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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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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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Short title: Quality of muscles from suckler bulls

Abstract

There is a requirement in some beef markets to slaughter bulls at under16 months of age. This requires high levels of concentrate feeding. Increasing the slaughter age of bulls to 19 months facilitates the inclusion of a grazing period thereby decreasing the cost of production. Recent data indicate few quality differences in *Longissimus thoracis* (LT) muscle from conventionally reared 16 month bulls and 19 months old bulls that had a grazing period prior to finishing on concentrates. The aim of the present study was to expand this observation to additional commercially important muscles/cuts. The production systems selected were concentrates offered *ad libitum* and slaughter at under 16 months of age (16-C) or at 19 months of age (19-CC) to examine the effect of age per se, and the cheaper alternative for 19 month bulls described above (19-GC). The results indicate that muscles from 19-CC were more red, had more intramuscular fat and higher cook loss than those from 16-C. No differences in muscle objective texture or sensory texture and acceptability were found between treatments. The expected differences in composition and quality between the muscles were generally consistent across the production systems examined. Therefore, for the type of animal and range of ages investigated, the effect of the production system on LT quality was generally representative of the effect on the other muscles analysed. In addition, the data do not support the under 16 month age restriction, based on meat acceptability, in commercial suckler bull production.

Keywords: beef, palatability, Gluteus medius, Semitendinosus, Longissimus thoracis

Implications

Recent data indicate few quality differences in striploin muscle from conventionally reared 16 month bulls and 19 month old bulls that had a grazing period prior to finishing on concentrates. Data from the present study indicate that the effect of the production system on striploin quality was generally representative of the effect on the sirloin and “eye of the round” cuts. Overall, the data do not support the under 16 month age restriction, based on meat acceptability, in some markets for commercially produced suckler bull beef.

Introduction

The majority of Irish male cattle are reared as steers in grass-based production systems and slaughtered at around 24 months of age. However, compared to steers, non-castrated male cattle grow faster, have higher feed conversion efficiency and lower carbon footprint and bull beef has a lower intramuscular fat (IF) concentration, as preferred by consumers (Murphy, et al., 2017; Carabante *et al*., 2018). Nevertheless, entire male production is discouraged in the context of a market specification that requires the slaughter of bulls at under 16 months with a carcass fatness score of 6 (1-15 scale) or 2+ (Teagasc, 2015). This commercial age/carcass limit was imposed due to a perception that meat quality of meat, particularly tenderness, from entire males deteriorates as they age (Dikeman *et al.*, 1986). The achievement of these specifications requires the use of expensive concentrate feedstuffs but increasing the slaughter age of bulls offers the opportunity to include a cheaper pasture grazing period (O’Riordan *et al.*, 2011). This may also add value to bull beef, since grass-fed beef is perceived by the consumer to be a “greener-healthier” option (Carabante *et al*., 2018). Recent studies that compared animals

differing in carcass weight/age and production systems showed few palatability differences in the *Longissimus thoracis* (LT) muscle when aged for 14 days (Mezgebo *et al.*, 2017a,b; 2019). It seems unreasonable therefore to maintain the under 16 month age limit for bulls. However, available studies are usually limited to LT, with little information available on the effect of age, across different production systems, on other commercially relevant muscles.

The meat quality characteristics of the LT muscle from the traditional under16 month bull production system were recently reported (Moran et al 2019). The objective of the present study was to compare the meat quality characteristics of LT, *Gluteus medius* (GM) and *Semitendinosus* (ST) muscles from these bulls with a 19 month production system in which similar bulls were offered a high concentrate diet indoors or a cheaper option in which a grazing period was included before finishing on a high concentrate diet indoors. The hypotheses tested were that animal age *per se* would not impair the quality of the muscles selected, and that there would be no difference in the quality of the three muscles when bulls were slaughtered at the same age from two systems differing in the cost of production.

Material and methods

Animals and treatments

Spring-born late-maturing breed entire male cattle (n=60 ca. 8 months age), were blocked on breed and weight and within block were randomly assigned to one of three production systems. During the winter, the animals were indoors and offered grass silage *ad libitum* plus 2 kg of concentrate. At the end of the winter they were offered a barley-based concentrate plus grass silage *ad libitum* indoors for 100 or

200 days until slaughter at under 16 (16-C) or 19 (19-CC) months of age, respectively. The third group grazed a perennial ryegrass pasture for 100 days and were then finished indoors on the same diet as 16-C and 19-CC for 100 days until slaughter at 19 months of age (19-GC).

Feeding management

For 16-C and 19-CC, an increasing amount of concentrates was offered in two equal feeds daily and then *ad libitum* once refusal occurred. Grass silage, a first harvest from a predominantly perennial ryegrass sward, mowed with a disc mower, wilted for 24 hours, harvested using a precision-chop harvester and stored in bunker silos was offered *ad libitum* for the duration of the finishing period. The silage, analysed as described by Moloney and O'Kiely (1995) had dry matter (DM) digestibility 688 g/kg, pH 4.1 and crude protein (CP) concentration 130 g/kg DM. Concentrates consisted of 862 g rolled barley, 60 g soya bean, 50 g cane molasses and 28 g vitamins and minerals premix per kg and contained 185 g neutral detergent fibre and an estimated 12.4 MJ metabolisable energy/kg DM.

For 19-GC, the total grazing area was a single block of 13.2 hectares (ha) and the area allowance was adjusted to ensure a constant herbage mass (2300 kg DM/ha). The grass, sampled periodically, had DM digestibility 757 g/kg and CP concentration 163 g/kg DM. Upon housing, these bulls were managed as described for 16-C and 19-CC. Indoor animals were accommodated in concrete-slatted floor pens (lying area c. 3.4 m^2 per bull).

Slaughter, sampling procedures, pH and colour.

