



# GROWTH PERFORMANCE, CARCASS TRAITS, MEAT QUALITY AND HISTOCHEMICAL CHARACTERISTICS OF MUSCLE FIBERS IN MEXICAN HAIRLESS FINISHING PIGS SUPPLEMENTED WITH ORGANIC COPPER †

## [EVALUACIÓN DEL CRECIMIENTO, RAZGOS DE CANAL, CALIDAD DE LA CARNE Y CARACTERÍSTICAS HISTOQUÍMICAS DE FIBRAS MUSCULARES EN CERDOS PELÓN MEXICANO EN FINALIZACIÓN SUPLEMENTADOS CON COBRE ORGÁNICO]

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### SUMMARY

**Background:** Pork meat is related to cardiovascular diseases, due to its high content of saturated fatty acids and cholesterol. But it has been shown that dietary supplementation of pigs with organic minerals benefits growth qualities and improves carcass and meat quality characteristics. **Objective:** The objective was to evaluate the effect of Cu proteinate supplementation of finishing Mexican Hairless pigs (MHP) on growth, carcass characteristics, meat quality and muscle fiber composition. **Methodology:** Twenty-eight castrated male MHP (62±5.2 kg BW) were randomly assigned to four levels of Cu in the diet (0, 75, 150, and 225 mg kg<sup>-1</sup> DM) for 46 d. The DMI, DWG, FC and FE were measured. At the end of fattening, the pigs were slaughtered and the characteristics of the carcass were evaluated. Tissue samples of *Longissimus thoracis* (LT) were obtained and muscle fibers, pH, color, temperature and drip loss were evaluated. **Results:** The DMI was reduced linearly ( $P \leq 0.05$ ) as Cu level supplementation increased in the diet. Growth performance was modified quadratically as Cu level supplementation was increased in the diet, as well as the highest DWG and the lowest FC values were found in barrows supplemented with 75 mg Cu kg<sup>-1</sup> DM. Pigs supplemented with 75 and 150 mg Cu kg<sup>-1</sup> DM had the highest ( $P \leq 0.05$ ) CW and LEA compared with the other treatments. BFT and DL decreased linearly ( $P \leq 0.05$ ) as Cu supplementation increased in the diet. The Cu supplementation affected ( $P \leq 0.05$ ) the color index *b* and *h* after 48h. The Cu affected ( $P \leq 0.05$ ) the number, composition and relative area of the type I, IIA and BII fibers of the LT. **Implications:** Organic Cu supplementation in the diet of Mexican Hairless pigs benefits growth efficiency, carcass yield and backfat reduction; however, its effect on content, FA profile, and oxidative stability of IF meat should be analyzed. **Conclusions:** It is concluded that Cu supplementation effected quadratically the values of Final BW, DWG, CW and LEA, and linearly Cu affected the values of DMI, FC, CCD, BFT, DL, pH, the color index *b* and *h* after 48h; treatments with Cu also influenced linearly or quadratically the number, composition and relative area of the type I, IIA and BII fibers of the LT post slaughter muscle metabolism and it may influence the parameters of quality of meat in finishing Mexican Hairless

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pigs. The production of MHP meat in an intensive fattening system is profitable and can be improved with the inclusion of Cu proteinate in the diet.

**Keywords:** Copper; Mexican Hairless pigs; performance; carcass; meat; muscle fibers; histochemistry.

## RESUMEN

**Antecedentes:** La carne de cerdo está relacionada a padecimientos de enfermedades cardiovasculares, debido a su alto contenido de ácidos grasos saturados y colesterol. Pero se ha demostrado que la suplementación en la dieta de cerdos con minerales orgánicos, beneficia las cualidades de crecimiento, y mejora las características de calidad de la canal y la carne. **Objetivo:** El objetivo fue evaluar el efecto de la suplementación con proteinato de Cu a cerdos Pelón Mexicano (CPM) en finalización sobre el crecimiento, características de la canal, calidad de la carne y composición de fibras musculares. **Metodología:** Veintiocho CPM, machos castrados ( $62 \pm 5.2$  kg de PV) fueron asignados al azar a cuatro niveles de Cu en la dieta (0, 75, 150 y 225 mg  $\text{kg}^{-1}$  MS) durante 46 d. Se midió el CMS, GDP y CA. Al término de la engorda, los cerdos fueron sacrificados y se evaluaron las características de la canal. Se obtuvieron muestras de tejido del músculo *Longissimus thoracis* y se evaluaron las fibras musculares, pH, color, temperatura y PPG. **Resultados:** El CMS se redujo linealmente ( $P \leq 0.05$ ) a medida que aumentó el nivel de Cu en la dieta. El comportamiento del crecimiento se modificó cuadráticamente a medida que se incrementó la suplementación de Cu en la dieta, así como los valores más altos de GPD y los valores más bajos de CA se encontraron en los cerdos con 75 mg  $\text{Cu kg}^{-1}$  MS. Los cerdos con 75 mg  $\text{Cu kg}^{-1}$  MS tuvieron mayor ( $P \leq 0.05$ ) peso de canal caliente, mientras que con 150 mg  $\text{Cu kg}^{-1}$  MS tuvieron mayor ( $P \leq 0.05$ ) ojo del área de la chuleta comparado con los otros tratamientos. Los valores de espesor de grasa dorsal y pérdida por goteo disminuyeron linealmente ( $P \leq 0.05$ ) a medida que aumentó la suplementación de Cu en la dieta. La suplementación con Cu afectó ( $P \leq 0.05$ ) los índices de color *b* y *h* a las 48 h. El Cu afectó ( $P \leq 0.05$ ) el número, composición y área relativa de las fibras tipo I, IIA y BII del músculo LT. **Implicaciones:** La suplementación de Cu orgánico en la dieta de cerdos pelón mexicano beneficia la eficiencia del crecimiento, el rendimiento de la canal y la reducción de la grasa dorsal, sin embargo, debe analizarse su efecto en el contenido, perfil de AG y la estabilidad oxidativa de la GI de la carne. **Conclusiones:** Se concluye que la suplementación con Cu afectó cuadráticamente los valores de PV Final, GDP, PC y AOCH, y linealmente Cu afectó los valores de CMS, CA, RC, GD, PPG, pH, índices de color *b* y *h* después de 48h; los tratamientos con Cu también influyeron lineal o cuadráticamente en el número, composición y área relativa de las fibras tipo I, IIA y BII del metabolismo muscular post sacrificio del LT y pueden influir en los parámetros de calidad de la carne en cerdos Pelón Mexicanos en finalización. La producción de carne de CPM en un sistema de engorde intensivo es rentable y puede mejorarse con la inclusión de proteinato de Cu en la dieta.

**Palabras clave:** Cobre; cerdos Pelón Mexicano; rendimiento; canal; carne; fibras musculares; histoquímica.

## INTRODUCTION

At present, worldwide, the final consumer demands healthy food, therefore, the pork meat chain is focused on producing healthy quality meat quickly and efficiently (Sang *et al.*, 2016). In Mexico, pork occupies second place in apparent national consumption, however, some sector of the population rejects it due to prejudices about its quality and health effects. Projections on world pork production indicate that it will grow at an average annual rate of 0.9% in the period from 2018 to 2027, with 130.9 million tons (FIRA, 2019). Cardiovascular diseases are among the main causes of high mortality worldwide, therefore health and nutrition professionals continue to research to find adequate recommendations for their prevention (Royo-Bordonada *et al.*, 2017). In humans, consumption of a healthy diet, such as the ingestion of animal products with lower saturated fat content, has health benefits by reducing the incidence of metabolic syndrome, diabetes mellitus, cardiovascular diseases, breast cancer, and cardiovascular problems of chronic degenerative diseases (Urquiga *et al.*, 2017).

