Influence of dietary cardoon meal on growth performance and selected meat quality parameters of lambs, and the antioxidant potential of cardoon extract in ovine muscle homogenates

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Abstract

Fatty acids and oxidative stability were determined in meat from lambs fed a diet containing 15% dehydrated alfalfa (CON, *n*=8) or cardoon meal (CMD, *n*=7). Furthermore, the antioxidant activity of a phenolic-rich cardoon meal extract (1.32 GAE mg/ml) was examined in muscle homogenates (0, 0.5, 1, 5% v/w) subjected to iron/ascorbate-induced oxidation. Feeding CMD did not affect lamb performances and carcass traits but reduced ($P < 0.05$) the vaccenic and rumenic acids and increased stearic acid in muscle. Lipid oxidation was higher in raw meat from the CMD-fed lambs after 7 days of storage ($P < 0.05$). Feeding CMD did not affect the colour stability of raw meat and the oxidative stability of cooked meat and of muscle homogenates incubated with pro-oxidant catalysts. Adding 5% cardoon extract in muscle homogenates increased (+114.3%; $P = 0.03$) the total phenolic content and reduced (-77.6%; $P < 0.01$) lipid oxidation, demonstrating the antioxidant potential of compounds present in cardoon meal.

Keywords: *Lamb meat, Growth performance, Fatty acids, Lipid oxidation, Cardoon, Polyphenols*

1. Introduction

Meat from ruminant animals (cattle, sheep, goat and buffalo) can be a viable dietary source of bioactive fatty acids including conjugated linoleic acids (CLA), monounsaturated fatty acids (MUFA) and *n*-3 long-chain polyunsaturated fatty acids (PUFA; Bessa, Alves, & Santos-Silva, 2015). However, ruminant meat also contains high levels of saturated fatty acids (SFA) and *trans*-fatty acids (TFA) linked to increased risk of cardio-metabolic diseases in humans (McAfee et al., 2010). The fatty acid profile of ruminant meat is significantly influenced by extensive conversion of dietary PUFA to SFA during biohydrogenation of lipids that occur in the fore-stomach (rumen) (Shingfield, Bonnet, & Scollan, 2013). However, feeding strategies such as supplementation of PUFA-rich vegetable oil could increase the intramuscular deposition of PUFA and CLA and reduce SFA levels in ruminant meat (Bessa et al., 2015).

Lipid oxidation is the main chemical process associated with oxidative deterioration of meat, resulting in undesirable rancid off-flavour and colour deterioration that limit shelf-life and negatively impact consumer acceptability of meat (Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998). The balance between antioxidant and pro-oxidant components in muscle tissues mainly influences lipid stability. Animal diets could influence the biochemical components of muscle tissues through enrichment with antioxidant compounds, such as α-tocopherol (vitamin E), that enhance the oxidative stability of meat (Luciano et al., 2013; Salami et al., 2016).

Phenolic compounds are secondary metabolites that are ubiquitous in several plant species and may be found in considerable amounts in agro-industrial by-products (AIBP; Balasundram, Sundram, & Samman, 2006). The AIBP can be a valuable and economical resource for animal feeding or for the extraction of bioactive phenolic compounds used in functional food

applications. Feeding phenolic-rich AIBP improves the fatty acid composition of ruminant meat through inhibition of ruminal biohydrogenation (Lanza et al., 2015) and may enhance the oxidative stability of meat through controversial antioxidant mechanisms (Vasta & Luciano, 2011). Phenolics could exhibit *in vivo* antioxidant activities through direct deposition of these compounds in tissues or through possible indirect antioxidant mechanisms including the sparing effect of polyphenols on other antioxidants, such as ascorbic acid and tocopherols (Valenti et al., 2018). Furthermore, phenolic-rich extracts obtained from AIBP have been directly incorporated into meat products during processing to inhibit oxidative deterioration and extend shelf-life (Balzan et al., 2017; Kanatt, Chander, Radhakrishna, & Sharma, 2005; Rodríguez-Carpena, Morcuende, & Estévez, 2011).

Cardoon meal is a by-product retained after the extraction of oil from the seeds of cultivated cardoon (*Cynara cardunculus* var. *altilis*), a perennial herb native to the Mediterranean region and widespread in parts of Europe, Americas and Oceania. Cardoon meal has been proposed as an alternative feed resource because of its potential as a valuable source of fibre, protein, amino acids and bioactive compounds such as polyphenols and unsaturated fatty acids (Genovese et al., 2015). Cardoon meal may also contain residual amount of other antioxidant compounds such as α-tocopherol found in considerable concentration in cardoon seed oil (Maccarone et al., 1999). Moreover, extracts obtained from different morphological parts (leaf, seed, stem and flower) of cardoon have demonstrated *in vitro* antioxidant and antimicrobial activities attributed to the phenolic constituents present (Falleh et al., 2008; Pandino, Lombardo, Mauromicale, & Williamson, 2011; Ramos et al., 2014). To our knowledge, no information has been published on the effect of dietary cardoon meal or cardoon extract on meat quality. Therefore, the first objective of this study was to determine the effect of dietary cardoon meal on lamb growth

performance, carcass characteristics, and meat fatty acid composition and oxidative stability. In addition, the antioxidant potential of a phenolic-rich cardoon meal extract on lipid oxidation in an ovine muscle model system was investigated.

2. Materials and Methods

2.1. Animals, diets and experimental design

The experiment was conducted indoors in the experimental farm of the University of Catania. The animals were handled by trained personnel according to the European Union legislation for the protection of animals used for scientific purposes (2010/63/ EU Directive) and the study was approved by the University of Catania (FIR-2014-PI/LB/Di3A). Fifteen male Sarda x Comisana lambs (age 75 d and average weight 19.58 ± 2.01 kg) were randomly assigned to two experimental groups. Each animal was reared in an individual pen and adapted to the experimental diets for a period of 9 d by progressive substitution of the weaning feed with the experimental feeds until a total replacement of the weaning diet was achieved. The control group (CON, n = 8), was raised on a commercial concentrate-based diet containing the following ingredients (*as-fed* basis): barley (48.0%), dehydrated alfalfa (15.0%), wheat bran (23.0%), soybean meal (10.0%), molasses (2.0%) and vitamin premix (2.0%). The cardoon meal group (CMD, n = 7), received the same diet as the CON lambs except that the 15% dehydrated alfalfa was completely replaced by cardoon meal. The chemical composition of the experimental diets is outlined in Table 1. The CON and CMD diets were supplied in form of pellets and lambs had *ad libitum* access to feeds and water for 75 days pre-slaughter. Experimental feed samples were collected at two-week intervals during the feeding trial and stored in vacuum packs at -30 °C prior to chemical analysis. Diets were supplied daily and the amount of refusal was measured before morning feeding to calculate dry matter intake (DMI). The body weight (BW) of the

lambs was measured at the start of the experiment and recorded weekly (at 09:00 h before providing fresh feed) to calculate average daily gain (ADG).

