

## EFFECTS OF HIGH DIETARY COPPER SUPPLEMENTATION ON THE COPPER ACCUMULATION AND TOTAL COPPER CONTENT IN FATTENING PIGS

ADAMS, S. – YANG, H. – CHE, D.\* – JIANG, H.\* – QIN, G.

*College of Animal Science and Technology, Jilin Agricultural University  
130118 Changchun, China*

*\*Corresponding authors*

*e-mail: hljiang@jlau.edu.cn; phone: +86-186-446-5676; chedongsheng@163.com; phone: +86-136-4431-9554*

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**Abstract.** The objective of this current study was to investigate the effects of high dietary copper supplementation on the tissue copper deposition, distribution, and total copper concentration in fattening pigs. A total of 24 (Landrace × Large white × Duroc) pigs with an average initial body weight (BW) of  $30 \pm 1.05$  kg were selected for the current experiment. At the beginning of the experiment, the pigs were randomly divided into four treatment groups with three replicate pens per treatment and two pigs per replicate and arranged in accordance to a completely randomized design based on the BW. The four treatment administered were as follows: 10 mg/kg, 45 mg/kg, 135 mg/kg, and 225 mg/kg of copper. The result indicates that the copper content in the liver, kidney, and heart increased with the increase in dietary copper composition. There was no significant difference in the copper content of the different visceral tissues in the 10 mg/kg treatment group. However, when the copper levels increased to 45 mg/kg and 135 mg/kg, the copper content in the liver was significantly higher than that in the other visceral tissues ( $P < 0.05$ ). At 225 mg/kg dietary copper, there was a significant increase in the copper content of the foreleg muscle. There was no significant difference in copper content of the rib bones, tibia bones, and femur bone marrow copper accumulation between the 10 mg/kg copper and the 45 mg/kg copper fed pigs. In addition, there was no significant difference in the brain, blood, cerebellum, and skin copper content within all the treatment groups. Therefore, dietary copper levels increased with the increase in total copper accumulation in pigs. Hence, higher dietary copper supplementation increased liver, kidney, heart, fur, bones, bone marrow copper accumulation and total copper levels in fattening pigs.

**Keywords:** *micronutrient, metabolism, bioavailability, visceral tissues, diet, supplementation*

### Introduction

Copper is an indispensable micronutrient that forms part of all animal tissues and is required for a variety of biological processes essential for the maintenance of life (Gaetke et al., 2014). It is a co-factor of cellular enzymes, such as catalase, cytochrome oxidase, dopamine-beta-hydroxylase, and peroxidases. The extreme concentrations of copper inhibit sulfhydryl groups on enzymes such as glucose-6-phosphatase and glutathione reductase which protect cells from damage by free radicals (Bremner and Beattie, 1995). The most frequently utilized dietary copper supplement in animals' diet is inorganic copper usually in the form of copper sulphate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ). Copper occurs in the organic forms of chelates, complexes, proteinases, and like other organic trace minerals is often considered as an alternative to inorganic sources in animals diets (Huang et al., 2010). The relative bioavailability estimates of organic copper sources range between 88% and 147% of cupric sulphate in poultry, swine, sheep, and cattle (Baker and Ammerman, 1995). Copper is recognized as a growth-promoting agent in non-ruminant animals but its use at high levels is considered to be detrimental to the

environment (Armstrong et al., 2004; Veum et al., 2004). Diverse concentration and forms of copper have different bioavailabilities and different effects on animals (Guo et al., 2001). For example, copper is widely distributed in different animals' tissues such as the heart, liver, spleen, lungs, kidneys, and other internal organs, at saturated copper concentrations, copper accumulates in the bones. The requirement of copper as a nutrient is low and NRC (1998) recommends three to six milligram per kilogram copper for nursery and grower-finisher pigs. Generally, copper sulphate (125 to 250 mg/kg) is routinely added to nursery pigs diet as a growth promoter and its benefits on feed intake and weight gain have been well documented (Zhao et al., 2014).

