Meat Science 186 (2022) 108731

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Effect of different levels of organic zinc supplementation on pork quality

Antonio Natalello [a], Hajer Khelil-Arfa [b], Giuseppe Luciano [a,*], Mieke Zoon [b], Ruggero Menci [a], Manuel Scerra [c], Alexandra Blanchard [b], Fabrizio Mangano [a], Luisa Biondi [a], Alessandro Priolo [a]

[a] University of Catania, Dipartimento di Agricoltura, Alimentazione e Ambiente (Di3A), Via Valdisavoia 5, 95123 Catania, Italy
[b] Pancosma ADM, A One Business Center, 1180 Rolle, Switzerland
[c] University of Reggio Calabria, Dipartimento di Agraria, Produzioni Animali, Via dell'Università, 25, 89124 Reggio Calabria, Italy

ARTICLE INFO

Keywords:
Intramuscular fatty acids
Antioxidant capacity
Fat-soluble vitamins
Antioxidant enzymes
Lipid oxidation
Meat shelf-life

ABSTRACT

This study investigated the effect of two supplementation levels of zinc glycinate (ZnGly) on performance, carcass characteristics, and meat quality of growing-finishing pigs. Thirty pigs (bodyweight: 61 ± 4.0 kg) were assigned to three treatments and fed *ad libitum* for 56 days a diet supplemented with 0 (control), 45 (Zn45), or 100 mg/kg (Zn100) of ZnGly. The highest ZnGly supplementation lowered the average daily gain ($P = 0.031$); while, cold carcass weight did not differ between treatments. Both ZnGly levels reduced carcass chill loss ($P < 0.001$). Micromineral content, color stability, and fatty acid profile of meat were not altered by ZnGly. Superoxide dismutase activity was lowered by Zn45 compared to control ($P = 0.007$); 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 ($P = 0.037$). Our results 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 needs to be further elucidated.

1. Introduction

Zinc is an essential microelement for the regular growth and development of all animal organisms (Sloup, Jankovská, Nechybová, Peřinková, & Langrová, 2017), as it plays a fundamental role in the activity of over 300 enzymes and is required in more than 2000 transcription factors involved in gene expression (Prasad & Kucuk, 2002). Due to these multiple and important biological functions, animal diets 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 (National Research Council, 2012). However, the innate zinc level of conventional swine diets is usually not sufficient to meet this requirement, mainly because of the presence of phytic acid in cereals, which reduces zinc availability by forming insoluble complexes (Oberleas, Muhrer, & O'Dell, 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.

Zinc supplementation raises concerns related to environmental issues (Monteiro, Lofts, & Boxall, 2010) which justifies limitations in the level 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 (Hill, Mahan, & Jolliff, 2014; Van Heugten, Spears, Kegley, Ward, & Qureshi, 2003).

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., 2021), little information is available on the quality of meat. Indeed, only a few aspects of pork quality, such as proximate composition, drip loss, pH, color, and fatty acids (FA), have been marginally investigated when the effect of zinc supplementation was assessed in growing-finishing pigs (Bučko, Hellová, & Debrecéni, 2013; Holen, Rambo, Hilbrands, & Johnston, 2018; Rekiel, Batorska, Wiécek, & Dziuba, 2005). Given the wide spectrum of biological activities of zinc, there is the potential to affect several meat quality traits, mediated by the growth-promoting activity and/or related to a 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-

* Corresponding author.
E-mail address: giuseppe.luciano@unict.it (G. Luciano).

https://doi.org/10.1016/j.meatsci.2021.108731
Received 13 September 2021; Received in revised form 27 December 2021; Accepted 28 December 2021
Available online 3 January 2022

oxidant metals or scavenge free radicals, such as hydroxyl radicals and singlet oxygen (Prasad & 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) in binding to the cell membrane (Oteiza, Olin, Fraga, & Keen, 1996).

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

2. Materials and methods

2.1. Animals and experimental design

The experiment 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* a basal diet supplemented with 0 mg/kg (control group, CON), 45 mg/kg (Zn45 treatment), or 100 mg/kg (Zn100 treatment) of zinc glycinate (ZnGly; Pancosma S.A., Rolle, Switzerland) for 56 days. 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, ZnGly was pre-mixed with 1:1 (w:w) sepiolite:calcium carbonate, and the obtained blend was then incorporated into the basal diet in the ratio of 1 kg/1000 kg before pelleting. The carrier (i.e., 1:1 sepiolite: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.

Table 1
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-mineral 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[3]*, mg/kg DM*	
α-Tocopherol	7.59
γ-Tocopherol	21.5
δ-Tocopherol	6.42
Microminerals[3]*, 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 (7000 mg); vitamin K3 (250 mg); vitamin B1 (250 mg); vitamin B2 (450 mg); vitamin B6 (350 mg); vitamin B12 (3 mg); niacinamide (2500 mg); calcium D-pantothenate (2000 mg); folic acid (100 mg); choline chloride (50,000 mg); iron(II) sulfate monohydrate (10,000 mg); manganous sulphate monohydrate (7500 mg); copper(II) sulphate pentahydrate (1500 mg); potassium iodide (100 mg); sodium selenite (30 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).

[3] Innate content in the basal diet before the addition of the vitamin-mineral premix.

2.2. Slaughter procedure and samplings

After 56 days of feeding trial, all the pigs were weighed and transported 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 postmortem 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, 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)* a portion was stored vacuum-packed at −80 °C for micromineral and FA determination; *ii)* a second portion 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; *iii*) the third portion 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 (1995). Neutral detergent fiber (NDF) was determined according to Van Soest, Robertson, and Lewis (1991). The content of zinc, iron, copper, and manganese in the diet was determined by atomic absorption spectrometry as described by Lombardo, Pandino, and Mauromicale (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 (AAnalyst 200 model, Perkin Elmer, Norwalk, USA).

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. One hundred mg of ground feed sample was weighed into the tube, and 1.5 mL of chloroform and 2.5 mL of sulfuric acid (2%) in methanol were added. Tubes were incubated for 2 h at 70 °C in a water bath. After cooling to room temperature, 1.5 mL of chloroform and 2.5 mL of 6% K_2CO_3 were added. Samples were centrifuged at 2500 ×*g* for 10 min at 4 °C and 1 mL of organic phase (bottom) was collected and evaporated under nitrogen flow. The dried residue was dissolved in 1 mL of hexane and analyzed through gas-chromatograph as later described for the analysis of intramuscular FA.

Tocopherols of the basal diet were extracted from 200 mg samples as reported by Rufino-Moya, Joy, Lobón, Bertolín, and Blanco (2020), using 3 mL of methanol:acetone:petroleum ether (1:1:1, v:v:v) with BHT (0.01%, w:v) and vortexing 1 min. The supernatant was collected after centrifugation at 1000 ×*g* for 5 min, repeating the extraction for a total of 3 times. The collected solvent was evaporated under nitrogen flow and the residue was dissolved in 1 mL of methanol. The extract was filtered by 0.22-μm PTFE filter and placed into a 2-mL vial. Tocopherols were determined through ultra-high performance liquid chromatograph (UHPLC) as later detailed for the analysis of muscle tocopherols.

