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Compositional, organoleptic, metabolic enzyme activity and fibre characteristics of muscle from bulls with different growth paths to a common carcass weight

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Short title: Effect of growth path on bull beef quality

Abstract. The proximate composition, organoleptic quality, metabolic enzyme activity and fibre characteristics of *longissimus thoracis* muscle from suckler bulls (n = 42) assigned to three target indoor winter growth rates (average daily gain of 0.6, 1.0 and 1.5 kg) were investigated. The feeding regimes to achieve the target average daily gains were 2, 4 and 6 kg concentrate daily, respectively, plus grass silage *ad libitum.* The duration of the winter feeding period was 123 d after which bulls were turned out to pasture and grazed for 99 d before re-housing and finishing on concentrates *ad libitum* plus grass silage until they reached a live weight to yield a target carcass weight of 380 kg. The average daily gain during the grazing period was higher ($P < 0.001$) for the 0.6 than for the 1.0 and 1.5 average daily gain groups, which did not differ. Proximate composition, collagen content, metabolic enzyme activity and fibre type distribution of the *longissimus thoracis* muscle were similar ($P > 0.05$) between the 0.6, 1.0 and 1.5 average daily gain groups. Apart from tenderness, which was rated higher ($P < 0.05$) for the 0.6 group than for the 1.0 average daily gain group, the sensory characteristics of the beef were not influenced by the different winter growth rates applied. Overall, restricting the growth rate during winter had little effect on subsequent beef appearance or eating quality.

Additional keywords*:* winter growth rate, compensatory growth, beef quality.

Introduction

In Ireland, an increase in the number of bulls is a recent feature of male beef production due to the superior feed utilization efficiency of bulls compared to steers (O'Riordan *et al.* 2011). Traditionally, after weaning, suckler bulls were raised indoors on concentrate-based diets. In temperate climates, grazed grass is cheaper than grass silage which in turn is cheaper than concentrates (Finneran *et al.* 2011). O'Riordan *et al.* (2012) described a modified sytem to improve the economics of bull beef production which incorporated a grass silage-based indoor winter feeding period followed by a grazing period prior to finishing indoors on a high concentrate ration. Mezgebo *et al.* (2016) reported that compared to the traditional system, the modified grass-based system led to a decrease in intramuscular fat concentration and collagen solubility associated with a decrease in tenderness, flavour liking and overall liking of the beef. A feature of the modified grass-based bull production system is the exploitation of compensatory growth (Hornick *et al.* 2000) at pasture subsequent to the winter feeding phase. The appropriate growth rate during the winter feeding phase to optimise compensatory growth at pasture without impairment of overall growth within the production system was investigated by Marren *et al.* (2013). Since the bulls were finished on a common diet subsequent to grazing, manipulation of the winter growth rate gave rise to different pathways of growth to slaughter.

If variations in the growth path of an animal influences muscle structure or characteristics, there may be a residual effect on meat quality when the animal is eventually slaughtered. There are several reports on the effects of growth path and/or early life growth rate on subsequent growth, carcass characteristics and meat quality of steers (Cassar-Malek *et al.* 2004; Greenwood *et al.* 2005; Tomkins *et al.* 2006). However, the extent to which growth

path to slaughter might affect the ultimate carcass characteristics and quality of beef from suckler bulls raised in a temperate grass-based production system, has not been widely studied. The aim of this study, therefore, was to determine the extent to which variation in winter growth rate of suckler bulls, followed by a period at pasture and then indoor finishing on concentrates, would affect selected carcass characteristics and biochemical and sensory quality of muscle. It was hypothesised that changing the growth path of suckler bulls to a common carcass weight by restricting the growth rate during the winter period has little influence on beef quality.

Materials and methods

Animals and management

As part of a larger study, described by Marren *et al.* (2013), 42 weaned Charolais and Limousin sired suckler bulls were purchased at livestock marts in Ireland at approximately 8 months of age during October/November, acclimatised to slatted floor accommodation and offered grass silage *ad libitum* plus 2 kg/head.day^{-1} of a barley-based concentrate. In early December animals were assigned at random to one of three winter growth rate treatments balanced for sire breed, birth date, initial weight and dam breed, with 14 bulls (9 Charolais-sired and 5 Limousin-sired) in each treatment group. The 3 target winter growth rates, expressed as average daily gain (ADG), were 0.6, 1.0 and 1.5 kg. The feeding regimes to achieve the target ADG of 0.6, 1.0 and 1.5 kg were 2, 4 and 6 kg concentrate (870 g/kg rolled barley, 60 g/kg soya bean meal, 50 g/kg molasses and 20 g/kg minerals/vitamins) daily, respectively, plus grass silage (dry matter digestibility 700 g/kg) *ad libitum*. There were 4 pens of animals (3 or 4 animals/pen) when indoors. The duration of the winter feeding period was 123 d after which, bulls were turned out to pasture, where they were rotationally grazed for 99 days and then re-housed. Following housing they were gradually adapted (during a 3-week period) to the barley-based concentrates (same formulation as above) *ad libitum* plus grass silage *ad libitum*. The bulls were slaughtered within treatment, at a commercial slaughter plant (Kepak Group, Clonee, Co. Meath, Ireland) on reaching the mean live weight to achieve a target carcass weight of 380 kg. The duration of the concentrate finishing periods were 71, 65 and 51 d for ADG 0.6, ADG 1.0 and ADG 1.5 kg, respectively. The bulls were managed to avoid pre-slaughter stress, i.e. they were accustomed to pre-slaughter handling due to regular weighing and to penning due to the indoor finishing period; in addition the animals were carefully managed during transport and lairage. The study was carried out under license from the Irish Government

Department of Health and Children (experimental licence number B100/2483) and with the approval of Teagasc, the Irish Agriculture and Food Development Authority. All procedures used complied with national regulations concerning experimentation on farm animals.

