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Coláiste na hOllscoile Corcaigh

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4 1 **Influence of dietary cardoon meal on growth performance and selected meat quality**
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6 2 **parameters of lambs, and the antioxidant potential of cardoon extract in ovine muscle**
7
8 3 **homogenates**
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59 **21 Abstract**
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61 22 Fatty acids and oxidative stability were determined in meat from lambs fed a diet containing
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63 23 15% dehydrated alfalfa (CON, $n=8$) or cardoon meal (CMD, $n=7$). Furthermore, the antioxidant
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65 24 activity of a phenolic-rich cardoon meal extract (1.32 GAE mg/ml) was examined in muscle
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67 25 homogenates (0, 0.5, 1, 5% v/w) subjected to iron/ascorbate-induced oxidation. Feeding CMD
68
69 26 did not affect lamb performances and carcass traits but reduced ($P < 0.05$) the vaccenic and
70
71 27 rumenic acids and increased stearic acid in muscle. Lipid oxidation was higher in raw meat from
72
73 28 the CMD-fed lambs after 7 days of storage ($P < 0.05$). Feeding CMD did not affect the colour
74
75 29 stability of raw meat and the oxidative stability of cooked meat and of muscle homogenates
76
77 30 incubated with pro-oxidant catalysts. Adding 5% cardoon extract in muscle homogenates
78
79 31 increased (+114.3%; $P = 0.03$) the total phenolic content and reduced (-77.6%; $P < 0.01$) lipid
80
81 32 oxidation, demonstrating the antioxidant potential of compounds present in cardoon meal.
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88 34 **Keywords:** *Lamb meat, Growth performance, Fatty acids, Lipid oxidation, Cardoon,*
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90 35 *Polyphenols*
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115 **41 1. Introduction**
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118 42 Meat from ruminant animals (cattle, sheep, goat and buffalo) can be a viable dietary source of
119
120 43 bioactive fatty acids including conjugated linoleic acids (CLA), monounsaturated fatty acids
121
122 44 (MUFA) and *n*-3 long-chain polyunsaturated fatty acids (PUFA; Bessa, Alves, & Santos-Silva,
123
124 45 2015). However, ruminant meat also contains high levels of saturated fatty acids (SFA) and
125
126 46 *trans*-fatty acids (TFA) linked to increased risk of cardio-metabolic diseases in humans (McAfee
127
128 47 et al., 2010). The fatty acid profile of ruminant meat is significantly influenced by extensive
129
130 48 conversion of dietary PUFA to SFA during biohydrogenation of lipids that occur in the fore-
131
132 49 stomach (rumen) (Shingfield, Bonnet, & Scollan, 2013). However, feeding strategies such as
133
134 50 supplementation of PUFA-rich vegetable oil could increase the intramuscular deposition of
135
136 51 PUFA and CLA and reduce SFA levels in ruminant meat (Bessa et al., 2015).
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140 52 Lipid oxidation is the main chemical process associated with oxidative deterioration of meat,
141
142 53 resulting in undesirable rancid off-flavour and colour deterioration that limit shelf-life and
143
144 54 negatively impact consumer acceptability of meat (Morrissey, Sheehy, Galvin, Kerry, &
145
146 55 Buckley, 1998). The balance between antioxidant and pro-oxidant components in muscle tissues
147
148 56 mainly influences lipid stability. Animal diets could influence the biochemical components of
149
150 57 muscle tissues through enrichment with antioxidant compounds, such as α -tocopherol (vitamin
151
152 58 E), that enhance the oxidative stability of meat (Luciano et al., 2013; Salami et al., 2016).
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155

156 59 Phenolic compounds are secondary metabolites that are ubiquitous in several plant species and
157
158 60 may be found in considerable amounts in agro-industrial by-products (AIBP; Balasundram,
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160 61 Sundram, & Samman, 2006). The AIBP can be a valuable and economical resource for animal
161
162 62 feeding or for the extraction of bioactive phenolic compounds used in functional food
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171 63 applications. Feeding phenolic-rich AIBP improves the fatty acid composition of ruminant meat
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173 64 through inhibition of ruminal biohydrogenation (Lanza et al., 2015) and may enhance the
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175 65 oxidative stability of meat through controversial antioxidant mechanisms (Vasta & Luciano,
176
177 66 2011). Phenolics could exhibit *in vivo* antioxidant activities through direct deposition of these
178
179 67 compounds in tissues or through possible indirect antioxidant mechanisms including the sparing
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181 68 effect of polyphenols on other antioxidants, such as ascorbic acid and tocopherols (Valenti et al.,
182
183 69 2018). Furthermore, phenolic-rich extracts obtained from AIBP have been directly incorporated
184
185 70 into meat products during processing to inhibit oxidative deterioration and extend shelf-life
186
187 71 (Balzan et al., 2017; Kanatt, Chander, Radhakrishna, & Sharma, 2005; Rodríguez-Carpena,
188
189 72 Morcuende, & Estévez, 2011).

192
193 73 Cardoon meal is a by-product retained after the extraction of oil from the seeds of cultivated
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195 74 cardoon (*Cynara cardunculus* var. *altilis*), a perennial herb native to the Mediterranean region
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197 75 and widespread in parts of Europe, Americas and Oceania. Cardoon meal has been proposed as
198
199 76 an alternative feed resource because of its potential as a valuable source of fibre, protein, amino
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201 77 acids and bioactive compounds such as polyphenols and unsaturated fatty acids (Genovese et al.,
202
203 78 2015). Cardoon meal may also contain residual amount of other antioxidant compounds such as
204
205 79 α -tocopherol found in considerable concentration in cardoon seed oil (Maccarone et al., 1999).
206
207 80 Moreover, extracts obtained from different morphological parts (leaf, seed, stem and flower) of
208
209 81 cardoon have demonstrated *in vitro* antioxidant and antimicrobial activities attributed to the
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211 82 phenolic constituents present (Falleh et al., 2008; Pandino, Lombardo, Mauromicale, &
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213 83 Williamson, 2011; Ramos et al., 2014). To our knowledge, no information has been published on
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215 84 the effect of dietary cardoon meal or cardoon extract on meat quality. Therefore, the first
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217 85 objective of this study was to determine the effect of dietary cardoon meal on lamb growth
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227 86 performance, carcass characteristics, and meat fatty acid composition and oxidative stability. In
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229 87 addition, the antioxidant potential of a phenolic-rich cardoon meal extract on lipid oxidation in
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231 88 an ovine muscle model system was investigated.
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234 89 **2. Materials and Methods**

