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University College Cork, Ireland Coláiste na hOllscoile Corcaigh

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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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# SCHOLARONE<sup>™</sup> Manuscripts

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## 20 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 C22:2*n*-6, C20:5*n*-3 and total *n*-3 polyunsaturated fatty acids whereas 75%CGF increased conjugated linoleic acids and C18:3*n*-3. Diet did not affect the oxidative stability and sensory attributes of beef patties.

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

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

#### 42 INTRODUCTION

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

Animal diet has a significant impact on meat quality traits including those related to nutritional value, shelf-life, and sensory characteristics, which influence consumer preference and acceptability. Research into the fatty acid composition of beef has attracted considerable attention in recent years due to its nutritional significance and relationship to other meat quality attributes (e.g. flavour and oxidative stability) which in turn influence retail value and eating quality<sup>3</sup>. Microbial transformation of dietary unsaturated fatty acids to saturated fatty acids (SFA) in the rumen is a major factor limiting the accumulation of polyunsaturated fatty acids (PUFA) in ruminant meat and dietary strategies designed to alter this process are of interest to animal/food scientists and the meat industry <sup>4</sup>. 

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

> compounds, such as vitamin E, in the muscle <sup>5</sup>. However, supplementation of grass-based diets with concentrate rations has a varied effect on meat quality indices depending on several factors including the nutrient composition of concentrate ingredients which influences the metabolic response of animals and the ultimate composition of the meat <sup>6</sup>. The lower starch and higher fibre content in CGF, compared to grains, decrease the negative effects of starchy concentrate-based feeds on ruminal pH and fibre digestion in cattle fed forage-based diets<sup>2</sup>. It is proposed that feeding CGF, compared to high-starch grains, in combination with grass silage could increase the ruminal outflow of PUFA for absorption into the muscle tissue and improve the fatty acid profile of the meat. Moreover, CGF consists of bran and steep liquor that are rich sources of phenolic antioxidants such as protocatechuic acid, vanillic acid, p-coumaric acid, ferulic acid, sinapic acid and quercetin <sup>7, 8</sup>. Ingestion and deposition of phenolic compounds in muscle tissues may ultimately enhance the oxidative stability of meat 1,9.

> Previous studies have shown that replacement of corn/soybean meal with CGF (25% of dry matter, DM) in a concentrate feedlot ration had a minimal effect on the fatty acid profile, retail shelf-life, sensory attributes and acceptability of beef from steers fed corn silage <sup>10, 11</sup>. To our knowledge, there is no information available to date on the quality of meat from beef cattle fed a grass silage-based diet supplemented with concentrate rations containing CGF. Therefore, the objective of this study was to examine the chemical composition, fatty acid profile, oxidative stability and sensory attributes of beef from steers offered *ad libitum* grass silage and a concentrate supplement in which rolled barley/soybean meal was replaced by varying levels (0, 25, 50, 75%) of dried CGF.

#### 88 MATERIALS AND METHODS

#### 89 Animals, diets and experimental design

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

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

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

Animals were slaughtered in a commercial abattoir on two consecutive weeks (balanced for treatment) to facilitate sample collection and measurements. Samples of *longissimus thoracis* muscle (LT) were removed from the left side of the carcass at 48 h post-mortem, vacuumpacked and aged for 14 days at 4 °C, and subsequently stored at -20 °C prior to further analysis. Information on animal intake, growth, carcass traits, LT drip loss, and colour (lightness  $L^*$ , redness  $a^*$  and yellowness  $b^*$ ) of subcutaneous fat and LT is presented in Kelly, et al. <sup>12</sup>.

#### 123 Chemical analysis of feed

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

Phenolic compounds were extracted from samples of concentrate rations using aqueous methanol (50:50, v/v) and acetone (70:30, v/v) solvents <sup>14</sup>. Polyphenol-rich extracts were analysed for total phenol content (TPC) using the Folin-Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventós, 1999) and absorbance measurements were recorded at 750 nm using a UV-vis spectrophotometer (Cary 300 Bio, UV-vis spectrophotometer, Varian Instruments, Palo Alto, CA, USA). Results were expressed as g of gallic acid equivalents (GAE)/kg of DM feed.

The fatty acid composition was determined in freeze-dried samples of concentrate rations by a one-step extraction-transesterification procedure using chloroform <sup>15</sup> and 2% (v/v) sulfuric acid in methanol <sup>16</sup>, with 19:0 nonadecanoate (Larodan, Solna, Sweden) added as an internal standard. Gas chromatographic analysis of fatty acid methyl esters (FAME) was performed as described by Cherif, et al. <sup>17</sup>. Individual fatty acids were expressed as g/kg of DM feed.