Carcass grading and muscle tissue collection

Post slaughter, carcasses (without electrical stimulation) were weighed and graded for conformation (15 point scale, classes E^+ (highest, 15) to P^- (lowest, 1) and fatness (15 point scale, scores 5^+ (highest) to 1^- (lowest), 5^+ is 15) according to the EU Beef Carcass Classification Scheme (Anon 2004). Carcasses were then maintained at 4ºC for approximately 8h, when the chills were set to zero. At 1 h post-slaughter, a sample (*ca.* 20 g) of *longissimus thoracis* (LT) muscle tissue (from the 9^{th} rib position) was taken, snap frozen in liquid nitrogen and maintained at -80ºC for metabolic enzyme activity and fibre typing analyses.

Muscle pH and temperature measurement

Muscle pH and temperature were measured at 2, 3.5, 5 and 48 h post-mortem by making a scalpel incision in the muscle at the $10/11^{th}$ rib and inserting a glass electrode (Model EC-2010-06, Amagruss Electrodes Ltd., Westport, Co. Mayo, Ireland) and a temperature probe attached to a portable pH and temperature meter (Model no. 250A, Orion Research Inc., Boston, MA) approximately 4.0 cm into the muscle. Before analysis the meter was calibrated using standard phosphate buffers (pH 4.01 and 7.00, Radiometer, Copenhagen, Denmark) after which the electrode was thoroughly rinsed. The electrode was also rinsed with distilled water between measurements of individual carcasses The meter was adjusted to allow temperature-compensated pH measurement.

Muscle colour measurement and colour grade assessment

At 48 h post-mortem, carcasses were cut at the 5/6th ribs. The cut surface of the muscle was allowed to bloom for 1 h and the muscle colour (*L, a, b*) was measured using a Miniscan XE Plus (Hunter Associates Laboratory Inc., Reston, Virginia, USA). The *L, a, b* colour coordinates represent lightness (scale 0 (black) to 100 (white)), redness (scale +a (red) to –a* (green)) and yellowness (scale +b (yellow) to –b (blue)) of the muscle respectively. A chroma (saturation) colour intensity, *C* (computed as $\sqrt{(a^2 + b^2)}$, where a higher *'C'* value indicates higher colour saturation) and hue angle, *H* (computed as $[\tan^{-1}(b/a)][180/\pi]$, where 0/360º is red, 90º is yellow, 180º is green and 270º is blue colour) were also determined

On the same day, a sample (*ca.* 11 cm in length) of LT muscle was excised (from the 10th rib area), vacuum packed, aged for a further 12 days at 2ºC, and finally frozen and stored at -18ºC prior to compositional, collagen and sensory analysis.

Compositional analysis

Frozen samples of LT were thawed in a refrigerator (4ºC overnight). The lean meat and exudate were homogenized using a Robot coupe blender (R301 Ultra, Robot coupe SA, Vincennes, France). Sub-samples (*ca.* 20 g) were dispensed into plastic tubes (100 x 150 mm, McDonnells, Dublin, Ireland) and re-frozen for subsequent collagen analysis. Moisture and intramuscular fat contents were determined using the SMART System 5 microwave moisture drying oven and NMR SMART Trac rapid fat analyser (CEM Corporation, Matthews, NC, USA) using AOAC Methods 985.14 and 985.26 (AOAC 1990), respectively. Protein concentration was determined using a LECO FP328 (LECO Corp., MI, USA) protein analyser based on the Dumas method and according to AOAC

method 992.15 (AOAC 1990). Ash was determined by incinerating samples in a furnace (540°C overnight).

Collagen analysis

All chemicals were obtained from Sigma-Aldrich, Ireland Ltd. Sulphuric acid (3.5M), acetate/citrate buffer (pH 6.0), oxidant solution (chloramine T), colour reagent (4-dimethylaminobenzaldehyde), NaOH (1M) and hydroxyproline standard solutions were prepared according to Kolar (1990). Total and soluble collagen measurement followed adaptations of the methods of Woessner (1961), Hill (1966), Kolar (1990) and Fang *et al.* (1999). Homogenized samples of LT were thawed in a refrigerator at 4^{o}C overnight and 5 g were placed in a 50 mL centrifuge tube (114 x 28 mm, PP, Sarstedt, Nümbrecht, Germany) with 12 mL acetate/citrate buffer, pH 6.0 (Fang *et al.* 1999). Following vortex mixing the tubes were placed in a water bath at 77°C for 65 min, agitated every 15 min and centrifuged for 10 min at 3990g (Rotofix 32A centrifuge, Hettich, Germany). The supernatant was collected and 8 mL of Ringer's solution was added to the precipitate which was centrifuged again (10 min at 3990g) following vortex mixing. The supernatants from the two centrifugations were combined. The supernatants and precipitates were hydrolysed separately, each in 30 mL of 3.5 M H_2SO_4 in an oven at 105°C for 16 h The hydrolysates were diluted to 250 mL and the hydroxyproline content was determined by a colorimetric reaction (Kolar 1990) after neutralising the solutions with 4.37 mL of 1 M NaOH (Woessner 1961). Absorbance was measured (UVMini-1240 Spectrophotometer, Shimadzu, Japan) at 558 nm. Using hydroxyproline as a standard, the soluble and insoluble collagen concentration were calculated from the hydroxyproline concentration in the supernatant and precipitate, respectively, using a conversion factor of 7.25 (Goll *et al.* 1963). Total collagen was calculated from the sum of the hydroxyproline concentration in the precipitate and in the supernatant, and expressed in mg/g of wet weight.

