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Meat quality from pigs fed tomato processing waste

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ARTICLE INFO	A B S T R A C T
<i>Keywords:</i> Tomato pomace Pork Fat Fatty acids Oxidative stability	Sixteen Nero Siciliano pigs were used to investigate the effect of dietary tomato processing waste on meat quality. During 86 days one group (CON, $n = 8$) received a pelleted conventional diet, while another group (TOM, $n = 8$) received the same diet in which tomato waste replaced 15% of corn. The dietary treatment did not affect growth performances. The TOM diet reduced intramuscular fat, SFA and MUFA content, while increasing the <i>n</i> -6: <i>n</i> -3 ratio in meat ($P < .05$). The TOM diet increased the concentration of PUFA, PUFA <i>n</i> -3, PUFA <i>n</i> -6 and the <i>n</i> -6: <i>n</i> -3 ratio ($P < .01$). The instrumental colour descriptors of backfat were unaffected by diet. The TOM diet increased deposition of retinol in meat ($P < .001$) but did not affect oxidative stability parameters measured in fresh meat and meat homogeneties with pro-oxidant catalysts. Concluding tomato pomace fed to pigs at

1. Introduction

Tomato pomace is the primary waste biomass that results from the industrial processing of tomato for the production of juice, paste and ketchup. Tomato pomace is composed of a variable proportion of seeds, peels and small amounts of residual pulp and discarded unprocessed fruits (Del Valle, Cámara, & Torija, 2006). As a waste, it is disposed following strict regulations, which represents a cost for the industries. However, its chemical composition makes tomato pomace suitable to be used in food and feed industries and for other purposes. Tomato pomace could be included in the formulation of antioxidant-enriched functional foods or as an additive to improve food shelf-life (Kalogeropoulos, Chiou, Pyriochou, Peristeraki, & Karathanos, 2012). Other studies suggested the use of tomato pomace for the production of biogas (Allison & Simmons, 2017) or for the sequential recovery of carotenoids, oil, protein and lignocellulosic matter in a multi-target biorefinery (Kehili et al., 2016). During the last decade, the inclusion of agro-industrial by-products in livestock feeding has been evaluated as a strategy to increase sustainability of food production systems. On the one hand, food-processing industries would profitably attenuate costs generated by disposing of processing wastes according to the regulations (Kasapidou, Sossidou, & Mitlianga, 2015). On the other hand, these biomasses could replace cereals and other conventional feeds,

contributing to reduce the feeding costs for livestock and the feed-tofood competition in livestock production (Wilkinson, 2011). Additionally, bioactive molecules that reside in several agro-industrial waste biomasses, such as unsaturated fatty acids and antioxidant compounds, may serve as functional ingredients in animal diets and could improve meat quality traits (Salami et al., 2019).

higher levels compared to previous reports had no adverse effects on the investigated meat quality traits.

Although the industrial processing technology determines variations in the chemical composition of tomato pomace, it is generally rich in fibre, as well as in protein and fat that are present in the seeds (Del Valle et al., 2006). Additionally, tomato peels and seeds are rich in essential amino acids (Kehili et al., 2016), the lack of which represents a limiting factor in the formulation of diets for pigs. The tomato seed oil is characterized by a high content of unsaturated fatty acids, mainly represented by linoleic and oleic acids (Peiretti, Gai, Rotolo, Brugiapaglia, & Gasco, 2013). The accumulation of polyunsaturated fatty acids (PUFA) in meat would be desirable for human health, although a high unsaturation degree of the intramuscular fat could have negative effects on the oxidative stability of meat (Bekhit, Hopkins, Fahri, & Ponnampalam, 2013). Noteworthy, tomato pomace is a natural source of carotenoids (mainly lycopene) and other molecules that could serve as dietary antioxidants when fed to animals (Rao, Waseem, & Agarwal, 1998), thus contrasting the potential pro-oxidative effect of an excess of PUFA in muscle. The effect of dietary tomato pomace on the quality

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traits of meat has been scarcely investigated, particularly in pigs. Feeding pigs with diets containing 3% or 5% of tomato by-products tended to increase the n-6 to n-3 fatty acid ratio with no effects on meat colour (Chung, Son Ah, Lee Su, & Kim, 2014). Correia et al. (2017) found that the inclusion of 5% tomato pomace in the diet of young pigs increased the content of α -tocopherol in muscle, but did not affect lipid oxidation of fresh meat; however, the authors did not investigate the oxidative stability of meat over a period of storage or display. Additionally, the favourable chemical composition of tomato pomace overall reported in literature may justify its inclusion at greater levels in the diet of pigs, which would allow a more effective exploitation of this by-product and could also exert more pronounced effects on meat quality. In light of the above, this study aimed at assessing if inclusion of 15% tomato pomace in pig diets is a viable strategy for: i) replacing conventional ingredients (such as corn) of a commercial concentratebased diet, and ii) improving meat quality traits with particular emphasis on the fatty acid composition and oxidative stability.

2. Materials and methods

2.1. Tomato pomace

Tomato pomace, comprising of peels, seeds and residual pulp, was obtained from a tomato sauce factory (Sicily, Italy). Within 3 h of collection, the fresh by-product was dried in approximately 24 h using a ventilated oven at 60 °C until constant weight and the dried pomace was stored at room temperature until its inclusion in the experimental diet (approximately 20 days).

2.2. Animals and dietary treatments

The experimental trial was conducted between July and October 2016 at a commercial farm located in Southern Italy (37,046818 N. 14.692325 E). The animals were handled in accordance with the European legislation (Directive 2010/63/EU) and the study was approved by the University of Catania (approval: PR16-18L2P4). Sixteen Nero Siciliano male pigs, born within two weeks, were selected at 7 months of age from 50 castrated hogs available at the farm. Subsequently, the animals were weighed (initial body weight 42.7 \pm 2.31 [SD] kg), housed in individual pens (3 m²) and randomly assigned to two feeding treatments: namely control (CON; n = 8) and tomato (TOM; n = 8). For 7 days, pigs were progressively adapted to their respective experimental diets. During an 86-day experimental period, the CON group was fed exclusively a corn- and barley-based commercial diet, while the diet given to the TOM group included 15% of tomato pomace in replacement of an equal amount of corn. All the ingredients of the diets were milled to a final particle size of 5 mm, mixed and pelleted at low-temperature (40 °C). Table 1 reports the ingredients and the chemical composition of the experimental diets. Each pen was equipped with a nipple water dispenser and feeder. Pigs were fed ad libitum with the respective experimental diet and feed was supplied every day (09:00 h), with the amount of feed offered and refused being recorded. At the end of the experimental trial, animals were weighed and transported to a commercial abattoir.