Therefore, it is justified to continue with studies on nutrition and feeding of pigs, with the aim of improving their productive efficiency, reducing production costs, improving the nutritional properties of meat, shelf life, fat and protein oxidation, sensory attributes such as flavor, tenderness, color, juiciness, among others, identifying the metabolic processes and events that occur in the muscle fibers of the pig in its transformation from muscle to meat. In this sense, different feeding programs and methods have been designed and tested, providing rations that include conventional and non-conventional foods and additives (Montero *et al.*, 2015). The results obtained indicate that the characteristics and performance of the carcass can be improved, thus modulating the quality of meat and meat products for consumption through the use in the diet of sources rich in fatty acids, antioxidants, organic minerals chelated with amino acids such as selenium, chromium, zinc, copper, etc. A substantial contribution of swine nutrition studies to the agri-food industry is to make meat a “functional food”, with a lower content of saturated fat and a higher content of fatty acids in the

intramuscular fat of the meat and subcutaneous fat of the carcass, thus achieving a healthier product for the final consumer (Valenzuela *et al.*, 2014).

A valued strategy is the use of copper (Cu) in pig nutrition, mainly as a growth-promoting additive; supplementation in the diet of piglets with levels of 100 to 250 mg of Cu kg<sup>-1</sup> Dry Matter (DM) significantly influences the microbial population of the digestive tract (Jensen, 2016). Recently, Diwa (2019), concluded that supplementation of 150 mg Cu kg<sup>-1</sup> DM, from Cu hydroxychloride, to diets for pigs positively influenced the immune system, changed intestinal health, energy metabolism, growth performance, and regulates some genes involved in post-absorptive metabolism of lipids. Likewise, Colín-Álvarez *et al.* (2019) indicated that Cu proteinate fed to finishing pigs affected ( $P \leq 0.01$ ) the final pH and color index *a* ( $P \leq 0.05$ ) of the *Longissimus thoracis* (LT) muscle, also Cu improved the daily weight gain, feed efficiency, area of chop and the yield of primary cuts, and it reduced the backfat thickness in the carcass. Studies in fattening cattle have shown that Cu affects lipid metabolism. Engle and Spears (2000) reported that in Angus cattle, a supplement of Cu reduced the level of cholesterol, increased the unsaturated fatty acids (UFA) and reduced saturated fatty acids (SFA) content in muscle. Betonha *et al.* (2012) found that Cu supplementation in Nellore cattle increased the level of unsaturated fatty acids and reduced the cholesterol in muscle; the above is due to changes in the reduced glutathione and oxidized glutathione form (GSH/GSSG) ratio. According to Kim *et al.* (1992), the reduced glutathione (GSH) stimulates the production of 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA), an enzyme that participates in the synthesis of cholesterol, and hepatic Cu indirectly regulates cholesterol biosynthesis by decreasing the activity of GSH and increasing that of oxidized glutathione (GSSG). Cheng *et al.* (2010) found that Cu dietary in growing sheep (20 mg kg<sup>-1</sup> DM), altered plasma insulin and leptin metabolism, decreased lipogenic enzymes activities, and increased lipolytic enzymes in subcutaneous adipose and liver tissues. The reduction in subcutaneous backfat may be due to Cu reducing synthesis and increasing lipolysis of adipose tissue.

Copper is an essential micronutrient, it participates in different biochemical pathways and functions; as a metal ion, it catalyzes lipid peroxidation, and it is a cofactor of the cytochrome oxidase enzyme in the respiratory chain that produces ATP (Eskin, 2021). The requirement of Cu in the diet of pigs is of 25 mg kg<sup>-1</sup> DM (NRC, 2012); however, for piglets over 12 weeks of age, the European Union authorized a maximum level of 170 mg Cu kg<sup>-1</sup> DM (EFSA, 2016).

The skeletal muscle has different types of fibers, according to its histochemical characteristics, they are classified in three types: i) slow oxidative fibers or type I ( $\beta$ -red), ii) fast oxidative-glycolytic fibers, or type IIA ( $\alpha$ -red), and iii) fast glycolytic or type IIB ( $\alpha$ -white). This classification represents both, end of the metabolic profile (type I and IIB) and intermediate energetic metabolism (type IIA) (Handel and Stickland, 1987; Klont *et al.*, 1998). Two factors that have an influence on the quality of pork meat, also related to the alteration in the postmortem metabolism of the muscle are fiber composition and the size of myocyte. This determines the biochemical course of the muscle, hence influencing the transformation of the muscle into meat (Fiedler *et al.*, 2003; Ryu and Kim, 2006). Therefore, changes in the degree or frequency of the glycolysis may produce a non-favorable muscle pH. The rapid decrease of the pH may reach a final low pH that results in the denaturation of the protein with less quality parameters (Henckel *et al.*, 1997; Henckel *et al.*, 2000). A decisive factor of the biochemical pathways of the muscle is the composition of the type of fibers, which results from the coordinated expression of different sets of structural proteins and metabolic enzymes (Chang *et al.*, 2003). For this reason, the variation of the type of muscle fiber can explain, partially, the variation in some of the characteristics of the meat quality (Essen-Gustavsson *et al.*, 1994); however, the relationship between the area of muscle fibers with the quantity and quality of meat is still controversial, but, despite this, the results reveal that a total number of muscle fibers and a larger cross-sectional area do not significantly deteriorate their quality (Sang *et al.*, 2016). The selection of the pig by its growth rate, lean tissue and muscle mass produced changes in the fibers, increasing the type IIB (Weiler *et al.*, 1995; Rehfeldt *et al.*, 1999). Lefaucheur (2010) has suggested that a way to improve lean meat production without affecting its quality can be done through a combination of increases in the total number of muscle fibers and a moderate increase in their cross-sectional area.

The Mexican Hairless pig (MHP) is a local creole breed, which derived from Mediterranean pigs that created the Celtic, Neapolitan and Iberian varieties pigs. This breed preserves genetic features (*loci* S0355, S0215 and SW632) of the Spanish Iberian pig (Canul *et al.*, 2005). The MHP pure is characterized by its slow growth, poor feed conversion, abundant subcutaneous fat and small muscle mass (Sierra-Vázquez *et al.*, 2016). The feed efficiency of MHP is good in the early stages of fattening, but, in general, its productive performance represents less than 50% of that obtained in improved pigs, despite supplying balanced feed. Therefore, the integration of MHP livestock production units as an initial part of the production chain has been suggested, to obtain greater

profitability, as well as the conservation of this valuable zoogenetic resource (Ramos-Canché *et al.*, 2020).