However, high dietary copper presents health and environmental concerns when excess copper is excreted in faeces (Kornegay et al., 1997) and accumulated in muscle tissues. The accumulation of copper in animals' tissues and its excretion into soil has implicated to decrease soil productivity and pose both health and environmental threats. It was demonstrated that high dietary copper from inorganic sources antagonises other nutrients utilisation, such as zinc (Zhao et al., 2008) and phosphorus (Banks et al., 2004). As a result of the negative impact of high dietary copper sulphate, the commission of the European Communities regulates maximum allowed total copper in a feed as 170 mg/kg in piglets up to 12 weeks of age and 25 mg/kg in all other pigs. However, the ban on antibiotics usage in some parts of the world has motivated some farmers to still adhere to high copper supplementation in monogastric feeds above the required standards especially in swine nutrition. Hence, we hypothesised that higher copper concentration in swine nutrition may decrease the rate of copper deposition in muscle tissues and organs. The aim of the present study was to investigate the effects of high dietary copper concentrations on tissues and organ copper distribution and deposition in fattening pigs.

## **Materials and methods**

### ***Experimental site and location***

This study was performed in the animal breeding station of Jilin Agricultural University located in Changchun city of the Jilin Province in the People's Republic of China. Jilin is found on latitude 43°42' N and longitude 126° 12' E, and Changchun is on latitude 43°88' N and longitude 125°35' E. The annual rainfall ranges between 350-1000 mm (March – August) and dry season between September - February. Winter ranges between November – March with temperatures between -8 °C (17 °F) – -20 °C (-4.6 °F) and summer temperatures between 16°C (61.4°F) and 28 °C (81.2 °F) around May-July.

### ***Experimental design, animals, housing and diet***

A total of 24 (Landrace × Large white × Duroc) pigs with an average initial BW of 30 ± 1.05 kg were selected for the current experiment. The experiment was conducted for 87 days including 7 days of pre-feeding trial. At the beginning of the experiment, the pigs were divided into four treatment groups, with three replicate pens per treatment and two pigs per replicate, in accordance with a completely randomized design based on the BW. The copper content in the treatments was formulated based on the national guidelines that stipulated that the body weight of fattening pigs between 30-60 kg should contain ≤ 150 mg/kg copper and body weight above 60 kg should possess ≤

25 mg/kg copper. The four treatment administered were as follows: 10 mg/kg, 45 mg/kg, 135 mg/kg, and 225 mg/kg. The control pigs were fed the basal diet and the experimental pigs were fed the basal diet with the different copper concentrations. Pigs were fed twice daily and provided with 4% of the total body weight. The composition of the basal diet is provided in *Table 1*. The diet was provided in a mash form and formulated in accordance with the (NRC, 1998) nutrients recommendation. The basal diet contains corn-soybean meal as the main raw material and dietary copper was supplemented as copper sulphate (CuSO<sub>4</sub>). Water was supplied *ad libitum* throughout the entire experimental period. The pigs were housed in an environmentally-controlled room with an average temperature of 26 °C. The pens were disinfected once a month, cleaned with a broom every day to keep a healthy and hygienic condition, and prevent disease infection among pigs.

**Table 1.** Composition of experimental diets and nutrient indexes (%DM basis) prepared using the guidelines of NRC (1998) with corn and soybean as the main energy and protein sources

Items	30–60 kg	60–120 kg
Ingredients		
Corn	64.5	70.0
Soybean meal	11.0	11.5
Bran	22.0	16.0
Bone meal	1.0	1.0
Limestone	0.4	0.5
Salt	0.4	0.5
Lysine-HCL	0.2	
Trace mineral premix	0.5	0.5
Total	100	100
Calculated nutrient level		
Digestibility energy (MJ/kg)	13.67	13.59
Crude protein (CP)	17.06	15.55
Lysine (Lys)	0.85	0.63
Methionine + Cysteine (Met+Cys)	4.6	4.6
Copper sulphate (CuSO <sub>4</sub> ) (mg/kg)	10.00	10.00
Phosphorus (P)	0.65	0.55
Calcium (Ca)	0.64	0.71

Note: per kilogram of premix contains: iron 10000 mg, zinc 1000 mg, manganese 1000 mg, selenium 30 mg, iodine 50 mg, VA2000000IU, VD20000IU vitamin D2000IU, 2000 mg niacin, folic acid, pantothenic acid 2000 mg 30 mg, VK50 mg, riboflavin 250 mg, VB1 200 mg VB12 1000 ug, choline chloride 100 g, antioxidant 20000 mg, biotin 5 mg, VB6100 mg

### **Organs and tissues sampling**

At the end of the feeding trial, three pigs from each group were slaughtered in accordance with the normal farming practice and various organs and tissues were sampled for the determination of copper. The sampling method was as follows: about 20 g of muscle tissues were taken from the forelegs, hind legs, and buttocks and about 0.05 cm<sup>2</sup> of fur was collected from the back skin of pigs immediately after slaughter.

