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ABSTRACT

This study investigated the effect of two levels of zinc chelate of glycine hydrate (ZnGly) on performance, carcass characteristics and meat quality of growing-finishing pigs. Thirty pigs (initial bodyweight: 61.0 ± 4.02 kg), were randomly assigned to three treatments and fed *ad libitum* for 56 days with a basal diet supplemented with 0 mg/kg (control group, CON), 45 mg/kg (Zn45 treatment) or 100 mg/kg (Zn100 treatment) of ZnGly. Both ZnGly levels strongly reduced chilling carcass loss ($P < 0.001$). Micromineral content, color stability and fatty acid profile of meat was not altered by ZnGly. Superoxide dismutase activity was lowered by Zn45 compared to CON ($P = 0.007$); while, catalase activity was enhanced by Zn100 ($P = 0.003$). Although ZnGly supplementation did not influence lipid oxidation in raw meat and in meat homogenates incubated with pro-oxidant catalysts, Zn45 limited lipid oxidation in cooked meat ($P = 0.037$). The results of this study demonstrated that supplementing pigs with 45 mg/kg of ZnGly could improve the oxidative stability of pork subjected to strong pro-oxidant conditions, but this effect need be further elucidated.

KEYWORDS: intramuscular fatty acids; antioxidant capacity; fat-soluble vitamins; antioxidant enzymes; lipid oxidation; meat shelf-life.

1. Introduction

Zinc is an essential microelement for the regular growth and development of all animal organisms (Sloup et al., 2017), as it plays a fundamental role in the activity of over 300 enzymes and is required in more than 2,000 transcription factors involved in gene expression (Prasad and Kucuk, 2002). Due to these multiple and important biological functions animal's diet need to contain an adequate amount of this trace mineral in order to avoid deficiency. A zinc level of about 50 mg/kg in the diet is recommended for growing-finishing pigs (NRC, 2012). However, the innate zinc level of conventional swine diets is usually not sufficient to meet this requirement, mainly due to the presence of phytic acid in cereals, which reduces zinc availability by forming insoluble complexes (Oberleas et al., 1962; Sloup et al., 2017). Therefore, zinc supplementation and/or the addition of the phytase enzyme, which releases zinc from the phytate complex, are necessary practices to prevent zinc deficiency.

However, zinc supplementation also raises concerns related to environmental issues (Monteiro et al., 2010) which justifies limitations in the levels of supplementation in different countries (e.g., European Union regulation, 2016/1095). In this context, organic zinc sources, such as amino acid chelates, increase the mineral bioavailability, thus allowing to reduce the level of supplementation in the diet (van Heugten et al., 2003; Hill et al., 2014).

Although several studies have investigated the effect of zinc supplementation on growth performance and carcass characteristics of growing-finishing pigs (Cemin et al., 2019; Villagómez-Estrada et al., 2020), little information is available on the quality of the meat. Indeed, only a few aspects of the pork quality, such as proximate composition, drip loss, pH, color and fatty acids, have been marginally investigated when the effect of zinc supplementation was assessed in growing-finishing pigs (Rekiel et al., 2005; Bučko et al., 2013; Holen et al., 2017). However, the wide spectrum of biological activities of zinc may have impacts on several meat quality traits mediated by the growth-promoting activity and/or related

to more direct effect. For example, zinc may exert a role in maintaining the integrity of cell membranes and may act as an antioxidant factor through different mechanisms (Prasad, 1998; Sloup et al., 2017). In particular, zinc could enhance the endogenous antioxidant defenses by acting on antioxidant enzymes and inducing the synthesis of the metallothionein proteins which are able to bind pro-oxidant metals or scavenge free radicals, such as hydroxyl radicals and singlet oxygen (Prasad and Kucuk, 2002; Sloup et al., 2017). Moreover, zinc is believed to decrease the formation of hydroxyl radicals, due to the competition with iron and copper (which catalyze the production of hydroxyl radicals) to bind to the cell membrane (Oteiza et al., 1996).

Due to these multiple biological roles of zinc, in the present study, we hypothesized that zinc supplementation may have some positive effect on the muscle antioxidant status and, in turn, an effect on color and oxidative stability of pork. Therefore, the objective of this study was to evaluate the effect of two levels (45 mg/kg and 100 mg/kg) of zinc chelate of glycine hydrate (ZnGly) on growth performance, carcass characteristics and meat quality in growing-finishing pigs.

2. Materials and methods

2.1. Animals and experimental design

The experimental trial was carried out at the facilities of the University of Catania and the experimental protocol was approved by the animal welfare committee (OPBA) of the University of Catania (No. 286946). Animals were handled by specialized personnel.

Thirty castrated male pigs (crossbred PIC × Piétrain) were selected for their age (125 ± 5 day) and bodyweight (61.0 ± 4.02 kg) from a large range of barrows in a local commercial farm. The animals were transported to the university facilities and allocated in individual pens fitted with metal trough and nipple water dispenser. All the pens were placed in the same room on a concrete floor and the barrows were bedded with wheat straw. Pigs were randomly

assigned to three dietary treatments (10 pigs/treatment) and fed *ad libitum* with a basal diet supplemented with 0 mg/kg (control group, CON), 45 mg/kg (Zn45 treatment) or 100 mg/kg (Zn100 treatment) of zinc chelate of glycine hydrate (ZnGly; Pancosma S.A., Rolle, Switzerland). The ingredients and chemical composition of basal diet are shown in Table 1. All the ingredients were ground, mixed thoroughly and pelleted. To ensure homogeneous distribution, the zinc additive was pre-mixed with sepiolite and calcium carbonate (50:50 w:w) and the obtained blend was then incorporated into the basal diet before pelleting in the ratio of 1 kg/1,000 kg. The carrier (i.e., sepiolite and calcium carbonate) without the addition of zinc additive was included in the control group at the same dosage. Barrows were allowed free access to feed and water during the entire experimental period. Offered concentrates and orts were recorded for each pig in order to determinate the feed consumption. Individual bodyweight was measured fortnightly during the trial. One pig from the Zn45 group died a few days before the end of feeding trial for reasons unrelated to the experiment.

2.2. Slaughter procedure and samplings

After 56 days of feeding trial, all the pigs were weighed and conducted to a commercial abattoir (transport duration of approximately 30 min) where they were slaughtered on the same day by electric stunning and exsanguination according to the European Union Regulation (council regulation (EC) No. 1099/2009). Liver samples were immediately collected after the evisceration, vacuum packaged and frozen at -80 °C pending micromineral analysis. Hot carcass weight (HCW) was recorded within 20 min from slaughter. Muscle pH was measured at 45 min post-mortem directly in the *longissimus thoracis and lumborum* (LTL) muscle using a pH-meter outfitted with a penetrating electrode (Orion 9106; Orion Research Incorporated, Boston, MA). After 24 h of storage at 4 °C, carcasses were weighed to determinate the cold carcass weight (CCW) and muscle pH was recorded as above. Then, a portion of LTL muscle

(between the 13th thoracic vertebra and the 3rd lumbar vertebra), together with backfat and rind stuck on it, was excised from each right-side carcass, vacuum packaged and transported refrigerated to the university laboratories. On the same day, muscle samples were divided into three portions: *i)* an aliquot was stored vacuum-packed at -80 °C for micromineral and fatty acid determination; *ii)* a second aliquot was chopped into small pieces, immediately frozen in liquid nitrogen and stored at -80 °C for analyses of hydrophilic antioxidant capacity, fat-soluble vitamins, cholesterol and antioxidant enzymes and *iii*) the third aliquot was aged vacuum-packed at 4 °C for 24 h and then used for oxidative stability measurements.

2.3. Feed analyses

Representative feed samples were collected three times over the feeding period, vacuum-packaged and stored at -20 °C pending analyses. Feed samples were ground in a hammer mill fitted with 1-mm screen and analyzed for dry matter (DM), crude protein, crude fat and ash according to the AOAC methods (1995). Neutral detergent fiber (NDF) was determined according to Van Soest et al. (1991). The content of zinc, iron, copper and manganese in the diet was determined by atomic absorption spectrometry as described in Lombardo et al. (2017) with some adaptations. Approximately 1.3 g of oven-dried diet was mixed with 2 drops of nitric acid and incinerated at 550 °C until a greyish-white ash was obtained (48 h). The ashes were dissolved in 10 mL of 37% HCl and filtered through a paper filter before analysis with the atomic absorption spectrometer.