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### Determination of muscle pH and proximate composition

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

### <sup>2</sup> 151 Analysis of vitamin E and fatty acids in LT muscle

The α-tocopherol (vitamin E) content in minced LT samples was determined by highperformance liquid chromatography (HPLC) as previously described by Salami, et al. <sup>18</sup>. In brief, HPLC analysis was carried out on a ProStar liquid chromatograph (Varian Analytical Instruments, Palo Alto, CA, USA) equipped with a ProStar autosampler (Model 410, Varian Instruments). Sample injection volume (partial loop fill) was 20 µl. The α-tocopherol was separated on a 250 x 4.6 mm Polaris C18-A 5u column (Metachem, Ansys<sup>®</sup> Technologies, CA, USA) and detected using a ProStar UV/Vis detector (Varian Instruments) at 292 nm. The

mobile phase was methanol/water (97:3) and isocratic elution took place at 2ml/min for a total run time of 10 min. A personal computer and Star LC workstation software (version 6.20, Varian Inc.) was used for calculation of peak areas. A standard curve was generated using a range of  $\alpha$ -tocopherol concentrations (7, 14, 21 µg/ml) and the concentration of  $\alpha$ -tocopherol in beef was expressed in  $\mu g/g$  of beef muscle. The percentage recovery of vitamin E from beef samples, through the extraction procedure, was determined by including vitamin E (0.2 ml of 22.8 µg/ml) as an internal standard. The percentage recovery (92.8%) was calculated by comparison of peak areas of vitamin E recovered through the extraction procedure with those obtained by direct injection of the vitamin E standard (22.8 µg/ml) onto the column. 

For fatty acids analysis, the lipid fraction in minced LT samples was extracted following the procedure described by Bligh and Dyer<sup>19</sup> and the lipid fraction was transesterified to FAME using BF<sub>3</sub> in methanol as a catalyst <sup>20</sup> and dissolved in isooctane. The gas-chromatographic analysis was conducted following the injection, pressure and temperature conditions described extensively by Salami, et al.<sup>21</sup>. Individual compounds were identified using FAME standards (a mixture of Supleco 37 component FAME mix, trans-11 vaccenic acid methyl ester and conjugated linoleic acid methyl ester; Sigma-Aldrich Ireland Ltd., Vale Road, Arklow, Wicklow, Ireland) and results were reported as g/100g of the total fatty acids. The atherogenic index (AI) and thrombogenic index (TI) were calculated according to Ulbricht and Southgate <sup>22</sup> as follows: 

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

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

where MUFA is monounsaturated fatty acids and PUFA is polyunsaturated fatty acids. 

#### Determination of total phenol content and in vitro antioxidant activity

#### **Preparation of muscle homogenates**

Beef homogenates (10% w/v) were prepared as described by Owele, et al. <sup>23</sup> for the determination of *in vitro* antioxidant activities. Briefly, minced LT (5 g) was homogenised in 0.05 M phosphate buffer (45 ml) using an Ultra-turrax T25 homogeniser for 3 min. Muscle homogenates were centrifuged at 7,800 g for 10 min at 4 °C using an Avanti<sup>®</sup> J-E Centrifuge (Beckman Coulter Inc., Palo Alto, CA, USA). The supernatant was filtered through Whatman No. 1 paper and the filtrate was analysed for ferric reducing antioxidant power (FRAP) and ferric ion chelating activity (FICA). For the determination of TPC and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assays, 10% trichloroacetic acid (5 ml) was added to muscle homogenates and the mixture was centrifuged at 7,800 g for 10 min at 4 °C. The supernatant was filtered through Whatman No. 1 paper and analysed for TPC and Z.C. DPPH activity. 

#### Measurement of the total phenol content

Muscle extracts were analysed for TPC using the Folin-Ciocalteu method <sup>24</sup> with minor modifications. Briefly, extracts (0.5 ml) were mixed with Folin-Ciocalteu reagent (2.5 ml, 20% in distilled water) and sodium carbonate (2 ml, 7.5% in distilled water) was added after 5 min. The mixture was stored in the dark for 2 h at room temperature and absorbance measurements were recorded at 750 nm on a UV-vis spectrophotometer (Cary 300 Bio, UV-vis spectrophotometer, Varian Instruments, CA, USA) against a blank containing all reagents and distilled water. A calibration curve using standard solutions of aqueous gallic acid (20 -

203 100 μg/ml) was constructed and results are expressed as mg of gallic acid equivalents
204 (GAE)/g of muscle.