The abdominal cavity was opened and 10 g of various organs were obtained. The adipose tissues were obtained by taken 20 g of the back muscles of pigs. The femur bone, femur bone marrow, ribs, and bones were collected from pigs for the determination of copper levels.

### Blood sampling

Blood samples were taken at the time of slaughtering into separate tubes and heparinized. The samples were immediately transferred to the laboratory where plasma and serum were subsequently separated by centrifuging the whole blood samples at  $2500 \times g$  at  $4^\circ\text{C}$  for 5 min. The heparinized plasma samples were frozen at  $-20^\circ\text{C}$  until analysis for copper concentrations.

### Chemical analysis

The chemical composition of the diets was analysed by standard methods. Dry matter was determined by drying feed samples at  $105^\circ\text{C}$  to constant weight. The crude protein by Kjeldahl and ether extract by Soxhlet fat analysis as described by (AOAC, 2000). The copper concentrations of feed, tissues, organs, faeces, urine, and plasma were analysed by the Flame Atomic Absorption Spectroscopy (Shimadzu Scientific Instruments, Kyoto, Japan) as previously described by (Wu et al., 2015).

### Statistical analysis

The data were analysed by one-way analysis of variance (ANOVA) using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). A probability value of  $P \leq 0.05$  was considered to be statistically significant and the multiple comparisons test was performed by LSD method, the test results were estimated as Mean  $\pm$  SE.

## Results

### Copper distribution and deposition in the internal organs

Table 2 shows that among the different tissues and organs analysed from the fattening pigs, the deposition of copper in the liver was higher.

**Table 2.** Effects of dietary copper levels on different tissues and organs (mg/kg)

Levels	Feed	Liver	Kidney	Heart	Lung	Stomach	Spleen	Pancreas	Lymph nodes
10 mg/kg	10	9.18 $\pm$ 0.16 <sup>ab</sup>	8.39 $\pm$ 0.34 <sup>a</sup>	5.84 $\pm$ 0.65 <sup>ab</sup>	3.68 $\pm$ 1.63 <sup>ab</sup>	1.10 $\pm$ 0.01 <sup>ab</sup>	0.95 $\pm$ 0.03 <sup>bc</sup>	4.54 $\pm$ 0.95 <sup>a</sup>	4.28 $\pm$ 1.12 <sup>abc</sup>
45 mg/kg	45	12.58 $\pm$ 0.14 <sup>a</sup>	10.16 $\pm$ 0.04 <sup>b</sup>	6.30 $\pm$ 0.20 <sup>b</sup>	3.55 $\pm$ 0.78 <sup>b</sup>	2.01 $\pm$ 0.06 <sup>b</sup>	1.94 $\pm$ 0.09 <sup>b</sup>	4.45 $\pm$ 0.98 <sup>a</sup>	4.45 $\pm$ 0.85 <sup>b</sup>
135 mg/kg	135	31.98 $\pm$ 2.20 <sup>a</sup>	13.51 $\pm$ 0.18 <sup>b</sup>	7.64 $\pm$ 0.12 <sup>c</sup>	3.37 $\pm$ 0.05 <sup>c</sup>	2.72 $\pm$ 0.2 <sup>c</sup>	2.68 $\pm$ 0.31 <sup>c</sup>	5.41 $\pm$ 1.76 <sup>a</sup>	3.77 $\pm$ 1.08 <sup>c</sup>
225 mg/kg	225	49.6 $\pm$ 21.37 <sup>a</sup>	20.38 $\pm$ 0.21 <sup>b</sup>	12.49 $\pm$ 0.2 <sup>b</sup>	3.21 $\pm$ 0.65 <sup>b</sup>	3.98 $\pm$ 0.04 <sup>b</sup>	3.88 $\pm$ 0.06 <sup>b</sup>	5.27 $\pm$ 0.96 <sup>ab</sup>	3.50 $\pm$ 0.21 <sup>b</sup>

Different letters in the same column the data before are significantly different at the level of  $P < 0.05$

We observed that the amount of copper deposition increased with the increase in copper concentration in the feed. The copper content in the liver, kidney, and heart showed an increasing trend with increasing copper levels in the feed. The kidney copper composition in the 225 mg/kg copper sulphate group was approximately 2.5 times higher than that of the kidney copper content in the control group. The lowers copper

accumulation in tissues and organs was registered in pigs supplemented with 10 mg/kg copper. There was no significant difference in copper content in the different visceral tissues at 10 mg/kg group. However, at 45 mg/kg and 135 mg/kg copper content there was a significantly ( $P < 0.05$ ) increase in the liver copper levels compared to other visceral tissues. We observed higher copper levels in the liver followed by the kidneys, heart, spleen, and the pancreas. However, at 225 mg/kg copper levels, there was an increased in liver copper content compared to the kidney copper level. There was a decreased trend in the lungs copper content with the increase in dietary copper levels.

