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Concentrate supplementation with dried corn gluten feed improves the fatty acid profile of beef from steers offered grass silage

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3 1 **Concentrate supplementation with dried corn gluten feed improves the fatty acid profile**
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5 2 **of *longissimus thoracis muscle* from steers offered grass silage**
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9 3 **Running title: Effects of feeding dried corn gluten feed on beef quality**
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Abstract

BACKGROUND: Concentrate supplementation of a grass silage-based ration is a typical practice employed for indoor winter finishing of beef cattle in many temperate countries. Plant by-products, such as dried corn gluten feed (CGF), can be utilised to replace conventional feedstuffs in a concentrate supplement to enhance the sustainability of ruminant production systems and to improve meat quality. This study examined the chemical composition, fatty acid profile, oxidative stability and sensory attributes of beef (*longissimus thoracis* muscle) from steers offered grass silage and concentrate supplements containing varying levels (0%, 25%, 50%, 75%) of CGF substituted for barley/soybean meal.

RESULTS: Feeding 50%CGF decreased the protein content and increased intramuscular fat compared to 25%CGF. Total phenol content and iron-reducing antioxidant power followed the order: 0%CGF > 50%CGF and 25%CGF > 0%CGF = 50%CGF, respectively. Compared to 0%CGF, 25%CGF and 75%CGF decreased C14:0 and increased C22:2n-6, C20:5n-3 and total n-3 polyunsaturated fatty acids whereas 75%CGF increased conjugated linoleic acids and C18:3n-3. Diet did not affect the oxidative stability and sensory attributes of beef patties.

CONCLUSION: The inclusion of up to 75%CGF in a supplementary concentrate for steers increased the proportion of health-promoting unsaturated fatty acids without negatively influencing the shelf-life and eating quality of *longissimus thoracis* muscle.

Keywords: corn gluten feed, beef, antioxidant potential, fatty acids, eating quality

42 INTRODUCTION

43 Concentrate supplementation of a grass silage-based ration is a typical practice employed for
44 indoor winter finishing of beef cattle in many temperate countries including Ireland. The
45 replacement of conventional feedstuffs (such as corn, barley and soybean meal) in
46 concentrate rations with less-expensive agro-industrial by-products, is an effective strategy
47 for improving the sustainability of ruminant meat production systems ¹. Corn gluten feed
48 (CGF) is a by-product of wet milling process containing bran and steep liquor obtained after
49 the removal of germ, gluten and starch from the corn kernel ². The rapid expansion of the
50 corn milling industry has increased the use of CGF as a dietary source of energy and protein
51 for beef cattle.

52 Animal diet has a significant impact on meat quality traits including those related to
53 nutritional value, shelf-life, and sensory characteristics, which influence consumer preference
54 and acceptability. Research into the fatty acid composition of beef has attracted considerable
55 attention in recent years due to its nutritional significance and relationship to other meat
56 quality attributes (e.g. flavour and oxidative stability) which in turn influence retail value and
57 eating quality³. Microbial transformation of dietary unsaturated fatty acids to saturated fatty
58 acids (SFA) in the rumen is a major factor limiting the accumulation of polyunsaturated fatty
59 acids (PUFA) in ruminant meat and dietary strategies designed to alter this process are of
60 interest to animal/food scientists and the meat industry ⁴.

61 Grass silage-based diets, compared to concentrate-based diets, have been particularly
62 effective in reducing ruminal biohydrogenation and improving beef nutritional value by
63 increasing the content of PUFA and conjugated linoleic acid (CLA) while decreasing SFA
64 levels in beef ⁵. Additionally, grass silage-based diets may enhance the antioxidant capacity
65 and extend the shelf-life stability of beef by increasing the deposition of antioxidant

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3 66 compounds, such as vitamin E, in the muscle ⁵. However, supplementation of grass-based
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5 67 diets with concentrate rations has a varied effect on meat quality indices depending on several
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7 68 factors including the nutrient composition of concentrate ingredients which influences the
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9 69 metabolic response of animals and the ultimate composition of the meat ⁶. The lower starch
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11 70 and higher fibre content in CGF, compared to grains, decrease the negative effects of starchy
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13 71 concentrate-based feeds on ruminal pH and fibre digestion in cattle fed forage-based diets ².
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15 72 It is proposed that feeding CGF, compared to high-starch grains, in combination with grass
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17 73 silage could increase the ruminal outflow of PUFA for absorption into the muscle tissue and
18
19 74 improve the fatty acid profile of the meat. Moreover, CGF consists of bran and steep liquor
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21 75 that are rich sources of phenolic antioxidants such as protocatechuic acid, vanillic acid, *p*-
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23 76 coumaric acid, ferulic acid, sinapic acid and quercetin ^{7, 8}. Ingestion and deposition of
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25 77 phenolic compounds in muscle tissues may ultimately enhance the oxidative stability of meat
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27 78 ^{1, 9}.

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34 79 Previous studies have shown that replacement of corn/soybean meal with CGF (25% of dry
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36 80 matter, DM) in a concentrate feedlot ration had a minimal effect on the fatty acid profile,
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38 81 retail shelf-life, sensory attributes and acceptability of beef from steers fed corn silage ^{10, 11}.
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40 82 To our knowledge, there is no information available to date on the quality of meat from beef
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42 83 cattle fed a grass silage-based diet supplemented with concentrate rations containing CGF.
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44 84 Therefore, the objective of this study was to examine the chemical composition, fatty acid
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46 85 profile, oxidative stability and sensory attributes of beef from steers offered *ad libitum* grass
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48 86 silage and a concentrate supplement in which rolled barley/soybean meal was replaced by
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50 87 varying levels (0, 25, 50, 75%) of dried CGF.
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88 MATERIALS AND METHODS

89 *Animals, diets and experimental design*

90 The experimental procedures used in this study were approved by the Teagasc animal ethics
91 committee and conducted under license from the Irish Government Department of Health and
92 Children. The animals were managed by trained personnel according to the European Union
93 legislation for the protection of animals used for scientific purposes (2010/63/EU Directive).
94 Forty-eight weaned, spring-born Charolais and Limousin-sired suckler bulls were purchased
95 directly from suckler farms at ~7 months of age and assembled at Teagasc Animal &
96 Grassland Research and Innovation Centre, Grange, Ireland. Following assembly, the bulls
97 were castrated and offered grass silage *ad libitum* plus 2 kg of a barley-based concentrate and
98 60 g of a mineral-vitamin supplement per head daily for a 187-d back-grounding period. All
99 animals had *ad libitum* access to clean water.

100 Steers were subsequently blocked by breed and live weight (424.0 ± 39.0 kg) and, from
101 within each block, randomly assigned to one of four concentrate rations ($n = 12$
102 steers/treatment) offered separately as a supplement to *ad libitum* grass (*Lolium perenne*)
103 silage. Concentrate rations contained either 0% CGF (control, rolled barley/soybean meal-
104 based ration), or 25% CGF, 50% CGF or 75% CGF as a replacement for rolled
105 barley/soybean meal (*as-fed* basis). The ingredient and chemical composition of the
106 experimental concentrate rations are outlined in Table 1. Representative samples of the
107 concentrate rations were obtained twice weekly and stored at -20 °C prior to chemical
108 analysis. The steers were housed in a slatted-floor building in groups of five or six animals
109 per pen with a Calan gate feeding system (American Calan Inc., Northwood, NH, USA)
110 allowing individual feed intake of steers to be recorded. During the feeding trial, one steer
111 from the 50% CGF treatment experienced constraint with the individual feeding system,

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3 112 resulting in measurements recorded for 11 steers in this group while measurements were
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5 113 recorded for 12 steers in the other dietary treatments. Steers were individually offered 4.0 kg
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7 114 DM daily (2 kg in the morning and afternoon feeding sessions) of their respective
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9 115 supplementary concentrates for 124 days pre-slaughter.

