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Performance and meat quality in pigs fed hydrolysable tannins from *Tara spinosa*

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ABSTRACT

This study aimed to assess the effect of dietary tara (*Tara spinosa* (Feuillée ex Molina) Britton & Rose) hydrolysable tannins on performance and meat quality of finishing pigs. Twenty barrows (crossbred PIC × Piétrain; age: 125 ± 5 d; bodyweight: 60.8 ± 3.89 kg) were randomly assigned to two groups and fed ad libitum for 7 weeks a control diet (CON) or a diet supplemented with 10 g/kg of tara tannins (TAT), respectively. No differences ($P > 0.10$) on growth performance and carcass traits were observed between the two groups. Meat fatty acid profile was not affected ($P > 0.10$) by the diet, but the content of C22:5 *n*-3 tended to be lower ($P = 0.079$) in TAT pork. Dietary tannins tended to reduce ($P = 0.095$) meat cholesterol. The diet had no effect ($P > 0.10$) on fat-soluble antioxidant vitamins, hydrophilic antioxidant capacity, catalase activity, and glutathione peroxidase activity. Superoxide dismutase activity tended to be lower ($P = 0.087$) in TAT meat than in CON meat. Dietary tannins did not affect ($P > 0.10$) backfat and meat color development during 6 days of refrigerated storage, but TAT meat tended to be darker ($P = 0.082$). Meat from pigs fed tara tannins showed lower ($P = 0.028$) hydroperoxides content and a tendency toward lower conjugated dienes ($P = 0.079$) and malondialdehyde ($P = 0.084$) contents. Also, dietary tannins delayed lipid oxidation in meat subjected to oxidative challenges such as catalysis and cooking ($P < 0.05$). The positive effect of dietary tara hydrolysable tannins on lipid oxidation was likely due to their antioxidant and anti-inflammatory capacity, but it may have been mitigated by the high α-tocopherol content in meat.

1. Introduction

With a world trend of population growth and consequent increasing consumption of animal products, livestock farming must adopt sustainable strategies to improve food system resilience and preserve the environment (FAO, 2017). Sustainability can be achieved by resorting to locally available feeds, such as legumes and agro-industrial by-products, as an alternative to conventional resource-intensive, human-edible feed crops (Hlatini, Ncobela, Zindove, & Chimonyo, 2018; Jezierny, Mosenthin, & Bauer, 2010; Salami et al., 2019). In addition, the use of natural alternatives to in-feed antimicrobials can help address health and environmental hazards associated with the use of pharmaceuticals and the emergence of antibiotic resistance (Caprarulo, Giromini, & Rossi, 2021; Huang, Liu, Zhao, Hu, & Wang, 2018). These strategies may also have economic relevance when considering the high cost of conventional feed crops, such as soybean or corn, and the increasing influence of ecological aspects on consumers' choice (Grunert, Sonntag, Glanz-Chanos, & Forum, 2018).

Tannins, phenolic compounds ubiquitous in the vegetable kingdom, play an intriguing role in this scenario. On the one hand, they have long been considered anti-nutritional factors due to their protein-binding ability and astringent taste, which discouraged the use of local crops and by-products precisely because of their tannin content (Jezierny et al., 2010; Salami et al., 2019). Nonetheless, recent studies have reported no negative effect of dietary tannins on pigs growth performance. For example, low doses (up to 30 g/kg) of chestnut (*Castanea sativa*) tannin extract did not affect average daily gain (ADG) or final bodyweight (BW) of finishing pigs (Bee et al., 2017; Čandek-Potokar et al., 2015). On the other hand, their antioxidant and antimicrobial activities make tannins bioactive molecules with positive effects on animal health. For instance, dietary tannins can be used in pig farming to contrast postweaning diarrhea (Girard et al., 2018; Xu et al., 2022), treat

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Table 1
Chemical composition of basal diet.

Item	Basal diet
Dry matter (DM), g/kg as fed	911
Crude Protein	169
Crude Fat	32.7
Neutral detergent fiber	164
Ash	41.4
Total phenols (TAeq[1])	3.71
Total tannins (TAeq[1])	1.12
Fatty acids, g/kg DM	
C16:0	2.50
C18:0	0.49
C18:1 *c*9	3.48
C18:2 *c*9*c*12	7.56
C18:3 *c*9*c*12*c*15	0.58
Tocopherols, mg/kg DM	
α-Tocopherol	7.54
γ-Tocopherol	21.4
δ-Tocopherol	6.38

The diet was supplemented with 1 kg/100 kg of vitamin-mineral mix, containing: vitamin A (650,000 U), vitamin D3 (200,000 U), 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), and sodium selenite (30 mg).
The diet was supplemented with 1 kg/500 kg of amino acid mix, containing: lysine (80,000 mg), threonine (280,000 mg), methionine (240,000 mg), tryptophan (120,000 mg), and L-valine (240,000 mg).
[1] TAeq, tannic acid equivalents.

nematode parasitism (Williams, Fryganas, Ramsay, Mueller-Harvey, & Thamsborg, 2014), and improve intestinal health (Caprarulo et al., 2021).

If tannins are to be considered as a functional natural ingredient in the diet, their effect on product quality must also be assessed. In recent years, research has focused on the effect of dietary tannins on meat quality, but information regarding pork is still poor and inconclusive. Ranucci et al. (2015) observed lower lipid oxidation in meat from pigs fed 1 g/kg of chestnut tannin extract in combination with 1 g/kg of oregano essential oil. On the contrary, O'Grady, Carpenter, Lynch, O'Brien, and Kerry (2008) reported no difference in color and lipid stability of meat from pigs fed 0.6 or 0.3 g/kg of tannins from grape seed or bearberry, respectively. Moreover, some authors observed different fatty acid (FA) profile in pork after feeding tannin-containing ingredients such as carob pulp (up to 150 g/kg; Inserra et al., 2015) or dehydrated sainfoin (up to 150 g/kg; Seoni, Battacone, Kragten, Dohme-Meier, & Bee, 2021).

The present study aims to evaluate, for the first time, the effect of dietary tannins from tara (*Tara spinosa* (Feuillée ex Molina) Britton & Rose) on pork quality. Tara is a leguminous tree native to South America whose pods are ground to extract hydrolysable tannins (HT), mainly gallotannins, for commercial purposes (Chambi et al., 2013). Feeding tara extract resulted in a synergistic antioxidant effect that increased the tocopherols content in lamb meat (Valenti et al., 2019). Our hypothesis was that supplementing the diet of finishing pigs with hydrolysable tannins from tara can improve meat quality, particularly oxidative stability, without affecting growth performance.