Fatty acids of the basal diet were determined in a one-step extraction-transesterification procedure using chloroform and methanolic sulfuric acid (Valenti et al., 2018). In brief, 1 mg/mL of internal standard (tridecanoic acid; C13:0) in hexane was placed into a glass tube and the solvent was evaporated under nitrogen flow. An amount of 100 mg of ground feed sample was weighted into the tube and 1.5 mL of chloroform and 2.5 mL of sulfuric acid (2%)

153 in methanol were added. Tubes were incubated for 2 h at 70 °C in a water bath. After cooling
154 to room temperature, 1.5 mL of chloroform and 2.5 mL of 6% K_2CO_3 were added. Samples
155 were centrifuged at 2,500 × *g* for 10 min at 4 °C and 1 mL of organic phase (bottom) was
156 collected and evaporated under nitrogen flow. The dried residue was dissolved with 1 mL of
157 hexane and analyzed through gas-chromatograph as later described for the analysis of
158 intramuscular fatty acids.
159 Tocopherols of the basal diet were extracted as reported by Rufino-Moya et al. (2020)
160 from 200 mg using 3 mL of methanol:acetone:petroleum ether (1:1:1, v:v:v) with BHT (0.01%,
161 w:v) and vortexing 1 min. The supernatant was collected after centrifugation at 1,000 × *g* for
162 5 min, repeating the extraction for a total of 3 times. The collected solvent was evaporated
163 under nitrogen flow and the residue was dissolved in 1 mL of methanol. Sample was filtered
164 by 0.22 µm PTFE filter and placed into a 2-mL vial. Tocopherols were determined through
165 ultra-high performance liquid chromatograph (UHPLC) as later detailed for the analysis of
166 muscle tocopherols.
167
168 *2.4. Micromineral and fatty acid analyses*
169 Micromineral content in liver and muscle samples were analyzed as described above
170 for the diet, with the only change being that 8 g of tissue was oven-dried and 8 drops of nitric
171 acid were used before ashing.
172 Intramuscular fat was extracted from 10 g of muscle using a mixture 2:1 (v:v) of
173 chloroform and methanol. Then, fatty acids were converted to fatty acid methyl esters (FAME)
174 by a base catalyzed transesterification using sodium methoxide in methanol. Methyl
175 nonadecanoate (C19:0) was used as internal standard. Fatty acids were separated through a gas-
176 chromatograph (model TRACE GC; Thermo Finnigan, Milan, Italy) in a 100-m high-polar
177 fused silica capillary column (25 mm i.d., 0.25-µm film thickness; SP. 24056; Supelco Inc.,

178 Bellefonte, PA) and identified by a flame ionization detector (FID). Gas-chromatography
179 conditions and identification of fatty acids was performed as reported in Natalello et al. (2019).
180 Atherogenicity (AI) and thrombogenicity (TI) indexes were calculated using the formulas
181 developed by Ulbricht and Southgate (1991), while the hypocholesterolemic to
182 Hypercholesterolemic ratio (h/H) was computed as reported in Fernández et al (2007), with
183 some minor changes as follows: h/H = [(sum of C18:1 *c*9, C18:1 *c*11, C18:2 *c*9 c12, C20:1
184 *c*11, C18:3 *c*9 *c*12 *c*15, C20:2 *c*11 *c*14, C20:3 *n*-6, C20:3 *n*-3, C20:4 *n*-6, C22:4 *n*-6, C22:5 *n*-
185 3, C22:6 *n*-3)/(sum of C14:0 and C16:0)].
186
187 *2.5. Hydrophilic antioxidant capacity assays*
188 The antioxidant capacity in aqueous muscle extract was estimated with three different
189 assays in order to evaluate the radical scavenging activity and the reducing capacity as reported
190 in Luciano et al. (2017). In brief, muscle samples were deprived of visible fat and finely minced
191 using a knife. One gram of minced muscle was homogenized in 10 mL of distilled water for 1
192 min at 9,000 rpm (Diax 900, Heidolph ElektroGmbH & Co. KG, Kelheim, Germany). The
193 tubes containing samples were constantly kept in a water/ice bath during the homogenization.
194 Samples were centrifuged at 2,500 × *g* for 20 min at 4°C and supernatant was filtered using
195 Whatman No. 541 filter paper. Filtrated samples were then aliquoted into 3 subsamples, each
196 for one of the following analyses, and stored at -80 °C.
197 The radical scavenging capacity was measured with the Trolox equivalent antioxidant
198 capacity (TEAC) assay, performed as described in Aouadi et al. (2014) with some
199 modifications. The $ABTS^{\bullet+}$ solution was prepared by allowing an equal volume of 14 mM
200 aqueous ABTS [2,2-azinobis-(3-ethylbenzothiazoline 6-sulphonate)] and 4.9 mM potassium
201 persulphate to react for 16 h at room temperature. Twenty µL of filtered sample was mixed
202 with a 2 mL volume of $ABTS^{\bullet+}$ solution and the absorbance at 734 nm was recorded after 60

min of incubation at 30 °C. A blank sample was prepared using 20 µL of distilled water instead of the muscle sample in order to account for the spontaneous discoloration. The calibration curve was made dissolving the Trolox standard (238813; Merck Life Science S.r.l., Milano, Italy) in 4.9 mM potassium persulphate to get four points at concentrations ranging from 100 to 400 µg/mL.

Ferric reducing antioxidant power (FRAP) assay was performed as a measurement of the reducing capacity, using the method developed by Benzie and Strain (1996) with minor adaptations. In short, 50 µL of filtered muscle sample were mixed with 150 µL of distilled water and 1.5 mL of a solution 10:1:1 (v:v:v) of 300 mM acetate buffer (pH 3.6), 10 mmol TPTZ solution (2,4,6-tripyridyl-s-triazine in 40 mM HCl) and 20 mM aqueous ferric chloride. The mixture was incubated at 37 °C for 60 min and the absorbance was then read at 593 nm as well as a reagent blank. Aqueous ferrous sulfate heptahydrate ($FeS0_4 \times 7H_2O$) was used to build a six-point calibration curve at concentrations ranging from 28 to 280 µg/mL.

Folin-Ciocalteu assay was performed as a further measurement of the reducing capacity, using the method described by Makkar et al (1993). In brief, 0.5 mL of 1N Folin-Ciocalteu reagent was mixed with 1 mL of filtered muscle sample previously diluted 1:2 with distilled water. Then, 2.5 mL of sodium carbonate (20% w:v) was added to the mixture. After 40 min of incubation at room temperature in the dark, the solution was centrifuged at 2,500 × *g* for 10 min at 4°C and the absorbance was read at 725 nm. A six-point calibration curve was made using aqueous tannic acid at concentrations ranging from 10 to 100 µg/mL.