#### 205 Measurement of in vitro antioxidant activities

Radical scavenging activity in muscle was measured using the DPPH assay following a minor modification of the method described by Yen and Wu<sup>25</sup>. Muscle extract (0.6 ml) and distilled water (2.4 ml) were mixed with 0.2 mM DPPH in methanol (3 ml) and incubated in the dark for 1 h at room temperature. Absorbance measurements were recorded at 517 nm on a UV-vis spectrophotometer (Cary 300 Bio) against a methanol blank. An assay blank containing distilled water (3 ml) and 0.2 mM DPPH in methanol (3 ml) was used for calculation purposes. A calibration curve using standard solutions of methanolic Trolox (10 -50 µg/ml) was constructed and results were expressed as mg of Trolox equivalents (TE)/g of muscle. 

Total antioxidant activity in muscle was determined using FRAP assay following a minor modification of the method described by Benzie and Strain <sup>26</sup>. Briefly, muscle extract (0.45 ml) was mixed with 8.55 ml FRAP reagent (a mixture of 30 mM acetate buffer (pH 3.6), 10 mM 2,4,6-Tris (2-pyridyl)-s-triazine in 40 mM HCl and 20 mM FeCl<sub>3</sub>.6H<sub>2</sub>O in distilled water in the ratio 10:1:1, respectively incubated at 37 °C for 10 min prior to use). The mixture was incubated for 30 min in the dark and absorbance was recorded at 593 nm on a UV-vis spectrophotometer (Cary 300 Bio) against a blank containing all reagents. A calibration curve using solutions of methanolic Trolox (0.033 - 0.1 mg/ml) was constructed and results are expressed as mg TE/g of muscle. 

The iron-chelating activity of muscle was measured using a minor modification of FICA assay described by Yen and Wu  $^{25}$ . Muscle extract (0.5 ml) was mixed with FeCl<sub>2</sub> (2 mM in distilled water, 0.1 ml), ferrozine solution (5 mM in distilled water, 0.2 ml) and distilled

water (4.2 ml). The assay control contained  $FeCl_2$  (0.1 m), ferrozine solution (0.2 ml) and distilled water (4.7 ml). The mixture was incubated for 1 h in the dark at room temperature and absorbance measurements were recorded at 562 nm against a water blank on a UV-vis spectrophotometer (Cary 300 Bio). The chelating activity was calculated as follows:

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

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

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

Muscle homogenates (25%) were prepared by homogenising 15 g of LT in a buffer (0.12 M KCL 5 mM histidine, pH 5.5) surrounded by crushed ice using an Ultra Turrax T25 homogeniser. Lipid oxidation in muscle homogenates (39.2 g) was initiated by the addition of pro-oxidants (45  $\mu$ M FeCl<sub>3</sub>/sodium ascorbate, 1:1) <sup>27</sup>. Lipid oxidation (2-thiobarbituric acid reactive substances, TBARS) and oxymyoglobin (OxyMb) content in muscle homogenate were measured at 1 and 4 h of storage at 4 °C as described by Hayes, et al. <sup>28</sup>.

240 Beef processing and packaging

The LT muscles were thawed overnight at 4 °C, trimmed of visible fat and connective tissue, and minced twice through a plate with 4 mm holes (Model P114L, Talsa, Valencia, Spain). The minced muscle was formed into beef patties (100 g portions) using a meat former (Ministeak burger maker, O.L Smith Co. Ltd., Italy). For the fresh beef study, patties were individually placed in a low oxygen permeable (<1  $cm^3/m^2/24$  h at STP) polystyrene/ethyl vinyl alcohol/polyethylene (PE) trays and flushed with 80% O<sub>2</sub>:20% CO<sub>2</sub> (modified atmosphere packs, MAP) using a vacuum-sealing unit (VS 100, Gustav Müller & Co. KG, Homburg, Germany) equipped with a gas mixer (Witt-Gasetechnik GmbH & Co. KG, Witten, Germany). Trays were covered and heat-sealed using a low oxygen permeable (3 

cm<sup>3</sup>/m<sup>2</sup>/24 h at STP) laminated barrier film with a polyolefin heat-sealable layer. Fresh beef patties in MAP were stored for up to 14 days under fluorescent lighting (660 lx) at 4 °C. The gas atmosphere (% O<sub>2</sub> and % CO<sub>2</sub>) in MAP was measured using a CheckMate 9900 (PBI-DanSensor, Denmark). The average gas composition in MAP was 79.53  $\pm$  0.39% O<sub>2</sub> and 20.77  $\pm$  0.23% CO<sub>2</sub> on day 1 of storage and 74.03  $\pm$  0.77% O<sub>2</sub> and 25.85  $\pm$  0.73% CO<sub>2</sub> on day 14 of storage.