236 90 **2.1. Animals, diets and experimental design**

238
239 91 The experiment was conducted indoors in the experimental farm of the University of Catania.
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241 92 The animals were handled by trained personnel according to the European Union legislation for
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243 93 the protection of animals used for scientific purposes (2010/63/ EU Directive) and the study was
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245 94 approved by the University of Catania (FIR-2014-PI/LB/Di3A). Fifteen male Sarda x Comisana
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247 95 lambs (age 75 d and average weight 19.58 ± 2.01 kg) were randomly assigned to two
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249 96 experimental groups. Each animal was reared in an individual pen and adapted to the
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251 97 experimental diets for a period of 9 d by progressive substitution of the weaning feed with the
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253 98 experimental feeds until a total replacement of the weaning diet was achieved. The control group
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255 99 (CON, $n = 8$), was raised on a commercial concentrate-based diet containing the following
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257 100 ingredients (*as-fed* basis): barley (48.0%), dehydrated alfalfa (15.0%), wheat bran (23.0%),
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259 101 soybean meal (10.0%), molasses (2.0%) and vitamin premix (2.0%). The cardoon meal group
260
261 102 (CMD, $n = 7$), received the same diet as the CON lambs except that the 15% dehydrated alfalfa
262
263 103 was completely replaced by cardoon meal. The chemical composition of the experimental diets is
264
265 104 outlined in Table 1. The CON and CMD diets were supplied in form of pellets and lambs had *ad*
266
267 105 *libitum* access to feeds and water for 75 days pre-slaughter. Experimental feed samples were
268
269 106 collected at two-week intervals during the feeding trial and stored in vacuum packs at -30 °C
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271 107 prior to chemical analysis. Diets were supplied daily and the amount of refusal was measured
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273 108 before morning feeding to calculate dry matter intake (DMI). The body weight (BW) of the
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283 109 lambs was measured at the start of the experiment and recorded weekly (at 09:00 h before
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285 110 providing fresh feed) to calculate average daily gain (ADG).
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289 290 112 ***2.2. Slaughter, and carcass sampling and measurement*** 291 292

293 113 The lambs were slaughtered (stunned by captive bolt before exsanguination) in a commercial
294
295 114 abattoir, where they had free access to the experimental diets and water until approximately 3 h
296
297 115 before slaughter. Carcass weight was recorded following removal of the visceral organs and
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299 116 dressing percentage was calculated as the percentage of carcass weight to final BW. Visual
300
301 117 appraisal of hot hanging carcasses was performed by a certified meat grader to determine the
302
303 118 conformation and fat cover scores according to the European Union's EUROP carcass
304
305 119 classification system (Commission Regulation (EC) No 823/98, 1998) . Carcass conformation
306
307 120 and fatness scores were based on a five-point scale and each score class was further classified
308
309 121 into high, medium and low to obtain a 15-point score for a more precise description of carcass
310
311 122 traits. Carcasses were stored at 4°C for 24 h *post-mortem* and ultimate pH (pH₂₄) was measured
312
313 123 on the *longissimus thoracis* muscle using a pH-meter (Orion 9106). Colour descriptors lightness
314
315 124 (L*), redness (a*), yellowness (b*), Chroma (C*), and Hue angle (H*) were recorded on the
316
317 125 *longissimus thoracis* muscle, using Minolta CM-2022 spectrophotometer (d/8° geometry;
318
319 126 Minolta Co., Ltd. Osaka, Japan) on specular components excluded (SCE) mode, illuminant A
320
321 127 and a 10° standard observer. After 24 h of storage at 4 °C, the entire *longissimus thoracis et*
322
323 128 *lumborum* (LTL) muscle from the right half-carcass was removed, packed under vacuum and
324
325 129 stored at -80 °C for analyses of intramuscular fatty acids and tocopherols. The entire LTL from
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327 130 the left half-carcass was vacuum-packed and stored at 4 °C for 3 days, pending oxidative
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329 131 stability measurements.
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339 132 **2.3. Feed analysis**
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342 133 **2.3.1. Analysis of chemical composition, fatty acids and vitamin E**
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345 134 Dry matter (DM), crude protein, ether extract and ash content were determined in the
346
347 135 experimental diets following the method of AOAC (1995). The neutral detergent fibre, acid
348
349 136 detergent fibre and acid detergent lignin were analysed according to Van Soest, Robertson, and
350
351 137 Lewis (1991). The fatty acid composition of the experimental diets was determined by a one-step
352
353 138 extraction–transesterification procedure using chloroform (Sukhija & Palmquist, 1988) and 2%
354
355 139 (v/v) sulfuric acid in methanol (Shingfield et al., 2003). Gas chromatographic (GC) analysis of
356
357 140 fatty acid methyl esters (FAME) was performed as described later (section 2.3) for fatty acid
358
359 141 profile of lamb meat. Individual fatty acids of experimental diets were expressed as mg/g of DM.
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361
362 142 Vitamin E (α -, γ - and δ -tocopherols) was analysed in the experimental diets as described by
363
364 143 Cherif et al. (2018). Briefly, freeze-dried samples were homogenized and saponified with
365
366 144 ethanolic KOH stabilized with BHT. Tocopherols were extracted using hexane/ethyl acetate (9/1,
367
368 145 v/v), dried under N₂ and dissolved in acetonitrile. Vitamin E was analysed by HPLC (Perkin
369
370 146 Elmer series 200), equipped with an autosampler (model AS 950-10, Tokyo, Japan) and a
371
372 147 Synergy Hydro-RP column (4 μ m, 4.6 \times 100 mm; Phenomenex, Bologna, Italy). Tocopherols
373
374 148 were eluted at a flow rate of 2 ml/min and identified using a fluorescence detector (model Jasco,
375
376 149 FP-1525) set at an excitation and emission λ of 295 nm and 328 nm, respectively. Quantification
377
378 150 was based on external calibration curves of pure standard compounds (Sigma-Aldrich, Bornem,
379
380 151 Belgium) solubilised in ethanol.
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395 152 **2.3.2. Analysis of total phenol content and in vitro antioxidant activity**
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398 153 Phenolic compounds were extracted from cardoon meal, dehydrated alfalfa and experimental
399
400 154 diets using aqueous methanol (50:50, v/v) and acetone (70:30, v/v) solvents (Jiménez-Escrig,
401
402 155 Jiménez-Jiménez, Pulido, & Saura-Calixto, 2001). Polyphenol-rich extracts were analysed for
403
404 156 total phenol content (TPC) using the Folin-Ciocalteu method (Singleton, Orthofer, & Lamuela-
405
406 157 Raventós, 1999) with minor modifications. Briefly, extracts (0.5 ml) were mixed with Folin-
407
408 158 Ciocalteu reagent (2.5 ml, 20% in distilled water) and sodium carbonate (2 ml, 7.5% in distilled
409
410 159 water) was added after 5 min. The mixture was stored in the dark for 2 h at room temperature
411
412 160 and absorbance measurements were recorded at 750 nm using a UV-vis spectrophotometer (Cary
413
414 161 300 Bio, UV-vis spectrophotometer, Varian Instruments, CA, USA) against a blank containing
415
416 162 all reagents and distilled water. A calibration curve using standard solutions of aqueous gallic
417
418 163 acid (20 – 100 µg/ml) was plotted and results were expressed as g of gallic acid equivalents
419
420 164 (GAE)/kg of DM feed.
421
422