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13 116 Animals were slaughtered in a commercial abattoir on two consecutive weeks (balanced for
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15 117 treatment) to facilitate sample collection and measurements. Samples of *longissimus thoracis*
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17 118 muscle (LT) were removed from the left side of the carcass at 48 h post-mortem, vacuum-
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19 119 packed and aged for 14 days at 4 °C, and subsequently stored at -20 °C prior to further
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21 120 analysis. Information on animal intake, growth, carcass traits, LT drip loss, and colour
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23 121 (lightness L^* , redness a^* and yellowness b^*) of subcutaneous fat and LT is presented in Kelly,
24
25 122 et al. ¹².

26 27 28 29 30 123 ***Chemical analysis of feed***

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33 124 Representative samples of concentrate rations were analyzed for dry matter, crude protein,
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35 125 ash, neutral detergent fibre, acid detergent fibre and starch concentrations as described by
36
37 126 O'Kiely ¹³. Total fat concentration or Oil-B (acid hydrolysis/ether extract) was measured
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39 127 using a Soxtec instrument (Tecator, Höganäs, Sweden).

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43 128 Phenolic compounds were extracted from samples of concentrate rations using aqueous
44
45 129 methanol (50:50, v/v) and acetone (70:30, v/v) solvents ¹⁴. Polyphenol-rich extracts were
46
47 130 analysed for total phenol content (TPC) using the Folin-Ciocalteu method (Singleton,
48
49 131 Orthofer, & Lamuela-Raventós, 1999) and absorbance measurements were recorded at 750
50
51 132 nm using a UV-vis spectrophotometer (Cary 300 Bio, UV-vis spectrophotometer, Varian
52
53 133 Instruments, Palo Alto, CA, USA). Results were expressed as g of gallic acid equivalents
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55 134 (GAE)/kg of DM feed.

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3 135 The fatty acid composition was determined in freeze-dried samples of concentrate rations by
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5 136 a one-step extraction–transesterification procedure using chloroform¹⁵ and 2% (v/v) sulfuric
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7 137 acid in methanol¹⁶, with 19:0 nonadecanoate (Larodan, Solna, Sweden) added as an internal
8
9 138 standard. Gas chromatographic analysis of fatty acid methyl esters (FAME) was performed as
10
11 139 described by Cherif, et al.¹⁷. Individual fatty acids were expressed as g/kg of DM feed.

15 140 ***Determination of muscle pH and proximate composition***

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18 141 Analysis of muscle pH and proximate composition was performed as described by Salami, et
19
20 142 al.¹⁸. Briefly, the LT muscle was thawed and trimmed of visible fat and connective tissue and
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22 143 minced twice through a plate with 4 mm holes (Model P114L, Talsa, Valencia, Spain). Raw
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24 144 minced LT samples (5 g) were homogenised for 3 min in 45 ml distilled water using an Ultra
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26 145 Turrax T25 homogeniser (Janke and Kunkel, IKA-Labortechnik, GmbH and Co., Staufen,
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28 146 Germany). The pH of the beef homogenates was measured at 20 °C using a pH meter (Seven
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30 147 Easy portable, Mettler-Toledo GmbH, Schweizenbach, Switzerland). Minced LT samples
31
32 148 were analysed for moisture and fat contents using a SMART Trac rapid moisture/fat analyser
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34 149 (CEM Corporation, Matthews, NC, USA). The ash content was determined using a muffle
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36 150 furnace (550 °C for 3 h) and protein content was determined by the Kjeldahl method.

42 151 ***Analysis of vitamin E and fatty acids in LT muscle***

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45 152 The α -tocopherol (vitamin E) content in minced LT samples was determined by high-
46
47 153 performance liquid chromatography (HPLC) as previously described by Salami, et al.¹⁸. In
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49 154 brief, HPLC analysis was carried out on a ProStar liquid chromatograph (Varian Analytical
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51 155 Instruments, Palo Alto, CA, USA) equipped with a ProStar autosampler (Model 410, Varian
52
53 156 Instruments). Sample injection volume (partial loop fill) was 20 μ l. The α -tocopherol was
54
55 157 separated on a 250 x 4.6 mm Polaris C18-A 5 μ column (Metachem, Ansys® Technologies,
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57 158 CA, USA) and detected using a ProStar UV/Vis detector (Varian Instruments) at 292 nm. The

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3 159 mobile phase was methanol/water (97:3) and isocratic elution took place at 2ml/min for a
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6 160 total run time of 10 min. A personal computer and Star LC workstation software (version
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8 161 6.20, Varian Inc.) was used for calculation of peak areas. A standard curve was generated
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10 162 using a range of α -tocopherol concentrations (7, 14, 21 $\mu\text{g/ml}$) and the concentration of α -
11
12 163 tocopherol in beef was expressed in $\mu\text{g/g}$ of beef muscle. The percentage recovery of vitamin
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15 164 E from beef samples, through the extraction procedure, was determined by including vitamin
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17 165 E (0.2 ml of 22.8 $\mu\text{g/ml}$) as an internal standard. The percentage recovery (92.8%) was
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19 166 calculated by comparison of peak areas of vitamin E recovered through the extraction
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21 167 procedure with those obtained by direct injection of the vitamin E standard (22.8 $\mu\text{g/ml}$) onto
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24 168 the column.

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27 169 For fatty acids analysis, the lipid fraction in minced LT samples was extracted following the
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29 170 procedure described by Bligh and Dyer¹⁹ and the lipid fraction was transesterified to FAME
30
31 171 using BF_3 in methanol as a catalyst²⁰ and dissolved in isooctane. The gas-chromatographic
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33 172 analysis was conducted following the injection, pressure and temperature conditions
34
35 173 described extensively by Salami, et al.²¹. Individual compounds were identified using
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37 174 FAME standards (a mixture of Supleco 37 component FAME mix, *trans*-11 vaccenic acid
38
39 175 methyl ester and conjugated linoleic acid methyl ester; Sigma-Aldrich Ireland Ltd., Vale
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41 176 Road, Arklow, Wicklow, Ireland) and results were reported as g/100g of the total fatty acids.
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43
44 177 The atherogenic index (AI) and thrombogenic index (TI) were calculated according to
45
46 178 Ulbricht and Southgate²² as follows:

$$179 \quad \text{AI} = \frac{\text{C12:0} + (4 \times \text{C14:0}) + \text{C16:0}}{n - 6 \text{ PUFA} + n - 3 \text{ PUFA} + \text{MUFA}}$$

$$180 \quad \text{TI} = \frac{\text{C14:0} + \text{C16:0} + \text{C18:0}}{(0.5 \times \text{MUFA}) + (0.5 \times n - 6 \text{ PUFA}) + (3 \times n - 3 \text{ PUFA}) + \left(\frac{n - 3 \text{ PUFA}}{n - 6 \text{ PUFA}}\right)}$$