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

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

 Lipid oxidation was measured in fresh beef patties on days 1, 4, 7, 11, and 14 of storage and days 1, 3, and 6 in cooked beef patties. Lipid oxidation measurements were carried out following the method described by Siu and Draper <sup>29</sup>. Results were expressed as TBARS in mg malondialdehyde (MDA)/kg meat.

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

#### 274 Measurement of textural properties of beef patties

The texture profile analysis (TPA) of fresh beef patties stored in MAP was measured on days 2 and 7 of storage. The TPA parameters (hardness (N), springiness (mm), cohesiveness (dimensionless), gumminess (N), chewiness (N  $\times$  mm), adhesiveness (N)) were measured using a 30 kg load cell texture analyser (TA.XT2i Texture Analyser, Stable Micro Systems, UK) as described by Moroney, et al. <sup>30</sup>.

#### 280 Sensory analysis of beef patties

Sensory acceptance testing of fresh beef patties (n = 8/treatment) stored in MAP was carried out by 40 untrained panellists in 2 sessions (20 panellists/session) on days 2 and 7 of storage as described by O'Sullivan, et al.<sup>31</sup>. Beef patties were cooked for sensory analysis in a Zanussi oven at 180 °C for 20 min until an internal temperature of 72 °C was reached. Following cooking, patties were cooled to room temperature and cut into  $2 \text{ cm} \times 2 \text{ cm}$  cubes, identified with random three-digit codes. On each day of evaluation, beef samples were served to panellists in two separate sessions (morning and afternoon sessions). Before serving to panellists, beef samples were re-heated in a microwave for 10 s to release the meat odour and flavour. Sensory evaluation was performed in the panel booths of the University's sensory laboratory according to international standard regulations <sup>32</sup>. Panellists were provided with water to cleanse their palates between samples. Each panellist received beef samples presented in a randomised order to prevent any flavour carryover effects <sup>33</sup>. Panellists were asked to indicate their degree of liking for appearance, odour, texture, juiciness, flavour and overall acceptability on a 10 cm line scale ranging from 0 (extremely dislike) to 10 (extremely like).

## 296 Statistical analysis

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

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

2 316 **RESULTS AND DISCUSSION** 

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

cattle <sup>2</sup>. There is no information on the impact of dietary CGF on beef quality when included in a concentrate supplement for finishing beef cattle fed grass silage. In this regard, the present study investigated quality indices of beef from steers fed grass silage and a concentrate supplement in which rolled barley/soybean meal was replaced with different levels (0%, 25%, 50% and 75%) of CGF. The inclusion of 25%, 50% and 75% CGF in concentrate supplement correspond to 10.9%, 21.5% and 33.7% of the total dietary intake (grass silage + concentrate supplement), respectively.

## *pH and proximate composition of muscle*

The pH and proximate composition of LT muscle are presented in Table 2. Values of muscle pH in all treatments (5.47 - 5.53) were within the normal range (5.4 - 5.8) for beef <sup>34</sup>. The moisture, IMF and protein content of LT muscles from the CGF treatments was not significantly different from the control (0% CGF). Similarly, it has been reported that the proximate composition of different muscles obtained from steers fed CGF (25% dietary DM) was not different from those fed corn/soybean meal <sup>10, 11</sup>. In comparison to 50% CGF, muscles from steers fed 25% CGF had greater protein content and lower IMF content (Table 2). The observed differences in IMF may not be related to the level of feed intake as steers had a similar DM intake (grass silage and concentrate) and growth rate across the dietary treatments <sup>12</sup>. However, it is possible that variation in IMF content is related to differences in the ruminal fermentation pattern, producing more of a glucogenic precursor (propionate), and/or extent of starch digestion in the small intestine <sup>35</sup>. In addition, the ash content in LT muscle decreased quadratically (P = 0.015) as CGF inclusion increased, with 25% CGF being lower (P < 0.05) than 0% CGF. Overall, the present results showed that inclusion of 75% CGF in supplementary concentrate ration did not affect the muscle protein level, IMF, moisture, and ash values compared to the control and other CGF treatments. 

