Title	Concentrate supplementation with dried corn gluten feed improves the fatty acid profile of longissimus thoracis muscle from steers offered grass silage
Authors	Salami, Saheed A.;O'Grady, Michael N.;Luciano, Giuseppe;Priolo, Alessandro;McGee, Mark;Moloney, Aidan P.;Kerry, Joseph P.
Publication date	2021-01-27
Original Citation	Salami, S. A., O'Grady, M. N., Luciano, G., Priolo, A., McGee, M., Moloney, A. P. and Kerry, J. P. (2021) 'Concentrate supplementation with dried corn gluten feed improves the fatty acid profile of longissimus thoracis muscle from steers offered grass silage', Journal of the Science of Food and Agriculture. doi: 10.1002/jsfa.11123
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.1002/jsfa.11123
Rights	© 2021, Society of Chemical Industry. Published by John Wiley & Sons, Inc. This is the peer reviewed version of the following item: Salami, S. A., O'Grady, M. N., Luciano, G., Priolo, A., McGee, M., Moloney, A. P. and Kerry, J. P. (2021) 'Concentrate supplementation with dried corn gluten feed improves the fatty acid profile of longissimus thoracis muscle from steers offered grass silage', Journal of the Science of Food and Agriculture, doi: 10.1002/jsfa.11123, which has been published in final form at https://doi.org/10.1002/jsfa.11123. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions.
Download date	2023-03-29 09:48:04
Item downloaded from	http://hdl.handle.net/10468/11118

University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

Journal of the
Science of
Food and Agriculture

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

Journal:	*Journal of the Science of Food and Agriculture*
Manuscript ID	JSFA-20-3622.R1
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	24-Jan-2021
Complete List of Authors:	Salami, Saheed; University College Cork, School of Food and Nutritional Sciences; University of Catania, Department Di3A, Animal Production Science O'Grady, Michael ; University College Cork, School of Food and Nutritional Sciences Luciano , Giuseppe ; University of Catania, DISPA - Unit of Animal Production Science Priolo, Alessandro; University of Catania, Department Di3A, Animal Production Science McGee, Mark; Teagasc, Animal & Grassland Research and Innovation Centre P. Moloney, Aidan; Teagasc, Animal & Grassland Research and Innovation Centre P. Kerry, Joseph; University College Cork, Ireland, Food and Nutritional Sciences;
Key Words:	Beef, Fatty acids, Animal feeding, eating quality, corn gluten feed, antioxidant potential

Concentrate supplementation with dried corn gluten feed improves the fatty acid profile of *longissimus thoracis* muscle from steers offered grass silage

Running title: Effects of feeding dried corn gluten feed on beef quality

Saheed A. Salami[a,b,*], Michael N. O'Grady[a], Giuseppe Luciano[b], Alessandro Priolo[b], Mark McGee[c], Aidan P. Moloney[c] and Joseph P. Kerry[a]

[a]*Food Packaging Group, School of Food and Nutritional Sciences, College of Science, Engineering and Food Science, University College Cork, Cork, Ireland*

[b]*Department Di3A, Animal Production Science, University of Catania, Via Valdisavoia 5, 95123 Catania, Italy*

[c]*Teagasc, Animal & Grassland Research and Innovation Centre, Grange, Dunsany, Co. Meath, Ireland*

[*]Corresponding author: Saheed A. Salami. E-mail address: s.salami@umail.ucc.ie

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.

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

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 [1]. 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 [2]. 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[3]. 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 [4].

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 [5]. 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 [5]. 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 [6]. 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 [2]. 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 [7, 8]. 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 [10, 11]. 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.

MATERIALS AND METHODS

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 ± 39.0 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 meal-based 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, vacuum-packed 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. [12].

Chemical analysis of feed

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

Phenolic compounds were extracted from samples of concentrate rations using aqueous methanol (50:50, v/v) and acetone (70:30, v/v) solvents [14]. 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 [15] and 2% (v/v) sulfuric acid in methanol [16], 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. [17]. Individual fatty acids were expressed as g/kg of DM feed.