2.2. Slaughter, and carcass sampling and measurement

The lambs were slaughtered (stunned by captive bolt before exsanguination) in a commercial abattoir, where they had free access to the experimental diets and water until approximately 3 h before slaughter. Carcass weight was recorded following removal of the visceral organs and dressing percentage was calculated as the percentage of carcass weight to final BW. Visual appraisal of hot hanging carcasses was performed by a certified meat grader to determine the conformation and fat cover scores according to the European Union's EUROP carcass classification system (Commission Regulation (EC) No 823/98, 1998) . Carcass conformation and fatness scores were based on a five-point scale and each score class was further classified into high, medium and low to obtain a 15-point score for a more precise description of carcass traits. Carcasses were stored at 4°C for 24 h *post-mortem* and ultimate pH (pH24) was measured on the *longissimus thoracis* muscle using a pH-meter (Orion 9106). Colour descriptors lightness (L*), redness (a*), yellowness (b*), Chroma (C*), and Hue angle (H*) were recorded on the *longissimus thoracis* muscle, using Minolta CM-2022 spectrophotometer (d/8° geometry; Minolta Co., Ltd. Osaka, Japan) on specular components excluded (SCE) mode, illuminant A and a 10° standard observer. After 24 h of storage at 4 °C, the entire *longissimus thoracis et lumborum* (LTL) muscle from the right half-carcass was removed, packed under vacuum and stored at -80 °C for analyses of intramuscular fatty acids and tocopherols. The entire LTL from the left half-carcass was vacuum-packed and stored at 4 °C for 3 days, pending oxidative stability measurements.

2.3. Feed analysis

2.3.1. Analysis of chemical composition, fatty acids and vitamin E

Dry matter (DM), crude protein, ether extract and ash content were determined in the experimental diets following the method of AOAC (1995). The neutral detergent fibre, acid detergent fibre and acid detergent lignin were analysed according to Van Soest, Robertson, and Lewis (1991). The fatty acid composition of the experimental diets was determined by a one-step extraction–transesterification procedure using chloroform (Sukhija & Palmquist, 1988) and 2% (v/v) sulfuric acid in methanol (Shingfield et al., 2003). Gas chromatographic (GC) analysis of fatty acid methyl esters (FAME) was performed as described later (section 2.3) for fatty acid profile of lamb meat. Individual fatty acids of experimental diets were expressed as mg/g of DM.

Vitamin E (α-, γ- and δ-tocopherols) was analysed in the experimental diets as described by Cherif et al. (2018). Briefly, freeze-dried samples were homogenized and saponified with ethanolic KOH stabilized with BHT. Tocopherols were extracted using hexane/ethyl acetate (9/1, v/v), dried under N_2 and dissolved in acetonitrile. Vitamin E was analysed by HPLC (Perkin Elmer series 200), equipped with an autosampler (model AS 950-10, Tokyo, Japan) and a Synergy Hydro-RP column (4 μm, 4.6 × 100 mm; Phenomenex, Bologna, Italy). Tocopherols were eluted at a flow rate of 2 ml/min and identified using a fluorescence detector (model Jasco, FP-1525) set at an excitation and emission λ of 295 nm and 328 nm, respectively. Quantification was based on external calibration curves of pure standard compounds (Sigma-Aldrich, Bornem, Belgium) solubilised in ethanol.

2.3.2. Analysis of total phenol content and in vitro antioxidant activity

Phenolic compounds were extracted from cardoon meal, dehydrated alfalfa and experimental diets 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) 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 using 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 μg/ml) was plotted and results were expressed as g of gallic acid equivalents (GAE)/kg of DM feed.

In vitro antioxidant activity of polyphenol-rich extracts was measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging (Yen & Wu, 1999) and ferric reducing antioxidant power (FRAP) total antioxidant assays (Benzie & Strain, 1999), with minor modifications. For the DPPH assay, extract (0.6 ml) and distilled water (2.4 ml) were mixed with 0.2 mM DPPH in methanol (3 ml) and stored in the dark for 1 h at room temperature. Absorbance measurements were recorded at 517 nm using 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 μg/ml) was plotted and results were expressed as g of Trolox equivalents (TE)/kg of DM feed.

For the FRAP assay, 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 $FeCl_3.6H_2O$ in distilled water in the ratio 10:1:1, respectively incubated at 37 °C for 10 min prior to use). The mixture was stored in the dark for 30 min at room temperature and absorbance measurements were recorded at 593 nm using a UV-vis spectrophotometer (Cary 300 Bio) against a blank containing all reagents. A calibration curve using standard solutions of methanolic Trolox (0.033 – 0.1 mg/ml) was plotted and results were expressed as g of Trolox equivalents (TE)/kg of DM feed.

2.4. Analysis of fatty acids and vitamin E in lamb meat

Intramuscular fat was extracted from 10 g of minced LTL with a mixture of methanol and chloroform (2:1, v/v) according to the method of Folch, Lees, and Sloane Stanley (1957). Lipids (30 mg) were converted to FAME by base-catalysed transesterification (Christie, 1982) using 0.5 mL of sodium methoxide in methanol 0.5 N and 1 mL of hexane containing 1mg /mL nonadecanoic acid (C19:0) as an internal standard. Gas chromatographic analysis was conducted as described by Valenti et al. (2018) using a GC 8000 Top ThermoQuest (Milan, Italy) gas-chromatograph equipped with a flame ionization detector and a high polar column (WCOT-fused silica CP-Select CB for FAME Varian, Middelburg, the Netherlands; 100m×0.25mm i.d.; film thickness 0.25 μm). Helium was the carrier gas at a constant flow of 1 ml/min. Total FAME profile in a 1 μL sample volume (2 μL for feed samples) at a split ratio of 1:80 was determined using the following conditions: the oven temperature was programmed at 40 °C and held for 4 min, then increased to 120 °C at 10 °C/min, held for 1 min, then increased up to 180 °C at 5 °C/min, held for 18 min, then increased up to 200 °C at 2 °C/min, held for 15 min, and then increased up to 230 °C at 2 °C/min, held for 19 min. The injector and detector temperatures were

set at 270 °C and 300 °C, respectively. The identification of individual FAME was based on the retention time comparison with commercially available standard mixture of FAME (Nu-Chek Prep Inc., Elysian, MN, USA; Larodan Fine Chemicals, Malmo, Sweden) and comparison with chromatograms published by Kramer, Hernandez, Hernandez, Kraft, and Dugan (2008) and Alves and Bessa (2007). Fatty acids were expressed as g/100 g of total fatty acids. The dietary risk for cardiovascular diseases was assessed by calculating the atherogenic index (the relationship between FA with pro-atherogenic and anti-atherogenic properties) and thrombogenic index (the relationship between FA with pro-thrombogenic and anti-thrombogenic properties). The atherogenic index (AI) and thrombogenic index (TI) were calculated as outlined by Ulbricht and Southgate (1991):

$$AI = \frac{C12:0 + (4 \times C14:0) + C16:0}{n\text{-}6\ PUFA + n\text{-}3\ PUFA + MUFA}$$

$$TI = \frac{C14:0 + C16:0 + C18:0}{(0.5 \times MUFA) + (0.5 \times n\text{-}6\ PUFA) + (3 \times n\text{-}3\ PUFA) + \left(\frac{n\text{-}3\ PUFA}{n\text{-}6\ PUFA}\right)}$$

Vitamin E (α-, γ- and δ-tocopherols) and retinol were extracted from lamb muscle according to the method of Schüep and Rettenmaier (1994). Chromatographic analysis of vitamin E was performed as described in section 2.2.1 for feeds. Retinol was analyzed in the same chromatographic run and identified using the UV-VIS detector set at λ 325 nm (Cherif et al., 2018). Identification and quantification were achieved using external calibration curves of standard compounds (Sigma-Aldrich) solubilised in ethanol.