2.3. Slaughtering procedures and sampling of meat and backfat

All the animals were slaughtered after an overnight fast according to European Regulation by electric stunning and exsanguination. Each carcass was immediately weighed and the Longissimus thoracis (LT) muscle (7th - 11st rib), together with backfat, were excised from the left side after 24-h storage of the carcass at 4 °C. The inner backfat (hereinafter backfat) was separated from the muscle using a scalpel. An aliquot of backfat was used fresh for instrumental colour measurement, while the remaining portion vacuum packaged and stored at -80 °C until analysis of fatty acid composition. The muscle pH was measured

Table 1

Ingredients and chemical composition of the experimental diets.

	Diet	
	CON	TOM
Ingredients, g/100 g as fed		
Corn	50	35
Barley	20	20
Soybean meal	12	12
Faba bean	5	5
Carob pulp	3	3
Tomato pomace	0	15
Wheat middlings	7	7
Mineral and vitamin premix	3	3
Chemical composition		
Dry matter (DM), g/100 g as fed	90.1	90.9
Crude Protein, g/100 g DM	14.7	16.8
Ether extract, g/100 g DM	2.66	4.64
NDF, g/100 g DM	15.9	20.8
Ash, $g/100 g DM$	4.67	5.07
NSC^{a} , $g/100 g DM$	62.1	52.7
DE^{b} , kcal/kg DM	3522	3459
Amino acids, g/100 g DM		
Aspartic Acid. Asp	1.13	1.32
Threenine. Thr	0.57	0.60
Serine. Ser	0.68	0.75
Glutamic Acid. Glu	2.67	2.97
Proline, Pro	0.98	1.11
Glycine, Gly	0.58	0.67
Alanine, Ala	0.73	0.79
Valine, Val	0.64	0.70
Isoleucine, Ile	0.56	0.63
Leucine, Leu	1.14	1.19
Tyrosine, Tyr	0.38	0.45
Phenylalanine, Phe	0.66	0.72
Histidine, His	0.43	0.51
Lysine, Lys	0.67	0.79
Arginine, Arg	0.87	1.09
Tryptophan, Trp	0.03	0.23
Cysteine, Cys	0.40	0.41
Methionine, Met	0.37	0.40
Total	13.8	15.6
Fat-soluble vitamins, $\mu g/g$ DM		
β-carotene	ND ^c	3.61
Lycopene	ND ^c	460
α-tocopherol	52.4	57.7
γ-tocopherol	1.56	2.97
Total fatty acids (FA), g/100 g DM	1.37	3.86
Individual FA, % of total FA		
14:0	0.29	0.14
16:0	19.7	15.0
16:1	0.40	0.25
18:0	3.44	4.84
c9 18:1	25.7	22.6
c9 c12 18:2	39.3	48.3
c9 c12 c15 18:3	2.34	2.53

^a NSC: non-structural carbohydrates = 100 - (CP + NDF + EE + Ash).

^b DE: digestible energy; calculated as reported by Kil, Kim, and Stein (2013). ^c ND, not detectable.

using an Orion 9106 pH-meter equipped with a penetrating electrode (Orion Research Incorporated, Boston, MA), which was calibrated at 4 °C using standard solutions at pH 4 and 7. The LT was then divided into two subsamples: one was vacuum packaged and stored at -80 °C for analyses of fatty acids and fat-soluble vitamins, while the other was kept vacuum packaged at 4 °C for 1 day pending oxidative stability analyses.

2.4. Sampling and analyses of feeds

A representative sample of tomato pomace was collected immediately after the above-described drying procedure, while samples of each diet were collected three times over the duration of the experiment. All the collected feed samples were vacuum-packed and stored at

-30 °C until analyses. Equal amounts of the three subsamples collected during the trial were pooled to create a representative sample of each diet. The CON and TOM diets were finely milled (0.5-mm screen) and analysed for neutral detergent fibre (NDF) according to Van Soest, Robertson, and Lewis (1991). Crude protein, crude fat (ether extract) and ash were determined according to AOAC (1995). Amino acids were analysed in the diets (1 g) using post-column derivatization using ninhydrin followed by analysis using an amino acid analyzer (model 3A29, Carlo Erba Strumentazione, Corsico, Italy), according to the method of Moore, Spackman, and Stein (1980).

Fatty acid methyl esters (FAME) from feed lipids were prepared in a one-step extraction-methylation procedure (Natalello et al., 2019). Methyl esters were separated and determined by a gas chromatograph as later described for meat samples. The vitamin E isoforms (α and γ tocopherols) and carotenoids (lycopene and β-carotene) from feedstuffs were extracted and analysed as described by Valenti et al. (2018). Briefly, after homogenization with an ethanolic butylated hydroxytoluene (BHT) solution (0.06%, w/v), feedstuff samples were saponified (potassium hydroxide 60%, w/v) at 70 °C for 30 min. Three washes with hexane/ethyl acetate (9:1, v/v) were used to extract vitamin E and carotenoids. After drying under a nitrogen stream, the residue was dissolved in acetonitrile and 50 µL was injected in a highperformance liquid chromatograph (Perkin Elmer series 200), equipped with an autosampler (AS 950-10, Tokyo, Japan) and a Synergy Hydro-RP column (4 μ m, 4.6 \times 100 mm; Phenomenex, Bologna, Italy). Flow rate was set at 2 mL/min and 1 mL/min for tocopherols and carotenoids, respectively. Acetonitrile/methanol/tetrahydrofuran/1% ammonium acetate solution (68/22/7/3, $\nu/\nu/\nu/\nu$) was used in isocratic condition as the mobile phase for tocopherol analysis. While, an elution gradient program was used for carotenoid determination, starting from 90% of mobile phase A (methanol/water/acetonitrile 10/05/85, v/v/v) to 100% of mobile phase B (methanol/ethyl-acetate 70/30, v/v) over a 20 min period. The tocopherols were identified using a fluorescence detector (model Jasco, FP-1525) set at excitation and emission wavelengths of 295 nm and 328 nm, respectively). Absorbances at 450 nm and 505 nm were used for detection of β-carotene and lycopene, respectively, using an UV-visible detector (Jasco UV2075 Plus, Tokyo, Japan). Quantification of tocopherols and carotenoids was achieved using external curves, with increasing concentrations of pure standards (Sigma-Aldrich, Bornem, Belgium).