Determination of muscle pH and proximate composition

Analysis of muscle pH and proximate composition was performed as described by Salami, et al. [18]. 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.

Analysis of vitamin E and fatty acids in LT muscle

The α-tocopherol (vitamin E) content in minced LT samples was determined by high-performance liquid chromatography (HPLC) as previously described by Salami, et al. [18]. 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® 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 α-tocopherol concentrations (7, 14, 21 μg/ml) and the concentration of α-tocopherol in beef was expressed in μg/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 [19] and the lipid fraction was transesterified to FAME using BF_3 in methanol as a catalyst [20] and dissolved in isooctane. The gas-chromatographic analysis was conducted following the injection, pressure and temperature conditions described extensively by Salami, et al. [21]. 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 [22] as follows:

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

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

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 Qwele, et al. [23] 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® 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 DPPH activity.

Measurement of the total phenol content

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

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

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 [25]. 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 [26]. 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 $FeCl_3.6H_2O$ in distilled water in the ratio 10:1:1, respectively incubated at 37 °C for 10 min prior to use). The mixture was 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 $FeCl_2$ (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:

Chelating activity (%) = [1 − (absorbance of sample)/(absorbance of control)] × 100.

Determination of the oxidative stability and sensory properties of beef

Measurement of lipid oxidation and oxymyoglobin in muscle homogenates

Muscle homogenates (25%) were prepared by homogenising 15 g of LT in a buffer (0.12 M KCL 5 mM histidine, pH 5.5) surrounded by crushed ice using an Ultra Turrax T25 homogeniser. Lipid oxidation in muscle homogenates (39.2 g) was initiated by the addition of pro-oxidants (45 μM $FeCl_3$/sodium ascorbate, 1:1) [27]. 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. [28].

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_2:20% CO_2 (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^3/m^2/24 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_2 and % CO_2) in MAP was measured using a CheckMate 9900 (PBI-DanSensor, Denmark). The average gas composition in MAP was 79.53 ± 0.39% O_2 and 20.77 ± 0.23% CO_2 on day 1 of storage and 74.03 ± 0.77% O_2 and 25.85 ± 0.73% CO_2 on day 14 of storage.

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

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 [29]. 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^*', 'a^*' and 'b^*' value represents lightness, redness and yellowness, respectively. Colour measurements were averaged for readings taken from four different locations on the surface of beef patties.

Measurement of textural properties of beef patties

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

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. [31]. Beef patties were cooked for sensory analysis in a Zanussi oven at 180 °C for 20 min until an internal temperature of 72 °C was reached. Following cooking, patties were cooled to room temperature and cut into 2 cm × 2 cm cubes, identified with random three-digit codes. On each day of 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 [32]. 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 [33]. 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).

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 $P \leq 0.05$ and a tendency for treatment effect was observed when $0.05 < P \leq 0.10$. All data analysis was performed using SPSS statistical software (IBM Statistics version 22).

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 co-product of wet milling of corn that is increasingly used in concentrate diets for finishing beef

cattle [2]. 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 [34]. 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 [10, 11]. 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 [12]. 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 [35]. 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.

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 [6], 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 [36]. 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 [9].

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^{3+} to Fe^{2+} [26]. 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 [9]. 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*

= -0.16; P = 0.288) between TPC and FRAP values. Nonetheless, the current results demonstrated that dietary inclusion of up to 75% CGF in the concentrate supplement did not compromise the antioxidant capacity of muscle.