2.6. Fat-soluble vitamins and cholesterol

Tocopherols, retinol and cholesterol were determined using the method developed by Bertolín et al. (2018) with minor adjustments as follows. Lyophilized muscle samples (500 mg) were placed in 15-mL plastic centrifuge tube together with 200 mg of L-ascorbic acid and 7.5

mL of saponification solution (10% w:v potassium hydroxide in 1:1 v:v ethanol:water solution). Samples were saponified overnight at 22 °C in an incubator shaker (KS 4000 i control; IKA®-Werke GmbH & Co. KG, Staufen, Germany) set at 250 rpm and protected from light. The following day, 5 mL of 9:1 (v:v) hexane:ethyl acetate containing 25 µg/mL of BHT was added and the tubes were vortexed for 60 s and then centrifuged at 2,000 × *g* for 5 min at 10 °C (Centrifuge 5810 R; Eppendorf s.r.l., Milano, Italy). The upper phase was collected and placed in a glass tube. This extraction procedure was repeated twice. The collected organic phases were evaporated under nitrogen flow at 40 °C using a sample concentrator and a block heater (SBHCONC/1 and SBH130D/3 Stuart®; Cole-Parmer, Stone, United Kingdom). The dry residues were dissolved in 1 mL of methanol (HPLC grade), warming tubes at 40 °C and vortexing thoroughly. The samples were then filtered by 0.2 µm-13 mm PTFE syringe filters and placed into 2-mL amber vials. The analytes were separated and quantified through a Nexera UHPLC (Shimadzu Corporation, Kyoto, Japan) equipped with a C18 phase column (Zorbax ODS; 25 cm × 4.6 mm, 5 µm; Supelco, Bellefonte, PA). A sample volume of 10 µL was injected into the system and the isocratic mobile phase was methanol at the flow rate of 1.3 mL/min. The temperature of the samples (SIL-40C XS Autosampler, Shimadzu) and column oven (CTO-40C, Shimadzu) were set at 25 °C and 40 °C, respectively. Retinol and cholesterol were detected by a photodiode array detector (PDA; SPD-M40, Shimadzu) at the absorbance of 325 nm and 220 nm, respectively. Tocopherols were detected using a spectrofluorometric detector (RF-20AXS, Shimadzu) at 295 nm excitation wavelength and 330 nm emission wavelength. The analytes were identified by comparison of retention times with those of pure standards (Merck Life Science S.r.l., Milano, Italy). The quantification was achieved by external calibration curves made with pure standards.

2.7. Antioxidant enzymes

Frozen muscle samples were deprived of visible fat and finely minced with a knife. Five grams of minced muscle was placed in a 50-mL centrifuge tube with 10 mL of ice-cold 50 mM phosphate buffer [pH 7.0; disodium phosphate dodecahydrate ($Na_2HPO_4 \times 12H_2O$) and KH_2PO_4] and homogenized at 13,000 rpm for 60 s (30 s + pause + 30 s) using a T18 digital Ultra-Turrax® (IKA®-Werke GmbH & Co. KG, Staufen, Germany). During the homogenization, the tubes containing samples were always kept in a water/ice bath to prevent enzymatic reactions and oxidations. Tubes were then centrifuged at 2,800 × g for 20 min at 4°C. Supernatants (1.8 mL) were collected in 2-mL microcentrifuge tubes and further centrifuged at 10,000 × g for 10 min at 4°C. The muscle extract thus obtained was aliquoted (0.5 mL) in 3 microtubes and immediately stored at -80 °C until enzymatic analyses.

The catalase (CAT) activity was determined following the method of Jin et al. (2013) with some modifications. An aliquot of muscle extract was thawed at room temperature for a few min, vortexed and 30 µL was placed in a UV cuvette. A volume of 1.74 mL of H_2O_2 solution (11 mM H_2O_2 in 50 mM phosphate buffer) was added and the cuvette was immediately capped and inverted 4 times. The changes in absorbance were monitored at 240 nm in kinetics mode (model UV-1601; Shimadzu corporation, Kyoto, Japan) over 3 min with reading interval of one second. A blank was prepared with 30 µL of 50 mM phosphate buffer and 1.74 mL of H_2O_2 solution. The molar extinction coefficient of H_2O_2 (39.5 M^{-1} cm^{-1}) was used to calculate the catalase activity and results were expressed as U/g of muscle. One unit (U) of catalase activity was defined as the amount of muscle extract needed to decompose 1 µmol of H_2O_2 per min.

Glutathione peroxidase (GSH-Px) activity was measured according to the procedure developed by Flohé and Gunzler (1984) with some adaptations. Briefly, 500 µL of the assay medium (100 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, 2 mM NaN_3), 100 µL of muscle extract (previously diluted 4-fold in 50 mM phosphate buffer), 100 µL of glutathione

reductase (2.4 U/mL), 100 µL of 10 mM L-glutathione and 100 µL of NADPH solution (1.5 mM NADPH in 0.1% $NaHCO_3$) were directly placed into a UV cuvette and mixed by inverting the cuvette. After 5 min of incubation at room temperature, the overall reaction was started by adding 100 µL of 1.5 mM H_2O_2 and the absorbance at 340 nm was monitored in kinetic mode every second for 5 min. A blank was prepared with 50 mM phosphate buffer instead of muscle extract. The extinction coefficient of 6.22 mM^{-1} cm^{-1} was used to calculate NADPH concentration. One U of GSH-Px activity was defined as the amount of muscle extract needed to oxidize 1 µmol of NADPH per min.

Superoxide dismutase (SOD) activity was evaluated according to the method described in Gatellier et al. (2004). In short, 760 µL of 50 mM Tris-HCl buffer (8.2 pH) were located into a UV cuvette. Then, 20 µL of muscle extract and 20 µL of 10 mM pyrogallol were added and the cuvette was inverted. The absorbance at 340 nm was monitored in kinetic mode for 300 seconds with reading interval of 1 second. One U of SOD activity was defined as the amount of muscle extract needed to inhibit the pyrogallol autoxidation by 50% through comparison with a blank (20 µL of 50 mM phosphate buffer in place of muscle extract). Results were expressed as U of enzyme activity per g of muscle.

2.8. Oxidative stability of backfat and meat

Oxidative stability was evaluated in fresh and cooked meat over aerobic storage as reported by Valenti et al. (2019). In brief, three 2-cm-thick slices were prepared from each LTL muscle sample, placed in polystyrene trays and over-wrapped with 2-layers of domestic cling film. Slices were stored in the dark at 4 °C for 0 (after 2 h of blooming), 3 and 6 days. After each storage time, one of the 3 slices was used to determined color and then frozen pending lipid oxidation analysis. Regarding the cooked meat, other 3 slices of muscle were prepared, vacuum packaged and directly cooked at 70 °C for 30 min. Then, one slice was immediately

303 frozen pending lipid oxidation analysis (day 0), whereas the other two slices were stored in
304 aerobic conditions, as for the raw meat, for 2 and 4 days. After each storage time, one of the 2
305 slices was frozen pending lipid oxidation analysis. Furthermore, as described by Biondi et al
306 (2020), three slices of backfat were cut to a thickness of 2 cm and stored in the same conditions
307 as for the raw meat for 0, 3 and 6 days and used to measure the color descriptors.

308 Color stability of raw meat and backfat was measured by a Minolta CM 2022
309 spectrophotometer (d/8° geometry; Minolta Co. Ltd. Osaka, Japan) set to operate in the
310 specular components excluded (SCE) mode and to measure with the illuminant A and 10°
311 standard observer. Three measurements were taken on the meat and backfat surface on non-
312 overlapping areas and the mean value was calculated. The color descriptors L* (lightness), a*
313 (redness), b* (yellowness), C (saturation) and h_{ab} (hue angle) were measured in the CIE L* a*
314 b* color space. Total color change (ΔE) between 3 or 6 days of storage and the day 0 was
315 calculated as $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. Where ΔL*, Δa* and Δb* are the differences
316 in L*, a* and b*, respectively, between day 0 and day 3 or day 6. Furthermore, the reflectance
317 spectra from 400 to 700 nm wavelength were also recorded in order to calculate the 630/580
318 nm ratio as indicator of myoglobin oxidation.

319 Lipid oxidation was determined in both raw and cooked slices by measuring the 2-
320 thiobarbituric acid reactive substances (TBARS) at the end of each storage time, as reported in
321 Natalello et al. (2020) with some modifications. In brief, 5 g of frozen meat was firstly minced
322 with a knife and then homogenized with 15 mL of 7.5% (w/v) trichloroacetic acid (TCA). The
323 homogenate was filtered through filter paper (Whatman No. 1) and 4 mL of clear filtrate was
324 mixed with 4 mL of 0.02 M aqueous thiobarbituric acid (TBA). After incubation at 80 °C for
325 90 min, the absorbance was read at 532 nm (UV-1601; Shimadzu Corporation, Milan, Italy).
326 A calibration curve was prepared with TEP (1,1,3,3,-tetraethoxypropane) in distilled water at

concentrations ranging from 5 to 65 nmoles/4 mL and results were expressed as mg of malondialdehyde (MDA) per kg of meat.