On the day of slaughter, the animals were transported to a commercial abattoir (30 km) and slaughtered immediately by bolt stunning followed by exsanguination from the jugular vein. Electrical stimulation was not applied and carcasses were hung by the Achilles tendon. Post-slaughter, carcasses were weighed and graded for conformation (15-point scale, classes E+ (highest) to P− (lowest), E+ is 15) and fatness (15-point scale, scores 5+ (highest) to 1− (lowest), 5+ is 15) according to the EU Beef Carcass Classification Scheme (Anon, 2004). Within 1 hour of slaughter, carcasses were placed in a chill set at 9°C and ambient temperature was monitored. The pH and temperature decline of the LT at the 10th rib were recorded in the left side carcass (Moran *et al.*, 2017). After approximately 10 h the chill temperature was decreased to 0 °C. At 48 h post mortem, subcutaneous fat colour was recorded (Moran *et al.*, 2017).The entire GM and ST muscles and the “cube roll” commercial cut (LT between 6^{th} and 10^{th} rib) were removed, vacuum packaged, transported to Teagasc, Food Research Centre, (Ashtown, Dublin) and stored at 2ºC. After a further 24 h, the ultimate pH was measured and the muscles were cut into individual steaks (thickness 25 mm). The first steak of each muscle was used for meat colour determination. Thus, steaks were wrapped in oxygen-permeable polyvinylchloride film (oxygen permeability of 580 ml/m^2 h at standard temperature and pressure) and allowed to bloom at 4 °C for 1 and 24 h. CIE L* (lightness), a* (redness) and b* (yellowness) were measured through the film at three locations on each muscle, using a dual beam spectrometer (UltraScan XE; Hunter Laboratories, Reston, VA, USA) and averaged. Hue angle (H*) and Chroma (C*) values were also recorded. The remaining steaks were vacuum packed after cutting. Samples for chemical composition and collagen determinations were then stored at -20 °C. Samples for Warner-Bratzler shear force (WBSF) variables, cook loss and sensory analysis were

wet-aged for 11 additional days (2°C) (total of 14 days ageing) and then frozen at –20 °C.

Meat chemical analysis and sarcomere length measurement

The proximate composition and total and soluble collagen concentrations of muscle were measured as described by Moran *et al*. (2017). Sarcomere length was measured by laser diffraction in unaged steaks (Koolmees *et al., 1*986). Details are provided in Supplementary Material S1.

Instrumental texture and sensory evaluation

WBSF was measured according to Shackelford *et al.* (1995). Details are provided in Supplementary Material S1. Three parameters were measured: N, the peak force required to shear through a meat sample; slope (Mpa), the slope in the 20% to 80% segment of the total peak and area (J), the total peak area (Mathoniere *et al.*, 2000).

Sensory testing was conducted using naïve assessors (n=15) (Stone *et al*., 2012a) who ranged in age from 20-50 and who consumed beef regularly. Steaks were grilled to an internal temperature of 72°C, assigned three-digit random codes and served under standard lighting (LUX, 1000) to assessors as 1cm² pieces, in randomised order (Stone *et al.,* 2012b). Each assessor was asked to rate the sensory qualities of steak from each animal according to the methodology of the American Meat Science Association (2005). The assessors rated five sensory qualities on a scale (8-point hedonic) from 1-8 for tenderness (3-5 chews) where 1=extremely tough and 8=extremely tender, overall flavour where 1=very poor and 8=extremely good, overall firmness where 1=extremely mushy and 8=extremely firm, overall texture where

1=very poor and 8=extremely good and overall acceptability where 1=not acceptable and 8=extremely acceptable. Distilled water and unsalted soda crackers were provided to purge the palate of residual flavour notes between samples. Additional details are provided in Supplementary Material S1.

Statistical analysis

The slope of the pH decline as the carcasses cooled was calculated according to a first order decay equation. All data were subjected to analysis of variance using the generalized linear mixed model (GLIMMIX) procedure of SAS (SAS Inst. Inc., Cary, NC, USA) with animal as experimental unit. The fixed factors were block, production system, muscle type and the production system by muscle type interaction. To establish a split plot analysis (i.e., the muscles within animals), animal ID * production system was included in the random statement to identify the correct variance for the main plot effect.

Assessor was also included in the random statement for analysis of the sensory data. For meat colour variables the fixed factors were block, production system, muscle type, blooming time (1h and 24 hours) and their interactions. To establish a split-split plot analysis (i.e., the muscles within animals and blooming time within muscle), animal ID * production system and animal ID * production system * muscle type were included in the random statement.When significant effects were detected, the post hoc Tukey test was used to determine the differences between means. All stated differences are statistically significant ($P < 0.05$, at least).

Results

Animal performance and carcass characteristics (Table 1)

During the finishing period, 16-C, 19-GC and 19-CC bulls had similar daily intakes (DM) of concentrate (9.3, 9.4 and 9.1 kg /animal, respectively) and silage (1.21, 1.35 and 1.21 kg /animal, respectively). Grass intake for 19-GC was estimated, based on the growth of the animals and their associated energy requirement (Agricultural and Food Research Council, 1993) as 9.1 kg DM/animal per day.

Growth during the finishing phase was higher for 16-C than19-GC and 19-CC which did not differ. During the grazing period for 19-GC, growth was lower than 19-CC indoors. Carcasses from16-C were the lightest and 19-CC the heaviest; there was no difference between the treatments for fat or conformation scores. For subcutaneous fat colour; the lightness (L value) was higher for 19-CC than 16-C and 19-GC which did not differ, the redness (a value) was higher for 19-GC than 19-CC which was higher than 16-C, the yellowness (b value) was lower for 16-C than 19-CC and 19-GC which did not differ.

The pH and colour of muscle (Table 2)

There were no interactions between production system and muscle type. Ultimate muscle pH was did not differ between production systems but was higher for GM than LT but similar to ST. The ultimate pH of ST and LT did not differ. The pattern of pH decline (for LT muscle only) is shown in Figure 1. The rate of pH decline between 1 to 7h was higher for 16-CC (slope= 0.09) than 19-CC and 19-GC which were similar (slope = 0.07 and slope = 0.06, respectively).

The muscle colour variables presented are averaged over 1h and 24 h blooming. A* and chroma* were higher for 19-CC than for 19-GC and 16-C which did not differ. B* was higher for 16-C than for 19-GC and 19-CC which did not differ. L* was lower for LT compared to GM and ST which did not differ; a* was higher for GM than LT which in turn was higher than ST; b* was higher for GM than ST which in turn was higher than LT; chroma* was higher for GM than LT and ST which did not differ, hue* was higher for ST than GM which in turn was higher than LT.