Fatty acid composition of muscle

Effect of diet on the fatty acid profile of LT muscle

Results of FA composition in LT muscle are presented in Table 3. Dietary treatment did not affect (P > 0.05) the relative amount of total SFA in LT muscle but influenced the proportion of individual SFA with nutritional significance. Muscle from steers fed 25% and 75% CGF had decreased (P < 0.05) percentages of C14:0 and C17:0 compared to those fed 0% and 50% CGF. In addition, CGF diets tended (P = 0.074) to decrease C16:0 in a cubic manner, with 25% CGF displaying the greatest decrease (-37.2%) compared to 0% CGF. Notably, the consumption of elevated levels of dietary C14:0 and C16:0 increases low-density lipoprotein cholesterol, linked to increased risk of cardiovascular diseases (CVD), obesity and insulin resistance in humans [4, 5]. Thus, it can be deduced that feeding 25% and 75% CGF decreased 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 [5].

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) C20:5*n*-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 α-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 [4]. It is well-documented that consumption of dietary PUFA and CLA exhibit biological activities that prevent CVD, cancer and metabolic syndromes in humans [5]. 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 [37]. 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 [10]. 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. [10]. 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. [10]. 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 [2]. Thus, it is possible that CGF exhibited a complementary effect in protecting dietary unsaturated fatty acids against ruminal biohydrogenation.

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 [38]. 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 [39]. The predictor variables were linearly

combined to obtain three canonical discriminant functions (CAN). The first two CAN described 97.1% (CAN 1 = 89.1% and CAN 2 = 8.0%) of the total variance associated with the multivariate structure on the CAN plot (Figure 1). Discriminant power of the canonical 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.

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 [10].

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 [40].

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 ($P < 0.05$) 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 × 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 [10, 11].

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 × 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 [41], 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 [11].

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, C22:2*n*-6 and C20:5*n*-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.

ACKNOWLEDGEMENTS

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

CONFLICT OF INTEREST

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

SUPPORTING INFORMATION

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

REFERENCES

1. Salami S, Luciano G, O'Grady M, Biondi L, Newbold C, Kerry J and Priolo A, Sustainability of feeding plant by-products: A review of the implications for ruminant meat production. *Animal Feed Science and Technology* **251**:37-55 (2019).

2. Stock R, Lewis J, Klopfenstein TJ and Milton C, Review of new information on the use of wet and dry milling feed by-products in feedlot diets. *Journal of Animal Science* **77**:1-12 (2000).

3. Wood J, Enser M, Fisher A, Nute G, Sheard P, Richardson R, Hughes S and Whittington F, Fat deposition, fatty acid composition and meat quality: A review. *Meat Science* **78**:343-358 (2008).

4. Bessa RJ, Alves SP and Santos-Silva J, Constraints and potentials for the nutritional modulation of the fatty acid composition of ruminant meat. *European Journal of Lipid Science and Technology* **117**:1325-1344 (2015).

5. Scollan ND, Dannenberger D, Nuernberg K, Richardson I, MacKintosh S, Hocquette J-F and Moloney AP, Enhancing the nutritional and health value of beef lipids and their relationship with meat quality. *Meat Science* **97**:384-394 (2014).

6. Luciano G, Moloney A, Priolo A, Röhrle F, Vasta V, Biondi L, López-Andrés P, Grasso S and Monahan F, Vitamin E and polyunsaturated fatty acids in bovine muscle and the oxidative stability of beef from cattle receiving grass or concentrate-based rations. *Journal of Animal Science* **89**:3759-3768 (2011).

7. Inglett GE and Chen D, Antioxidant activity and phenolic content of air-classified corn bran. *Cereal Chemistry* **88**:36-40 (2011).

8. Rodríguez-López L, Vecino X, Barbosa-Pereira L, Moldes A and Cruz J, A multifunctional extract from corn steep liquor: antioxidant and surfactant activities. *Food & Function* **7**:3724-3732 (2016).

9. Salami S, Guinguina A, Agboola J, Omede A, Agbonlahor E and Tayyab U, In vivo and postmortem effects of feed antioxidants in livestock: a review of the implications on authorization of antioxidant feed additives. *Animal* **10**:1375-1390 (2016).