2.5. Fatty acids and fat-soluble vitamins in meat

Lipids from 10 g LT were extracted and methylated as described by (Natalello et al., 2019). The FAME in 1 µL sample were separated by gas chromatography in a 100-m high-polar fused silica capillary column (SP – 2560 fused silica, Supelco, Bellafonte, PA, 100 m \times 0.25 mm i.d.; film thickness $0.25\,\mu\text{m})$ using a Thermo Finnigan Trace GC equipped with a flame ionization detector (ThermoQuest, Milan, Italy) and the GC conditions reported in detail by Natalello et al. (2019). Commercial pure standard mixes of FAME (Nu-Chek Prep Inc., Elysian, MN, USA; Larodan Fine Chemicals, Malmo, Sweden) and individual FAME (Sigma-Aldrich, Bornem, Belgium) were used for identification of each compound, while nonadecanoic acid was used as internal standard for quantification. Fatty acids were expressed as mg/g muscle. The atherogenic (AI) and thromogenic index (AI) were calculated to assess the risk of cardiovascular diseases (Ulbricht and Southgate (1991). The peroxidability index (PI) was calculated as described by Valenti et al. (2019) in order to estimate the potential susceptibility of meat to lipid oxidation, based on the unsaturation degree of the intramuscular fatty acids. Vitamin E (α - and γ -tocopherol) and retinol were extracted from 2 g of muscle according to Schüep and Rettenmaier (1994) and identification and quantification was carried out by HPLC following the same procedure used for the feedstuffs. Retinol was identified in the same chromatographic run using a UV-vis detector (Jasco UV2075 Plus) set at λ 325 nm and quantified by means of external calibration using a

pure commercial standard (Sigma-Aldrich, Steinheim, Germany) in ethanol.

2.6. Meat oxidative stability

Oxidative stability was measured in fresh meat over aerobic storage and in meat homogenates incubated with pro-oxidant catalysts, as described in details by Valenti et al. (2019). Briefly, for fresh meat, three slices (2 cm thickness) were prepared from each muscle and placed in polystyrene trays, covered with PVC film and stored in the dark at 4 °C. Each slice was used for measuring lipid oxidation and colour stability at days: 0 (after 2 h of blooming), 3 and 5. Meat colour was measured using a Minolta CM 2022 spectrophotometer (d/8° geometry; Minolta Co. Ltd. Osaka, Japan) operating with the specular components excluded (SCE) mode, illuminant A and 10° standard observer. Duplicate measurements were performed on each slice and the mean value was calculated. The colour descriptors L* (lightness), a* (redness), b* (yellowness), C* (saturation) and H* (hue angle) were measured in the CIE L* a* b* colour space. The reflectance spectrum of the meat surface was also measured from 400 to 700 nm wavelength. The accumulation of metmyoglobin on the meat surface over storage duration was monitored by calculating the ratio (K/S)572 ÷ (K/S)525 (Luciano et al., 2011), whereby this ratio decreases with increasing proportion of MetMb. The ratio (K/S) between the absorption (K) and the scattering (S) coefficients at the selected wavelengths was calculated as:

 $(K/S)_{\lambda} = (1-R_{\lambda})^2/2R_{\lambda}$

Lipid oxidation was determined by measuring the 2-thiobarbituric acid reactive substances (TBARS) using the method described by Siu and Draper (1978). Meat samples (2.5 g) were homogenized with distilled water (12.5 ml) using a Heidolph Diax 900 tissue homogenizer (Heidolph ElektroGmbH & Co. KG, Kelheim, Germany) and kept in a water/ice bath during homogenization. Trichloroacetic acid (12.5 ml; 10% w/v) was added and samples were filtered through filter paper to remove precipitated proteins. The filtrate was incubated with thiobarbituric acid (0.06 M) for 90 min at 80 °C and the absorbance at 532 nm was measured using a Shimadzu UV/vis spectrophotometer (UV-1601; Shimadzu Corporation, Milan, Italy). A calibration curve was prepared using standard solutions of 1,1,3,3,-tetraethoxypropane (0 to 65 nmoles/4 mL) and was used to express TBARS as mg of malonaldehyde (MDA)/kg of meat.

Oxidative stability parameters were also measured in meat homogenates incubated with ferric chloride/sodium ascorbate (Fe/Asc) as pro-oxidant catalysts using the method described by Valenti et al. (2019). Briefly, meat (7.5 g) was homogenized with 37.5 g MES (2-(Nmorpholino) ethanesulfonic acid) buffer (4 °C, pH 5.7). Two aliquots were collected immediately, after which 45 μ M (final concentration) ferric chloride hexahydrate and L-sodium ascorbate were added. The homogenates were incubated at 37 °C under continuous shaking (IKA KS-4000 thermostatic shaker; IKA-Werke GmbH & Co. KG, Staufen, Germany) and the same volumes as above were collected at 30 and 60 min. For each time, one aliquot (3 ml) was used for measuring lipid oxidation as described for fresh meat, while the other aliquot (4 ml) was used for measuring the concentration of myoglobin (Mb, mg/g of meat) and the proportion of metmyoglobin (MetMb%; Krzywicki, 1982; Tang, Faustman, & Hoagland, 2004).

2.7. Fatty acid composition and colour of backfat

Lipids from 5 g of backfat were extracted and fatty acids were methylated and analysed as described above for meat samples. The same colour descriptors evaluated above for the meat (L*, a*, b*, C* and H* values) were measured on the backfat. Two colour measurements were taken on the surface of each backfat sample and the average value was calculated.