### ***Distribution and accumulation of copper in muscle tissue of fattening pigs***

The accumulation of copper in pig muscle tissue and adipose tissue is detailed in *Table 3*. From *Table 3*, there was no significant difference in the copper concentration of pig muscle tissue as the copper content of the feed increases. At 225 mg/kg dietary copper levels, there was a significant increase in the copper content of the foreleg muscle. Although the copper concentration of most muscle tissues increased as the dietary copper content increases, the hind leg muscle observed a slightly decreased in copper content at higher dietary copper levels. Conversely, the copper content of the foreleg muscle was relatively higher than the copper content in the loin and the hip muscle tissues.

**Table 3.** Dietary copper supplementation on the copper contents in the muscle tissues and fat in pigs fed different copper feeds

Levels	Feed	Foreleg muscle	Hindleg muscle	Loin	Hip muscle	Adipose tissue
10 mg/kg copper	10	0.510±0.004 <sup>a</sup>	0.100±0.144 <sup>a</sup>	0.072±0.09 <sup>a</sup>	0.057±0.003 <sup>a</sup>	0.076±0.005 <sup>a</sup>
45 mg/kg copper	45	0.610±0.050 <sup>a</sup>	0.100±1.017 <sup>a</sup>	0.05±0.026 <sup>ab</sup>	0.060±0.020 <sup>ab</sup>	0.131±0.021 <sup>b</sup>
135 mg/kg copper	135	0.630±0.070 <sup>a</sup>	0.270±1.091 <sup>a</sup>	0.064±0.05 <sup>a</sup>	0.057±0.120 <sup>a</sup>	0.147±0.364 <sup>a</sup>
225 mg/kg copper	225	0.750±0.020 <sup>b</sup>	0.370±0.585 <sup>ab</sup>	0.073±0.034 <sup>b</sup>	0.041±0.065 <sup>b</sup>	0.276±0.420 <sup>a</sup>

Different letters in the same column the data before are significantly different at the level of  $P < 0.05$

### ***Distribution and accumulation of copper in porcine bones***

From *Table 4* it can be observed that as the content of copper in the feed increases, the accumulation of copper in swine bones also increases.

**Table 4.** Copper supplementation on the copper content in pigs' bones with different copper feeding doses

Levels	Feed	Rib bone	Tibia bone	Femur bone	Femur bone marrow
10 mg/kg copper	10	4.11±1.05 <sup>a</sup>	4.38±0.83 <sup>a</sup>	4.15±0.02 <sup>a</sup>	0.52±2.96 <sup>a</sup>
45 mg/kg copper	45	5.12±0.25 <sup>a</sup>	4.59±0.68 <sup>a</sup>	18.25± 3.21 <sup>b</sup>	2.52±2.68 <sup>a</sup>
135 mg/kg copper	135	22.28±0.85 <sup>b</sup>	22.13±0.24 <sup>b</sup>	57.36±8.21 <sup>c</sup>	8.32±1.32 <sup>b</sup>
225 mg/kg copper	225	22.45±0.24 <sup>b</sup>	22.22±0.35 <sup>b</sup>	62.32±3.25 <sup>d</sup>	10.25±2.35 <sup>c</sup>

Different letters in the same column the data before are significantly different at the level of  $P < 0.05$

The degree of copper accumulation in the tibia bones, rib bones, femur bones, and femur bone marrow varies greatly. However, the degree of copper enrichment in the bone marrow was lower in all the treatment groups. There was no significant difference in the copper levels of the rib bones, tibia bones, and femur bone marrow copper

accumulation between the 10 mg/kg copper and the 45 mg/kg copper fed pigs. The content of copper in the femur bone was significantly higher ( $P < 0.05$ ) than the copper content in the femur bone marrow, ribs, and the tibia bones at 45 mg/kg copper levels. In addition, there was a significant difference in the copper content of the rib bones and the tibia bones at 135 mg/kg copper and 225 mg/kg copper fed pigs. However, the 225 mg/kg treatment groups registered the highest copper content in the tibia bone, femur bone, and femur bone marrow.