2.5. Measurement of lamb meat oxidative stability

Oxidative stability parameters were measured in raw and cooked lamb meat, as well as in LTL homogenates incubated with pro-oxidant catalysts, as described by Valenti et al. (2018). Briefly, for analyses on raw and cooked meat, 6 slices (2 cm thickness) were cut from the LTL which was previously stored under vacuum at 4 °C as described in section 2.1. Three slices were placed in polystyrene trays, covered with PVC film and stored at 4 °C. Each slice was used for measuring lipid oxidation and colour stability at one of three-time points: day 0 (after 2 hours of blooming), and days 4 and 7. The remaining 3 slices were packed under vacuum and cooked for 30 min at 70°C in a water bath. After cooling in a cold-water bath, one slice was used immediately for measurement of lipid oxidation, while the other 2 slices were stored at 4°C in the same conditions described for the raw meat samples, and lipid oxidation was measured after 2 and 4 days. For both raw and cooked meat, lipid oxidation was measured as thiobarbituric acid reactive substances (TBARS) values according to the procedure of Siu and Draper (1978) and results were expressed as μg malonaldehyde (MDA)/g of meat. Colour was measured in raw meat using a Minolta CM-2022 spectrophotometer (d/8° geometry; Minolta Co., Ltd. Osaka, Japan) set in the specular components excluded (SCE) mode, illuminant A and a 10° standard observer. The colour descriptors lightness (L^*), redness (a^*), yellowness (b^*), Chroma (C^*), and Hue angle (H^*) were recorded, as well as the reflectance spectra from 400 to 700 nm. The ratio $(K/S)_{572} \div (K/S)_{525}$ was calculated to monitor the accumulation of metmyoglobin (MetMb) on the meat surface over time, with values of the ratio decreasing 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:

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

The resistance of meat to lipid and myoglobin oxidation was also assessed in LTL homogenates incubated in the presence of Fe^{3+} and ascorbate (Fe/Asc) as catalysts of oxidative reactions. Briefly, the minced LTL (7.5 g) was homogenized with 37.5 g of MES buffer (pH 5.6). Homogenates were equilibrated to 37 °C and two aliquots (3 ml and 4 ml) were collected for measuring the initial extent of lipid and myoglobin oxidation (0 minutes). Ferric chloride hexahydrate and L-sodium ascorbate were added at equimolar concentration to reach the final concentration of 45 μM. The homogenates were incubated under continuous stirring in a temperature controlled IKA KS-4000 orbital shaker (IKA-Werke GmbH & Co. KG, Staufen, Germany) set at 37°C and 190 rpm. After 30 and 60 minutes of incubation, two aliquots (3 ml and 4 ml) were collected for lipid and myoglobin oxidation analyses. Lipid oxidation was measured in the 3 ml aliquots using the method of Siu and Draper (1978). The 4 ml aliquots were centrifuged at 6800 × g at 4 °C, filtered through Whatman 541 filter paper and directly scanned in a UV/VIS spectrophotometer (UV-1601, Shimadzu Co., Milan, Italy). The absorbances at 503, 525, 557, 582, and 730 nm were used to calculate the proportion of metmyoglobin (MetMb %; Tang, Faustman, & Hoagland, 2004).

2.6. Determination of antioxidant potential of cardoon extract in a muscle model system

2.6.1. Preparation of cardoon extract

Phenolic compounds were extracted in triplicate by suspending finely-ground cardoon meal (0.5 g) in 100% methanol (20 ml) and incubated at room temperature in an orbital shaker (Max Q 6000 Shaker Thermo Fisher Scientific, Ireland) at 200 rpm for 4 h. After 4 h, the mixture was filtered through Whatman No. 1 filter paper. Pooled solvent extracts were concentrated by placing in a 50 ml round-bottomed flask and solvent (methanol) was removed by rotary evaporation (Labo-Rota C-311, Resona Technics, Switzerland) for 1 h at 55 °C. The dried

extract was re-suspended in 20 ml methanol and analysed for TPC using the Folin-Ciocalteu reagent as described for experimental feeds (section 2.2.1.). Result was expressed as mg GAE/ml extract.

2.6.2. Preparation of muscle homogenates

Fresh lamb LTL (n = 3) was obtained from a meat retail outlet (Cork, Ireland) and stored at 4 °C prior to analysis. Muscle homogenates (25%) were prepared in triplicate following a minor modification of the method described by O'Grady, Monahan, and Brunton (2001). Briefly, LTL (15 g) was chopped and homogenised in 0.12 M KCL 5 mM histidine (45 ml), pH 5.5, using an Ultra-turrax T25 homogeniser (Janke & Kunkel GmbH, IKA® Labortechnik, Staufen, Germany) at 24,000 rpm for 5 min. The muscle tissue and buffer were surrounded by crushed ice to control the temperature during homogenisation. Cardoon extract (stock concentration of 1.32 GAE mg/ml) was added to the homogenates (39.2 g) at 0, 0.5, 1 and 5% v/w of the final volume (40 ml). Lipid oxidation in muscle homogenate samples was initiated by the addition of equimolar $FeCl_3$/sodium ascorbate (45 μM) pro-oxidants. The TPC and lipid oxidation in muscle homogenate were measured after 4 h of storage at 4 °C.

2.6.3. Measurement of TPC and lipid oxidation in muscle homogenate

The total phenolic content in muscle homogenates was measured in triplicate by mixing 10% TCA (0.5 ml), 0.05 M phosphate buffer (3 ml) and muscle homogenate (2 g). The mixture was 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 TPC using the Folin-Ciocalteu reagent as described for experimental feeds (section 2.2.2). Results were expressed as g GAE/g muscle. Lipid oxidation was measured

in triplicate following the TBARS assay described by Siu and Draper (1978) and results were expressed as μg MDA/g meat.

2.7. Statistical analysis

The average data of DMI, BW and feed efficiency for individual lambs were subjected to one-way ANOVA test to analyse the effect of dietary treatment on growth performance parameters. The effect of dietary treatment on carcass traits, intramuscular fatty acids and fat-soluble vitamins, were also analysed with one-way ANOVA test, using individual lambs as the experimental units. Data on the oxidative stability parameters (raw, cooked and muscle homogenates) were analysed with a full-repeated measures ANOVA. Effects of diet represented the 'between-subjects' factor and the effect of storage time/incubation was measured using the 'within-subjects' factor and the interaction between diet and storage time/incubation was tested. Individual lambs were considered as the experimental units in all the statistical analyses performed for the effect of dietary cardoon meal on growth performance and meat quality. Analyses relating to the effect of cardoon extract addition on TPC and lipid oxidation (TBARS) in LTL homogenates was performed in triplicate and mean sample values (n = 3) for each of the four treatment groups (CON, CE0.5, CE1.0 and CE5.0) were subjected to a one-way ANOVA. Pearson's correlation analysis was performed to assess the relationship between the TPC and TBARS in LTL homogenates. Significance was declared when $P \leq 0.05$, while a tendency for effects were considered when $0.05 < P \leq 0.10$. All statistical analyses were performed using the SPSS software (IBM Statistics version 22).

3. Results and Discussion

3.1. Antioxidant activity of experimental feedstuffs and diets

The TPC of cardoon meal was 4-fold higher ($P < 0.01$; 60.4 *vs* 15.5 g GAE/kg DM) than dehydrated alfalfa, which may explain the 2.5-fold increase in TPC when dehydrated alfalfa was replaced by cardoon meal in CMD, compared to CON (Table 1). The phenolic concentration of cardoon meal was 5-fold higher than previously reported for cardoon press cake (Genovese et al., 2015), a similar by-product obtained from the mechanical extraction of oil from cardoon seeds. Variation in the TPC of cardoon extracts may be related to factors such as plant geographical origin, varieties and maturity stage, agricultural methodologies, and phenol extraction procedures (Ramos et al., 2014). As expected, assessment of the *in vitro* antioxidant activities showed that cardoon meal exhibited a higher ($P < 0.01$) DPPH free radical scavenging activity and FRAP total antioxidant activity compared to dehydrated alfalfa (Figure 1a). Similarly, replacement of dehydrated alfalfa with cardoon meal in the experimental diets resulted in higher ($P < 0.01$) antioxidant activities in CMD compared to CON (Figure 1b).