2. Material and methods

2.1. Animals and diet

The experiment was approved by the animal welfare committee "Organismo Preposto al Benessere degli Animali (OP BA)" of the University of Catania (protocol No. 286946).

Twenty crossbred PIC × Piétrain barrows homogeneous for age (125 ± 5 d) and bodyweight (60.8 ± 3.9 kg) were selected from a local farm and transferred to the experimental farm of the University of Catania (37°24′35.3" N, 15°03′34.9″ E). The pigs were housed in a shed with concrete floor in individual pens. Each pen was equipped with water nipple dispenser, metal trough, and straw bedding. The pigs were randomly assigned to two balanced groups (n = 10), namely control (CON) and tara tannin (TAT). Both groups received ad libitum a commercial pelleted diet for finishing pigs composed of maize (420 g/kg), barley (200 g/kg), wheat bran (152 g/kg), soybean meal (134 g/kg), fava bean (74 g/kg), vitamin/mineral supplement (10 g/kg), sodium carbonate (8 g/kg), and amino acid supplement (2 g/kg). The chemical composition of the basal diet is reported in Table 1. The diet of TAT group was supplemented with 11.5 g/kg of commercial tara tannin extract (SilvaTeam). The tannin extract was mixed with the other supplements directly at the feed company before being added to the basal diet. Considering 87% purity, as analyzed by Folin-Ciocalteau method (Luciano et al., 2017), this resulted in a dietary supplementation of 10 g/kg of tara hydrolysable tannins. The inclusion level was chosen basing on previous findings in the scientific literature in order to avoid detrimental effects on pig health. Feed offer and refusal were recorded individually to assess voluntary feed intake. Individual bodyweight was recorded fortnightly. The trial lasted 56 days.

2.2. Slaughtering, carcass traits, and sampling

At the end of the trial, on the same day, all the pigs were transferred to a commercial abattoir (30 min away) and immediately slaughtered according to the European Union regulation (EC No. 1099/2009), with electrical stunning. Hot carcass weight was measured within 20 min of the slaughter. After 45 min post mortem, pH_1 was determined in the *longissimus thoracis et lumborum* muscle (LTL) using a pH-meter equipped with temperature probe and penetrating electrode (Orion 9106; Orion Research Incorporated), calibrated with pH -4 and pH -7 buffer solutions. Carcasses were stored at 0–4 °C, and weight and ultimate pH (pH_u) were recorded again at 24 h post-mortem. Right-side LTL (together with backfat and rind) was excised between the 13th thoracic vertebra and 3rd lumbar vertebra, and prepared for the analyses. A first muscle aliquot was vacuum-packaged and stored at −80 °C pending FA profile analysis. A second muscle aliquot was chopped, frozen with liquid nitrogen, and stored at −80 °C pending cholesterol, vitamins, hydrophilic antioxidant capacity, and enzymes analyses. A third aliquot was vacuum-packaged and aged at 0–4 °C for additional 24 h pending shelf-life and oxidative challenges trials.

Basal diet sub-samples were collected at the beginning, middle and end of the trial, vacuum-packaged, and stored at −20 °C.

2.3. Feed analyses

Before the analyses, the sub-samples were mixed and ground (1-mm screen hammer mill) to obtain a representative sample of the basal diet used during the trial. Dry matter, crude protein, crude fat, and ash were analyzed according to AOAC (1995), and neutral detergent fiber was analyzed according to Van Soest, Robertson, and Lewis (1991). Phenolic compounds and tannins were quantified following the Folin-Ciocalteau method (as modified by Luciano et al., 2017). A 100 mg sample was used to assess FA profile trough one-step extraction-transesterification and gas-chromatographic analysis (Valenti et al., 2018). A 200 mg sample was used to assess tocopherols content trough methanol:acetone:petroleum ether (1:1:1, v:v:v) extraction (Bertolín, Joy, Rufino-Moya, Lobón, & Blanco, 2018) and ultra-high-performance liquid chromatograph (UHPLC) analysis (set as described in chapter 2.4.2).

2.4. Meat analyses

2.4.1. Intramuscular fat and fatty acid profile

Intramuscular fat was extracted from 10 g of meat using 2:1 (v:v) chloroform:methanol (Folch, Lees, & Sloane Stanley, 1957). Meat FA profile was then determined through gas-chromatography. Methanolic sodium methoxide was used to convert FA in methyl esters (FAME) according to the procedure of Natalello et al. (2019). FAME were collected in GC-grade hexane and stored at −20 °C. Gas-chromatographic analysis was performed using a TRACE GC (Thermo Finnigan) fitted with a flame ionization detector and a high-polar fused silica capillary column (length: 100 m; i.d.: 25 mm; film thickness: 0.25 μm; SP. 24,056; Supelco Inc.). Injection volume was 1 μL of sample. Time and temperature were set as described by Natalello et al. (2019). The internal standard was methyl-C19:0 and individual FA were identified using commercial pure standard (Nu-Chek Prep Inc.; Larodan Fine Chemicals). After calculating the proportional content of each FA basing on the internal standard, FA profile was expressed as mg per 100 g of muscle according to measured intramuscular fat content. The results were used to calculate atherogenicity and thrombogenicity indices (Ulbricht & Southgate, 1991) and hypo- to hyper-cholesterolemic ratio (Santos-Silva, Bessa, & Santos-Silva, 2002).

2.4.2. Cholesterol and fat-soluble antioxidant vitamins

Cholesterol, tocopherols, and retinol contents were assessed by UHPLC using the method described by Bertolín et al. (2018), as modified by Menci et al. (2022). Briefly, samples (0.5 g) were added with L-ascorbic acid (0.2 g) and 10% KOH in 1:1 ethanol:water (7.5 mL) and saponified overnight in an incubator shaker. A double extraction was performed with 5 mL of 9:1 hexane:ethyl acetate with BHT (25 mg/L) and centrifugation (2000 ×*g*, 5 min, 10 °C). Supernatants were evaporated at 40 °C under nitrogen flow, dissolved in 1 mL of HPLC-grade methanol, and filtered through PTFE syringe filters. The whole procedure was carried out protecting samples from light. Analytes were quantified using a Nexera UHPLC (Shimadzu Corporation) equipped with a C18 phase column (Zorbax ODS; length: 25 cm; i.d.: 4.6 mm; particle size: 5 μm; Supelco). Ten μL of sample (kept at 25 °C) was injected with methanol as isocratic mobile phase (column temperature: 40 °C). A photodiode array detector (SPD-M40, Shimadzu) was used to analyze cholesterol (absorbance wavelength: 220 nm) and retinol (325 nm). A fluorescence detector (RF-20AXS, Shimadzu) was used to analyze tocopherols (excitation wavelength: 295 nm; emission wavelength: 330 nm). Pure standards (Merck Life Science s.r.l.) of each analyte were injected to compare retention times and to build calibration curves.