On the other hand, a benefit of Creole breeds, such as the MHP, is their adaptation to different environmental and climatic conditions and their greater rusticity (Ramos-Canché *et al.*, 2020), with greater resistance to diseases and lower nutrient requirements vs pigs commercial ones, which are less resistant to high temperatures that compromise voluntary feed intake, daily weight gain and fertility (Linares *et al.*, 2011). The meat of the MHP has an excellent flavor and tenderness, associated to the type of backfat thickness (75.6% UFA) and intramuscular fat (13.45%) with (68.1% UFA), with high lipogenic activity during its development and metabolic ability to synthesize and deposit unsaturated fatty acids, and it is suitable to elaborate Iberian style cold cuts (Pacheco, 2019; Dzib-Cauich *et al.*, 2021). Currently, some factor that favors Creole breeds is related to the quality demands of final consumers of animal meat, who prefer animal products without antibiotics and hormones, coming from small-scale systems (Ramos-Canché *et al.*, 2020); in the case of MHP, consumers perceive greater flavor, juiciness, nutritional contribution and stimulation of taste. The above has led to greater acceptance and demand vs pork from specialized breeds (Cardozo and Rodríguez, 2010; Linares *et al.*, 2011). In addition, the content and profile of lipids affect the nutritional, organoleptic and technological attributes of pork meat and its products (Stachowiak *et al.*, 2013). However, according to the studies of Pozo-Leyva *et al.* (2022), Creole pig producers require greater technical training in productive, reproductive aspects and management of the livestock production unit. These authors have also identified that the use of technologies not appropriate for Creole pig production is a critical problem that strongly and directly limits the breeding of Creole pigs.

Few studies have evaluated the effect of organic Cu in the quality of the carcass and meat of pigs, and there has been even less studies addressed to evaluate the effect of Cu in the type of fibers in the LT muscle. The hypothesis was to test whether the dietary supplementation of 0, 75, 150 and 225 mg of Cu kg<sup>-1</sup> DM in finishing pigs influences their growth efficiency, carcass characteristics, muscle fiber type composition, the biochemical processes of muscle maturation and its relationship with meat quality. The objective of this study was to assess the growth performance, characteristics of the carcass, quality of meat and composition and type of fibers in the *Longissimus thoracis* muscle in Mexican Hairless finishing pigs supplemented with different levels of organic Cu in the diet.

## MATERIALS AND METHODS

This research was approved by the Bioethics and Animal Welfare Committee of the Facultad de Medicina Veterinaria y Zootecnia of the Universidad Autónoma del Estado de México and it was carried out in the Experimental Unit on Animal Production of the same institution. The handling procedures were carried out following the official guidelines for animal care in Mexico, NOM-051-ZOO-1995: humanitarian care of animals during transportation (SAGARPA, 1995a) and, NOM-033-ZOO-1995: humanitarian slaughter of domestic and wild animals (SAGARPA, 1995b).

### Animals, handling and treatments

A total of twenty-eight barrows of the ¾ Mexican Hairless pigs breed (62±5.2 kg body weight (BW) and 120±4 days old), coming from four litters of the “Mesa Rica” Livestock Production Unit, backyard level, located in the town of Tehuastepec, municipality of Valle de Bravo, Estado de México were lodged for 46-d in individual pens of 2.5 x 3 m, with automatic drinkers and feeders, in addition to an adaptation time of 15-d on a basal diet (BD) that covered fattening pig nutritional requirements (Table 1). The feeds used to prepare the basal diet supplied to the pigs were purchased from a supplier of the Universidad Autónoma del Estado de México, located in the municipality of Ixtlahuaca, Estado de México. The cost of the diet is indicated in Table 1. The “Mesa Rica” Livestock Production Unit was originally established with MHP animals, reproductive females and males, from a farm located in Mizantla, Veracruz, México. The selection of the pigs is according to the recording of their weights in each productive phase lasting 4 weeks each. Pigs were weighted individually, vitaminized with vigantol ADE-F (2.0 mL), dewormed with ivomec (1.0 mL) and assigned randomly to the treatments (0, 75, 125 and 225 mg Cu kg<sup>-1</sup> DM) in a completely random experimental design. The Cu (copper proteinate) was provided through the premix (Bioplex Cu, Alltech, Inc®, Nicholasville, KY), which contains 1000 mg of Cu kg<sup>-1</sup>, dispersed using the “top dressing” method, which consists of mixing the product with a little of the top part of the feed provided (basal diet), to ensure its daily intake. During the experimental phase of evaluating the productive response, the pigs remained clinically healthy and no antibiotic treatment was applied.

### Animal growth performance

The finishing phase lasted 46-d and the BD was provided *ad libitum*. The feed that was offered and the little feed refused was weighted and registered daily to calculate the voluntary feed intake. The

chemical composition (g kg<sup>-1</sup> DM) of the refused feed was: crude protein (CP) 143.0; ether extract (EE) 36.0; Crude fiber (CF), 33.5; ash, 46.0; calcium (Ca), 7.5; Phosphorus (P), 7.0 y Cu, 14.0 (AOAC, 2007; Fick *et al.*, 1979). Samples of the feed were taken twice a week, a composited sample was dried in a forced-air furnace at 60 °C for 24 h and its chemical composition was analyzed in laboratory: DM, ash, ether extract, crude protein and crude fiber, according to AOAC (2007) method number 930.15, 942.05, 945.16, 984.13 y 962.09), as well as the content of Ca and Cu by atomic absorption spectrophotometry, and P by colorimetric method (Fick *et al.*, 1979) (Table 1.). Pigs were weighed every 15-d; at the end of the study, they were transported to the municipal slaughterhouse in Toluca, Estado de México, where they were weighed and slaughtered, after a fasting period of 12 h, according to the slaughterhouse regulations.

**Table 1. Composition and proximal analysis of the basal diet for fattening pigs.**

Ingredient	(g kg <sup>-1</sup> DM)
Sorghum grain	789.0
Soybean meal	176.0
Minerals and vitamins <sup>1</sup>	30.0
Calcium phosphate	5.0
Total	1000.0
<b>Proximal analysis of laboratory</b>	<b>(g kg<sup>-1</sup> DM)</b>
Metabolizable energy, Mcal kg <sup>-1</sup> DM	3.52
Crude protein	145.0
Ether extract	37.0
Crude fiber	34.0
Ash	45.0
Calcium	8.8
Phosphorus	7.6
Copper, ppm	15.0
Cost: \$/kg feed base	6.94

<sup>1</sup>Premix of minerals and vitamins (Multitec, Malta Cleyton®) (per kg): Ca, 4500 g; Zn, 1.5 g; Cu, 10 g; Fe, 140 g; K, 90 g; Co, 500 g; Mg, 36 g; I, 500 mg; Se, 90 mg; Na, 125 g; Vit. A, 3,000 UI/Kg; Vit. D<sub>3</sub>, 750 UI/Kg; Vit. E, 25 UI/Kg.

**Evaluation of the carcass**

The pigs were transported, for 1 h the day before slaughter, to a public slaughterhouse, with a rest time greater than 16 h. The slaughter of the pigs was made through humanitarian methods (NOM-033-ZOO-1995: humanitarian slaughter of domestic and wild animals) (SAGARPA, 1995b). After the electrical stunning, the carcasses were bled and scalded in water at 65°C. After that, their weight was recorded within 15 min after the slaughter and evisceration. The carcass and viscera inspection tests were carried out, established at the slaughterhouse, and carried out by the veterinary personnel of the slaughter

establishment; no abnormalities, parasites, lesions, etc. were detected, both in the carcass and in the red and digestive viscera. Thirty minutes after the slaughter, a cross-cut was performed on the carcass, at the last rib, a portion of LT was obtained and the area of loin eye was measured through the planimetric method. Back fat thickness (BFT) was measured in the mid-line of the right half of the carcass, on the 10th rib. Drip loss (DL) was measured using the Apple and Jensey (2013) method. Meat samples were weighed and suspended in nylon bags for 2 d at a temperature of 4 °C; weight at day 0 and day 2 was carried out to obtain the shrinkage of meat. Drip loss was expressed as the percentage of the initial sample weight.