2.4. Micromineral and fatty acid analyses

The micromineral content in liver and muscle samples was analyzed as described above for the diet, starting with oven-drying 8 g of fresh tissue and adding 8 drops of nitric acid before ashing.

Intramuscular fat was extracted from 10 g of muscle using a mixture 2:1 (v:v) of chloroform and methanol. Then, FA were converted to fatty acid methyl esters (FAME) by a base catalyzed transesterification using sodium methoxide in methanol. Methyl nonadecanoate (C19:0) was used as internal standard. Fatty acids were separated through a gas-chromatograph (model TRACE GC; Thermo Finnigan, Milan, Italy) in a 100-m high-polar fused silica capillary column (25-mm i.d., 0.25-μm film thickness; SP. 24056; Supelco Inc., Bellefonte, PA) and identified by a flame ionization detector (FID). Gas-chromatography conditions and identification of FAME was performed as reported by Natalello et al. (2019). Atherogenicity index (AI) and thrombogenicity index (TI) were calculated using the formulas developed by Ulbricht and Southgate (1991), while the hypocholesterolemic to hypercholesterolemic ratio (h/H) was computed as reported by Fernández et al. (2007), with some minor changes as follows: h/H = [(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)].

2.5. Hydrophilic antioxidant capacity assays

The antioxidant capacity in aqueous muscle extract was estimated with three different assays in order to evaluate the radical scavenging activity and the reducing capacity as reported by Luciano et al. (2017). In brief, muscle samples were deprived of visible fat and finely minced using a knife. One gram of minced muscle was homogenized in 10 mL of distilled water for 1 min at 9000 rpm (Diax 900, Heidolph ElektroGmbH & Co. KG, Kelheim, Germany). The tubes containing samples were constantly kept in a water/ice bath during the homogenization. The samples were centrifuged at 2500 ×*g* for 20 min at 4 °C and supernatant was filtered using Whatman No. 541 filter paper. Filtrated samples were then aliquoted into 3 subsamples, each for one of the following analyses, and stored at −80 °C.

The radical scavenging capacity was measured with the Trolox equivalent antioxidant capacity (TEAC) assay, performed as described in Aouadi et al. (2014) with some modifications. The $ABTS^{\bullet+}$ solution was prepared by allowing an equal volume of 14 mM aqueous ABTS [2,2-azinobis-(3-ethylbenzothiazoline 6-sulphonate)] and 4.9 mM potassium persulphate to react for 16 h at room temperature. Twenty μL of filtered sample was mixed with 2 mL 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 by dissolving the Trolox standard (238813; Merck Life Science S.r.l., Milano, Italy) in phosphate buffer saline (pH 7.4) 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, as well as a reagent blank, was incubated at 37 °C for 60 min and the absorbance was then read at 593 nm. Aqueous ferrous sulfate heptahydrate ($FeSO_4 \times 7H_2O$) 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, Blümmel, Borowy, and Becker (1993). In brief, 0.5 mL of 1 N 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 2500 ×*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, Joy, Rufino-Moya, Lobón, and Blanco (2018) with minor adjustments as follows. Lyophilized muscle samples (500 mg) were placed in 15-mL plastic centrifuge tubes 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 2000 ×*g* for 5 min at 10 °C (Centrifuge 5810 R; Eppendorf s.r.l., Milano, Italy). The upper phases were collected and placed in glass tubes. The extraction procedure was repeated twice in total. 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 their 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 tube containing sample was always kept in a water/ice bath to prevent enzymatic reactions and oxidation. The tube was then centrifuged at 2800 ×*g* for 20 min at 4 °C. Then, 1.8 mL of supernatant was collected in a 2-mL microcentrifuge tube and further centrifuged at 10,000 ×*g* for 10 min at 4 °C. The muscle extract 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, He, Yu, Zhang, and Ma (2013) with some modifications. An aliquot of muscle extract was thawed at room temperature for a few minutes, 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, 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 1 s. 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 Günzler (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 times 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, Mercier, and Renerre (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 s with reading interval of 1 s. 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).

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 determine color parameters and then frozen pending lipid oxidation analysis. Regarding the cooked meat, other 3 slices of muscle were prepared, vacuum packaged, and directly cooked in water bath at 70 °C for 30 min. Then, one slice was immediately frozen pending lipid oxidation analysis (day 0), whereas the other two slices were stored in aerobic conditions, as for the raw meat, for 2 and 4 days. After each storage time, one of the 2 slices was frozen pending lipid oxidation analysis. Furthermore, as described by Biondi et al. (2020), three slices of backfat were cut to a thickness of 2 cm and stored in the same conditions as for the raw meat for 0, 3, and 6 days and used to measure the color descriptors.

Color stability of raw meat and backfat was measured by a Minolta CM 2022 spectrophotometer (d/8° geometry; Minolta Co. Ltd. Osaka, Japan) set to operate in the specular components excluded (SCE) mode and to measure with the illuminant A and 10° standard observer. For both meat and backfat, 3 measurements were taken on the slice surface on non-overlapping areas and averaged. The color descriptors L* (lightness), a* (redness), b* (yellowness), C (saturation), and h_{ab} (hue angle) were measured in the CIE L* a* b* color space. Total color change (ΔE) between 3 or 6 days of storage and the day 0 was 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 day 6. Furthermore, the reflectance spectrum from 400 to 700 nm wavelength was recorded in order to calculate the 630/580 nm ratio as indicator of myoglobin oxidation.

Lipid oxidation was determined in both raw and cooked meat slices by measuring the 2-thiobarbituric acid reactive substances (TBARS) at the end of each storage time, as reported by Natalello et al. (2020) with some modifications. In brief, 5 g of frozen meat was minced with a knife and then homogenized with 15 mL of 7.5% (*w/v*) trichloroacetic acid (TCA). The homogenate was filtered through filter paper (Whatman No. 1), and 4 mL of clear filtrate was mixed with 4 mL of 0.02 M aqueous thiobarbituric acid (TBA). After incubation at 80 °C for 90 min, the absorbance was read at 532 nm (UV-1601; Shimadzu Corporation, Milan, Italy). A calibration curve was prepared with TEP (1,1,3,3,-tetraethoxypropane) in distilled water at concentrations ranging from 5 to 65 nmol/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. 1 paper. The filtered sample (2 mL) was reacted with 2 mL of 0.02 M 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 nmol/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 FA profile, overall antioxidant capacity, and 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.

Significance was declared at $P \leq 0.05$, while trends toward significance were considered when $0.05 < P \leq 0.10$. Differences between

means were assessed using the Tukey's Honest Significant Difference test. Moreover, orthogonal polynomial contrasts were used in order to test for linear and quadratic trends of the dependent variables across the ZnGly levels, using SPSS "One-Way ANOVA" feature, which automatically balances when treatments are unequally spaced.