2.8. Statistical analysis

Data on animal performance parameters, meat pH, intramuscular fatty acid composition and concentration of antioxidants, as well as the backfat colour and fatty acids were analysed in a completely randomized design to test the effect of the dietary treatment (CON *vs* TOM), with the model:

$$y_{ii} = \mu + Di + e_{ij}$$

where: y_{ij} is the observation; μ is the overall mean; D_i is the diet (i = CON; TOM) and e_{ij} the residual error.

The oxidative stability data in fresh meat and meat homogenates were analysed as repeated measures, using a mixed model to test the effects of the dietary treatment (Diet), the time of storage (Time) and the Diet \times Time interaction as fixed factors. The individual pig was included as a random effect. The following model was adopted:

$$Y_{ijkl} = \mu + D_i + T_j + I_k(D) + (D \times T)_{ij} + e_{ijkl}$$

where y_{ijkl} is the observation; μ is the overall mean; D_i is the fixed effect of diet (i = CON; TOM); T_j the fixed effect of time (j = 0, 3, 5 days for fresh meat; 0, 30, 60 min for meat homogenates); $I_k(D)$ is the random effect of the individual pig nested with the dietary treatment; $(D \times T)_{ij}$ is the interaction between silage and time; e_{ijkl} is the residual error. Differences between means were assessed using Tukey's test for multiple comparisons.

Effects and differences were declared significant when $P \le .05$, while trends toward significance where considered when $.05 < P \le .1$. Statistical analyses were performed with the statistical software Minitab, version 16 (Minitab Inc., State College, PA).

3. Results

3.1. Feeds and animal performances

Table 1 reports the chemical composition of the two diets. The substitution of corn by an equal amount of tomato pomace increased the concentration of crude protein, ether extract and NDF in the TOM diet compared to the CON diet. The composition of amino acids and the content of the main essential amino acids was overall comparable between the experimental diets, with the exception of tryptophan, which was found at a greater concentration in the TOM diet compared to CON. Regarding the fatty acid composition, the concentration of linoleic acid was greater in the TOM diet compared to CON. Detectable amounts of β -carotene and lycopene were found only in the TOM diet, while small differences were observed for tocopherols between the two diets.

The dietary treatment did not affect the dry matter intake (2.86 vs 2.87 kg/day for CON and TOM, respectively; P = .947), average daily gain (449 vs 453 g/d for CON and TOM, respectively; P = .902), final live weight (84.5 vs 84.8 kg for CON and TOM, respectively; P = .929), carcass weight (64.7 vs 67.3 kg for CON and TOM, respectively; P = .491) and carcass yield (76.7 vs 79.3% for CON and TOM, respectively; P = .268).

3.2. Fatty acid composition of meat

Table 2 reports the effect of dietary treatment on the content of intramuscular fat (IMF), fatty acids and antioxidants in the muscle of pigs. The content of IMF was greater (P = .048) in the meat of CON pigs as compared to TOM meat. The sum of saturated fatty acids (SFA) was lower (P = .035) in meat from animals fed the TOM diet. Within the SFA class, the TOM diet specifically reduced the concentration of 16:0, 18:0 and 20:0 (P < .05), while tending to increase the concentration of 10:0 (P = .069), 12:0 (P = .091) and 14:0 (P = .065). The sum of monounsaturated fatty acids (MUFA) was reduced by feeding the TOM diet (P = .015). In particular, *c*9 14:1, *c*9 16:1, *c*9 18:1, *c*11 18:1 and

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Table 2

Effect of the dietary treatment on intramuscular fat, fatty acids and fat-soluble vitamins.

	Dietary t	reatment ^a	SEM	P value ^b
	CON	TOM		
Intramuscular fat (IMF), g/100 g muscle	4.65	3.47	0.305	0.048
Fatty acids, mg/g muscle				
10:0	0.04	0.03	0.004	0.069
12:0	0.03	0.02	0.003	0.091
14:0	0.48	0.32	0.044	0.065
c9 14:1	0.01	0.01	0.001	0.043
15:0	0.02	0.02	0.001	0.247
16:0	9.50	6.45	0.754	0.038
c9 16:1	1.04	0.62	0.106	0.042
17:0	0.09	0.08	0.006	0.312
18:0	5.25	3.61	0.392	0.030
c9 18:1	16.1	10.6	1.220	0.016
c11 18:1	1.36	0.89	0.107	0.021
c9 c12 18:2	3.14	3.87	0.228	0.109
c9 c12 c15 18:3	0.16	0.17	0.012	0.526
20:0	0.07	0.05	0.006	0.050
c11 20:1	0.27	0.19	0.021	0.041
20:2 <i>n</i> -6	0.10	0.14	0.009	0.016
20:3 <i>n</i> -6	0.06	0.06	0.002	0.345
20:3 n-3	0.02	0.03	0.001	0.090
20:4 <i>n</i> -6	0.35	0.33	0.037	0.803
20:5 n-3	0.02	0.01	0.001	0.240
22:0	0.01	0.01	0.001	0.465
22:4 <i>n</i> -6	0.07	0.08	0.003	0.523
22:5 n-6	0.01	0.02	0.000	0.042
22:5 n-3	0.04	0.05	0.002	0.469
22:6 n-3	0.01	0.01	0.002	0.090
SFA	15.4	10.5	1.200	0.035
MUFA	18.2	11.9	1.450	0.018
PUFA	4.00	4.78	0.243	0.113
PUFA n-3	0.25	0.26	0.016	0.638
PUFA n-6	3.74	4.50	0.229	0.097
n-6 / n-3	15.2	17.2	0.390	0.005
Atherogenic Index ^c	0.49	0.46	0.012	0.126
Trombogenic Index ^d	1.24	1.13	0.034	0.094
Peroxidability Index ^e	5.36	6.10	0.263	0.166
Fat-soluble vitamins, $\mu g/g$ of meat				
α-tocopherol	1.00	1.05	0.063	0.701
γ-tocopherol	0.01	0.01	0.002	0.957
Retinol	0.25	1.14	0.141	< 0.001

^a Treatments were: control diet (CON); diet containing 15% of tomato pomace (TOM).

^b *P* value of the effect of the dietary treatment.

 $^{\rm c}$ Atherogenic index (C12:0 + 4*C14:0 + C16:0) / (MUFA + PUFA n

6 + PUFA n-3).

