Quality of three muscles from suckler bulls finished on concentrates and slaughtered at 16 months of age or slaughtered at 19 months of age from two production systems

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**Supplementary Material S1: Materials and methods.**

For direct analysis (e.g proximate composition, pH, etc), all instruments were calibrated according to manufacturer instructions and using reference material provided.

The pH meters were calibrated with fresh standard buffers before each measurement event and a reading of both standards was taken afterwards as an external validation to ensure the quality of the measurement during the trial.

Moisture and intramuscular fat concentrations were measured using the Smart System 5 microwave moisture drying oven and NMR Smart Trac rapid fat analyser (CEM Corporation, Matthews, NC, USA) using AOAC Official Method 985.14.19. Protein concentration was determined using a LECO FP328 (LECO Corp., St Joseph, MI, USA) protein analyser based on the Dumas method according to AOAC method 992.15.20. All analyses were carried out in duplicate with a standard deviation between replicates <1.00. A commercial reference material (BB501b, European Reference Materials, LGC, Middlesex, UK) was used as a quality control for proximate composition once daily.

For colour measurement, the instrument was standardized prior to analysis following manufacturer’s instructions by using the original light trap and white tile covered with a clean sample of the packaging material used (polyvinylchloride film). After every 10 samples, a reading of the white and green tiles was taken to ensure the correct performance of the instrument. When a deviation from the initial values was found the instrument was re-standardized.

*Sarcomere length measurement*

From each steak triplicate pieces of meat were excised (2.0 x 1.0 x 1.0 cm) with the 2 cm length running parallel to the fibre direction, and subsequently fixed with glutaraldehyde solution (5% glutaraldehyde in 0.1M NaHPO4 at 7.2 pH) for 4 hours at 4ºC. Samples were then removed, dried and placed in a sucrose solution (0.2M sucrose in 0.1M NaHPO4 at pH 7.2) overnight. On the day of analysis, the fibres were separated using tweezers, blended and kept in sucrose buffer. From each cube, sarcomere lengths of three subsamples samples (2 drops in a glass slide) were observed by laser diffraction, recording a total of 10 sarcomere measurements per subsample.

The length (µm) was calculated using the equation determined by Cross et al., (1981).

where D= Distance from the specimen to the diffraction pattern screen in mm. Preferably 100 mm, T= spacing between diffraction bands in mm. The band is 2T so divide your measurement. 0.6328 is the wavelength of the laser in meters. The coefficient of variation between the 3 slides (10 readings per slide) was 4.47%

Reference:

Cross H, West R and Dutson T 1981. Comparison of methods for measuring sarcomere length in beef semitendinosus muscle. Meat Science 5,261–266.

*Collagen determination*

Samples which had been aged for 3 days were freeze dried and then milled to a fine homogenate. Approximately 4 g of muscle homogenate was defatted using 20 mL of diethyl ether overnight and re-dried. The heat-soluble collagen was extracted as described by Hill (1966) with slight modifications. Briefly, 2.5 g of fat-free dry (FFD) muscle hydrolysate was heated in a water bath for 2 h at 90 °C with 15 mL of Ringer's solution and then centrifuged (LYNX 6000, Thermo Scientific, Waltham, MA, USA) twice at 3 990g for 10 min at room temperature. The supernatants from the two centrifugations were combined. Then 100 μL of final supernatant and 3 mg of FFD (total collagen) of each muscle (in triplicate) were hydrolysed using 2 mL of 6 M HCl under nitrogen in sealed vials at 110 °C overnight. Following hydrolysis, the vials were cooled and centrifuged (5174C/R, Eppendorf, Stevenage, UK) at 18 187g for 1 min at room temperature to remove particulate matter.

Quantitative analysis of hydroxyproline in FFD muscle hydrolysates was carried out using LC-MS/MS with slight modifications of the method reported by Colgrave et al. (2008). Briefly, 100 μL aliquots of the hydrolysates were dried under nitrogen and reconstituted in 1 mL of 0.1% formic acid. 100 μL of 0.1% formic acid was added to 100 μL of the reconstituted sample and then 5 μL of the final reconstituted sample was injected into a Waters Acquity UPLC system with an ACQUITY UPLC@BEH C18 (50 mm × 2.1 mm, particle size 1.7 μm) column coupled to tandem mass spectrometry (Waters Corp, MA, USA). The flow rate was 0.5 mL/min using an isocratic flow of 95% solvent A (0.1% formic acid in HPLC water) and 5% solvent B (0.1% formic acid in Acetonitrile). Data acquisition and processing were performed using the Target Lynx Software (Waters Corp, MA, USA).