3. Results

3.1. Growth performance and carcass traits

The growth performance and carcass traits of the experimental pigs 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 CON treatment, while Zn45 group did not statistically differ from the other two treatments. In addition, linear reductions were observed for the daily intake, final body weight, and ADG when polynomial contrasts were tested ($P < 0.05$). 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 ($P = 0.042$) and a significant linear response was also observed ($P < 0.05$), while CCW 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. Moreover, cold carcass yield responded quadratically to dietary ZnGly levels ($P < 0.01$) and carcass chill loss decreased in linear and quadratic manners ($P < 0.05$). Cooking loss, pH, and color parameters of muscle as well as color parameters of backfat were not influenced by the ZnGly supplementation ($P > 0.05$).

Table 2
Effect of zinc glycinate (ZnGly) supplementation on growth performance and carcass traits.

	Dietary treatment [1]			SEM[2]	*P*-value
	CON	Zn45	Zn100		
Growth performance					
Daily intake, kg/d	3.17	3.06	2.95	0.041	0.069[3]
Final body weight, kg	115	111	107	1.380	0.063[3]
ADG[4], kg/d	0.97[a]	0.88[ab]	0.83[b]	0.022	0.031[3]
FCR[4]	3.29	3.52	3.56	0.065	0.179
Carcass traits					
HCW[4], kg	92.7[a]	89.8[ab]	86.3[b]	1.080	0.042[3]
CCW[4], kg	88.2	86.4	83.2	1.000	0.107
Hot carcass yield, %	80.6	81.0	80.4	0.261	0.612
Cold carcass yield, %	76.6	78.0	77.5	0.258	0.094[5]
Carcass chill loss, %	4.85[a]	3.70[b]	3.59[b]	0.156	<0.001[6]
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	0.440	0.469

[a, b] Means in the same row lacking a common superscript differ ($P < 0.05$).
[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] Linear response to dietary ZnGly concentrations ($P < 0.05$).
[4] ADG, average daily gain; FCR, feed conversion ratio (daily intake/ADG); HCW, hot carcass weight; CCW, cold carcass weight.
[5] Quadratic response to dietary ZnGly concentrations ($P < 0.01$).
[6] Linear and quadratic response to dietary ZnGly concentrations ($P < 0.05$).

3.2. Microminerals, 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 Zn45 group than in CON and Zn100 groups ($P = 0.006$). In addition, the iron concentration of liver responded in quadratic manner to the ZnGly supplementation ($P < 0.05$).

Table 4 shows the effect of ZnGly supplementation on intramuscular fat content, FA profile, and cholesterol of muscle. Dietary treatment did not affect the intramuscular fat content and the individual FA ($P > 0.05$), except for C22:5 *n*-3 that was lower in both Zn45 and Zn100 treatments 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., AI, TI, and h/H) were not altered by ZnGly supplementation ($P > 0.05$), as well as the 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 groups ($P = 0.06$) and it showed a linear response to dietary treatment ($P < 0.05$).

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 ($P = 0.007$). Furthermore, when polynomial contrasts were performed, catalase activity showed a linear ($P < 0.05$) increase and SOD activity displayed both linear ($P < 0.05$) and quadratic ($P < 0.01$) responses to ZnGly concentrations. 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

Table 3
Effect of zinc glycinate (ZnGly) supplementation on the 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[3]
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

[a, b] Means in the same row lacking a common superscript differ ($P < 0.05$).
[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] Quadratic response to dietary ZnGly concentrations ($P < 0.05$).

Table 4
Effect of zinc glycinate (ZnGly) supplementation on the intramuscular fat, cholesterol, and fatty acid profile of meat.

	Dietary treatment[1]			SEM[2]	P-value
	CON	Zn45	Zn100		
Intramuscular fat, g/100 g muscle	1.65	1.68	1.92	0.105	0.534
Cholesterol, mg/g muscle	0.60	0.52	0.54	0.015	0.060[3]
Fatty acids, mg/100 g muscle					
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[3]
C22:6 *n*-3	0.86	1.24	0.98	0.130	0.500
Sums and calculations					
SFA[4]	621	630	719	40.20	0.552
MUFA[4]	779	814	925	54.80	0.531
PUFA[4]	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[5]	0.46	0.47	0.46	0.005	0.626
TI[6]	1.08	1.09	1.10	0.014	0.822
h/H[7]	2.38	2.32	2.39	0.027	0.527
HP-PUFA[8]	50.6	47.8	49.2	1.580	0.782
Peroxidability index[9]	328	312	342	14.10	0.700

[a, b] Means in the same row lacking a common superscript differ ($P < 0.05$).
[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] Linear response to dietary ZnGly concentrations ($P < 0.05$).
[4] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
[5] Atherogenicity index = (C12:0 + 4 × C14:0 + C16:0)/(MUFA + PUFA *n*-6 + PUFA *n*-3).
[6] 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).
[7] 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)/(C14:0 + C16:0).
[8] Highly peroxidizable-PUFA = calculated as the sum of PUFA with three or more unsaturated bonds.
[9] Calculated as: peroxidability index = (Σdienoic × 1) + (Σtrienoic × 2) + (Σtetraenoic × 3) + (Σpentaenoic × 4) + (Σhexaenoic × 5).

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* values of backfat increased after 3 days of storage and stabilized thereafter between day 3 and day 6. Instead, a*, b*, and C* 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 after 3 days of storage ($P = 0.001$). 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

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 muscle					
Catalase (CAT)	141[b]	150[b]	170[a]	3.84	0.003[3]
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[4]
Fat-soluble vitamins					
α-tocopherol, μg/g muscle	3.16	2.96	3.09	0.085	0.646
retinol, ng/g muscle	15.1	14.9	14.1	0.672	0.828
Hydrophilic antioxidant capacity					
TEAC[5]	49.9	60.5	61.3	4.42	0.512
FRAP[6]	32.7	34.3	31.8	1.24	0.726
Folin–Ciocalteu[7]	0.69	0.66	0.66	0.014	0.468

[a, b] Means in the same row lacking a common superscript differ ($P < 0.05$).
[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] Linear response to dietary ZnGly concentrations ($P < 0.05$).
[4] Linear ($P < 0.05$) and quadratic ($P < 0.01$) response to dietary ZnGly concentrations.
[5] Trolox equivalent antioxidant capacity. Expressed as mg of Trolox equivalents per g of muscle.
[6] Ferric reducing antioxidant power. Expressed as mg of Fe^{2+} equivalents per g of muscle.
[7] Expressed as mg of tannic acid equivalents per g of muscle.