423
424 165 *In vitro* antioxidant activity of polyphenol-rich extracts was measured using 1,1-diphenyl-2-
425
426 166 picrylhydrazyl (DPPH) free radical scavenging (Yen & Wu, 1999) and ferric reducing
427
428 167 antioxidant power (FRAP) total antioxidant assays (Benzie & Strain, 1999), with minor
429
430 168 modifications. For the DPPH assay, extract (0.6 ml) and distilled water (2.4 ml) were mixed with
431
432 169 0.2 mM DPPH in methanol (3 ml) and stored in the dark for 1 h at room temperature.
433
434 170 Absorbance measurements were recorded at 517 nm using a UV-vis spectrophotometer (Cary
435
436 171 300 Bio) against a methanol blank. An assay blank containing distilled water (3 ml) and 0.2 mM
437
438 172 DPPH in methanol (3 ml) was used for calculation purposes. A calibration curve using standard
439
440 173 solutions of methanolic Trolox (10 – 50 µg/ml) was plotted and results were expressed as g of
441
442 174 Trolox equivalents (TE)/kg of DM feed.
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450
451 175 For the FRAP assay, extract (0.45 ml) was mixed with 8.55 ml FRAP reagent (a mixture of 30
452
453 176 mM acetate buffer (pH 3.6), 10 mM 2,4,6-Tris (2-pyridyl)-s-triazine in 40 mM HCl and 20 mM
454
455 177 FeCl₃.6H₂O in distilled water in the ratio 10:1:1, respectively incubated at 37 °C for 10 min prior
456
457
458 178 to use). The mixture was stored in the dark for 30 min at room temperature and absorbance
459
460 179 measurements were recorded at 593 nm using a UV-vis spectrophotometer (Cary 300 Bio)
461
462 180 against a blank containing all reagents. A calibration curve using standard solutions of
463
464 181 methanolic Trolox (0.033 – 0.1 mg/ml) was plotted and results were expressed as g of Trolox
465
466 182 equivalents (TE)/kg of DM feed.

468 469 183 ***2.4. Analysis of fatty acids and vitamin E in lamb meat***

470
471
472 184 Intramuscular fat was extracted from 10 g of minced LTL with a mixture of methanol and
473
474 185 chloroform (2:1, v/v) according to the method of Folch, Lees, and Sloane Stanley (1957). Lipids
475
476 186 (30 mg) were converted to FAME by base-catalysed transesterification (Christie, 1982) using 0.5
477
478 187 mL of sodium methoxide in methanol 0.5 N and 1 mL of hexane containing 1mg /mL
479
480 188 nonadecanoic acid (C19:0) as an internal standard. Gas chromatographic analysis was conducted
481
482 189 as described by Valenti et al. (2018) using a GC 8000 Top ThermoQuest (Milan, Italy) gas-
483
484 190 chromatograph equipped with a flame ionization detector and a high polar column (WCOT-fused
485
486 191 silica CP-Select CB for FAME Varian, Middelburg, the Netherlands; 100m×0.25mm i.d.; film
487
488 192 thickness 0.25 µm). Helium was the carrier gas at a constant flow of 1 ml/min. Total FAME
489
490 193 profile in a 1 µL sample volume (2 µL for feed samples) at a split ratio of 1:80 was determined
491
492 194 using the following conditions: the oven temperature was programmed at 40 °C and held for 4
493
494 195 min, then increased to 120 °C at 10 °C/min, held for 1 min, then increased up to 180 °C at 5
495
496 196 °C/min, held for 18 min, then increased up to 200 °C at 2 °C/min, held for 15 min, and then
497
498 197 increased up to 230 °C at 2 °C/min, held for 19 min. The injector and detector temperatures were
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198 set at 270 °C and 300 °C, respectively. The identification of individual FAME was based on the
 199 retention time comparison with commercially available standard mixture of FAME (Nu-Chek
 200 Prep Inc., Elysian, MN, USA; Larodan Fine Chemicals, Malmo, Sweden) and comparison with
 201 chromatograms published by Kramer, Hernandez, Hernandez, Kraft, and Dugan (2008) and
 202 Alves and Bessa (2007). Fatty acids were expressed as g/100 g of total fatty acids. The dietary
 203 risk for cardiovascular diseases was assessed by calculating the atherogenic index (the
 204 relationship between FA with pro-atherogenic and anti-atherogenic properties) and thrombogenic
 205 index (the relationship between FA with pro-thrombogenic and anti-thrombogenic properties).
 206 The atherogenic index (AI) and thrombogenic index (TI) were calculated as outlined by Ulbricht
 207 and Southgate (1991):

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

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

210 Vitamin E (α -, γ - and δ -tocopherols) and retinol were extracted from lamb muscle according to
 211 the method of Schüep and Rettenmaier (1994). Chromatographic analysis of vitamin E was
 212 performed as described in section 2.2.1 for feeds. Retinol was analyzed in the same
 213 chromatographic run and identified using the UV-VIS detector set at λ 325 nm (Cherif et al.,
 214 2018). Identification and quantification were achieved using external calibration curves of
 215 standard compounds (Sigma-Aldrich) solubilised in ethanol.

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562
563 216 **2.5. Measurement of lamb meat oxidative stability**
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566 217 Oxidative stability parameters were measured in raw and cooked lamb meat, as well as in LTL
567
568 218 homogenates incubated with pro-oxidant catalysts, as described by Valenti et al. (2018). Briefly,
569
570 219 for analyses on raw and cooked meat, 6 slices (2 cm thickness) were cut from the LTL which
571
572 220 was previously stored under vacuum at 4 °C as described in section 2.1. Three slices were placed
573
574 221 in polystyrene trays, covered with PVC film and stored at 4 °C. Each slice was used for
575
576 222 measuring lipid oxidation and colour stability at one of three-time points: day 0 (after 2 hours of
577
578 223 blooming), and days 4 and 7. The remaining 3 slices were packed under vacuum and cooked for
579
580 224 30 min at 70°C in a water bath. After cooling in a cold-water bath, one slice was used
581
582 225 immediately for measurement of lipid oxidation, while the other 2 slices were stored at 4°C in
583
584 226 the same conditions described for the raw meat samples, and lipid oxidation was measured after
585
586 227 2 and 4 days. For both raw and cooked meat, lipid oxidation was measured as thiobarbituric acid
587
588 228 reactive substances (TBARS) values according to the procedure of Siu and Draper (1978) and
589
590 229 results were expressed as µg malonaldehyde (MDA)/g of meat. Colour was measured in raw
591
592 230 meat using a Minolta CM-2022 spectrophotometer (d/8° geometry; Minolta Co., Ltd. Osaka,
593
594 231 Japan) set in the specular components excluded (SCE) mode, illuminant A and a 10° standard
595
596 232 observer. The colour descriptors lightness (L*), redness (a*), yellowness (b*), Chroma (C*), and
597
598 233 Hue angle (H*) were recorded, as well as the reflectance spectra from 400 to 700 nm. The ratio
599
600 234 $(K/S)_{572} \div (K/S)_{525}$ was calculated to monitor the accumulation of metmyoglobin (MetMb) on the
601
602 235 meat surface over time, with values of the ratio decreasing with increasing proportion of MetMb.
603
604 236 The ratio (K/S) between the absorption (K) and the scattering (S) coefficients at the selected
605
606 237 wavelengths was calculated as:
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608
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611 238
$$(K/S)_\lambda = (1 - R_\lambda)^2 / 2R_\lambda$$