Lipid oxidation was also assessed in meat homogenates incubated with Fe^{3+} and ascorbate (Fe/Asc) as catalyst of oxidative reactions. The analytic procedures used were adapted from Luciano et al. (2019). Briefly, 10 g of meat was homogenized with 40 mL of 2-(N-morpholino)ethanesulfonic acid (MES) buffer (pH 5.7). The homogenate was equilibrated at 4 °C and then 4 mL was collected for measuring the initial extent of lipid oxidation (0 min). Subsequently, 40 μL of an equimolar solution of ferric chloride hexahydrate and L-sodium ascorbate (45 mM) was added and samples were incubated at room temperature. After 30 and 60 min of incubation, 4 mL of sample was collected, mixed with 4 mL of 15% TCA and filtered through Whatman No. 541 paper. The filtered sample (2 mL) was reacted with 2 mL of 0.02M aqueous TBA at 80 °C for 90 min. The absorbance was read at 532 and a calibration curve was prepared using standard solutions of TEP (2.5 to 32.5 nmoles/2 mL).

2.9. Statistical analysis

All the data were analyzed with the SPSS software (SPSS For Analytics, version 26; IBM corporation, Armonk, NY). The single animal was used as statistical unit. The effect of dietary additive on animal performance, carcass traits, micromineral content of liver and muscle, intramuscular fat, meat fatty acid profile, overall antioxidant capacity, enzymatic and non-enzymatic antioxidants in muscle was statistically analyzed using one-way ANOVA. Data of color and lipid stability were analyzed using a mixed model for repeated measures. The terms in the model were: dietary treatment (D), time of storage or incubation (T) and their interaction (D × T) as fixed factors and each animal as random factor. When P-value was ≤ of 0.05, multiple comparisons were performed using the Tukey post hoc test.

3. Results

3.1. Animal performance and carcass traits

The animal performance and carcass traits of pigs fed diets supplemented with ZnGly are shown in Table 2. The highest ZnGly supplementation (i.e., Zn100) reduced the average daily gain (ADG; $P = 0.031$) or tended to reduce the daily intake ($P = 0.069$) and final body weight ($P = 0.063$) compared to the CON treatment, while Zn45 group did not statistically differ from the other two treatments. Conversely, the feed conversion ratio was not affected by dietary Zn ($P > 0.05$). Hot carcass weight was greater in the control treatment compared only to Zn100 treatment ($P = 0.042$), while the cold carcass weight was statistically comparable between treatments ($P > 0.05$). Both levels of ZnGly supplementation reduced ($P < 0.001$) carcass weight loss occurring within 24 h of chilling storage and tended to increase ($P = 0.094$) the cold carcass yield as compared with CON treatment. Cooking loss, pH and color parameters of muscle as well as color parameters of backfat were not influenced by the ZnGly supplementation ($P > 0.05$).

3.2. Liver and muscle microminerals and intramuscular fatty acids and cholesterol

The contents of copper, iron, manganese and zinc in liver and muscle are reported in Table 3. No differences were observed for all microminerals ($P > 0.05$) with the sole exception of the iron concentration in the liver, which was higher in the Zn45 group than in CON and Zn100 groups ($P = 0.006$).

Table 4 shows the effect of ZnGly supplementation on intramuscular fat content, fatty acid (FA) profile and cholesterol of muscle. Dietary treatment did not affect the intramuscular fat content and the individual fatty acids ($P > 0.05$), except for C22:5 *n*-3 that was lower in both Zn45 and Zn100 compared to CON treatment ($P = 0.001$). In turn, the principal FA classes (i.e., saturated, monounsaturated and polyunsaturated FA) and health indices (i.e.,

atherogenicity and thrombogenicity indices and hypocholesterolemic to hypercholesterolemic ratio) were not altered by ZnGly supplementation ($P > 0.05$), as well as highly peroxidizable polyunsaturated FA (HP-PUFA) with at least three double bonds and the peroxidability index. Cholesterol concentration in muscle tended to be greater in CON group than Zn45 and Zn100 ($P = 0.06$).

3.3. Antioxidant status of muscle

As shown in Table 5, the highest dose of ZnGly supplementation (i.e., Zn100) increased the catalase activity compared to CON and Zn45 treatments ($P = 0.003$), while Zn45 group showed a lower SOD activity than CON, with an intermediate value for Zn100 treatment ($P = 0.007$). No differences between the three treatments were observed for the fat-soluble vitamins (i.e., α-tocopherol and retinol) and the activity of GSH-Px ($P > 0.05$). Similarly, hydrophilic antioxidant capacity, measured with TEAC, FRAP and Folin-Ciocalteu assays, were statistically comparable for all the treatments ($P > 0.05$).

3.4. Color stability of backfat and meat

The effects of the mineral supplementation and the time of storage on color stability of backfat and meat are reported in Table 6. Neither backfat color descriptors nor color descriptors of raw meat were influenced by ZnGly supplementation ($P > 0.05$). All the color parameters measured in both backfat and meat slices were affected by the time of storage, except for h_{ab} in the backfat ($P = 0.174$). Specifically, L* (lightness) values of backfat increased after 3 days of storage and stabilized thereafter between day 3 and day 6. Instead, a* (redness), b* (yellowness) and C* (saturation) values were lower in the backfat after 6 days of storage compared to day 0 and day 3 ($P < 0.001$). Consequently, ΔE was higher in backfat after 6 days of storage than compared to 3-day storage time ($P = 0.001$). Concerning the meat color, L*

values increased, while a*, b*, C* and 630/580 ratio decreased along the 6 days of refrigerated storage ($P < 0.001$). The meat slices stored 3 days had higher values of h_{ab} compared to day 0 and day 6 ($P < 0.001$). Similar to backfat, ΔE values of meat were greater after 6 days of chilling storage than after 3 days ($P < 0.001$). No significant interaction was found between the dietary treatment and the storage time for all the color parameters ($P > 0.05$).

3.5. Meat lipid stability

There were no significant interactions between ZnGly supplementation and time of storage/incubation for lipid stability analysis ($P > 0.05$). Therefore, figure 1 shows the main effects of (a) the ZnGly supplementation and (b) the time of storage/incubation on meat lipid oxidation as evaluated by TBARS assay. The dietary treatment did not significantly affect the lipid oxidation in raw meat ($P = 0.250$) and homogenized meat incubated with pro-oxidant catalysts ($P = 0.531$). Whereas the lower dose of ZnGly supplementation (i.e., Zn45) reduced the lipid oxidation in cooked meat ($P = 0.037$; Fig. 1a). As expected, lipid oxidation increased ($P < 0.001$) over the time of refrigerated storage or incubation (Fig. 1b), for the raw and cooked meat or for the homogenized meat, respectively. In raw meat, TBARS values at day 0 and day 3 were similar and lower compared to day 6. In homogenized and cooked meat, the lowest and the highest TBARS values were observed at the first and last time point, respectively, and intermediate values for the center time point.

4. Discussion

4.1. Animal performance

Although high levels of zinc (Zn) supplementation have long been reported to stimulate voluntary feed intake and weight gain in young pigs (Hahn and Baker, 1993; Case and Carlson, 2002; Barszcz et al., 2019), this effect seems less clear for growing-finishing pigs. Indeed, early

studies demonstrated that feeding growing pigs with increasing levels of Zn oxide (from 0 to 200 mg/kg) had no effect on growth performance (Poulsen and Larsen, 1995; Larsen and Poulsen, 1996). Similarly, D'Souza et al. (2012) observed no improvement in feed intake and daily gain when growing pigs were supplemented with 250 mg/kg of Zn. The reason for this discrepancy between young and heavy pigs might be due to the different physiological period, as the growing phase is a more stable and regular physiological period than post-weaning one, being that weaning causes stress, reduces feed intake and increases susceptibility to infections (Blaabjerg and Poulsen, 2017). As a result, growing-finishing pigs are less demanding than post-weaning piglet in terms of Zn requirement which decreases from 100 to 50 mg/kg for young to finishing pigs (NRC, 2012).