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 ($P > 0.05$). Fig. 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 and homogenized meat incubated with pro-oxidant catalysts ($P > 0.05$). The lowest dose of ZnGly supplementation (i.e., Zn45) reduced the lipid oxidation in cooked meat ($P = 0.037$; Fig. 1a). As expected, lipid oxidation increased ($P < 0.001$; Fig. 1b) over the time of refrigerated storage (for the raw and cooked meat) or incubation (for the homogenized meat). 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 central time point.

4. Discussion

4.1. Growth performance

Although high levels of zinc (Zn) supplementation have long been reported to stimulate voluntary feed intake and weight gain in young pigs (Barszcz, Taciak, Tuśnio, Čobanová, & Grešáková, 2019; Case & Carlson, 2002; Hahn & Baker, 1993), this effect seems less evident 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 (Larsen & Poulsen, 1996; Poulsen & Larsen, 1995). Similarly, D'souza, Mullan, Pethick, Pluske, and Dunshea (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

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}	4.50^{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

[a, b, c] Means in the same row lacking a common superscript differ ($P < 0.05$).
[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.

to the different physiological period, as the growing phase is a more stable and regular period than the post-weaning one, being that weaning causes stress, reduces feed intake and increases susceptibility to infections (Blaabjerg & Poulsen, 2017). As a result, growing-finishing pigs are less demanding than post-weaning piglets in terms of Zn requirement, which decreases from 100 to 50 mg/kg for young to finishing pigs (National Research Council, 2012).

In the present study, the Zn supplementation did not improve growth performance. On the contrary, a negative linear trend was observed for feed intake, final body weight, and ADG with increasing levels of Zn supplementation and the highest ZnGly level (i.e., Zn100) reduced ADG and tended to reduce feed intake and final body weight compared to control treatment. There is no simple explanation for this effect. Reynolds, Forbes, and Miller (2010) concluded that pharmacological inclusion (i.e., 3100 mg/kg) of Zn oxide strongly reduced the feed intake of weaned piglets due to palatability issue. However, to the best of our knowledge, no issues related to the palatability of Zn supplementation for growing-finishing pigs have been reported in the literature. In addition, the low levels of Zn supplementation used in our study would hardly affect diet palatability, also considering that organic Zn sources are known to be more palatable than inorganic ones. A possible explanation for our results may be that the Zn level in the control diet (i.e., 22.3 mg/kg) was below the NRC requirements. It may be supposed that control pigs increased the ingestion to achieve an adequate daily intake of Zn. In our study, this could have been possible because animals were fed ad libitum, whereas, in other studies about Zn supplementation, growing pigs were fed restrictively (e.g., Larsen & Poulsen, 1996; Poulsen & Larsen, 1995). This hypothesis may partially explain why the Zn content in muscle and liver was not different between the groups despite the Zn deficiency of the control diet. On the other hand, some studies demonstrated that omitting the supply of microminerals, and thus Zn, in the diets of pigs had no negative impact on animal growth performance (Gowanlock, Mahan, Jolliff, Moeller, & Hill, 2013; Shelton, Southern, LeMieux, Bidner, & Page, 2004). In addition, a possible effect of Zn stored in tissues, deriving from previous feeding, on the results we observed cannot be ruled out. Indeed, before starting the present experiment, all the pigs were fed a diet identical to the experimental diet but with a Zn supplementation of 100 mg/kg. 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 pigs, especially in commercial farm conditions, where there are likely to be several challenges for pigs.

4.2. Carcass traits and meat quality

Interestingly, CCW was comparable between the three treatments, despite the fact that HCW was reduced by the highest level of ZnGly supplementation. The effect on HCW could be a direct consequence of the lower ADG observed for the Zn100 treatment. Instead, the lack of effect on CCW was clearly due to the lower carcass weight loss for the ZnGly treatments that occurred during the 24 h post-mortem of refrigerated storage. Indeed, carcass chill loss was reduced by more than one percentage point by dietary Zn additive and, in turn, cold carcass yield tended to be higher in ZnGly supplemented pigs.

Moisture loss during refrigerated storage reduces the overall weight of the carcass, which contributes to economic loss by reducing the weight of the salable product. In addition, water loss may have a huge effect on meat quality as it is closely related to color, taste, tenderness, and juiciness (Warner, 2017). The rate and extent of pH fall are the main factors affecting the ability of the muscle to retain fluids; indeed, a rapid decline of pH after slaughter or a high ultimate pH usually lead to defects in raw meat, such as PSE (pale, soft, and exudative) or DFD (dark, firm, and dry) meats (Warner, 2017), respectively. However, the reduction of carcass chill loss by ZnGly supplementation observed in the present study should be due to other mechanisms not related to muscle pH. Indeed, muscle pH values measured at 45 min or 24 h post-mortem were not affected by Zn supplementation and can be considered as normal values for pork (Matarneh, England, Scheffler, & Gerrard, 2017).

The effect of Zn supplementation on pork quality has been rarely investigated. 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 is a number of poultry studies that observed a positive effect of Zn supplementation on meat moisture retention. For instance, Liu et al. (2011) demonstrated that different Zn levels in broiler's diet were able to reduce drip loss of both breast and thigh muscles, regardless of the Zn source. Likewise, cooking loss of breast and thigh was linearly reduced by increasing inclusions of Zn bearing palygorskite in broiler's 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, Salarmoini, & Asadi-Karam, 2014). Similar to our