Metabolic enzyme activities

Glycolytic enzyme activities (lactate dehydrogenase (LDH) and phosphofructokinase (PFK)) and oxidative enzyme activities (isocitrate dehydrogenase (ICDH), citrate synthase (CS) and cytochrome *c* oxidase (COX)) were quantified spectrophotometrically as described by Jurie *et al.* (2006) with slight modifications. Muscle samples were homogenized with a polytron (PT-MR 2100, Kinematica, Lucerne, Switzerland) in a 5% (w/v) solution with 10 mM Tris base (pH 8.0), sucrose 0.25 M and 2 mM EDTA. An aliquot of the homogenate was centrifuged at 6000g for 15 min at 4°C for the determination of PFK, LDH and ICDH activities. The remainder of the homogenate was frozen and stored at -20°C for determination of CS and COX activities.

The PFK and LDH activities were measured by following the disappearance of nicotinamide adenine dinucleotide (reduced form, NADH) at 340 nm, and ICDH activity was measured by following the reduction of nicotinamide adenine dinucleotide phosphate (NADP) at 340 nm. The PFK activity was determined according to Beutler (1971), using 10 µL of supernatant in 1 mL of a reaction mixture that contained 50 mM Tris-HCl, 5 mM $MgCl_2$, 0.05 mM NADH, 1 mM ATP, 10 mM fructose-6-phosphate, 0.7 U/mL aldolase, 6.7 U/mL triose phosphate isomerise and 0.7 U/mL α-glycerophosphate dehydrogenase. The LDH activity was determined according to Ansay (1974), using 10 µL of diluted (four-fold) supernatant in 2.9 mL of a reaction mixture that contained 48.2 mM triethanolamine, 4.82 mM EDTA, 0.23 mM NADH and 2 mM pyruvate. The ICDH activity was determined according to Briand *et al.* (1981), using 200 µL of supernatant in 2.7 mL of a reaction mixture that contained 36 mM Na_2HPO_4, 0.5 mM $MnCl_2$, 0.05% Triton X-100, 0.3 mM NADP and 1.3 mM isocitrate.

The CS activity was determined by measuring the rate of initial reaction at 412 nm using the DTNB (5,5'-dithiobis-(2-nitrobenzoic acid)) assay as previously described by Shepherd and Garland (1969). The reaction mixture contained 94.8 mM Tris base, 95.6 µM DTNB, 50 mM acetyl-CoA, 1.2 mM oxaloacetate and 30 µL of supernatant in a total volume of 1 mL. The COX activity was determined according to Smith and Conrad (1956) with 20 µL of homogenate in 1 mL of a reaction mixture that contained 90 µM reduced cytochrome *C* as substrate and 50 mM potassium phosphate. The oxidation of cytochrome *c* was measured at 550 nm. Muscle protein content was determined spectrophotometrically according to Lowry *et al.* (1951). All enzyme activities were measured at 25°C, in duplicate, and expressed micro moles of substrate converted per minute per gram of wet muscle (µmol/min.g of tissue) and per gram of protein (µmol/min.g of protein).

Muscle fibre typing

Muscle fibre types (type I, IIA, IIX and IIB) were identified by separating myosin heavy chain (MyHC) isoforms (MyHC I, IIa, IIx and IIb) using high-resolution mini-gel electrophoresis as described by Picard *et al.* (2011). Myofibrillar proteins were extracted from 200 mg of muscle using a buffer containing 0.5 M NaCl, 20 mM sodium pyrophosphate, 50 mM Tris, 1 mM EDTA and 1 mM dithiothreitol. Samples were homogenised with a polytron (PT-MR 2100, Kinematica, Lucerne, Switzerland) and were centrifuged at 2500g for 10 min at 4°C. Supernatants were diluted 1:1 (v/v) with glycerol.

Protein concentration was determined according to Bradford (1976). Samples were then suspended in 1:1 (v/v) in basic 2x Laemmli buffer containing 4% (w/v) sodium dodecyl sulphate (SDS), 10% (v/v) β-mercaptoethanol, 20% (v/v) glycerol, 125 mM Tris (pH 6.8) and 0.01% (w/v) pyronin Y, incubated at room temperature for 10 min and then heated (70°C) for 10 min. The separating gel consisted of 35% (v/v) glycerol, 9% (w/v) acrylamide-Bis (50:1), 230 mM Tris (pH 8.8), 115 mM glycine and 0.4% (w/v) SDS. The

stacking gel consisted of 47% (v/v) glycerol, 6% (w/v) acrylamide-Bis (50:1), 110 mM Tris (pH 6.8), 6 mM EDTA and 0.4% (w/v) SDS. The gel constituents were prepared from stock solutions, and polymerisation was initiated with 0.05% (v/v) TEMED and 0.1% (w/v) ammonium persulphate. Five µg of protein were loaded per well onto 0.75-mm-thick gels set on a Mini-Protean II Dual Slab Cell electrophoretic system (Bio-Rad). The entire gel unit was placed in an incubator at 4°C and electrophoresis was performed at a constant voltage of 70 V for 30 h. After migration, the gels were stained with Coomassie blue, and relative amounts of different MyHC isoforms were quantified using ImageQuant TL v2003 software (Amersham) as described in Picard *et al*. (2011).