In the present study, the Zn supplementation did not improve growth performance. On the contrary, the highest Zn level (i.e., Zn100) reduced the average daily gain (ADG) and tended to reduce feed intake and final body weight compared to control treatment. A possible explanation for our results may be due to the fact that pigs from control group tended to increase feed intake to compensate for the lack of Zn supplementation and thus to meet their daily Zn requirement. In turn, this may have led to an increase of ADG. This hypothesized compensatory effect may not have been observed in the studies of Poulsen and Larsen (Poulsen and Larsen, 1995; Larsen and Poulsen, 1996) because growing pigs were fed restrictively (i.e., approximately 10% below the expected maximum intake) and thus not allowing them to increase their intake to meet Zn requirements. On the other hand, some studies provided evidences that omitting the supply of microminerals, including Zn, in diets of pigs had no negative impact on animal growth performance, even when growing pigs were fed *ad libitum* (Shelton et al., 2004; Gowanlock et al., 2013). Nevertheless, it should be stressed that, unlike young pigs, only a few studies have investigated the Zn supplementation in heavy pigs. Hence, further investigations are required to fully explicate the effect of Zn supplementation in heavy

452 pigs, especially in commercial farm conditions, where there are likely to be several challenges
453 for pigs.
454
455 *4.2. Carcass traits and meat quality*
456 Interestingly, cold carcass weight (CCW) was comparable between the three
457 treatments, despite the fact that the hot carcass weight (HCW) was reduced by the highest level
458 of Zn supplementation. The difference for the HCW could be a direct consequence of the
459 observed trend for the final body weight, which were slightly lowered by the highest Zn level
460 (i.e., Zn100). While the lack of effect on CCW was clearly due to the lower carcass weight loss
461 for the Zn treatments that occurred during the 24 hours post-mortem of refrigerated storage.
462 Indeed, chilling carcass loss was reduced by more than one percentage point by dietary Zn
463 additive and, in turn, cold carcass yield tended to be higher in Zn pigs.
464 Moisture loss during refrigerated storage reduces the overall weight of the carcass,
465 which contributes to economic loss by reducing the weight of the salable product. In addition,
466 water loss may have a huge effect on meat quality as it is closely related to color, taste,
467 tenderness and juiciness (Warner, 2017). The rate and extent of pH fall are the main factors
468 affecting the ability of the muscle to retain fluids; indeed, a rapid decline of pH after slaughter
469 or a high ultimate pH usually lead to defects in raw meat, such as PSE (pale, soft, and
470 exudative) or DFD (dark, firm, and dry) meats (Warner, 2017). In the present study, muscle
471 pH values measured at 45 minutes or 24 hours post-mortem were not affected by Zn
472 supplementation and can be considered as normal values for pork (Matarneh et al., 2017).
473 Therefore, it would appear that the reduction of chilling carcass loss should be due to other
474 mechanisms not related to muscle pH.
475 The effect of Zn supplementation on pork quality has been rarely investigated.
476 However, the few studies conducted so far have reported no effect of Zn on pork water loss

(Bučko et al., 2013; Gowanlock et al., 2013; Holen et al., 2018). While, there are a number of poultry studies that have observed a positive effect of Zn supplementation on meat moisture retention. For instance, Liu et al. (2011) demonstrated that different Zn levels in broiler diets were able to reduce drip loss of both breast and thigh muscles regardless the Zn source. Likewise, cooking loss of breast and thigh was linearly reduced by increasing inclusions of Zn bearing palygorskite in a broiler diet, but drip loss was not affected (Yang et al., 2016). Moreover, meat from quails fed diets supplemented with 40 and 80 mg/kg of Zn showed lower drip and cooking losses (Rouhalamini et al., 2014). Similar to our findings, in a recent study (Chang et al., 2021) a reduction of breast water loss was reported after 24 h of refrigerated storage and no difference for pH values when ducks received a basal diet supplemented with increasing levels of zinc glycine chelate. To explain these findings, most of the above studies on poultry meat (Liu et al., 2011; Yang et al., 2016; Chang et al., 2021) speculated that Zn supplementation may have enhanced the antioxidant capacity of muscle and consequently reduced lipid oxidation, which has a negative influence on the integrity of cell membranes and thus on the ability to retain intracellular fluids. In the present study, we might rule out this hypothesis as no significant effects on the lipid oxidation of raw meat were observed. Indeed, as better explained in the following paragraphs, lipid oxidation in raw meat developed to a limited extent even after 6 days of storage in aerobic conditions and was not affected by the dietary treatment. Therefore, it seems implausible that during the first 24 hours after slaughter there was such a strong lipid oxidation able to significantly influence the chilling carcass loss. Further studies are needed to elucidate the biological mechanisms involved in reducing meat water loss when animals are supplemented with Zn additives.

In the current study, the Zn supplementation did not influence neither color of muscle and backfat nor meat fatty acid profile. To the best of our knowledge, there are hardly any studies in which the effect of dietary Zn on pork color and fatty acid have been evaluated.

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Bučko et al. (2013) observed that fresh meat, from pigs supplemented with 66 mg/kg of organic Zn, showed lower values of L* (lightness), b* (yellowness) and polyunsaturated FA (PUFA). Whereas, Rekiel et al. (2005) did not find any significant effect of 0.05% Zn oxide on the main FA classes (i.e., saturated, monounsaturated and polyunsaturated FA) and Holen et al. (2018) noticed no meaningful effect on color characteristics of pork when pigs were supplemented with organic and inorganic Zn under crowded housing conditions.

In our experiment, Zn supplementation tended to reduce the cholesterol content of pork, which was in agreement with Dukare et al. (2021) who reported decrease of thigh and breast cholesterol content in broiler meat by supplementing different sources of Zn up to 80 mg/kg level. However, differently from our findings, these authors reported also a reduction of fat percentages of thigh and breast by dietary Zn that might explain the lower content of cholesterol.

It is well known that liver is the main storage site of Zn (Hill et al., 2014) and thus it is mostly responsible for Zn homeostatic regulation. Indeed, several studies have found higher Zn content in liver compared to other organs (van Heugten et al., 2003; Untea et al., 2017). In line with that, we observed here a greater concentration of Zn in the liver than muscle tissue. A previous study has shown that liver Zn content of nursery pigs increased linearly as the level of Zn supplementation increased from 25 to 100 mg/kg (Hill et al., 2014); while Case and Carlson (2002) detected higher Zn content in the liver of pigs supplemented with an excessively high level (i.e., 3,000 mg/kg) of Zn but not in pigs supplemented with lower levels (i.e., 150 and 500 mg/kg). In the present study, no differences in hepatic Zn content were observed when pig diets were supplemented with 45 and 100 mg/kg of Zn. From our results it would appear that the supply of Zn at low levels has no meaningful effects on Zn accumulation in liver of growing-finishing pigs. It should be recalled that Zn can compete with other microminerals, such as iron (Fe) and copper (Cu), for absorption and high levels of dietary Zn could lead to

deficiencies of these elements (Jensen-Waern et al., 1998). An early study of Cox and Hale (1962) demonstrated that Fe concentration in pig liver was lowered by Zn supplementation at the level of 4,000 mg/kg but not with 2,000 mg/kg. Whereas, Carlson et al. (1999) observed no difference in Fe concentration of liver from pigs supplemented with 3,000 mg/kg. Interestingly, in our study, a significant higher concentration of Fe was found in the liver form Zn45 treatment. Hence, our finding seems to suggest that low levels of zinc glycinate (ZnGly) have not limited the absorption and the accumulation of micromineral, but rather Fe content in the liver was enhanced by 45 mg/kg of ZnGly.

In the present study, micromineral content in the muscle was not affected by Zn treatments. Our results indicated that pork from pigs given ZnGly up to 100 mg/kg should not raise any concerns for consumption as muscular Zn concentration was comparable with control treatment. This was in accordance with a previous report (Jensen-Waern et al., 1998), in which the supplementation of Zn did not influence microelement contents in pig muscle despite the high dosage used (i.e., 2500 mg/kg).