Flavonoids and hydroxycinnamic acids are the main classes of phenolic compounds, which contribute to the antioxidant effect of extracts obtained from cardoon leaf, seed, stem and flower (Falleh et al., 2008; Pandino et al., 2011; Pinelli et al., 2007; Ramos et al., 2014). It has been shown that the TPC of cardoon extracts strongly correlated with DPPH antiradical activity (Falleh et al., 2008; Ramos et al., 2014) and FRAP total antioxidant capacity (Pandino et al., 2011). In addition, α-tocopherol is another potent antioxidant which can be found in high residual levels in cardoon meal as cardoon oil has been shown to contain considerable amounts of vitamin E (Maccarone et al., 1999). This is consistent with the 4-fold increase in α-tocopherol content found in CMD compared to CON (Table 1).

3.2. Growth performance and carcass traits

Lambs fed CMD exhibited lower ($P < 0.05$) DMI compared to CON-fed lambs. Lower DMI in lambs fed CMD may be related to low feed palatability due to the high content of dietary phenolic compounds. Cajarville, González, Repetto, Rodríguez, and Martínez (1999) reported a similar decrease in the voluntary intake of sheep fed *ad libitum* green forage of cardoon possibly due to the presence of high concentration of phenolic compounds in cardoon leaves (Kukić et al., 2008). Phenolic compounds, such as tannins, may confer unpleasant taste or bind to salivary proteins forming a polyphenol-protein complex that induce astringency sensations and trigger low feed intake in animals (Makkar, 2003). However, dietary treatment did not affect ($P > 0.05$) growth performance parameters in terms of final BW, ADG and feed efficiency (Table 2). Differences in DMI did not reflect on growth performance parameters possibly due to the low number of experimental units or that reduced DMI in CMD-fed lambs did not compromise nutrient digestibility and utilization. Accordingly, Cajarville, González, Repetto, Alvir, and Rodríguez (2000) reported that up to 25% cardoon seeds could be included in ruminant diets without negative effect on ruminal fermentation pattern and nutrient digestibility. Furthermore, there was no effect of dietary treatment on carcass characteristics such as carcass weight, dressing percentage, carcass conformation, fatness scores, muscle ultimate pH and colour characteristics (Table 2). Notably, values of ultimate pH were slightly higher than the normal pH values (5.4 – 5.8) for post-mortem muscle (Faustman & Cassens, 1990) particularly for lambs fed CON due to the fact that one carcass displayed an ultimate pH of 6.3. Nonetheless, the values of colour variables are within the satisfactory range for average consumer acceptance of fresh meat, particularly in terms of lightness (L^*) and redness (a^*) (Khliji, Van de Ven, Lamb, Lanza, & Hopkins, 2010).

3.3. Effect of dietary cardoon meal on lamb meat fatty acid composition and fat-soluble vitamin levels

Intramuscular fat, fatty acid profile and fat-soluble vitamin contents in LTL muscle from lambs fed CON and CMD diets are presented in Table 3. In general, dietary treatment did not influence ($P > 0.05$) intramuscular fat content or the total composition of SFA, MUFA, PUFA, odd- and branched-chain fatty acids (OBCFA). Dietary treatment did not influence ($P > 0.05$) the concentration of individual SFA (C12:0, C14:0 and C16:0) that results in hypercholesterolemic effects in humans (Mensink, 2005). This could in part explain why meat from lambs fed CON and CMD had similar ($P > 0.05$) nutritional indices (atherogenic index and thrombogenic index) for risk of cardiovascular diseases. However, lambs fed CMD displayed higher concentration ($P < 0.05$) of intramuscular C18:0 (stearic acid), and lower proportions ($P < 0.05$) of total *trans*-18:1, *trans*-10 C18:1, *trans*-11 C18:1 (vaccenic acid) and *cis*-9 *trans*-11 C18:2 CLA (rumenic acid) compared to CON-fed lambs. In addition, the proportion of C18:3 *n*-3 (α-linolenic acid) tended ($P = 0.070$) to be greater in the muscle of lambs fed CON compared to CMD-fed lambs. Consequently, the ratio of *n*-6:*n*-3 PUFA ($P = 0.052$) tended to be lower in CON-fed lambs compared to CMD-fed lambs. Regarding fat-soluble vitamins in LTL, the concentration of retinol ($P = 0.048$) was greater in muscle from lambs fed CMD relative to CON but diet did not affect ($P > 0.05$) vitamin E (α-, γ- and δ-tocopherols) concentration (Table 3).

Indeed, changes in intramuscular fatty acids might be due to possible differences in ruminal biohydrogenation. The present study showed that dietary treatment significantly affected the intramuscular concentration of individual fatty acids (stearic, *trans*-10 C18:1, vaccenic and rumenic acids) with potential nutritional implications. It has been suggested that dietary consumption of stearic acid does not increase plasma low-density lipoprotein and cholesterol

levels in humans in contrast to the negative effect of other SFA that are risk factors for cardio-metabolic diseases (Mensink, 2005). Thus, the greater amount of stearic acid in lambs fed CMD may not have detrimental effects on human health. Muscle from lambs fed CON exhibited a higher proportion of vaccenic and rumenic acids that are known for their potential health benefits in humans (Bessa et al., 2015). Both vaccenic and rumenic acids are intermediate FA synthesized during ruminal biohydrogenation but rumenic acid can be further synthesized in muscle tissues through endogenous desaturation of vaccenic acid by the enzyme Δ-9-desaturase (Corl et al., 2001). Thus, higher concentration of intramuscular rumenic acid in CON-fed lambs may be due to greater ruminal outflow and absorption of vaccenic acid that serves as the main precursor for the endogenous synthesis of rumenic acid in muscle tissues. Rumenic acid is the major naturally occurring CLA isomer found in ruminant meat and milk (Bessa et al., 2015) and dietary consumption of this FA potentially prevent human diseases including cancer, cardiovascular diseases, obesity, bone density loss, and diabetes (McGuire & McGuire, 2000).

From a nutritional perspective, the accumulation *t*-10 C18:1 in ruminant edible products is undesirable due to the potential cytotoxic effect of this fatty acid (Vahmani et al., 2016). Thus, a lower concentration of *t*-10 C18:1 in meat of lambs fed CMD may be viewed as a positive outcome compared to CON. Concentrate feeding systems are known to promote the alternative ruminal biohydrogenation pathway that increase the synthesis of intermediates such as *t*-10 18;1, at the expense of *t*-11 18:1, referred to as the *t*-10 shift (Bessa et al. 2015). Bessa et al. (2015) postulated that the ratio of *t*-10/*t*-11 18:1 >1 in ruminant meat or milk is an indicator of occurrence of *trans*-10 shift during ruminal biohydrogenation. Considering that concentrate diets were fed in this study, it is not surprising that *t*-10/*t*-11 18:1 was > 1 in meat from lambs fed CON and CMD, suggesting that both diets induced *t*-10 shift. However, feeding CMD tended (*P*

= 0.084) to reduce the occurrence of the *t*-10 shift, suggesting that the presence of phenolics in CMD might have modified the ruminal biohydrogenation pathway.