612
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618
619 239 The resistance of meat to lipid and myoglobin oxidation was also assessed in LTL homogenates
620
621 240 incubated in the presence of Fe³⁺ and ascorbate (Fe/Asc) as catalysts of oxidative reactions.
622
623 241 Briefly, the minced LTL (7.5 g) was homogenized with 37.5 g of MES buffer (pH 5.6).
624
625 242 Homogenates were equilibrated to 37 °C and two aliquots (3 ml and 4 ml) were collected for
626
627 243 measuring the initial extent of lipid and myoglobin oxidation (0 minutes). Ferric chloride
628
629 244 hexahydrate and L-sodium ascorbate were added at equimolar concentration to reach the final
630
631 245 concentration of 45 µM. The homogenates were incubated under continuous stirring in a
632
633 246 temperature controlled IKA KS-4000 orbital shaker (IKA-Werke GmbH & Co. KG, Staufen,
634
635 247 Germany) set at 37°C and 190 rpm. After 30 and 60 minutes of incubation, two aliquots (3 ml
636
637 248 and 4 ml) were collected for lipid and myoglobin oxidation analyses. Lipid oxidation was
638
639 249 measured in the 3 ml aliquots using the method of Siu and Draper (1978). The 4 ml aliquots were
640
641 250 centrifuged at 6800 × g at 4 °C, filtered through Whatman 541 filter paper and directly scanned
642
643 251 in a UV/VIS spectrophotometer (UV-1601, Shimadzu Co., Milan, Italy). The absorbances at 503,
644
645 252 525, 557, 582, and 730 nm were used to calculate the proportion of metmyoglobin (MetMb %;
646
647 253 Tang, Faustman, & Hoagland, 2004).
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652 254 ***2.6. Determination of antioxidant potential of cardoon extract in a muscle model system***

653 654 255 ***2.6.1. Preparation of cardoon extract***

656
657 256 Phenolic compounds were extracted in triplicate by suspending finely-ground cardoon meal (0.5
658
659 257 g) in 100% methanol (20 ml) and incubated at room temperature in an orbital shaker (Max Q
660
661 258 6000 Shaker Thermo Fisher Scientific, Ireland) at 200 rpm for 4 h. After 4 h, the mixture was
662
663 259 filtered through Whatman No. 1 filter paper. Pooled solvent extracts were concentrated by
664
665 260 placing in a 50 ml round-bottomed flask and solvent (methanol) was removed by rotary
666
667 261 evaporation (Labo-Rota C-311, Resona Technics, Switzerland) for 1 h at 55 °C. The dried
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674
675 262 extract was re-suspended in 20 ml methanol and analysed for TPC using the Folin-Ciocalteu
676
677 263 reagent as described for experimental feeds (section 2.2.1.). Result was expressed as mg GAE/ml
678
679
680 264 extract.

682 265 **2.6.2. Preparation of muscle homogenates**

684
685 266 Fresh lamb LTL ($n = 3$) was obtained from a meat retail outlet (Cork, Ireland) and stored at 4 °C
686
687 267 prior to analysis. Muscle homogenates (25%) were prepared in triplicate following a minor
688
689 268 modification of the method described by O'Grady, Monahan, and Brunton (2001). Briefly, LTL
690
691 269 (15 g) was chopped and homogenised in 0.12 M KCL 5 mM histidine (45 ml), pH 5.5, using an
692
693 270 Ultra-turrax T25 homogeniser (Janke & Kunkel GmbH, IKA® Labortechnik, Staufen, Germany)
694
695 271 at 24,000 rpm for 5 min. The muscle tissue and buffer were surrounded by crushed ice to control
696
697 272 the temperature during homogenisation. Cardoon extract (stock concentration of 1.32 GAE
698
699 273 mg/ml) was added to the homogenates (39.2 g) at 0, 0.5, 1 and 5% v/w of the final volume (40
700
701 274 ml). Lipid oxidation in muscle homogenate samples was initiated by the addition of equimolar
702
703 275 FeCl₃/sodium ascorbate (45 µM) pro-oxidants. The TPC and lipid oxidation in muscle
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705 276 homogenate were measured after 4 h of storage at 4 °C.

708 277 **2.6.3. Measurement of TPC and lipid oxidation in muscle homogenate**

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711 278 The total phenolic content in muscle homogenates was measured in triplicate by mixing 10%
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713 279 TCA (0.5 ml), 0.05 M phosphate buffer (3 ml) and muscle homogenate (2 g). The mixture was
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715 280 centrifuged at 7800 ×g for 10 min at 4 °C using an Avanti® J-E Centrifuge (Beckman Coulter
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717 281 Inc., Palo Alto, CA, USA). The supernatant was filtered through Whatman No. 1 paper and the
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719 282 filtrate was analysed for TPC using the Folin-Ciocalteu reagent as described for experimental
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721 283 feeds (section 2.2.2). Results were expressed as g GAE/g muscle. Lipid oxidation was measured
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731 284 in triplicate following the TBARS assay described by Siu and Draper (1978) and results were
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733 285 expressed as $\mu\text{g MDA/g meat}$.
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736 286 **2.7. Statistical analysis**

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739 287 The average data of DMI, BW and feed efficiency for individual lambs were subjected to one-
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741 288 way ANOVA test to analyse the effect of dietary treatment on growth performance parameters.
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743 289 The effect of dietary treatment on carcass traits, intramuscular fatty acids and fat-soluble
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745 290 vitamins, were also analysed with one-way ANOVA test, using individual lambs as the
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747 291 experimental units. Data on the oxidative stability parameters (raw, cooked and muscle
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749 292 homogenates) were analysed with a full-repeated measures ANOVA. Effects of diet represented
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751 293 the ‘between-subjects’ factor and the effect of storage time/incubation was measured using the
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753 294 ‘within-subjects’ factor and the interaction between diet and storage time/incubation was tested.
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755 295 Individual lambs were considered as the experimental units in all the statistical analyses
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757 296 performed for the effect of dietary cardoon meal on growth performance and meat quality.
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759 297 Analyses relating to the effect of cardoon extract addition on TPC and lipid oxidation (TBARS)
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761 298 in LTL homogenates was performed in triplicate and mean sample values ($n = 3$) for each of the
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763 299 four treatment groups (CON, CE0.5, CE1.0 and CE5.0) were subjected to a one-way ANOVA.
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765 300 Pearson’s correlation analysis was performed to assess the relationship between the TPC and
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767 301 TBARS in LTL homogenates. Significance was declared when $P \leq 0.05$, while a tendency for
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769 302 effects were considered when $0.05 < P \leq 0.10$. All statistical analyses were performed using the
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771 303 SPSS software (IBM Statistics version 22).
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788 304 **3. Results and Discussion**

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790 305 **3.1. Antioxidant activity of experimental feedstuffs and diets**

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792 306 The TPC of cardoon meal was 4-fold higher ($P < 0.01$; 60.4 vs 15.5 g GAE/kg DM) than
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794 307 dehydrated alfalfa, which may explain the 2.5-fold increase in TPC when dehydrated alfalfa was
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796 308 replaced by cardoon meal in CMD, compared to CON (Table 1). The phenolic concentration of
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798 309 cardoon meal was 5-fold higher than previously reported for cardoon press cake (Genovese et al.,
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800 310 2015), a similar by-product obtained from the mechanical extraction of oil from cardoon seeds.
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802 311 Variation in the TPC of cardoon extracts may be related to factors such as plant geographical
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804 312 origin, varieties and maturity stage, agricultural methodologies, and phenol extraction procedures
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806 313 (Ramos et al., 2014). As expected, assessment of the *in vitro* antioxidant activities showed that
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808 314 cardoon meal exhibited a higher ($P < 0.01$) DPPH free radical scavenging activity and FRAP
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810 315 total antioxidant activity compared to dehydrated alfalfa (Figure 1a). Similarly, replacement of
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812 316 dehydrated alfalfa with cardoon meal in the experimental diets resulted in higher ($P < 0.01$)
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814 317 antioxidant activities in CMD compared to CON (Figure 1b).