2.4.3. Hydrophilic antioxidant capacity

Three different antioxidant assays were performed, namely Trolox equivalent antioxidant capacity (TEAC), ferric reducing antioxidant power (FRAP), and Folin-Ciocalteau. First, meat aqueous extract was obtained from 1-g samples by homogenization (Diax 900, Heidolph ElektroGmbH & Co. KG) at 9000 r/min for 1 min in distilled water (10 mL). Overheating was prevented by keeping samples in a water-ice bath during homogenization. Samples were then centrifuged (2500 ×*g*, 20 min, 4 °C) and supernatants were filtered (Whatman paper, grade 541). The obtained aqueous extract was divided into exact aliquots to be used for the antioxidant assays and stored at −80 °C.

For the TEAC assay (Aouadi et al., 2014), 20 μL of meat extract was incubated in water bath at 30 °C for 60 min with 2 mL of ABTS radical solution. The absorbance at 734 nm was then measured using a UV-1601 spectrophotometer (Shimadzu Corporation). To account for spontaneous discoloration, a blank was prepared with distilled water in stead of meat extract. A Trolox (238,813; Merck Life Science s.r.l.) solution in pH -7 phosphate buffer ($Na_2HPO_4 \times 12H_2O$ and KH_2PO_4) was used to build a calibration curve with concentration up to 400 μg/mL.

For the FRAP assay (Benzie & Strain, 1996), 50 μL of meat extract was diluted with 150 μL of distilled water and incubated in water bath at 37 °C for 60 min with 1.5 mL of a 10:1:1 (v:v:v) solution of 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-*s*-triazine (in 40 mM HCl), and 20 mM aqueous $FeCl_3$. The absorbance at 593 nm was then measured. A solution of ferrous sulfate heptahydrate in distilled water was used to build a calibration curve with concentration up to 280 μg/mL.

For the Folin-Ciocalteu assay (Aouadi et al., 2014), 1 mL of meat extract was diluted with 2 mL of distilled water, mixed with 0.5 mL of 1 N Folin-Ciocalteu reagent and 2.5 mL of 20% sodium carbonate, and incubated in the dark for 40 min. The sample was then centrifuged (2500 ×*g*, 10 min, 4 °C) and the absorbance at 725 nm was measured. A solution of tannic acid in distilled water was used to build a calibration curve with concentration up to 100 μg/mL.

2.4.4. Antioxidant enzymes

The activity of antioxidant enzymes was assessed on meat extract as described by Natalello et al. (2022). Meat extract was obtained from 5-g samples by homogenization (T18 digital Ultra-Turrax, IKA-Werke GmbH & Co. KG) at 13,000 r/min for 1 min in ice-cold 50 mM phosphate buffer (pH 7.0). During homogenization, the tubes containing samples were always kept in a water-ice bath. Samples were then centrifuged (2800 ×*g*, 20 min, 4 °C) and supernatants were collected in microtubes and centrifuged (10,000 ×*g*, 10 min, 4 °C) again. The obtained extract was divided into exact aliquots to be used for the antioxidant enzymes analyses and stored at −80 °C.

Catalase (CAT) activity was assessed following the protocol described by Jin, He, Yu, Zhang, and Ma (2013). Thirty μL of meat extract was placed in a UV cuvette with 1.74 mL of a H_2O_2 solution (11 mM H_2O_2 in 50 mM phosphate buffer) and the cuvette was immediately inverted four times. The absorbance at 240 nm was monitored in kinetics mode, with reading interval of 1 s, over 3 min (UV-1601; Shimadzu Corporation). Phosphate buffer was used to prepare a blank. Results were calculated using the molar extinction coefficient of H_2O_2 (39.5 L/mol/cm) and expressed as U/g, where U corresponds to the amount of enzyme needed to decompose 1 μmol of H_2O_2 per min.

Glutathione peroxidase (GPx) activity was assessed following the method of Flohé and Günzler (1984). Twenty μL of meat extract was placed in a UV cuvette with 80 μL 50 mM phosphate buffer, 500 μL of assay medium (100 mM pH 7.0 potassium phosphate buffer, 1 mM EDTA, 2 mM NaN_3), 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% $NaHCO_3$). After exactly 5 min, 100 μL of 1.5 mM H_2O_2 was added and the absorbance at 340 nm was monitored in kinetics mode, with reading interval of 1 s, over 5 min. Phosphate buffer was used to prepare a blank. Results were calculated using the molar extinction coefficient of NADPH (6220 L/mol/cm) and expressed as U/g, where U corresponds to the amount of enzyme needed to oxidize 1 μmol of NADPH per min.

Superoxide dismutase (SOD) activity was assessed following the method of Gatellier, Mercier, and Renerre (2004). Twenty μL of meat extract was placed in a UV cuvette with 20 μL of 10 mM pyrogallol and 760 μL of 50 mM tris-HCl buffer (pH 8.2) and the cuvette was immediately inverted. The absorbance at 340 nm was recorded in kinetics mode, with reading interval of 1 s, over 5 min. Phosphate buffer was used to prepare a blank. Results were compared with the blank and expressed as U/g, where U corresponds to the amount of enzyme needed to inhibit the autoxidation of pyrogallol by 50%.

2.4.5. Oxidative challenges and color

For the shelf-life trial on fresh meat, three 2-cm thick slices were cut from each LTL samples and placed in polystyrene trays, which were overwrapped with plastic wrap and stored in the dark at 0–4 °C (Valenti et al., 2019). Color parameters were assessed after 2 h (to allow blooming), 3 d, and 6 d of storage, using a different slice for each time. A portable spectrophotometer (CM-2022, Minolta Co. Ltd.) was used (SCE mode, illuminant A, 10° standard observer) to measure the color

descriptors L^* (lightness), a^* (redness), b^* (yellowness), C^* (chroma), and hue angle, as well as the reflectance spectrum between 400 nm and 700 nm. Three measurements were taken on non-overlapping areas of lean meat and backfat. The slices were then trimmed of backfat and frozen pending lipid oxidation analysis.