10. Segers J, Stewart R, Lents C, Pringle T, Froetschel M, Lowe B, McKeith R and Stelzleni A, Effect of long-term corn by-product feeding on beef quality, strip loin fatty acid profiles, and shelf life. *Journal of Animal Science* **89**:3792-3802 (2011).

11. Stelzleni A, Segers J and Stewart R, Long-term use of corn coproducts as a source of protein in beef finishing diets and the effects on carcass characteristics and round muscle quality. *Journal of Animal Science* **94**:1227-1237 (2016).

12. Kelly M, Moloney A, Kelly A and McGee M, Intake, growth, carcass and selected meat quality traits of steers offered grass silage and supplementary concentrates with increasing levels of dried corn gluten feed. *Advances in Animal Biosciences* **9**:150 (2018).

13. O'Kiely P, Intake, growth and feed conversion efficiency of finishing beef cattle offered diets based on triticale, maize or grass silages, or ad libitum concentrate. *Irish Journal of Agricultural and Food Research*:189-207 (2011).

14. Jiménez-Escrig A, Jiménez-Jiménez I, Pulido R and Saura-Calixto F, Antioxidant activity of fresh and processed edible seaweeds. *Journal of the Science of Food and Agriculture* **81**:530-534 (2001).

15. Sukhija PS and Palmquist D, Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. *Journal of Agricultural and Food Chemistry* **36**:1202-1206 (1988).

16. Shingfield K, Ahvenjärvi S, Toivonen V, Ärölä A, Nurmela K, Huhtanen P and Griinari JM, Effect of dietary fish oil on biohydrogenation of fatty acids and milk fatty acid content in cows. *Animal Science* **77**:165-179 (2003).

17. Cherif M, Valenti B, Abidi S, Luciano G, Mattioli S, Pauselli M, Bouzarraa I, Priolo A and Salem HB, Supplementation of Nigella sativa seeds to Barbarine lambs raised on low- or high-concentrate diets: Effects on meat fatty acid composition and oxidative stability. *Meat Science* **139**:134-141 (2018).

18. Salami SA, O'Grady MN, Luciano G, Priolo A, McGee M, Moloney AP and Kerry JP, Quality indices and sensory attributes of beef from steers offered grass silage and a concentrate supplemented with dried citrus pulp. *Meat Science* **168**:108181 (2020).

19. Bligh EG and Dyer WJ, A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* **37**:911-917 (1959).

20. Park P and Goins R, In situ preparation of fatty acid methyl esters for analysis of fatty acid composition in foods. *Journal of Food Science* **59**:1262-1266 (1994).

21. Salami S, O'Grady M, Luciano G, Priolo A, McGee M, Moloney A and Kerry J, Quality indices and sensory attributes of beef from steers offered grass silage and a concentrate supplemented with dried citrus pulp. *Meat Science* **168**:108181 (2020).

22. Ulbricht T and Southgate D, Coronary heart disease: seven dietary factors. *The Lancet* **338**:985-992 (1991).

23. Qwele K, Hugo A, Oyedemi S, Moyo B, Masika P and Muchenje V, Chemical composition, fatty acid content and antioxidant potential of meat from goats supplemented

with Moringa (Moringa oleifera) leaves, sunflower cake and grass hay. *Meat Science* **93**:455-462 (2013).

24. Singleton VL, Orthofer R and Lamuela-Raventós RM, Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent, In *Methods in Enzymology,* ed. by Packer L. Elsevier Science Publishers, Amsterdam, pp. 152-178 (1999).

25. Yen GC and Wu JY, Antioxidant and radical scavenging properties of extracts from Ganoderma tsugae. *Food Chemistry* **65**:375-379 (1999).

26. Benzie IF and Strain J, Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration, In Methods in Enzymology, ed. by Packer L. Elsevier Science Publishers, Amsterdam, pp. 15-27 (1999).

27. O'Grady M, Monahan F and Brunton N, Oxymyoglobin oxidation and lipid oxidation in bovine muscle—mechanistic studies. *Journal of Food Science* **66**:386-392 (2001).