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818 318 Flavonoids and hydroxycinnamic acids are the main classes of phenolic compounds, which
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820 319 contribute to the antioxidant effect of extracts obtained from cardoon leaf, seed, stem and flower
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822 320 (Falleh et al., 2008; Pandino et al., 2011; Pinelli et al., 2007; Ramos et al., 2014). It has been
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824 321 shown that the TPC of cardoon extracts strongly correlated with DPPH antiradical activity
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826 322 (Falleh et al., 2008; Ramos et al., 2014) and FRAP total antioxidant capacity (Pandino et al.,
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828 323 2011). In addition, α -tocopherol is another potent antioxidant which can be found in high
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830 324 residual levels in cardoon meal as cardoon oil has been shown to contain considerable amounts
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832 325 of vitamin E (Maccarone et al., 1999). This is consistent with the 4-fold increase in α -tocopherol
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834 326 content found in CMD compared to CON (Table 1).
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843 327 **3.2. Growth performance and carcass traits**
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846 328 Lambs fed CMD exhibited lower ($P < 0.05$) DMI compared to CON-fed lambs. Lower DMI in
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848 329 lambs fed CMD may be related to low feed palatability due to the high content of dietary
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850 330 phenolic compounds. Cajarville, González, Repetto, Rodríguez, and Martínez (1999) reported a
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852 331 similar decrease in the voluntary intake of sheep fed *ad libitum* green forage of cardoon possibly
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854 332 due to the presence of high concentration of phenolic compounds in cardoon leaves (Kukić et al.,
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856 333 2008). Phenolic compounds, such as tannins, may confer unpleasant taste or bind to salivary
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858 334 proteins forming a polyphenol-protein complex that induce astringency sensations and trigger
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860 335 low feed intake in animals (Makkar, 2003). However, dietary treatment did not affect ($P > 0.05$)
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862 336 growth performance parameters in terms of final BW, ADG and feed efficiency (Table 2).
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864 337 Differences in DMI did not reflect on growth performance parameters possibly due to the low
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866 338 number of experimental units or that reduced DMI in CMD-fed lambs did not compromise
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868 339 nutrient digestibility and utilization. Accordingly, Cajarville, González, Repetto, Alvir, and
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870 340 Rodríguez (2000) reported that up to 25% cardoon seeds could be included in ruminant diets
871
872 341 without negative effect on ruminal fermentation pattern and nutrient digestibility. Furthermore,
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874 342 there was no effect of dietary treatment on carcass characteristics such as carcass weight,
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876 343 dressing percentage, carcass conformation, fatness scores, muscle ultimate pH and colour
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878 344 characteristics (Table 2). Notably, values of ultimate pH were slightly higher than the normal pH
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880 345 values (5.4 – 5.8) for post-mortem muscle (Faustman & Cassens, 1990) particularly for lambs
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882 346 fed CON due to the fact that one carcass displayed an ultimate pH of 6.3. Nonetheless, the values
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884 347 of colour variables are within the satisfactory range for average consumer acceptance of fresh
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886 348 meat, particularly in terms of lightness (L^*) and redness (a^*) (Khlijji, Van de Ven, Lamb, Lanza,
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888 349 & Hopkins, 2010).
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899 350 **3.3. Effect of dietary cardoon meal on lamb meat fatty acid composition and fat-soluble**
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901 **351 vitamin levels**

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904 352 Intramuscular fat, fatty acid profile and fat-soluble vitamin contents in LTL muscle from lambs
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906 353 fed CON and CMD diets are presented in Table 3. In general, dietary treatment did not influence
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908 354 ($P > 0.05$) intramuscular fat content or the total composition of SFA, MUFA, PUFA, odd- and
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910 355 branched-chain fatty acids (OBCFA). Dietary treatment did not influence ($P > 0.05$) the
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912 356 concentration of individual SFA (C12:0, C14:0 and C16:0) that results in hypercholesterolemic
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914 357 effects in humans (Mensink, 2005). This could in part explain why meat from lambs fed CON
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916 358 and CMD had similar ($P > 0.05$) nutritional indices (atherogenic index and thrombogenic index)
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918 359 for risk of cardiovascular diseases. However, lambs fed CMD displayed higher concentration (P
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920 360 < 0.05) of intramuscular C18:0 (stearic acid), and lower proportions ($P < 0.05$) of total *trans*-
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922 361 18:1, *trans*-10 C18:1, *trans*-11 C18:1 (vaccenic acid) and *cis*-9 *trans*-11 C18:2 CLA (rumenic
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924 362 acid) compared to CON-fed lambs. In addition, the proportion of C18:3 *n*-3 (α -linolenic acid)
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926 363 tended ($P = 0.070$) to be greater in the muscle of lambs fed CON compared to CMD-fed lambs.
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928 364 Consequently, the ratio of *n*-6:*n*-3 PUFA ($P = 0.052$) tended to be lower in CON-fed lambs
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930 365 compared to CMD-fed lambs. Regarding fat-soluble vitamins in LTL, the concentration of
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932 366 retinol ($P = 0.048$) was greater in muscle from lambs fed CMD relative to CON but diet did not
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934 367 affect ($P > 0.05$) vitamin E (α -, γ - and δ -tocopherols) concentration (Table 3).

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939 368 Indeed, changes in intramuscular fatty acids might be due to possible differences in ruminal
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941 369 biohydrogenation. The present study showed that dietary treatment significantly affected the
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943 370 intramuscular concentration of individual fatty acids (stearic, *trans*-10 C18:1, vaccenic and
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945 371 rumenic acids) with potential nutritional implications. It has been suggested that dietary
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947 372 consumption of stearic acid does not increase plasma low-density lipoprotein and cholesterol
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955 373 levels in humans in contrast to the negative effect of other SFA that are risk factors for cardio-
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957 374 metabolic diseases (Mensink, 2005). Thus, the greater amount of stearic acid in lambs fed CMD
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959 375 may not have detrimental effects on human health. Muscle from lambs fed CON exhibited a
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961 376 higher proportion of vaccenic and rumenic acids that are known for their potential health benefits
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963 377 in humans (Bessa et al., 2015). Both vaccenic and rumenic acids are intermediate FA synthesized
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965 378 during ruminal biohydrogenation but rumenic acid can be further synthesized in muscle tissues
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967 379 through endogenous desaturation of vaccenic acid by the enzyme Δ -9-desaturase (Corl et al.,
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969 380 2001). Thus, higher concentration of intramuscular rumenic acid in CON-fed lambs may be due
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971 381 to greater ruminal outflow and absorption of vaccenic acid that serves as the main precursor for
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973 382 the endogenous synthesis of rumenic acid in muscle tissues. Rumenic acid is the major naturally
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975 383 occurring CLA isomer found in ruminant meat and milk (Bessa et al., 2015) and dietary
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977 384 consumption of this FA potentially prevent human diseases including cancer, cardiovascular
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979 385 diseases, obesity, bone density loss, and diabetes (McGuire & McGuire, 2000).

