**The effects of graded levels of concentrate supplementation of pasture finished late maturing bulls on the colour and lipid stability of beef**

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**Materials and methods**

*Reagents*

2-Thiobarbituric acid, trichloroacetic acid, potassium hydroxide, sodium chloride hexane, heptane, pyrogallol, α-tocopherol standard and tetra-ethoxypropane were purchased from Sigma Aldrich (Dublin, Ireland). Tricosanoic acid methyl ester, ethanol and methanol were purchased from Fisher Scientific (Dublin, Ireland).

*Proximate analysis*

A Robot coupe blender (R301 Ultra, Robot coupe SA, Vincennes, France) was used to homogenise thawed muscles (4°C, overnight). Intramuscular fat and moisture content were analysed using the SMART System 5 microwave moisture drying oven and NMR Smart Trac rapid fat analyser (CEM Corporation, Matthews, NC, USA) following AOAC Methods 985.14 and 985.26, respectively (AOAC, 2000). Protein concentration was determined by combustion using a LECO FP328 (LECO Corp., MI, USA) protein analyser based on the Dumas method and according to AOAC method 992.15 (AOAC, 2000). Ash was determined by incinerating samples in a furnace (540°C overnight).

*Fatty acid composition*

Fatty acid methyl esters (FAME) were prepared using the rapid microwave assisted method described by Brunton *et al*. (2015). Briefly, 100 µl of 10 mg/ml internal standard (IS) (tricosanoic acid methyl ester in heptane) was added to 1 g sample (muscle/feed). The samples were saponified in 2.5% methanolic potassium hydroxide in a microwave set at 120°C. The samples were cooled to room temperature and thereafter esterified using 5% methanolic acetyl chloride. Extraction of FAME was done using 10 ml of pentane and 20 ml of saturated NaCl solution. Separation and quantification of FAME was done on a CP-Sil 88 capillary 100 m × 0.25 mm (ID) × 0.2 μm (film thickness) column using a Clarus 580 GC (Perkin Elmer, Singapore) equipped with a flame ionisation detector. Hydrogen was used as carrier gas at a flow rate of 1.25 ml/min. Sample injection volume was 0.5 μl, at a split ratio of 10:1. The oven temperature was set initially at 80°C, increased to 220°C at 6.2°C/min, held for 3.2 min, further increased to 240°C at 6.3°C/min, and held for 6.5 min. The injector and detector temperatures were 250 and 270°C, respectively. FAs were identified by comparing their retention times with standards (Supelco™ FAME mix). Peaks were integrated using TotalChrom 6.3.2 software (PerkinElmer, Waltham, MA, USA) and quantification of FAME was based on the IS method. The FA were expressed as mg/100g muscle and mg/kg DM of feed.

*α-Tocopherol concentration*

Vitamin E (α-tocopherol) in feed was measured following the method of Fratianni *et al.* (2002) with minor modifications. In brief, 2 ml ethanolic pyrogallol (6%), 0.8 ml ethanol, 0.8 ml sodium chloride (1%) and 0.8 ml potassium hydroxide (60%) were added to 250 mg of feed sample which had been milled through a 1 mm mesh screen. Samples were flushed with nitrogen and placed in a water bath at 70°C for 45 min. The samples were vortexed every 10 min. Thereafter, the samples were cooled in an ice bath and 2 ml of sodium chloride solution (1%) was added after which α-tocopherol was extracted three times using 2 ml of hexane/ethyl acetate (9:1 v/v). The organic layers were dried under nitrogen and the residue was dissolved in 2 ml of ethanol. α-Tocopherol in muscle was extracted as described in Dunne *et al*. (2005a). Analysis was carried out on a reversed phase HPLC system using an Agilent 1200 series instrument (Agilent Technologies Inc., Santa Clara, CA) fitted with a fluorescence detector (λexcitation = 295nm and λemision = 330nm; Agilent 1260 Infinity) and a Zorbax Eclipse XDB-C18 column (4.6x150 mm 5 μm) with corresponding guard column (Agilent Technologies). The mobile phase was methanol at a flow rate 1 ml/min. The injection volume was 20 µl and elution time was set at 14 min with the column maintained at a temperature of 25°C. For identification and quantification of α-tocopherol, peak areas of samples were compared with external α-tocopherol standards made up to the following concentrations: 0, 0.25, 0.5, 1, 2.5, 5, and 6 μg/ml in methanol. Results were expressed as μg α-tocopherol/g muscle or mg α-tocopherol/kg feed.

**References**

AOAC 2000. Association of Official Analytical Chemists, Official Methods 985.14, 985.26, 992.15. AOAC International, Gaithersburg, MD, United States.