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3 181 where MUFA is monounsaturated fatty acids and PUFA is polyunsaturated fatty acids.
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6 182 ***Determination of total phenol content and in vitro antioxidant activity***
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9 183 ***Preparation of muscle homogenates***
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12 184 Beef homogenates (10% w/v) were prepared as described by Qwele, et al. ²³ for the
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14 185 determination of *in vitro* antioxidant activities. Briefly, minced LT (5 g) was homogenised in
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16 186 0.05 M phosphate buffer (45 ml) using an Ultra-turrax T25 homogeniser for 3 min. Muscle
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18 187 homogenates were centrifuged at 7,800 g for 10 min at 4 °C using an Avanti® J-E Centrifuge
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20 188 (Beckman Coulter Inc., Palo Alto, CA, USA). The supernatant was filtered through Whatman
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22 189 No. 1 paper and the filtrate was analysed for ferric reducing antioxidant power (FRAP) and
23
24 190 ferric ion chelating activity (FICA). For the determination of TPC and 1,1-diphenyl-2-
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26 191 picrylhydrazyl (DPPH) radical scavenging activity assays, 10% trichloroacetic acid (5 ml)
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28 192 was added to muscle homogenates and the mixture was centrifuged at 7,800 g for 10 min at 4
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30 193 °C. The supernatant was filtered through Whatman No. 1 paper and analysed for TPC and
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32 194 DPPH activity.
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39 195 ***Measurement of the total phenol content***
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42 196 Muscle extracts were analysed for TPC using the Folin-Ciocalteu method ²⁴ with minor
43
44 197 modifications. Briefly, extracts (0.5 ml) were mixed with Folin-Ciocalteu reagent (2.5 ml,
45
46 198 20% in distilled water) and sodium carbonate (2 ml, 7.5% in distilled water) was added after
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48 199 5 min. The mixture was stored in the dark for 2 h at room temperature and absorbance
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50 200 measurements were recorded at 750 nm on a UV-vis spectrophotometer (Cary 300 Bio, UV-
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52 201 vis spectrophotometer, Varian Instruments, CA, USA) against a blank containing all reagents
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54 202 and distilled water. A calibration curve using standard solutions of aqueous gallic acid (20 –
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3 203 100 µg/ml) was constructed and results are expressed as mg of gallic acid equivalents
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5 204 (GAE)/g of muscle.

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9 205 ***Measurement of in vitro antioxidant activities***

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11 206 Radical scavenging activity in muscle was measured using the DPPH assay following a
12
13 207 minor modification of the method described by Yen and Wu ²⁵. Muscle extract (0.6 ml) and
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15 208 distilled water (2.4 ml) were mixed with 0.2 mM DPPH in methanol (3 ml) and incubated in
16
17 209 the dark for 1 h at room temperature. Absorbance measurements were recorded at 517 nm on
18
19 210 a UV-vis spectrophotometer (Cary 300 Bio) against a methanol blank. An assay blank
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21 211 containing distilled water (3 ml) and 0.2 mM DPPH in methanol (3 ml) was used for
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23 212 calculation purposes. A calibration curve using standard solutions of methanolic Trolox (10 –
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25 213 50 µg/ml) was constructed and results were expressed as mg of Trolox equivalents (TE)/g of
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27 214 muscle.

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33 215 Total antioxidant activity in muscle was determined using FRAP assay following a minor
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35 216 modification of the method described by Benzie and Strain ²⁶. Briefly, muscle extract (0.45
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37 217 ml) was mixed with 8.55 ml FRAP reagent (a mixture of 30 mM acetate buffer (pH 3.6), 10
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39 218 mM 2,4,6-Tris (2-pyridyl)-s-triazine in 40 mM HCl and 20 mM FeCl₃.6H₂O in distilled
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41 219 water in the ratio 10:1:1, respectively incubated at 37 °C for 10 min prior to use). The mixture
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43 220 was incubated for 30 min in the dark and absorbance was recorded at 593 nm on a UV-vis
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45 221 spectrophotometer (Cary 300 Bio) against a blank containing all reagents. A calibration curve
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47 222 using solutions of methanolic Trolox (0.033 – 0.1 mg/ml) was constructed and results are
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49 223 expressed as mg TE/g of muscle.

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53
54 224 The iron-chelating activity of muscle was measured using a minor modification of FICA
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56 225 assay described by Yen and Wu ²⁵. Muscle extract (0.5 ml) was mixed with FeCl₂ (2 mM in
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58 226 distilled water, 0.1 ml), ferrozine solution (5 mM in distilled water, 0.2 ml) and distilled
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227 water (4.2 ml). The assay control contained FeCl₂ (0.1 m), ferrozine solution (0.2 ml) and
228 distilled water (4.7 ml). The mixture was incubated for 1 h in the dark at room temperature
229 and absorbance measurements were recorded at 562 nm against a water blank on a UV-vis
230 spectrophotometer (Cary 300 Bio). The chelating activity was calculated as follows:

231 $\text{Chelating activity (\%)} = [1 - (\text{absorbance of sample})/(\text{absorbance of control})] \times 100.$

232 *Determination of the oxidative stability and sensory properties of beef*

233 *Measurement of lipid oxidation and oxymyoglobin in muscle homogenates*

234 Muscle homogenates (25%) were prepared by homogenising 15 g of LT in a buffer (0.12 M
235 KCL 5 mM histidine, pH 5.5) surrounded by crushed ice using an Ultra Turrax T25
236 homogeniser. Lipid oxidation in muscle homogenates (39.2 g) was initiated by the addition of
237 pro-oxidants (45 µM FeCl₃/sodium ascorbate, 1:1)²⁷. Lipid oxidation (2-thiobarbituric acid
238 reactive substances, TBARS) and oxymyoglobin (OxyMb) content in muscle homogenate
239 were measured at 1 and 4 h of storage at 4 °C as described by Hayes, et al.²⁸.

240 *Beef processing and packaging*

241 The LT muscles were thawed overnight at 4 °C, trimmed of visible fat and connective tissue,
242 and minced twice through a plate with 4 mm holes (Model P114L, Talsa, Valencia, Spain).
243 The minced muscle was formed into beef patties (100 g portions) using a meat former
244 (Ministek burger maker, O.L Smith Co. Ltd., Italy). For the fresh beef study, patties were
245 individually placed in a low oxygen permeable (<1 cm³/m²/24 h at STP) polystyrene/ethyl
246 vinyl alcohol/polyethylene (PE) trays and flushed with 80% O₂:20% CO₂ (modified
247 atmosphere packs, MAP) using a vacuum-sealing unit (VS 100, Gustav Müller & Co. KG,
248 Homburg, Germany) equipped with a gas mixer (Witt-Gasetechnik GmbH & Co. KG,
249 Witten, Germany). Trays were covered and heat-sealed using a low oxygen permeable (3

250 cm³/m²/24 h at STP) laminated barrier film with a polyolefin heat-sealable layer. Fresh beef
251 patties in MAP were stored for up to 14 days under fluorescent lighting (660 lx) at 4 °C. The
252 gas atmosphere (% O₂ and % CO₂) in MAP was measured using a CheckMate 9900 (PBI-
253 DanSensor, Denmark). The average gas composition in MAP was 79.53 ± 0.39% O₂ and
254 20.77 ± 0.23% CO₂ on day 1 of storage and 74.03 ± 0.77% O₂ and 25.85 ± 0.73% CO₂ on day
255 14 of storage.

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

261 *Measurement of lipid oxidation and surface colour of beef patties*

262 Lipid oxidation was measured in fresh beef patties on days 1, 4, 7, 11, and 14 of storage and
263 days 1, 3, and 6 in cooked beef patties. Lipid oxidation measurements were carried out
264 following the method described by Siu and Draper²⁹. Results were expressed as TBARS in
265 mg malondialdehyde (MDA)/kg meat.

266 The surface colour of fresh beef patties on days 1, 4, 7, 11, and 14 of storage was measured
267 using a Konica Minolta CR-400 Chroma-Meter (Minolta Camera Co., Osaka, Japan). The
268 Chroma-Meter consisted of a measuring head (CR-400), with an 8 mm diameter measuring
269 area, illuminant D65, a 2° standard observer, and a data processor (DP-400). The Chrom-
270 Meter was calibrated on the CIE LAB colour space system using a white tile (D_c: L = 97.79,
271 a = -0.11, b = 2.69). The 'L*', 'a*' and 'b*' value represents lightness, redness and
272 yellowness, respectively. Colour measurements were averaged for readings taken from four
273 different locations on the surface of beef patties.

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3 274 ***Measurement of textural properties of beef patties***
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6 275 The texture profile analysis (TPA) of fresh beef patties stored in MAP was measured on days
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8 276 2 and 7 of storage. The TPA parameters (hardness (N), springiness (mm), cohesiveness
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10 277 (dimensionless), gumminess (N), chewiness (N × mm), adhesiveness (N)) were measured
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13 278 using a 30 kg load cell texture analyser (TA.XT2i Texture Analyser, Stable Micro Systems,
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15 279 UK) as described by Moroney, et al. ³⁰.