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983 386 From a nutritional perspective, the accumulation *t*-10 C18:1 in ruminant edible products is
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985 387 undesirable due to the potential cytotoxic effect of this fatty acid (Vahmani et al., 2016). Thus, a
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987 388 lower concentration of *t*-10 C18:1 in meat of lambs fed CMD may be viewed as a positive
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989 389 outcome compared to CON. Concentrate feeding systems are known to promote the alternative
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991 390 ruminal biohydrogenation pathway that increase the synthesis of intermediates such as *t*-10 18:1,
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993 391 at the expense of *t*-11 18:1, referred to as the *t*-10 shift (Bessa et al. 2015). Bessa et al. (2015)
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995 392 postulated that the ratio of *t*-10/*t*-11 18:1 >1 in ruminant meat or milk is an indicator of
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997 393 occurrence of *trans*-10 shift during ruminal biohydrogenation. Considering that concentrate diets
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999 394 were fed in this study, it is not surprising that *t*-10/*t*-11 18:1 was > 1 in meat from lambs fed
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1001 395 CON and CMD, suggesting that both diets induced *t*-10 shift. However, feeding CMD tended (*P*

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1011 396 = 0.084) to reduce the occurrence of the *t*-10 shift, suggesting that the presence of phenolics in
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1013 397 CMD might have modified the ruminal biohydrogenation pathway.
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1016 398 A number of factors may account for the lack of positive effect of CMD to inhibit ruminal
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1018 399 biohydrogenation and increase PUFA and CLA contents in meat. It is possible that
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1020 400 hydroxycinnamic acids, the major cardoon phenolic compounds, were metabolised by the
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1022 401 consortium of microbes residing in the fore-stomach (rumen) of lambs as shown with human
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1024 402 faecal microbiota (Gonthier et al., 2006). Furthermore, effects of dietary phenolics to increase
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1026 403 PUFA and CLA contents in ruminant meat have been largely reported for diets containing a high
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1028 404 concentration of tannins (Vasta, Nudda, Cannas, Lanza, & Priolo, 2008). Though the tannin
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1030 405 content of diets was not measured in the present study, it has been shown that cardoon extracts
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1032 406 contain a very low concentration of tannins (Falleh et al., 2008). Thus, results from the present
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1034 407 study emphasized the variation in the effect of diets containing a high concentration of bioactive
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1036 408 compounds, which may be dependent on the type and/or concentration of phenolic compounds
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1038 409 present. In contrast, alfalfa fed in various processed forms (fresh, hay, dehydrated, silage) has
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1040 410 been documented to enrich lamb muscle and offal with lower SFA and higher content of PUFA
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1042 411 and rumenic acid (Cerci, Ciftci, Bahsi, & Kilinc, 2011; Ciftci et al., 2010; Realini, Bianchi,
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1044 412 Bentancur, & Garibotto, 2017). The positive effects of dietary alfalfa on the fatty acid profile of
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1046 413 ruminant meat may be due to the presence of saponins and flavonoids which inhibit ruminal
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1048 414 biohydrogenation (Petersen & Jensen, 2014).
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1053 415 ***3.4. Effect of dietary cardoon meal on oxidative stability of lamb meat***

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1056 416 The results of oxidative stability of raw meat, cooked meat and muscle homogenates are
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1058 417 presented in Table 4. As expected, lipid oxidation (TBARS values) significantly increased ($P <$
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1060 418 0.001) in raw meat as a function of storage time. Although in raw meat an overall effect of the
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1067 419 dietary treatment was not found on lipid oxidation, and interactive effect of the dietary treatment
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1069 420 and time of storage was found ($P < 0.05$), with higher TBARS values measured in raw meat
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1071 slices from the CMD-fed lambs compared to the CON treatment after 7 days of storage (Figure
1072 421
1073 2). This result is not easy to explain, considering the lack of effect of the dietary treatment on the
1074 422
1075 concentration of PUFA and α -tocopherol in muscle. It can be speculated that a higher content of
1076 423
1077 CLA (cis-9 trans-11 18:2) in the meat from CON-fed lambs could be responsible for the reduced
1078 424
1079 lipid oxidation as CLA may exert a protective effect on muscle oxidation (Joo, Lee, Ha, & Park,
1080 425
1081 2002). Hur et al. (2004) also reported that the CLA present in meat does not participate in
1082 426
1083 oxidation processes and reduces the formation of fatty acid free radicals, which results in
1084 427
1085 reduced lipid oxidation. Noteworthy, as shown in Table 4, neither the dietary, nor the diet \times time
1086 428
1087 interaction affected the colour stability of raw lamb meat measured using instrumental colour
1088 429
1089 descriptors (lightness L^* , redness a^* , yellowness b^* , saturation C^* and hue angle H^*) and an index
1090 430
1091 for metmyoglobin accumulation ($(K/S)_{572} \div (K/S)_{525}$). The measured parameters mostly related
1092 431
1093 to meat browning were only affected by the storage period, with redness, saturation and the
1094 432
1095 $(K/S)_{572} \div (K/S)_{525}$ decreasing over time ($P < 0.05$), while hue increased ($P < 0.05$).
1096 433
1097 Additionally, as shown in Table 4, the use of stronger pro-oxidant conditions (i.e. cooking and
1098 434
1099 incubation of muscle homogenates with $FeCl_3$ /sodium ascorbate pro-oxidants) greatly promoted
1100 435
1101 lipid and myoglobin oxidation which markedly increased over time ($P < 0.001$). However, the
1102 436
1103 different susceptibility to lipid oxidation observed in raw meat was not evident in cooked meat
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1105 and in muscle homogenates, which suggests that such oxidative challenges might have overcome
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1107 inherent differences between treatments in the susceptibility to oxidation.
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1113 440 In general, feeding CMD did not improve the oxidative stability of meat despite the greater
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1115 441 content of phenolic compounds and the consequently higher antioxidant capacity of the CMD
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442 diet compared to CON (Table 1 and Figure 1b). Indeed, the *in vivo* antioxidant potential of
443 dietary phenolics is still controversial. There are evidences that phenolics may exhibit
444 antioxidant effect through direct absorption and deposition into muscle tissues or through
445 indirect antioxidant mechanisms including the sparing effect of polyphenols on other
446 antioxidants, such as ascorbic acid and tocopherols (Valenti et al., 2018). As previously
447 highlighted in this study, possible microbial metabolism of cardoon phenolics in the gut of lambs
448 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

450 Antioxidant effect of the phenolic-rich extract obtained from cardoon meal was tested in LTL
451 homogenates subjected to FeCl₃/sodium ascorbate-induced oxidation by incubating with
452 iron/ascorbate pro-oxidants for 4 h. Addition of 5% cardoon extract significantly increased
453 (+114.3%; *P* < 0.05) the total phenolic content (TPC) in LTL homogenates compared to the
454 control (Figure 3a). Similarly, addition of 5% extract inhibited (-77.6%; *P* < 0.001) lipid
455 oxidation (TBARS values) relative to the control (Figure 3b). Pearson's correlation analysis
456 showed that TPC negatively correlated (*r* = -0.724; *P* = 0.008) with TBARS values, suggesting
457 that cardoon phenolics contributed to the inhibition of lipid oxidation in LTL homogenates. The
458 present study suggests that the antioxidant effect of cardoon extract in an ovine muscle system is
459 dose-dependent as only the addition of 5% cardoon extract significantly inhibited lipid oxidation
460 relative to the control. Similarly, Falleh et al. (2008) demonstrated that cardoon extracts
461 exhibited *in vitro* antioxidant activities (DPPH radical and superoxide anion scavenging
462 activities) in a concentration-dependent manner.