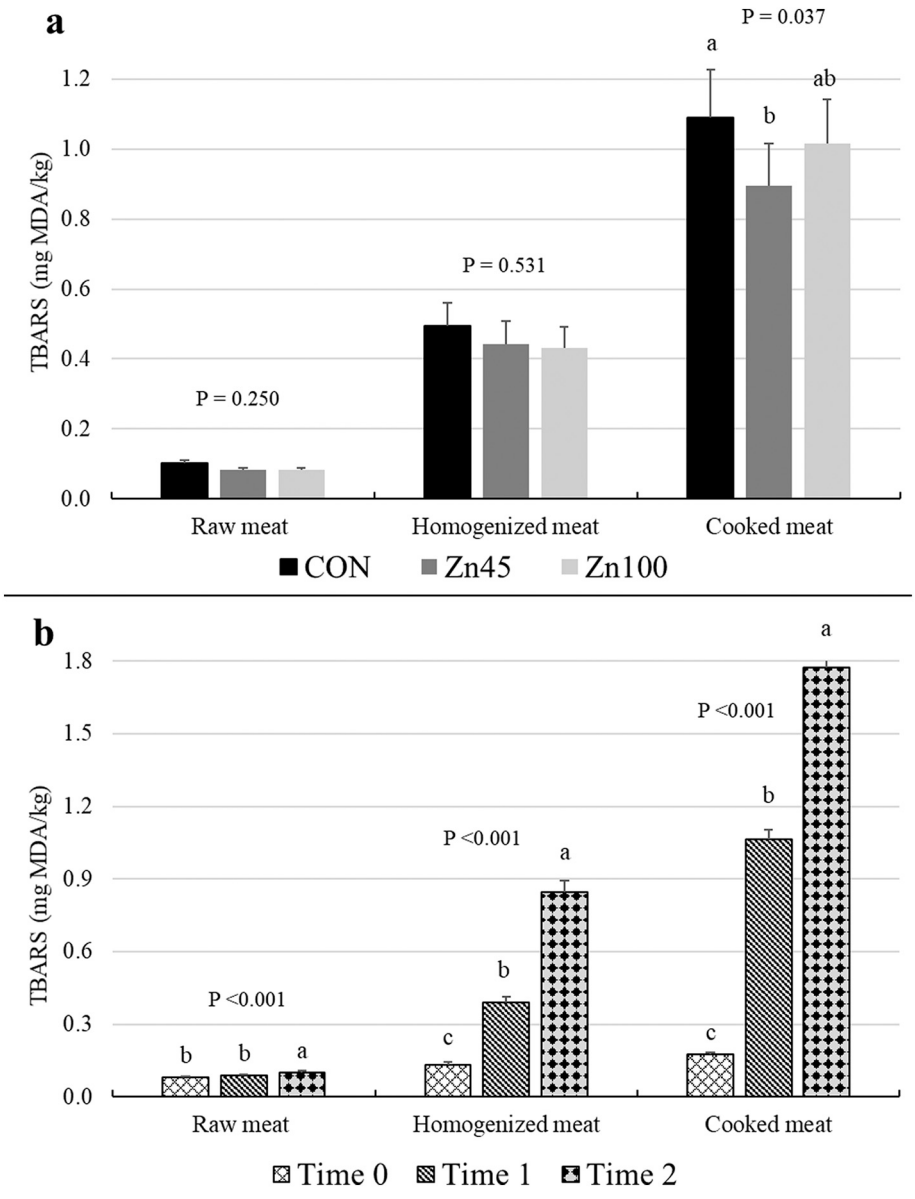

Fig. 1. Main effect of (a) zinc glycinate (ZnGly) supplementation or (b) time of storage/incubation on lipid oxidation (TBARS assay) measured in raw and cooked meat slices stored aerobically at +4 °C or in homogenized meat incubated with Fe^{3+} and ascorbate. Columns are the mean values and error bars 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, for raw meat; minutes 0, 30, 60, for homogenized meat incubated with Fe^{3+} and ascorbate; days 0, 2, 4, for cooked meat.
[a, b, c] Different letters, within each class (raw meat, homogenized meat, or cooked meat), indicate differences between means ($P \leq 0.05$) tested using the Tukey's adjustment for multiple comparisons.

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 ZnGly. To explain these findings, most of the above studies on poultry meat (Chang et al., 2021; Liu et al., 2011; Yang et al., 2016) 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 unlikely that during the first 24 h after slaughter there was such a strong lipid oxidation able to significantly influence the carcass chill 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 the color of meat and backfat nor meat FA profile. To the best of our knowledge, there are hardly any studies in which the effects of dietary Zn on pork color and FA have been evaluated. Bučko et al. (2013) observed that fresh meat from pigs supplemented with 66 mg/kg of organic Zn showed lower values of L*, b*, and polyunsaturated FA (PUFA). On the contrary, Rekiel et al. (2005) did not find any significant effect of 0.05% dietary 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, ZnGly supplementation tended to reduce the cholesterol content of pork, in agreement with Dukare et al. (2021), who reported a decrease of thigh and breast cholesterol content in broiler meat by supplementing different sources of Zn up to 80 mg/kg. However, in contrast to our findings, these authors reported 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 (Untea, Panaite, Saracila, & Soica, 2017; Van Heugten

et al., 2003). In line with that, we observed here a greater concentration of Zn in liver than in muscle. A previous study (Hill et al., 2014) showed that the liver Zn content of nursery pigs increased linearly as the level of Zn supplementation increased from 25 to 100 mg/kg, whereas Case and Carlson (2002) detected higher Zn content in the liver of pigs supplemented with an excessively high level (i.e., 3000 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 ZnGly. From our results it would appear that the supply of Zn at relative low levels has no meaningful effects on Zn accumulation in liver of growing-finishing pigs. However, as stated above, we cannot exclude a possible influence of Zn deriving from feeding preceding the experimental trial, which may have been stored in liver. 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 4000 mg/kg, but not at 2000 mg/kg. Whereas, Carlson, Hill, and Link (1999) observed no difference in Fe concentration of liver from pigs supplemented with 3000 mg/kg. Interestingly, in our study, a significant higher concentration of Fe was found in the liver from Zn45 treatment. Hence, our finding seems to suggest that low levels of zinc 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, the micromineral content of meat was not affected by the dietary treatment. Despite the increasing level of Zn content in the three diets, the Zn content of meat was similar between treatments. Our result was in accordance with an early study (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). Similarly, Paulk et al. (2015) observed no differences in loin Zn concentration of finishing pigs supplemented with organic or inorganic sources of Zn (from 75 to 225 mg/kg) and with ractopamine HCl. In our study, muscular Zn content ranged between 19.4 and 19.8 mg/kg, which is in line with these previous reports (Jensen-Waern et al., 1998; Paulk et al., 2015).

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, Hopkins, Fahri, & Ponnampalam, 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 manganese (Mn); EC-SOD, present in the extracellular space and containing also Zn and Cu (Zelko, Mariani, & Folz, 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 of Zn (Bun, Guo, Guo, Ji, & Cao, 2011; Dukare et al., 2021), it is believed that SOD activity is mainly regulated by the availability of Cu rather than Zn (Carlson et al., 1999; Harris, 1992). In the present study, we measured the total activity of the three forms of SOD as whole and observed a lower total SOD activity in the muscle of pigs supplemented with 45 mg/kg of ZnGly. This result might be derived from some interaction of the Zn with the other cofactors of SOD (i.e., Cu and Mn), as Zn – competing for absorption and for binding to cell membranes – can reduce the availability of Cu and Mn. Similarly, Zn supplementation may also have some interactive effect with Fe, which is a cofactor of catalase enzyme (Nicholls, 2012). Indeed, in the present experiment, catalase activity was higher in the muscle of pigs supplemented with 100 mg/kg of ZnGly. Consistently, Wen et al. (2019) showed that muscle from Pekin ducks supplemented with 240 mg/kg of Zn had a higher catalase activity compared with 0, 15, and 30 mg/kg of Zn supplementation. However, this hypothesis seems to contrast our result of micromineral contents of muscle, which were unaffected by Zn supplementation.