All colour variables increased between 1 and 24 hours of blooming. There was an interaction between production system and blooming time for a*, b* and chroma* (Supplementary Table S1). Thus, after 1 hour, a* was higher for 19-CC than 19-GC and 16-C which did not differ but after 24 hours, there was no difference between production systems. After 1 hour, there was no difference between production systems for b* but after 24 hours, b* for 16-C was similar to 19-CC and higher than 19-GC which did not differ from 19-CC. After 1 hour chroma* was similar for 19-CC and 19-GC but 19-CC was higher than 16-C which did not differ from 19-GC. After 24 hours there was no difference between production systems.

There was an interaction between muscle type and blooming time for all colour variables (Supplementary Table 2). Thus, after 1 hour, L* was lower for LT than GM and ST which did not differ. After 24 hours, L* for LT was similar to GM but lower than ST; GM was similar to ST. After 1 hour, a* was similar for LT and ST and both were higher than GM. After 24 hours, a* was similar for LT and GM but both were higher than ST. After 1 hour, b* was higher for GM than ST which in turn was higher

than LT. After 24 hours, b* was similar for GM and ST and both were higher than LT. After 1 hour, chroma* was higher for GM than ST which in turn was higher than LT but there were no differences between muscles after 24 hours. After 1 hour, hue* was higher for ST than LT and GM which did not differ. After 24 hours, hue* was higher for ST than GM which in turn was higher than LT.

Chemical composition (Table 3)

Other than IF concentration, there were no interactions between production system and muscle type. Muscle moisture concentration was higher for 16-C than 19-CC but similar to 19-GC. Muscle moisture concentration was similar for 19-CC and 19-GC. LT had similar protein concentration to ST and both were higher than GM, ST had higher moisture concentration than GM which in turn was higher than LT. LT had lower total collagen concentration than ST and similar to GM, which did not differ from ST. GM had higher soluble collagen concentration than LT which did not differ from ST, GM did not differ from ST. LT had similar collagen solubility to GM and both were higher than ST.

There was an interaction between production system and muscle type for IF concentration (Figure 2). Thus, LT IF concentration for 19-CC was similar to 19-GC but higher than 16-C. LT IF concentration for 19-GC was similar to 16-C. GM IF concentration was similar for 19-CC and 19-GC and both were higher than 16-C. ST IF concentration was similar for all production systems.

Texture related measurements (Table 4)

Other than sarcomere length there were no interactions between production system and muscle type. Muscle cook loss and WBSF area were lower for 16-C than for 19-CC and 19-GC which did not differ. Cook loss was lower for LT than GM and ST which did not differ. WBSF was higher for ST than LT and GM which did not differ while WBSF area was similar for GM and ST and both were higher than LT. There was an interaction between production system and muscle type for sarcomere length (Figure 3). Thus both LT and GM sarcomere lengths were similar across production systems whereas ST sarcomere length was higher for 16-C compared to GM and ST which did not differ.

Sensory characteristics (Table 5)

There were no interactions between production system and muscle type and production system did not affect any of the variables evaluated. ST was rated less tender and as having poorer texture and less acceptable than LT and GM which did not differ. LT had better flavour than GM and ST which did not differ.

Discussion

Context

We have recently shown that an age limit of under 16 months for suckler bulls in premium beef markets is not justified on the basis of sensory characteristics and that a cheaper system whereby bulls spend a period at pasture prior to finishing indoors at 19 months of age approximately, should be an option for producers. These findings were restricted to the LT and can be criticized by industry on that basis. While there are some studies that indicate that results obtained with the LT may be applicable to other muscles, little information is available with respect to suckler bulls.

The primary objective of this study therefore was to expand our previous findings to additional muscles chosen to represent different chemical and structural characteristics, anatomical regions, and economic value. The production systems were chosen to represent the industry standard (16-C) and a cheaper alternative (19-GC) but also to allow a comparison of the effect of age *per se* within one production system not confounded by changes in animal management or ration composition. The general lack of significant interactions for the variables examined indicate that production system effects are consistent across the muscles chosen. Accordingly, the emphasis in the discussion is on the main effects of production system and interactions with muscle type are referenced where appropriate.

Effect of production system

It is recognised that the growth path to slaughter differed among the production systems which may influence aspects of muscle quality. The carcass weights were as expected and all carcasses achieved the desired fatness score of > 6 (1-15 scale). The colour of the subcutaneous fat, especially the yellowness is important in some markets. Based on the factors influencing fat colour (Dunne *et al.,* 2006) we hypothesised that fat yellowness would be higher in 19-GC due to deposition of carotenoids from the grass during the grazing period. The data do not support this hypothesis. If the fat was more yellow due to grazing it seems the length of the finishing period on concentrates was sufficient to remove this effect. The differences in subcutaneous fat “redness” seem related to the lower subcutaneous fat cover causing transparency and increasing the influence of the underlying meat colour (Knight *et al.*, 1999).

Animals were transported to the abattoir in their farm groupings to avoid aggressive behaviour that could occur due to mixing unfamiliar animals and they were slaughtered immediately upon arrival. For ease of access, the early post-mortem phase of carcass management was monitored only in LT. All carcasses were within the desired pH/temperature zone to avoid either heat or cold shortening (Meat and Livestock Australia, 2017). The slower rate of pH decline relative to temperature in the older bulls may reflect the heavier carcasses. The lack of difference in the rate of pH decline between the two groups of older bulls likely reflects similar glycolytic reserves even though carcass weight differed. Despite the difference in post-mortem pH decline, however, the ultimate LT pH values were within the normally acceptable range of pH (5.4-5.8) to avoid “darker meat” (Viljoen *et al.*, 2002).

Surface colour is the first attribute perceived by the consumer and a major influence on the decision to purchase meat (Issanchou 1996). In general, muscle becomes darker as animals mature. The data in the present study (lower L*) tend to support this. In general, muscle also becomes more red (higher a*) as animals mature. The data in the present study are also consistent with this (albeit the difference between 16-C and 19-GC was not statistically significant) and with Mezgebo *et al.* (2019). The higher redness in muscle from 19-CC compared to 19-GC may be related to higher myoglobin concentration, but myoglobin concentration was not measured. Differences in the individual colour parameters led to differences in C* and H* values between the groups as described in the results section.

