



Quality indices and sensory attributes of beef from steers offered grass silage and a concentrate supplemented with dried citrus pulp

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ARTICLE INFO

Keywords:

Beef
Citrus pulp
Oxidative stability
Sensory analysis
Fatty acids

ABSTRACT

This study investigated the quality composition, oxidative stability and sensory attributes of beef (*longissimus thoracis*, LT) from steers offered grass silage and a concentrate supplement in which barley was replaced by 40% and 80% (*as-fed* basis) of dried citrus pulp (DCP). Dietary treatment did not influence the antioxidant status (α -tocopherol and total phenolic contents) and activities of LT (radical scavenging activity, ferric reducing antioxidant power and iron chelating activity). Feeding DCP significantly increased the proportion of conjugated linoleic acids and polyunsaturated fatty acids in beef. Lipid and colour stability of fresh beef patties stored in modified atmosphere packs (MAP) were unaffected by dietary treatment but feeding 40% DCP reduced ($P < .05$) lipid oxidation in aerobically-stored cooked beef patties. Beef patties stored in MAP for up to 7 days were assessed by sensory panellists to be juicier for those fed 40% DCP compared to 0% and 80% DCP. Results indicated that substitution of barley with DCP improved the fatty acid profiles of beef without negatively influencing the eating quality of beef.

1. Introduction

Concentrate feeding in combination with grass silage is a strategy typically used for winter-finishing of beef cattle in temperate countries like Ireland (McGee, 2005). The substitution of cereals with agro-industrial by-products in concentrate-based rations reduces feed cost, mitigates food-feed-fuel competition and enhances resource efficiency of livestock production (Salami et al., 2019). Citrus pulp is a whole by-product obtained after the extraction of juice from citrus fruits consisting of peel, pulp and seed residues (Martinez-Pascual & Fernandez-Carmona, 1980). Due to the high amount of digestible fibre in citrus pulp, this by-product can be used as a high-energy feedstuff, compared to starch-rich cereals, to avoid a decline in ruminal pH and negative effects on forage digestibility in ruminants (Bampidis & Robinson, 2006). A recent study indicated that dried citrus pulp (DCP) can be used to replace up to 80% barley in a concentrate supplement offered to grass silage-fed bulls, without detrimental effects on animal performance (Lenehan, Moloney, O'Riordan, Kelly, & McGee, 2017).

Dried citrus pulp may contain a high residual amount of bioactive compounds such as phenolics, carotenoids, ascorbic acids, and

tocopherols (Fernández-López et al., 2004). By-products rich in phenolic compounds have been employed to replace cereals in different feeding strategies to inhibit ruminal biohydrogenation of unsaturated fatty acids and thus, promoting greater incorporation of unsaturated fatty acids in muscle tissues (Salami et al., 2019). Moreover, feeding diets containing bioactive compounds such as phenolics and α -tocopherol could enrich muscle tissues with natural antioxidant compounds that could improve the oxidative stability of meat (Salami et al., 2019).

Fatty acid composition in ruminant-derived foods (meat and meat) is characterized by a low content of health-promoting polyunsaturated fatty acid (PUFA) and a high proportion of undesirable saturated fatty acids (SFA) and *trans* fatty acids (TFA), mainly due to the biohydrogenation of dietary PUFA in the rumen (Bessa, Alves, & Santos-Silva, 2015). Concentrate-based rations in which cereals (corn and barley) are replaced with DCP may alter ruminal biohydrogenation possibly due to a reduced dietary starch content (Santos-Silva et al., 2016) or a high concentration of phenolic compounds in DCP (Lanza et al., 2015). This alteration in ruminal biohydrogenation might explain the effect of dietary DCP in improving the fatty acid profile of ovine meat and milk by increasing the concentration of *t*-11 18:1 (vaccenic acid), *c*-9, *t*-11

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<https://doi.org/10.1016/j.meatsci.2020.108181>

Received 3 January 2020; Received in revised form 2 May 2020; Accepted 6 May 2020

Available online 08 May 2020

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conjugated linoleic acid (CLA) and PUFA while decreasing *trans* 18:1 isomers and the *n*-6:*n*-3 PUFA ratio (Lanza et al., 2015; Oliveira, Alves, Santos-Silva, & Bessa, 2017; Santos-Silva et al., 2016).

Lipid oxidation is the primary factor responsible for discolouration and rancid off-flavour development during the shelf-life of meat, resulting in quality deterioration and reduced acceptability (Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998). Increased PUFA content potentially increases the susceptibility of meat to lipid oxidation and dietary supplementation with antioxidant compounds enhance the oxidative stability of meat (Bekhit, Hopkins, Fahri, & Ponnampalam, 2013). Citrus fruits and their by-products are known to contain a substantial amount of phenolic compounds, predominantly flavonoids (such as naringin, hesperidin, quercetin, rutin and luteolin), that exhibit potent antioxidant activities (Balasundram, Sundram, & Samman, 2006; Benavente-García, Castillo, Marin, Ortuño, & Del Río, 1997). Previous studies have shown that feeding DCP as a source of dietary phenolic antioxidants in a concentrate-based ration improved the oxidative stability of aerobically-stored lamb meat (Gravador et al., 2014; Insera et al., 2014) and beef (Tayengwa et al., 2020). Moreover, dietary supplementation of the major citrus flavonoid compounds (naringin and hesperidin) reduced lipid oxidation in aerobically-stored broiler meat (Goliomytis et al., 2015) and lamb meat (Simitzis et al., 2019).

To our knowledge, the effect of dietary DCP on beef quality traits has not been reported in a grass silage feeding system. Thus, it was hypothesised in this study that replacing cereals with bioactive-rich DCP in a concentrate supplement offered to grass silage-fed cattle may improve beef quality by increasing the proportion of beneficial unsaturated fatty acids without negatively influencing the oxidative stability and acceptability of beef. The objective of this study was to examine the chemical composition, fatty acid profile, oxidative stability, texture characteristics and consumer acceptability of beef from steers offered grass silage and a concentrate supplement in which barley was substituted with increasing levels of DCP.

2. Materials and methods

2.1. Animals, diets and experimental design

Animal experimental procedures were approved by the Teagasc animal ethics committee and conducted under license from the Irish Government Department of Health and Children. The animals were managed by trained personnel according to the European Union legislation for the protection of animals used for scientific purposes (2010/63/EU Directive). Thirty-six weaned, spring-born Charolais- ($n = 21$) and Limousin-sired ($n = 15$) suckler bulls were purchased directly from suckler farms at ~7 months of age and assembled at Teagasc Animal & Grassland Research and Innovation Centre, Grange, Ireland. Following assembly, the bulls were castrated and offered grass silage *ad libitum* plus 2 kg of a barley-based concentrate and 60 g of a mineral-vitamin supplement per head daily for a 187-d back-grounding period. All animals had *ad libitum* access to clean water.

Steers were subsequently blocked by breed and live weight (427.2 ± 37.2 kg) and, from within each block, randomly assigned to one of three concentrate rations ($n = 12$ steers/treatment) offered separately as a supplement to *ad libitum* grass (*Lolium perenne*) silage. Concentrate rations contained either 0% DCP (control, barley-based ration), or 40% DCP or 80% DCP as a replacement for the barley (*as-fed* basis). Table 1 indicates the ingredient and chemical composition of the experimental concentrate rations. Representative samples of the concentrate rations were obtained twice weekly and stored at -20 °C prior to chemical analysis. The steers were housed in a slatted-floor building in groups of five or six animals per pen with a Calan gate feeding system (American Calan Inc., Northwood, NH, USA) allowing individual feed intake of steers to be recorded. During the feeding trial, one steer from the 40% DCP treatment experienced constraint with the individual feeding system, resulting in measurements recorded for 11 steers in

Table 1

Ingredient and chemical composition of experimental concentrate diets containing increasing levels of dried citrus pulp (DCP).