A number of factors may account for the lack of positive effect of CMD to inhibit ruminal biohydrogenation and increase PUFA and CLA contents in meat. It is possible that hydroxycinnamic acids, the major cardoon phenolic compounds, were metabolised by the consortium of microbes residing in the fore-stomach (rumen) of lambs as shown with human faecal microbiota (Gonthier et al., 2006). Furthermore, effects of dietary phenolics to increase PUFA and CLA contents in ruminant meat have been largely reported for diets containing a high concentration of tannins (Vasta, Nudda, Cannas, Lanza, & Priolo, 2008). Though the tannin content of diets was not measured in the present study, it has been shown that cardoon extracts contain a very low concentration of tannins (Falleh et al., 2008). Thus, results from the present study emphasized the variation in the effect of diets containing a high concentration of bioactive compounds, which may be dependent on the type and/or concentration of phenolic compounds present. In contrast, alfalfa fed in various processed forms (fresh, hay, dehydrated, silage) has been documented to enrich lamb muscle and offal with lower SFA and higher content of PUFA and rumenic acid (Cerci, Ciftci, Bahsi, & Kilinc, 2011; Ciftci et al., 2010; Realini, Bianchi, Bentancur, & Garibotto, 2017). The positive effects of dietary alfalfa on the fatty acid profile of ruminant meat may be due to the presence of saponins and flavonoids which inhibit ruminal biohydrogenation (Petersen & Jensen, 2014).

3.4. Effect of dietary cardoon meal on oxidative stability of lamb meat

The results of oxidative stability of raw meat, cooked meat and muscle homogenates are presented in Table 4. As expected, lipid oxidation (TBARS values) significantly increased ($P < 0.001$) in raw meat as a function of storage time. Although in raw meat an overall effect of the

dietary treatment was not found on lipid oxidation, and interactive effect of the dietary treatment and time of storage was found ($P < 0.05$), with higher TBARS values measured in raw meat slices from the CMD-fed lambs compared to the CON treatment after 7 days of storage (Figure 2). This result is not easy to explain, considering the lack of effect of the dietary treatment on the concentration of PUFA and α-tocopherol in muscle. It can be speculated that a higher content of CLA (cis-9 trans-11 18:2) in the meat from CON-fed lambs could be responsible for the reduced lipid oxidation as CLA may exert a protective effect on muscle oxidation (Joo, Lee, Ha, & Park, 2002). Hur et al. (2004) also reported that the CLA present in meat does not participate in oxidation processes and reduces the formation of fatty acid free radicals, which results in reduced lipid oxidation. Noteworthy, as shown in Table 4, neither the dietary, nor the diet × time interaction affected the colour stability of raw lamb meat measured using instrumental colour descriptors (lightness L^*, redness a^*, yellowness b^*, saturation C^* and hue angle H^*) and an index for metmyoglobin accumulation ($(K/S)_{572} \div (K/S)_{525}$). The measured parameters mostly related to meat browning were only affected by the storage period, with redness, saturation and the $(K/S)_{572} \div (K/S)_{525}$ decreasing over time ($P < 0.05$), while hue increased ($P < 0.05$). Additionally, as shown in Table 4, the use of stronger pro-oxidant conditions (i.e. cooking and incubation of muscle homogenates with $FeCl_3$/sodium ascorbate pro-oxidants) greatly promoted lipid and myoglobin oxidation which markedly increased over time ($P < 0.001$). However, the different susceptibility to lipid oxidation observed in raw meat was not evident in cooked meat and in muscle homogenates, which suggests that such oxidative challenges might have overcome inherent differences between treatments in the susceptibility to oxidation.

In general, feeding CMD did not improve the oxidative stability of meat despite the greater content of phenolic compounds and the consequently higher antioxidant capacity of the CMD

diet compared to CON (Table 1 and Figure 1b). Indeed, the *in vivo* antioxidant potential of dietary phenolics is still controversial. There are evidences that phenolics may exhibit antioxidant effect through direct absorption and deposition into muscle tissues or through indirect antioxidant mechanisms including the sparing effect of polyphenols on other antioxidants, such as ascorbic acid and tocopherols (Valenti et al., 2018). As previously highlighted in this study, possible microbial metabolism of cardoon phenolics in the gut of lambs may also account for the lack of functional *in vivo* effect in lambs fed CMD.

3.5. Effect of cardoon extract on lipid oxidation in a muscle-based system

Antioxidant effect of the phenolic-rich extract obtained from cardoon meal was tested in LTL homogenates subjected to $FeCl_3$/sodium ascorbate-induced oxidation by incubating with iron/ascorbate pro-oxidants for 4 h. Addition of 5% cardoon extract significantly increased (+114.3%; $P < 0.05$) the total phenolic content (TPC) in LTL homogenates compared to the control (Figure 3a). Similarly, addition of 5% extract inhibited (-77.6%; $P < 0.001$) lipid oxidation (TBARS values) relative to the control (Figure 3b). Pearson's correlation analysis showed that TPC negatively correlated ($r = -0.724$; $P = 0.008$) with TBARS values, suggesting that cardoon phenolics contributed to the inhibition of lipid oxidation in LTL homogenates. The present study suggests that the antioxidant effect of cardoon extract in an ovine muscle system is dose-dependent as only the addition of 5% cardoon extract significantly inhibited lipid oxidation relative to the control. Similarly, Falleh et al. (2008) demonstrated that cardoon extracts exhibited *in vitro* antioxidant activities (DPPH radical and superoxide anion scavenging activities) in a concentration-dependent manner.

Several plant extracts have been shown to exhibit antioxidant efficacy with potential application as natural alternatives to potentially-toxic synthetic antioxidants in meat products (Jiang &

Xiong, 2016). Indeed, the present study is the first to demonstrate the antioxidant potential of cardoon extract in muscle model systems. Moreover, the addition of a cardoon extract can enhance the healthiness of meat products as cardoon phenolics may exert functional therapeutic properties such as antimicrobial, antimutagenic, hepatoprotective, choleretic and anti-cholestatic actions in humans (Adzet, Camarasa, & Laguna, 1987; Falleh et al., 2008). Therefore, further research is required to examine the use of cardoon extract as a natural additive for developing functional meat products with extended shelf-life and health-promoting properties.

4. Conclusions

The present study demonstrated that cardoon meal is a rich source of phenolic compounds with potent antioxidant activity. The inclusion of 15% cardoon meal replacing dehydrated alfalfa in a concentrate diet had no adverse effect on lamb growth performance but depressed feed intake. Dietary cardoon meal did not influence the intramuscular composition of SFA, MUFA, PUFA and nutritional indices (atherogenic index and thrombogenic index) but presented a lower concentration of potentially health-promoting fatty acids (vaccenic and rumenic acids) in lamb meat. Moreover, dietary inclusion of cardoon meal did not improve the oxidative stability of raw and cooked meat stored aerobically at 4 ºC for up to 7 and 4 days, respectively. In addition, a phenolic-rich extract obtained from cardoon meal exhibited potent antioxidant activity against lipid oxidation in an ovine muscle model system. Further research is required to evaluate the antioxidant effect of cardoon extract on the shelf-life and quality parameters of meat products.