^d Thrombogenic 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)].

^e Peroxidability Index (Σ dienoic×1) + (Σ trienoic×2) + (Σ tetraenoic×3) + (Σ pentaenoic×4) + (Σ hexaenoic×5).

c11 20:1 were found at lower concentration in the meat from the TOMfed animals (P < .05). The sum of polyunsaturated fatty acids (PUFA) was not affected by the dietary treatment. Among the individual PUFA, the TOM diet increased the concentration in muscle of 20:2 *n*-6 and 22:5 *n*-3 (P < .05) and tended to increase that of 20:3 *n*-3 (P = .090). Conversely, the concentration of 22:6 *n*-3 tended to be increased by feeding the CON diet (P = .090). The dietary treatment tended to affect the sum of PUFA *n*-6, with a greater concentration found in meat from the TOM group (P = .097); consequently, the TOM diet increased the *n*-6 to *n*-3 ratio (P = .005) compared to the CON treatment. The thrombogenic index tended to be lower in TOM meat (P = .094). The peroxidability index (PI) of the intramuscular fat was not affected by the dietary treatment.

Table 3

Effect of the dietary treatment on total fat, fatty acid composition and colour of backfat.

Die	Dietary treatment ^a		SEM	P value ^b
CO	N	ТОМ		
Total fat, g/100 g backfat 74.	.3	76.1	0.945	0.378
Fatty acids, mg/g backfat				
10:0 0.2	9	0.30	0.016	0.773
12:0 0.3	6	0.39	0.020	0.468
14:0 6.7	7	6.69	0.215	0.841
c9 14:1 0.0	6	0.03	0.007	0.009
15:0 0.3	0	0.39	0.034	0.200
16:0 14	4.6	143.2	4.712	0.887
c9 16:1 10.	.44	9.05	0.420	0.124
17:0 2.1	9	2.47	0.184	0.449
18:0 83	.07	81.86	4.734	0.902
c9 18:1 25	9.1	237.1	8.394	0.229
c11 18:1 15	.22	12.70	0.763	0.106
<i>c</i> 9 <i>c</i> 12 18:2 66	.36	124.1	8.881	< 0.001
<i>c9 c</i> 12 <i>c</i> 15 18:3 3.6	0	5.91	0.396	0.001
20:0 1.3	32	1.35	0.100	0.885
c11 20:1 6.4	7	5.51	0.327	0.145
20:2 <i>n</i> -6 2.9	5	5.44	0.372	< 0.001
20:3 <i>n</i> -6 0.4	7	0.63	0.046	0.076
20:3 n-3 0.5	54	0.76	0.038	0.002
20:4 <i>n</i> -6 1.2	26	1.48	0.095	0.267
22:4 <i>n</i> -6 0.4	3	0.62	0.038	0.004
22:5 n-3 0.2	.7	0.36	0.030	0.120
22:6 n-3 0.1	4	0.13	0.024	0.837
SFA 23	6.4	233.8	9.180	0.894
MUFA 293	3.8	267.3	9.403	0.196
PUFA 76	.09	139.5	9.778	< 0.001
PUFA n-3 4.5	5	7.16	0.449	< 0.001
PUFA n-6 71.	.47	132.2	9.346	< 0.001
<i>n</i> -6/ <i>n</i> -3 15.	.69	18.58	0.501	< 0.001
Colour descriptors				
L* values 74	.7	73.9	0.287	0.197
a* values 3.2	.6	3.44	0.109	0.423
b* values 4.5	3	4.81	0.137	0.331
C* values 5.5	9	5.92	0.157	0.302
H* values 54.	.2	54.4	0.793	0.891

^a Treatments were: control diet (CON); diet containing 15% of tomato pomace (TOM).

^b *P* value of the effect of the dietary treatment.

3.3. Fatty acid composition and colour of backfat

Table 3 reports the effect of the dietary treatment on the fatty acid composition and colour of backfat. The inclusion of tomato pomace in the diet did not affect the total fat and the sum of SFA and MUFA. Among the individual MUFA, only the concentration of c9 14:1 was affected by the dietary treatment and was lower in the backfat from the TOM-fed animals (P = .009). Feeding the TOM diet increased the concentration of total PUFA, PUFA n-3, PUFA n-6 and the ratio between n-6 and n-3 PUFA (P < .001) in the backfat. Particularly, within the PUFA, the TOM diet increased the concentration of *c9* 12 18:2, *c9* c12 c15 18:3, 20:2 *n*-6, 20:3 *n*-3 and 22:4 *n*-6 (P < .01). The colour descriptors (L*, a*, b*, C* and H* values) measured on the backfat samples were not significantly different between the CON and TOM groups.

3.4. Fat-soluble vitamins and oxidative stability of meat

As shown in Table 2, the dietary treatment did not affect the concentration of vitamin E (α - and γ -tocopherol; P > .05) in meat. Conversely, feeding the TOM diet increased the concentration of retinol in meat compared to the CON treatment (P < .001). Table 4 reports the oxidative stability parameters measured in fresh meat over 5 days of aerobic refrigerated storage, as well in meat homogenates incubated with pro-oxidant catalysts for 30 and 60 min at 37 °C. In fresh meat, lipid oxidation (TBARS values) was not affected by the dietary treatment and only tended to increase over the 5 days of storage (P = .060). The redness (a*) and saturation (C*) descriptors were not affected by the time of storage or by the dietary treatment (P > .05). Meat lightness (L*), yellowness (b*) and hue angle (H*) values were affected by the time of storage (P < .01), with values overall increasing from 0 to 3 days and stabilizing thereafter. Similarly, metmyoglobin accumulated on the meat surface from day 0 to day 3 of storage, as indicated by the decreasing values of the (K/S)572 ÷ (K/S)525 ratio, while no further change was observed from 3 to 5 days. The average L* values measured over the storage period were lower (P = .037) in the meat from the TOM-fed animals, while the vellowness descriptor (b*) only tended to be lower (P = .087) as compared to the CON group. The hue angle (H^{*}) descriptor was not affected by the dietary treatment, while the (K/S)₅₇₂ ÷ (K/S)₅₂₅ ratio was overall higher in meat from animals fed the TOM diet.