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18 280 ***Sensory analysis of beef patties***
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21 281 Sensory acceptance testing of fresh beef patties ($n = 8/\text{treatment}$) stored in MAP was carried
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23 282 out by 40 untrained panellists in 2 sessions (20 panellists/session) on days 2 and 7 of storage
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25 283 as described by O'Sullivan, et al. ³¹. Beef patties were cooked for sensory analysis in a
26
27 284 Zanussi oven at 180 °C for 20 min until an internal temperature of 72 °C was reached.
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29 285 Following cooking, patties were cooled to room temperature and cut into 2 cm × 2 cm cubes,
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31 286 identified with random three-digit codes. On each day of evaluation, beef samples were
32
33 287 served to panellists in two separate sessions (morning and afternoon sessions). Before serving
34
35 288 to panellists, beef samples were re-heated in a microwave for 10 s to release the meat odour
36
37 289 and flavour. Sensory evaluation was performed in the panel booths of the University's
38
39 290 sensory laboratory according to international standard regulations ³². Panellists were provided
40
41 291 with water to cleanse their palates between samples. Each panellist received beef samples
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43 292 presented in a randomised order to prevent any flavour carryover effects ³³. Panellists were
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45 293 asked to indicate their degree of liking for appearance, odour, texture, juiciness, flavour and
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47 294 overall acceptability on a 10 cm line scale ranging from 0 (extremely dislike) to 10
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49 295 (extremely like).
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296 ***Statistical analysis***

297 Data relating to proximate composition, antioxidant capacity and fatty acid profile analyses
298 were analysed using a general linear model including dietary treatment as a fixed factor and
299 block as a random factor. The intramuscular fat content was included as a covariate in the
300 model used for the analysis of fatty acid profiles in the muscle. The orthogonal polynomial
301 contrast was used to determine the linear, quadratic, and cubic responses for dietary inclusion
302 levels of CGF. Linear discriminant analysis was employed to determine the muscle FA
303 variables that best differentiate the dietary treatments. The statistical significance of the
304 discriminant model was assessed using Wilks' lambda test and the accuracy of the model in
305 assigning individual animals to their respective dietary group was cross-validated using the
306 "leave-one-out" classification method.

307 Data on the oxidative stability and sensory attributes were analysed using a mixed model in
308 which the effects of diet and storage/incubation time were included as fixed factors and the
309 interaction between diet and storage/incubation time was determined. The effects of panellist
310 and session were included as random terms in the model used for the analysis of sensory
311 eating attributes. The effect of slaughter day was included as a covariate term in all models
312 but was removed from the models due to non-significance ($P > 0.05$). Tukey's HSD test was
313 used for multiple comparisons of treatment means when significance was detected at $P \leq 0.05$
314 and a tendency for treatment effect was observed when $0.05 < P \leq 0.10$. All data analysis was
315 performed using SPSS statistical software (IBM Statistics version 22).

316 **RESULTS AND DISCUSSION**

317 The nutrition of animals plays a significant role in regulating physicochemical and metabolic
318 traits of muscles which in turn influences meat quality. Corn gluten feed (CGF) is a co-
319 product of wet milling of corn that is increasingly used in concentrate diets for finishing beef

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3 320 cattle ². There is no information on the impact of dietary CGF on beef quality when included
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5 321 in a concentrate supplement for finishing beef cattle fed grass silage. In this regard, the
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7 322 present study investigated quality indices of beef from steers fed grass silage and a
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9 323 concentrate supplement in which rolled barley/soybean meal was replaced with different
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11 324 levels (0%, 25%, 50% and 75%) of CGF. The inclusion of 25%, 50% and 75% CGF in
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13 325 concentrate supplement correspond to 10.9%, 21.5% and 33.7% of the total dietary intake
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15 326 (grass silage + concentrate supplement), respectively.
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20 327 *pH and proximate composition of muscle*

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23 328 The pH and proximate composition of LT muscle are presented in Table 2. Values of muscle
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25 329 pH in all treatments (5.47 – 5.53) were within the normal range (5.4 – 5.8) for beef ³⁴. The
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27 330 moisture, IMF and protein content of LT muscles from the CGF treatments was not
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29 331 significantly different from the control (0% CGF). Similarly, it has been reported that the
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31 332 proximate composition of different muscles obtained from steers fed CGF (25% dietary DM)
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33 333 was not different from those fed corn/soybean meal ^{10, 11}. In comparison to 50% CGF,
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35 334 muscles from steers fed 25% CGF had greater protein content and lower IMF content (Table
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37 335 2). The observed differences in IMF may not be related to the level of feed intake as steers
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39 336 had a similar DM intake (grass silage and concentrate) and growth rate across the dietary
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41 337 treatments ¹². However, it is possible that variation in IMF content is related to differences in
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43 338 the ruminal fermentation pattern, producing more of a glucogenic precursor (propionate),
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45 339 and/or extent of starch digestion in the small intestine ³⁵. In addition, the ash content in LT
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47 340 muscle decreased quadratically ($P = 0.015$) as CGF inclusion increased, with 25% CGF being
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49 341 lower ($P < 0.05$) than 0% CGF. Overall, the present results showed that inclusion of 75%
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51 342 CGF in supplementary concentrate ration did not affect the muscle protein level, IMF,
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53 343 moisture, and ash values compared to the control and other CGF treatments.
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344 *Antioxidant capacity of muscle*

345 The antioxidant status of muscle was assessed by measuring the concentration of vitamin E
346 and TPC. Dietary treatment did not affect the concentration of vitamin E in LT muscle as
347 shown in Table 2. Muscle vitamin E levels are similar to those reported for beef cattle grazed
348 entirely on pasture ⁶, due to the inclusion of grass silage in the diet fed in the present study.
349 The inclusion levels of CGF decreased ($P < 0.05$) the TPC in muscle in a quadratic manner,
350 with a significantly lower TPC in steers fed 50% CGF compared to those fed 0% CGF. This
351 observation was contrary to the higher amount of TPC found in CGF diets (Table 1),
352 suggesting that CGF phenolics were not deposited in the muscle. This may be due to low
353 bioavailability of CGF phenolics as shown in rats fed phenolic compounds from corn bran, a
354 major constituent of CGF ³⁶. Moreover, the deposition of dietary phenolics in ruminant meat
355 is influenced by the interaction of polyphenols with other dietary components, microbial
356 metabolism in the digestive tract or limitations for the absorption of phenolic compounds into
357 muscle tissues ⁹.

358 Regardless of the differences in muscle TPC, dietary treatment did not influence ($P > 0.05$)
359 the radical scavenging activity and chelating activity of the LT muscle (Table 2). However,
360 the FRAP value was greater in cattle fed 25% CGF compared to those fed 0% and 50% CGF,
361 which was inconsistent with the pattern of dietary effects observed for muscle TPC. The
362 FRAP is an indicator of total antioxidant capacity estimated via the reduction of Fe^{3+} to Fe^{2+}
363 ²⁶. Beef muscle is a complex biological system comprising of enzymatic (glutathione
364 peroxidase, catalase, superoxide dismutase etc.) and non-enzymatic (selenium, vitamins E, C
365 and A, etc.) factors contributing to the overall total antioxidant activity of meat ⁹. Thus, the
366 discrepancy between the pattern of dietary effect on TPC and FRAP values may be related to
367 the contribution of other compounds, apart from polyphenols, to the overall antioxidant
368 capacity of muscle. This speculation may be supported by a lack of significant correlation (r

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3 369 = -0.16; $P = 0.288$) between TPC and FRAP values. Nonetheless, the current results
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5 370 demonstrated that dietary inclusion of up to 75% CGF in the concentrate supplement did not
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8 371 compromise the antioxidant capacity of muscle.
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11 372 *Fatty acid composition of muscle*