**Meat quality**

The color of the chilled meat (4 °C) was measured, after cutting, with a calibrated chromameter (Minolta Chromameter CR-300 Osaka, Japan), 24 and 48 h postmortem, in five randomly representative homogeneous areas, with no intramuscular fat and blood splashes. To do this, a portion of the LT was extracted in the last rib, according to the recommendations of the American Meat Science Association (Hunt *et al.*, 1991; King *et al.*, 2023). Illuminant C and standard observer position of 2 and standard observer position of 2° were used to detect differences in redness in the meat samples between the applied treatments. The following coordinates were determined and the results were expressed as C.I.E. Commission International de l’Eclairage (CIE) lightness (L\*), redness (a\*, red±green) and yellowness (b\*, yellow±blue) values. The saturation or chroma index (C\*), which refers to the clarity or liveliness of the color and the hue angle was calculated using the following equations (Albertí *et al.*, 2016):

$$C^* = (-a^{*2} + b^{*2})^{1/2}$$
$$h = \arctan(b^*/a^*)$$

**Histochemical analysis**

Within 45 min postmortem, 5 samples of 1 cm<sup>3</sup> were taken from the last rib, in a parallel cut to the microfiber package of the LT for histochemical analysis. Samples were placed in 2-methyl butane alcohol, cooled with liquid nitrogen; then they were frozen and preserved at -80 °C until the next analyses. Ten serial sections were cross cut from the muscle (10 µm in thickness) in a cryostat microtome (CM1800 Leica, Germany) at -20 °C and placed in glass slides. The sections were colored by histochemical reaction of the enzyme activity of myosin adenosine triphosphatase (Myosin ATPase) after acid pre-incubation (pH 4.6) (Lind and Kernell, 1991), combining the analysis with the staining of the nicotinamide adenine dinucleotide (reduced)

tetrazolium reductase (NADH-TR) (Gil *et al.*, 2001), to prove the metabolic activity of the fibers. The number of fibers per mm<sup>2</sup> and the area of every type of fiber were measured with the colored sections, using the system of image analysis Sigma Scan Pro (Ver. 4 for Windows, Systat Software Inc, USA). The portions of the sections that were analyzed did not suffer damage due to freezing. A minimum of 300 fibers were evaluated per sample (1500 per pig). With this information, the proportions of the three types of fibers, the average area and the relative area surface occupied by every fiber were calculated. This last data was obtained by adding the individual area of every type of fiber.

### Economic analysis

An economic analysis was carried out, using the partial budget method, with information on the cost of the diets supplied (basal diet without and with different doses of copper proteinate), the feed conversion of each treatment, cost per kg of weight gain and price (\$) per kg of live pork (Harper *et al.*, 2014). A price (\$) per kg of live pig sold was considered 20% higher than that of commercial white breeds.

### Experimental design and statistical model

The experimental design was completely randomized considering the statistical model (Steel *et al.*, 1997):  $Y_{ij} = \mu + T_i + \xi_{ij}$ , where  $Y_{ij}$  = response variable in the  $j^{\text{th}}$  replication and  $i^{\text{th}}$  treatment;  $\mu$  = general mean;  $T_i$  = effect of treatment  $i$ ;  $\xi_{ij}$  = experimental error, i.i.d.  $N(0, \sigma^2)$ . Moreover, normality (Shapiro-Wilk's  $W$ ), independence (Durbin-Watson) and homogeneity of variance (Levene) of the data were proved. The growth variables of the pigs (Dry matter intake (DMI), weight gain (WG), and feed conversion (FC)) and meat quality (pH, color) were analyzed with PROC MIXED, considering treatments as fixed effects and the pigs as random effects within the measurement periods under a Toeplitz correlation model (TOEP) with AR covariance structure (1) and REML 1 estimation method (Littell *et al.*, 2006). Levels of Cu supplementation were partitioned into linear, quadratic, and cubic orthogonal polynomials for four unequally spaced levels with the statements LSMEANS and ESTIMATE. Differences between means were examined with the Tukey's test ( $P \leq 0.05$ ). All the statistical analyses were carried out with SAS v. 9.0 (SAS Institute Inc., 2016).

## RESULTS AND DISCUSSION

### Evaluation of the animal growth performance

The final body weight (FBW) and daily weight gain (DWG) improved quadratically ( $P \leq 0.05$ ) as dietary

Cu supplementation level was increased. This agrees with Diwa (2019) who found that the DWG in growing pigs with 150 mg of Cu kg<sup>-1</sup> DM, as Cu hydroxychloride, was higher ( $P \leq 0.05$ ) compared to the DWG in pigs that were not supplemented with Cu in diet. The DMI was reduced linearly ( $P \leq 0.05$ ) as Cu supplementation level increased in the diet; feed conversion (FC) improved linearly ( $P \leq 0.05$ ) as Cu level increased in the diet (Table 2). Treatment with 75 mg of Cu kg<sup>-1</sup> DM showed greater FBW and DWG, and less feed:gain ratio than the control; this means that DWG was 26.2% higher than the control group, and FC with 75 mg of Cu kg<sup>-1</sup> DM required 31.6% less feed to increase one kg of live weight. The pigs' DMI was higher for control group compared to treatments with 150 and 225 mg of Cu kg<sup>-1</sup> DM; thus, 225 mg of Cu kg<sup>-1</sup> DM seems to have a greater effect on DMI, since this was reduced by 36.3 % compared to control group and this might have affected their growth rate. In addition, Diwa (2019) reported that growing pigs improved energy metabolism and FC, this associated with improvements in intestinal health when supplementing their diet with 150 mg Cu kg<sup>-1</sup> DM as Cu hydroxychloride. Similarly, Colín-Álvarez *et al.* (2019) indicated that finishing pigs improved the DWG and FE as dietary Cu proteinate was increased. One advantage of supplying organic sources of Cu to fattening pigs is the reduction of fecal excretion, and, therefore, greater retention and use of Cu for biological purposes. Most of the literature mentioned above indicates little or no effect among Cu sources, on the weight gain or feed efficiency of pigs; however, in several studies it has been indicated that content of Cu in diets can be reduced in growing and finishing pigs when organic Cu is supplied, without affecting their growth performance.

The quadratic effect of Cu on the final LW and DGW showed a significant increase ( $P < 0.05$ ) in both variables with the dose of 75 mg of Cu kg<sup>-1</sup> MS, but with the dose of 150 mg of Cu kg<sup>-1</sup> MS the effect was smaller, and even with the dose of 225 mg of Cu kg<sup>-1</sup> DM the values of both variables were lower than with respect to the group without Cu. It should be noted that by increasing the dose of Cu, the DMI and FC values decreased linearly ( $P < 0.05$ ), this coincides with the linear effect ( $P < 0.05$ ) of Cu in reducing BFT as the Cu dose increased in the diet. The behavior of the variables described above could influence the yield of the carcass; in this regard, in the present study, the increase in Cu in the diet produced a tendency ( $P < 0.06$ ) to increase the yield of the carcass. The performance of the productive response variables, described above, may be associated with the function of Cu in energy metabolism and specifically with lipid metabolism (Engle and Spears, 2000). According to the studies by Diwa (2019), in pigs, supplementary dietary doses of 150 mg of Cu

kg<sup>-1</sup> DM can positively influence the energy metabolism and productive performance of pigs; likewise, in sheep Cheng et al. (2010) observed lower BFT deposition associated with lower activity of lipogenic enzymes and higher activity of lipolytic enzymes in both subcutaneous adipose tissue and liver.