For the shelf-life trial on cooked meat, three 2-cm thick slices were cut from each LTL samples, vacuum-packaged and cooked in water bath at 70 °C for 30 min in a single batch (Valenti et al., 2019). Samples were then unpacked, gently dabbed with paper towel and weighted to measure cooking weight loss. One cooked slice was then frozen pending lipid oxidation analyses, whereas the remaining two were first stored in polystyrene trays overwrapped with plastic wrap for 2 d and 4 d, respectively.

For catalyzed oxidation (Luciano et al., 2017), 10 g of frozen raw meat was homogenized with 40 mL of MES (2-(N-morpholino)ethanesulfonic acid) buffer (pH 5.7) and equilibrated at 0–4 °C. Three 4-mL aliquots were collected immediately or after 30 min or 60 min of incubation with 40 μL of a ferric chloride hexahydrate and L-sodium ascorbate equimolar solution, at room temperature. The 3 aliquots were immediately used for lipid oxidation assessment, as later described.

2.4.6. Lipid oxidation

Meat lipid oxidation was measured via 3 different analyses, on different substrates. Conjugated dienes and hydroperoxides were assessed on raw fresh meat, whereas thiobarbituric reactive substances (TBARS) were assessed on fresh meat, cooked meat, and catalyzed homogenates.

Conjugated dienes were measured following the procedure of Peña-Ramos and Xiong (2003). In short, 500 mg of meat was homogenized with 5 mL of distilled water. Then, 0.5 mL of sample was incubated for 1 min with 3:2 hexane:methanol and the resulting extract was centrifuged at 2000 ×*g* for 5 min. The absorbance at 233 nm was measured, and the results were calculated using the molar extinction coefficient of 25,200 L/mol/cm.

Hydroperoxides were determined following the procedure described by Maqsood, Benjakul, and Balange (2012). Briefly, 1 g of meat was homogenized for 1 min with 15 mL of 2:1 chloroform:methanol and then filtered (Whatman No. 1 filter paper). A 7-mL aliquot of filtrate was mixed with 2 mL of 0.5% NaCl and centrifuged (2800 ×*g*, 5 min, 4 °C). Then, 750 μL of the underlying phase was diluted 1 time with chloroform, and mixed with a solution of 1 mL of 2:1 chloroform:methanol, 12.5 μL of ammonium thiocyanate, and 12.5 μL of iron chloride. The sample was incubated in the dark for 20 min, and the absorbance at 500 nm was measured. Cumene hydroperoxide in 2:1 chloroform:methanol was used to build a calibration curve with concentration up to 3 ppm.

Secondary products of lipid oxidation were assessed through TBARS assay (Siu & Draper, 1978). For meat from shelf-life and cooking trials, 5-g samples were mixed with 15 mL of 7.5% trichloroacetic acid, homogenized and filtered (Whatman paper grade 1). For catalyzed homogenates, the 4-mL aliquots (chapter 2.4.5) were mixed with 4 mL of 15% trichloroacetic acid and filtered (Whatman paper grade 1). Regardless of the starting sample, 4 mL of filtrate was mixed with 4 mL of 0.02 M aqueous thiobarbituric and incubated in water bath at 80 °C for 90 min. Then, the absorbance at 532 nm was measured and the results were expressed as mg/kg of malondialdehyde (MDA) through comparison with a TEP (1,1,3,3-tetraethoxypropane) calibration curve.

2.5. Statistical analysis

Data was analyzed with the SPSS For Analytics (version 26, IBM corporation) using the animal as statistical unit. *Z*-score test showed the absence of outliers (all data was $< |3|$). The effect of diet on performance, carcass traits, intramuscular fat, FA profile, cholesterol, vitamins, antioxidant capacity, enzymes, conjugated dienes, and hydroperoxides was statistically analyzed using one-way ANOVA (number of observations = 20). Significance was declared when $P \leq 0.05$, while trends were considered for $0.05 < P \leq 0.10$. Data of color and TBARS were statistically analyzed using a mixed model for repeated measures (number of observations = 60). The fixed factors in the model were the diet, the time of storage/incubation, and their interaction, with the animal as random factor. The Tukey post hoc test for multiple comparisons was performed when $P \leq 0.050$.

Table 2
Performance and carcass traits of pigs.

Item	Dietary treatment[1]		SEM[2]	*P*-value
	CON	TAT		
Growth performance				
DMI[3], g/d	2862	3018	55.1	0.161
Final bodyweight, kg	116	120	2.2	0.248
ADG[3], g/d	956	1025	30.5	0.268
Feed conversion ratio (DMI/ADG)	3.00	2.99	0.065	0.909
Carcass traits				
pH_1,45 min post mortem	6.15	6.25	0.06	0.512
pH_u, 24 h post mortem	5.52	5.61	0.03	0.119
Hot carcass weight, kg	93.4	96.1	1.84	0.369
Hot carcass yield, %	81.7	79.8	0.37	0.340
Cold carcass weight, kg	89.8	91.6	1.77	0.352
Cold carcass yield, %	76.8	76.1	0.33	0.408
Chilling weight loss, %	4.78	4.69	0.14	0.585

[1] CON, control diet; TAT, diet supplemented with 10 g/kg of tara (*Tara spinosa*) tannins.
[2] SEM, standard error of the mean.
[3] ADG, average daily gain; DMI, dry matter intake.