Few publications have related objective measurements of colour with consumer preference. The data of Holman *et al.* (2016) indicate that a* provides the most

simple and robust prediction of beef colour acceptability. When a* was equal to or above 14.5, samples were acceptable to the consumer. L* and b* were also related with consumer preference, and acceptability increased linearly with L* and b* ranging from 34-50 and 13-22, respectively. From these data, we can conclude that all samples in the present study would be acceptable to the consumer (100% of the samples with a* value >14.5), however meat from 16-C bulls may be the most visually appealing, since it was higher in both L* and b* (49.10 and 15.16 respectively). Consumer studies are needed to confirm this suggestion.

Short and long term aerobic exposure was employed to adequately examine colour, particularly as different muscles were being studied. The increase in a* and b* with increased blooming time is in line with previous experiments (Nian *et al.*, 2017). That differences in a* between production systems disappeared and differences in b* only appeared after 24h of blooming indicates that comparison of colour between production systems at one time point, should be made with caution. Nian *et al.* (2017) also found that differences in colour variables between dairy origin animals differing in age were blooming time dependent.

The higher IF concentration as carcass weight increased was as expected (Mezgebo *et al.*, 2017b). The trend for higher IF concentration in 19-CC compared to 19-GC is similar to that observed by Moran *et al.* (2017) reflecting the differences in carcass weight. The interaction between production system and muscle type for IF concentration suggests that the difference between 16-C and 19 month bulls is more pronounced for GM than LT or ST. Collagen solubility is considered more important than concentration *per se* and decreases with animal maturity (Blanco *et al.*, 2013).

The lack of difference in collagen solubility between 16-C and 19 month bulls is consistent with Mezgebo *et al.* (2019) and likely reflects the absolute difference in age since an increase in the age difference in the latter study decreased collagen solubility. The similar collagen solubility in muscle from bulls slaughtered at the same age from the different production system was expected (Moran *et al.*, 2017), The lack of an effect of production system on sarcomere length in LT and GM is consistent with previous findings for LT in a similar study (Moran *et al.,* 2017). The longer sarcomere length in ST from 16-C compared to 19 month bulls was unexpected since any variations in carcass management between the two slaughter events would be expected to impact all three muscles. To our knowledge, there is no evidence that age per se decreases sarcomere length. This difference in sarcomere length did not affect tenderness (below).

Tenderness in one of the most important eating quality characteristics of beef (O'Quinn *et al.,* 2018).The perception that bull beef becomes less tender and less acceptable as an animal become older is an important contributor to the inclusion of age limits in market specifications for bull beef. To investigate texture-related differences between treatments, three characteristics were extracted from the WBSF measurement. WBSF (N) is indicative of the firmness of the muscle fibres while WBSF area and slope are related to the total energy needed to chew the meat and meat elasticity, respectively (Mathoniere *et al.*, 2000). The effect of production system on WBSF characteristics after 14 days post-mortem ageing was small and only the WBSF area was affected indicating that meat from 16 month bulls may be considered less chewy compared with meat from 19 month bulls. Non-significant changes in WBSF values in the age range in the present study are in agreement with

399 previous studies of 12-24 month (Dikeman *et al.*, 1986) and 8-24 month (Boccard *et*
400 *al.*, 1979) old bulls, respectively. The lack of WBSF differences between meat from
401 19 month bulls pre-finished on grass or concentrates and aged for 14 days agrees
402 with Moran *et al.* (2017).
403
404 Dikeman *et al.* (1986) observed no difference in sensory tenderness in LT from early
405 maturing bulls as age at slaughter increased from 12 to 24 months. Similarly, a
406 literature review of mainly French production systems indicated that as age at
407 slaughter increased from 12 to 24 months there was little evidence of an increase in
408 shear force or decrease in sensory tenderness in meat from bulls (Oury *et al*., 2007).
409 The findings in the present study support these studies and those of Mezgebo *et al.*
410 (2017a) who found no difference in LT tenderness (aged for 14 days) between 16-C
411 and 19-CC bulls. In the present study and in that of Moran *et al.* (2017), the lack of
412 difference in sensory characteristics of muscle when GC and CC bulls were
413 slaughtered at the same age may be related to the absence of differences in
414 components considered to contribute to cooked meat toughness (IF concentration,
415 collagen solubility and sarcomere length). However, when averaged across three
416 different carcass weights, sensory quality was poorer for LT from bulls on a GC
417 production system (Mezgebo *et al.,* 2017a). In that study the GC bulls were on
418 average 3 months older than those in the present but clearly factors other than just
419 age contributed to this observation.
420
421 No differences in cooking loss from LT from young bulls due to age were observed in
422 previous studies (Nian *et al.*, 2017). In the present study the lower cook loss from 16-

C despite the higher moisture concentration may be related to the longer time taken by the bigger steaks from the 19 month bulls to reach the target internal temperature.

Effect of muscle and muscle by age interactions

The highest ultimate pH for GM contrasts with Torrescano *et al*. (2003) who reported higher ultimate pH for LT compared with ST and GM. Generally, differences between the muscles in chemical composition were as expected e.g. (Keith *et al.*,1985) and reflect their inherent biochemical characteristics. The lower a* for “white” muscles like ST and higher a* for “red” muscles like GM, with intermediate values for LT described by Vestergaard *et al*. (2000) were also observed in the present study. The interaction of muscle type and blooming time indicates that despite 1h being the reference blooming time (Wulf and Wise, 1999), this is not appropriate for all muscles. Characterization of the time required for the colour of GM and ST to stabilise during display merits investigation.

While the total collagen concentration tended to be higher for GM than LT, soluble concentration was also higher such that collagen solubility was similar for both muscles similar to Torrescano *et al*. (2003). The higher insoluble collagen concentration (38.7 v 30.7 for GM and LT, respectively) did not negatively affect tenderness. While slightly shorter sarcomeres were detected in the present study, the differences between muscles were largely as reported by Keith *et al*. (1995). The higher shear force for ST than LT and GM, reflecting the collagen data, is similar to that reported previously (Torrescano *et al.*, 2003). Consistent with the instrumental texture, the sensory panel rated ST lowest for tenderness related parameters in line with previous experiments (Keith *et al.,* 1985). The higher overall flavour rating for LT

than GM and ST, possibly due to the higher IF concentration, also supports the findings of Keith *et al.* (1985).