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

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

472 **4. Conclusions**

473 The present study demonstrated that cardoon meal is a rich source of phenolic compounds with
474 potent antioxidant activity. The inclusion of 15% cardoon meal replacing dehydrated alfalfa in a
475 concentrate diet had no adverse effect on lamb growth performance but depressed feed intake.
476 Dietary cardoon meal did not influence the intramuscular composition of SFA, MUFA, PUFA
477 and nutritional indices (atherogenic index and thrombogenic index) but presented a lower
478 concentration of potentially health-promoting fatty acids (vaccenic and rumenic acids) in lamb
479 meat. Moreover, dietary inclusion of cardoon meal did not improve the oxidative stability of raw
480 and cooked meat stored aerobically at 4 °C for up to 7 and 4 days, respectively. In addition, a
481 phenolic-rich extract obtained from cardoon meal exhibited potent antioxidant activity against
482 lipid oxidation in an ovine muscle model system. Further research is required to evaluate the
483 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

| Parameter | Diets ¹ | |
|-------------------------------------|--------------------|--------|
| | CON | CMD |
| Dry matter (DM), % <i>as-fed</i> | 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 |
| <i>Fatty acids (mg/g DM)</i> | | |
| C14:0 | 0.034 | 0.034 |
| C16:0 | 4.357 | 5.062 |
| <i>cis</i> -9 C16:1 | 0.035 | 0.034 |
| C18:0 | 0.454 | 0.705 |
| C18:1 <i>n</i> -9 | 3.855 | 5.362 |
| <i>cis</i> -11 C18:1 | 0.209 | 0.206 |
| C18:2 <i>n</i> -6 | 12.190 | 16.852 |
| C18:3 <i>n</i> -3 | 1.255 | 1.065 |
| C20:0 | 0.088 | 0.086 |
| <i>Vitamins (µg/g DM)</i> | | |
| α-Tocopherol | 5.267 | 20.877 |
| γ-Tocopherol | 0.155 | 0.119 |
| δ-Tocopherol | 0.012 | 0.005 |

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

²Expressed as grams gallic acid equivalents/kg DM

NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin

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

| Parameter | Dietary ¹ | | SEM | P-value |
|---------------------------------|----------------------|-------|-------|---------|
| | CON | CMD | | |
| <i>Growth performance</i> | | | | |
| 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 |
| <i>Carcass traits</i> | | | | |
| 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 |

¹Diets were: CON (control diet), CMD (cardoos meal diet)

²Calculated as: g BW gain/kg DMI

³Conformation score: E = 5, excellent shape and muscularity; U = 4; R = 3; O = 2; P = 1, poor shape and muscularity.

⁴Fatness score: 1 = low, 5 = very high

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

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

¹Diets 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

⁶Atherogenic index = (C12:0 + [4 \times C14:0] + C16:0)/(*n*-3 PUFA + *n*-6 PUFA + MUFA).

⁷Thrombogenic index = (C14:0 + C16:0 + C18:0)/([0.5 \times MUFA] + [0.5 \times *n*-6 PUFA] + [3 \times *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 |
| <i>Raw meat sclices</i> | | | | | | | | | |
| 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 ÷ (K/S) ₅₂₅ | 0.93 | 0.92 | 0.99 ^a | 0.91 ^b | 0.87 ^c | 0.009 | 0.392 | < 0.001 | 0.880 |
| <i>Muscle homogenates</i> | | | | | | | | | |
| 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 |
| <i>Cooked meat slices</i> | | | | | | | | | |
| TBARS, µg/g | 3.58 | 3.78 | 1.42 ^c | 3.85 ^b | 5.76 ^a | 0.281 | 0.337 | < 0.001 | 0.930 |

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

²Times 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)

³P 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:

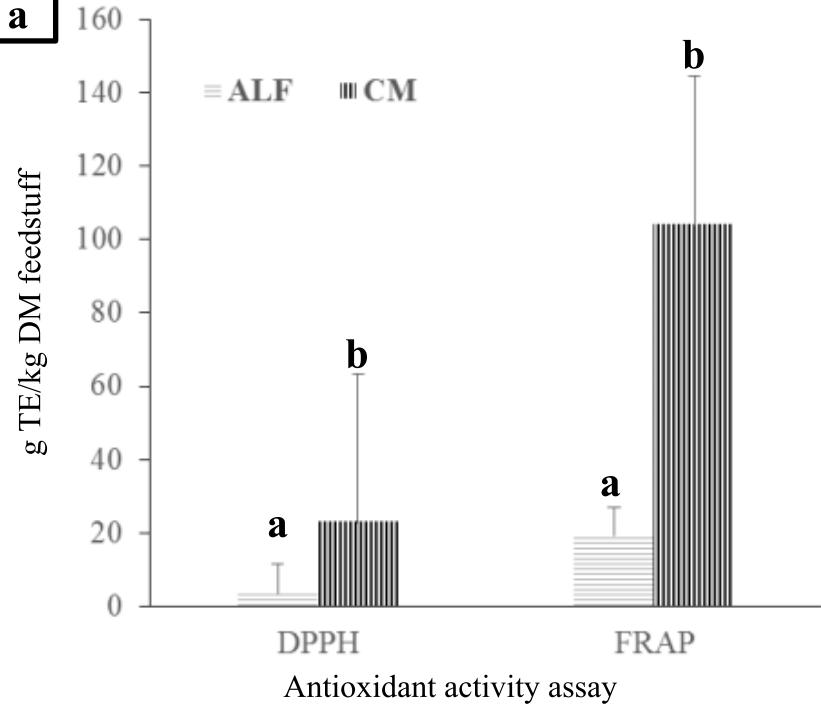
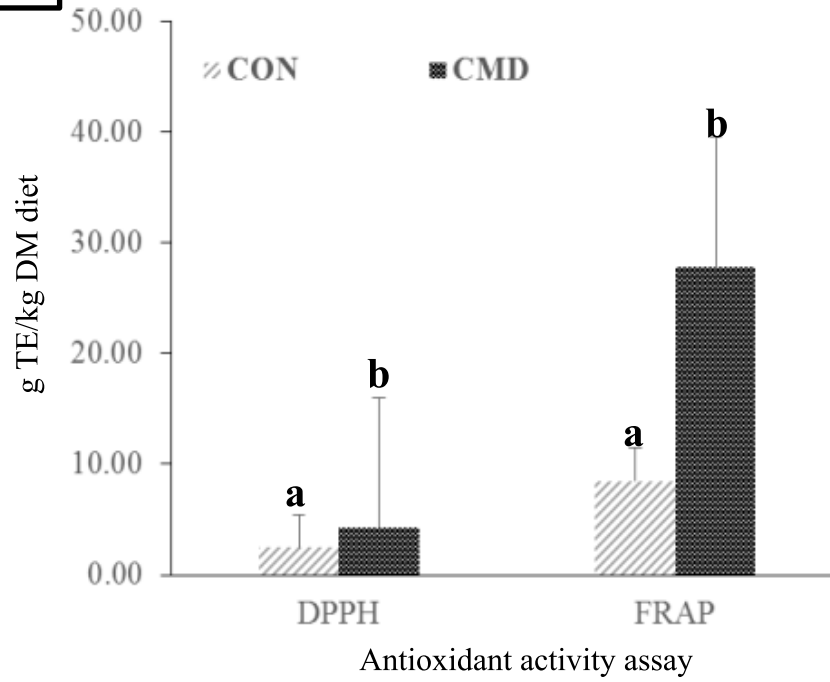
Figure 1. Antioxidant activity of **(a)** dietary test ingredients (feedstuff): dehydrated alfalfa (ALF) and cardoon meal (CM) **(b)** experimental diets: control (CON) and cardoon meal diet (CMD). Values are presented as means with standard error bars. ^{a,b}For antioxidant activity assay, mean values 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.