Sensory analysis

The sensory analysis was carried out at the University of Bristol, UK, using a 10-person trained taste panel who had been selected for their sensory acuity using the methods outlined in BSI (1993). The samples were thawed overnight at 4°C, cut into 20 mm thick steaks and grilled on pre-coded foil-lined grill pans under preheated, domestic low level grills, turning every 3 min until the desired centre temperature of 74°C (measured by a thermocouple probe at the geometrical centre of the sample) was reached. All fat and connective tissue was trimmed and the muscle cut into blocks 2 cm^3, which were wrapped in pre-labelled foils and placed in a heated incubator. Assessors tasted the samples in an order based on the designs outlined by MacFie *et al.* (1989) for balancing carryover effects between samples.

All sensory assessments were completed under red light in a purpose-built sensory suite where each tasting booth was equipped with computer terminals linked to a fileserver running a sensory software programme (Fizz v 2.20h, Biosystemes, Couternon, France).

Panellists assessed each steak using 0 - 100 mm unstructured intensity line scales for a consensually agreed texture profile, where 0 = nil and 100 = extreme, and 8-point category scales for tenderness (1 = extremely tough to 8 = extremely tender), juiciness (1 = extremely dry to 8 = extremely juicy), beefy flavour and abnormal beef flavour intensities (1 = extremely weak to 8 = extremely strong).

Statistical analysis

Data were subjected to analysis of variance using the General Linear Model (GLM) procedure of SPSS (IBM SPSS Statistics Version 20) where winter growth rate was regarded as a fixed factor, and differences between means were considered significant at $P < 0.05$. Assessor and session were also regarded as fixed factors when analysing the sensory panel data. Muscle pH and temperature data were analysed using the GLM repeated measures (GLM REP) procedure of SPSS.

Results

The growth pattern of the bulls is shown in Fig. 1. All treatment groups had similar live weight (368.3 kg on average) at the beginning of the winter feeding period. At the end of the winter period, live weight was higher ($P < 0.05$) for the ADG 1.5 than for the ADG 1.0 and 0.6 groups which did not differ. At the end of the grazing period, live weight was higher ($P < 0.05$) for the ADG 1.5 than for the ADG 1.0 and 0.6 groups, which did not differ. By the end of the indoor finishing period, all groups had similar ($P > 0.05$) live weight.

Production and carcass trait data are presented in Table 1. Mean age at slaughter was not significantly affected by winter growth rate albeit age at slaughter was numerically higher

for the ADG 0.6 group than for the ADG 1.0 group which in turn was higher than the ADG 1.5 group. The ADG achieved during the winter period was higher ($P < 0.001$) for the ADG 1.5 than for the ADG 1.0 and 0.6 groups, which did not differ significantly (but numerically). Average daily gain during the grazing period was higher ($P < 0.01$) for the ADG 0.6 group than for the ADG 1.0 and 1.5 groups, which did not differ. During the finishing period, all groups had a similar ($P > 0.05$) ADG. The bulls in each group had similar mean carcass weight and carcass conformation and fat scores.

Early post-mortem pH and temperature data for LT muscle are presented in Table 2. There was an interaction ($P < 0.05$) between ADG during the winter and time post-mortem with respect to muscle pH. Thus, at 2 h post-mortem, muscle pH was lower ($P < 0.05$) for the ADG 0.6 group than for the ADG 1.0 group but similar to the ADG 1.5 group which in turn was similar to ADG 1.0 group. At 3.5 h post-mortem, muscle pH was lower ($P < 0.05$) for the ADG 0.6 group than for the ADG 1.0 and ADG 1.5 groups which did not differ whereas muscle pH was similar between the groups at 5 h post-mortem. Muscle temperature was similar between the ADG groups at 2, 3.5 and 5 h post-mortem.

Ultimate pH (pH at 48 h post-mortem), colour, proximate composition and collagen data for LT muscle are presented in Table 3. Ultimate pH was higher ($P < 0.001$) for the ADG 1.5 group than for the ADG 1.0 group which in turn was higher ($P < 0.001$) than the ADG 0.6 group. Muscle *'L', 'a', 'b',* '*C'* and '*H'* values, proximate composition and collagen content were similar ($P > 0.05$) between the winter growth rate groups.

Muscle metabolic enzyme activity and fibre type distribution were similar ($P > 0.05$) between the groups (Table 4). The proportion of IIX fibres tended ($P = 0.1$) to be higher in the ADG 1.5 group and lower in the ADG 0.6 group. Fibre Type IIB was identified in only 5 animals (2 Charolais and 2 Limousin sired animals in the ADG 0.6, none in the ADG 1.0 group and in 1 Limousin sired animal in the ADG 1.5 group).

Data on sensory characteristics are presented in Table 5. Tenderness and ease of cutting were higher for the ADG 0.6 group ($P < 0.05$) than for ADG 1.0 group, but similar to the ADG 1.5 group, which in turn was similar to the ADG 1.0 group. During eating, toughness was lower ($P < 0.05$) for the ADG 0.6 group than for the ADG 1.5 group, but similar to the ADG 1.0 group, which in turn was similar to the ADG 1.5 group.

Discussion

Compensatory growth is the phenomenon whereby an animal experiences a period of increased growth following a period of nutritional restriction, relative to the growth of an unrestricted animal (Hornick *et al.* 2000). Exploitation of compensatory growth at pasture is a key element of grass-based beef production. In Spring-born, grass-based steer production systems in temperate climates a post-weaning/first winter indoors growth rate of 0.5-0.6 kg/day is advised, prior to finishing on a higher energy ration usually indoors (Teagasc 2015). Because of the higher growth potential of bulls compared to steers, this restriction on growth in the post-weaning phase might not be appropriate to maximize productivity and profitability in a grass-based bull production system. Accordingly, Marren *et al.* (2013) examined the impact of variation in winter growth on these metrics in this system. A target carcass weight of 380 kg was chosen as this weight is a key criterion of suitability for some international markets for Irish beef (Bord Bia 2011). As a consequence of the variation in winter growth rate, bulls experienced different growth paths to slaughter. The main objective of the present study was to determine the consequences of these different growth trajectories on aspects of beef quality.