## 344 Antioxidant capacity of muscle

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

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

369 = -0.16; P = 0.288) between TPC and FRAP values. Nonetheless, the current results370demonstrated that dietary inclusion of up to 75% CGF in the concentrate supplement did not371compromise the antioxidant capacity of muscle.372*Fatty acid composition of muscle*373*Effect of diet on the fatty acid profile of LT muscle*374Results of FA composition in LT muscle are presented in Table 3. Dietary treatment did not375affect (P > 0.05) the relative amount of total SFA in LT muscle but influenced the proportion376of individual SFA with nutritional significance. Muscle from steers fed 25% and 75% CGF377had decreased (P < 0.05) percentages of C14:0 and C17:0 compared to those fed 0% and 50%378CGF. In addition, CGF diets tended (P = 0.074) to decrease C16:0 in a cubic manner, with37925% CGF displaying the greatest decrease (-37.2%) compared to 0% CGF. Notably, the380consumption of elevated levels of dietary C14:0 and C16:0 increases low-density lipoprotein381cholesterol, linked to increased risk of cardiovascular diseases (CVD), obesity and insulin382resistance in humans 4.5. Thus, it can be deduced that feeding 25% and 75% CGF decreased

383 the proportion of hypercholesterolemic SFA in beef.

The proportion of total MUFA was unaffected (P > 0.05) by feeding CGF and no dietary effect was observed (P > 0.05) on the relative amount of *c*-9 C18:1, the predominant MUFA in beef. Compared to the CGF treatments, steers fed 0% CGF had higher (P < 0.05) C16:1 and *c*-10 C17:1, monounsaturated derivatives of C16:0 and C17:0, respectively. The accumulation of C16:1 and *c*-10 C17:1 suggests that *de novo* lipid synthesis may play a role in the alteration of LT muscle fatty acid profile in this study. A linear decrease in the percentage of total *trans* fatty acids (TFA) was mainly influenced by a linear decrease (P <0.05) of *t*-9+10 18:1. However, the proportion of *t*-11 18:1 (*trans*-vaccenic acid) was not affected (P > 0.05) by dietary treatment. The observed changes in muscle TFA may be of

minimal implication because current evidence suggests that dietary consumption of ruminant
 TFA may have a limited health impact in contrast to the increased CVD risks associated with
 industrial TFA <sup>5</sup>.

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

Different FA indices were used to evaluate the nutritional importance of beef fat and the contribution to healthy human diets. Dietary treatment did not influence (P > 0.05) PUFA to SFA ratio and the thrombogenic index. However, the *n*-6:*n*-3 ratio tended to be lower (P =0.093) in the CGF treatments, with values of 25% and 50% CGF desirably lower than the maximum nutritional recommendation of 4.0<sup>37</sup>. The inclusion levels of CGF tended to favourably decrease (P = 0.098) muscle atherogenicity index in a cubic response, due to a lower proportion of C14:0 and C16:0, and higher *n*-3 PUFA.

Overall, it is noteworthy that dietary CGF favourably altered the FA composition of beef in the present study contrary to a previous study which reported that CGF had a minimal impact on the fatty acid profile of LT muscle <sup>10</sup>. Differences in feed fatty acid composition could be one of the reasons for the discrepancies between the results presented and those of Segers, et al. <sup>10</sup>. The present study showed that increasing the inclusion levels of CGF resulted in corresponding increases in dietary PUFA (oleic acid and linoleic acid) in experimental diets (Table 1) whereas information on feed fatty acid composition was not reported by Segers, et al. <sup>10</sup>. Additionally, the inclusion of CGF in the present study decreased dietary starch levels which may mitigate the impairment of ruminal digestion that can occur from supplementation of forage with cereals<sup>2</sup>. Thus, it is possible that CGF exhibited a complementary effect in protecting dietary unsaturated fatty acids against ruminal biohydrogenation. 