Ingredient (<i>as-fed</i> basis, g/kg)	0% DCP	40% DCP	80% DCP
Rolled barley	862	467	58
Soybean meal	60	70	80
Dried citrus pulp (DCP)	–	400	800
Cane molasses	50	50	50
Minerals and vitamins	28	13	12
Chemical composition			
Dry matter (DM, g/kg)	801	828	854
Crude protein ^a	131	130	113
Ash ^a	58	67	87
Total fat ^a	28	28	21
Neutral detergent fibre ^a	201	200	234
Acid detergent fibre ^a	62	132	237
Starch ^a	502	315	63
Total phenol content ^b	6.55	12.41	19.04
Fatty acid (g/kg dry matter (DM))			
C12:0	0.10	0.30	0.20
C14:0	0.10	0.10	0.10
C16:0	3.30	4.30	3.60
C18:0	0.20	0.50	0.60
c-9 C18:1	1.90	3.00	2.70
c-9,12 C18:2	6.50	7.60	4.50
c-9,12,15 C18:3	0.60	0.80	0.70

^a Expressed as g/kg DM.

^b Expressed as g gallic acid equivalents/kg DM.

40% DCP group while measurements were recorded for the 12 steers in the 0% DCP and 80% DCP groups. Steers were individually offered 4.0 kg DM daily (2 kg in the morning and afternoon feeding sessions) of their respective supplementary concentrates for 124 days pre-slaughter.

Animals were slaughtered in a commercial abattoir on two consecutive weeks (balanced for treatment) to facilitate sample collection and measurements. Samples of *longissimus thoracis* muscle (LT) were removed from the left side of the carcass at 48 h post-mortem, vacuum-packed and aged for 14 days at 4 °C, and subsequently stored at -20 °C prior to further analysis. Information relating to production performance and carcass characteristics is presented in Kelly, Moloney, Kelly, and McGee (2017).

2.2. Feed analysis

Concentrate rations were analysed for dry matter, crude protein, ash, neutral detergent fibre, acid detergent fibre and starch concentrations as described by O'Kiely (2011). Total fat concentration or Oil-B (acid hydrolysis/ether extract) was measured using a Soxtec instrument (Tecator, Höganäs, Sweden).

Phenolic compounds were extracted from samples of concentrate rations using aqueous methanol (50:50, v/v) and acetone (70:30, v/v) solvents (Jiménez-Escrig, Jiménez-Jiménez, Pulido, & Saura-Calixto, 2001). Polyphenol-rich extracts were analysed for total phenol content (TPC) using the Folin-Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventós, 1999) and absorbance measurements were recorded at 750 nm using a UV-vis spectrophotometer (Cary 300 Bio, UV-vis spectrophotometer, Varian Instruments, Palo Alto, CA, USA). Results were expressed as g of gallic acid equivalents (GAE)/kg of DM feed.

The fatty acid composition was determined in freeze-dried samples of concentrate rations by a one-step extraction–transesterification procedure using chloroform (Sukhija & Palmquist, 1988) and 2% (v/v) sulfuric acid in methanol (Shingfield et al., 2003), with 19:0 nonadecanoate (Larodan, Solna, Sweden) added as an internal standard. Gas chromatographic analysis of fatty acid methyl esters (FAME) was performed as described by Cherif et al. (2018). Individual fatty acid was expressed as g/kg of DM feed.

2.3. Determination of muscle pH and proximate composition

The LT muscle was thawed and trimmed of visible fat and connective tissue and minced twice through a plate with 4 mm holes (Model P114L, Talsa, Valencia, Spain). Minced LT samples (5 g) were homogenised for 3 min at 24,000 rpm in 45 ml distilled water using an Ultra Turrax T25 homogeniser (Janke and Kunkel, IKA-Labortechnik, GmbH and Co., Staufen, Germany). The pH of the beef homogenates was measured at 20 °C using a pH meter (Seven Easy portable, Mettler-Toledo GmbH, Schweizbach, Switzerland). Raw minced LT samples were analysed for moisture and fat contents using a SMART Trac rapid moisture/fat analyser (CEM Corporation, Matthews, NC, USA). The ash content was determined using a muffle furnace (550 °C for 3 h) and protein content was determined by the Kjeldahl method.

2.4. Determination of vitamin E in LT muscle

The α -tocopherol (vitamin E) content in raw minced LT samples was determined by high-performance liquid chromatography (HPLC) following the extraction procedure described by [Buttriss and Diplock \(1984\)](#). HPLC analysis was carried out on a ProStar liquid chromatograph (Varian Analytical Instruments, Palo Alto, CA, USA) equipped with a ProStar autosampler (Model 410, Varian Instruments). Sample injection volume (partial loop fill) was 20 μ l. The α -tocopherol was separated on a 250 \times 4.6 mm Polaris C18-A 5 μ column (Metachem, Ansys[®] Technologies, CA, USA) and detected using a ProStar UV/Vis detector (Varian Instruments) at 292 nm. The mobile phase was methanol/water (97:3) and isocratic elution took place at 2 ml/min for a total run time of 10 min. A personal computer and Star LC workstation software (version 6.20, Varian Inc.) was used for calculation of peak areas. A standard curve was generated using a range of α -tocopherol concentrations (7, 14, 21 μ g/ml) and the concentration of α -tocopherol in beef was expressed in μ g/g of beef muscle. The percentage recovery of vitamin E from beef samples, through the extraction procedure, was determined by including vitamin E (0.2 ml of 22.8 μ g/ml) as an internal standard. The percentage recovery was calculated by comparison of peak areas of a standard amount of vitamin E recovered through the extraction procedure with those obtained by direct injection of the vitamin E standard (22.8 μ g/ml) onto the column. The average percentage recovery was 92.8% and values of α -tocopherol in muscle samples were adjusted to account for percentage recovery.

2.5. Determination of fatty acids in LT muscle

Lipid fraction in raw minced LT samples was extracted following the method described by [Bligh and Dyer \(1959\)](#). The lipid fraction was transesterified to fatty acid methyl esters (FAME) using BF₃ in methanol as a catalyst following the method described by [Park and Goins \(1994\)](#). FAMES were dissolved with isoctane, dried over anhydrous sodium sulphate (0.3 g) for 15 min and stored at -20 °C prior to gas chromatography analysis. The separation of FAME was carried out using a Varian 3800 gas chromatograph (Varian, Walnut Creek, CA, USA) using a WCOT fused silica capillary column (Varian CP-SIL 88 Tailor-Made FAME, 60 m \times 0.25 mm i.d. \times 0.20 μ m film thickness) and a flame ionisation detector. The column oven temperature was held at 150 °C for 25 min and programmed to increase from 150 °C to 240 °C at 4 °C/min and held for 2 min. The injector and detector temperatures were 270 °C and 260 °C respectively. Helium was used as the carrier gas at a pressure of 30 psi. The injection was carried out using a Combi PAL (CTC Analytics AG, Zwingen, Switzerland) auto-injector. The injection volumes and split ratios for FAMES were 1 μ l and 1:2 split, respectively. Individual fatty acids were identified by comparing relative retention times with pure FAME standards (a mixture of Supleco 37 component FAME mix, *trans*-11 vaccenic acid methyl ester and conjugated linoleic acid methyl ester; Sigma-Aldrich Ireland Ltd., Vale Road, Arklow, Wicklow, Ireland). Results were reported as individual fatty acids

expressed as a percentage of the total fatty acids ((peak area of individual FAME/total peak area of FAME examined) \times 100). Considering the complexity of the fatty acid profile in ruminant tissues, it is noted that only the major fatty acids and some minor isomers (representing over 90% of the total fatty acids present) relevant to this study are identified and reported to evaluate the effect of dietary treatment on the main changes in muscle fatty acid composition. In particular, isomers of 18:1 and 18:2 are incompletely resolved by the chromatography procedure, therefore some of the isomers listed herein may contain co-elution with other isomers. The atherogenic index (AI) and thrombogenic index (TI) were calculated according to [Ulbricht and Southgate \(1991\)](#).