The incubation of meat homogenates with pro-oxidants (Fe/Asc) produced a strong oxidative challenge, whereby lipid oxidation sharply increased over incubation time (P < .001) and TBARS reached the value of 2.21 mg/g after 60 min of incubation compared to 0.23 mg/g measured in fresh meat after 5 days of aerobic storage. Similarly, myoglobin oxidised readily in the homogenates over time of incubation, with the proportion of metmyoglobin (MetMb%) increasing from 17.3% to 65.0% after 60 min. The concentration of myoglobin was not affected by the time of incubation with pro-oxidant catalysts (P > .05), with an average value of 2.44 mg/g of meat. Overall, all the measured parameters in meat homogenates were not affected by the dietary treatment (P > .05).

4. Discussion

4.1. Fatty acid composition of meat and backfat

In the present study, the dietary administration of 15% tomato pomace reduced the accumulation of intramuscular fat (IMF) in meat. A reduction of the IMF content below certain limits could impair some meat quality traits such as juiciness, tenderness and flavour, with a negative impact on consumers' acceptability (Lawrie, 1998). Although we did not assess the sensory traits of meat, the concentration of IMF found in TOM meat (3.47 g/100 g of meat) is over the limits suggested by Lawrie (1998) and is comparable to the fat content reported in literature for the same breed and for other local breeds (Franco, Vazquez, & Lorenzo, 2014; Pugliese & Sirtori, 2012). The effect on IMF concentration could be attributed to the chemical composition of the two experimental diets. The TOM diet had a greater content of linoleic acid compared to the CON diet and a reduction of IMF deposition has been observed when pigs received a diet rich in linoleic acid (Hernández-López, Rodríguez-Carpena, Lemus-Flores, Grageola-Nuñez, & Estévez, 2016). Also, Teye et al. (2006) suggested that reducing the level of dietary protein and essential amino acids may limit the endogenous biosynthesis of protein in pigs with a consequent increase of the energy available for the deposition of intramuscular fat. Therefore, the greater concentration of protein and essential amino acids found in the TOM diet might partially explain the lower concentration of IMF in meat from the TOM-fed animals. Additionally, the TOM diet contained βcarotene and lycopene, which may have further contributed to reduce the IMF in meat. It has been reported that the dietary administration of lycopene reduced the amount of intramuscular fat in lambs (Jiang et al., 2015). Finally, feeding tomato pomace increased the concentration of retinol in meat. From a nutritional perspective, the concentration of retinol found in meat from TOM-fed animals ($114 \mu g / 100 g$) is below the recommended dietary intake of vitamin A (spanning from 600 to 1000 µg; Olson, 1987; Troesch, Hoeft, McBurney, Eggersdorfer, & Weber, 2012). However, the 4.6-fold increase in the content of retinol produced by the TOM diet highlights the potential of feeding tomato pomace to improve this meat quality trait. This result is in agreement

Table 4

Effect of the dietary treatment and time of storage on the oxidative stability parameters of meat.

	Dietary treatm	Dietary treatment (D) ¹		Time (T) ²		SEM	P values ³		
	CON	TOM	0	1	2		D	Т	$\mathrm{D} imes \mathrm{T}$
Meat slices									
TBARS, mg/kg	0.19	0.22	0.19	0.20	0.23	0.007	0.177	0.060	0.180
L* values	53.5	50.3	49.1 ^b	53.4 ^a	53.1 ^a	0.591	0.037	< 0.001	0.215
a* values	16.7	15.7	16.3	16.2	16.0	0.346	0.322	0.874	0.644
b* values	16.1	14.4	13.4 ^b	16.1 ^a	16.1 ^a	0.406	0.087	0.001	0.639
C* values	23.2	21.3	21.1	22.9	22.7	0.497	0.158	0.176	0.695
H* values	43.8	42.3	39.1 ^b	44.8 ^a	45.1 ^a	0.516	0.112	< 0.001	0.193
$(K/S)_{572} \div (K/S)_{525}$	0.91	0.94	0.98 ^a	0.91 ^b	0.90 ^b	0.007	0.006	< 0.001	0.201
Meat homogenates with Fe/Asc									
TBARS, mg/kg	1.19	1.37	0.24 ^c	1.40 ^b	2.21 ^a	0.163	0.583	< 0.001	0.727
Mb, mg/g	2.37	2.49	2.50	2.50	2.31	0.083	0.694	0.604	0.312
MetMb, % of Mb	42.5	43.4	17.3 ^c	46.6 ^b	65.0 ^a	3.101	0.779	< 0.001	0.879

^{a, b, c} Within row, different superscript letters indicate differences (P < .05) between times of storage tested using the Tukey's adjustment for multiple comparisons. ¹ Dietary treatments were: control diet (CON); diet containing 15% of tomato pomace (TOM).

² Times 0, 1, 2 = days 0, 3, 5 at 4 °C under aerobic conditions (meat slices); minutes 0, 30, 60 at 37 °C (meat homogenates incubated with Fe³⁺/ascorbate).

³ *P* values for the effects of the dietary treatment (D), time of storage (T) and of the D \times T interaction.

with previous reports (Valenti et al., 2018) and was likely due to the carotenoids present in this by-product. Pigs absorb dietary carotenoids inefficiently, but are able to convert pro-vitamin A carotenoids (such as β -carotene) into vitamin A (Álvarez, Meléndez-Martínez, Vicario, & Alcalde, 2015). Moreover, although lycopene does not possess direct pro-vitamin A activity, Aydemir et al. (2016) reported that it shows a partial indirect pro-vitamin A activity in mice and the dietary administration of lycopene increased the levels of vitamin A in chicken leg muscles (Englmaierová, Bubancová, Vít, & Skřivan, 2011). As retinoids may affect lipid metabolism, leading to a reduction of fat deposition (Bonet, Ribot, & Palou, 2011), it cannot be excluded that the effect of the dietary treatment on the concentration of retinol in muscle might have further contributed to the lower IMF content in meat from the TOM-fed animals.