Brunton NP, Mason C and Collins MJ 2015. Rapid microwave assisted preparation of fatty acid methyl esters for the analysis of fatty acid profiles in foods. Journal of Analytical Chemistry 70, 1218-1224.

Fratianni A, Caboni M, Irano M and Panfili G 2002. A critical comparison between traditional methods and supercritical carbon dioxide extraction for the determination of tocochromanols in cereals. European Food Research and Technology 215, 353-358.

**Supplementary table S1** *Fatty acid proportion (\* 100) in intramuscular fat from M. longissimus thoracis et lumborum muscle of late maturing suckler-bred bulls from four production systems (PS).*

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Day 0 | | | |  | Day 14 | | | |  |  |  | *P-Values* | |
|  |  | C | P | PC25 | PC50 |  | C | P | PC25 | PC50 |  | S.E.M. |  | PS | Day |
| *Fatty acids proportion \*100* | | | | | | | | | | | | | | | |
| C10:0 |  | 0.03b | 0.01ab | 0.01ab | 0.01a |  | 0.03b | 0.00a | 0.02ab | 0.00a |  | 0.00 |  | \*\*\* | n.s |
| C12:0 |  | 0.06 | 0.06 | 0.05 | 0.04 |  | 0.06 | 0.03 | 0.06 | 0.03 |  | 0.01 |  | n.s | n.s |
| C14:0 |  | 2.75b | 1.38a | 1.72a | 1.77a |  | 2.90b | 1.48a | 1.91a | 1.97a |  | 0.13 |  | \*\*\* | n.s |
| C14:1 |  | 0.48b | 0.11a | 0.23a | 0.26ab |  | 0.48b | 0.13a | 0.21ab | 0.22ab |  | 0.03 |  | \*\*\* | n.s |
| C15:0 |  | 0.47 | 0.47 | 0.44 | 0.51 |  | 0.44 | 0.58 | 0.56 | 0.62 |  | 0.03 |  | n.s | \* |
| C15:1 |  | 0.15a | 0.53b | 0.39ab | 0.41b |  | 0.09a | 0.43b | 0.31b | 0.33b |  | 0.03 |  | \*\*\* | \* |
| C16:0 |  | 27.4b | 21.0a | 23.2a | 22.9a |  | 26.6b | 22.3a | 25.0ab | 25.0ab |  | 0.53 |  | \*\*\* | \* |
| C16:1 |  | 1.17b | 0.61a | 0.79a | 0.70a |  | 1.21b | 0.69a | 0.82a | 0.77a |  | 0.05 |  | \*\*\* | n.s |
| C17:0 |  | 1.21 | 0.96 | 0.93 | 1.14 |  | 1.08 | 1.13 | 1.13 | 1.24 |  | 0.08 |  | n.s | n.s |
| C17:1 |  | 0.10 | 0.26 | 0.15 | 0.19 |  | 0.33 | 0.01 | 0.15 | 0.21 |  | 0.09 |  | n.s | n.s |
| C18:0 |  | 16.2a | 19.8b | 18.0ab | 18.6ab |  | 17.6a | 21.6b | 20.4ab | 20.8b |  | 0.47 |  | \*\*\* | \*\* |
| C18:1*n*-9c |  | 36.7b | 25.9a | 27.9a | 26.3a |  | 38.7b | 28.2a | 29.7a | 28.3a |  | 0.72 |  | \*\*\* | \*\* |
| C18:1*n*-7 |  | 1.89 | 2.04 | 2.07 | 1.87 |  | 1.77 | 2.00 | 2.01 | 1.88 |  | 0.08 |  | n.s | n.s |
| C18:2*n*-6t |  | 0.01 | 0.02 | 0.01 | 0.02 |  | 0.02 | 0.02 | 0.05 | 0.03 |  | 0.01 |  | n.s | n.s |
| C18:2*n*-6c |  | 5.17a | 11.07b | 10.61b | 11.65b |  | 3.87a | 8.90b | 8.02b | 8.90b |  | 0.70 |  | \*\*\* | \*\* |
| C20:0 |  | 0.12 | 0.21 | 0.16 | 0.30 |  | 0.12 | 0.15 | 0.23 | 0.29 |  | 0.05 |  | n.s | n.s |
| C18:3*n*-6 |  | 0.03a | 0.05ab | 0.06ab | 0.07b |  | 0.01a | 0.06b | 0.03ab | 0.04ab |  | 0.01 |  | \*\* | \*\* |
| C20:1*n*-9 |  | 0.15 | 0.13 | 0.17 | 0.14 |  | 0.14 | 0.13 | 0.11 | 0.11 |  | 0.02 |  | n.s | n.s |