2.6. Determination of total phenol content and in vitro antioxidant activity

2.6.1. Preparation of muscle homogenates

Beef homogenates (10% w/v) were prepared as described by [Qwele et al. \(2013\)](#) for the determination of *in vitro* antioxidant activities. In brief, raw minced LT (5 g) was homogenised in 0.05 M phosphate buffer (45 ml) using an Ultra-turrax T25 homogeniser at 24,000 rpm for 3 min. Muscle homogenates were centrifuged at 7800 g for 10 min at 4 °C using an Avanti[®] J-E Centrifuge (Beckman Coulter Inc., Palo Alto, CA, USA). The supernatant was filtered through Whatman No. 1 paper and the filtrate was analysed for ferric reducing antioxidant power (FRAP) and ferric ion chelating activity (FICA). For the determination of TPC and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assays, 10% trichloroacetic acid (5 ml) was added to muscle homogenates and the mixture was centrifuged at 7800 g for 10 min at 4 °C. The supernatant was filtered through Whatman No. 1 paper and analysed for TPC and DPPH assays.

2.6.2. Measurement of the total phenol content

Muscle extracts were analysed for TPC using the Folin-Ciocalteu method ([Singleton et al., 1999](#)) with minor modifications. Briefly, extracts (0.5 ml) were mixed with Folin-Ciocalteu reagent (2.5 ml, 20% in distilled water) and sodium carbonate (2 ml, 7.5% in distilled water) was added after 5 min. The mixture was stored in the dark for 2 h at room temperature and absorbance measurements were recorded at 750 nm on a UV-vis spectrophotometer (Cary 300 Bio, UV-vis spectrophotometer, Varian Instruments, CA, USA) against a blank containing all reagents and distilled water. A calibration curve using standard solutions of aqueous gallic acid (20–100 μ g/ml) was constructed and results were expressed as mg of gallic acid equivalents (GAE)/g of muscle.

2.6.3. Measurement of in vitro antioxidant activities

Radical scavenging activity in muscle was measured using the DPPH assay following a minor modification of the method described by [Yen and Wu \(1999\)](#). Muscle extract (0.6 ml) and distilled water (2.4 ml) were mixed with 0.2 mM DPPH in methanol (3 ml) and incubated in the dark for 1 h at room temperature. Absorbance measurements were recorded at 517 nm on a UV-vis spectrophotometer (Cary 300 Bio) against a methanol blank. An assay blank containing distilled water (3 ml) and 0.2 mM DPPH in methanol (3 ml) was used for calculation purposes. A calibration curve using standard solutions of methanolic Trolox (10–50 μ g/ml) was constructed and results were expressed as mg of Trolox equivalents (TE)/g of muscle.

Total antioxidant activity in muscle was determined using FRAP assay following a minor modification of the method described by [Benzie and Strain \(1999\)](#). Briefly, muscle extract (0.45 ml) was mixed with 8.55 ml FRAP reagent (a mixture of 30 mM acetate buffer (pH 3.6), 10 mM 2,4,6-Tris (2-pyridyl)-s-triazine in 40 mM HCl and 20 mM FeCl₃·6H₂O in distilled water in the ratio 10:1:1, respectively) incubated at 37 °C for 10 min prior to use). The mixture was incubated for 30 min in the dark and absorbance was recorded at 593 nm on a UV-vis spectrophotometer (Cary 300 Bio) against a blank containing all

reagents. A calibration curve using solutions of methanolic Trolox (0.033–0.1 mg/ml) was constructed and results were expressed as mg TE/g of muscle.

The iron-chelating activity of muscle was measured using a minor modification of FICA assay described by Yen and Wu (1999). Muscle extract (0.5 ml) was mixed with FeCl₂ (2 mM in distilled water, 0.1 ml), ferrozine solution (5 mM in distilled water, 0.2 ml) and distilled water (4.2 ml). The assay control contained FeCl₂ (0.1 mM), ferrozine solution (0.2 ml) and distilled water (4.7 ml). The mixture was incubated for 1 h in the dark at room temperature and absorbance measurements were recorded at 562 nm against a water blank on a UV-vis spectrophotometer (Cary 300 Bio). The chelating activity was calculated as follows:

Chelating activity (%) = $[1 - (\text{absorbance of sample})/(\text{absorbance of control})] \times 100$.

2.7. Determination of the oxidative stability and sensory properties of beef

2.7.1. Measurement of lipid oxidation and oxymyoglobin in muscle homogenates

Muscle homogenates (25%) were prepared by homogenising 15 g of LT in a buffer (0.12 M KCl, 5 mM histidine, pH 5.5) surrounded by crushed ice using an Ultra Turrax T25 homogeniser. Lipid oxidation in muscle homogenates (39.2 g) was initiated by the addition of pro-oxidants (45 µM FeCl₃/sodium ascorbate, 1:1) (O'Grady, Monahan, & Brunton, 2001). Lipid oxidation (2-thiobarbituric acid reactive substances, TBARS) and oxymyoglobin (OxyMb) content in muscle homogenate were measured at 1 and 4 h of storage at 4 °C as described by Hayes et al. (2009).

2.7.2. Meat processing and packaging

The LT muscles were thawed overnight at 4 °C, trimmed of visible fat and connective tissue, and minced twice through a plate with 4 mm holes (Model P114L, Talsa, Valencia, Spain). The minced muscle was formed into beef patties (100 g portions) using a meat former (Ministek burger maker, O.L Smith Co. Ltd., Italy). For the fresh beef study, patties were individually placed in a low oxygen permeable (<1 cm³/m²/24 h at STP) polystyrene/ethyl vinyl alcohol/polyethylene (PE) trays and flushed with 80% O₂:20% CO₂ (modified atmosphere packs, MAP) using a vacuum-sealing unit (VS 100, Gustav Müller & Co. KG, Homburg, Germany) equipped with a gas mixer (Witt-Gasetechnik GmbH & Co. KG, Witten, Germany). Trays were covered and heat-sealed using a low oxygen permeable (3 cm³/m²/24 h at STP) laminated barrier film with a polyolefin heat-sealable layer. Fresh beef patties in MAP were stored for up to 14 days under fluorescent lighting (660 lx) at 4 °C. The gas atmosphere (% O₂ and % CO₂) in MAP was measured using a CheckMate 9900 (PBI-DanSensor, Denmark). The average gas composition in MAP was 79.87 ± 0.98% O₂ and 20.71 ± 0.16% CO₂ on day 1 of storage and contained 74.22 ± 0.88% O₂ and 25.63 ± 0.71% CO₂ on day 14 of storage.

For the cooked beef study, minced patties were individually placed on an aluminium foil-lined trays and cooked at 180 °C for 20 min in a fan-assisted convection oven (Zanussi Professional, Model 10 GN1/1, Conegliano, Italy) until an internal temperature of 72 °C was reached. Cooked beef patties were placed in PE trays over-wrapped with oxygen-permeable film and stored for up to 6 days at 4 °C.

2.7.3. Measurement of lipid oxidation and surface colour

Lipid oxidation was measured in fresh beef patties on days 1, 4, 7, 11, and 14 of storage and on days 1, 3, and 6 in cooked beef patties (one beef patty/muscle/day). Lipid oxidation measurements were carried out following the method described by Siu and Draper (1978). Results were expressed as TBARS in mg malondialdehyde (MDA)/kg meat.

The surface colour of fresh beef patties was measured on days 1, 4, 7, 11, and 14 of storage (one beef patty/muscle/day) using a Konica Minolta CR-400 Chroma-Meter (Minolta Camera Co., Osaka, Japan).

The Chroma-Meter consisted of a measuring head (CR-400), with an 8 mm diameter measuring area, illuminant D65, a 2° standard observer, and a data processor (DP-400). The Chrom-Meter was calibrated on the CIE LAB colour space system using a white tile (D_c: L = 97.79, a = -0.11, b = 2.69). The 'L*', 'a*' and 'b*' value represents lightness, redness and yellowness, respectively. Colour measurements were averaged for readings taken from four different locations on the surface of beef patties. Chroma (C*) and hue angle (H*) were calculated as $[(a^{*2} + b^{*2})^{1/2}]$ and $[\tan^{-1}b^*/a^*]$, respectively.