427 Multivariate analysis of muscle fatty acid profile

The application of multivariate statistical analysis to chemical constituents (such as fatty acids) in muscle tissues can be used to discriminate meat from different feeding provenance, geographical origins or production systems <sup>38</sup>. This approach may be relevant to authenticate meat produced from sustainable feeding systems that promote the substitution of by-products for cereal grains in animal rations. In the present study, linear discriminant function analysis was performed to identify the fatty acids that better contribute to the differentiation of beef from animals fed diets in which rolled barley/soybean meal was replaced by CGF in concentrate rations. Six fatty acids (C15:0, C16:1, C17:0, *c*-10 C17:1, *t*-11 C18:1 and C22:0) were retained as the best quantitative predictor variables after a stepwise selection procedure was applied to the muscle fatty acid dataset. The predictor variables follow a similar observation suggesting that a combination of fatty acids derived from ruminal biohydrogenation intermediates and *de novo* fatty acid synthesis contribute to the discrimination of the feeding provenance of beef <sup>39</sup>. The predictor variables were linearly

441 combined to obtain three canonical discriminant functions (CAN). The first two CAN 442 described 97.1% (CAN 1 = 89.1% and CAN 2 = 8.0%) of the total variance associated with 443 the multivariate structure on the CAN plot (Figure 1). Discriminant power of the canonical 444 model was significant (P < 0.01) based on Wilks' lambda test of significance.

The scattered distribution on the CAN plot indicated that muscles from animals fed 25% and 75% CGF (located on the left side of the quadrant) were not clearly differentiated from each other but were discriminated by CAN 1 from those fed 0% and 50% CGF (located on the right side of the quadrant). The discriminant power of CAN 1 was maximized by c-10 C17:1, C17:0, t-11 C18:1 and C22:0 as shown with higher values of standardized coefficients of variables (Supplementary Table 1). However, CAN 2 distinguished beef from animals fed 0% CGF and 50% CGF, located on the upper and lower right side of the quadrant, respectively. The discriminant power of CAN 2 was highly influenced by C16:1 and c-10 C17:1 (Supplementary Table 1). Cross-validation of the discriminant model revealed that 10 steers were correctly classified to each of 0%, 25% or 50% CGF while 9 steers were correctly assigned to 75% CGF. The model inaccuracy showed that steers belonging to 0% (n = 2), 25% (n = 2), 50% (n = 1) and 75% (n = 3) CGF were wrongly assigned to 50%, 75%, 75% and 25% CGF, respectively, indicating that none of the CGF-fed steers was wrongly assigned to the control (0% CGF) group. Therefore, this data suggests that muscle fatty acid profile may be a potential chemical marker for discriminating beef from cattle finished on a forage-based diet and concentrate supplement containing CGF. Also, among the entire set of the identified fatty acids, the discriminant analysis allows for highlighting fatty acids which are more linked to feeding CGF. 

#### 463 Oxidative stability of beef

Feeding CGF diets did not affect (P > 0.05) lipid oxidation (TBARS) and colour (lightness  $L^*$ , redness  $a^*$ , yellowness  $b^*$ ) stability of fresh beef patties stored in high-oxygen modified atmosphere packs (MAP) for up to 14 days at 4 °C (Table 4). Significant effects (P < 0.01) of storage time was observed on measured parameters for lipid and colour stability. However, the interaction between treatment and storage time did not affect (P > 0.05) the lipid and colour stability parameters. In agreement with the current results, previous research has shown that substitution of corn/soybean meal with CGF (25% dietary DM) did not affect lipid oxidation and subjective colour acceptance of aerobically-stored fresh beef steaks subjected to refrigerated retail display over a 9-d period <sup>10</sup>. 

Furthermore, dietary treatment did not affect (P > 0.05) the levels of lipid oxidation in cooked beef patties stored in aerobic packs for up to 6 days at 4 °C (Table 4). The effect of storage time on lipid oxidation was similar between day 1 and 3 (P > 0.05) and differs when compared to day 6 (P < 0.05) (Table 4). However, there was no diet × storage time effect on the lipid oxidation of cooked beef patties. Therefore, the current results demonstrate that feeding CGF did not negatively influence the stability of beef patties evaluated under different oxidative conditions (MAP and aerobic packs).

Indeed, dietary CGF did not negatively influence the oxidative stability of beef patties stored in MAP and aerobic packs despite the increased amount of highly-peroxidizable PUFA in meat from steers fed 25% and 75% CGF. Furthermore, the extent of lipid oxidation and OxyMb oxidation were similar (P > 0.05) among dietary treatments when muscle homogenates were subjected to a more vigorous oxidative condition by incubating with iron/ascorbate pro-oxidants (Table 4). This observation further confirms that dietary CGF did not enhance the oxidative stability of beef possibly due to the lack of deposition of antioxidant phenolics in the muscle of CGF-fed steers. In agreement with this assertion, it has
been shown that dietary supplementation of ferulic acid, a major phenolic compound in CGF,
did not enhance the oxidative stability of beef <sup>40</sup>.