14 373 *Effect of diet on the fatty acid profile of LT muscle*

17 374 Results of FA composition in LT muscle are presented in Table 3. Dietary treatment did not
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19 375 affect ($P > 0.05$) the relative amount of total SFA in LT muscle but influenced the proportion
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21 376 of individual SFA with nutritional significance. Muscle from steers fed 25% and 75% CGF
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23 377 had decreased ($P < 0.05$) percentages of C14:0 and C17:0 compared to those fed 0% and 50%
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26 378 CGF. In addition, CGF diets tended ($P = 0.074$) to decrease C16:0 in a cubic manner, with
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28 379 25% CGF displaying the greatest decrease (-37.2%) compared to 0% CGF. Notably, the
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30 380 consumption of elevated levels of dietary C14:0 and C16:0 increases low-density lipoprotein
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32 381 cholesterol, linked to increased risk of cardiovascular diseases (CVD), obesity and insulin
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34 382 resistance in humans^{4,5}. Thus, it can be deduced that feeding 25% and 75% CGF decreased
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36 383 the proportion of hypercholesterolemic SFA in beef.
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40 384 The proportion of total MUFA was unaffected ($P > 0.05$) by feeding CGF and no dietary
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42 385 effect was observed ($P > 0.05$) on the relative amount of *c*-9 C18:1, the predominant MUFA
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44 386 in beef. Compared to the CGF treatments, steers fed 0% CGF had higher ($P < 0.05$) C16:1
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46 387 and *c*-10 C17:1, monounsaturated derivatives of C16:0 and C17:0, respectively. The
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48 388 accumulation of C16:1 and *c*-10 C17:1 suggests that *de novo* lipid synthesis may play a role
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50 389 in the alteration of LT muscle fatty acid profile in this study. A linear decrease in the
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52 390 percentage of total *trans* fatty acids (TFA) was mainly influenced by a linear decrease ($P <$
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54 391 0.05) of *t*-9+10 18:1. However, the proportion of *t*-11 18:1 (*trans*-vaccenic acid) was not
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56 392 affected ($P > 0.05$) by dietary treatment. The observed changes in muscle TFA may be of
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3 393 minimal implication because current evidence suggests that dietary consumption of ruminant
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5 394 TFA may have a limited health impact in contrast to the increased CVD risks associated with
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8 395 industrial TFA ⁵.

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10 396 Dietary treatment tended ($P = 0.063$) to influence the percentage of total PUFA in a cubic
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12 397 response, with the highest increase (+37.6%) observed in the 75% CGF compared to those
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14 398 fed 0% CGF. Feeding CGF diets increased ($P < 0.05$) C20:5 $n-3$ in cubic manner with
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16 399 correspondingly higher ($P < 0.01$) total $n-3$ PUFA. In comparison to 0% CGF, feeding 25%
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18 400 and 75% CGF increased ($P < 0.01$) the relative amount of long-chain PUFA (C22:2 $n-6$)
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20 401 whereas 75% CGF increased CLA ($c-9,t-11 + t-9,c-11 + t-7,c-9$ 18:2) and α -linolenic acid
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22 402 (C18:3 $n-3$). Ruminant meat and milk represent the main dietary source of $c-9,t-11$ CLA in the
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24 403 human diet, where CLA is the major isomer synthesized during ruminal biohydrogenation
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26 404 and from endogenous desaturation of *trans*-vaccenic acid in muscle or mammary tissues ⁴. It
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28 405 is well-documented that consumption of dietary PUFA and CLA exhibit biological activities
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30 406 that prevent CVD, cancer and metabolic syndromes in humans ⁵. Thus, the effect of dietary
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32 407 CGF in increasing the proportion of these fatty acids in LT muscle can be considered as a
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34 408 promising feeding strategy for enhancing the proportion of healthy fat in beef.

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36 409 Different FA indices were used to evaluate the nutritional importance of beef fat and the
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38 410 contribution to healthy human diets. Dietary treatment did not influence ($P > 0.05$) PUFA to
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40 411 SFA ratio and the thrombogenic index. However, the $n-6:n-3$ ratio tended to be lower ($P =$
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42 412 0.093) in the CGF treatments, with values of 25% and 50% CGF desirably lower than the
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44 413 maximum nutritional recommendation of 4.0 ³⁷. The inclusion levels of CGF tended to
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46 414 favourably decrease ($P = 0.098$) muscle atherogenicity index in a cubic response, due to a
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48 415 lower proportion of C14:0 and C16:0, and higher $n-3$ PUFA.
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3 416 Overall, it is noteworthy that dietary CGF favourably altered the FA composition of beef in
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5 417 the present study contrary to a previous study which reported that CGF had a minimal impact
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7 418 on the fatty acid profile of LT muscle ¹⁰. Differences in feed fatty acid composition could be
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10 419 one of the reasons for the discrepancies between the results presented and those of Segers, et
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12 420 al. ¹⁰. The present study showed that increasing the inclusion levels of CGF resulted in
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14 421 corresponding increases in dietary PUFA (oleic acid and linoleic acid) in experimental diets
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16 422 (Table 1) whereas information on feed fatty acid composition was not reported by Segers, et
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18 423 al. ¹⁰. Additionally, the inclusion of CGF in the present study decreased dietary starch levels
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20 424 which may mitigate the impairment of ruminal digestion that can occur from supplementation
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22 425 of forage with cereals ². Thus, it is possible that CGF exhibited a complementary effect in
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24 426 protecting dietary unsaturated fatty acids against ruminal biohydrogenation.
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29 427 ***Multivariate analysis of muscle fatty acid profile***

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32 428 The application of multivariate statistical analysis to chemical constituents (such as fatty
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34 429 acids) in muscle tissues can be used to discriminate meat from different feeding provenance,
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36 430 geographical origins or production systems ³⁸. This approach may be relevant to authenticate
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38 431 meat produced from sustainable feeding systems that promote the substitution of by-products
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40 432 for cereal grains in animal rations. In the present study, linear discriminant function analysis
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42 433 was performed to identify the fatty acids that better contribute to the differentiation of beef
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44 434 from animals fed diets in which rolled barley/soybean meal was replaced by CGF in
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46 435 concentrate rations. Six fatty acids (C15:0, C16:1, C17:0, *c*-10 C17:1, *t*-11 C18:1 and C22:0)
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48 436 were retained as the best quantitative predictor variables after a stepwise selection procedure
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50 437 was applied to the muscle fatty acid dataset. The predictor variables follow a similar
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52 438 observation suggesting that a combination of fatty acids derived from ruminal
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54 439 biohydrogenation intermediates and *de novo* fatty acid synthesis contribute to the
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56 440 discrimination of the feeding provenance of beef ³⁹. The predictor variables were linearly
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3 441 combined to obtain three canonical discriminant functions (CAN). The first two CAN
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5 442 described 97.1% (CAN 1 = 89.1% and CAN 2 = 8.0%) of the total variance associated with
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7 443 the multivariate structure on the CAN plot (Figure 1). Discriminant power of the canonical
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9 444 model was significant ($P < 0.01$) based on Wilks' lambda test of significance.

