the TBCC source compared to  $\text{CuSO}_4$  source ( $P < 0.10$ , Table 3). In the piglets that received the source  $\text{CuSO}_4$  was observed marginal enhance in 12.42% CD in duodenum segment in comparison to TBCC source ( $P < 0.10$ , Table 3).

**Table 2.** Effects of association between copper levels and source on growth performance of weaned piglets (from 21 to 63 days of age).<sup>1</sup>

Item <sup>2</sup>	Treatments				P value		
	Level		Source		Level	Source	L × S
	125 mg/kg	200 mg/kg	$\text{CuSO}_4$	TBCC			
BW, kg							
21 days	6.08±0.63	6.14±0.64	6.08±0.64	6.14±0.63	0.803	0.827	0.814
63 days	23.13±2.26	23.45±2.12	23.03±2.31	23.51±2.09	0.660	0.547	0.463
21 to 63 days of age							
ADG, g/d	0.50±0.08	0.46±0.04	0.46±0.06	0.50±0.06	0.293	0.305	0.496
ADFI, g/d	0.94±0.12	0.87±0.10	0.90±0.11	0.92±0.12	0.109	0.645	0.488
FCR, g/d	1.92±0.22	1.87±0.14	1.95±0.17	1.85±0.19	0.573	0.211	0.759

<sup>1</sup>Treatments: Level, 125 or 200 mg/kg; source,  $\text{CuSO}_4$  or TBCC.

<sup>2</sup> Abbreviations: BW, Body weight; ADG, Average Daily Gain; ADFI, Average Daily Feed Intake; FCR, Feed Conversion Ratio;  $\text{CuSO}_4$ , copper sulfate; TBCC, tribasic copper chloride; L × S, interaction level and source.



<sup>a,b</sup> Means with different superscript differ (Chi-square,  $P < 0.05$ )

**Figure 1.** Frequency of diarrhea of piglets from 21 to 63 days of age.

**Table 3.** Effects of association between copper levels and source on the intestinal morphology of weaned piglets (from 21 to 63 days of age).<sup>1</sup>

Item <sup>2</sup>	Treatments				P value		
	Level 125 mg/kg	Level 200 mg/kg	Source CuSO <sub>4</sub>	Source TBCC	Level	Source	L × S
<b>Duodenum (µm)</b>							
VH	377.78±62.87 <sup>a</sup>	308.03±64.08 <sup>b</sup>	343.62±68.86	338.96±77.67	0.025	0.900	0.846
CD	442.41±98.69	456.06±48.78	478.98±66.30 <sup>A</sup>	419.49±76.78 <sup>B</sup>	0.656	0.064	0.653
VH:CD	0.96±0.27 <sup>a</sup>	0.68±0.15 <sup>b</sup>	0.72±0.15 <sup>B</sup>	0.91±0.31 <sup>A</sup>	0.019	0.074	0.199
<b>Jejunum (µm)</b>							
VH	317.76±68.49	290.74±67.01	294.15±53.18	314.35±80.80	0.275	0.540	0.440
CD	368.87±75.10	374.79±85.50	381.04±61.45	362.62±94.91	0.886	0.838	0.683
VH:CD	0.90±0.29	0.82±0.29	0.80±0.25	0.92±0.32	0.510	0.361	0.228
<b>Ileum (µm)</b>							
VH	299.22±62.40	261.30±45.16	302.98±58.02 <sup>A</sup>	253.75±43.23 <sup>B</sup>	0.207	0.099	0.108
CD	325.49±77.94 <sup>A</sup>	262.19±66.00 <sup>B</sup>	292.67±68.93	295.01±88.67	0.063	0.942	0.478
VH:CD	0.99±0.17	1.06±0.36	1.07±0.23	0.99±0.32	0.573	0.515	0.997

<sup>ab</sup>Means without a common superscript letter differ (PDIFF, P<0.05).

<sup>AB</sup>Means without a common superscript letter trend differ (PDIFF, P<0.10).

<sup>1</sup>Treatments: Level, 125 or 200 mg/kg; source, CuSO<sub>4</sub> or TBCC.

<sup>2</sup>Abbreviations: VH, Villus Height; CD, Crypt Depth; VH:CD, relation villus height: crypt depth; CuSO<sub>4</sub>, copper sulfate; TBCC, tribasic copper chloride; L × S, interaction level and source.