The economic analysis indicates that the inclusion of Cu proteinate in the MHP diet in the finishing phase slightly increased the cost but due to the better feed conversion of the pigs supplemented with Cu there was a significant feed savings, therefore the cost for producing one kg of weight gain in these groups of pigs with Cu was lower; likewise, diets with Cu produced greater income per kg of pig sold live compared to the control group without Cu (Table 2). The above indicates that the production of MHP meat in an intensive fattening system is profitable and can be improved with the inclusion of Cu proteinate in the diet. In this regard (Ramos-Canché *et al.*, 2020) stated that the integration of Mexican hairless pig livestock production units into the meat production chain is a very important action to obtain greater income and profitability for the producer, as well as to preserve this invaluable zoogenetic resource. However, according to Pozo-Leyva *et al.* (2022), the lack of comprehensive training in technical, productive and reproductive aspects of animal management for creole pig producers is a serious limitation to integrating them into the value-added meat pork production chain.

### Carcass productivity traits

Copper supplementation increased carcass weight (CW) and loin eye area (LEA) quadratically ( $P \leq 0.05$ ) (Table 3). The CW was 9.9% more in the pigs

supplemented with 75 mg of Cu kg<sup>-1</sup> DM, compared to the control group. As a contrast, there was no effect ( $P > 0.05$ ) of Cu in the chilled carcass dressing (CCD). Sierra-Vásquez *et al.* (2016) indicated that in carcass weighing 32.3 kg, the CCD of the Mexican Hairless finishing male pig was 71.1%, a higher value compared to the general average (59.4%) of the CCD of the four treatments in this study. Regarding the LEA, this was higher ( $P \leq 0.05$ ) in the pigs supplemented with 150 mg of Cu kg<sup>-1</sup> DM (35 vs 32 cm<sup>2</sup>), compared to control treatment. Lighter carcasses (32.3 kg) of Mexican Hairless pigs, normally have lower values of LEA (6.5 to 8.5 cm<sup>2</sup>) (Sierra-Vásquez *et al.*, 2016). In this research, the BFT was reduced linearly ( $P \leq 0.05$ ) due to the effect of the Cu supplementation in the diet (Table 3). The BFT decreased ( $P \leq 0.05$ ) as Cu supplementation increased, showing the lowest value in pigs fed 225 mg of Cu kg<sup>-1</sup> DM; compared to control group, it was reduced by 13.7%. Myer *et al.* (1992) found that the addition of 250 mg Cu kg<sup>-1</sup> DM as Cu sulfate to diets for growing-finishing pigs containing canola oil did not have an additive influence on pig performance, pork fat modification, or carcass characteristics. In another study, Lauridsen *et al.* (1999), found that dietary Cu supplementation (35 and 175 mg kg<sup>-1</sup> DM) to growing pigs did not affect the intramuscular fat content of LD, but affected the content of long chain unsaturated fatty acids (FA) (C18:2, C18:3, C20:0, C20:1 and C22:5). Myer *et al.* (1992) concluded that there was no evidence of an additive influence of dietary copper sulfate addition, at a level of 250 ppm of Cu on fatty acid content in fat carcass. In the Colín-Álvarez *et al.* (2019) study, the Cu fed to commercial pigs improved ( $P \leq 0.05$ ) the area of chop and the yield of primary cuts, and it reduced the back fat thickness in the carcass as organic Cu supplementation increased.

**Table 2. Effect of organic copper in the growth performance and economic profitability of Mexican Hairless pig in finishing stage.**

Item	Supplemental Cu (mg kg <sup>-1</sup> DM)				SEM <sup>§</sup>	Effect <sup>‡</sup>
	0	75	150	225		
Initial BW <sup>1</sup> , kg	61.75	62.25	61.00	61.25	1.85	ns
Final BW <sup>1</sup> , kg	92.25 <sup>ab</sup>	99.00 <sup>a</sup>	93.00 <sup>ab</sup>	87.50 <sup>c</sup>	2.52	Q*
DMI <sup>2</sup> , kg d <sup>-1</sup>	4.43 <sup>a</sup>	3.65 <sup>ab</sup>	3.54 <sup>bc</sup>	2.82 <sup>c</sup>	0.20	L*
DWG <sup>3</sup> , kg d <sup>-1</sup>	0.663 <sup>b</sup>	0.799 <sup>a</sup>	0.696 <sup>b</sup>	0.571 <sup>b</sup>	0.06	Q*
Feed conversion <sup>4</sup>	6.68 <sup>a</sup>	4.57 <sup>b</sup>	5.08 <sup>ab</sup>	4.94 <sup>ab</sup>	0.41	L*
Feed cost, \$ kg <sup>-1</sup>	6.94	7.08	7.23	7.37	-----	-----
Cost kg of WG, \$ kg <sup>-1</sup>	46.35	32.35	36.72	36.40	-----	-----
Price per kg of live pork, \$ kg <sup>-1</sup>	55.0	55.0	55.0	55.0	-----	-----
Difference:income (kg pork sales) less expenses (feed cost)	8.65	22.75	18.25	18.60	-----	-----

<sup>§</sup>Standard error of the mean. <sup>‡</sup>Polynomial: ns= non-significant, Q= Quadratic, L=Linear ( $P \leq 0.05$ ).

<sup>1</sup>Body weight. <sup>2</sup>Dry matter intake. <sup>3</sup>Daily weight gain. <sup>4</sup>Feed conversion: Ratio between the DMI and the daily weight gain. <sup>abc</sup>Means with different letter in the same row are different ( $P \leq 0.05$ ).

**Table 3. Effect of organic copper in the traits of the carcass of Mexican Hairless pig in finishing stage.**

	Supplemental Cu (mg kg <sup>-1</sup> DM)				SEM <sup>‡</sup>	Effect <sup>£</sup>
	0	75	150	225		
Carcass weight, kg	53.25 <sup>b</sup>	58.50 <sup>a</sup>	55.75 <sup>b</sup>	53.25 <sup>b</sup>	1.93	Q*
CCD <sup>1</sup> , %	57.76 <sup>b</sup>	59.15 <sup>b</sup>	59.94 <sup>a</sup>	60.80 <sup>a</sup>	2.16	L <sup>+</sup>
LEA <sup>2</sup> , cm <sup>2</sup>	32.00 <sup>c</sup>	33.25 <sup>ab</sup>	35.00 <sup>a</sup>	33.00 <sup>ab</sup>	3.87	Q*
BFT <sup>3</sup> , cm	4.75 <sup>a</sup>	4.50 <sup>a</sup>	4.40 <sup>ab</sup>	4.10 <sup>b</sup>	0.28	L*

<sup>‡</sup>Standard error of the mean. <sup>£</sup>Polynomial: ns= non-significant, Q= Quadratic, L=Linear ( $P \leq 0.05$ )\*; Trend ( $P > 0.05$  to  $P < 0.10$ )\*. <sup>1</sup>Chilled carcass dressing. <sup>2</sup>Loin eye area. <sup>3</sup>Backfat thickness.