The manipulation of meat fatty acid composition, by increasing PUFA at expense of SFA, would be desirable for human health. Differently from ruminants, dietary fatty acids do not undergo substantial changes along the digestive tract of monogastrics and, after absorption, accumulate in animal tissues (Wood et al., 2008). For this reason, the results found in the present study for the fatty acid composition of meat can be largely explained by the fatty acid profile of the two experimental diets. In agreement with our findings, feeding tomato processing by-products has been reported to decrease the concentration of SFA and increase linoleic acid and total PUFA in other animal species (Botsoglou et al., 2004; Peiretti et al., 2013; Valenti et al., 2018). In the present study, dietary tomato pomace reduced the concentration of 16:0, 18:0, total SFA and tended to reduce the thrombogenic index. These findings are of relevance as it is well recognized that humans should limit the ingestion of saturated fatty acids in order to prevent cardiovascular diseases (Aranceta & Pérez-Rodrigo, 2012). The results found here for the fatty acid composition should be also linked to the effect of the diet on the IMF content. De Smet, Raes, and Demeyer (2004) reviewed that in pigs and ruminants the content of SFA and MUFA increases faster in the intramuscular fat with increasing fatness than does the content of PUFA. As the IMF is mainly composed of triacylglycerols, which are richer in SFA and MUFA than PUFA, an increase in fatness often leads to a reduction of the PUFA to SFA ratio. Taking into account the fatty acid composition of the CON and TOM diets, a greater concentration of linoleic, α -linolenic acid and total PUFA in the meat of TOM pigs would have been expected. In contrast, these fatty acids were found at comparable amounts between the two groups, which might be explained by the above commented differences in muscle IMF between the TOM and CON groups. It should be stressed that the fatty acid composition was expressed as concentration per unit of meat (mg/g muscle), which gives relevant information on the nutritional value of meat, but is greatly influenced by variations in IMF content. Expressing the fatty acids as the normalized percentage of each fatty acid relative to the total fatty acids (Supplementary Table S1) revealed the expected greater percentage of PUFA, linoleic and a-linolenic acid in the IMF of TOM meat. Regarding other fatty acids with potential health benefits, the proportion of eicosapentaenoic acid (EPA) was greater in the TOM than CON meat. Literature reports that diets rich in linoleic acid may result in an increased level of EPA and docosahesaenoic acid (DHA) in pork (Durand-Montgé, Realini, Barroeta, Lizardo, & Esteve-Garcia, 2010; Hernández-López et al., 2016), due to the endogenous elongation and desaturation of dietary linoleic acid to form EPA and DHA. Similarly to IMF, feeding diets high in linoleic and α -linolenic acids to pigs generally results in the accumulation of these fatty acids and their long-chain derivatives in the backfat. Consistent with this, we found a greater concentration of linoleic and α -linolenic acid, total PUFA, PUFA n-3 and PUFA n-6 in the backfat from the TOMfed animals and these results were confirmed when fatty acids were expressed as g/100 g total fatty acids (Supplementary Table S2).

4.2. Backfat colour

Fat is an important component of the pork carcass, with colour representing a sensory quality trait of major significance (Hallenstvedt, Kjos, Øverland, & Thomassen, 2012). Several studies have identified that fatty acid composition is among the most important factors contributing to the colour of pork fat. Maw, Fowler, Hamilton, and Petchey (2003) demonstrated that the sensory appreciation of fat yellowness in pork was positively correlated with linolenic and linoleic acids and similar results were provided by studies in which colour was measured instrumentally (Carrapiso & García, 2005). Therefore, the diet of animals can affect colour parameters of fat, through modifications of its fatty acid composition. Consistently, instrumental colour coordinates measured on the backfat have been proposed as markers for the authentication of the dietary background of pigs (Álvarez et al., 2015). It was initially proposed that, since fatty acids are colourless, the positive relation between PUFA and pigmentation in fat might be explained by the fact that diets promoting the accumulation of PUFA are generally rich in carotenoids that would be directly responsible for the yellow colour (Maw et al., 2003). However, it has been shown that the absorption of dietary carotenoids is very poor in pigs and that the administration of diets rich in carotenoids does not increase the deposition of carotenoids in pork fat (Álvarez et al., 2015). Therefore, the changes of fat colour associated with fatty acid composition might be

likely related to the well-known effect of fatty acid composition on physical properties of the backfat, such as firmness, which ultimately affect its light reflection properties (Carrapiso & García, 2005). In the present study, it is of note that differences in fat colour descriptors were not found between the CON and TOM treatments, despite the greater concentration of total PUFA in the backfat from TOM-fed animals. It will be of interest in future studies to more deeply assess the effects of tomato pomace on the quality traits of fat, including textural properties, susceptibility to oxidation and colour stability especially if meat is destined for further processing and ham production.

4.3. Meat oxidative stability

The deterioration of colour and the development of rancid off-flavours in meat over time of storage or display is primarily affected by the oxidation of lipids and myoglobin (Faustman, Sun, Mancini, & Suman, 2010). The inherent susceptibility of meat to oxidation is the result of a complex balance in muscle between anti-oxidant and pro-oxidant factors. Among the latter, heme iron in myoglobin may act as catalyst for lipid peroxidation, while highly unsaturated fatty acids are particularly susceptible to oxidation (Baron & Andersen, 2002; Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998). Nevertheless, endogenous antioxidant defences and exogenous antioxidant compounds of dietary origin operate in muscle to combat oxidative processes, thus contributing to extend meat shelf-life (Bekhit et al., 2013). Therefore, animal diets can greatly influence meat oxidative stability, as it can modify both the concentration of pro-oxidants (*e.g.* PUFA) and anti-oxidants (*e.g.* dietary antioxidant vitamins).