In the piglets that received the source CuSO<sub>4</sub> was observed marginal enhance in 12.42% CD in duodenum segment in comparison to TBCC source (P<0.10, Table 3). No effect to level of Cu was observed in CD, as well as no effect of Cu source was verified to VH and VH:CD ratio in this segment (P>0.05).

In ileum of animals that received the CuSO<sub>4</sub> source, it was verified marginal increase in 16.25% VH compared to TBCC source (P<0.10, Table 3). It also was observed in the piglets that received 125 mg/kg Cu marginally greater CD (19.45%) to 200 mg/kg (P<0.10, Table 3). No effect to Cu source was verified to CD and VH:CD ratio, as well as no effect to level was observed to VH and VH:CD ratio in this segment (P>0.05).

## DISCUSSION

The copper (Cu) is an essential trace element and is involved in several metabolic processes. The supplementation in piglets' diet at higher concentrations (100-250 mg/kg) to improve growth performance, and reduce the diarrhea incidence (Cromwell *et al.*, 1998, Hill *et al.*, 2000, Davis *et al.*, 2002; Shelton *et al.*, 2011; Ma *et al.*, 2015, Carpenter *et al.*, 2019, Lin *et al.*, 2020), and the use this mineral become essential especially in the context of the antibiotic ban, as growth promoters.

In the present study, the supplementation of Cu (level or source) did not affect growth performance piglets. These results were similar to those reported by Comwell *et al.* (1998), Veum *et al.* (2004), Pérez *et al.* (2011), Shelton *et al.* (2011), Fry *et al.* (2012)

and Bikker et al. (2016). The researches mentioned above verified differences between pig supplemented with high concentration compared to the control group (low level of Cu), but the growth performance was similar among the animals that received high concentration Cu, as in our study.

Others previous studies have found opposite responses with improve in BW and ADG (Zhao et al., 2014, 170 mg/kg copper 2-hydroxy-4-(methylthio) butanoic acid (Cu(HMTBa)<sub>2</sub>)), and it also enhances in G:F was reported by Carpernter et al. (2019) with 225 mg/kg Cu, and Lin et al. (2020) with 160-200 mg/kg TBCC or 80-160mg/kg copper proteinate (CuPro). This considerable variation observed in the literature results can be partially attributed to chemical variations inherent to different mineral sources, resulting in differences in efficacy, bioavailability and probably different absorption routes (Aldridge et al., 2007). Other factors may also contribute to inconsistent results, such as the duration and levels of supplementation (Hill et al., 2000), ingredients in the diet (Coble et al., 2018) and health condition of the animals (Zhao et al., 2007; Debski, 2016).

In this study, the animals that received 200 mg/kg Cu from TBCC showed the lowest diarrhea frequency in the overall period. A reduction of 75.00% in diarrhea was observed due to an increase in concentration Cu (from 125 to 200 mg/kg). Decreased diarrhea (75.75%) was also verified when CuSO<sub>4</sub> compared to the TBCC source. Our findings disagree with previous studies in which the percentage of animals with loose feces or diarrhea was similar between pig that received 120 and 160 mg/kg Cu from CuSO<sub>4</sub>·5H<sub>2</sub>O in almost overall period. Only 40-54 days of age, the increase of concentration from 120 to 160 mg/kg Cu led to a reduction of 61.19% in diarrhea (Bikker et al., 2016). Lin et al. (2020) also reported a reduction in diarrhea frequency when pig diets were supplemented with more than 80 mg/kg of TBCC or 40 mg/kg of CuPro, but no difference was observed between 80 and 200 mg/kg. A reduction of 8.37% diarrhea incidence was observed in animals that received 80 mg/kg Cu as CuPro compared to 80 mg/kg TBCC.

Recent studies have suggested that the reduction diarrhea frequency was likely due to high levels Cu (160 - 300 mg/kg) which might be mediated by an effect on gut microbiota (Hill et al., 2000; Bikker et al., 2016; Zhang et al., 2017; 2019; Lin et al., 2020). Besides, the supplementation with high concentrations Cu in the diet may lead to a decrease of the level of amines and consequently reduce the diarrhea incidence (Zhang et al., 2019), since Cu can act as a cofactor of enzyme diamine oxidase, which is responsible for catalyzing of the oxidation of diamines and putrescine to aldehydes (Festa and Thiele 2011; Kim et al. 2008). Biogenic amines are produced by fermentation of

amino acids by intestine microbiota from high dietary protein levels or reduced digestion and absorption in the small intestine (Neis *et al.*, 2015). The total crude protein level used in our study is following the recommendations of Rostagno *et al.* (2017) to the nursery period (18.06 to 21.42%). Thus, we believe that the diarrhea frequency results were not affected by the protein levels in the diet, but due to level and source Cu. These data showed different mineral sources, resulting in differences in efficacy and bioavailability (Aldridge *et al.*, 2007), and thereby, the association among level, source, and duration of supplementation can lead to divergence in results.