On the other hand, an increase in enzyme activity is often interpreted as an adaptive response to oxidative stress (Renerre, Dumont, & Gatellier, 1996). This is supported by Bun et al. (2011) and Stukelj, Toplak, and Svete (2013), who reported an increase in hepatic SOD activity in broilers challenged with a pathogen, and in serum SOD activity in pigs infected with a virus, respectively. Therefore, our finding may indicate that the lowest Zn supplementation led to a better oxidative condition status of animal and therefore a lower need to increase the response of the enzymatic defenses. Nevertheless, it should be underlined that the pigs of the present study were not subjected to any type of stress, as the animals were raised in ideal environmental and nutritional conditions throughout the experiment as well as kept in individual pens, thus avoiding any competition between pigs. Moreover, it is worth mentioning that the muscle from the present study had a high concentration of α-tocopherol (approximately 3 μg/g of muscle), regardless of the dietary treatment. Hence, considering that α-tocopherol (vitamin E) is a powerful antioxidant in muscle systems (Bekhit et al., 2013; Pettigrew & Esnaola, 2001), the high content may have adequately protected the muscle against natural oxidative processes and consequently limited the enzymatic defense response.

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

However, when pork slices were subjected to a much stronger oxidative challenge such as cooking, TBARS values increased to nearly 1.8 mg MDA/kg after 4 days of refrigerated aerobic storage (Fig. 1b). Interestingly, cooked meat from pigs supplemented with 45 mg/kg of ZnGly showed a higher resistance to lipid oxidation than control pork, with intermediate TBARS values for Zn100 treatment (Fig. 1a). Thus, it may be speculated that the effect of Zn on lipid oxidation was partially hidden by the strong protection exerted by vitamin E when the pork was exposed to low or medium oxidizing stressors (i.e., refrigerated aerobic storage or incubation with pro-oxidants), while this effect was evident in highly stressful conditions.

These results demonstrated that ZnGly may enhance the oxidative stability of pork under strong oxidative challenge, even if the antioxidant action mechanism of Zn remains to be elucidated. Indeed, according to our findings, the Zn supplementation would appear to have no effect on the fat-soluble vitamins or on the hydrophilic antioxidant capacity – evaluated by TEAC, FRAC and Folin-Ciocalteu assays – of the muscle. Moreover, the lack of response on the SOD activity would seem

more an indirect effect on the overall oxidative state of animal than a direct effect, as the lowest Zn level decreased the activity of this Zn-containing enzyme. Although in the present study a series of analyzes was carried out to better study the antioxidant effects of Zn supplementation on pork oxidative stability, further research is essential to clarify the antioxidant effect of dietary Zn by also evaluating other aspects related to Zn, such as its role in the induction of metallothionein proteins and in the inhibition of NADPH oxidase (Prasad & Kucuk, 2002). Furthermore, future studies should be designed to evaluate the effect of Zn in oxidative challenge conditions, such as diets deficient in vitamin E or supplemented with PUFA, or physiological stress conditions.

5. Conclusions

So far, little has been done to assess the effects of Zn supplementation on the performance and quality of the carcass and meat for pigs in the growing-finishing stage. The results obtained in this study suggest that supplementing the diet of growing-finishing pigs with 45 or 100 mg/kg of ZnGly may reduce carcass chill 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 the value of the salable carcass. 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 ZnGly showed a lower SOD activity compared to control muscle, while the highest level of ZnGly 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 ZnGly 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 rearing and meat storage conditions to better capture the antioxidant potential of Zn and its influence on the shelf-life of pork.

Author statement

Antonio Natalello: Conceptualization, Data Curation, Methodology, Writing - Original Draft, Writing - Review & Editing; Hajer Khelil-Arfa: Conceptualization, Resources; Giuseppe Luciano: Conceptualization, Data Curation, Writing - Review & Editing, Supervision, Funding acquisition; Mieke Zoon: Conceptualization, Resources; Ruggero Menci: Data Curation, Methodology, Software, Writing - Review & Editing; Manuel Scerra: Visualization, Investigation; Alexandra Blanchard: Conceptualization, Resources; Fabrizio Mangano: Methodology; Luisa Biondi: Conceptualization, Resources, Funding acquisition; Alessandro Priolo: Supervision, Visualization, Project administration;

Declaration of Competing 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.

Acknowledgements

Authors would like to thank Dr. Gaetano Pandino (University of Catania) for his help with the micro-mineral analyses. Part of the analyses on meat quality was funded by the University of Catania, Department of Agriculture, Food and Environment (project "QUALIGEN"; Linea 2–Piano di Incentivi per la Ricerca di Ateneo 2020/2022).

References

AOAC. (1995). *Association of Official Analytical Chemists. Official methods of analysis* (16th ed.). Washington, DC: AOAC.

Aouadi, D., Luciano, G., Vasta, V., Nasri, S., Brogna, D. M. R., Abidi, S., … Ben Salem, H. (2014). The antioxidant status and oxidative stability of muscle from lambs receiving oral administration of *Artemisia herba alba* and *Rosmarinus officinalis* essential oils. *Meat Science, 97*, 237–243.

Barszcz, M., Taciak, M., Tuśnio, A., Čobanová, K., & Grešáková, L. U. (2019). The effect of organic and inorganic zinc source, used in combination with potato fiber, on growth, nutrient digestibility and biochemical blood profile in growing pigs. *Livestock Science, 227*, 37–43.

Bekhit, A. E. D. A., Hopkins, D. L., Fahri, F. T., & Ponnampalam, E. N. (2013). Oxidative processes in muscle systems and fresh meat: Sources, markers, and remedies. *Comprehensive Reviews in Food Science and Food Safety, 12*(5), 565–597.

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry, 239*, 70–76.

Bertolín, J. R., Joy, M., Rufino-Moya, P. J., Lobón, S., & Blanco, M. (2018). Simultaneous determination of carotenoids, tocopherols, retinol and cholesterol in ovine lyophilised samples of milk, meat, and liver and in unprocessed/raw samples of fat. *Food Chemistry, 257*, 182–188.

Biondi, L., Luciano, G., Cutello, D., Natalello, A., Mattioli, S., Priolo, A., Lanza, M., Morbidini, L., Gallo, A., & Valenti, B. (2020). Meat quality from pigs fed tomato processing waste. *Meat Science, 159*, Article 107940.

Blaabjerg, K., & Poulsen, H. D. (2017). The use of zinc and copper in pig production. *DCA Natl. Cent. Jordbrug Fødevarer, 23*, 1–17.

Bučko, O., Hellová, D., & Debrecéni, O. (2013). Effect of organic zinc of pork quality, chemical composition and fatty acid profile of musculus longissimus thoracis in large white breed. *Research in Pig Breeding, 7*(2), 1–6.

Bun, S. D., Guo, Y. M., Guo, F. C., Ji, F. J., & Cao, H. (2011). Influence of organic zinc supplementation on the antioxidant status and immune responses of broilers challenged with Eimeria tenella. *Poultry Science, 90*(6), 1220–1226.

Carlson, M. S., Hill, G. M., & Link, J. E. (1999). Early-and traditionally weaned nursery pigs benefit from phase-feeding pharmacological concentrations of zinc oxide: Effect on metallothionein and mineral concentrations. *Journal of Animal Science, 77*(5), 1199–1207.