3. Results and discussion

3.1. Performance, carcass traits, and meat cooking loss

Supplementing pigs' diet with 10 g/kg of tara tannins for 8 weeks did not have any effect ($P > 0.10$) on ADG, final BW, carcass weight and yield, and muscle pH (Table 2). In agreement with our results, studies on dietary supplementation of chestnut tannins (up to 30 g/kg) to growing/finishing pigs consistently reported no detrimental effect on growth performance (Bee et al., 2017; Čandek-Potokar et al., 2015). However, digestibility trials on the use of tannin-containing crops in pig farming often led to the conclusion that tannins are antinutritional factors. For instance, feeding pigs with sorghum varieties high in tannins reduce energy digestibility (Pan et al., 2016) and aminoacids digestibility (Reis de Souza, Ávila Árres, Ramírez Rodríguez, & Mariscal-Landín, 2019). In particular, Pan et al. (2016) observed a reduction in energy digestibility with levels of dietary tannin similar to that used in the present experiment (i.e., 10 g/kg). A first explanation for these contrasting results could be due to the variability in tannins structure and activity. Tara and chestnut contain HT, which are simpler molecules compared to the condensed tannins contained in fava bean, pea, and sorghum (Jezierny et al., 2010; Reis de Souza et al., 2019). Thus, our results support the hypothesis that HT have a lower impact on diet digestibility and pig performance. Indeed, in some traditional free-range systems, pigs are well able to feed on acorns, tolerating the HT contained therein thanks to the neutralizing effect of endogenous proline (Cappai, Wolf, Pinna, & Kamphues, 2013). Nonetheless, some authors successfully fed up to 150 g/kg of sainfoin (Seoni et al., 2021) or carob pulp (Inserra et al., 2015), both sources of condensed tannins, without any negative effect on pig performance. Probably, the actual suitability of tannins in pig nutrition should be considered in relation to several variables, such as tannin structure and dose, breed, age, feeding strategy, and diet formulation, which affect animal response. For example, the results of Nyende, Wang, Zijlstra, and Beltranena (2023) suggest that the concurrence of other anti-nutritional compounds, such as vicine, rather than the sole presence of tannins impairs the digestibility of fava beans. Furthermore, the antinutritional effect of tannins could be counterbalanced by their positive effect on gut health (Xu et al., 2022).

Table 3
Intramuscular fat, cholesterol content, and fatty acids profile of pork.

Item	Dietary treatment[1]		SEM[2]	*P*-value
	CON	TAT		
Intramuscular fat, g/100 g of muscle	1.55	1.82	0.12	0.496
Cholesterol, mg/g	0.61	0.54	0.02	0.095
Fatty acids, mg/100 g of muscle				
C10:0	2.20	2.08	0.16	0.670
C12:0	1.50	1.59	0.12	0.696
C14:0	20.5	22.1	1.55	0.554
C16:0	393	429	29.4	0.490
C17:0 *anteiso*	4.73	5.24	0.36	0.518
C16:1 *c*9	51.6	54.5	3.70	0.651
C17:0	2.37	2.69	0.25	0.508
C18:0	205	223	17.6	0.515
C18:1 *t*9	2.32	2.24	0.21	0.976
C18:1 *c*9	660	740	52.6	0.408
C18:1 *c*11	66.3	71.7	4.34	0.478
C18:2 *c*9*c*12	176	170	9.22	0.706
C18:3 *c*9*c*12*c*15	11.1	13.5	1.06	0.240
C20:0	2.85	3.49	0.37	0.295
C20:1 *c*11	6.31	5.79	0.56	0.628
C20:2 *c*11*c*14	5.02	5.77	0.41	0.426
C20:3 *n*-6	4.50	4.66	0.25	0.728
C20:3 *n*-3	0.71	0.69	0.15	0.725
C20:4 *n*-6	29.6	29.6	1.46	0.986
C22:4 *n*-6	4.35	4.52	0.35	0.837
C22:5 *n*-3	4.23	3.38	0.23	0.079
C22:6 *n*-3	0.96	1.11	0.15	0.421
Sums and calculations				
SFA[3]	632	690	49.4	0.498
MUFA[3]	775	774	53.0	0.997
PUFA[3]	238	231	9.84	0.734
PUFA[3] *n*-6	220	215	11.0	0.787
PUFA[3] *n*-3	16.9	16.4	0.97	0.801
PUFA[3] *n*-6/*n*-3	13.6	13.5	0.61	0.945
AI[3]	0.46	0.47	0.01	0.436
TI[3]	1.07	1.10	0.01	0.506
h/H[3]	2.39	2.40	0.02	0.876

[1] CON, control diet; TAT, diet supplemented with 10 g/kg of tara (*Tara spinosa*) tannins.
[2] SEM, standard error of the mean.
[3] AI, atherogenicity index; h/H, hypocholesterolemic to hypercholesterolemic ratio; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TI, thrombogenicity index.

Table 4
Antioxidant capacity of pork.

Item	Dietary treatment[1]		SEM[2]	*P*-value
	CON	TAT		
Fat-soluble antioxidant vitamins				
α-tocopherol, μg/g meat	3.25	3.33	0.10	0.419
γ-tocopherol, μg/g meat	0.203	0.165	0.01	0.176
Retinol, ng/g meat	14.7	13.6	0.82	0.361
Hydrophilic antioxidant capacity, mg/g				
TEAC[3] (Trolox eq.)	47.3	61.0	5.910	0.360
FRAP[3] (Fe^{2+} eq.)	31.9	35.8	2.100	0.466
Folin-Ciocalteu (tannic acid eq.)	0.702	0.678	0.017	0.645
Antioxidant enzymes, U/g				
Catalase (CAT)	145	156	4.58	0.119
Glutathione peroxidase (GPx)	0.263	0.272	0.012	0.746
Superoxide dismutase (SOD)	130	117	4.40	0.087

[1] CON, control diet; TAT, diet supplemented with 10 g/kg of tara (*Tara spinosa*) tannins.
[2] SEM, standard error of the mean.
[3] FRAP, ferric reducing antioxidant power; TEAC, Trolox equivalent antioxidant capacity.

No difference ($P = 0.228$) was observed in meat cooking loss between the CON group (30.2 ± 2.4%) and the TAT group (28.4 ± 3.2%). Studies in which the effect of dietary phenolic compounds on pork water-holding capacity was assessed showed controversial results. Seoni et al. (2021) found higher drip loss in meat from pigs fed up to 150 g/kg of dehydrated sainfoin, while Rezar et al. (2017) observed higher thawing loss in meat from pigs fed up to 30 g/kg of chestnut HT. Instead, Xia et al. (2017) reported lower meat drip loss after feeding pigs 25 g/kg of sugar cane phenolic extract, which is rich in flavonoids and phenolic acids. It is still unclear how dietary tannins can affect meat water-holding capacity, and the diversity of experimental designs adopted so far (e.g., breed, diet, type and dose of tannins) does not allow an easy comparison among studies. However, in agreement with our results, feeding tannins to pigs is consistently reported to have no effect on meat cooking loss (Rezar et al., 2017; Seoni et al., 2021).