2.7.4. Measurement of textural properties of beef patties

The texture profile analysis (TPA) of fresh beef patties stored in MAP was measured on days 2 and 7 of storage. The TPA parameters (hardness (N), springiness (mm), cohesiveness (dimensionless), gumminess (N), chewiness (N × mm), adhesiveness (N)) were measured using a 30 kg load cell texture analyser (TA.XT2i Texture Analyser, Stable Micro Systems, UK) as described by Moroney, O'Grady, O'Doherty, and Kerry (2013).

2.7.5. Sensory acceptance testing of beef patties

Sensory acceptance of fresh beef patties (n = 8/treatment) stored in MAP was carried out by 40 untrained panellists in 2 sessions (20 panellists/session) on days 2 and 7 of storage as described by O'Sullivan, Byrne, and Martens (2003). Beef patties were cooked for sensory analysis in a Zanussi oven at 180 °C for 20 min until an internal temperature of 72 °C was reached. Following cooking, patties were cooled to room temperature and cut into 2 cm × 2 cm cubes, identified with random three-digit codes. On each day of sensory analysis, beef samples were served to panellists in two separate sessions (morning and afternoon sessions). Prior to serving to panellists, beef samples were re-heated in a microwave for 10 s to release the meat odour and flavour. Sensory evaluation was performed in the panel booths of the University's sensory laboratory according to international standard regulations (ISO, 2007). Sample presentation was randomised to prevent any flavour carryover effects (MacFie, Bratchell, Greenhoff, & Vallis, 1989). Panellists were asked to indicate their degree of liking for appearance, odour, texture, juiciness, flavour and overall acceptability on a 10 cm line scale ranging from 0 (extremely dislike) to 10 (extremely like). Panellists were provided with water to cleanse their palates between samples.

2.8. Statistical analysis

All analyses were performed in duplicate. Data on proximate composition, antioxidant capacity and fatty acid profiles analyses were analysed using a general linear model including dietary treatment as a fixed factor and block as a random factor. The orthogonal polynomial contrast was used to determine the linear and quadratic responses of dietary inclusion levels of DCP. Data on oxidative stability and sensory attributes were analysed using a mixed model to test the effect of dietary treatment and storage/incubation time as fixed factors and the interaction between dietary treatment and storage/incubation time. The effects of panellist and session were included as random terms in the model used for the analysis of sensory eating attributes. Tukey's HSD test was used for multiple comparisons of treatment means when significance was detected at $P < .05$ and a tendency for treatment effect was observed when $0.05 < P \leq .10$. All data analysis was performed using SPSS statistical software (IBM Statistics version 22).

3. Results and discussion

Dietary strategies provide feasible opportunities to enhance the nutritional quality of beef by increasing the PUFA and CLA content, linked to positive health effects such as decreased incidence of cardiovascular diseases (CVD) and cancer (Vahmani et al., 2015). Feeding DCP, compared to cereals, may improve the fatty acid profiles of

ruminant meat or milk by manipulating ruminal biohydrogenation due to its high concentration of phenolic compounds and lower starch content (Lanza et al., 2015; Santos et al., 2014). In an extensive review of literature, Vasta et al. (2019) highlighted that dietary polyphenols exhibit inhibitory or modulatory effects on ruminal biohydrogenation of unsaturated fatty acids through perturbation of the rumen microbiome involving the main bacterial species linked to the biohydrogenation process. Moreover, higher dietary starch content and decreased fibre content may alter the rate of ruminal lipolysis and biohydrogenation through changes in microbial ecology caused by the induction of low rumen pH or by the provision of higher starch availability for ruminal fermentation (Gerson, John, & King, 1985; Jenkins, Wallace, Moate, & Mosley, 2008). Moreover, high-starch concentrate diets may cause a shift towards the formation of *t*-10 18:1 at the expense of *t*-11 18:1 as the major biohydrogenation intermediate (Bessa et al., 2015).

The phenolic content in the DCP fed in the present study is approximately 2-fold (18.34 vs. 10.87 g gallic acid equivalent/kg DM) greater than reported in a study by Luciano et al. (2017). This highlights the considerable variation in the chemical composition of citrus pulp, which can be influenced by factors such as the fruit source and type of processing (Bampidis & Robinson, 2006). As expected, the substitution of barley with increasing levels (40% and 80%) of DCP substantially decreased the dietary starch content and increased the concentration of phenolic compounds in concentrate supplements (Table 1). The inclusion of 40% and 80% DCP in concentrate supplement represents 17.7% and 35.3% of the total dietary intake (grass silage + concentrate), respectively. Replacing barley with DCP in a concentrate supplement offered to grass silage-fed steers may increase the proportion of beneficial unsaturated fatty acids in beef through the inhibition of ruminal biohydrogenation because of the DCP phenolics and/or lower starch content in the DCP diets. In addition, DCP may increase the supply of dietary phenolic antioxidants which could enhance the antioxidant capacity and oxidative stability of beef without negatively influencing consumer acceptability.

3.1. Proximate composition of muscle

The proximate composition of LT beef is presented in Table 2. There was a quadratic response ($P = .015$) in muscle pH due to DCP inclusion levels. However, muscle pH measured in all dietary treatments was within the normal pH range (5.4–5.8) associated with *post-mortem*

muscle (Faustman & Cassens, 1990). Intramuscular fat (IMF) is an important meat quality trait considering its influence on the sensory characteristics and fatty acid composition of meat (Wood et al., 2008). It is known that high-starch diets promote the deposition of IMF because of increased availability of glucose used for lipogenesis in intramuscular adipocytes (Pethick, Harper, & Oddy, 2004). However, the present study demonstrated that the IMF was unaffected ($P > .05$) by the reduction in dietary starch content when barley was replaced with DCP in concentrate supplements (Table 1). Previous studies have also shown that replacing barley with DCP in concentrate diets did not affect the IMF content of lamb meat (Costa et al., 2017; Lanza et al., 2015). This is possibly due to the lack of a detrimental impact of dietary DCP on the production of ruminal volatile fatty acids used as the main energy sources in the tissues of ruminant animals (Bampidis & Robinson, 2006). Beef produced from the present study can be considered as lean meat according to the labelling criteria (<5% fat) stipulated by the Food Advisory Committee (1990). Furthermore, moisture contents were unaffected ($P > .05$) by dietary treatment. However, protein and ash contents responded quadratically ($P < .01$) to increasing levels of DCP, with 80% DCP displaying the highest values for both parameters (Table 2). Although the amino acid profile of the diets was not measured in the current study, citrus residues can contain desirable essential amino acid composition that may vary in proportion depending on the juice processing methods (Coleman & Shaw, 1977; Fernández-López et al., 2004). Postprandial, dietary-derived essential amino acids can act as signalling molecules for inducing muscle protein synthesis (van Vliet, Burd, & van Loon, 2015), which suggest that possibly higher essential amino acid contents in 80% DCP might have played a role in promoting greater muscle protein content. Furthermore, the higher ash content present in the 80% DCP diet (Table 1) is reflective of the presence of many vitamins (mainly carotenoids, vitamin B complex, vitamin C) and minerals (potassium and calcium) present in DCP (Bampidis & Robinson, 2006). Presumably, DCP vitamins and minerals were subsequently deposited in LT muscle, which could explain the higher ash contents found in the muscle of steers fed 80% DCP.

3.2. Antioxidant capacity of muscle

The biological antioxidant system comprises of enzymatic (such as glutathione peroxidase, superoxide dismutase, catalase) and non-enzymatic (such as vitamins A, E and C, and phenolic compounds) factors that are important to delay the onset of lipid peroxidation in *post-*

Table 2

Effect of increasing levels of dried citrus pulp (DCP) in supplementary concentrates on the pH, proximate composition, antioxidant status and antioxidant activity of *longissimus thoracis* muscle of steers.