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Table 1. Chemical composition of experimental diets

	Diets[1]	
Parameter	**CON**	**CMD**
Dry matter (DM), % *as-fed*	89.65	89.63
Crude protein, % DM	15.67	16.45
Ether extract, % DM	2.68	3.84
Ash, % DM	7.01	6.31
NDF, % DM	30.36	27.32
ADF, % DM	15.97	12.39
ADL, % DM	3.62	4.15
Total phenolic content[2]	5.21	13.08
Fatty acids (mg/g DM)		
C14:0	0.034	0.034
C16:0	4.357	5.062
cis-9 C16:1	0.035	0.034
C18:0	0.454	0.705
C18:1 *n*-9	3.855	5.362
cis-11 C18:1	0.209	0.206
C18:2 *n*-6	12.190	16.852
C18:3 *n*-3	1.255	1.065
C20:0	0.088	0.086
Vitamins (µg/g DM)		
α-Tocopherol	5.267	20.877
γ-Tocopherol	0.155	0.119
δ-Tocopherol	0.012	0.005

[1]Diets were: CON (control diet), CMD (cardoon meal diet)
[2]Expressed as grams gallic acid equivalents/kg DM
NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin

Table 2. Effect of dietary treatment on growth performance, carcass characteristics and colour of *longissimus thoracis et lumborum* muscle of lambs.

	Dietary[1]			
Parameter	**CON**	**CMD**	**SEM**	***P*-value**
Growth performance				
Dry matter intake, g/d	1078.3	932.2	31.15	0.013
Initial body weight, kg	20.1	20.1	0.54	0.974
Final body weight, kg	35.5	33.9	0.74	0.303
Average daily gain, g/d	204.5	184.0	8.04	0.215
Feed efficiency[2]	190.8	198.7	8.24	0.649
Carcass traits				
Carcass weight, kg	17.1	16.1	0.41	0.264
Dressing percent, %	48.1	47.5	0.29	0.333
Conformation score[3]	2.3	1.9	0.13	0.102
Fatness score[4]	2.5	2.4	0.04	0.738
Ultimate pH	5.91	5.83	0.04	0.310
Lightness, L^*	37.95	38.72	0.58	0.528
Redness, a^*	11.90	12.53	0.43	0.481
Yellowness, b^*	6.93	7.32	0.34	0.588
Saturation, C^*	13.78	14.51	0.53	0.512
Hue angle, H^*	29.96	30.23	0.56	0.817

[1]Diets were: CON (control diet), CMD (cardoon meal diet)
[2]Calculated as: g BW gain/kg DMI
[3]Conformation score: E = 5, excellent shape and muscularity; U = 4; R = 3; O = 2; P = 1, poor shape and muscularity.
[4]Fatness score: 1 = low, 5 = very high

661

Table 3. Effect of dietary treatment on the intramuscular fat content, fatty acid composition and fat soluble vitamins in the *longissimus thoracis et lumborum* muscle of lambs

	Dietary[1]			
Parameter	**CON**	**CMD**	**SEM**	***P*-value**
IMF (g/100g of muscle)	2.07	2.06	0.117	0.946
Fatty acids (g/100 g of total fatty acids)				
C12:0	0.13	0.13	0.007	0.920
C14:0	2.90	2.81	0.121	0.727
cis-9 C14:1	0.11	0.10	0.007	0.367
C15:0	0.36	0.36	0.011	0.848
iso C15:0	0.07	0.06	0.002	0.146
anteiso C15:0	0.11	0.11	0.005	0.907
C16:0	23.60	22.81	0.245	0.110
cis-9 C16:1	1.77	1.57	0.075	0.174
C17:0	1.15	1.22	0.052	0.544
iso C17:0	0.37	0.33	0.010	0.065
anteiso C17:0	0.49	0.45	0.011	0.060
C18:0	12.13	13.80	0.267	<0.001
cis-9 C18:1	38.78	39.56	0.447	0.403
cis-11 C18:1	1.58	1.58	0.047	0.994
trans-6 + 7 + 8 18:1	0.28	0.24	0.011	0.027
trans-9 C18:1	0.26	0.22	0.011	0.066
trans-10 C18:1	1.58	0.72	0.149	0.001
trans-11 C18:1	0.72	0.48	0.055	0.006
C18:2 *n*-6	6.85	6.89	0.323	0.944
cis-9 *trans*-11 C18:2*	0.43	0.30	0.024	0.002
cis-11 *trans*-13 C18:2	0.02	0.01	0.002	0.064
C18:3 *n*-3	0.53	0.47	0.015	0.070
C20:0	0.09	0.10	0.004	0.183
C20:3 *n*-6	0.15	0.15	0.013	0.992
C20:4 *n*-6	1.40	1.39	0.136	0.963
C20:5 *n*-3	0.08	0.07	0.010	0.589
C22:4 *n*-6	0.13	0.15	0.014	0.624
C22:5 *n*-6	0.04	0.04	0.005	0.541
C22:5 *n*-3	0.22	0.21	0.024	0.878
C22:6 *n*-3	0.05	0.06	0.006	0.577
Summary				
Σ SFA[2]	41.80	42.59	0.255	0.128
Σ MUFA[3]	47.21	46.35	0.444	0.357
Σ PUFA[4]	10.71	10.67	0.524	0.975
Σ OBCFA[5]	2.80	2.80	0.060	0.978
trans-10/*trans*-11 18:1	2.27	1.49	0.226	0.084
Total *trans* 18:1	3.041	1.821	0.201	<0.001
Atherogenic index[6]	0.62	0.61	0.014	0.716
Thrombogenic index[7]	1.24	1.29	0.019	0.196

Fat soluble vitamins, ng/g muscle				
Retinol	229.05	311.21	21.131	0.048
α-Tocopherol	276.94	365.51	34.200	0.207
γ-Tocopherol	1.99	1.48	0.191	0.191
δ-Tocopherol	19.90	26.54	1.970	0.093

[1]Diets were: CON (control diet), CMD (cardoon meal diet)
[2]Σ SFA = C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0
[3]Σ MUFA = *c*-9 C14:1 + *c*-9 C16:1 + *c*-9 C18:1 + *c*-11 C18:1 + *t*-9 C18:1 + *t*-10 C18:1 + *t*-11 C18:1
[4]Σ PUFA = C18:2 *n*-6 + *c*-9, *t*-11 C18:2 + *c*-11, *t*-13 C18:2 + C18:3 *n*-3 + C20:3 n-6 + C20:4 *n*-6 + C20:5 *n*-3 + C22:4 *n*-6 + C22:5 *n*-6 + C22:5 *n*-3 + C22:6 *n*-3
[5]Σ OBCFA = *iso* C15:0 + *anteiso* C15:0 + *iso* C17:0 + *anteiso* C17:0
[6]Atherogenic index = (C12:0 + [4 × C14:0] + C16:0)/(*n*-3 PUFA + *n*-6 PUFA + MUFA).
[7]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]).
IMF: intramuscular fat; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; OBCFA: Odd-and branched-chain fatty acids
[*]*c*-9, *t*-11 C18:2 co-eluted with *t*-8,*c*-10 C18:2 and *t*-7,*c*-9 C18:2.