Rat tail (α-1 (1) chain) (Enzo Life Sciences, Farmingdale, NY, USA) was used as the quality control collagen standard for validation. An aliquot of 100 μL of rat tail solution was hydrolysed and reconstituted using the same procedure used for test samples, then diluted with 0.1% formic acid in order to obtain three different standards in the high, medium and low levels of hydroxyproline. The concentration of hydroxyproline (nmol/L) was determined from integration of the area under the curve against a standard curve with a linear range from 100 to 5 000 nmol/L (R2 = 0.99). The conversion of area to mass of collagen was as previously described (Colgrave et al., 2008).

Quality control results were:

|  |  |  |
| --- | --- | --- |
|  | **Rat tail (259.9 nM)** | **Rat tail (2599 nM)** |
| **Mean** | 253.94 | 2233.42 |
| **Precision (%) RSD** | 13.13 | 2.59 |
| **Accuracy (%)** | 103.72 | 116.79 |

Percentage solubility was calculated as soluble hydroxyproline divided by total hydroxyproline multiplied by 100. All collagen properties were determined in triplicate for each sample and averaged. Between sample replicates the coefficient of variation (%) was 13% for total collagen and 10% for soluble collagen.

References:

Hill F 1966. The solubility of intramuscular collagen in meat animals of various ages. Journal of Food Science 3, 1161–166.

Colgrave ML, Allingham PG and Jones A 2008. Hydroxyproline quantification for the estimation of collagen in tissue using multiple reaction monitoring mass spectrometry. Journal of Chromatography *A* 1212, 150–153.

*Warner Bratzler shear force (WBSF) and cooking loss*

Steak (2.54cm thick), cut from a standardised location (same across all animals within a muscle) were used. All external fat from the steaks was removed. If steaks were two small, two steaks were used. Weight before cooking was recorded. The temperature of the sample at the initiation of the cooking was standardized by placing the samples in a bag (vacuum bags) and then in a circulating water bath (Model no. Y38, Grant Instruments Ltd., Barrington, Cambridge, UK) set to 20ºC for 10 min. Samples were then transferred to be cooked in a water bath (Model no. Y38, Grant Instruments Ltd., Barrington, Cambridge, UK) set at 72ºC and cooked until an internal temperature of 70ºC was achieved. This was monitored by a temperature probe (HI 904, Hanna Foodcare Instruments, Bedfordshire, UK) placed in the geometric centre of each steak. A consistent number of samples per water bath on each batch of analysis (n= 8) was used in order to minimise variation due to cooking time, avoid bath over filling and ensure water circulation around the samples. The time of cooking needed to reach 70ºC was recorded.

Cooking was stopped by immersing the bag for 3 min. in iced water. All the juices were poured out of the bag after removal from the water bath and once room temperature was reached the cooked weight was recorded. The samples were kept in the fridge in a properly closed bag to avoid dehydration overnight.

The WBSF analysis was done on 1.25 cm diameter cores (Cores 8) cut parallel to the longitudinal orientation of fibres. In all cases, 6 to 8 representative cores were taken from each sample. When the cores reached room temperature, they were sheared using the Warner-Bratzler shear blade attached to an Instron Universal Testing Machine (Models 5543, Instron (UK) Ltd., High Wycombe, UK). A 500 N load cell was used with a crosshead speed 50 mm/min. The average maximum shear force was calculated by excluding the two extreme values from eight acquisitions. In addition, the slope between 20 and 80% of the maximum force was measured as well as the total area of the curve. The Instron was calibrated daily following suppliers instructions and once calibrated blades were not touched at any stage. Before the start and before every sample blanks were run and no measurement was done if at least 3 of them were not below 1N, if this value was not reached the blade was replaced and the Instron calibrated to ensure the quality of the analysis.