Item	Dietary treatment (% DCP)			SEM	P-value	Contrast effect	
	0	40	80			Linear	Quadratic
Muscle pH	5.47 ^a	5.52 ^b	5.49 ^a	0.009	0.019	0.133	0.015
<i>Proximate composition (g/100 g wet weight)</i>							
Protein	23.67 ^a	22.91 ^b	23.79 ^a	0.118	0.003	0.612	0.001
Intramuscular fat	2.57	2.54	2.31	0.098	0.511	0.287	0.660
Moisture	73.12	73.16	73.21	0.090	0.914	0.674	0.987
Ash	1.11 ^a	0.94 ^b	1.37 ^c	0.031	<0.001	<0.001	<0.001
<i>Antioxidant status</i>							
α-tocopherol (μg/g muscle)	2.38	2.51	2.69	0.107	0.510	0.251	0.914
TPC (mg GAE/g muscle)	0.94	0.90	0.92	0.020	0.767	0.729	0.525
<i>Antioxidant activity</i>							
DPPH (mg TE/g muscle)	0.25	0.25	0.24	0.004	0.367	0.162	0.898
FRAP (mg TE/g muscle)	0.38	0.39	0.38	0.004	0.355	0.416	0.236
FICA (%)	55.22	56.29	53.81	1.294	0.749	0.662	0.537

a,bMeans within the same row bearing different superscripts are significantly different ($P < .05$).

SEM: Standard error of mean.

TPC: Total phenol content DPPH: 1,1-diphenyl-2-picrylhydrazyl; FRAP: Ferric reducing antioxidant power; FICA: Ferric ion chelating activity. GAE: gallic acid equivalent; TE: trolox equivalent.

mortem muscle (Salami et al., 2016). The concentration of α -tocopherol (vitamin E) and phenolic compounds in meat primarily depend on their dietary intakes (Castillo, Pereira, Abuelo, & Hernández, 2013). In the present study, the α -tocopherol concentration and TPC in LT were unaffected ($P > .05$) by the inclusion of DCP in concentrate supplements (Table 2). This was consistent with the lack of differences ($P > .05$) in muscle antioxidant activities (DPPH-radical scavenging, FRAP and FICA) between treatments (Table 2).

Feeding DCP as a source of dietary phenolic compounds in a concentrate-based ration has been shown to enhance the antioxidant activity, measured as FRAP, of beef muscle tissues from steers (Tayengwa et al., 2020). The current result is contrary to the expectation that a high concentration of phenolic compounds in dietary DCP would enhance the antioxidant capacity of beef. It is noteworthy that the inclusion of 40% and 80% DCP in concentrate supplements increased dietary TPC by 2-fold and 3-fold, respectively. Despite the high phenolic content in DCP diets, the lack of differences in muscle TPC suggests that absorption of citrus polyphenols into the muscle was restricted which could explain the lack of antioxidant effect in the muscle. In agreement with this theory, Bodas et al. (2012) showed that dietary supplementation of a citrus flavonoid, naringin, resulted in the accumulation of naringenin (aglycone fraction of naringin) and subsequent antioxidant activity in the liver but not in the muscle of lambs. The liver is regarded as the main organ involved in the metabolism of dietary polyphenols, resulting in the formation of several metabolites before being absorbed for excretion (Bravo, 1998; Spencer, El Mohsen, Minihane, & Mathers, 2008). Thus, the role of the liver in the metabolism of polyphenols might have promoted a greater concentration of phenolic metabolites in this organ whereas no or low concentration of the phenolic compounds are absorbed into the muscles.

Furthermore, the inclusion of 35% DCP in a concentrate diet of lambs resulted in a 2-fold increase in dietary TPC without affecting the TPC or antioxidant activities of plasma, liver and muscle, measured by radical scavenging and reducing power assays (Luciano et al., 2017). Similarly, supplementation of PUFA-rich concentrate diets with 0.5 or 5 g/kg of citrus extract did not affect the radical scavenging activity in plasma, muscle and liver of rats (Gladine et al., 2007; Gladine, Morand, Rock, Bauchart, & Durand, 2007). However, other studies have shown that feeding 18% pelleted CP increased the TPC and reducing power in milk (Santos et al., 2014) while the inclusion of up to 21% DCP in the concentrate diet of rabbits increased the total antioxidant capacity of the liver (Lu et al., 2018). Thus, it is possible that differences in the absorption and *in vivo* antioxidant effect of citrus polyphenols is influenced by factors such as animal species, interaction with other dietary ingredients and the tissue of measurement.

Luciano et al. (2017) and Tayengwa et al. (2020) indicated that dietary DCP increased the concentration of α -tocopherol in lamb and beef muscle tissues, respectively. This observation contradicts results presented in the current study, where no effect of dietary DCP on α -tocopherol concentration was observed. It should be emphasised that the TPC, DPPH, FRAP and FICA assays used in the present study are not specific for assessing the antioxidant activity related to only polyphenols but rather provide different information about the mechanism of total antioxidant activity in a particular substance or biological tissue (Niki, 2010). Thus, the combination of these assays is robust to detect any direct or indirect changes in the antioxidant capacity of muscle that may arise from the intake of dietary citrus polyphenols or other bioactive molecules and their *in vivo* metabolites. In this regard, the current results suggest that inclusion of DCP in concentrate supplements fed with grass silage did not enhance the antioxidant capacity of beef. Further studies are required to investigate other factors (including the metabolism of DCP phenolics, and the sparing effect of DCP phenolics on other antioxidants such as α -tocopherol) that might play a role in the *in vivo* antioxidant efficacy of DCP including potential effects on antioxidant enzyme activities.

3.3. Fatty acid profiles of muscle

The fatty acid composition of ruminant meat, including beef, is influenced by the fatty acid composition of the feed, metabolism (lipolysis and subsequent biohydrogenation) of dietary lipids in the rumen and endogenous fatty acid metabolism and synthesis in the muscle (Bessa et al., 2015). The replacement of barley with DCP in a concentrate supplement did not result in substantial differences in feed fatty acid composition (Table 1). Total SFA was similar ($P > .05$) between dietary treatments and this was consistent with the lack of dietary differences ($P > .05$) in C14:0 and the two most abundant individual SFA (C16:0 and C18:0) found in beef (Table 3). Costa et al. (2017) also reported that substituting barley with 42% DCP in concentrate diet did not affect the concentration of these SFA in the *longissimus* muscle of lambs and this effect was consistent with the lack of changes in the concentration of these fatty acids in the rumen of lambs (Lanza et al., 2015).

The percentages of total MUFA and TFA were unaffected ($P > .05$) by dietary treatment. However, dietary DCP levels linearly decreased the percentage of *c*-10 17:1 ($P < .01$). Dietary treatment tended ($P = .065$) to increase the percentage of the predominant MUFA, *c*-9 C18:1 (oleic acid), with a quadratic response as the dietary level of DCP increased. A similar tendency for a higher percentage of oleic acid in lamb meat was associated with the lower starch content in concentrate diets in which cereals (barley, corn and wheat) were replaced with a combination of DCP, hominy feed and palm kernel meal (Oliveira et al., 2017).

Furthermore, the diet tended ($P = .096$) to influence the proportion of *t*-11 18:1, with a linear increase as the dietary level of DCP increased. The presence of *t*-11 18:1 in food products potentially exhibits health-promoting properties in humans coupled with its role as the primary precursor for the *de novo* synthesis of bioactive *c*-9, *t*-11 CLA (Bessa et al., 2015; Vahmani et al., 2015). The presence of *t*-11 18:1 in ruminant meat or milk is associated with its synthesis from dietary linoleic and linolenic acid during ruminal biohydrogenation before being absorbed into the muscle or mammary tissues (Bessa et al., 2015). In our agreement with our observation, previous studies have also demonstrated that feeding concentrate diets containing DCP increased *t*-11 18:1 concentration in lamb meat (Costa et al., 2017) and ewe milk (Santos-Silva et al., 2016). However, Lanza et al. (2015) demonstrated that substituting barley with up to 35% DCP in concentrate diet of lambs increased the percentage of *t*-11 18:1 in the plasma but no difference was observed in the rumen and muscle tissue. Variation in the accumulation of *t*-11 18:1 in meat or milk from ruminants fed reduced dietary starch may be attributed to the formation of a competing intermediate (*t*-10 18:1) at the expense of *t*-11 18:1 in the ruminal biohydrogenation pathway (Costa et al., 2017). Further studies would be useful to investigate strategies that could maximize the effect of DCP on the synthesis of *t*-11 18:1 during ruminal biohydrogenation.