Table 4. Effect of the dietary treatment and time of storage or incubation on the oxidative stability parameters of meat

	Diet (D)[1]		Storage/incubation time (T)[2]			SEM	*P* values[3]		
	CON	CMD	1	2	3		D	T	D × T
Raw meat sclices									
TBARS, µg/g	0.92	1.13	0.18^{c}	0.83^{b}	2.03^{a}	0.136	0.129	< 0.001	0.036
Lightness, *L**	43.70	42.96	43.87	43.41	42.79	0.299	0.229	0.364	0.872
Redness, *a**	12.45	13.19	15.09^{a}	12.67^{b}	10.62^{c}	0.340	0.075	< 0.001	0.635
Yellowness, *b**	11.82	12.20	12.42	12.14	11.43	0.194	0.133	0.098	0.626
Saturation, *C**	17.21	17.99	19.55^{a}	17.56^{b}	15.62^{c}	0.356	0.146	< 0.001	0.630
Hue Angle, *H**	43.78	43.06	39.33^{c}	43.81^{b}	47.19^{a}	0.550	0.183	< 0.001	0.549
$(K/S)_{572} \div (K/S)_{525}$	0.93	0.92	0.99^{a}	0.91^{b}	0.87^{c}	0.009	0.392	< 0.001	0.880
Muscle homogenates									
TBARS, µg/g	2.43	2.73	0.15^{b}	3.63^{a}	3.96^{a}	0.271	0.102	< 0.001	0.437
Metmyoglobin, %	55.41	55.56	12.11^{c}	65.10^{b}	89.21^{a}	4.911	0.925	< 0.001	0.747
Cooked meat slices									
TBARS, µg/g	3.58	3.78	1.42^{c}	3.85^{b}	5.76^{a}	0.281	0.337	< 0.001	0.930

[1]Diets were: CON (control diet), CMD (cardoon meal diet)

[2]Times 1, 2, 3 correspond to: 0, 4, 7 days (raw meat stored at 4°C under aerobic conditions), 0, 30 and 60 minutes (muscle homogenates incubated with Fe/Asc at 37°C under continuous stirring) and 0, 2, 4 days (cooked meat stored at 4°C under aerobic conditions)

[3]*P* values for the effects of the dietary treatment (D), time of storage or incubation (T) and of the D × T interaction.

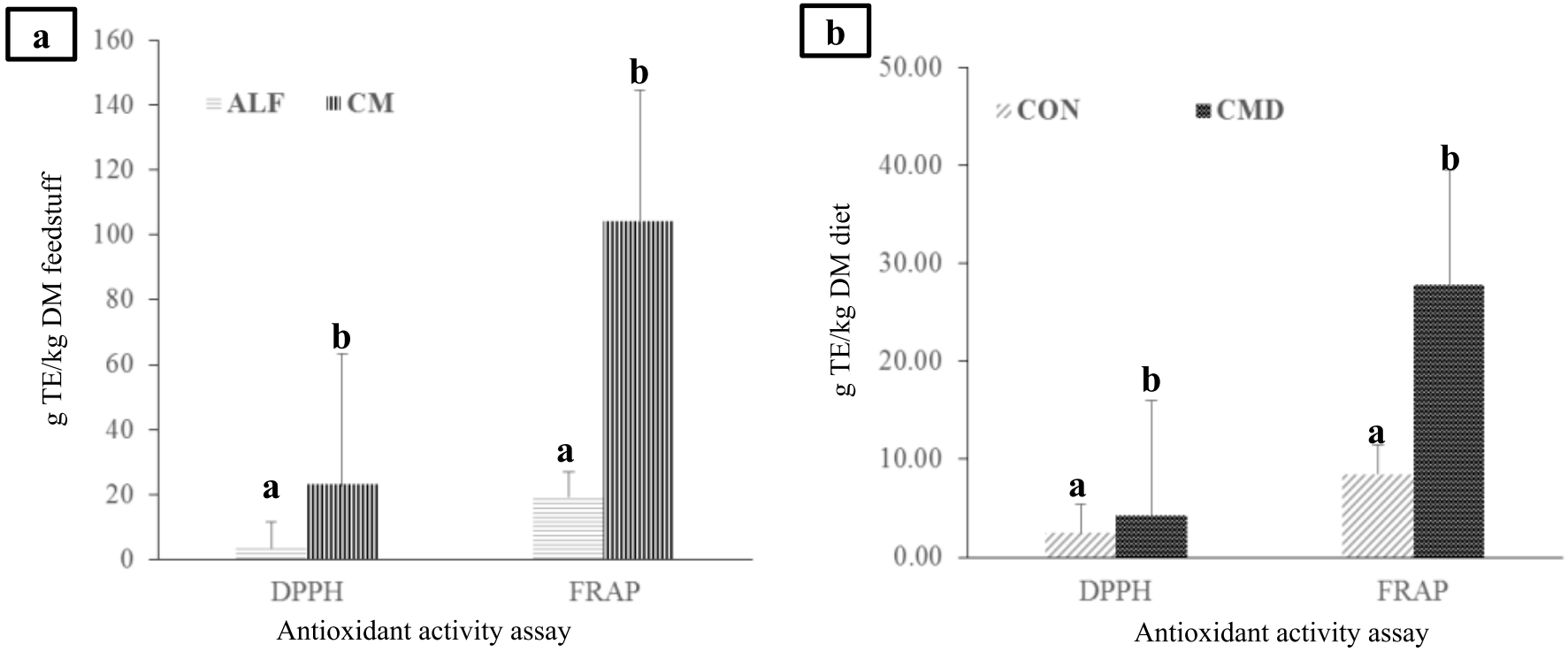
[a, b, c] Within row, different superscript letter indicate differences ($P < 0.05$) between times of storage

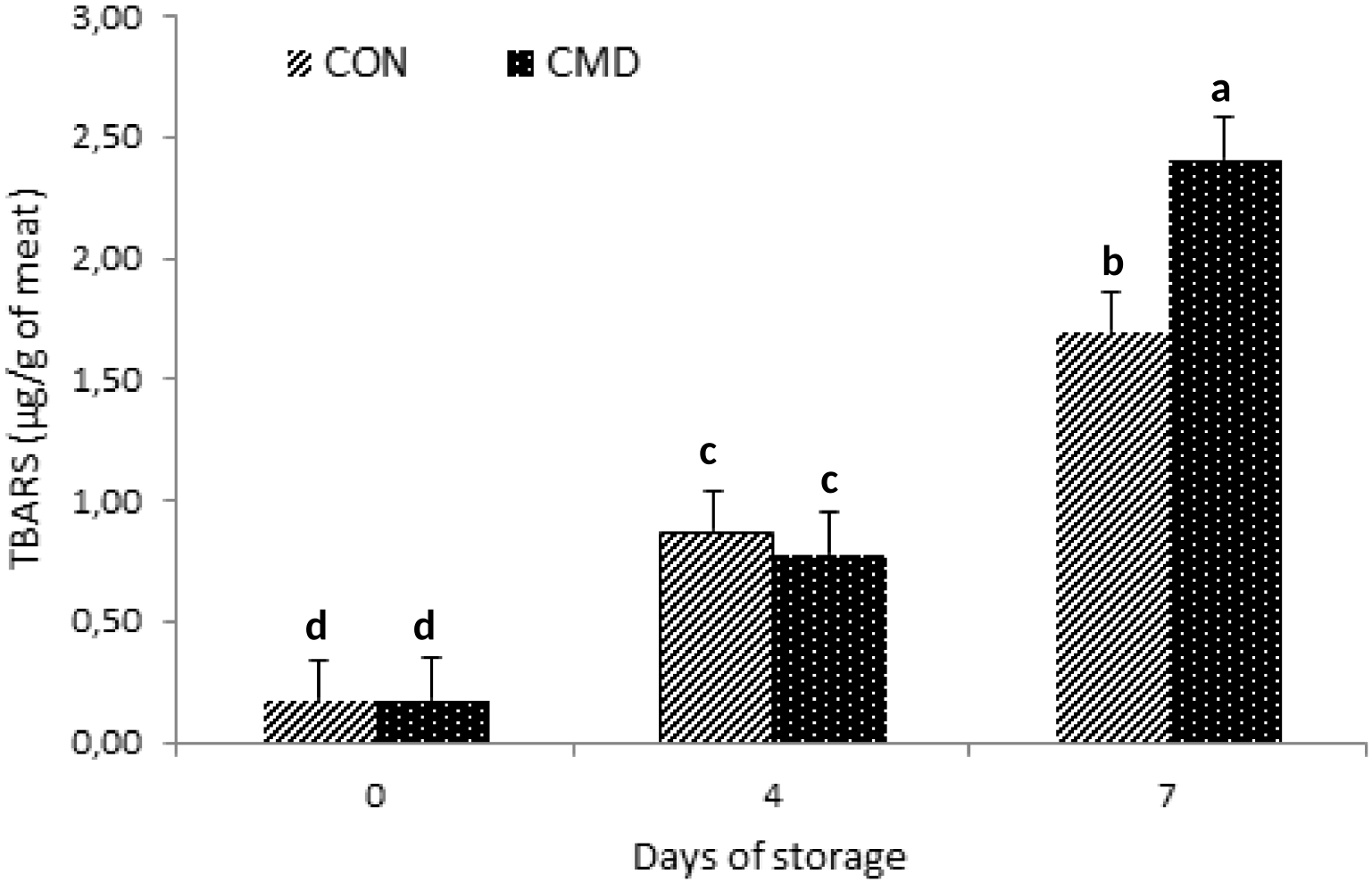
Figure captions:

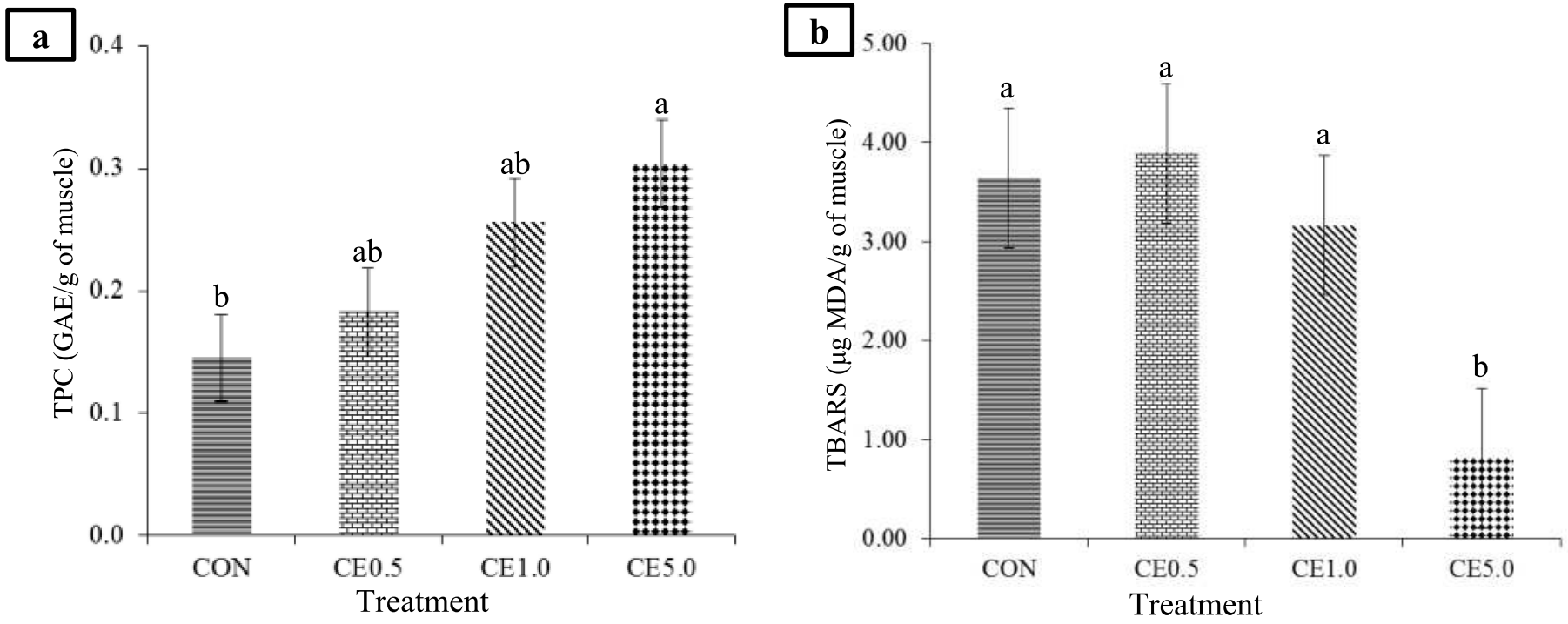
Figure 1. Antioxidant activity of **(a)** dietary test ingredients (feedstuff): dehydrated alfalfa (ALF) and cardoon meal (CM) **(b)** experimental diets: control (CON) and cardoon meal diet (CMD). Values are presented as means with standard error bars. [a,b]For antioxidant activity assay, mean values with different letters are significantly different ($P < 0.05$).
DPPH: 2,2-diphenyl-1-picrylhydrazyl assay (free radical scavenging activity); FRAP: Ferric reducing antioxidant power assay (total antioxidant activity); TE: trolox equivalent.

Figure 2. Interactive effect of the diet and time of storage (D × T) on the lipid oxidation (TBARS, µg MDA/g of muscle) measured in fresh *longissimus thoracis et lumborum* (LTL) muscle slices stored aerobically at 4°C for 7 days. Diets were: CON (control diet), CMD (cardoon meal diet). Values are presented as means with standard error bars. [a, b, c, d] Mean values with different letters are significantly different ($P < 0.05$)

Figure 3. Effect of cardoon extract (CE) addition on **(a)** total phenolic contents (TPC, GAE/g of muscle) **(b)** lipid oxidation (TBARS, µg MDA/g of muscle) of ovine *longissimus thoracis et lumborum* (LTL) muscle homogenates after 4 h of incubation with a $FeCl_3$/sodium ascorbate pro-oxidant system.
Treatments are as follows: CON, CE0.5, CE1.0 and CE5.0 represent addition of 0, 0.5%, 1.0%, and 5.0% of cardoon extract to muscle homogenates, respectively. Values are presented as means with standard error bars. [a,b]For each treatment, bars with different letters are significantly different ($P < 0.05$).
GAE: gallic acid equivalents; TBARS: thiobarbituric acid reactive substances.


a
160
140
120
100
80
60
40
20
0
ALF
CM
b
b
a
a
g TE/kg DM feedstuff
DPPH
FRAP
Antioxidant activity assay
b
50.00
40.00
30.00
20.00
10.00
0.00
CON
CMD
b
b
a
a
g TE/kg DM diet
DPPH
FRAP
Antioxidant activity assay


3,00
2,50
2,00
1,50
1,00
0,50
0,00
TBARS (µg/g of meat)
CON
CMD
a
b
c
c
d
d
0
4
7
Days of storage


a
0.4
0.3
0.2
0.1
0.0
TPC (GAE/g of muscle)
b
ab
ab
a
CON
CE0.5
CE1.0
CE5.0
Treatment
b
5.00
4.00
3.00
2.00
1.00
0.00
TBARS (μg MDA/g of muscle)
a
a
a
b
CON
CE0.5
CE1.0
CE5.0
Treatment