3.2. Intramuscular fat

In the present study, dietary tara HT did not affect ($P = 0.496$) intramuscular fat content, but tended to reduce ($P = 0.095$) cholesterol content (Table 3). A similar trend was observed in the cholesterol content of meat from pigs supplemented with 0.33 g/kg of magnolia bark extract, which is rich in neolignanes (Menci et al., 2022). Interestingly, Taranu et al. (2018) reported lower plasma cholesterol content in pigs fed 50 g/kg of grape seed cake, and Ao et al. (2011) observed lower low-density lipoprotein content in the plasma of pigs fed 2 g/kg of *Saurus chinensis* phenolic extract. In both these studies, the effects were attributed to the phenolic compounds contained in the dietary supplements, although is unclear whether they acted as stimulators of bile acids secretion or as inhibitors of pancreatic cholesterol esterase (Ao et al., 2011; Taranu et al., 2018). We suppose that tara HT as well may have affected cholesterol metabolism in the pigs of our experiment and that this tended to reduce meat cholesterol content. However, considering the low extent of the reduction, this would hardly have any implication on the nutritional quality of meat.

The FA profile of intramuscular fat was not affected ($P > 0.10$) by the dietary supplementation of 10 g/kg of tara HT, except for C22:5 *n*-3 content, which tended to be lower ($P = 0.079$) in TAT pork than in CON pork (Table 3). The FA profile plays a major role in characterizing both nutritional and technological quality of meat. In ruminants, dietary tannins are able to affect the FA profile of products through the modulation of ruminal biohydrogenation (Frutos et al., 2020). This does not apply to monogastric animals, in which meat FA profile is rather a direct consequence of the FA profile of the diet, especially for PUFA (Alfaia et al., 2019). Indeed, in some studies reporting an effect of a tannin-containing diet on pork FA profile, animals were not fed isolated phenolic compounds or tannin extracts, but rather received diets including tannin-containing feeds such as sainfoin (Seoni et al., 2021) or carob pulp (Inserra et al., 2015), which markedly changed the FA profile of the diet. However, the results of Rezar et al. (2017), who supplemented the diet of finishing pigs with chestnut tannin extract, suggest that tannin doses higher than 20 g/kg may affect the FA profile of intramuscular fat, particularly PUFA. Probably, the beneficial effect of tannins on intestinal health may actually modify the absorption of certain fatty acids, as hypothesized by Kafantaris et al. (2018) after observing the health status and meat FA profile of piglets fed grape pomace, which is rich in condensed tannins.

3.3. Meat antioxidant capacity

Meat antioxidant capacity is the set of biochemical defenses against oxidation, consisting in several antioxidant systems, including enzymatic defenses and antioxidant molecules. In the present study, we assessed the content of fat-soluble antioxidant vitamins, the antioxidant capacity of hydrophilic antioxidants, and the activity of antioxidant enzymes (Table 4). α-tocopherol is the most important fat-soluble antioxidant in meat, whereas γ-tocopherol and retinol play a minor role in protecting lipids from oxidation. Feeding 10 g/kg of tara HT had no effect ($P > 0.10$) on the content of these fat-soluble vitamins in pork (Table 4). There is almost no information about the effect of dietary

Table 5
Color stability of backfat and meat.

Item	Dietary treatment[1]		Storage time (T), d			SEM[2]	*P*-value		
	CON	TAT	0	3	6		D	T	D × T
Backfat									
L* (lightness)	74.3	76.0	75.2[b]	75.3[b]	77.3[a]	0.35	0.898	0.008	0.301
a* (redness)	4.90	5.15	5.54[a]	5.79[a]	3.89[b]	0.175	0.689	<0.001	0.495
b* (yellowness)	6.34	6.10	6.23[b]	7.29[a]	3.84[c]	0.251	0.129	<0.001	0.162
C* (saturation)	7.76	7.99	8.35[a]	9.32[a]	5.54[b]	0.295	0.343	<0.001	0.382
Hue angle, deg	56.1	49.1	48.3	51.3	60.6	3.67	0.270	0.348	0.182
Meat									
L* (lightness)	54.7	53.4	52.0[b]	55.1[a]	55.5[a]	0.384	0.082	<0.001	0.148
a* (redness)	5.77	5.82	7.98[a]	5.40[b]	4.12[c]	0.233	0.923	<0.001	0.150
b* (yellowness)	6.29	6.23	8.20[a]	6.54[b]	4.22[c]	0.249	0.610	<0.001	0.142
C* (saturation)	8.55	8.54	11.6[a]	8.49[b]	5.91[c]	0.336	0.732	<0.001	0.131
Hue angle, deg	47.4	46.6	45.6[b]	50.3[a]	45.4[b]	0.403	0.238	<0.001	0.149
630/580 ratio	1.24	1.25	1.35[a]	1.22[b]	1.16[c]	0.011	0.173	<0.001	0.267

[a,b,c]In each row, means that do not share a superscript letter are significantly different.
[1] CON, control diet; TAT, diet supplemented with 10 g/kg of tara (*Tara spinosa*) tannins.
[2] SEM, standard error of the mean.

tannins on the tocopherol content of pork. Rezar et al. (2017) reported a reduction in meat α-tocopherol after feeding pigs 30 g/kg of chestnut HT, but the authors themselves question the reliability of their results. A sparing effect toward vitamin E and other antioxidants has been proposed for phenolic compounds, which may exert their antioxidant activity in the gut allowing a greater quota of antioxidant molecules to be absorbed and accumulated in the tissues (Iglesias, Pazos, Torres, & Medina, 2012). However, consistently with our results, this was not observed in pigs whose diets were supplemented with tannins from grape (Gessner et al., 2013) or chestnut (González & Tejeda, 2007).

To account for the antioxidant activity of hydrophilic compounds we performed different laboratory assays on meat aqueous extract. These assays are based on radical scavenging activity (i.e., TEAC) or reducing power (i.e., FRAP and Folin-Ciocalteu). According to our results, dietary tara HT had no effect ($P > 0.10$) on TEAC, FRAP, and Folin-Ciocalteu assays in pork (Table 4). To the best of our knowledge, this is the first study assessing the effect of dietary tannins on hydrophilic antioxidants in pork. Considering other types of phenolic compounds, Zhang et al. (2015) found up to 0.6 g/kg of dietary resveratrol to increase pork antioxidant capacity, whereas Menci et al. (2022) observed no effect on meat hydrophilic antioxidant capacity after feeding pigs 0.33 mg/kg of dietary magnolia bark extract, which is rich in neolignanes. Our results agree with the general opinion that any effect of dietary tannins on meat antioxidant capacity is not directly due to their presence. Indeed, polyphenols have low bioavailability and the efficiency of absorption in animals is insufficient to allow significant accumulation in tissues (Surai, 2014). Further studies should investigate whether higher supplementation levels of tannins could increase the antioxidant capacity of hydrophilic compounds in meat.