An increase in the proportion of PUFA, particularly *n*-3 PUFA and *c*-9,*t*-11 CLA, in beef fat would improve the nutritional quality of beef and contribute to a healthy human diet considering the physiological effects of these fatty acids in preventing or reducing the risk of human diseases such as CVD, cancer and obesity (Bessa et al., 2015; Vahmani et al., 2015). The inclusion of dietary DCP linearly increased ($P < .01$) the proportion of total PUFA driven by a linear increase in *c*-9,12 C18:2 (linoleic acid), CLA (*c*-9,*t*-11 C18:2 + *t*-9,*c*-11 C18:2) and C22:2 *n*-6, and a quadratic increase in C20:5 *n*-3 (Table 3). Feeding 40% and 80% DCP increased ($P < .01$) the percentage of CLA compared to 0% DCP. Quantitative analyses have shown that *c*-9,*t*-11 C18:2 is the major CLA isomer (60–85% of the total CLA) in beef fat (Fritsche et al., 2000), indicating that *c*-9,*t*-11 C18:2 is the predominant fatty acid in the co-eluted CLA peak identified in this study. In agreement with this observation, a similar increase in *c*-9,*t*-11 CLA has been reported in lamb meat (Lanza et al., 2015) and ewe milk (Santos-Silva et al., 2016) when cereals (barley and corn) were replaced with up to 35% DCP in concentrate diets. However, this effect was inconsistent as shown with a

Table 3Effect of increasing levels of dried citrus pulp (DCP) in supplementary concentrates to grass silage-fed steers on the fatty acid composition of *longissimus thoracis* muscle of steers.

Fatty acids (% of total fatty acids)	Dietary treatment (% DCP)			Contrast effect			
	0	40	80	SEM	P-value	Linear	Quadratic
C12:0	0.07	0.08	0.07	0.009	0.753	0.903	0.461
C14:0	2.51	2.75	2.45	0.084	0.314	0.765	0.138
c-9 C14:1	0.44	0.35	0.37	0.041	0.676	0.529	0.538
C15:0	0.39	0.42	0.41	0.019	0.831	0.696	0.644
C16:0	22.99	20.43	21.82	1.610	0.822	0.770	0.583
C16:1	1.85	1.58	1.68	0.135	0.741	0.630	0.549
C17:0	0.86	0.85	0.85	0.024	0.980	0.848	0.957
c-9 C17:1	0.50 ^a	0.27 ^b	0.24 ^b	0.032	<0.0001	<0.0001	0.073
C18:0	13.32	12.22	12.31	0.331	0.325	0.212	0.408
t-9 18:1	2.07	2.42	1.97	0.128	0.350	0.747	0.160
t-11 C18:1	0.95	1.00	1.15	0.041	0.096	0.039	0.529
c-9 C18:1	29.11	34.66	31.55	0.973	0.065	0.281	0.037
t-9,12 C18:2	0.28	0.40	0.40	0.052	0.559	0.349	0.601
c-9,12 C18:2	2.29	2.72	3.18	0.160	0.067	0.021	0.970
C20:0	0.02	0.01	0.10	0.032	0.414	0.291	0.423
c-9,12,15 C18:3	0.35	0.40	0.37	0.013	0.193	0.480	0.095
¹ CLA	0.14 ^a	0.34 ^b	0.41 ^b	0.033	0.001	<0.0001	0.292
C22:0	0.52	0.52	0.47	0.026	0.742	0.504	0.704
C20:4 n-6	1.10	1.19	1.08	0.028	0.116	0.432	0.639
C20:5 n-3	0.18 ^a	0.38 ^b	0.25 ^{ab}	0.031	0.021	0.277	0.010
C22:5 n-3	0.44	0.46	0.47	0.043	0.216	0.771	0.086
Summary							
ΣSFA	41.15	37.63	38.52	1.687	0.688	0.531	0.555
ΣMUFA	36.98	40.69	37.65	0.912	0.221	0.653	0.125
ΣPUFA	4.97 ^a	6.04 ^b	6.52 ^b	0.185	0.002	0.001	0.357
Total trans	3.02	3.42	3.13	0.106	0.309	0.681	0.142
Σn-6 PUFA	3.87 ^a	4.62 ^{ab}	5.02 ^b	0.159	0.020	0.006	0.825
Σn-3 PUFA	0.96 ^a	1.24 ^b	1.09 ^{ab}	0.037	0.004	0.178	0.003
n-6:n-3	4.03	3.73	4.61	0.416	0.220	0.691	0.095
PUFA:SFA	0.12	0.16	0.17	0.011	0.458	0.321	0.443
² Atherogenicity index	0.82	0.70	0.76	0.045	0.562	0.568	0.365
³ Thrombogenicity index	1.78	1.43	1.62	0.080	0.211	0.387	0.124

a,bMeans in the same row bearing different superscripts are significantly different ($P \leq .05$).

SEM: Standard error of mean.

CLA: conjugated linoleic acid; SFA: saturated fatty acids; MUFA: mono-unsaturated fatty acids; PUFA: poly-unsaturated fatty acids.

¹ CLA: c-9 t-11 18:2 + t-9 c-11 18:2.² Atherogenic index: (C12:0 + [4 × C14:0] + C16:0)/(n-3 PUFA + n-6 PUFA + MUFA).³ Thrombogenic index: (C14:0 + C16:0 + C18:0)/([0.5 × MUFA] + [0.5 × n-6 PUFA] + [3 × n-3 PUFA] + [n-3/n-6 PUFA]).

lack of change in the concentration of c-9,t-11 CLA in lamb meat when barley was substituted with 42% DCP in a concentrate diet (Costa et al., 2017).

The availability of c-9,t-11 CLA in ruminant meat and milk is derived from its synthesis during ruminal biohydrogenation and endogenous desaturation of t-11 18:1 via Δ -9-desaturase activity in ruminant tissues (Bessa et al., 2015). However, endogenous synthesis is considered as the predominant source, accounting for up to 95% of c-9,t-11 CLA in ruminant meat and milk (Palmquist, Lock, Shingfield, & Bauman, 2005). In the present study, it is noteworthy that a linear increase in c-9,t-11 CLA is consistent with that of t-11 18:1. In addition to the possible inhibitory effect of DCP on ruminal biohydrogenation, high phenolic content in DCP may enhance endogenous desaturation due to the effect of dietary phenolics, such as tannins, in stimulating the expression of Δ -9-desaturase in the muscle (Vasta et al., 2009).

Feeding 40% DCP treatment increased the percentage of C20:5 n-3 in LT muscle. This is consistent with the increased proportion of long-chain PUFA (C20:5 n-3 and C22:5 n-3) observed in the muscle of lambs fed concentrate diets containing DCP as a substitute for barley (Lanza et al., 2015). Furthermore, the percentage of total n-6 PUFA linearly increased ($P < .05$) as the dietary level of DCP increased, with muscle from steers fed 80% DCP higher in total n-6 PUFA relative to 0% DCP. However, increasing levels of DCP resulted in a quadratic increase ($P < .05$) in total n-3 PUFA, with 40% DCP being higher compared to 0% DCP. Overall, the measurements of nutritional indices (n-6:n-3

PUFA, PUFA:SFA, atherogenicity index and thrombogenicity index) did not differ ($P > .05$) between dietary treatments.