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13 445 The scattered distribution on the CAN plot indicated that muscles from animals fed 25% and
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15 446 75% CGF (located on the left side of the quadrant) were not clearly differentiated from each
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17 447 other but were discriminated by CAN 1 from those fed 0% and 50% CGF (located on the
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19 448 right side of the quadrant). The discriminant power of CAN 1 was maximized by *c*-10 C17:1,
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21 449 C17:0, *t*-11 C18:1 and C22:0 as shown with higher values of standardized coefficients of
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23 450 variables (Supplementary Table 1). However, CAN 2 distinguished beef from animals fed 0%
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25 451 CGF and 50% CGF, located on the upper and lower right side of the quadrant, respectively.
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27 452 The discriminant power of CAN 2 was highly influenced by C16:1 and *c*-10 C17:1
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29 453 (Supplementary Table 1). Cross-validation of the discriminant model revealed that 10 steers
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31 454 were correctly classified to each of 0%, 25% or 50% CGF while 9 steers were correctly
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33 455 assigned to 75% CGF. The model inaccuracy showed that steers belonging to 0% ($n = 2$),
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35 456 25% ($n = 2$), 50% ($n = 1$) and 75% ($n = 3$) CGF were wrongly assigned to 50%, 75%, 75%
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37 457 and 25% CGF, respectively, indicating that none of the CGF-fed steers was wrongly assigned
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39 458 to the control (0% CGF) group. Therefore, this data suggests that muscle fatty acid profile
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41 459 may be a potential chemical marker for discriminating beef from cattle finished on a forage-
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43 460 based diet and concentrate supplement containing CGF. Also, among the entire set of the
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45 461 identified fatty acids, the discriminant analysis allows for highlighting fatty acids which are
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47 462 more linked to feeding CGF.
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3 463 ***Oxidative stability of beef***
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6 464 Feeding CGF diets did not affect ($P > 0.05$) lipid oxidation (TBARS) and colour (lightness
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8 465 L^* , redness a^* , yellowness b^*) stability of fresh beef patties stored in high-oxygen modified
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10 466 atmosphere packs (MAP) for up to 14 days at 4 °C (Table 4). Significant effects ($P < 0.01$) of
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12 467 storage time was observed on measured parameters for lipid and colour stability. However,
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14 468 the interaction between treatment and storage time did not affect ($P > 0.05$) the lipid and
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16 469 colour stability parameters. In agreement with the current results, previous research has
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18 470 shown that substitution of corn/soybean meal with CGF (25% dietary DM) did not affect
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20 471 lipid oxidation and subjective colour acceptance of aerobically-stored fresh beef steaks
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22 472 subjected to refrigerated retail display over a 9-d period¹⁰.
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28 473 Furthermore, dietary treatment did not affect ($P > 0.05$) the levels of lipid oxidation in
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30 474 cooked beef patties stored in aerobic packs for up to 6 days at 4 °C (Table 4). The effect of
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32 475 storage time on lipid oxidation was similar between day 1 and 3 ($P > 0.05$) and differs when
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34 476 compared to day 6 ($P < 0.05$) (Table 4). However, there was no diet × storage time effect on
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36 477 the lipid oxidation of cooked beef patties. Therefore, the current results demonstrate that
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38 478 feeding CGF did not negatively influence the stability of beef patties evaluated under
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40 479 different oxidative conditions (MAP and aerobic packs).
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45 480 Indeed, dietary CGF did not negatively influence the oxidative stability of beef patties stored
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47 481 in MAP and aerobic packs despite the increased amount of highly-peroxidizable PUFA in
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49 482 meat from steers fed 25% and 75% CGF. Furthermore, the extent of lipid oxidation and
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51 483 OxyMb oxidation were similar ($P > 0.05$) among dietary treatments when muscle
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53 484 homogenates were subjected to a more vigorous oxidative condition by incubating with
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55 485 iron/ascorbate pro-oxidants (Table 4). This observation further confirms that dietary CGF did
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57 486 not enhance the oxidative stability of beef possibly due to the lack of deposition of
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3 487 antioxidant phenolics in the muscle of CGF-fed steers. In agreement with this assertion, it has
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5 488 been shown that dietary supplementation of ferulic acid, a major phenolic compound in CGF,
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7 489 did not enhance the oxidative stability of beef⁴⁰.

490 *Instrumental texture properties and sensory eating attributes of beef*

14 491 Dietary treatment did not influence ($P > 0.05$) texture profile analysis (TPA) parameters
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16 492 (hardness, springiness, cohesiveness, gumminess, chewiness and adhesiveness) of beef
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18 493 patties on days 2 and 7 of storage in MAP (Table 5). A significant effect of storage time ($P <$
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20 494 0.05) was observed on hardness, gumminess and chewiness while springiness, cohesiveness
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22 495 and adhesiveness did not differ ($P > 0.05$) over the storage time. No significant interaction (P
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24 496 > 0.05) was found between diet \times storage time. Previous studies have demonstrated that
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26 497 inclusion of CGF in a concentrate finishing ration of steers did not affect the instrumental
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28 498 texture (Warner-Bratzler shear force) of beef^{10, 11}.

33 499 Sensory naïve panellists did not detect differences ($P > 0.05$) between dietary treatments in
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35 500 the eating quality characteristics (appearance, odour, texture, juiciness, flavour, and overall
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37 501 acceptability) of beef patties at days 2 and 7 of storage in MAP (Table 5). Storage time did
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39 502 not influence ($P > 0.05$) eating quality characteristics except the liking of texture which was
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41 503 decreased ($P < 0.05$) in 50% CGF beef patties between days 2 and 7 of storage. Moreover, no
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43 504 significant effect ($P > 0.05$) of diet \times storage time was observed on all the eating quality
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45 505 attributes rated by the panellists. It has been shown that TPA parameters correlate with the
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47 506 assessment of subjective sensory tenderness of beef⁴¹, which may explain why the lack of
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49 507 dietary effect on TPA parameters was consistent with the similarity in consumer liking for
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51 508 beef texture. Overall, the current results indicate that dietary inclusion of CGF did not
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53 509 negatively influence the consumer acceptance of beef in agreement with a similar result
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55 510 reported with trained sensory panellists¹¹.

511 CONCLUSIONS

512 The replacement of rolled barley/soybean meal with CGF in concentrate supplement had a
513 minimal effect on the chemical composition and antioxidant capacity of **LT muscle** from
514 steers offered grass silage. The inclusion of up to 75% CGF in supplementary concentrate
515 diet improved the fatty acid profile of **LT muscle** by decreasing the proportion of undesirable
516 hypercholesterolemic SFA (C14:0) and increasing health-promoting PUFA (CLA, C18:3 *n*-3,
517 C22:2*n*-6 and C20:5*n*-3). Moreover, the muscle fatty acid profile showed potential to
518 discriminate **LT muscle** from cattle offered grass silage and supplementary concentrate in
519 which the rolled barley/soybean meal was replaced with CGF. The improved fatty acid
520 profile of **LT muscle** did not negatively influence the oxidative stability, textural attributes,
521 and sensory eating quality of beef patties.

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530 CONFLICT OF INTEREST

531 The authors wish to confirm that there are no known conflicts of interest associated with this
532 publication.

533 **SUPPORTING INFORMATION**

534 Supplementary Table 1. Summary of standardized coefficient of variables and variance
535 structure described by the canonical discriminant function (CAN).

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3 653 **FIGURE LEGENDS**
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5 654 Figure 1. Plot showing the discrimination of dietary treatments following canonical
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8 655 discriminant function (CAN) analysis of the fatty acid profiles of longissimus thoracis
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10 656 muscles from steers offered grass silage and a supplementary concentrate containing varying
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12 657 levels (0%, 25%, 50% and 75%) of dried corn gluten feed (CGF).
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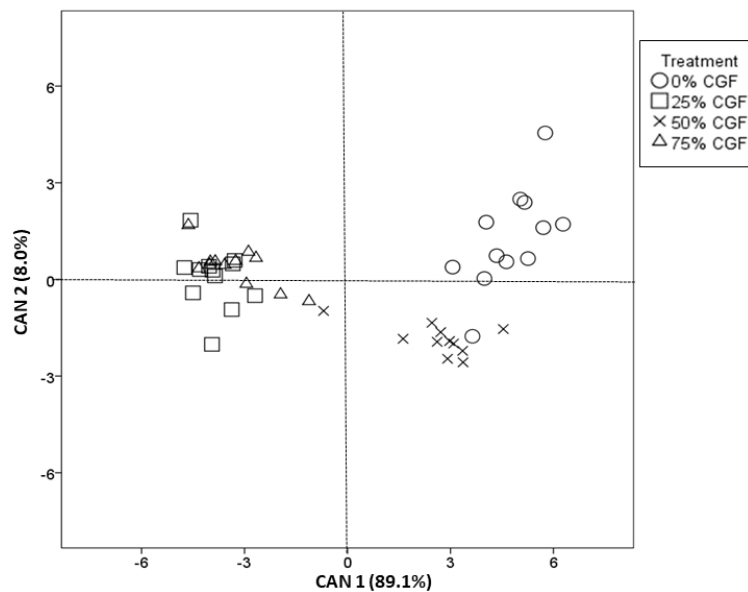


Figure 1. Plot showing the discrimination of dietary treatments following canonical discriminant function (CAN) analysis of the fatty acid profiles of longissimus thoracis muscles from steers offered grass silage and a supplementary concentrate containing varying levels (0%, 25%, 50% and 75%) of dried corn gluten feed (CGF).