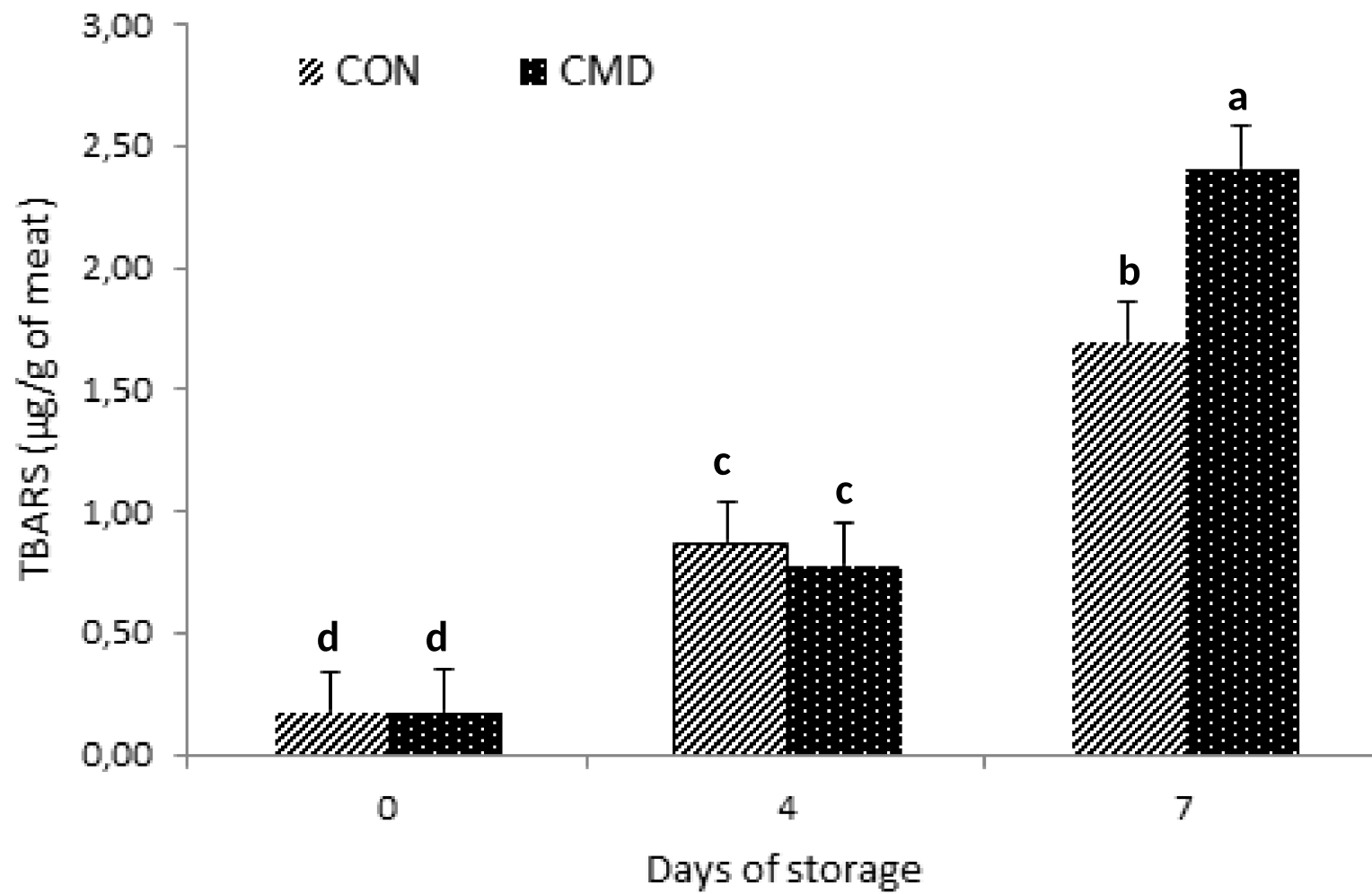
Figure 2. Interactive effect of the diet and time of storage ($D \times T$) on the lipid oxidation (TBARS, $\mu\text{g MDA/g}$ of muscle) measured in fresh *longissimus thoracis et lumborum* (LTL) muscle slices stored aerobically at 4°C for 7 days. Diets were: CON (control diet), CMD (cardoon meal diet).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

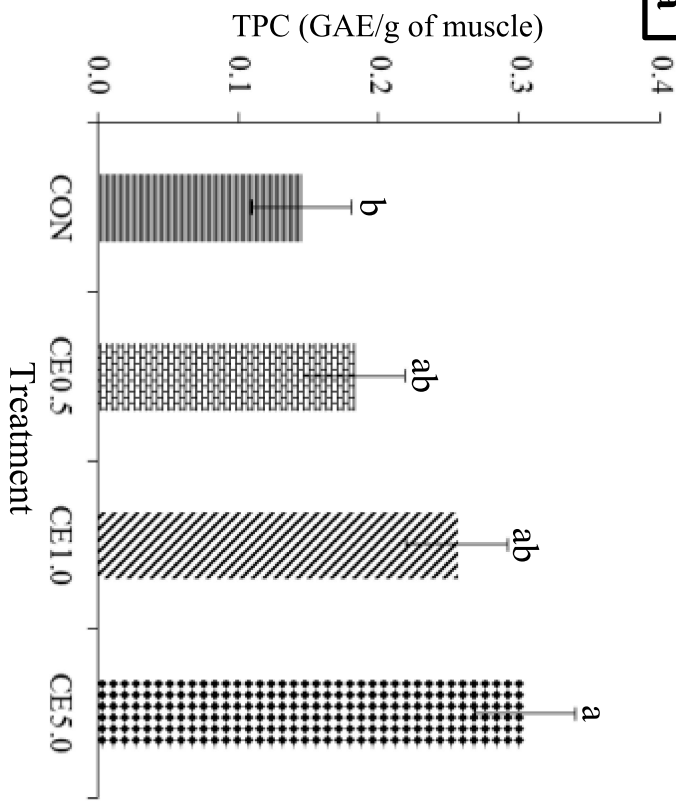
Figure 3. Effect of cardoon extract (CE) addition on **(a)** total phenolic contents (TPC, GAE/g of muscle) **(b)** lipid oxidation (TBARS, $\mu\text{g MDA/g}$ of muscle) of ovine *longissimus thoracis et lumborum* (LTL) muscle homogenates after 4 h of incubation with a FeCl_3 /sodium ascorbate pro-oxidant 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**b**



a**b**