### ***Accumulation and distribution of copper in various organs***

From *Table 5*, we have observed that as the concentration of copper in the feed gradually increased, the copper content in the pig's brain, cerebellum, blood, skin, and fur shows an increasing trend. The blood and the skin registered the lowest copper accumulation among the treatment groups. There was no significant difference in the brain, blood, cerebellum, and skin copper content within all the treatment groups. However, there was a significant difference in the fur copper content of the 225 mg/kg copper fed pigs in comparison with the control. The fur copper content of the 225 mg/kg copper diet was significantly different from the 135 mg/kg copper diet, but, not significantly different from the 10 mg/kg and 45 mg/kg copper treated groups. Although the copper content of the brain was high, its rate of accumulation was lower compared to the fur.

**Table 5.** Dietary copper supplementation on the copper content in the brain, skin blood, and fur of pigs fed different copper levels

Treatments	Feed	Brain	Cerebellum	Skin	Blood	Fur
10 mg/kg copper	10	12.137±4.274 <sup>a</sup>	6.794±0.987 <sup>a</sup>	0.039±0.021 <sup>a</sup>	1.604±0.080 <sup>a</sup>	3.791±0.554 <sup>a</sup>
45 mg/kg copper	45	12.037±0.837 <sup>a</sup>	7.255±0.125 <sup>a</sup>	0.059±0.087 <sup>a</sup>	1.684±0.114 <sup>a</sup>	4.474±0.821 <sup>a</sup>
135 mg/kg copper	135	12.261±3.374 <sup>a</sup>	8.321±0.125 <sup>a</sup>	0.071±0.067 <sup>a</sup>	1.890±0.212 <sup>a</sup>	10.560±8.454 <sup>b</sup>
225 mg/kg copper	225	12.354±2.354 <sup>a</sup>	9.321±0.102 <sup>a</sup>	0.084±0.517 <sup>a</sup>	1.684±0.162 <sup>a</sup>	12.540±7.211 <sup>c</sup>

Different letters in the same column the data before are significantly different at the level of  $P < 0.05$

### ***Effect of dietary copper levels on the total copper content in pigs***

From *Table 6*, we have observed that as the content of copper in the feed increased, the total copper accumulation in the pigs also increased. The total copper content in the 225 mg/kg copper treated group was significantly higher in comparison with the 10 mg/kg, 45 mg/kg, and 135 mg/kg. The copper content in the liver, kidney, femur bone, femur bone marrow, ribs, and fur increased with the increase in copper content of the feed. The copper levels in the muscle tissues, lungs, and other tissues of the fattening pigs body follow a similar trend as other parts of the visceral organs.

**Table 6.** Effects of dietary copper supplementation on the total copper content in the tissues and organs of pigs

Copper content (mg/kg)	Total copper in tissues and organs of pigs (mg/kg)
10 mg/kg copper	76.501±0.321 <sup>Aa</sup>
45 mg/kg copper	102.654±1.880 <sup>Aa</sup>
135 mg/kg copper	215.393±1.325 <sup>AB</sup>
225 mg/kg copper	256.826±21.201 <sup>Bb</sup>

Lowercase letters indicate that there is a significant difference between the data and the control group at the level of ( $P < 0.05$ ). Capital letters indicate that there is a significant difference between the data and the control group at the level of ( $P < 0.01$ )

## Discussion

Dietary supplementation of feed additives has recently gain scientific interest due to the current ban on antibiotics usage as feed supplement in most countries (Adams et al., 2019; Adams et al., 2018a, b; Che et al., 2018). Most animal species have limited access to copper from the environment. The concentration of dietary copper varies greatly because feed materials from different sources have different copper content. The main sources of copper for animals are the food, drinking water, and copper containing supplementations (de Romaña et al., 2011; Tomaszewska et al., 2014). Copper has been used in the formation and maintenance of myelin and is needed for the synthesis of melanin in the eyes, hair, and skin. It is a constituent of cytochrome c oxidase, vital in cellular respiration, and forms a complex relationship with zinc-superoxide dismutase (Letelier et al., 2009; Gaetke et al., 2014). The quantity of copper absorbed from food and water is reasonably low, and the body controls excess amounts of copper by either reducing the absorption or increasing the excretion of copper under normal conditions. The absorption rate of dietary copper is affected by several factors such as sex, age, type of feed, and quantity of copper supplemented (de Romaña et al., 2011). However, the control of internal copper homeostasis prevents the excess accumulation of copper in body tissues and organs (Gaetke et al., 2014). The distribution and accumulation of copper in most tissues of the body have been linked to the total amount of copper supplemented in the diet and the total copper ingested. The supplementation of high copper diets in growing pigs may result in the increased in copper accumulation in organs and excretion in manure, which poses both health and environmental risk (Kornegay and Versteegen, 2001). The excretion of high copper levels in swine manure is reported elsewhere (Yin et al., 2018; Zheng et al., 2018; Liao et al., 2018). Thus, it is important to examine the effects of dietary supplementation of copper on the relative distribution and accumulation of copper in organs and total copper content in fattening pigs.