254x190mm (96 x 96 DPI)

Table 1. Ingredient and chemical composition of experimental concentrate diets

Parameter	0% CGF	25% CGF	50% CGF	75% CGF
<i>Ingredient (as-fed basis, g kg⁻¹)</i>				
Rolled barley	862	673	424	175
Soybean meal	60	-	-	-
Dried corn gluten feed (CGF)	-	250	500	750
Cane molasses	50	50	50	50
Minerals and vitamins	28	27	26	25
<i>Chemical composition</i>				
Crude protein ¹	131	136	162	191
Ash ¹	58	67	80	82
Total fat ¹	28	30	26	35
Neutral detergent fibre ¹	201	184	245	314
Acid detergent fibre ¹	62	76	88	100
Starch ¹	502	422	341	211
Total phenol content ²	6.55	10.16	13.60	14.69
<i>Fatty acid (g kg⁻¹ dry matter (DM))</i>				
C12:0	0.10	-	0.20	0.10
C14:0	0.10	0.10	0.10	0.10
C16:0	3.30	4.60	5.70	6.70
C18:0	0.20	0.40	0.50	0.80
<i>c</i> -9 C18:1	1.90	3.30	4.90	7.10
<i>c</i> -9,12 C18:2	6.50	10.40	13.7	16.9
<i>c</i> -9,12,15 C18:3	0.60	0.80	0.90	0.80

¹Expressed as g kg⁻¹ DM²Expressed as g gallic acid equivalents kg⁻¹ DM

Table 2. Effect of feeding steers with grass silage and a supplementary concentrate containing varying levels of dried corn gluten feed (CGF) on the pH, proximate composition, antioxidant status and antioxidant activity of *longissimus thoracis* muscle

Item	Dietary treatment (% CGF)				SEM	P- value	Contrast effect		
	0	25	50	75			Linear	Quadratic	Cubic
Muscle pH	5.47 ^b	5.53 ^a	5.50 ^{ab}	5.51 ^a	0.007	0.002	0.052	0.045	0.006
<i>Proximate composition (g kg⁻¹ wet weight)</i>									
Protein	236.7 ^{ab}	238.7 ^a	227.5 ^b	236.4 ^{ab}	1.390	0.020	0.297	0.191	0.006
Intramuscular fat	25.7 ^{ab}	21.1 ^b	28.4 ^a	22.6 ^{ab}	0.890	0.016	0.786	0.709	0.002
Moisture	731.2	731.9	725.9	730.4	1.020	0.170	0.361	0.348	0.062
Ash	11.1 ^a	10.3 ^b	10.5 ^{ab}	10.5 ^{ab}	0.090	0.006	0.040	0.017	0.082
<i>Antioxidant status</i>									
α -tocopherol ($\mu\text{g g}^{-1}$ muscle)	2.38	2.59	2.66	2.66	0.065	0.397	0.129	0.433	0.877
TPC (mg GAE g ⁻¹ muscle)	0.94 ^a	0.88 ^{ab}	0.72 ^b	0.85 ^{ab}	0.025	0.015	0.049	0.037	0.069
<i>Antioxidant activity</i>									
DPPH (mg TE g ⁻¹ muscle)	0.25	0.25	0.25	0.25	0.002	0.621	0.584	0.228	0.997
FRAP (mg TE g ⁻¹ muscle)	0.38 ^b	0.44 ^a	0.38 ^b	0.41 ^{ab}	0.007	<0.001	0.446	0.070	<0.001
FICA (%)	55.22	57.87	56.01	62.34	1.284	0.199	0.088	0.469	0.270

^{a,b}Means within the same row bearing different superscripts are significantly different ($P < 0.05$).

SEM: Standard error of mean

TPC: total phenol content; DPPH: 1,1-diphenyl-2-picrylhydrazyl; FRAP: ferric reducing antioxidant power; FICA: ferric ion chelating activity.

GAE: gallic acid equivalent; TE: trolox equivalent

Table 3. Effect of feeding steers with grass silage and a supplementary concentrate containing varying levels of dried corn gluten feed (CGF) on the fatty acid (FA) composition of *longissimus thoracis* muscle

Fatty acid (% of total fatty acids)	Dietary treatment (% CGF)				SEM	P-value	¹ Contrast effect		
	0	25	50	75			L	Q	C
C12:0	0.07	0.04	0.06	0.07	0.007	0.241	0.669	0.179	0.158
C14:0	2.51 ^a	1.59 ^b	2.21 ^a	1.66 ^b	0.076	<0.001	<0.001	0.095	<0.001
<i>c</i> -9 C14:1	0.44	0.37	0.35	0.36	0.026	0.483	0.223	0.506	0.484
C15:0	0.39	0.94	0.45	0.56	0.077	0.073	0.960	0.143	0.032
C16:0	22.99	14.44	20.23	17.12	1.124	0.074	0.222	0.202	0.052
C16:1	1.85 ^a	0.39 ^b	0.54 ^b	0.42 ^b	0.107	<0.001	<0.001	<0.001	0.004
C17:0	0.86 ^a	0.54 ^b	0.88 ^a	0.63 ^b	0.031	<0.001	0.076	0.471	<0.001
<i>c</i> -9 C17:1	0.50 ^a	0.05 ^b	0.44 ^a	0.04 ^b	0.035	<0.001	<0.001	0.499	<0.001
C18:0	13.32	14.13	13.95	13.13	0.268	0.379	0.805	0.156	0.362
<i>t</i> -9+10 18:1	2.07 ^a	1.25 ^{ab}	1.72 ^{ab}	0.99 ^b	0.132	0.037	0.014	0.811	0.113
<i>t</i> -11 C18:1	0.95	0.77	0.97	0.98	0.036	0.080	0.363	0.190	0.045
<i>c</i> -9 C18:1	29.11	29.64	31.77	29.83	0.710	0.507	0.530	0.375	0.268
<i>t</i> -9, <i>t</i> -12 C18:2	0.28	0.11	0.29	0.37	0.060	0.475	0.431	0.326	0.376
<i>c</i> -9, <i>c</i> -12 C18:2	2.29	1.83	1.47	2.50	0.208	0.328	0.886	0.081	0.507
C20:0	0.02 ^b	0.11 ^a	0.10 ^{ab}	0.08 ^{ab}	0.012	0.024	0.111	0.008	0.916
<i>c</i> -11 C20:1	0.46	0.55	0.40	0.51	0.386	0.371	0.169	0.318	0.645
<i>c</i> -9,12,15 C18:3	0.34 ^b	0.35 ^{ab}	0.35 ^{ab}	0.45 ^a	0.014	0.032	0.013	0.115	0.554
² CLA	0.14 ^b	0.33 ^{ab}	0.17 ^{ab}	0.37 ^a	0.030	0.015	0.038	0.955	0.010
C22:0	0.52	0.42	0.41	0.61	0.034	0.116	0.340	0.026	0.838
C20:4 <i>n</i> -6	1.10	0.98	1.01	0.89	0.038	0.176	0.140	0.238	<0.201
C20:5 <i>n</i> -3	0.18 ^a	0.46 ^b	0.38 ^b	0.44 ^b	0.031	0.002	0.003	0.054	0.043
C22:5 <i>n</i> -3	0.44 ^b	0.71 ^{ab}	0.45 ^b	0.79 ^a	0.045	0.021	0.041	0.679	0.015
<i>Summary</i>									
∑SFA	41.15	32.98	38.51	34.34	1.200	0.128	0.157	0.369	0.091
∑MUFA	36.98	33.02	36.19	33.12	0.750	0.138	0.235	0.786	0.045
∑PUFA	4.97	5.59	4.32	5.61	0.254	0.067	0.157	0.161	0.059
Total <i>trans</i>	3.02 ^a	2.02 ^b	2.69 ^{ab}	1.97 ^b	0.129	0.015	0.020	0.518	0.025
∑ <i>n</i> -6 PUFA	3.87	3.74	2.97	4.35	0.245	0.177	0.623	0.086	0.182
∑ <i>n</i> -3 PUFA	0.96 ^a	1.52 ^b	1.18 ^b	1.68 ^b	0.033	<0.001	<0.001	0.229	0.021
<i>n</i> -6: <i>n</i> -3	4.03	2.46	2.52	2.59	0.487	0.091	0.221	0.042	0.753
PUFA:SFA	0.12	0.17	0.11	0.16	0.012	0.245	0.565	0.283	0.098
AI	0.82	0.57	0.76	0.63	0.035	0.098	0.212	0.334	0.053
TI	1.78	1.45	1.70	1.47	0.061	0.346	0.217	0.615	0.222