The winter growth rates achieved reflected the different amounts of supplementary concentrates offered to the different groups during the winter. While the trend was largely as planned, the range in difference was a little narrower than anticipated. Nevertheless, when the animals were turned out to pasture after the indoor winter period, the ADG 0.6 group exhibited compensatory growth (1.24 kg/day) relative to the ADG 1.5 group (1.02 kg/day) whereas the ADG 1.0 group did not (0.98 kg/day). The more pronounced differences in growth path in this study were therefore between ADG 0.6 and ADG 1.5. The higher winter growth rate for the ADG 1.5 group was not recovered subsequently and

this group was on average, 20 days younger at slaughter, which we recognise could mask differences due to growth path *per se*. However, Mazgebo *et al* (2016) observed few differences in meat quality when similar bulls were slaughtered at a range of ages across different production systems,

All groups had similar pre-slaughter handling and were finished indoors, making them unlikely to suffer from pre-slaughter stress-related loss of glycogen (Pethick *et al.* 1994). While the mean pHu value was statistically higher for the ADG 1.5 group, the differences between groups were small in absolute terms and within the 'normal' pH range (i.e. 5.4 – 5.8) (Page *et al.* 2001; Viljoen *et al.* 2002). The lack of pre-slaughter stress was further supported by the similar muscle colour and no carcasses were deemed "dark cutters" by abattoir personnel. With regard to post-mortem LT pH and temperature profiles (Fig. 2), the pattern of pH decrease was not in the cold or heat shortening window ((Thompson, 2002).

The relationship between growth rate and tenderness was reviewed by Oddy *et al.* (2001). The authors indicated that there was evidence that differences in growth rate could lead to changes in collagen properties (its structure, cross-linking and matrix). A decrease in growth rate resulting from a severe nutritional restriction can increase the connective tissue contribution to beef toughness (Harper 1999; Oddy *et al.* 2001), as lower growth rate results in a decrease in the amount of newly formed collagen (i.e. more soluble) which then leads to the increase in the proportion of the existing (i.e. more older, cross-linked and therefore less soluble) collagen (McCormick 1994). Harper (1999) also indicated that compensatory growth could result in a restructuring of connective tissues (i.e. replacing

the existing (older) collagen with a newly synthesised (more soluble) collagen) In the present study, if an alteration in collagen structure occurred during the period of relative nutritional restriction it was reversed during the subsequent grazing and finishing periods as there were no significant differences in total collagen of the proportion of soluble collagen. Silva *et al*. (2017) reported a similar finding for Nellore bulls grown at different rates post-weaning but offered a high concentrate ration prior to slaughter.

The lack of difference in carcass fat score and muscle composition likely reflects the similar mean carcass weight for each treatment group. Similarly, Lancaster *et al*. (2014) in a meta-analysis of published studies concluded, that despite undergoing a variety of growth paths, there was no relationship between stocker/backgrounding growth rate and carcass fat variables if cattle were slaughtered at a similar carcass weight.

Variations in the growth rate of cattle can also lead to changes in metabolic characteristics, proteolytic enzyme activities and muscle fibre characteristics of beef (Geay *et al.* 2001; Oddy *et al.* 2001). Yambayamba and Price (1991) suggested that a decrease in the energy intake of the animal leads to muscle with more oxidative fibres than glycolytic fibres. The studies of Picard *et al*. (2007, 2014) demonstrated that in French beef breeds, bulls that had the more tender LT also had a lower proportion of IIX fibres. The results of the present study are in accordance with these data as LT from the 0.6 group contained a numerically lower proportion of IIX fibres and was rated more tender. Possible differences in muscle fibres due to different winter growth rates were lost in the subsequent periods by accelerated growth during the grazing period (for ADG 0.6 group in particular) and similar (and higher) growth rates during the finishing period for all ADG groups. Even though this

was not directly tested in the present study, a study by Greenwood *et al.* (2009) on muscle fibres and Tomkins *et al.* (2006) on eating quality of beef from steers managed to grow differently (i.e. fast, slow or display weight loss) prior to finishing period supports this hypothesis. From their study, it was concluded that early (post-weaning) nutritional restriction results in a decrease in glycolytic fibres and an increase in oxidative fibres but the effect did not persist after the nutritional restriction was lifted during the subsequent recovery and finishing periods (Greenwood *et al.* 2009) and the quality of the beef was not affected by the post-weaning nutritional restrictions prior to the finishing period (Tomkins *et al.* 2006).

The scientific literature on the Type IIB isoform is not consistent as some authors indicate possible expression of type IIB fibre in certain bovine muscles (Maccatrozzo *et al.* 2009) while others (Chikuni *et al.* 2004; Toniolo *et al.* 2005) dispute its presence. Picard and Cassar-Malek (2009) demonstrated that while the mRNA is generally present in the protein is only present in the muscle ofsome cattle. These authors also showed that the expression of Type IIB fibre is breed specific (higher frequency in purebred Limousin compared to purebred Charolais cattle, personal communication).

Maltin *et al.* (2001) showed that an increase in metabolizable energy intake, which led to increased live weight gain, resulted in increased glycolytic properties of the muscle. In our study, the glycolytic enzymes, LDH and PFK, representative of the glycolytic potential and oxidative enzymes, ICDH, CS and COX, markers of oxidative potential of the muscle, were not affected by the differences in the winter growth rate of the bulls and these similarities could be attributed to the similar growth rates during the finishing period.