3.4. Oxidative stability of beef

Lipid oxidation is a major cause of quality deterioration and reduced acceptability during the shelf life of meat. This is attributed to rancid flavour development and colour deterioration resulting from the conversion of oxymyoglobin (red) to metmyoglobin (brown) (Morrissey et al., 1998). Animal diets can influence the oxidative resistance of meat by altering the balance between pro-oxidant and antioxidant compounds in muscle especially by increasing the amount of oxidizable PUFA in the phospholipid membrane (Faustman, Sun, Mancini, & Suman, 2010). In the present study, the effect of dietary replacement of barley with DCP on beef oxidation was examined under three different oxidative conditions (MAP storage, iron/ascorbate-induced lipid oxidation and aerobic storage). Lipid oxidation, measured as TBARS, increased ($P < .01$) in fresh beef patties stored in MAP (80% O₂:20% CO₂) over 14 days (Fig. 1a). Despite the higher proportion of PUFA present in the muscle from steers fed DCP diets, dietary treatment did not affect ($P > .05$) the concentration of TBARS in fresh beef patties during the storage duration. As expected, surface discoloration progressively occurred in fresh beef patties stored in MAP over the 14-day storage period (Table 4). This is evident with a decrease ($P < .05$) in redness (a*), yellowness (b*) and chroma (C*, colour vividness) and an

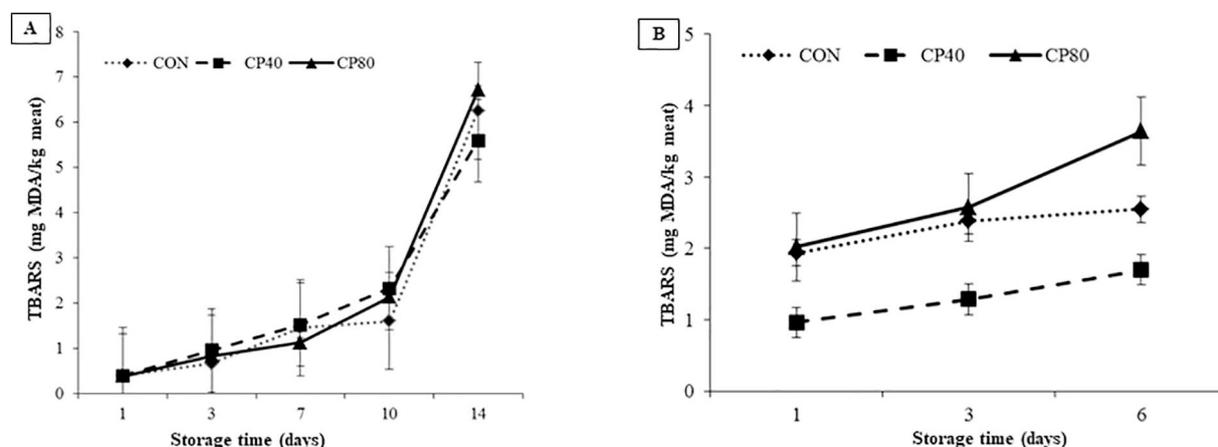


Fig. 1. Effect of increasing levels of dried citrus pulp (DCP) in supplementary concentrates to grass silage-fed steers on the lipid oxidation (thiobarbituric acid reactive substances (TBARS)) of (A) fresh beef patties stored in modified atmosphere packs (80% O₂:20% CO₂) at 4 °C for up to 14 days (B) cooked beef patties stored in aerobic packs at 4 °C for up to 6 days.

increase in hue angle (H^* , the trueness of red). However, the instrumental colour variables (L^* , a^* , b^* , C^* , H^*) were unaffected by dietary treatment and no diet \times storage time interaction was observed over the duration of storage. The values of $a^* = 12$ and $C^* = 16$ are considered as thresholds for visual discolouration that limits consumer acceptability of beef (Van Rooyen, Allen, Crawley, & O'Connor, 2017). The current data indicated that values of a^* and C^* measured up to 10 days were greater than the threshold values, suggesting that visual acceptability of barley- and DCP-fed beef may extend up to 10 days of storage.

There is a close interaction between processes involved in lipid oxidation and myoglobin oxidation due to the ability of free radicals generated in their biochemical reactions to influence these oxidative processes in a reciprocal manner (Faustman et al., 2010). This may partly explain why the lack of dietary effect on lipid oxidation was consistent with that of colour stability when fresh beef patties were stored in MAP. This is further supported by the lack of dietary effect ($P > .05$) on lipid and oxymyoglobin oxidation when muscle homogenates were subjected to a greater oxidative challenge by incubating with iron/ascorbate pro-oxidants (Table 4). To our knowledge, the present study is the first to report the effect of dietary DCP on the oxidative stability of MAP-stored beef. Tayengwa et al. (2020) recently showed that the inclusion of 150 g/kg DM of DCP in a concentrate-based ration of steers increased the antioxidant activity and reduced

lipid oxidation in beef steaks stored in air-permeable packs for up to 9 days. Moreover, previous studies have shown that dietary supplementation of DCP or citrus flavonoids enhanced the oxidative stability of fresh meat from other animal species. The inclusion of up to 35% DCP in concentrate diet reduced lipid and protein oxidation in lamb meat stored aerobically at 4 °C for up to 6 days (Gravador et al., 2014; Inserra et al., 2014). Similarly, dietary supplementation of flavonoid compounds (naringin or hesperidin) reduced lipid oxidation and discolouration in aerobically-stored fresh broiler meat (Goliomytis et al., 2015) and lamb meat (Simitzis et al., 2019). In the present study, the inability of dietary DCP to enhance the oxidative stability of MAP-stored beef patties can be explained by the similarity in muscle antioxidant capacity as earlier discussed.

Heat processing induces the formation of free radicals which accelerate oxidative reactions and increase the susceptibility of meat to lipid peroxidation (Bekhit et al., 2013). In this regard, the effect of feeding DCP was further examined in cooked beef patties stored in aerobic packs at 4 °C for up to 6 days (Fig. 1b). Lipid oxidation increased ($P < .01$) as a function of storage time. In comparison to 0% and 80% DCP, feeding 40% DCP decreased ($P < .05$) lipid oxidation on day 1 and 3 of storage and a tendency ($P = .096$) for reduced levels of lipid oxidation was observed on day 6 of storage. The reason for the antioxidant effect of 40% DCP in cooked beef is unclear considering

Table 4

Effect of increasing levels of dried citrus pulp (DCP) in supplementary concentrates to grass silage-fed steers on the colour stability of fresh beef patties stored in modified atmosphere packs and lipid oxidation of muscle homogenates incubated with a ferric chloride/sodium ascorbate pro-oxidant system.

Parameter	Diet (D)			Storage/incubation time (T) ¹					SEM	P-value ²		
	0% DCP	40% DCP	80% DCP	1	2	3	4	5		D	T	D \times T
<i>Fresh beef patties</i>												
Lightness, L^*	49.24	48.92	49.30	48.31 ^a	48.47 ^a	48.22 ^a	48.86 ^a	51.92 ^b	0.340	0.868	0.004	0.999
Redness, a^*	16.72	16.31	16.64	22.75 ^a	20.95 ^b	18.97 ^c	14.33 ^d	5.79 ^e	0.928	0.576	<0.001	0.751
Yellowness, b^*	15.24	14.95	15.32	16.69 ^a	15.90 ^b	15.06 ^c	4.45 ^c	13.73 ^d	0.171	0.083	<0.001	0.617
Chroma, C^*	22.99	22.52	23.01	28.22 ^a	26.31 ^b	24.22 ^c	19.87 ^d	15.58 ^e	0.702	0.286	<0.001	0.519
Hue angle, H^*	44.52	44.93	45.03	36.29 ^a	37.23 ^a	38.48 ^a	43.96 ^b	68.17 ^c	1.827	0.804	<0.001	0.986
<i>Muscle homogenates</i>												
TBARS ³	4.21	4.17	4.44	2.293 ^a	6.255 ^b				0.260	0.537	<0.001	0.977
OxyMb ⁴ (%)	65.31	69.64	69.86	86.819 ^a	49.648 ^b				2.477	0.146	<0.001	0.529

a,b,c,d,e Within row, different superscript letters indicate differences ($P < .05$) between storage/incubation time.

SEM: Standard error of mean.

¹ Times 1, 2, 3, 4, 5 correspond to: 1, 4, 7, 10 and 14 days (fresh beef patties stored at 4 °C in modified atmosphere packs); 1 and 4 h (muscle homogenates incubated with Fe/Ascorbate at 4 °C).

² P-values for the effects of the dietary treatment (D), time of storage or incubation (T) and D \times T interaction.

³ TBARS: 2-thiobarbituric acid reactive substances expressed as mg malondialdehyde/kg meat.