The results of the present study demonstrate that the inclusion of tomato pomace in the diet did not alter the concentration of measured pro-oxidant components in meat. Indeed, the concentration of heme iron (myoglobin; Mb) was not different between groups and the dietary treatment did not affect the concentration of PUFA in meat and the peroxidability index of IMF, which was calculated to take into account that the susceptibility of PUFA to oxidation increases with increasing degree of unsaturation (Scislowski, Bauchart, Gruffat, Laplaud, & Durand, 2005). Regarding the potential antioxidants, as commented above, feeding tomato pomace increased the concentration of retinol in muscle. Although a number of studies have demonstrated that vitamin A possesses antioxidant properties, its antioxidant activity in the animal tissues and its contribution to the oxidative stability of meat are still controversial. For example, Bartov, Sklan, and Friedman (1997) reported no effect of feeding supranutritional doses of vitamin A to broilers on the oxidative stability of meat. It has also been demonstrated that the oral administration of vitamin A did not exert any antioxidant effect in rats and induced oxidative stress in trained subjects (Petiz et al., 2017) and similar findings were reported by other authors. Recently, we found that feeding dried tomato pomace to lambs produced a 2-fold increase in the concentration of retinol in muscle, but did not affect the resistance of meat to oxidation (Valenti et al., 2018). If the effect of retinol on meat oxidative stability is still under debate, vitamin E is unequivocally recognized as the most effective exogenous antioxidant in muscle able to delay oxidative deterioration and extend the shelf-life of meat (Ponnampalam et al., 2014). The concentration of vitamin E in muscle readily responds to modifications of its content in the diet (Bellés, Campo, Roncalés, & Beltrán, 2019; Sales & Koukolová, 2011; Trefan et al., 2011). Therefore, in the present study, the lack of difference in the concentration of vitamin E in meat can be easily explained by the comparable concentration of a-tocopherol in the experimental diets.

These results suggest that the dietary treatment had no appreciable effect on the balance between pro- and anti-oxidants in muscle, which may be the reason for the minimal differences in meat shelf life parameters observed between the CON and TOM treatments. In fresh meat, we did not observe an effect of diet on lipid oxidation. Similarly, colour coordinates mostly related to meat browning (a*, C* and H* values) did

not differ between treatments. We only observed a higher $(K/S)_{572}$ ÷ (K/S)₅₂₅ ratio overall measured across the storage period in meat from pigs fed the TOM diet. This ratio reflects the accumulation of metmyogobin (MMb) on the meat surface, with higher values indicating lower proportions of MMb relative to the total pigment concentration (Luciano et al., 2011). Therefore, this result indicates that feeding the TOM diet reduced the oxidation of myoglobin in meat over 5 days of refrigerated storage. Lipid and myoglobin oxidation in meat are considered to be linked and factors reducing the extent of lipid oxidation generally result in lower myoglobin oxidation (Faustman et al., 2010). However, in the present study, the diet did not affect lipid oxidation; therefore it is not easy to propose a plausible explanation for the observed effect on MMb accumulation. It might be hypothesised that tomato pomace may have a selective antioxidant effects against myoglobin oxidation, which could be due to the occurrence of antioxidant compounds other than fat-soluble vitamins. Tomato processing wastes contain appreciable amounts of phenolic compounds with pronounced antioxidant activity (Valdez-Morales, Espinosa-Alonso, Espinoza-Torres, Delgado-Vargas, & Medina-Godoy, 2014). Although inconsistent results have been provided in literature on the effect of dietary phenolic compounds on meat oxidative stability, it has been demonstrated that dietary phenolic compounds can increase the resistance of myoglobin to oxidation in lamb meat independent from lipid oxidation (Luciano et al., 2009, 2011).

It should be stressed that all the oxidative stability parameters measured indicate that fresh meat underwent very low oxidative deterioration over refrigerated storage. Indeed, lipid oxidation only tended to increase with time of storage and the range of TBARS values measured from 0 to 5 days (0.19 to 0.23 mg MDA/kg of meat) suggesting a high stability of fresh meat to lipid oxidation. In addition, it is worth mentioning that these values are below the threshold limits proposed for the sensory detection of rancid flavours (from 0.5 to 1 mg MDA/kg of meat; Lanari, Schaefer, & Scheller, 1995; Rossi et al., 2013). Similarly, regardless of the above-mentioned differences between groups, all the colour stability parameters that were affected by the time of storage were subjected to numerically little variations over time and changed only from day 0 to day 3, with no further changes being measured up to 5 days. These results agree with the results reported for fresh pork stored in similar conditions for up to 9 days (Inserra et al., 2015). Aerobic storage of fresh meat in darkness may represent a lowoxidative challenge for meat to fully express its resistance to oxidative deterioration. Indeed, it has been reported that differences in oxidative stability between meat samples were masked under refrigerated storage of fresh meat, but were evident when meat was subjected to stronger oxidative challenges (cooking or incubation of meat homogenates with pro-oxidant catalysts; Luciano et al., 2019). Therefore, in the present study, we also assessed oxidative stability in meat homogenates incubated with Fe/Asc catalysts in order to assess possible diet-related differences of meat oxidative stability under more pro-oxidant conditions. As expected, Fe/Asc induced a dramatic increase in both lipid and myoglobin oxidation compared to the aerobic refrigerated storage. However, under these conditions, no effect of dietary treatment was observed in any of the measured parameters and this result further confirms that feeding tomato pomace did not produce noticeable effects on meat oxidative stability.

5. Conclusions

The results of the present study demonstrated that replacing 15% corn with tomato pomace did not affect animal performance, which suggests that tomato pomace could be potentially included in diets for pigs at higher levels compared to previous reports. This would allow replacement of a remarkable amount of conventional diet ingredients, thus reducing feeding costs and attenuating the problems linked to the disposal of tomato wastes. Clearly, the use of tomato pomace and other alternative feed ingredients, while maintaining acceptable performance

parameters, should not negatively impact meat quality parameters. The present study demonstrated that feeding tomato pomace affected some of the measured meat quality parameters. The inclusion of this byproduct in the diet increased the concentration of vitamin A in meat, reduced the deposition of intramuscular fat and modified its fatty acid composition. The effect of tomato pomace on the fatty acid composition was particularly evident in the backfat, where the concentration of linoleic acid and polyunsaturated fatty acids were increased. Despite the observed effects, the inclusion of 15% tomato pomace in the diet did not affect the colour of the backfat. In addition, feeding tomato pomace did not affect the oxidative stability of meat. It will now be necessary to further investigate the effects of comparable levels of tomato pomace on other meat quality parameters. Among these, special attention should be given to the sensory properties of meat, due to the observed effect of tomato pomace on the intramuscular fat content. Also, future studies should be conducted to assess the effects of increasing levels of tomato wastes in diets for pigs in order to exploit this alternative feed resource with confidence.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meatsci.2019.107940.

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