Case, C. L., & Carlson, M. S. (2002). Effect of feeding organic and inorganic sources of additional zinc on growth performance and zinc balance in nursery pigs. *Journal of Animal Science, 80*(7), 1917–1924.

Cemin, H. S., Carpenter, C. B., Woodworth, J. C., Tokach, M. D., Dritz, S. S., DeRouchey, J. M., … Usry, J. L. (2019). Effects of zinc source and level on growth performance and carcass characteristics of finishing pigs. *Translational Animal Science, 3*(2), 742–748.

Chang, Y., Zhang, Z., Wu, B., Zhao, H., Liu, G., Chen, X., … Jia, G. (2021). Evaluating zinc glycine chelate in Cherry Valley ducks: Responses of growth performance, nutrient utilization, serum parameters, antioxidant status, meat quality and zinc accumulation. *Animal Feed Science and Technology, 275*, Article 114875.

Cox, D. H., & Hale, O. M. (1962). Liver iron depletion without copper loss in swine fed excess zinc. *The Journal of Nutrition, 77*, 225–228.

D'souza, D. N., Mullan, B. P., Pethick, D. W., Pluske, J. R., & Dunshea, F. R. (2012). Nutritional strategies affect carcass and pork quality but have no effect on intramuscular fat content of pork. *Animal Production Science, 52*(4), 276–282.

Dukare, S., Mir, N. A., Mandal, A. B., Dev, K., Begum, J., Rokade, J. J., … Bhanja, S. K. (2021). A comparative study on the antioxidant status, meat quality, and mineral deposition in broiler chicken fed dietary nano zinc viz-a-viz inorganic zinc. *Journal of Food Science and Technology, 58*(3), 834–843.

Fernández, M., Ordóñez, J. A., Cambero, I., Santos, C., Pin, C., & de la Hoz, L. (2007). Fatty acid compositions of selected varieties of Spanish dry ham related to their nutritional implications. *Food Chemistry, 101*(1), 107–112.

Flohé, L., & Günzler, W. A. (1984). [12] assays of glutathione peroxidase. *Methods in Enzymology, 105*, 114–120.

Gatellier, P., Mercier, Y., & Renerre, M. (2004). Effect of diet finishing mode (pasture or mixed diet) on antioxidant status of Charolais bovine meat. *Meat Science, 67*(3), 385–394.

Gowanlock, D. W., Mahan, D. C., Jolliff, J. S., Moeller, S. J., & Hill, G. M. (2013). Evaluating the NRC levels of cu, Fe, Mn, and Zn using organic minerals for grower-finisher swine. *Journal of Animal Science, 91*(12), 5680–5686.

Hahn, J. D., & Baker, D. H. (1993). Growth and plasma zinc responses of young pigs fed pharmacologic levels of zinc. *Journal of Animal Science, 71*(11), 3020–3024.

Harris, E. D. (1992). Copper as a cofactor and regulator of copper, zinc superoxide dismutase. *The Journal of Nutrition, 122*(suppl_3), 636–640.

Hill, G. M., Mahan, D. C., & Jolliff, J. S. (2014). Comparison of organic and inorganic zinc sources to maximize growth and meet the zinc needs of the nursery pig. *Journal of Animal Science, 92*(4), 1582–1594.

Holen, J. P., Rambo, Z., Hilbrands, A. M., & Johnston, L. J. (2018). Effects of dietary zinc source and concentration on performance of growing-finishing pigs reared with reduced floor space. *The Professional Animal Scientist, 34*(2), 133–143.

Jensen-Waern, M., Melin, L., Lindberg, R., Johannisson, A., Petersson, L., & Wallgren, P. (1998). Dietary zinc oxide in weaned pigs—Effects on performance, tissue