### Meat quality traits

Drip loss was reduced linearly ( $P \leq 0.05$ ) due the effect of increasing Cu supplementation in diet (Table 4), which increased water retention capacity of meat. The DL value was lower in the meat of pigs supplemented with 150 and 225 mg Cu kg<sup>-1</sup> DM. Comparing the dose of 225 with control group, the shrinkage was reduced 52% as an effect of Cu. This suggest that the copper might produce less protein denaturation, with less DL (Apple and Yancey, 2013). Within the process, the slower loss of ATP, helps to maintain the protein structural integrity, which delays their denaturation and it increases the carcass weight. This might be due to the function of the Cu as a cofactor of the enzyme cytochrome oxidase, which is essential in the production of ATP in respiratory chain (Lim and Paik, 2006).

Post-slaughter pH, measured at 45 min, 24 and 48 h in LT muscle increased ( $P \leq 0.05$ ) linearly and muscle temperature measured at 45 min decreased ( $P \leq 0.05$ ) linearly as Cu supplementation increased (Table 4). The pH values in LT muscle with 75 and 150 mg Cu kg<sup>-1</sup> DM, measured at 24 and 48 h slaughter, coincides with the average value of pH (5.88) observed by Becerril (2009) in *Longissimus dorsi* muscle of Mexican Hairless finishing pigs. Hernández *et al.* (2008) did not observe the effect of the level of Cu inclusion in the diet of fattening pigs (27 vs 156 mg kg<sup>-1</sup> DM) on the LT muscle pH value (5.49); however, Colín-Álvarez *et al.* (2019) indicated that when increasing the concentration of organic Cu in the diet of pigs in finishing (0, 75, 150 and 225 mg kg<sup>-1</sup> DM) increased the muscle mean value pH, from 5.54 to 6.12, at 45 min and 24 h post-slaughter.

**Table 4. Effect of organic copper in the quality of *Longissimus thoracis* muscle of Mexican Hairless pig in finishing stage.**

	Supplemental Cu (mg kg <sup>-1</sup> DM)				SEM <sup>‡</sup>	Effect <sup>£</sup>
	0	75	150	225		
Drip loss, %	1.25 <sup>a</sup>	1.22 <sup>a</sup>	0.92 <sup>b</sup>	0.60 <sup>c</sup>	0.03	L*
pH 45 min	5.65 <sup>c</sup>	5.97 <sup>bc</sup>	6.03 <sup>b</sup>	6.20 <sup>a</sup>	0.03	L*
pH 24 h	5.53 <sup>c</sup>	5.82 <sup>b</sup>	5.88 <sup>b</sup>	6.15 <sup>a</sup>	0.03	L*
pH 48 h	5.45 <sup>c</sup>	5.77 <sup>b</sup>	5.84 <sup>b</sup>	6.01 <sup>a</sup>	0.03	L*
T <sup>1</sup> 45 min	34.92 <sup>a</sup>	34.10 <sup>ab</sup>	34.13 <sup>ab</sup>	33.53 <sup>c</sup>	0.39	L*
Color index						
L 24 h	33.62	33.65	34.17	37.97	1.87	ns
L 48 h	34	34.21	35.27	37.83	1.56	ns
a 24 h	8.65	8.66	8.05	10.36	0.78	ns
a 48 h	8.90	9.06	8.85	11.24	0.95	ns
b 24 h	3.75	3.74	3.92	5.31	0.68	ns
b 48 h	4.57 <sup>b</sup>	4.83 <sup>b</sup>	4.91 <sup>b</sup>	7.23 <sup>a</sup>	0.49	L*
C*24 h	8.75	9.45	8.97	11.68	0.62	ns
C*48 h	10.12	10.41	9.79	13.44	0.86	ns
h 24 h	22.25 <sup>b</sup>	23.00 <sup>b</sup>	26.19 <sup>a</sup>	28.31 <sup>a</sup>	0.13	L*
h 48 h	27.25 <sup>c</sup>	28.49 <sup>b</sup>	29.59 <sup>b</sup>	32.59 <sup>a</sup>	0.20	L*

<sup>‡</sup>Standard error of the mean. <sup>£</sup>Polynomial: ns= non-significant, Q= Quadratic, L=Linear ( $P \leq 0.05$ )\*. <sup>1</sup>Temperature.



According to the NMX-EF.081-2003 standard (SAGARPA, 2003) if the pH value of the LT muscle at 45 min post slaughter is less than 5.8 points, the acceptance by the consumer is low, but if the pH value at 45 min post slaughter varies from 5.9 to 6.8, the degree of acceptance is good. Likewise, if the pH of the muscle declines very fast (less than 5.8) in the first h post-slaughter due to the excess of accumulated lactic acid, combined with a high temperature of the carcass, it can cause an excess of denaturation of the proteins and predispose to the development of pale soft exudative (PSE) meat, soft in texture, pale in color and exudative; rapid decrease in muscle pH can be attributed to genetic predisposition, pre slaughter stress, or combination of both factors (Apple, 2002). The main problem of PSE meat is its high pH value and greater content of water, which makes it more susceptible to the proliferation of microorganisms, reducing its shelf life.

The intramuscular excess of lactic acid can cause the final pH to be less than 5.5, so, virtually, the meat is normal, red in color, smooth and exudative in texture; however, higher acidity causes muscle proteins to lose their affinity for water and their ability to retain water, consequently, there is greater loss of water in meat. Conversely, when the muscle glycogen reserve is low, the accumulated lactic acid content is lower, consequently, the pH value a few hours post slaughter is greater than 6, this predisposes to the development of a dark firm dry (DFD) meat, of dark color, texture firm and dry (Apple, 2002). Therefore, pH parameter is an indicator of meat quality, reflecting a better color, shelf life and juiciness, with less water loss, without drastic decrease in size when cooked. In contrast, it should be noted that pH average value of the LT muscle of pigs supplemented with 225 mg Cu kg<sup>-1</sup> DM was greater than 6, this can affect the quality of the meat, predisposing towards a dark, firm and dry meat.

The coloration indexes *b* and *h* at 48 h changed linearly ( $P \leq 0.05$ ), and the index *h* increased linearly ( $P \leq 0.05$ ) at 24 h due to the effect of increasing dietary Cu supplementation (Table 4). In the three treatments with Cu, with three measurements times (45 min, 24 and 48 h) the pH of the carcass was higher than the pH in the control group, particularly when supplemented 225 mg Cu kg<sup>-1</sup> DM. Huaigang (2019) mentioned that evolution and final value of pH is related genetically and phenotypically with the criteria of meat quality such as color, DL and sensory features like tenderness, juiciness, flavor and smell.

The temperature of the carcasses of the control group at 45 min was higher ( $P \leq 0.05$ ) than the carcasses in the treatment with 225 mg Cu kg<sup>-1</sup> DM. The combination of high temperature on the carcass (>32° C) with a very fast drop in pH value (<5.5 in 45 min),

may increase the denaturation of the sarcoplasmic and myofibrillar proteins, which may reduce DL and change the meat color, this can predispose the appearance of meat that is PSE after 18 to 24 h of refrigeration. This also reduces the performance when processing the meat product (Warris and Brown, 1987).