In conclusion, meat from bulls produced at 16 and 19 months of age on a similar diet and at 19 months of age from the economically more attractive option, when aged for 14 days, had similar quality characteristics. For the type of animal and range of ages investigated in the present study, the effect of production system on LT quality was generally representative of the effect on the other muscles examined. The findings of the study do not support the under 16 month age restriction, based on meat quality and acceptability, in commercial suckler bull production.

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Declaration of interest

The authors declare no conflict of interest.

Ethics statement

This study was licensed by the Irish Government Department of Health and Children (B100/2483). All procedures complied with national regulations concerning experimentation on farm animals.

Software and data repository resources

None of the data were deposited in an official repository.

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Table 1 *Performance and carcass fat colour of 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).*

Performance	16-C	19-CC	19-GC	SED	p-value
Growth rate (kg/day)					
Pasture phase	-	1.97[a]	1.49[b]	0.109	0.001
Finishing phase	1.85[a]	1.26[b]	1.45[b]	0.154	0.003
Carcass weight (kg)	358[c]	437[a]	399[b]	14.8	0.000
Fat Score (1-15)	7.27	7.40	7.53	0.49	0.860
Conformation (1-15)	9.93	10.99	10.40	0.46	0.094
Fat colour L	67.9[b]	71.8[a]	67.1[b]	1.04	0.000
Fat colour a	5.18[c]	7.12[b]	8.54[a]	0.516	0.000
Fat colour b	11.7[b]	14.0[a]	14.3[a]	0.37	0.000

SED = standard error of the difference between means. L, a, b = lightness, redness and yellowness, respectively. [a. b. c] Least square means within a row with different superscripts differ significantly at $P < 0.05$.

Table 2. *Colour and pH of Longissimus thoracis (LT), Gluteus medius (GM) and Semitendinosus (ST) muscles of 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).*

	Production system (P)				Muscle (M)				p-value		
	16-C	19-CC	19-GC	SED	LT	GM	ST	SED	P	M	P*M
L*	49.10	47.71	47.55	0.755	46.74^{b}	48.39^{a}	49.22^{a}	0.351	0.093	0.000	0.381
a*	16.84^{b}	18.32^{a}	17.29^{b}	0.314	17.17^{b}	18.76^{a}	16.52^{c}	0.237	0.000	0.000	0.477
b*	15.15	15.01	14.42	0.386	13.66^{c}	15.84^{a}	15.09^{b}	0.224	0.141	0.000	0.196
C*	22.68^{b}	23.72^{a}	22.55^{b}	0.394	21.97^{b}	24.58^{a}	22.40^{b}	0.292	0.012	0.000	0.362
h*	42.11^{a}	39.34^{b}	39.96^{b}	0.771	38.71^{c}	40.13^{b}	42.57^{a}	0.338	0.003	0.000	0.105
pH	5.52	5.54	5.54	0.011	5.52^{b}	5.55^{a}	5.53^{ab}	0.008	0.164	0.003	0.395

SED = standard error of the difference between means; L, a, b = lightness, redness and yellowness, respectively C* = chroma; h* = hue. $^{a.\ b.\ c}$ Least square means within P or M with different superscripts differ significantly at $P<0.05$.

664

665 **Table 3.** *Composition and collagen characteristics of* Longissimus thoracis *(LT),* Gluteus
666 medius *(GM) and* Semitendinosus *(ST) muscles of bulls offered concentrates* ad libitum *until*
667 *slaughter at 16 (16-C) or 19 (19-CC) months of age or at 19 months of age after finishing on*
668 *concentrates subsequent to a period at pasture (19-GC).*

Composition	Production system (P)				Muscle (M)				p-value		
	16-C	19-CC	19-GC	SED	LT	GM	ST	SED	P	M	P*M
Protein (%)	22.8	22.9	22.9	0.263	23.2^{a}	22.5^{b}	23.0^{a}	0.186	0.943	0.004	0.743
IF (%)	0.77^{b}	2.12^{a}	1.57^{a}	0.259	1.95^{a}	1.63^{a}	0.84^{b}	0.172	0.000	0.000	0.006
Moisture (%)	75.2^{a}	74.1^{b}	74.9^{ab}	0.360	74.2^{c}	74.7^{b}	75.4^{a}	0.20	0.014	0.000	0.149
T collagen	55.6	50.1	56.8	5.19	45.0^{b}	56.7^{a}	60.7^{a}	5.54	0.425	0.007	0.622
HS collagen	16.8	14.2	15.8	1.28	14.3^{b}	18.0^{a}	14.6^{ab}	1.60	0.143	0.040	0.210
Solubility (%)	32.0	31.9	31.5	3.39	35.0^{a}	34.6^{a}	25.8^{b}	3.29	0.989	0.001	0.507

669 SED = standard error of the difference between means; IF = intramuscular fat; T = total and
670 HS = heat soluble collagen (mg/g dry defatted meat), respectively.
671 $^{a, b}$ Least square means within P or M with different superscripts differ significantly at $P<0.05$.
672

673 **Table 4.** *Texture related variables of* Longissimus thoracis *(LT),* Gluteus medius *(GM) and*
674 Semitendinosus *(ST) muscles of bulls offered concentrates* ad libitum *until slaughter at 16*
675 *(16-C) or 19 (19-CC) months of age or at 19 months of age after finishing on concentrates*
676 *subsequent to a period at pasture (19-GC).*

	Production system (P)				Muscle (M)				p-value		
	16-C	19-CC	19-GC	SED	LT	GM	ST	SED	P	M	P*M
SL	1.59	1.56	1.57	0.032	1.50[b]	1.47[b]	1.75[a]	0.031	0.638	0.000	0.001
CL (%)	27.2[b]	29.4[a]	29.9[a]	0.430	27.3[b]	29.2[a]	30.0[a]	0.51	0.000	0.000	0.983
WBSF	37.0	36.6	37.3	1.67	34.5[b]	36.5[b]	39.8[a]	1.31	0.914	0.000	0.233
Slope	0.794	0.810	0.798	0.041	0.778	0.770	0.854	0.035	0.938	0.060	0.757
Area	0.274[b]	0.325[a]	0.329[a]	0.016	0.264[b]	0.317[a]	0.348[a]	0.015	0.003	0.000	0.799