concentrations, morphology, neutrophil functions and faecal microflora. *Research in Veterinary Science, 64*(3), 225–231.
Jin, G., He, L., Yu, X., Zhang, J., & Ma, M. (2013). Antioxidant enzyme activities are affected by salt content and temperature and influence muscle lipid oxidation during dry-salted bacon processing. *Food Chemistry, 141*(3), 2751–2756.
Larsen, T., & Poulsen, H. D. (1996). The relationship between mineral and nitrogen balances in growing pigs fed diets supplemented with zinc oxide. *Canadian Journal of Animal Science, 76*(3), 409–415.
Liu, Z. H., Lu, L., Li, S. F., Zhang, L. Y., Xi, L., Zhang, K. Y., & Luo, X. G. (2011). Effects of supplemental zinc source and level on growth performance, carcass traits, and meat quality of broilers. *Poultry Science, 90*(8), 1782–1790.
Lombardo, S., Pandino, G., & Mauromicale, G. (2017). Minerals profile of two globe artichoke cultivars as affected by NPK fertilizer regimes. *Food Research International, 100*, 95–99.
Luciano, G., Natalello, A., Mattioli, S., Pauselli, M., Sebastiani, B., Niderkorn, V., Copani, G., Benhissi, H., Amanpour, A., & Valenti, B. (2019). Feeding lambs with silage mixtures of grass, sainfoin and red clover improves meat oxidative stability under high oxidative challenge. *Meat Science, 156*, 59–67.
Luciano, G., Roscini, V., Mattioli, S., Ruggeri, S., Gravador, R. S., Natalello, A., … Priolo, A. (2017). Vitamin E is the major contributor to the antioxidant capacity in lambs fed whole dried citrus pulp. *Animal, 11*(3), 411–417.
Makkar, H. P., Blümmel, M., Borowy, N. K., & Becker, K. (1993). Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. *Journal of the Science of Food and Agriculture, 61*(2), 161–165.
Marklund, S. L. (1984). Extracellular superoxide dismutase and other superoxide dismutase isoenzymes in tissues from nine mammalian species. *Biochemical Journal, 222*(3), 649–655.
Matarneh, S. K., England, E. M., Scheffler, T. L., & Gerrard, D. E. (2017). The conversion of muscle to meat. In *Lawrie's Meat Science* (pp. 159–185). Woodhead Publishing.
Monteiro, S. C., Lofts, S., & Boxall, A. B. (2010). Pre-assessment of environmental impact of zinc and copper used in animal nutrition. *EFSA Supporting Publications, 7*(9), 74E.
Natalello, A., Luciano, G., Morbidini, L., Valenti, B., Pauselli, M., Frutos, P., … Priolo, A. (2019). Effect of feeding pomegranate by-product on fatty acid composition of ruminal digesta, liver and muscle in lambs. *Journal of Agricultural and Food Chemistry, 67*, 4472–4482.
Natalello, A., Priolo, A., Valenti, B., Codini, M., Mattioli, S., Pauselli, M., Puccio, M., Lanza, M., Stergiadis, S., & Luciano, G. (2020). Dietary pomegranate by-product improves oxidative stability of lamb meat. *Meat Science, 162*, Article 108037.
National Research Council. (2012). *Nutrient requirements of swine* (11th revised ed.). Washington, DC: National Academy Press. https://doi.org/10.17226/13298
Nicholls, P. (2012). Classical catalase: Ancient and modern. *Archives of Biochemistry and Biophysics, 525*(2), 95–101.
Oberleas, D., Muhrer, M. E., & O'Dell, B. L. (1962). Effects of phytic acid on zinc availability and parakeratosis in swine. *Journal of Animal Science, 21*(1), 57–61.
Oteiza, P. L., Olin, K. L., Fraga, C. G., & Keen, C. L. (1996). Oxidant defense systems in testes from zinc-deficient rats. *Proceedings of the Society for Experimental Biology and Medicine, 213*(1), 85–91.
Paulk, C. B., Burnett, D. D., Tokach, M. D., Nelssen, J. L., Dritz, S. S., DeRouchey, J. M., … Gonzalez, J. M. (2015). Effect of added zinc in diets with ractopamine hydrochloride on growth performance, carcass characteristics, and ileal mucosal inflammation mRNA expression of finishing pigs. *Journal of Animal Science, 93*(1), 185–196.
Pettigrew, J. E., & Esnaola, M. A. (2001). Swine nutrition and pork quality: A review. *Journal of Animal Science, 79*(suppl_E), E316–E342.
Poulsen, H. D., & Larsen, T. (1995). Zinc excretion and retention in growing pigs fed increasing levels of zinc oxide. *Livestock Production Science, 43*(3), 235–242.
Prasad, A. S. (1998). Zinc in human health: An update. *The Journal of Trace Elements in Experimental Medicine, 11*, 63–87.
Prasad, A. S., & Kucuk, O. (2002). Zinc in cancer prevention. *Cancer Metastasis Reviews, 21*(3), 291–295.
Rekiel, A., Batorska, M., Wiêcek, J., & Dziuba, M. (2005). Slaughter value and meat quality in pigs fed diets with different feed additives. *Polish Journal of Food and Nutrition Sciences, 14*(1), 27–30.
Renerre, M., Dumont, F., & Gatellier, P. (1996). Antioxidant enzyme activities in beef in relation to oxidation of lipid and myoglobin. *Meat Science, 43*(2), 111–121.
Reynolds, F. H., Forbes, J. M., & Miller, H. M. (2010). Does the newly weaned piglet select a zinc oxide supplemented feed, when given the choice? *Animal, 4*(8), 1359–1367.
Rouhalamini, S. M., Salarmoini, M., & Asadi-Karam, G. (2014). Effect of zinc sulfate and organic chromium supplementation on the performance, meat quality and immune response of Japanese quails under heat stress conditions. *Poultry Science Journal, 2*(2), 165–181.
Rufino-Moya, P. J., Joy, M., Lobón, S., Bertolín, J. R., & Blanco, M. (2020). Carotenoids and liposoluble vitamins in the plasma and tissues of light lambs given different maternal feedings and fattening concentrates. *Animals, 10*(10), 1813.
Sheard, P. R., Enser, M., Wood, J. D., Nute, G. R., Gill, B. P., & Richardson, R. I. (2000). Shelf life and quality of pork and pork products with raised n-3 PUFA. *Meat Science, 55*(2), 213–221.
Shelton, J. L., Southern, L. L., LeMieux, F. M., Bidner, T. D., & Page, T. G. (2004). Effects of microbial phytase, low calcium and phosphorus, and removing the dietary trace mineral premix on carcass traits, pork quality, plasma metabolites, and tissue mineral content in growing-finishing pigs. *Journal of Animal Science, 82*(9), 2630–2639.
Sloup, V., Jankovská, I., Nechybová, S., Peřinková, P., & Langrová, I. (2017). Zinc in the animal organism: A review. *Scientia Agriculturae Bohemica, 48*(1), 13–21.
Stukelj, M., Toplak, I., & Svete, A. N. (2013). Blood antioxidant enzymes (SOD, GPX), biochemical and haematological parameters in pigs naturally infected with porcine reproductive and respiratory syndrome virus. *Polish Journal of Veterinary Sciences, 16*(2).
Ulbricht, T. L. V., & Southgate, D. A. T. (1991). Coronary heart disease: Seven dietary factors. *Lancet, 338*, 985–992.
Untea, A. E., Panaite, T. D., Saracila, M., & Soica, C. (2017). Effects of dietary symbiotics and organic zinc on trace minerals composition of pork. In *Scientific Papers: Series D, Animal Science-The International Session of Scientific Communications of the Faculty of Animal Science* (p. 60).
Valenti, B., Luciano, G., Pauselli, M., Mattioli, S., Biondi, L., Priolo, A., Natalello, A., Morbidini, L., & Lanza, M. (2018). Dried tomato pomace supplementation to reduce lamb concentrate intake: Effects on growth performance and meat quality. *Meat Science, 145*, 63–70.
Valenti, B., Natalello, A., Vasta, V., Campidonico, L., Roscini, V., Mattioli, S., Pauselli, M., Priolo, A., Lanza, M., & Luciano, G. (2019). Effect of different dietary tannin extracts on lamb growth performances and meat oxidative stability: Comparison between mimosa, chestnut and tara. *Animal, 13*(2), 435–443.
Van Heugten, E., Spears, J. W., Kegley, E. B., Ward, J. D., & Qureshi, M. A. (2003). Effects of organic forms of zinc on growth performance, tissue zinc distribution, and immune response of weanling pigs. *Journal of Animal Science, 81*(8), 2063–2071.
Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science, 74*(10), 3583–3597.
Villagómez-Estrada, S., Pérez, J. F., van Kuijk, S., Melo-Durán, D., Karimirad, R., & Solà-Oriol, D. (2021). Effects of two zinc supplementation levels and two zinc and copper sources with different solubility characteristics on the growth performance, carcass characteristics and digestibility of growing-finishing pigs. *Journal of Animal Physiology and Animal Nutrition, 105*(1), 59–71.
Warner, R. D. (2017). The eating quality of meat—IV water-holding capacity and juiciness. In *Lawrie's meat science* (pp. 419–459). Woodhead Publishing.
Wen, M., Wu, B., Zhao, H., Liu, G., Chen, X., Tian, G., & Jia, G. (2019). Effects of dietary zinc on carcass traits, meat quality, antioxidant status, and tissue zinc accumulation of Pekin ducks. *Biological Trace Element Research, 190*(1), 187–196.
Yang, W. L., Chen, Y. P., Cheng, Y. F., Li, X. H., Zhang, R. Q., Wen, C., & Zhou, Y. M. (2016). An evaluation of zinc bearing palygorskite inclusion on the growth performance, mineral content, meat quality, and antioxidant status of broilers. *Poultry Science, 95*(4), 878–885.
Zelko, I. N., Mariani, T. J., & Folz, R. J. (2002). Superoxide dismutase multigene family: A comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. *Free Radical Biology & Medicine, 33*(3), 337–349.