⁴ OxyMb: Oxymyoglobin, % of total myoglobin.

that dietary DCP did not enhance the antioxidant capacity or oxidative stability of fresh LT muscle. However, heat treatment has been shown to enhance the liberation and activation of bound phenolic compounds from citrus resulting in increased antioxidant activity (Hayat et al., 2010; Jeong et al., 2004). Thus, it can be suggested that heating might have enhanced the antioxidant activity of DCP phenolics deposited in the muscle which could partly explain the discrepancy between the antioxidant effects observed in cooked beef patties from steers fed 40% DCP but not in fresh beef patties or muscle homogenates. In relation to this hypothesis, however, it is unclear why the cooked beef patties from steers fed 80% DCP did not exhibit a similar antioxidant effect. This discrepancy in antioxidative property may be due to factors such as variation in the affinity of phenolic compounds to lipid bilayers and uneven distribution of phenolic accumulation in the muscle tissues (Tang, Kerry, Sheehan, Buckley, & Morrissey, 2000, 2001). Indeed, further studies are required to investigate the possible mechanisms through which citrus phenolic compounds, or their *in vivo* metabolites may exert an antioxidant effect in cooked meat.

3.5. Texture profile and consumer acceptability of beef

Lipid and protein oxidation in *post-mortem* muscle potentially impact tenderness properties which influence the palatability and consumer acceptability of meat (Bekhit et al., 2013). Previous studies have demonstrated that feeding DCP did not affect the tenderness, measured by Warner–Bratzler shear force, of fresh beef (De Souza Duarte et al., 2011; Maia Filho et al., 2016). However, no information has been reported on the effect of dietary DCP on meat tenderness relative to the effect of oxidation during storage. Texture profile analysis provides a detailed characterization of textural attributes (hardness, springiness, cohesiveness, gumminess, chewiness and adhesiveness) of meat. Hardness (force required to attain a given deformation at first bite) is considered as the most important textural attribute because of its direct relationship with the objective tenderness of meat (Caine, Aalhus, Best, Dugan, & Jeremiah, 2003). There was no significant effect of dietary treatment on all the texture profile parameters over the storage duration. The lack of dietary effect on the lipid oxidation of beef may partly explain why the texture profile parameters did not differ between dietary treatments. Hardness, gumminess and chewiness increased ($P < .05$) in CON beef patties over the 7 days of storage (Table 5), in agreement with the effect of high-oxygen MAP (80% O₂:20% CO₂) in reducing beef tenderness due to crosslinking/aggregation of myosin by

protein oxidation (Kim, Huff-Lonerger, Sebranek, & Lonergan, 2010). However, there was no interactive effect of diet and storage time on the texture profile parameters of beef patties.

The effect of diet on muscle composition such as fatty acids could influence oxidative development and consequently affect sensory perception and consumer acceptability of meat (Wood et al., 2008). There is limited information on the effect of dietary DCP on the eating quality of meat. Previous sensory evaluation studies have shown that feeding orange pulp did not affect the sensory characteristics and overall acceptance of meat from guinea pigs (Mínguez & Calvo, 2018) and lambs (Lanza, Priolo, Biondi, Bella, & Salem, 2001). Similarly, the present study showed that naïve panellists did not detect differences in the eating quality descriptors (appearance, odour, texture, flavour and overall acceptability) of cooked beef patties stored in MAP for up to 7 days (Table 5). However, cooked beef patties from steers fed 40% DCP diets were assessed to be juicier than those fed 0% DCP and 80% DCP diets. Interestingly, this outcome is consistent with the observation highlighted earlier that feeding 40% DCP diet significantly reduced lipid oxidation in cooked beef patties. Moreover, 40% DCP numerically decreased the hardness of beef patties which may suggest the effect of slightly lower lipid oxidation. Lipid oxidation stimulates protein oxidation which reduced the protein solubility and water holding capacity of meat (Huang & Ahn, 2019). Indeed, water holding capacity correlates with the juiciness likeness of meat. Dietary antioxidants improved the water-holding capacity of meat by preserving the integrity of muscle cell membranes through inhibition of the oxidation of membrane phospholipids during oxidative conditions such as cooking and storage (Gray, Gomaa, & Buckley, 1996). Thus, there is a possibility that cooking stimulated the antioxidant phenolics in 40% DCP-fed beef patties which could explain the reduced lipid oxidation and a potential increase in water holding capacity resulting in higher juiciness likeness.

4. Conclusions

The inclusion of up to 80% DCP in concentrate supplement did not influence the antioxidant capacity of beef from steers offered grass silage. From a human nutrition perspective, dietary DCP improved the fatty acid composition of beef by increasing the percentage of CLA and PUFA. Feeding DCP did not compromise the oxidative stability of fresh beef stored in MAP while 40% DCP reduced the lipid oxidation in aerobically-stored cooked beef patties. Moreover, dietary DCP did not negatively influence the texture characteristics and consumer

Table 5

Effect of increasing levels of dried citrus pulp (DCP) in supplementary concentrates to grass silage-fed steers on the texture profile parameters and eating attributes of beef patties stored in modified atmosphere packs at 4 °C for up to 7 days.

Parameter	Diet (D)			Storage time (T, day)		P-value ¹			
	0% DCP	40% DCP	80% DCP	2	7	SEM	D	T	D x T
Textural attributes									
Hardness	20.26	19.73	20.82	17.55 ^a	22.99 ^b	0.913	0.715	<0.001	0.076
Springiness	0.84	0.84	0.86	0.84	0.85	0.005	0.286	0.261	0.204
Cohesiveness	0.60	0.57	0.62	0.59	0.60	0.009	0.063	0.368	0.588
Gumminess	12.19	11.25	13.01	10.33 ^a	13.97 ^b	0.677	0.346	0.002	0.096
Chewiness	10.33	9.43	11.18	8.71 ^a	11.93 ^b	0.607	0.270	0.002	0.081
Adhesiveness	-1.48	-1.50	-0.98	-1.26	-1.38	0.103	0.061	0.538	0.249
Eating quality									
Appearance	5.72	6.13	6.26	5.83	6.24	0.145	0.273	0.147	0.763
Odour	6.01	6.05	6.20	6.02	6.15	0.124	0.802	0.618	0.801
Texture	4.45	5.20	4.54	4.80	4.66	0.151	0.091	0.663	0.956
Juiciness	3.30 ^x	4.21 ^y	3.21 ^x	3.65	3.49	0.143	0.007	0.581	0.903
Flavour	5.77	6.18	5.71	5.79	5.99	0.130	0.262	0.434	0.684
Overall acceptability	5.07	5.67	5.08	5.25	5.29	0.128	0.085	0.866	0.961

x,yWithin row, different superscript letters indicate differences ($P < .05$) between dietary treatment.

a,bWithin row, different superscript letters indicate differences ($P < .05$) between storage/incubation time.

SEM: Standard error of mean.

¹ P-values for the effects of the dietary treatment (D), time of storage (T) and D × T interaction.

acceptability of beef.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgements

This work was conducted as part of a PhD thesis project supported by the Agricultural Transformation by Innovation (AGTRAIN) Erasmus Mundus Joint Doctorate Program, funded by the EACEA (Education, Audiovisual and Culture Executive Agency) of the European Commission. The technical assistance provided by Bernardo Valenti (University of Catania, Italy) is gratefully acknowledged. The animal production component of the study was funded by the Department of Agriculture, Food and the Marine Competitive Research Programme (11/S/122, FEFAN).

Authorship statement

Saheed A. Salami: Conceptualization, Methodology, Investigation, Data Curation, Software, Writing - Original Draft, Writing - Review & Editing, Project administration. Michael N. O'Grady: Investigation, Methodology, Supervision, Writing - Review & Editing. Giuseppe Luciano: Writing - Review & Editing, Supervision. Alessandro Priolo: Writing - Review & Editing, Supervision, Funding acquisition. Mark McGee: Methodology, Writing - Review & Editing; Funding acquisition. Aidan P. Moloney: Methodology, Writing - Review & Editing, Funding acquisition. Joseph P. Kerry: Writing - Review & Editing, Supervision, Funding acquisition.

Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in the Meat Science Journal.

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