4.3. Oxidative stability

Among the endogenous defense systems against oxidative processes in muscle, SOD is one of the most important enzymes and is responsible for the dismutation of superoxide anions into hydrogen peroxide (Bekhit et al., 2013). In mammals, SOD enzyme contains different cofactors and exists in three different forms: CuZn-SOD, present in the cytoplasm and containing Cu and Zn; Mn-SOD, present exclusively in the mitochondrial spaces and containing Mn; EC-SOD, containing also Zn and Cu but operates in the extracellular space (Zelko et al., 2002). In pigs as well as in other mammal species, CuZn-SOD shows much greater activity than other forms (Marklund, 1984). Although some studies have observed an increase in SOD activity in the serum or tissues of animals supplemented with increasing levels

552 of Zn (Bun et al., 2011; Dukare et al., 2021), it is believed that SOD activity is mainly regulated
553 by the availability of Cu rather than Zn (Harris, 1992; Carlson et., 1999). In the present study,
554 we measured the total activity of the three forms of SOD as whole and observed a lower total
555 SOD activity in the muscle of pigs supplemented with 45 mg/kg of ZnGly. This result might
556 be derived from some interaction of the Zn with the other cofactors of SOD (i.e., Cu and Mn),
557 since Zn – competing for adsorbent and for binding to cell membranes – can reduce the
558 availability of Cu and Mn. Similarly, Zn supplementation may also have some interactive effect
559 with Fe, which is a cofactor of catalase enzyme (Nicholls, 2012). Indeed, in the present
560 experiment, catalase activity was higher in the muscle of pigs supplemented with 100 mg/kg
561 of Zn. Consistently, Wen et al. (2011) showed that muscle from Pekin ducks supplemented
562 with 240 mg/kg of Zn had a higher catalase activity compared with 0, 15 and 30 mg/kg.
563 However, this hypothesis seems to contrast our result of micromineral contents of muscle,
564 which were unaffected by Zn supplementation.

565 On the other hand, an increase in enzyme activity is often interpreted as an adaptive
566 response to oxidative stress (Renerre et al., 1996). This belief is supported by the studies of
567 Bun et al. (2011) and Stukelj et al. (2013), which reported increased hepatic or serum SOD
568 activity in broilers challenged with a pathogen or pigs infected with a virus, respectively.
569 Therefore, our finding may indicate that the lowest Zn supplementation led to a better oxidative
570 condition status of animal and therefore a lower need to increase the response of the enzymatic
571 defenses. Nevertheless, it should be underlined that the pigs of the present study were not
572 subjected to any type of stress, since the animals were raised in ideal environmental and
573 nutritional conditions throughout the experiment as well as kept in individual pens, thus
574 avoiding any competitions problem between pigs. Moreover, it is worth mentioning that the
575 muscle from the present study had a remarkable concentration of α-tocopherol (approximately
576 3 μg/g of muscle) regardless the dietary treatment. Hence, considering that α-tocopherol

577 (vitamin E) is a powerful antioxidant in muscle systems (Pettigrew and Esnaola, 2001; Bekhit
578 et al., 2013), its high content may have adequately protected the muscle against natural
579 oxidative processes and consequently limiting the enzymatic defense response.

580 In the current study, color stability of meat and backfat was not affected by Zn
581 supplementation. Whereas, the effect of the storage days on the color parameters was evident
582 as expected. Yet, it is important to emphasize that the color variations over time were
583 numerically small, indicating that the backfat and muscle of the present experiment were not
584 prone to oxidation even after 6 days of aerobic storage. This low propensity to oxidation was
585 consistent with the values of secondary products of lipid oxidation assessed by the TBARS
586 assay. Indeed, the TBARS values of raw meat did not statistically differ between meat at time
587 0 and after 3 days of storage, but increased only after 6 days of aerobic storage at 4 ° C and in
588 any case with values always lower than 0.1 mg MDA per kg of meat (Fig.1b). It should be
589 remembered that these values were far below the threshold at which consumers may detect off-
590 flavors in pork (Sheard et al., 2000). Even when the meat was subjected to a strong oxidative
591 stressor, such as incubation with pro-oxidant catalysts (Fe^{3+} and ascorbate), the observed values
592 were always below 0.9 mg MDA per kg of meat (Fig.1b). A plausible explanation for this
593 scarce propensity of pork to lipid oxidation could be due to the fairly high concentration of α-
594 tocopherol in the muscles of the present study regardless the dietary treatment, as already
595 mentioned above; indeed, vitamin E has been proven to be particularly effective in delaying
596 lipid oxidation and reducing secondary lipid oxidation products in pork as well reviewed by
597 Pettigrew and Esnaola (2001).

598 However, when pork slices were subjected to a much stronger oxidative challenge, such
599 as cooking, TBARS values from cooked pork slices increased to nearly 1.8 mg MDA/kg after
600 4 days of refrigerated aerobic storage (Fig.1b). Interestingly, cooked pork from pigs
601 supplemented with 45 mg/kg of ZnGly showed a higher resistance to lipid oxidation than

602 control treatment, with intermediate TBARS values for Zn100 treatment (Fig.1a). Thus, it may
603 be speculated that the effect of Zn on lipid oxidation was partially hidden by the strong
604 protection exerted by vitamin E when the pork was exposed to low or medium oxidized
605 stressors (i.e., refrigerated aerobic storage or incubation with pro-oxidants), while this effect
606 was evident in highly stressful conditions.

607 These results demonstrated that ZnGly may enhance the oxidative stability of pork
608 under strong oxidative challenge, even if the antioxidant action mechanism of Zn remains to
609 be elucidated. Indeed, according to our findings, the Zn supplementation would appear to have
610 no effect on the fat-soluble vitamins or on the hydrophilic antioxidant capacity – evaluated by
611 TEAC, FRAC and Folin-Ciocalteu assays – of the muscle. Moreover, the lack of response on
612 the SOD activity would seem more an indirect effect on the overall oxidative state of animal
613 than a direct effect since the lowest Zn level decreased the activity of this Zn-containing
614 enzyme. Although in the present study a series of analyzes was carried out to better study the
615 antioxidant effects of Zn supplementation on pork oxidative stability, further research is
616 essential to clarify the antioxidant effect of dietary Zn by also evaluating other aspects related
617 to Zn such as its role in the induction of metallothionein proteins and in the inhibition of
618 NADPH oxidase (Prasad and Kucuk, 2002). Furthermore, future studies should be designed to
619 evaluate the effect of Zn in oxidative challenge conditions such as diets deficient in vitamin E
620 or supplemented with polyunsaturated FA, or physiological stress conditions.

621

622 **5. Conclusions**

623 So far, little has been done to assess the effects of Zn supplementation on the
624 performance and quality of the carcass and meat for pigs in the growing-finishing stage. The
625 results obtained in this study suggest that supplementing the diet of growing-finishing pigs with
626 45 or 100 mg/kg of ZnGly may reduce chilling carcass loss and cholesterol content of meat.

Even though the highest level of Zn supplementation slightly lowered the average daily gain, cold carcass weight did not statistically differ between treatments, indicating that ZnGly has no detrimental effect on economic productivity for swine industries. Both levels of Zn supplementation did not alter the micromineral content, fatty acid profile, fat-soluble vitamins and hydrophilic antioxidant capacity of muscle. Muscle from pigs supplemented 45 mg/kg of Zn showed a lower SOD activity compared to control muscle, while the highest level of Zn increased the catalase activity. This result certainly needs further investigation to establish the role of Zn in regulation of the activity of these enzymes.

Our results showed that the Zn supplementation level of 45 mg/kg improved the meat oxidative stability when pork was subjected to strong oxidative challenges such as cooking, while no effects were observed under aerobic storage of fresh meat or incubation of meat homogenates with pro-oxidant catalysts. Therefore, in these experimental conditions, an antioxidant effect of Zn supplementation may be not evident under milder oxidizing conditions and may be hidden by the effective protection of vitamin E against lipid oxidation. Future studies should be addressed to examine the Zn supplementation in different breeding and meat storage conditions to better capture the antioxidant potential of Zn and its influence on the shelf-life of pork.

Conflict of interest

The authors Hajer Khelil-Arfa, Mieke Zoon and Alexandra Blanchard are employees at Pancosma S.A. and they played no role in collection, analysis and interpretation of data. All authors declare no potential conflicts of interest.