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

Dietary treatment did not influence (P > 0.05) texture profile analysis (TPA) parameters (hardness, springiness, cohesiveness, gumminess, chewiness and adhesiveness) of beef patties on days 2 and 7 of storage in MAP (Table 5). A significant effect of storage time (P < 10.05) was observed on hardness, gumminess and chewiness while springiness, cohesiveness and adhesiveness did not differ (P > 0.05) over the storage time. No significant interaction (P> 0.05) was found between diet  $\times$  storage time. Previous studies have demonstrated that inclusion of CGF in a concentrate finishing ration of steers did not affect the instrumental texture (Warner-Bratzler shear force) of beef<sup>10, 11</sup>. 

Sensory naïve panellists did not detect differences (P > 0.05) between dietary treatments in the eating quality characteristics (appearance, odour, texture, juiciness, flavour, and overall acceptability) of beef patties at days 2 and 7 of storage in MAP (Table 5). Storage time did not influence (P > 0.05) eating quality characteristics except the liking of texture which was decreased (P < 0.05) in 50% CGF beef patties between days 2 and 7 of storage. Moreover, no significant effect (P > 0.05) of diet  $\times$  storage time was observed on all the eating quality attributes rated by the panellists. It has been shown that TPA parameters correlate with the assessment of subjective sensory tenderness of beef <sup>41</sup>, which may explain why the lack of dietary effect on TPA parameters was consistent with the similarity in consumer liking for beef texture. Overall, the current results indicate that dietary inclusion of CGF did not negatively influence the consumer acceptance of beef in agreement with a similar result reported with trained sensory panellists <sup>11</sup>. 

#### 511 CONCLUSIONS

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

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530 CONFLICT OF INTEREST

The authors wish to confirm that there are no known conflicts of interest associated with thispublication.

## 533 SUPPORTING INFORMATION

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

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## 653 FIGURE LEGENDS

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).

to per period



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)

JSFA@wiley.com

Parameter	0% CGF	25% CGF	50% CGF	75% CG
Ingredient (as-fed basis, g kg <sup>-1</sup> )				
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
Chemical composition				
Crude protein <sup>1</sup>	131	136	162	191
Ash <sup>1</sup>	58	67	80	82
Total fat <sup>1</sup>	28	30	26	35
Neutral detergent fibre <sup>1</sup>	201	184	245	314
Acid detergent fibre <sup>1</sup>	62	76	88	100
Starch <sup>1</sup>	502	422	341	211
Total phenol content <sup>2</sup>	6.55	10.16	13.60	14.69
Fatty acid (g kg <sup>-1</sup> dry matter (DM	))			
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
<sup>1</sup> Expressed as g kg <sup>-1</sup> DM <sup>2</sup> Expressed as g gallic acid equivalents kg	-1 DM	R.		

**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

	Diet	ary treat	ment (%	CGF)			(	Contrast effe	et
Item	0	25	50	75	SEM	<i>P</i> - value	Linear	Quadratic	Cubic
Muscle pH	5.47 <sup>b</sup>	5.53ª	5.50 <sup>ab</sup>	5.51ª	0.007	0.002	0.052	0.045	0.006
Proximate composition (g kg-	wet weigl	ht)							
Protein	236.7 <sup>ab</sup>	238.7ª	227.5 <sup>b</sup>	236.4 <sup>ab</sup>	1.390	0.020	0.297	0.191	0.006
Intramuscular fat	25.7 <sup>ab</sup>	21.1 <sup>b</sup>	28.4ª	22.6 <sup>ab</sup>	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	11.1ª	10.3 <sup>b</sup>	10.5 <sup>ab</sup>	10.5 <sup>ab</sup>	0.090	0.006	0.040	0.017	0.082
Antioxidant status									
$\alpha$ -tocopherol (µg g <sup>-1</sup> muscle)	2.38	2.59	2.66	2.66	0.065	0.397	0.129	0.433	0.877
TPC (mg GAE g <sup>-1</sup> muscle)	0.94ª	0.88 <sup>ab</sup>	0.72 <sup>b</sup>	0.85 <sup>ab</sup>	0.025	0.015	0.049	0.037	0.069
Antioxidant activity									
DPPH (mg TE g <sup>-1</sup> muscle)	0.25	0.25	0.25	0.25	0.002	0.621	0.584	0.228	0.997
FRAP (mg TE g <sup>-1</sup> muscle)	0.38 <sup>b</sup>	0.44ª	0.38 <sup>b</sup>	0.41 <sup>ab</sup>	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

<sup>a,b</sup>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: erric reducing antioxidant power; FICA: ferric ion chelating activity.