Collection of biopsies at the end of each treatment period for muscle fibre type and metabolic enzyme activity analysis would be interesting in future studies.

The higher tenderness rating for muscle from the ADG 0.6 group could be related to the higher growth rate during the grazing period (i.e. compensatory growth) in the ADG 0.6 group compared to the other groups. Higher protein turnover associated with higher growth rate has been suggested to lead to an increase in post-mortem proteolytic activity in muscle which in turn can increase tenderness (Koohmaraie *et al.* 2002; Perry and Thompson 2005). The literature on the effect of pre-slaughter growth rate on tenderness *per se* is equivocal (Moloney *et al*. 2008) and given the relatively long re-alimentation period (when the grazing and finishing phases are considered together) in the present study, the difference in tenderness was unexpected. Differences in tenderness due to post-weaning growth in the literature seem to relate to the size of the interval between the end of the post-weaning phase and slaughter. In this regard, Tomkins *et al*. (2006) concluded that nutrient restriction of steers in the immediate post-weaning period followed by pasture re-alimentation for 192 days "did not influence final beef quality". In addition, Costa *et al*. (2015) reported that the effect of growth path of Alentejana bulls on tenderness was muscle dependent. In any event, the size of the difference in tenderness rating in the present study, a difference of 0.3 units on an 8 point scale, is unlikely to be detected by an untrained consumer and did not influence the rating for overall liking by trained sensory panellists.

Conclusion

The study has shown that in a grass-based production system, increasing the growth of bulls during the winter, above that advised for steers, had no commercially relevant effect on the sensory characteristics of beef. In this production system, muscle proximate composition, collagen content, metabolic enzyme activities and fibre type distribution were not affected by variations in growth rate during the winter or in the overall growth path to slaughter. Producers therefore can economically optimise the growth path of bulls without concern for a deleterious effect on beef quality.

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Table 1. Production and carcass traits of bulls that had different winter growth rates

ADG = Average daily live weight gain. [A] *Conformation score: E^+ (highest, 15) to P^- (lowest, 1).* [B] *Fat score: 5^+ (highest, 15) to 1^- (lowest, 1).* [a, b] *means within rows, assigned different superscripts differ significantly ; ** $P < 0.01$; *** $P < 0.001$.*

	Winter growth rate (kg/day)				
Traits	0.6	1.0	1.5	s.e.m	Significance
Number of animals	14	14	14		
Age at slaughter (months)	18.3	18.1	17.9	0.29	0.60
ADG winter (kg/day)	0.76[a]	0.92[a]	1.30[b]	0.068	***
ADG grazing (kg/day)	1.24[b]	0.98[a]	1.02[a]	0.051	**
ADG finishing (kg/day)	2.04	2.33	2.06	0.108	0.12
ADG overall (kg/day)	1.10	1.07	1.02	0.038	0.34
Slaughter weight (kg)	693	686	695	15.2	0.92
Carcass weight (kg)	387	384	383	9.3	0.95
Conformation score[A]	9.71	9.43	9.14	0.326	0.47
Fat score[B]	6.64	6.71	6.64	0.282	0.98

Table 2. Early post-mortem pH and temperature profile of longissimus thoracis muscle from bulls that had different winter growth rates (kg/day)

*[a, b, c] means within columns (effect of winter growth rate), assigned different superscripts differ significantly; * P < 0.05; *** P < 0.001.*

	Growth rate (ADG)	Time, post-mortem (h)			s.e.m.	Significance		
		2	3.5	5		ADG	Time	ADG x Time
pH	0.6	6.28[a]	5.97[a]	5.85 [a]	0.067	0.124	***	*
	1.0	6.51[b]	6.18[b]	5.90 [a]				
	1.5	6.42[a,b]	6.17[b]	5.99 [a]				
Temperature	0.6	32.39	27.30	21.86	0.658	0.09	***	0.46
	1.0	30.33	25.81	20.96				
	1.5	30.73	25.14	20.86				

Table 3. Ultimate pH, colour, proximate composition and collagen content of longissimus thoracis muscle from bulls that had different winter growth rates

[A] Muscle colour coordinates: L = lightness, a = redness, b = yellowness, C = chroma and H = hue angle. $^{a, b, c}$ *means within a row assigned different superscripts differ significantly; *** P < 0.001.*

	Winter growth rate (kg/day)				
	0.6	1.0	1.5	s.e.m.	Significance
Ultimate pH	5.62[a]	5.68[b]	5.75[c]	0.021	***
Muscle colour[A]					
'L'	28.25	28.61	28.84	0.565	0.76
'a'	21.15	21.79	21.76	0.318	0.29
'b'	12.91	13.12	13.18	0.207	0.63
'C'	24.78	25.44	25.45	0.347	0.31
'H'	31.40	31.08	31.20	0.345	0.81
Proximate composition (g/kg)					
Fat	10.2	14.7	12.7	1.88	0.25
Moisture	748.7	748.7	753.8	3.04	0.40
Protein	230.7	227.7	232.6	2.11	0.28
Ash	11.3	11.1	11.0	0.32	0.73
Collagen content					
Total collagen (mg/g)	3.86	3.76	3.87	0.155	0.86
Soluble collagen (%)	9.50	11.28	11.34	0.718	0.13

Table 4. Metabolic enzyme activity and fibre characteristics of longissimus thoracis muscle from bulls that had different winter growth rates

[A] LDH: lactate dehydrogenase; PFK: phosphofructokinase; ICDH: isocitrate dehydrogenase; COX: cytochrome c oxidase; CS: citrate synthase; [B] Muscle fibre types: I = oxidative, IIA = oxido-glycolytic and IIX = glycolytic.