The main antioxidant enzymes that cells deploy to neutralize reactive oxygen species are CAT, GPx, and SOD, which are thus found in muscle and meat. Some studies suggest that dietary phenolic compounds may exert an indirect antioxidant effect by activating the endogenous enzymatic defenses (Masella, Di Benedetto, Varì, Filesi, & Giovannini, 2005). This is in contrast with our results, as meat from pigs fed 10 g/kg of tara HT showed similar ($P > 0.10$) CAT and GPx activities and even a lower ($P = 0.087$) SOD activity compared with the control group (Table 4). However, research on this aspect is still incomplete and opposite results were obtained by feeding pigs different phenolic compounds. For example, dietary resveratrol increased muscle GPx activity (Zhang et al., 2015), whereas dietary flavonoids and phenolic acids from sugar cane reduced SOD activity (Xia et al., 2017). Explanation for the levels of antioxidant enzymes activity in muscle may be ambiguous. On the one hand, the higher the activity, the higher the antioxidant capacity of meat. On the other hand, it can be argued that higher enzymatic activities are the result of higher levels of oxidation (Milani, Gagliardi, Cova, & Cereda, 2011), which must therefore be determined to properly assess the oxidative status of the meat. Moreover, Zou, Xiang, Wang, Wei, and Peng (2016) reported an increase in serum SOD and GPx activities and liver GPx activity after supplementing pigs' diet with 25 mg/kg of quercetin, a flavonoid, although no effect was observed on muscle antioxidant enzymes activity. Therefore, the activity of antioxidant enzymes in muscle may not represent the overall oxidative status of the animal.

3.4. Meat oxidative stability

Oxidative stability is a major characteristic of meat quality, as it determines its shelf life and sensory attributes. Among many oxidative processes of meat, in this study we focused on meat color stability and lipid oxidation, as they can directly influence the consumer through the development of discoloration and off-flavor. Dietary tara HT did not affect ($P > 0.10$) backfat and meat color, but TAT pork tended to be darker ($P = 0.082$) than CON pork (Table 5). Feeding pigs with tannins has generally no effect on meat color (Rezar et al., 2017; Seoni et al., 2021), although some phenolic compounds such as resveratrol (Zhang et al., 2015) or magnolol (Menci et al., 2022) are reported to reduce pork L^* value. However, the slight difference found in the present study would hardly have any effect on consumer acceptance. As expected, meat and backfat color changed over time during refrigerated storage (Table 5). In backfat, L^* value increased ($P = 0.008$), whereas a^* and C* values decreased ($P < 0.001$) at the 6th day of storage. Yellowness followed a curve, reaching the highest level after 3 days and the lowest level after 6 days of storage ($P < 0.001$). In meat, L^* increased ($P < 0.001$), whereas a^*, b^*, and C* decreased ($P < 0.001$) over time. Hue angle was higher ($P < 0.001$) at day 3 of storage compared to day 0 and day 6. The ratio of 630 nm to 580 nm absorbance, representative of oxymyoglobin proportion, linearly decreased ($P < 0.001$) over 6 days of refrigerated storage. No interaction between diet and storage time was observed ($P > 0.10$). According to our results, dietary tara HT at 10 g/kg supplementation dose have no effect on raw pork color stability over 6 days of refrigerated storage.

Interestingly, feeding pigs with tara HT had a positive effect on meat lipid stability. During lipid oxidation, unsaturated fatty acids react with oxygen in a chain reaction that starts with the production of conjugated dienes and hydroperoxides and ends with the production of volatile molecules such as aldehydes and ketones (Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998). Fresh meat obtained from pigs fed with tara HT

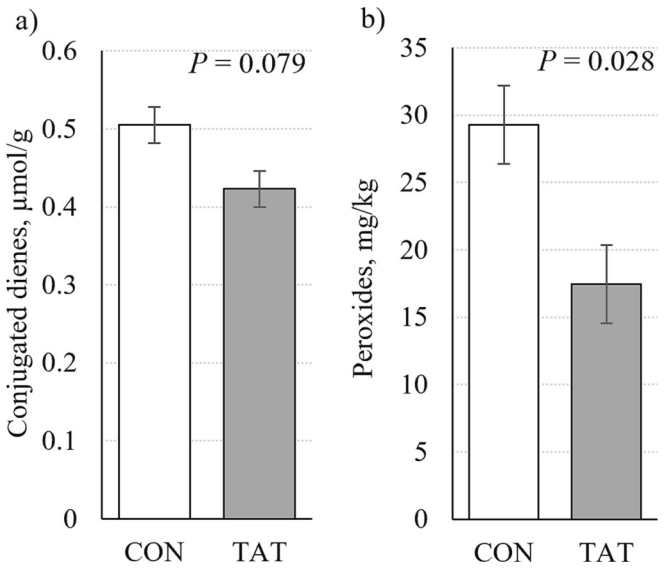

Fig. 1. Effect of dietary treatment on conjugated dienes (a) and hydroperoxides (b) in raw fresh meat. Error bars represent the standard error of the mean. CON, control diet; TAT, diet supplemented with 10 g/kg of tara (*Tara spinosa*) tannins.

showed a trend toward lower ($P = 0.079$) concentrations of conjugated dienes and a lower ($P = 0.028$) content of hydroperoxides, compared with the control meat (Fig. 1). This resulted in a reduced production of secondary compounds of lipid oxidation, as TAT raw pork tended to have lower ($P = 0.084$) MDA content throughout 6 days of refrigerated storage compared to CON pork (Table 6). When meat was subjected to oxidative challenges such as catalysis and cooking, a diet × time interaction occurred (Table 6): lipid oxidation linearly increased ($P < 0.001$) over time in both groups, but TAT pork had lower ($P < 0.05$) TBARS values than CON pork at the latest time point (Fig. 2). The protective effect of dietary phenolic compounds toward meat lipids was already observed in pigs fed resveratrol (Zhang et al., 2015), quercetin (Zou et al., 2016), or magnolia bark extract (Menci et al., 2022). Inserra et al. (2015) reported higher TBARS value in pork after feeding 150 g/kg of tannin-containing carob pulp, but in their study the diet increased the content of oxidizable *n*-3 PUFA in meat. The effect on lipid oxidation observed in the present study cannot be explained by different substrate availability, as meat FA profile was not affected by the diet, nor by different antioxidant capacity. Indeed, the basal diet used in the present study ensured a good level of α-tocopherol in the meat of both groups, considering that 3 µg/g can provide effective protection against lipid oxidation (Sales & Koukolová, 2011). We hypothesize that tara HT, thanks to their antioxidant activity, acted as an added protection against oxidative stress in the animal. For instance, this effect was observed in the intestine of pigs fed tannin-containing grape by-products (Gessner et al., 2013) and in the serum and liver of pigs fed quercetin (Zou et al., 2016). The resulting lower inflammatory state would have slowed down the initiation of lipid oxidation in muscle. This may also explain the lower SOD activity in the meat of TAT pigs compared to CON pigs. Interestingly, a similar effect was observed in the meat from pigs fed sugar cane phenolic extract, in which a lower SOD activity was associated with a lower MDA content (Xia et al., 2017).