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855 **Table 1.**

856 Ingredients and chemical composition of the basal diet.

	Basal diet
Ingredients, g/kg as fed basis	
Maize	420
Barley	200
Wheat bran	152
Soybean meal (48% crude protein)	134
Fava bean	74
Vitamin premix[1]	10
Sodium carbonate	8
Amino acid premix[2]	2
Chemical composition, g/kg DM	
DM, g/kg as fed	905
Crude Protein	170
Crude Fat	32.9
Neutral detergent fiber	165
Ash	41.7
Fatty acids, g/kg DM	

C16:0	2.52
C18:0	0.49
C18:1 *c*9	3.50
C18:2 *c*9 *c*12	7.61
C18:3 *c*9 *c*12 *c*15	0.58
Tocopherols, mg/kg DM	
α-Tocopherol	7.59
γ-Tocopherol	21.5
δ-Tocopherol	6.42
Microminerals, mg/kg DM	
Copper	16.8
Iron	94.6
Manganese	1.17
Zinc	22.3

[1] One kg of premix contained: vitamin A (650,000 U.I.); vitamin D3 (200,000 U.I.); vitamin E (7,000 mg); vitamin K3 (250 mg); vitamin B1 (250 mg); vitamin B2 (450 mg); vitamin B6 (350 mg); vitamin B12 (3 mg); niacinamide (2,500 mg); calcium D-pantothenate (2,000 mg); folic acid (100 mg); choline chloride (50,000 mg).

[2] One kg of premix contained: lysine (80,000 mg); threonine (280,000 mg); methionine (240,000 mg); tryptophan (120,000 mg); L-valine (240,000 mg).

865 **Table 2.**

866 Effect of zinc glycinate (ZnGly) supplementation on animal performances and carcass traits.

	Dietary treatment [1]			SEM[2]	P-value
	CON	Zn45	Zn100		
Growth performance					
Average daily intake, kg/d	3.17	3.06	2.95	0.041	0.069
Final body weight, kg	115	111	107	1.380	0.063
ADG[3], kg/d	0.97[a]	0.88[ab]	0.83[b]	0.022	0.031
FCR[3]	3.29	3.52	3.56	0.065	0.179
Carcass traits					
HCW[3], kg	92.7[a]	89.8[ab]	86.3[b]	1.080	0.042
CCW[3], kg	88.2	86.4	83.2	1.000	0.107
Carcass yield (hot), %	80.6	81.0	80.4	0.261	0.612
Carcass yield (cold), %	76.6	78.0	77.5	0.258	0.094
Chilling carcass loss, %	4.85[a]	3.70[b]	3.59[b]	0.156	<0.001
Cooking loss, %	29.7	28.8	26.9	0.588	0.126
Muscle pH at 45 min	6.18	6.21	6.27	0.032	0.509
Muscle pH at 24 h	5.50	5.49	5.50	0.026	0.986
Muscle color descriptors					
L* (lightness)	52.5	53.2	52.0	0.583	0.700
a* (redness)	7.73	8.12	7.56	0.210	0.567
b* (yellowness)	7.98	8.44	7.72	0.270	0.562
C* (saturation)	11.1	11.7	10.8	0.334	0.551
h_{ab} (hue angle)	45.6	46.1	45.3	0.379	0.736
Backfat color descriptors					
L* (lightness)	75.1	74.5	76.0	0.431	0.361
a* (redness)	5.73	6.22	5.72	0.205	0.546
b* (yellowness)	6.35	7.16	6.57	0.257	0.438
C* (saturation)	8.57	9.50	8.72	0.322	0.476
h_{ab} (hue angle)	47.7	48.9	48.8	0.44	0.469

867

868 [1] CON, control; Zn45, diet containing 45 mg/kg of ZnGly; Zn100, diet containing 100 mg/kg
869 of ZnGly.
870 [2] SEM, standard error of the mean.
871 [3] ADG, average daily gain; FCR, feed conversion ratio (daily intake/ADG); HCW, hot carcass
872 weight; CCW, cold carcass weight.
873

Table 3.

Effect of zinc glycinate (ZnGly) supplementation on micromineral content in liver and muscle (mg/kg fresh tissue).

	Dietary treatment [1]			SEM[2]	P-value
	CON	Zn45	Zn100		
Liver					
Copper	10.8	11.7	11.1	0.217	0.281
Iron	281[b]	335[a]	296[b]	7.510	0.006
Manganese	3.53	3.61	3.63	0.027	0.286
Zinc	58.4	58.1	59.1	0.336	0.467
Muscle					
Copper	1.88	1.90	1.93	0.027	0.757
Iron	22.9	24.7	27.2	1.130	0.302
Manganese	0.20	0.20	0.20	0.002	0.573
Zinc	19.4	19.6	19.8	0.299	0.867

[1] CON, control; Zn45, diet containing 45 mg/kg of ZnGly; Zn100, diet containing 100 mg/kg of ZnGly.

[2] SEM, standard error of the mean.

882 **Table 4.**

883 Effect of zinc glycinate (ZnGly) supplementation on intramuscular fat, cholesterol and meat
884 fatty acid profile.

885

	Dietary treatment[1]			SEM[2]	P-value
	CON	Zn45	Zn100		
Intramuscular fat, g/100 g	1.65	1.68	1.92	0.105	0.534
Cholesterol, mg/g	0.60	0.52	0.54	0.015	0.060
Fatty acids, mg/100 g					
C10:0	2.22	2.06	2.29	0.143	0.820
C12:0	1.49	1.51	1.83	0.122	0.451
C14:0	20.2	21.2	24.2	1.470	0.524
C16:0	387	399	449	25.00	0.571
C17:0 *anteiso*	4.76	4.82	5.93	0.367	0.348
C16:1 *c*9	51.0	54.8	56.5	3.450	0.809
C17:0	2.35	2.81	2.73	0.201	0.613
C18:0	200	196	230	13.30	0.526
C18:1 *t*9	2.23	2.47	2.35	0.175	0.865
C18:1 *c*9	650	676	778	46.80	0.505
C18:1 *c*11	65.4	68.8	74.8	4.150	0.654
C18:2 *c*9 *c*12	177	168	195	10.20	0.570
C20:0	2.69	2.74	3.31	0.232	0.484
C20:1 *c*11	11.0	12.1	14.2	0.856	0.308
C18:3 *c*9 *c*12 *c*15	6.35	5.19	6.70	0.573	0.557
C20:2 *c*11 *c*14	5.10	5.23	6.90	0.480	0.231
C20:3 *n*-6	4.48	4.51	4.38	0.185	0.963
C20:3 *n*-3	0.80	0.82	0.93	0.110	0.884
C20:4 *n*-6	29.5	28.4	28.6	0.804	0.832
C22:4 *n*-6	4.37	4.56	4.72	0.181	0.743
C22:5 *n*-3	4.20[a]	3.06[b]	2.86[b]	0.171	0.001
C22:6 *n*-3	0.86	1.24	0.98	0.130	0.500
SFA[3]	621	630	719	40.20	0.552
MUFA[3]	779	814	925	54.80	0.531
PUFA[3]	233	221	251	12.00	0.612
PUFA *n*-6	221	211	240	11.40	0.601
PUFA *n*-3	12.2	10.3	11.5	0.662	0.526
PUFA *n*-6/*n*-3	18.4	21.5	21.4	0.765	0.174
AI[4]	0.46	0.47	0.46	0.005	0.626
TI[5]	1.08	1.09	1.10	0.014	0.822
h/H[6]	2.38	2.32	2.39	0.027	0.527
HP-PUFA[7]	50.6	47.8	49.2	1.58	0.782
Peroxidability index[8]	328	312	342	14.1	0.700

886

887 [1] CON, control; Zn45, diet containing 45 mg/kg of ZnGly; Zn100, diet containing 100 mg/kg
888 of ZnGly.

[2] SEM, standard error of the mean.

[3] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

[4] Atherogenicity index = (C12:0 + 4 × C14:0 + C16:0)/(MUFA + PUFA *n*−6 + PUFA *n*-3).

[5] Thrombogenicity index = (C14:0 + C16:0 + C18:0)/(0.5 × C18:1) + (0.5 × other MUFA) + (0.5 × PUFA *n*-6) + (3 × PUFA *n*-3) + (PUFA *n*-3/PUFA *n*-6).