	Winter growth rate (kg/day)				
	0.6	1.0	1.5	s.e.m.	Significance
Metabolic enzyme activity[A]					
(µmol/min.g of tissue)					
LDH	968.7	936.3	930.2	21.11	0.39
PFK	112.4	109.8	98.9	6.26	0.28
ICDH	1.02	1.09	1.03	0.055	0.61
COX	15.21	16.85	13.58	1.338	0.24
CS	4.58	4.36	5.32	0.482	0.35
(µmol/min.g of protein)					
LDH	5478	5118	4917	314	0.45
PFK	636.2	596.5	521.0	45.98	0.21
ICDH	5.68	5.95	5.41	0.350	0.55
COX	87.06	91.50	72.05	8.953	0.29
CS	26.23	24.10	28.75	3.339	0.62
Muscle fibre types[B] (%)					
I	17.08	14.89	16.73	1.169	0.37
IIA	46.77	47.03	41.05	2.863	0.26
IIX	29.83	38.08	40.17	3.518	0.10

Table 5. Sensory characteristics of longissimus thoracis muscle from bulls that had different winter growth rates

*[A]scale 1-8 where 8 = extremely tender, juicy, strong beefy flavour, strong abnormal flavour, liked. [B]scale 0-100 where 100 - extremely easy to cut ,clean cut, tough, crispy, juicy, spongy, moisture, chewy, greasy, fibrous, gristly, pulpy, dissoluble, easy, lots of fine particles, wet .[a, b] means within rows, assigned different superscripts differ significantly; * P < 0.05.*

Sensory characteristics	Winter growth rate (kg/day)			s.e.m	Significance
	0.6	1.0	1.5		
Basic tastes[A]					
Tenderness	4.31[b]	4.01[a]	4.08[ab]	0.085	*
Juiciness	4.72	4.76	4.78	0.064	0.80
Beefy flavour	4.38	4.42	4.38	0.056	0.86
Abnormal flavour	2.41	2.51	2.51	0.071	0.47
Flavour liking	5.06	4.95	4.97	0.071	0.49
Overall liking	4.55	4.44	4.50	0.069	0.55
On-cut[B]					
Ease of Cut	50.24[b]	44.54[a]	46.35[ab]	1.295	*
Clean Cut	56.58	53.61	55.74	1.158	0.17
In-bite[B]					
Toughness	53.41	55.98	56.52	1.186	0.14
Crispness	23.53	24.64	24.93	0.998	0.58
Juiciness	42.84	44.94	45.47	0.981	0.14
Sponginess	26.28	25.71	26.37	0.848	0.84

Eating[B]					
Toughness	50.69[a]	53.98[ab]	54.95[b]	1.264	*
Moisture	43.95	44.53	46.22	1.035	0.28
Chewiness	47.63	51.04	50.98	1.364	0.13
Greasiness	16.05	15.7	15.58	0.725	0.89
Fibres	45.91	46.7	45.75	0.846	0.70
Gristle	7.27	8.09	7.15	0.721	0.60
Pulpy	49.11	50.43	50.87	1.011	0.44
Dissolubility	43.62	41.08	40.25	1.117	0.09
Residual[B]					
Greasiness	16.51	15.24	15.32	0.633	0.28
Ease of swallow	52.29	50.73	52.23	1.192	0.58
Pulpy	48.78	47.9	49.34	1.119	0.66
Particles	51.84	52.35	52.2	0.926	0.93
Mouthfeel	48.66	50.45	49.35	1.003	0.52

Figure captions

Fig. 1. Growth pattern (average live weight by month) of bulls that had different winter growth rates (average daily live weight gain (ADG) of 0.6, 1.0 and 1.5 kg/day), n = 14 per treatment.