28. Hayes J, Stepanyan V, Allen P, O'Grady M, O'Brien N and Kerry J, The effect of lutein, sesamol, ellagic acid and olive leaf extract on lipid oxidation and oxymyoglobin oxidation in bovine and porcine muscle model systems. *Meat Science* **83**:201-208 (2009).

29. Siu G and Draper H, A survey of the malonaldehyde content of retail meats and fish. *Journal of Food Science* **43**:1147-1149 (1978).

30. Moroney N, O'Grady M, O'Doherty J and Kerry J, Effect of a brown seaweed (Laminaria digitata) extract containing laminarin and fucoidan on the quality and shelf-life of fresh and cooked minced pork patties. *Meat Science* **94**:304-311 (2013).

31. O'Sullivan M, Byrne D and Martens M, Evaluation of pork colour: sensory colour assessment using trained and untrained sensory panellists. *Meat Science* **63**:119-129 (2003).

32. ISO, *International Standard ISO 8589: Sensory Analysis: General Guidance for the Design of Test Rooms*. ISO (2007).

33. MacFie HJ, Bratchell N, Greenhoff K and Vallis LV, Designs to balance the effect of order of presentation and first-order carry-over effects in hall tests. *Journal of Sensory Studies* **4**:129-148 (1989).

34. Faustman C and Cassens R, The biochemical basis for discoloration in fresh meat: a review. *Journal of Muscle Foods* **1**:217-243 (1990).

35. Pethick DW, Harper GS and Oddy VH, Growth, development and nutritional manipulation of marbling in cattle: a review. *Australian Journal of Experimental Agriculture* **44**:705-715 (2004).

36. Zhao Z, Egashira Y and Sanada H, Phenolic antioxidants richly contained in corn bran are slightly bioavailable in rats. *Journal of Agricultural and Food Chemistry* **53**:5030-5035 (2005).

37. HMSO, Nutritional aspects of cardiovascular disease (report on health and social subjects No. 46). *Her Majesty's Stationery Office (HMSO), London, United Kingdom* (1994).

38. Monahan FJ, Schmidt O and Moloney AP, Meat provenance: Authentication of geographical origin and dietary background of meat. *Meat Science* (2018).

39. Alfaia CP, Alves SP, Martins SI, Costa AS, Fontes CM, Lemos JP, Bessa RJ and Prates JA, Effect of the feeding system on intramuscular fatty acids and conjugated linoleic acid isomers of beef cattle, with emphasis on their nutritional value and discriminatory ability. *Food Chemistry* **114**:939-946 (2009).

40. Torres EP, Rios HG, Lagarda TI, Melendres MV, Ramos AP, Garcia LZ, Saavedra AP and Ramirez JD, Ferulic acid in diets of heifers and its effect on the oxidative stability of meat stored in refrigeration. *Journal of Animal Science* **94**:431-432 (2016).

41. Caine W, Aalhus J, Best D, Dugan M and Jeremiah L, Relationship of texture profile analysis and Warner-Bratzler shear force with sensory characteristics of beef rib steaks. *Meat Science* **64**:333-339 (2003).

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).