There was a tendency for paler meat in the control group, although the effect of Cu supplementation on the main meat color traits ( $L^*$ ,  $a^*$ ,  $C^*$ ) was not significant. Only for  $h^*$  index there were significant changes related to Cu supplementation, that produced increase of meat tone at 24 and 48 h post slaughter. According to the system for qualitative classification of lean meat from the hot carcass of pigs, the standard NMX-EF-081-2003 (SAGARPA, 2003), if the color of the LT muscle is pale and greyish pink, the degree of acceptance by the consumer is good, and if the color is dark red, light red or slightly pink, its degree of acceptance is low, that is, it is rejected. The increase in the dose of Cu in the diet of the pigs did not influence the values of the index  $a^*$  in muscle; therefore, in the present study, the increasing doses of Cu could have influenced the color for a higher degree of acceptance of the meat by the consumer. Hernández *et al.* (2008) did not observe effect of the source and level of Cu (27 vs 156 mg kg<sup>-1</sup> DM) in the diet of pigs on the indexes of coloration  $L^*$ ,  $a^*$  and  $b^*$  of the muscle. In contrast, Colín-Álvarez *et al.* (2019) indicated that the increase of organic Cu (0, 75, 150 and 225 mg kg<sup>-1</sup> DM) in the diet of F1 Landrace X Duroc finishing pigs reduced ( $P \leq 0.05$ ) the values of the  $a^*$  and  $b^*$  indexes in LT muscle a 24 and 48 h post slaughter.

The color of the meat is determined by its myoglobin content; in adult pigs, due to the higher concentration of myoglobin, the meat is redder in color. The  $C^*$  index (color intensity) is determined by the pigment content of the muscle (myoglobin) and normally ranges from pale pink to deep red; respect to the  $h^*$  index (true color or tone), it indicates the chemical state of the myoglobin pigment, if the meat is exposed to air, the myoglobin binds to oxygen and its color is bright red (oxymyoglobin), but if the meat is packed under high vacuum, without the presence of oxygen, its color turns purple (deoxymyoglobin) and, when the meat spoils, due to the passage of time, the myoglobin loses its ability to bind oxygen, and the color turns brown (metmyoglobin) (Albertí *et al.*, 2016). During the time that the meat is on the shelf it oxidizes and its color varies, the luminosity ( $L^*$ ) remains more or less stable, and the intensity of the color ( $C^*$ ) decreases gradually, while the tone ( $h^*$ ), first it descends and then it increases sharply, producing an aspect that predisposes it to be rejected by the consumer. Therefore, the stability of the color over time determines the shelf life of the meat (Ripoll

*et al.*, 2012); in the present study, a dose equal to or greater than 150 mg Cu kg<sup>-1</sup> DM in the diet of pigs can predispose to a shorter shelf life of the meat. Additionally, a higher final pH at 48 h post slaughter is associated to a minor DL, with better performance on the meat (Rubio, 2013), as it may have happened with 225 mg Cu kg<sup>-1</sup> DM in this study.

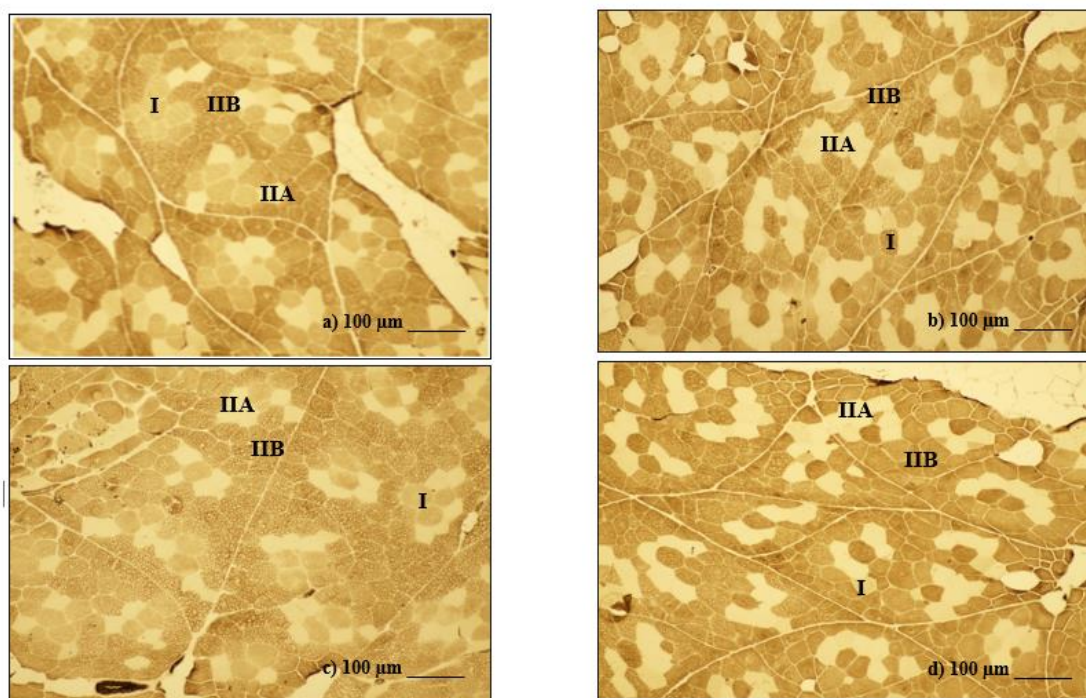
### Histochemical analysis

In the LT muscle of adult pigs from commercial lines, the fiber areas increased in the following order: type IIA, I and IIB (Lefaucheur *et al.*, 1991). Ryu and Kim (2006) reported in commercial lines pigs that fibers increased in the order I, IIA and IIB. In this study the number of type IIB fiber increased quadratically ( $P \leq 0.05$ ) and the composition (%) of the type I fiber decreased linearly ( $P \leq 0.05$ ) as Cu supplementation increased 150 and 225 mg Cu kg<sup>-1</sup> DM (Table 5). The histochemical analysis shows the typical distribution of the fast glycolytic muscle fibers (type IIB) of LT muscle of the pig; in this study we found more type IIB fibers than type I and IIA (Figure 1).

The Cu supplementation did not affect ( $P > 0.05$ ) total number of muscle fibers, but it affected ( $P \leq 0.05$ ) the muscle composition of fiber type (Table 5). The number, percentage and relative area occupied by type I fibers reduced linearly ( $P \leq 0.05$ ) as Cu supplementation increased. These results coincide

with those obtained by Lefaucheur *et al.* (1991), but differ from what was observed by Ryu and Kim, 2006.

In pigs supplemented with 150 and 225 mg Cu kg<sup>-1</sup> DM, the number and relative area occupied by type I fibers were reduced as Cu increased in the diet, but the average area number of type I and IIA fibers was increased linearly. The control treatment had a higher proportion of type I fibers compared to the treatments supplemented with Cu. Additionally, the values of average area and relative occupied area of type IIB fibers increased significantly when Cu was increased in the diet. In the LT muscle, the pH values of the control, 75 and 150 mg Cu kg<sup>-1</sup> DM treatments, in the three times of measurement, may be explained by the number, composition, average area and relative area occupied of the type of fibers, I, IIA and IIB, found in this genotype of pigs without genetic selection; the LT muscle had a larger number, proportion and relative area occupied by the type IIB fibers, these fibers are anaerobic by nature, have fast glycolysis and highest activity of the ATPase, therefore, in the early post-mortem period, continue to break down the glycogen into lactic acid, and consequently the reduction of the pH occurs in the process of transformation from muscle to meat (Bowker *et al.*, 2004).