The present study showed that with the increased in dietary copper levels, there was an increase in the copper content in the tissues and organs of pigs. However, the copper levels in different tissues and organs varied greatly. Similarly, Peña et al. (1999), who indicated that the highest concentration of copper was in the liver, kidneys, brain, heart, and the lowest concentration was in the bones and muscles. The authors indicated that the average copper content in adult animals was three times lower than growing animals. This was due to the high metabolic requirements of copper in growing animals. Also, Bremner and Beattie (1995) indicated that the liver is the main storage site for copper deposition following higher dietary copper consumption and the copper concentrations in a normal adult liver is 18–45 mg copper per gram dry weight. Moreover, the concentration and distribution of copper varied throughout the animal's life. We observed that the total amount of copper retained increased as the copper intake increases, reaching a plateau with dietary copper levels of 225 mg/kg. The accumulation of copper in the liver may increase to concentrations that can affect the normal functioning of the liver causing toxicosis in stress conditions. The adverse accumulation of copper in the liver could lead to liver degradation and damage in situations of Wilson's diseases (Guo et al., 2001; Roberts and Schilsky, 2008). Meanwhile, the blockage of copper absorption by the formation of copper complexes with the intestinal cells and the redistribution of copper in the liver accounts for the use of zinc in the treatment of this Wilson's disease (Bremner and Beattie, 1995). In contrast, Tomaszewska et al. (2014) fed growing rats with 5 mg/kg of organic and inorganic

copper levels per day and observed no copper deposition in the plasma and liver between the control and the experimentally treated group. Yelin et al. (1987), who noted that during liver necrosis, the liver discharges higher concentrations of copper (50 mg/g dry weight) into the blood hence causing rapid accumulation of copper in the erythrocytes and successively causing oxidative injury to the red blood cell.

Conversely, supported by the notion that copper absorption and deposition increased with increasing dietary copper levels (Barceloux and Barceloux, 1999). Copper is an essential trace mineral; little information is available on its absorption in the lungs. The National Research Council in 1977 noted that copper can be absorbed in the lungs due to the manifestation of metal fume fever for the duration of copper volatilization but the occurrence of copper to stimulate metal fume fever was low due to the high temperature requirement for copper volatilization. In this current experiment, we observed significantly decreased in the copper content of the lungs as the concentration of dietary copper levels increases. The reason for the decreased in lungs copper levels at higher dietary copper levels is not established. The absorption of copper in the lymphatic nodes follows similar trend as the absorption of copper in the lungs. The absorption of copper in the stomach depends on several factors such as dietary components, the chemical forms of copper, the interaction of copper with other metals like zinc, selenium, cadmium, and the proportion of the quantity of copper in the stomach (Stern et al., 2007). The absorption of copper in the stomach increased with the increase in dietary copper concentrations. However, the quantity of copper stored in the body does not affect the absorption of copper in the body tissues and the clinical reduction in copper absorption is not affected by the consumption of high concentrations of dietary zinc (Sandström, 2007). The present study indicates that the concentration of copper in the femur bone, femur bone marrow, kidney, and other tissues was much higher than that in muscle tissue. Hence, high dietary copper supplementation does not increase muscle copper deposition.

## Conclusion

The result of this study indicated that dietary copper supplementation in the diets of growing pigs increased the copper deposition and accumulation in different body tissues and organs. However, these concentrations of copper were lower in the muscles and other visceral organ. Therefore, indicating that pigs can metabolise higher copper levels above the current standards without significant copper deposition in muscles and other parts.

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**Conflict of interests.** The authors declare that there is no conflict of interests.



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