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GAE: gallic acid equivalent; TE: trolox equivalent

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<b>Table 3.</b> 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	Die	tary treat	ment (%	CGF)	_		<sup>1</sup> Contrast effect		ffect
total fatty acids)	0	25	50	75	SEM	<b>P</b> -value	L	Q	С
C12:0	0.07	0.04	0.06	0.07	0.007	0.241	0.669	0.179	0.158
C14:0	2.51ª	1.59 <sup>b</sup>	2.21ª	1.66 <sup>b</sup>	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 <sup>a</sup>	0.39 <sup>b</sup>	0.54 <sup>b</sup>	0.42 <sup>b</sup>	0.107	< 0.001	< 0.001	< 0.001	0.004
C17:0	0.86 <sup>a</sup>	0.54 <sup>b</sup>	0.88 <sup>a</sup>	0.63 <sup>b</sup>	0.031	< 0.001	0.076	0.471	< 0.001
<i>c</i> -9 C17:1	0.50 <sup>a</sup>	0.05 <sup>b</sup>	0.44 <sup>a</sup>	0.04 <sup>b</sup>	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 <sup>a</sup>	1.25 <sup>ab</sup>	1.72 <sup>ab</sup>	0.99 <sup>b</sup>	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 <sup>b</sup>	0.11ª	0.10 <sup>ab</sup>	0.08 <sup>ab</sup>	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 <sup>b</sup>	0.35 <sup>ab</sup>	0.35 <sup>ab</sup>	0.45ª	0.014	0.032	0.013	0.115	0.554
<sup>2</sup> CLA	0.14 <sup>b</sup>	0.33 <sup>ab</sup>	0.17 <sup>ab</sup>	0.37ª	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 <sup>a</sup>	0.46 <sup>b</sup>	0.38 <sup>b</sup>	0.44 <sup>b</sup>	0.031	0.002	0.003	0.054	0.043
C22:5 <i>n</i> -3	0.44 <sup>b</sup>	0.71 <sup>ab</sup>	0.45 <sup>b</sup>	0.79ª	0.045	0.021	0.041	0.679	0.015
Summary									
∑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 <sup>a</sup>	2.02 <sup>b</sup>	2.69 <sup>ab</sup>	1.97 <sup>b</sup>	0.129	0.015	0.020	0.518	0.025
$\sum n-6$ PUFA	3.87	3.74	2.97	4.35	0.245	0.177	0.623	0.086	0.182
∑ <i>n</i> -3 PUFA	0.96ª	1.52 <sup>b</sup>	1.18 <sup>b</sup>	1.68 <sup>b</sup>	0.033	< 0.001	< 0.001	0.229	0.021
n-6:n-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

<sup>a,b</sup>Means in the same row bearing different superscripts are significantly different ( $P \le 0.05$ ). SEM: Standard error of mean.

<sup>1</sup>Contrast effect: L: Linear; Q: Quadratic; C: Cubic. <sup>2</sup>CLA: *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.

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

**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)

x,yWithin row, different superscript letters indicate differences (P < 0.05) between dietary treatment.

a,b,c,d,eWithin row, different superscript letters indicate differences (P < 0.05) between storage/incubation time.

SEM: Standard error of mean.

<sup>1</sup>Times 1, 2, 3, 4, 5 correspond to: 1, 4, 7, 10 and 14 days (fresh beef patties stored at 4°C in modified atmosphere packs); 1, 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.

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

<sup>4</sup>Chroma (colour vividness), higher values indicate greater saturation of red.

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

<sup>6</sup>OxyMb: 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

	Diet (D, % CGF)				Storage time (T, day)			<i>P</i> -value <sup>1</sup>		
Parameter	0	25	50	75	2	7	SEM	D	Т	D x T
Textural attributes										
Hardness	20.26	21.40	21.98	21.06	17.34 <sup>a</sup>	25.01 <sup>b</sup>	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 <sup>a</sup>	14.91 <sup>b</sup>	0.629	0.936	< 0.001	0.400
Chewiness	10.33	10.73	11.12	10.67	8.58 <sup>a</sup>	12.85 <sup>b</sup>	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
Eating quality										
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

<sup>a,b</sup>Within row, different superscript letters indicate differences (P < 0.05) between storage time.

<sup>1</sup>*P*-values for the effects of the dietary treatment (D), time of storage (T) and D×T interaction. SEM: Standard error of mean.

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
Statistics			
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

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