**Figure 1.** Micrographs that show the three types of muscle fibers (I, IIA y IIB) in a crosscut of the *Longissimus thoracis*. Coloring was carried out with the Acid Myosin ATPase technique. These micrographs are from Mexican Hairless pigs in finishing stage which were given a dietary supplement of a) 0, b) 75, c) 150, and d) 225 mg of Cu kg<sup>-1</sup> DM. Scale bar=100 µm.

**Table 5. Effect of organic copper in the composition of *Longissimus thoracis* fiber types of Mexican Hairless pigs in finishing stage.**

Item	Supplemental Cu (mg kg <sup>-1</sup> DM)				SEM <sup>¥</sup>	Effect <sup>£</sup>
	0	75	150	225		
Muscle fibers (number/mm2)						
Total	287.5	285	290.7	253.8	17.4	ns
Type I	82.5 <sup>a</sup>	77.2 <sup>a</sup>	56.7 <sup>b</sup>	31.3 <sup>c</sup>	5.4	L*
Type IIA	63.5	63.0	64.0	69.5	2.8	ns
Type IIB	141.5 <sup>b</sup>	144.8 <sup>b</sup>	170.0 <sup>a</sup>	153.0 <sup>a</sup>	12.7	Q*
Composition of the type of fiber (%)						
Type I	28.7 <sup>a</sup>	27.1 <sup>b</sup>	19.5 <sup>b</sup>	12.3 <sup>c</sup>	3.8	L*
Type IIA	22.1	22.1	22.0	27.4	2.5	ns
Type IIB	49.2 <sup>b</sup>	50.8 <sup>b</sup>	58.5 <sup>a</sup>	60.3 <sup>a</sup>	4.3	L*
Fibers average area (µm <sup>2</sup> )						
Type I	1290.8 <sup>b</sup>	1317.8 <sup>b</sup>	1657.0 <sup>a</sup>	1640.0 <sup>a</sup>	199.2	L*
Type IIA	1115.4 <sup>b</sup>	1116.4 <sup>b</sup>	1472.6 <sup>a</sup>	1524.3 <sup>a</sup>	223.6	L*
Type IIB	2800.1 <sup>b</sup>	3014.1 <sup>a</sup>	2956.4 <sup>a</sup>	2972.6 <sup>a</sup>	222.7	Q*
Relative area occupied (µm <sup>2</sup> )						
Type I	24.0 <sup>a</sup>	21.6 <sup>a</sup>	15.2 <sup>b</sup>	10.8 <sup>c</sup>	6.7	L*
Type IIA	2.5 <sup>c</sup>	2.9 <sup>c</sup>	5.4 <sup>b</sup>	17.6 <sup>a</sup>	1.1	L*
Type IIB	73.5 <sup>b</sup>	75.5 <sup>b</sup>	79.4 <sup>a</sup>	79.6 <sup>a</sup>	3.3	L*

<sup>¥</sup>Standard Error of the Mean.

<sup>£</sup>Polynomial: ns= non-significant, Q= Quadratic, L=Linear ( $P \leq 0.05$ )\*.

<sup>abc</sup>Means with different letter in the same row are different ( $P \leq 0.05$ )\*.

### Muscle fiber composition and meat quality traits

Efficient lean meat production has been related to the total number (hyperplasia) and size (hypertrophy) of muscle fibers (Larzul *et al.*, 1997; Rehfeldt *et al.*, 2000). Initial studies by Cameron (1990) and Cannon *et al.* (1995) indicated that rapid lean meat production was significantly related to poor meat quality in some pig breeds, mainly affected by muscle fiber size; however, Rehfeldt *et al.* (2000); Kim *et al.* (2008) reported that the total number of muscle fibers was positively correlated with muscle mass and quality of pork. More recent studies by Sang *et al.* (2016) confirmed that higher total number of muscle fibers in combination with higher muscle fiber cross-sectional area improved final lean meat production, ensuring normal quality, with increases in both total number and area of muscle fibers type I and type IIA muscle fibers, and decrease in the number and area ratio of type IIB muscle fibers. Recently, Valenzuela-Grijalva *et al.* (2021) carried out a study on ferulic acid and ractopamine supplementation in diets of finishing pigs and found positive effects on growth performance, daily weight gain, backfat thickness and *Longissimus dorsi* muscle area; likewise, they indicated that the histochemical analysis showed that the applied treatments induced changes in the muscle fibers types, from fast fibers to intermediate (alkaline ATPase) and from oxidative to glycolytic fibers, with a smaller cross-sectional area, and a greater number of muscle fibers per area.

In relation to the loss of water (drip loss) and pH value of the meat Larzul *et al.* (1997) and Ryu and Kim (2006) reported that these variables are not associated with the parameters of the muscle fibers, however, in a study by Sang *et al.* (2016) showed that the proportion of type I fiber muscular area was positively related to meat pH value at 45 min, while the proportion of type IIB fiber muscular area was negatively related to the pH value at 45 min and 24 h. In the present study should be noted that histological changes due to the effect of 225 mg Cu kg<sup>-1</sup> DM dose on type IIB muscle fibers were not sufficient to reduce the pH value of LT muscle; therefore, when the muscle does not reach an acidic pH rapidly, its proteins do not approach its isoelectric point, consequently there is greater water retention and greater performance in meat, which benefits the color and reduces the risk of a pale appearance. According to Sang *et al.* (2016), in pork, water loss and protein denaturation had a strong positive correlation with the proportion of the area or total number of type IIB muscle fibers. The PSE meat represents significant economic losses, it is not attractive to the consumer and its low water retention capacity produces exudate that the microorganisms take advantage of to contaminate it (Ryu and Kim, 2006). Therefore, in Mexican Hairless pigs it is possible to expect changes in the initial post-slaughter energetic metabolism, which will affect the decreased rate of muscle and the final pH of meat (Ryu and Kim, 2006). In this study, the faster pH meat decrease at 45 min postmortem,

the lower final pH at 24 and 48 h of the control pig treatment had the pH value lower compared to pigs supplemented with 75 and 150 mg Cu kg<sup>-1</sup> DM, but it was found the slowest rate of pH decrease in the *LT* muscle was for pigs with 225 mg Cu kg<sup>-1</sup> DM.

## CONCLUSION

It is concluded that Cu supplementation effected quadratically the values of Final BW, DWG, CW and LEA, and linearly Cu affected the values of DMI, FC, CCD, BFT, DL, pH, the color index *b* and *h* after 48h; treatments with Cu also influenced linearly or quadratically the number, composition and relative area of the type I, IIA and BII fibers of the *LT* post slaughter. Our findings support the hypothesis that changes caused in the growth performance, carcass characteristics, muscle fiber type composition and post-slaughter muscle metabolism by dietary organic copper supplementation may influence the parameters of quality of meat in finishing Mexican Hairless pigs. The economic analysis indicates that the production of MHP meat in an intensive fattening system is profitable and can be improved with the inclusion of Cu proteinate in the diet.

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**Compliance with ethical standards.** The pigs were slaughtered according to the protocols and procedures for animal care in México were complied with (NOM-051-ZOO-1995, Trato humanitario en la movilización de animales; NOM-033-SAG/ZOO-2014, Métodos para dar muerte a los animales domésticos y silvestres).

**Data availability.** Data are available with I.A. Domínguez-Vara igy92@hotmail.com, upon reasonable request.

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