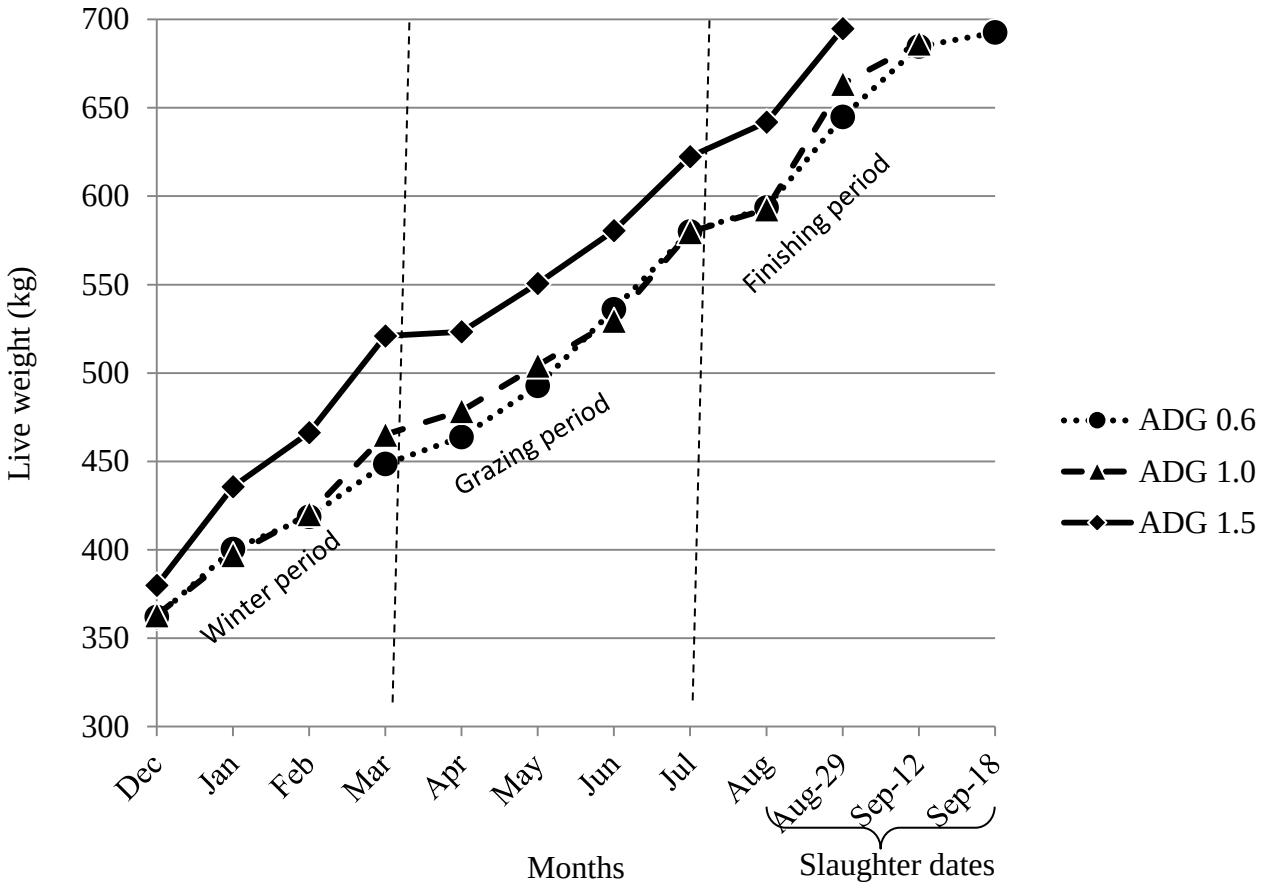

Fig. 2. Post-mortem muscle pH relative to muscle temperature for bulls that had different winter growth rates (average daily live weight gain (ADG) of 0.6, 1.0 and 1.5 kg/day). Measurements were made at 2, 3.5, 5 and 48 h post-mortem (PM).

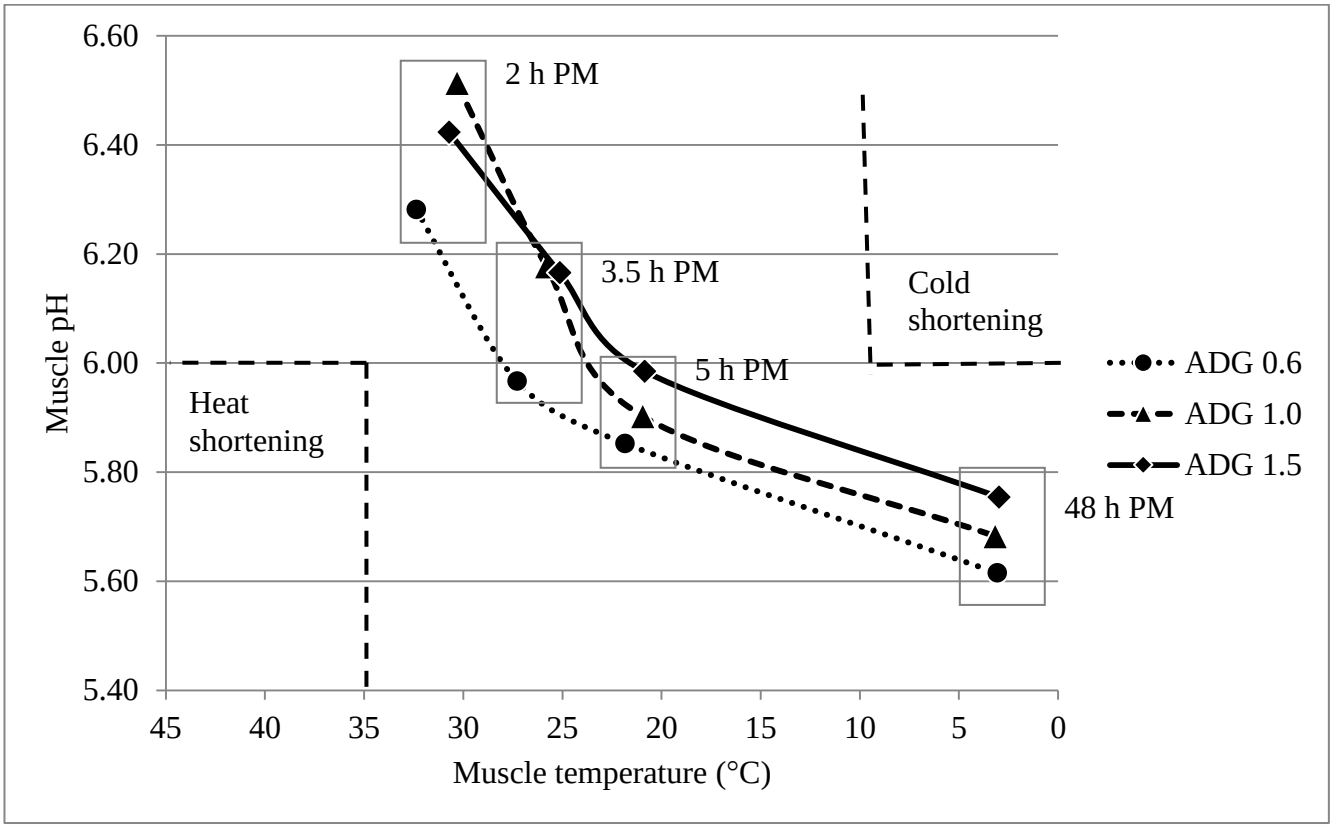

Fig. 2.