4. Conclusions

Supplementing finishing pigs' diet with 10 g/kg of tara hydrolysable tannins reduced lipid oxidation in meat. However, the moderate extent of this effect would hardly affect the organoleptic quality of meat. The good level of α-tocopherol in the meat ensured by the diet we used may have hidden any potential positive effect of tannins. Remarkably, dietary tara tannins had no adverse effect on pig performance. Further research should investigate the effect of dietary tannins on meat oxidative stability with low in-feed levels of vitamin E. Targeted analyses should be included to understand whether the indirect antioxidant effect of dietary tannins is related to a lower inflammatory status of the animal.

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CRediT authorship contribution statement

Ruggero Menci: Investigation, Formal analysis, Visualization, Writing – original draft. **Giuseppe Luciano:** Conceptualization, Funding acquisition, Methodology, Writing – review & editing. **Antonio Natalello:** Conceptualization, Methodology, Investigation, Formal analysis, Project administration, Writing – review & editing. **Alessandro Priolo:** Conceptualization, Funding acquisition, Methodology, Supervision. **Fabrizio Mangano:** Investigation. **Luisa Biondi:** Validation. **Marco Bella:** Resources. **Manuel Scerra:** Writing – review & editing. **Massimiliano Lanza:** Supervision, Writing – original draft.

Table 6
Secondary products of lipid oxidation in pork (TBARS[1]).

Item	Dietary treatment[2] (D)		Time[3] (T)			SEM[4]	*P*-value		
	CON	TAN	0	1	2		D	T	D × T
Raw	0.106	0.081	0.086[b]	0.087[b]	0.103[a]	0.004	0.084	<0.001	0.540
Catalyzed	0.494	0.380	0.147[c]	0.370[b]	0.793[a]	0.041	0.047	<0.001	0.033
Cooked	1.058	0.860	0.176[c]	1.028[b]	1.720[a]	0.089	0.007	<0.001	0.013

a, b, c, means that do not share a superscript letter are significantly different.
[1] TBARS, thiobarbituric acid reactive substances (expressed as mg of malondialdehyde per kg of meat).
[2] CON, control diet; TAN, diet supplemented with 10 g/kg of tara (*Tara spinosa*) tannins.
[3] Times 0, 1, and 2 correspond to: 0, 3, and 6 d in raw meat; 0, 30, and 60 min in catalyzed meat; 0, 2, and 4 d in cooked meat.
[4] SEM, standard error of the mean.

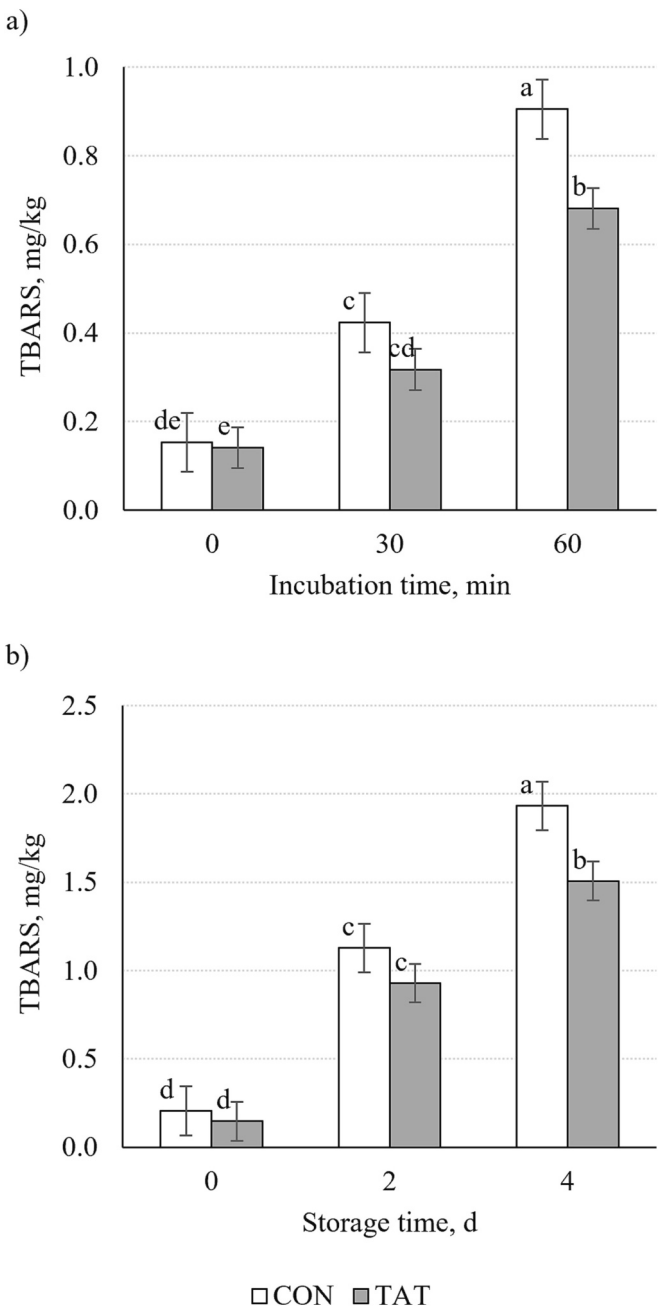

Fig. 2. Interaction between dietary treatment and time on lipid oxidation in catalyzed meat (a) and cooked meat (b). Error bars represent the standard error of the mean.
CON, control diet; TAT, diet supplemented with 10 g/kg of tara (*Tara spinosa*) tannins; TBARS, thiobarbituric acid reactive substances (expressed as mg of malondialdehyde per kg of meat).
a–e, in each graphic, bars that do not share a letter are different at $P < 0.05$.

Declaration of Competing Interest

None.

Data availability

Data will be made available on request.

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