[6] hypocholesterolemic to hypercholesterolemic ratio = [(sum of C18:1 *c*9, C18:1 *c*11, C18:2 *c*9 c12, C20:1 *c*11, C18:3 *c*9 *c*12 *c*15, C20:2 *c*11 *c*14, C20:3 *n*-6, C20:3 *n*-3, C20:4 *n*-6, C22:4 *n*-6, C22:5 *n*-3, C22:6 *n*-3)/(sum of C14:0 and C16:0)].

[7] Highly peroxidizable-PUFA = calculated as the sum of PUFA with three or more unsaturated bonds.

[8] Calculated as: peroxidability index = (Σdienoic × 1) + (Σtrienoic × 2) + (Σtetraenoic × 3) + (Σpentaenoic × 4) + (Σhexaenoic × 5).

Table 5. Effect of zinc glycinate (ZnGly) supplementation on the overall antioxidant capacity, enzymatic and non-enzymatic antioxidants in muscle.

	Dietary treatment[1]			SEM[2]	P-value
	CON	Zn45	Zn100		
Antioxidant enzymes, U/g					
Catalase (CAT)	141[b]	150[b]	170[a]	3.84	0.003
Glutathione peroxidase (GSH-Px)	0.26	0.28	0.28	0.011	0.835
Superoxide dismutase (SOD)	132[a]	111[b]	121[ab]	2.76	0.007
Fat-soluble vitamins					
α-tocopherol, µg/g of meat	3.16	2.96	3.09	0.085	0.646
retinol, ng/g of meat	15.1	14.9	14.1	0.672	0.828
Hydrophilic antioxidant capacity					
TEAC[3]	49.9	60.5	61.3	4.42	0.512
FRAP[4]	32.7	34.3	31.8	1.24	0.726
Folin–Ciocalteu[5]	0.69	0.66	0.66	0.014	0.468

[1] CON, control; Zn45, diet containing 45 mg/kg of ZnGly; Zn100, diet containing 100 mg/kg of ZnGly.

[2] SEM, standard error of the mean.

[3]Trolox equivalent antioxidant capacity (TEAC). Expressed as mg of Trolox equivalents/g of muscle.

[4]Ferric reducing antioxidant power (FRAP). Expressed as mg of Fe^{+2} equivalents/g of muscle.

[5]Expressed as mg of tannic acid equivalents (TAE)/g of muscle.

Table 6.

Effect of zinc glycinate (ZnGly) supplementation on color stability of backfat and meat.

	Dietary treatment (D)[1]			Time (T)[2]			SEM[3]	P-value		
	CON	Zn45	Zn100	Day 0	Day 3	Day 6		D	T	D x T
Backfat color descriptors										
L* (lightness)	75.9	76.4	77.0	75.2^{b}	76.7^{a}	77.4^{a}	0.233	0.107	<0.001	0.059
a* (redness)	5.00	5.38	5.23	5.88^{a}	5.44^{a}	4.28^{b}	0.126	0.521	<0.001	0.768
b* (yellowness)	5.47	6.22	6.02	6.68^{a}	6.66^{a}	4.34^{b}	0.186	0.123	<0.001	0.381
C* (saturation)	7.48	8.27	7.99	8.91^{a}	8.62^{a}	6.18^{b}	0.212	0.266	<0.001	0.582
h_{ab} (hue angle)	57.6	54.1	48.4	48.5	50.4	61.2	2.97	0.451	0.174	0.527
ΔE[4]	4.41	4.23	3.35	-	3.25	4.73	0.293	0.421	0.001	0.956
Meat color descriptors										
L* (lightness)	55.0	55.0	53.9	52.5^{c}	54.6^{b}	56.7^{a}	0.332	0.399	<0.001	0.752
a* (redness)	5.85	5.93	5.55	7.79^{a}	5.28^{b}	4.23^{c}	0.187	0.475	<0.001	0.692
b* (yellowness)	6.41	6.46	5.97	8.03^{a}	6.28^{b}	4.5^{c}	0.196	0.436	<0.001	0.616
C* (saturation)	8.69	8.78	8.16	11.2^{a}	8.22^{b}	6.19^{c}	0.267	0.439	<0.001	0.618
h_{ab} (hue angle)	47.5	47.5	47.0	45.6^{b}	49.7^{a}	46.7^{b}	0.290	0.678	<0.001	0.877
630/580 ratio	1.24	1.24	1.23	1.34^{a}	1.21^{b}	1.15^{c}	0.009	0.617	<0.001	0.927
ΔE[4]	5.98	5.91	5.81	-	4.68	7.12	0.267	0.966	<0.001	0.740

[1] CON, control; Zn45, diet containing 45 mg/kg of ZnGly; Zn100, diet containing 100 mg/kg of ZnGly.

[2] Days of refrigerated aerobic storage.

[3] SEM, standard error of the mean.[4] Total color change between each day of storage and the day 0. Calculated as $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. Where, ΔL^*, Δa^* and Δb^* are the differences in L*, a* and b*, respectively, between day 0 and day 3 or 6.

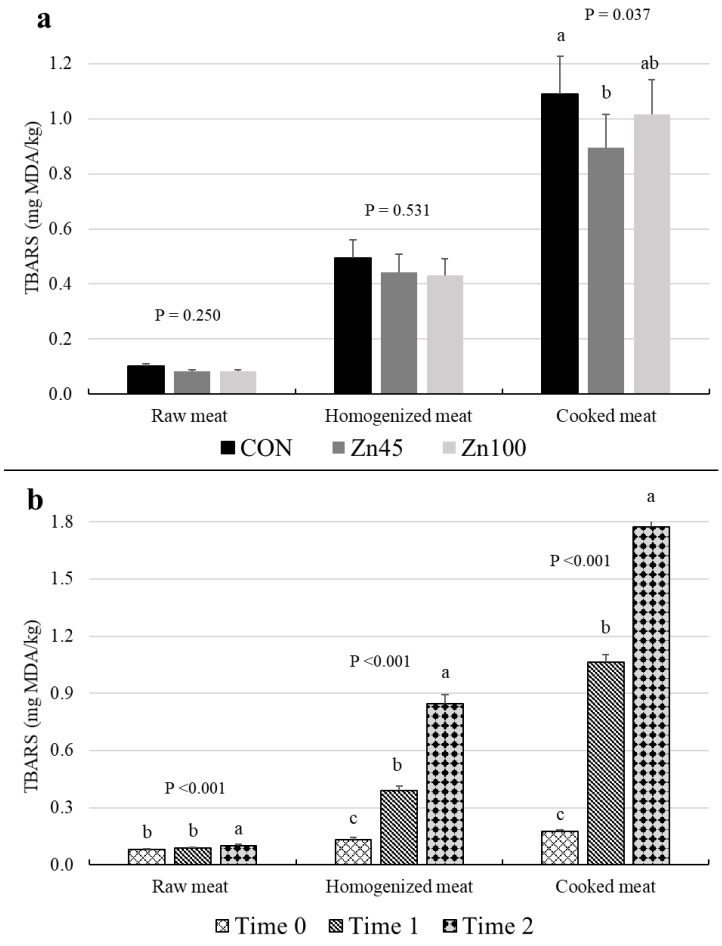

Fig. 1. Main effect of (a) zinc glycinate (ZnGly) supplementation or (b) time of storage/incubation on lipid oxidation (TBARS assay) measured in raw and cooked meat slices stored aerobically at +4 °C or in homogenized meat incubated with Fe^{3+} and ascorbate. Columns are the mean values and error bars represent the standard error of the mean.

CON, control; Zn45, diet containing 45 mg/kg of ZnGly; Zn100, diet containing 100 mg/kg of ZnGly.

0, 1, 2 correspond to: days 0, 3, 6 (raw meat); minutes 0, 30, 60 (homogenized meat incubated with Fe^{3+} and ascorbate); days 0, 2, 4 (cooked meat).

[a, b, c] Different superscript letters, within each class (raw meat or homogenized meat or cooked meat), indicate differences between means ($P \leq 0.05$) tested using the Tukey's adjustment for multiple comparisons.