677 SED = standard error of the difference between means; SL = sarcomere length (µm): CL =
678 cook loss; WBSF = Warner Brazler shear force, measured in N, slope in MPa, area in J;
679 [a. b] Least square means within P or M with different superscripts differ significantly at $P<0.05$.
680
681
682

683 **Table 5.** *Sensory characteristics of* Longissimus thoracis *(LT),* Gluteus medius *(GM) and*
684 Semitendinosus *(ST) muscles of bulls offered concentrates* ad libitum *until slaughter at 16*
685 *(16-C) or 19 (19-CC) months of age or at 19 months of age after finishing on concentrates*
686 *subsequent to a period at pasture (19-GC).*

Attributes[1]	Production system (P)				Muscle (M)				p-value		
	16-C	19-CC	19-GC	SED	LT	GM	ST	SED	P	M	P*M
Tenderness	4.34	4.71	4.57	0.198	4.81[a]	4.64[a]	4.19[b]	0.175	0.186	0.002	0.941
Flavour	4.89	5.10	4.93	0.162	5.35[a]	4.91[b]	4.67[b]	0.132	0.439	0.000	0.408
Firmness	5.40	5.08	5.25	0.138	5.14	5.22	5.37	0.139	0.093	0.258	0.513
Acceptability	4.70	4.98	4.81	0.173	5.10[a]	4.94[a]	4.47[b]	0.137	0.276	0.000	0.127
Texture	4.62	4.96	4.71	0.187	5.01[a]	4.93[a]	4.34[b]	0.138	0.176	0.000	0.188

687 SED = standard error of the difference between means. [1]Scale: tenderness (1=extremely
688 tough, 8=extremely tender), overall flavour (1=very poor, 8=very good), firmness (1=very
689 mushy, 8=very firm), overall acceptability (1=not acceptable, 8=extremely acceptable).
690 overall texture (1=very poor, 8=very good),
691 [a, b] Least square means within P or M with different superscripts differ significantly at $P<0.05$.
692

Figure captions

Figure 1. pH/temperature decline post-mortem in *longissimus thoracis* 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). Data are presented as mean value per group.

Figure 2. Sarcomere length (± SD) of *Longissimus thoracis* (LT), *Gluteus medius* (GM) and *Semitendinosus* (ST) muscles 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). Different letters indicate differences between production systems within muscle.

Figure 3. Intramuscular fat concentration (± SD) of *Longissimus thoracis* (LT), *Gluteus medius* (GM) and *Semitendinosus* (ST) muscles 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). Different letters indicate differences between production systems within muscle.