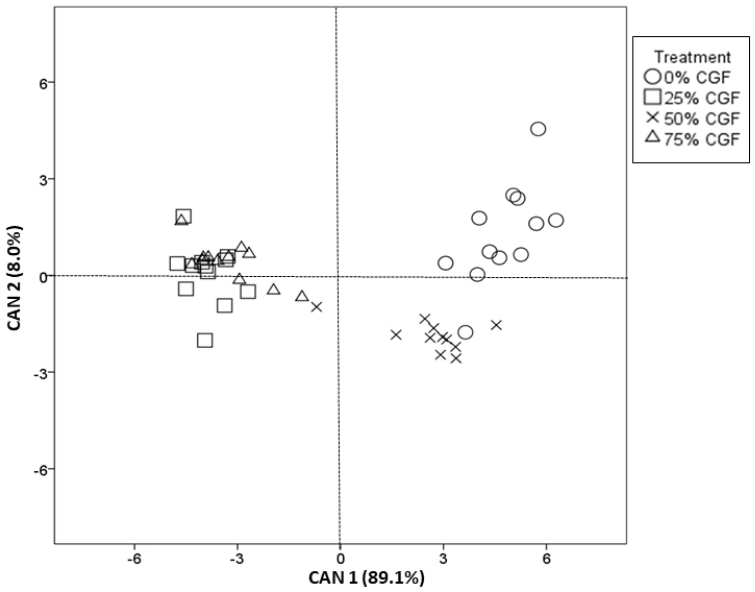

Figure 1. Plot showing the discrimination of dietary treatments following canonical discriminant function (CAN) analysis of the fatty acid profiles of longissimus thoracis muscles from steers offered grass silage and a supplementary concentrate containing varying levels (0%, 25%, 50% and 75%) of dried 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**
Ingredient (as-fed basis, g kg^{-1})				
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[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
Fatty acid (g kg^{-1} 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
c-9 C18:1	1.90	3.30	4.90	7.10
c-9,12 C18:2	6.50	10.40	13.7	16.9
c-9,12,15 C18:3	0.60	0.80	0.90	0.80

[1]Expressed as g kg^{-1} DM
[2]Expressed 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
Proximate composition (g kg^{-1} wet weight)									
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
Antioxidant status									
α-tocopherol (μg 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
Antioxidant activity									
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: erric 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
c-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
c-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
t-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
t-11 C18:1	0.95	0.77	0.97	0.98	0.036	0.080	0.363	0.190	0.045
c-9 C18:1	29.11	29.64	31.77	29.83	0.710	0.507	0.530	0.375	0.268
t-9, *t*-12 C18:2	0.28	0.11	0.29	0.37	0.060	0.475	0.431	0.326	0.376
c-9, *c*-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
c-11 C20:1	0.46	0.55	0.40	0.51	0.386	0.371	0.169	0.318	0.645
c-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 *n*-6	1.10	0.98	1.01	0.89	0.038	0.176	0.140	0.238	<0.201
C20:5 *n*-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 *n*-3	0.44^{b}	0.71^{ab}	0.45^{b}	0.79^{a}	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 *trans*	3.02^{a}	2.02^{b}	2.69^{ab}	1.97^{b}	0.129	0.015	0.020	0.518	0.025
∑*n*-6 PUFA	3.87	3.74	2.97	4.35	0.245	0.177	0.623	0.086	0.182
∑*n*-3 PUFA	0.96^{a}	1.52^{b}	1.18^{b}	1.68^{b}	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

[a,b]Means in the same row bearing different superscripts are significantly different ($P \leq 0.05$).
SEM: Standard error of mean.
[1]Contrast effect: L: Linear; Q: Quadratic; C: Cubic.
[2]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.

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	*P*-value[2]		
	0	25	50	75	1	2	3	4	5		D	T	D x T
Fresh beef patties													
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, L^*	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, a^*	16.72	16.83	16.57	16.60	22.86^{a}	20.93^{b}	18.59^{c}	14.98^{d}	6.05^{e}	2.154	0.905	<0.001	0.748
Yellowness, b^*	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], C^*	22.99	22.92	22.82	22.93	28.27^{a}	26.28^{b}	23.90^{c}	20.50^{d}	15.63^{e}	2.958	0.970	<0.001	0.748
Hue angle[5], H^*	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
Cooked beef patties													
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
Muscle homogenates													
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.

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

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

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

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

[6]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)			*P*-value[1]		
Parameter	**0**	**25**	**50**	**75**	**2**	**7**	**SEM**	**D**	**T**	**D x T**
Textural attributes										
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
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

[a,b]Within row, different superscript letters indicate differences ($P < 0.05$) between storage time.
[1]*P*-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
c-10 C17:1	1.310	-0.564	-0.260
t-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