^{a,b}Means in the same row bearing different superscripts are significantly different ($P \leq 0.05$).

SEM: Standard error of mean.

¹Contrast effect: L: Linear; Q: Quadratic; C: Cubic.

²CLA: *c*-9 *t*-11 18:2 + *t*-9 *c*-11 18:2 + *t*-7 *c*-9 18:2

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

AI: Atherogenicity index; TI: Thrombogenicity index.

Table 4. Effect of feeding steers with grass silage and a supplementary concentrate containing varying levels of dried corn gluten feed (CGF) on the oxidative stability of beef patties (*longissimus thoracis* muscle)

Parameter	Diet (D, % CGF)				Storage/incubation time (T) ¹					SEM	<i>P</i> -value ²		
	0	25	50	75	1	2	3	4	5		D	T	D x T
<i>Fresh beef patties</i>													
TBARS ³	2.07	2.15	1.66	2.51	0.35 ^a	0.76 ^{ab}	1.56 ^{bc}	1.98 ^c	5.84 ^d	0.271	0.070	<0.001	0.150
Lightness, <i>L</i> *	49.24	49.00	48.97	49.42	47.64 ^a	48.50 ^a	48.88 ^a	48.61 ^a	52.17 ^b	6.347	0.772	<0.001	0.881
Redness, <i>a</i> *	16.72	16.83	16.57	16.60	22.86 ^a	20.93 ^b	18.59 ^c	14.98 ^d	6.05 ^c	2.154	0.905	<0.001	0.748
Yellowness, <i>b</i> *	15.24	15.13	15.11	15.22	16.63 ^a	15.89 ^b	15.02 ^c	13.96 ^d	14.38 ^d	1.959	0.875	<0.001	0.818
Chroma ⁴ , <i>C</i> *	22.99	22.92	22.82	22.93	28.27 ^a	26.28 ^b	23.90 ^c	20.50 ^d	15.63 ^c	2.958	0.970	<0.001	0.748
Hue angle ⁵ , <i>H</i> *	44.52	43.92	44.66	44.96	36.04 ^a	37.21 ^{ab}	38.97 ^b	43.18 ^c	67.17 ^d	5.747	0.601	<0.001	0.700
<i>Cooked beef patties</i>													
TBARS ³	2.29	2.33	2.06	2.10	1.59 ^a	1.96 ^a	2.43 ^b			0.332	0.110	<0.001	0.981
<i>Muscle homogenates</i>													
TBARS ³	4.21	4.28	4.31	4.51	2.27 ^a	6.13 ^b				0.221	0.145	<0.001	0.947
OxyMb ⁶ (%)	65.31	68.50	68.90	67.00	86.02 ^a	48.77 ^b				2.187	0.615	<0.001	0.661

^{x,y}Within row, different superscript letters indicate differences ($P < 0.05$) between dietary treatment.

^{a,b,c,d,e}Within row, different superscript letters indicate differences ($P < 0.05$) between storage/incubation time.

SEM: Standard error of mean.

¹Times 1, 2, 3, 4, 5 correspond to: 1, 4, 7, 10 and 14 days (fresh beef patties stored at 4°C in modified atmosphere packs); 1, 3, 6 days (cooked beef patties stored at 4°C in aerobic packs); 1 and 4 hours (muscle homogenates incubated with Fe/Ascorbate at 4°C)

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

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

⁴Chroma (colour vividness), higher values indicate greater saturation of red.

⁵Hue angle (trueness of red), lower values indicate a redder colour.

⁶OxyMb: Oxymyoglobin, % of total myoglobin.

Table 5. Effect of feeding steers with grass silage and a supplementary concentrate containing varying levels of dried corn gluten feed (CGF) on texture profile parameters and the eating quality of beef patties (*longissimus thoracis* muscle) stored in modified atmosphere packs at 4 °C for up to 7 days

Parameter	Diet (D, % CGF)				Storage time (T, day)			P-value ¹		
	0	25	50	75	2	7	SEM	D	T	D x T
<i>Textural attributes</i>										
Hardness	20.26	21.40	21.98	21.06	17.34 ^a	25.01 ^b	0.948	0.724	<0.001	0.364
Springiness	0.84	0.85	0.86	0.86	0.85	0.86	0.004	0.243	0.051	0.337
Cohesiveness	0.60	0.58	0.58	0.59	0.58	0.59	0.006	0.833	0.398	0.630
Gumminess	12.19	12.61	12.90	12.37	10.12 ^a	14.91 ^b	0.629	0.936	<0.001	0.400
Chewiness	10.33	10.73	11.12	10.67	8.58 ^a	12.85 ^b	0.555	0.889	<0.001	0.342
Adhesiveness	-1.48	-0.91	-1.37	-1.05	-1.17	-1.23	0.103	0.186	0.776	0.423
<i>Eating quality</i>										
Appearance	5.72	6.34	6.12	6.18	5.93	6.25	0.123	0.323	0.185	0.849
Odour	6.01	6.07	6.07	6.15	6.02	6.13	0.105	0.973	0.613	0.956
Texture	4.45	4.94	4.70	4.92	4.93	4.58	0.122	0.458	0.156	0.784
Juiciness	3.30	3.98	3.76	3.55	3.75	3.55	0.119	0.226	0.411	0.894
Flavour	5.77	5.97	5.97	5.66	5.85	5.83	0.111	0.700	0.921	0.821
Overall acceptability	5.07	5.61	5.38	5.25	5.35	5.31	0.105	0.295	0.853	0.891

^{a,b}Within row, different superscript letters indicate differences ($P < 0.05$) between storage time.

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

SEM: Standard error of mean.

Supplementary Table 1. Summary of standardized coefficient of variables and variance structure described by the canonical discriminant function (CAN)

Variables	CAN 1	CAN 2	CAN 3
C15:0	-0.505	-0.043	-0.456
C16:1	0.297	1.028	-0.111
C17:0	0.804	-0.127	0.441
<i>c</i> -10 C17:1	1.310	-0.564	-0.260
<i>t</i> -11 C18:1	-0.710	0.018	0.710
C22:0	0.709	0.223	0.692
<i>Statistics</i>			
Eigen value	15.242	1.370	0.498
Variance (%)	89.1	8.0	2.9
Cumulative variance (%)	89.1	97.1	100
Canonical correlation	0.969	0.760	0.577