712

Figure 1 converted by EO

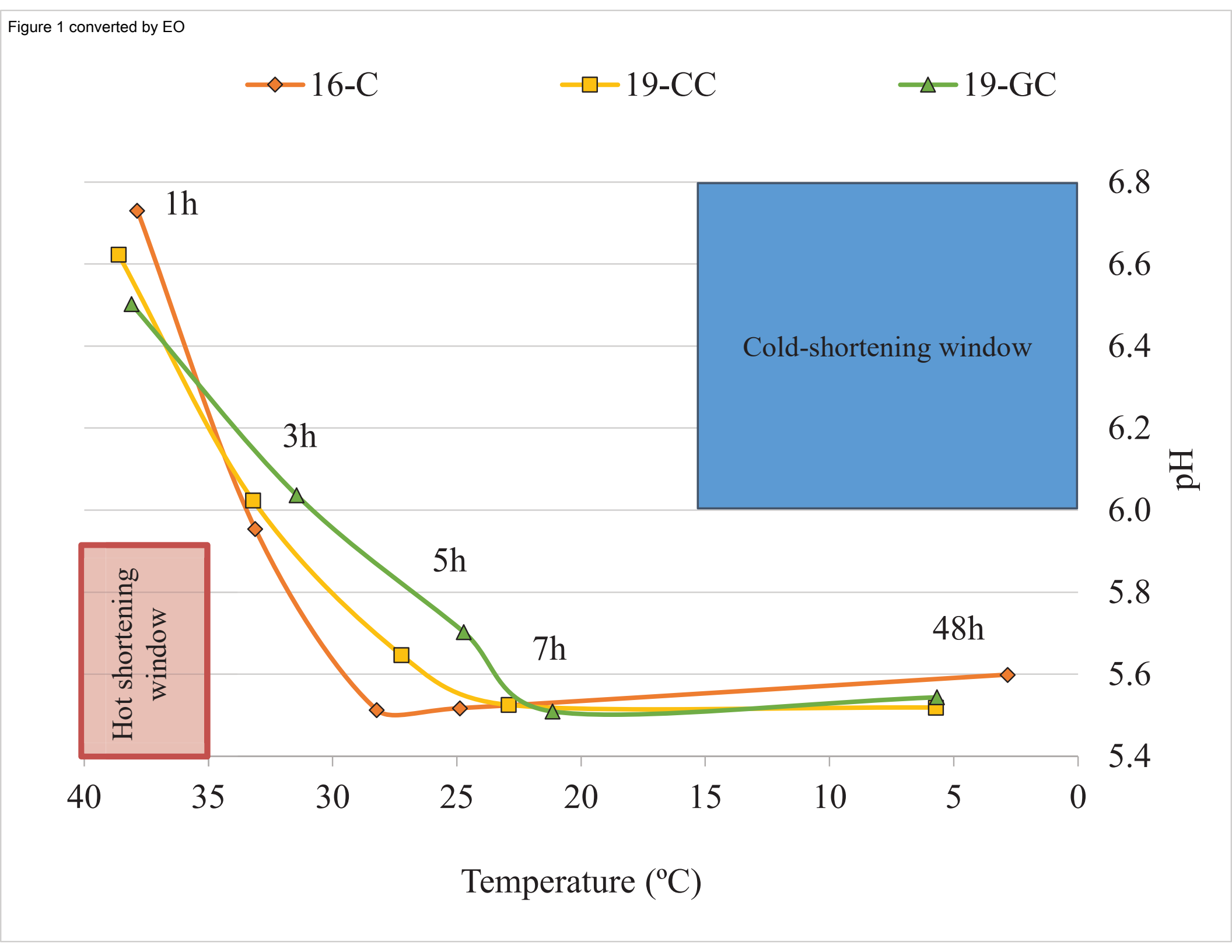


Figure 2 converted by EO

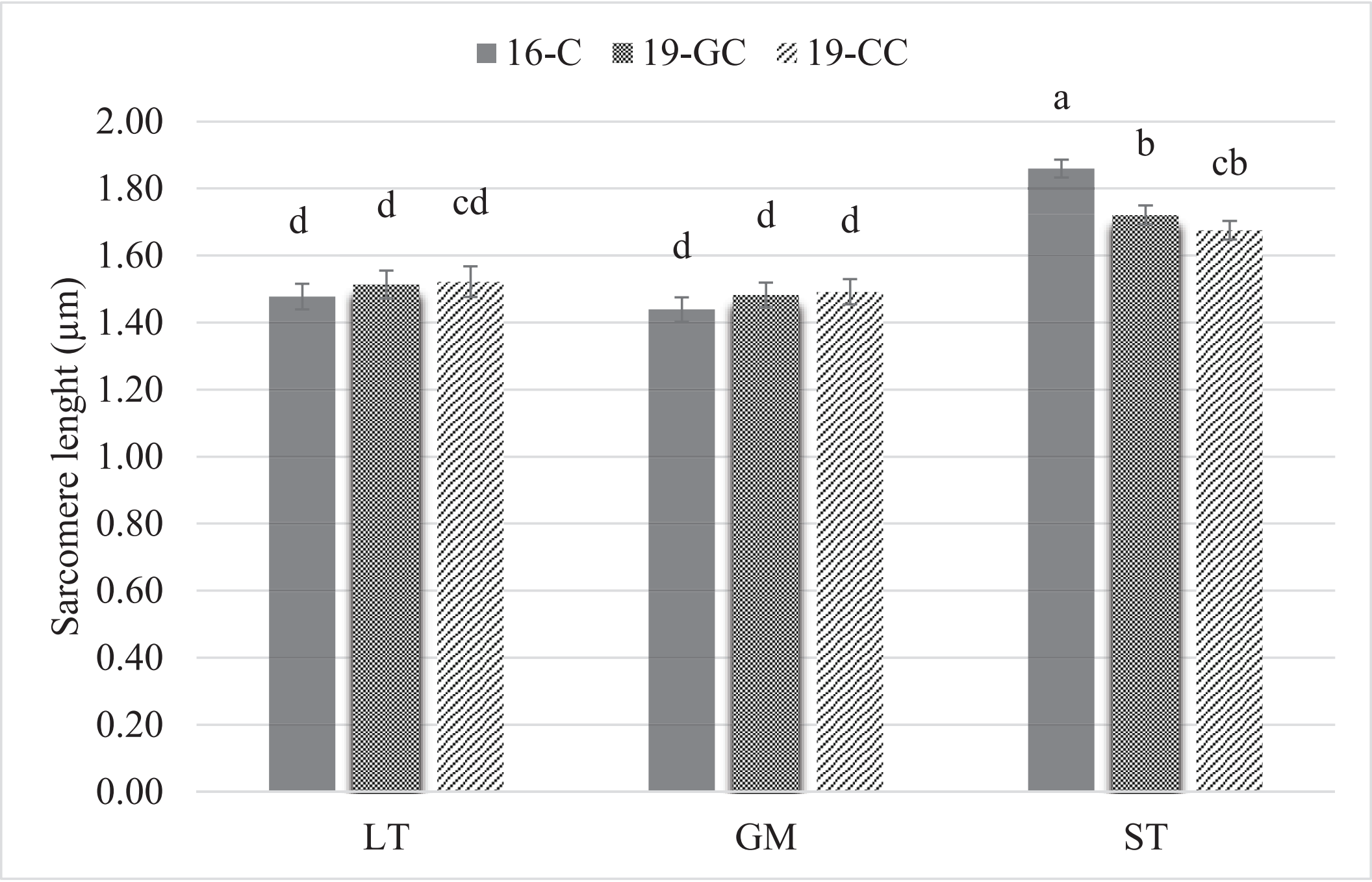
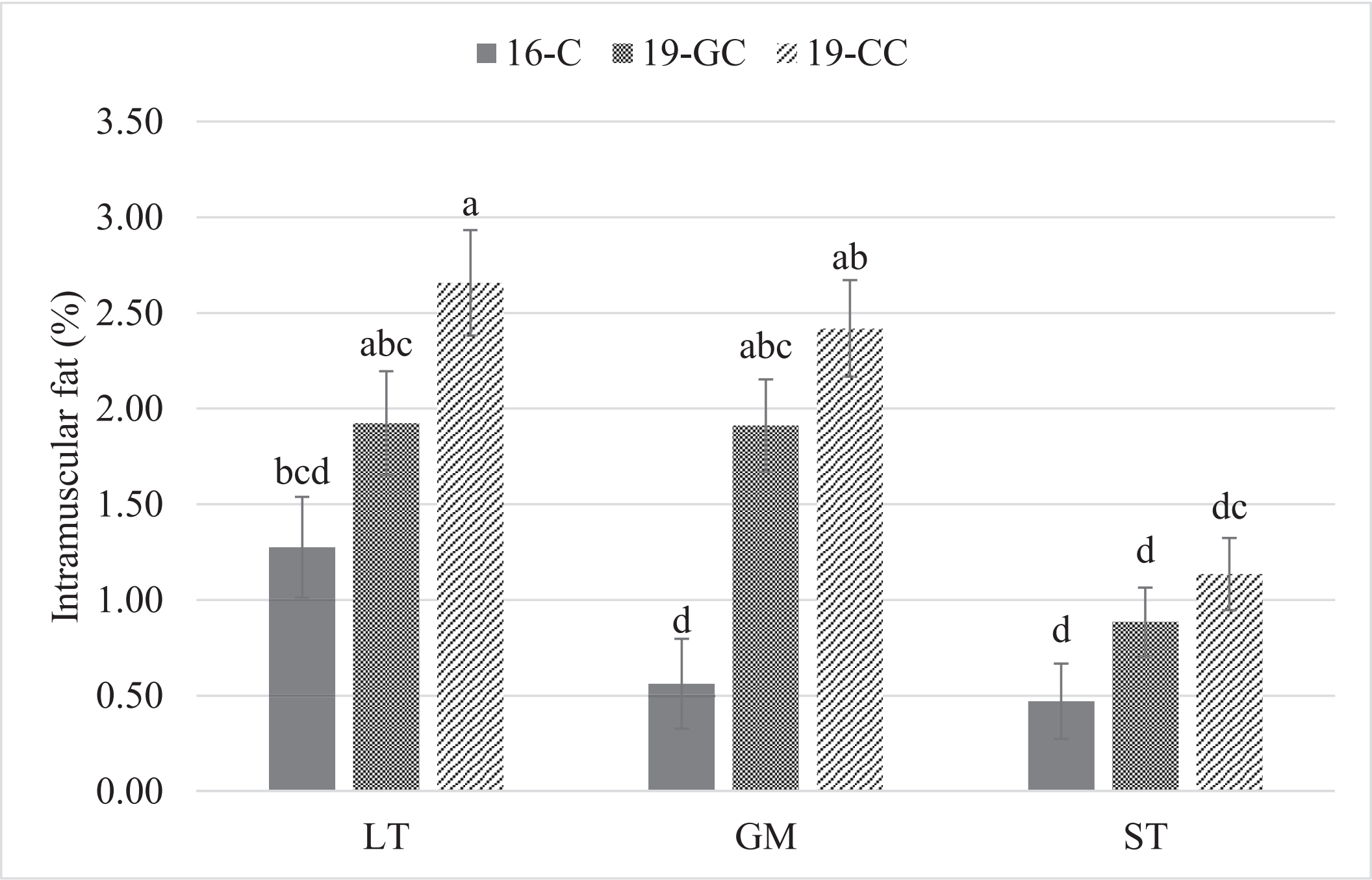