The duodenum of animals supplemented with 125 mg/kg Cu showed better villus height and VH:CD ratio and marginal improve VH:CD ratio was observed in the piglets that received Cu as TBCC. In the ileum, there was marginal enhance in villus height when the piglets were supplemented with CuSO<sub>4</sub> and higher CD when it received 125 mg/kg Cu. The present study provides conflicting results on whether level or source affect intestinal morphology.

There are contradictory studies regarding Cu in high concentrations (200 – 225 mg/kg) improve VH and VH:CD ratio in duodenum and jejunum (Zhao *et al.*, 2007; Fry *et al.*, 2012). Besides, Fry *et al.* (2012) also showed that the TBCC source was more efficient than CuSO<sub>4</sub> to enhance VH in the duodenum (25.44%) and jejunum (16.91%). There are limited studies regarding the association of different sources with high concentrations of Cu on intestinal morphology.

The piglet newly weaned usually shows reduced growth performance and increased diarrhea frequency, which is commonly affected by adverse changes in intestinal morphology (decrease in VH and enhance in CD), limiting animal growth (Ma *et al.*, 2015). The results of these sudden changes are a temporary reduction in the ability of villus to absorb and digest nutrients, and the occurrence of villus atrophy and crypt hyperplasia in the gut of piglets after weaning (Li *et al.*, 2001).

Copper is favorable for the development of the intestinal morphology, and the increase in VH:CD ratio generally corresponds to a greater absorptive area and fewer epithelial cells needed for cell renewal (Pluske *et al.*, 1997; Zhao *et al.*, 2007). However, the action of Cu may be affected by the levels of zinc provided in the diet.

Hill *et al.* (2000) reported low plasma Cu concentration when animals were fed high levels of Cu (250 mg/kg) and Zn (3,000 mg/kg). It also was observed better fecal consistency score in weaned pigs receiving a dietary Cu supplement of 250 mg/kg, but this study did not have analyzed intestinal morphology. On the other hand, Fry *et al.* (2012) evaluate pig fed 225 mg/kg Cu from TBCC or CuSO<sub>4</sub>, without supplementation of

Zn, it verified that enhanced VH in the duodenum in animals that received TBCC compared to CuSO<sub>4</sub>.

In the present study, Zn was provided in the diet of all treatments as ZnO at 2,500 mg/kg from 21 to 35 days of age (pre-starter periods I and II). Based on a previous study of Hill et al. (2000) and Fry et al. (2012) we suggested that high level of Zn and Cu provided in the diet of pig affected the absorption Cu and the excess this mineral in feces lead to alteration on microbial composition and reduction in diarrhea frequency. However, due to the low absorption Cu, there was no effect in intestinal morphology.

The better intestinal morphology observed in piglets supplemented with 125 mg/kg Cu allows the possibility of Cu inclusion to be made at lower levels than usual (200 to 250 mg/kg). This result is important because high levels of minerals such as Cu can lead to environmental pollution (Heo et al., 2013) due to excess of them in the feces and may promote selective pressure on antimicrobial resistant bacteria (Slifierz et al., 2014). This accumulation of metal in soils is likely to have several adverse effects on the environment, and the reduction of Cu's dietary supply is one of the possible approaches to prevent this environmental risk (Jondreville et al., 2003). Besides, fed 125 mg/kg of Cu had a decreased fecal Cu excretion compared with 250 mg/kg, which demonstrate that 125 mg/kg dietary Cu, regardless of source, may be an effective environmental alternative to 250 mg/kg of CuSO<sub>4</sub> in the diets for nursery pigs (Armstrong et al., 2004).

## **CONCLUSION**

From the results obtained in this work, it can be concluded that the supplementation dietary with 125 mg/kg of copper can reach better villus height and villus height to crypt depth ratio in the duodenum at 63 days, with satisfactory growth performance of piglets.

## **Acknowledgements**

The authors would like to thank the company De Heus for the support in the execution of this research.

## **Informative notes**

The procedures and experimental use of animals were approved by the Ethic Committee on Animal Use (CEUA) with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), protocol number 6255070515.

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