*Sensory analysis*

Sensory analysis was carried out in the sensory kitchen in University College Cork which features well-ventilated and portioned panel sensory booths and conforms to the standards of the International Organization for Standardization (1998). Meat samples of varying texture (tough, tender and very tender) were used to calibrate the panel in accordance with the method used by Conroy et al., (2017) to determine sensory acuity and consistency. It was observed that panellists had a consistently similar sensory response and scores were correlated to WBSF values.

References:

Conroy P, O’Sullivan MG, Hamill RH and Kerry JP 2017. Sensory capability of young, middle aged and elderly Irish assessors to identify beef steaks of varying texture. Meat Science 132, 125-130.

International Standards Organisation 1998. ISO 67.240.Sensory analysis. Geneva, Switzerland.

*Statistical analyses*

SAS syntaxes (Where PROD is production system and ID is animal)

*For performance*

proc glimmix nobound plots=(residualpanel studentpanel);

class ID block PROD;

model measurement= block PROD / ddfm=kr ;

lsmeans PROD/pdiff adj=tukey lines;

run;

*For chemical composition, instrumental texture and sarcomere length:*

proc glimmix nobound plots=(residualpanel studentpanel);

class ID block PROD muscle;

model measurement= block muscle|PROD / ddfm=kr ;

random PROD\*ID;

random residual/group=muscle;

lsmeans muscle|PROD/pdiff adj=tukey lines;

run;

*For sensory data*

proc glimmix nobound plots=(residualpanel studentpanel);

class ID block PROD muscle evaluator;

model sensory value= block muscle|PROD / ddfm=kr ;

random PROD\*ID PROD\*muscle\*ID evaluator;

lsmeans muscle|PROD pdiff adj=tukey lines;

run;

For meat colour (time represents measurement taken after 1 and 24h of blooming)

proc glimmix data=lara nobound plots=(residualpanel studentpanel);

class ID Block PROD muscle time;

model L= Block muscle|PROD|time / ddfm=kr ;

random PROD\*ID PROD\*muscle\*ID;

random residual/group=time;

lsmeans muscle|PROD|time/pdiff adj=tukey lines;

run;

**Supplementary Table S1**. *Colour of muscle after either 1 or 24 hours of blooming, from bulls offered concentrates ad libitum until slaughter at 16 (16-C) or 19 (19-CC) months of age or at 19 months of age after finishing on concentrates subsequent to a period at pasture (19-GC).*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Variable1 | 16-C | | 19-CC | | 19-GC | |
| 1 h | 24 h | 1 h | 24 h | 1 h | 24 h |
| L\* | 48.7 | 49.5 | 47.3 | 48.1 | 47.3 | 47.9 |
| a\* | 14.6c | 19.0a | 16.6b | 20.0a | 15.5c | 19.1a |
| b\* | 13.8c | 16.5a | 14.1c | 16.0ab | 13.5c | 15.3b |
| Chroma | 20.2c | 25.2a | 21.8b | 25.6a | 20.6bc | 24.5a |
| Hue | 43.3 | 40.9 | 40.2 | 38.5 | 41.1 | 38.8 |

1 L\*, a\*, b\* = lightness, redness and yellowness, respectively.

Least square means within a row, with different superscripts differ significantly at *P*<0.05.

**Supplementary Table S2**. *Colour of Longissimus thoracis (LT), Gluteus medius (GM) and Semitendinosus (ST) muscles, after either 1 or 24 hours of blooming, from bulls offered concentrates ad libitum until slaughter at 16 (16-C) or 19 (19-CC) months of age or at 19 months of age after finishing on concentrates subsequent to a period at pasture (19-GC).*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Variable | LT | | GM | | ST | |
| 1 h | 24 h | 1 h | 24 h | 1 h | 24 h |
| L\* | 46.2d | 47.3c | 48.3bc | 48.5abc | 48.8ab | 49.7a |
| a\* | 14.2c | 20.2a | 18.0b | 19.5a | 14.6c | 18.5b |
| b\* | 12.0e | 15.4c | 15.4bc | 16.3a | 14.0d | 16.1ab |
| Chroma | 18.6d | 25.4a | 23.7b | 25.4a | 20.3c | 24.5ab |
| Hue | 40.1bc | 37.3d | 40.4bc | 39.8c | 44.0a | 41.2b |

1 L\*, a\*, b\* = lightness, redness and yellowness, respectively.

Least square means within a row with different superscripts differ significantly at *P*<0.05.