Figure 3 converted by EO



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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***animal* Journal**

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 $NaHPO_4$ 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 $NaHPO_4$ 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).

$$\mu m = \frac{0.6328\ x\ D\ x\ \sqrt{\left(\frac{T}{D}\right)^2 + 1}}{T}$$

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 (R^2 = 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).*

Variable[1]	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*	14.6^{c}	19.0^{a}	16.6^{b}	20.0^{a}	15.5^{c}	19.1^{a}
b*	13.8^{c}	16.5^{a}	14.1^{c}	16.0^{ab}	13.5^{c}	15.3^{b}
Chroma	20.2^{c}	25.2^{a}	21.8^{b}	25.6^{a}	20.6^{bc}	24.5^{a}
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*	46.2^{d}	47.3^{c}	48.3^{bc}	48.5^{abc}	48.8^{ab}	49.7^{a}
a*	14.2^{c}	20.2^{a}	18.0^{b}	19.5^{a}	14.6^{c}	18.5^{b}
b*	12.0^{e}	15.4^{c}	15.4^{bc}	16.3^{a}	14.0^{d}	16.1^{ab}
Chroma	18.6^{d}	25.4^{a}	23.7^{b}	25.4^{a}	20.3^{c}	24.5^{ab}
Hue	40.1^{bc}	37.3^{d}	40.4^{bc}	39.8^{c}	44.0^{a}	41.2^{b}

[1] L*, a*, b* = lightness, redness and yellowness, respectively.
Least square means within a row with different superscripts differ significantly at $P<0.05$.

animal minor technical revision checklist

Last updated January 2018

Manuscript number: 19-61033R1
Title in Editorial Manager: 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
Corresponding author: Aidan Moloney

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- Berry DP, Wall E and Pryce JE 2014. Genetics and genomics of reproductive performance in dairy and beef cattle. Animal 8 (suppl. 1), 115–121.
- Knowles TG, Kestin SC, Haslam SM, Brown SN, Green LE, Butterworth A, Pope SJ, Dirk Pfeiffer D and Nicol CJ 2008. Leg disorders in broiler chickens: prevalence, risk factors and prevention. PLoS ONE 3, e1545.
- Pérez-Enciso M, Rincón JC and Legarra A 2015. Sequence- vs. chip-assisted genomic selection: accurate biological information is advised. Genetics Selection Evolution 47, 43. doi:10.1186/s12711-015-0117-5.
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Examples:

- Association of Official Analytical Chemists (AOAC) 2004. Official methods of analysis, volume 2, 18th edition. AOAC, Arlington, VA, USA.
- Littell RC, Milliken GA, Stroup WW and Wolfinger RD 1996. SAS system for mixed models. Statistical Analysis Systems Institute Inc., Cary, NC, USA.
- Martin P and Bateson P 2007. Measuring behaviour. Cambridge University Press, Cambridge, UK.
- National Research Council (NRC) 2012. Nutrient requirements of swine, 11th revised edition. National Academy Press, Washington, DC, USA.

Book chapter (or official report part) directions

- Author(s) Year. Chapter title. In Title of book (ed. A Editor and B Editor), pp. first-last page numbers. Publisher's name, City, State (2-letter abbreviation) for US places, Country.
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- Nozière P and Hoch T 2006. Modelling fluxes of volatile fatty acids from rumen to portal blood. In Nutrient digestion and utilization in farm animals (ed. E Kebreab, J Dijkstra, A Bannink, WJJ Gerrits and J France), pp. 40–47. CABI Publishing, Wallingford, UK.

Proceedings (or Conference papers) directions

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- Bispo E, Franco D, Monserrat L, González L, Pérez N and Moreno T 2007. Economic considerations of cull dairy cows fattened for a special market. In Proceedings of the 53rd International Congress of Meat Science and Technology, 5-10 August 2007, Beijing, China, pp. 581–582.
- Martuzzi F, Summer A, Malacarne M and Mariani P 2001. Main protein fractions and fatty acids composition of mare milk: some nutritional remarks with reference to woman and cow milk. Paper presented at the 52nd Annual Meeting of the European Association for Animal Production, 26-29 August 2001, Budapest, Hungary.

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- Bryant P 1999. Biodiversity and Conservation. Retrieved on 4 October 1999, from http://darwin.bio.uci.edu/~sustain/bio65/Titlpage.htm

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- Author AB Year. Thesis title. Type of thesis, University with English name, City, State (2-letter abbreviation) for US places, Country (i.e. location of the University).

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- Vlaeminck B 2006. Milk odd- and branched-chain fatty acids: indicators of rumen digestion for optimisation of dairy cattle feeding. PhD thesis, Ghent University, Ghent, Belgium.