| C18:3*n*-3 |  | 0.78a | 3.83b | 2.88b | 2.91b |  | 0.58a | 3.01b | 2.00b | 2.13b |  | 0.26 |  | \*\*\* | \*\* |
| C18:2*c*9 *t*11 |  | 0.16a | 0.25b | 0.25b | 0.27b |  | 0.15a | 0.28b | 0.22b | 0.26b |  | 0.03 |  | \* | n.s |
| C20:2 |  | 0.07 | 0.06 | 0.08 | 0.11 |  | 0.05 | 0.07 | 0.06 | 0.05 |  | 0.01 |  | n.s | n.s |
| C22:0 |  | 0.10 | 0.35 | 0.19 | 0.19 |  | 0.08a | 0.37b | 0.14ab | 0.16ab |  | 0.04 |  | \*\* | n.s |
| C20:3*n*-6 |  | 0.29a | 0. 83b | 0.77b | 0.80b |  | 0.17a | 0.61b | 0.53b | 0.52b |  | 0.05 |  | \*\*\* | \*\*\* |
| C20:3*n*-3 |  | 0.02 | 0.03 | 0.06 | 0.03 |  | 0.01 | 0.05 | 0.02 | 0.02 |  | 0.01 |  | n.s | n.s |
| C20:4*n*-6 |  | 1.19a | 4.02b | 3.51b | 3.37b |  | 0.65a | 2.80b | 2.18b | 2.17b |  | 0.23 |  | \*\*\* | \*\*\* |
| C20:4*n-*3 |  | 0.02 | 0.10 | 0.06 | 0.04 |  | 0.01 | 0.07 | 0.04 | 0.07 |  | 0.02 |  | \* | n.s |
| C22:2 |  | 0.08a | 0.33b | 0.32b | 0.30b |  | 0.05 | 0.23 | 0.19 | 0.18 |  | 0.04 |  | \*\* | \*\* |
| C24:0 |  | 0.02 | 0.03 | 0.03 | 0.03 |  | 0.01 | 0.04 | 0.03 | 0.03 |  | 0.01 |  | n.s | n.s |
| C20:5*n*-3 |  | 0.30a | 1.63b | 1.18b | 1.25b |  | 0.19a | 1.07b | 0.69b | 0.72b |  | 0.09 |  | \*\*\* | \*\*\* |
| C22:5*n*-3 |  | 0.59a | 2.09b | 1.71by | 1.71b |  | 0.29a | 1.60b | 1.02bx | 1.07b |  | 0.11 |  | \*\*\* | \*\*\* |
| C22:6*n*-3 |  | 0.05a | 0.21b | 0.20b | 0.20b |  | 0.02a | 0.14b | 0.12b | 0.12b |  | 0.02 |  | \*\*\* | \*\* |
| Others |  | 0.18 | 0.35 | 0.34 | 0.44 |  | 0.18 | 0.47 | 0.39 | 0.36 |  | 0.05 |  | \* | n.s |
| SFA |  | 48.3 | 44.3 | 44.7 | 45.5 |  | 48.8 | 47.7 | 49.5 | 50.1 |  | 0.82 |  | n.s | \*\* |
| MUFA |  | 42.8b | 30.9a | 33.2a | 31.3a |  | 44.9b | 32.9a | 34.9a | 33.3a |  | 0.78 |  | \*\*\* | \* |
| PUFA |  | 8.74a | 24.5b | 21.7b | 22.7b |  | 6.07a | 18.9b | 15.2b | 16.3b |  | 1.30 |  | \*\*\* | \*\*\* |
| *n*-6 PUFA |  | 7.00a | 16.6b | 15.6b | 16.6b |  | 4.98a | 13.0b | 11.3b | 12.2b |  | 0.97 |  | \*\*\* | \*\* |
| *n*-3 PUFA |  | 1.30a | 2.78b | 2.44b | 2.43b |  | 1.02a | 2.39b | 1.94b | 1.99b |  | 0.08 |  | \*\*\* | \*\*\* |
| *HP-PUFA* |  | 3.25a | 12.79b | 10.42by | 10.38b |  | 1.94a | 9.40b | 6.63bx | 6.84b |  | 0.62 |  | \*\*\* | \*\*\* |

C: rolled barley, P: grass only, PC25: grass with 25% concentrate dry matter (DM) and PC50: grass with 50% concentrate DM

Samples were stored in modified atmosphere (O2:CO2; 80:20) and subjected to simulated retail display (4°C,1000 lux for 12 h out of 24 h) for 14 days.

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids

HP-PUFA: highly peroxidizable PUFA. Calculated as the sum of PUFA with 3 or more double bonds

a,b,c Treatment means within day, assigned different superscripts differ significantly (P < 0.05)

x,y Treatment means within rows on different days of storage, assigned different superscripts differ significantly (P < 0.05)

\*: P < 0.05; \*\*: P < 0.01; \*\*\*: P < 0.001