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Consumption of high energy maize diets is associated with increased soluble collagen in muscle of Holstein bulls

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ABSTRACT

The aim was to investigate whether energy supplementation of gazing bulls can influence collagen concentration and solubility in three different muscles: *longissimus dorsi* (LD), *semitendinosus* (ST) and *supraspinatus* (SS). Thirty three Holstein bulls aged 15 months and reared on grass, were randomly assigned to three treatment groups for 85 days: fed grass *ad libitum* (G1-control); fed grass *ad libitum* and supplemented with 4 kg day^{-1} of ground maize (G2); fed grass *ad libitum* and supplemented with 8 kg day^{-1} of ground maize (G3). Total collagen content did not differ ($P>0.05$) among treatments, however, soluble (heat-labile) collagen was significantly ($P<0.001$) higher in meat from G3 (34%, 23% and 25% for LD, ST and SS, respectively), compared with G1-control (24%, 18% and 17% in LD, ST and SS, respectively). Overall, these results suggest that meat tenderness of grazing bulls may be improved by supplementing with ground maize (8 kg day^{-1}).

1. Introduction

Tenderness is one of the most appreciated characteristics of meat quality. Meat tenderness depends on various factors such as breed, gender, age and slaughter and maturation conditions. However, the recent progress and standardization of slaughter and storage procedures made by the meat industry implies that the feeding management of animals will become one of the major causes of inconsistency in meat quality.

Results of several studies indicate that tenderness and other sensory characteristics of meat are influenced by collagen content and solubility (Renand, Picard, Touraille, Berge, & Lepetit, 2001; Purslow, 2005; Lepetit, 2007). Other authors (Dransfield et al., 2003; Torrescano, Sanchez-Escalante, Gimenez, Roncales, & Beltran, 2003; Riley et al., 2005; Stolowski et al., 2006) found strong correlations between insoluble collagen content of beef muscles and raw Warner–Bratzler peak shear force values. Concerning cooked meat, results are less consistent with reports going from strong to, more usually low correlations between either muscle collagen amount or solubility and meat toughness, which may be dependent on interactions with muscle fibres, as suggested by Lepetit (2007, 2008).

Nutritional status prior to slaughter may influence fibre type and collagen content and solubility which determine meat tenderness (Sami, Augustini, & Schwarz, 2004; Thénard et al., 2006). However, the effect of diet on meat tenderness has yielded conflicting results. Some studies reported a reduction of total collagen and/or increase of collagen solubility of animals fed high energy diets (Miller, Cross, Crouse, & Jenkins, 1987; Cranwell, Unruh, Brethour, & Simms, 1996; Schnell, Belk, Tatum, Miller, & Smith, 1997) while others failed to demonstrate such effects. (Dikeman et al., 1986; Sami et al., 2004; Thénard et al., 2006; Serrano et al., 2007).

In the Azores, beef is mainly produced from Holstein bulls which are reared almost exclusively on grass, grazing all year round. Grazing cattle are known to produce meat with higher dietetic/nutritional quality than concentrate fed cattle (French et al., 2000; Padre et al., 2006). However, high levels of grass intake with consequent lower daily weight gain and protein turnover associated with the constant movement of these animals, are thought to increase collagen content and decrease collagen solubility, resulting in beef with lower commercial value due to its toughness (Jurie, Picard, & Geay, 1998). The aim of the current study was, therefore, to evaluate the effect of energy supplementation of grazing bulls on collagen content and solubility in three different carcass muscles: *longissimus dorsi* (LD), *semitendinosus* (ST) and *supraspinatus* (SS).

2. Materials and methods

2.1. Animals and treatments

Animal handling followed the EU directive 86/609/EEC concerning animal care. Thirty three Holstein bulls aged 15.4 ± 1.9 (SD) months and weighing 388 ± 51.5 (SD) kg which had been reared exclusively on grass pasture were blocked according to live weight and age and assigned at random to one of the following three dietary

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treatments corresponding to a finishing period of 85 days: finished exclusively on grass pasture (control); finished on grass pasture supplemented with 4 kg ground maize/head/day (+4 kg day^{-1} maize); and finished on grass pasture supplemented with 8 kg ground maize/head day^{-1} (+8 kg day^{-1} maize). Animals were gradually introduced to the total amount of maize during an adaptation period of 21 days. Ground maize was offered individually once daily at 07:30 using 2 specially built feeding trailers. The cereal was generally consumed within 15–30 min without refusals. Groups of bulls grazed rotationally in paddocks of approximately 2 ha at a stocking rate of 1.8 head per ha. Pasture was composed of a mixture of *Poa pratensis*, *Lolium perenne* and *Trifolium repens*. Table 1 shows the chemical composition of the maize and grass. Bulls were allowed free access to water and trace mineralized salt blocks throughout the experiment. The animals were weighed in the morning, always before receiving the cereal, at the start of the trial and at 21-day intervals until slaughter.

The experiment was carried out at the Azorean archipelago during spring (maximum vegetative growth) starting on 27^{th} March and ending on 20^{th} June.

2.2. Slaughter and carcass evaluation

On the day before slaughter, bulls were weighed on the farm before transportation (10 km) to the official abattoir of "Terceira island" where humane slaughter, according to standard procedures, occurred following 24 h of fasting with water *ad libitum*. One hour post-mortem, carcasses were graded visually for fatness by a supervisory grading officer of the Ministry of Agriculture, according to the SEUROP beef carcass grading system, using a scale ranging from 1 (very low fat cover) to 5 (very high fat cover). Twenty four hours post-mortem, measurements of the subcutaneous fat depth were made with callipers on the left side of carcasses (measured at the interface between 10^{th} and 11^{th} ribs at ¼, ½ and ¾ positions over the LD across the maximum cross-sectional width of the muscle). Samples (2×50 g) from (LD) – taken at the 10^{th} rib – ST and SS were also taken from the left side of carcasses. After being trimmed of all visible subcutaneous fat and intermuscular connective tissue, all meat cuts were vacuum-packed in oxygen impermeable bags, frozen and stored at −18 °C prior to analyses. ST and SS muscles were only used for collagen analysis whereas LD muscles were used for both collagen analysis and measurements of intramuscular fat and protein.

2.3. Analytical procedures

Plucked samples of pastures were randomly taken fortnightly over the duration of the experiment. The samples were stored at −18 °C and finally mixed to obtain a composite sample. Maize meal was also sampled fortnightly following the same procedure as for the pasture samples. The samples of feed were analysed for DM, crude protein (CP), crude fat (CF) and ash according to AOAC (1990) and neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) according to Robertson and Van Soest (1981).

Table 1
The chemical composition of the grass and maize.

Composition	Grass	Maize
Dry matter (%)	15.9	85.5
Crude protein	23.0	9.8
Crude fat	1.5	2.8
Ash	8.8	2.9
ADF	31.3	2.5
ADL	3.0	–
NDF	64.8	–

Values other than dry matter are expressed as percentage of dry matter.
ADF: acid detergent fibre; ADL: acid detergent lignin; NDF: neutral detergent fibre.

Ash and dry matter (DM) were determined in all muscles according to AOAC procedures (AOAC, 1990). Total protein of the LD was determined by the Kjeldahl method according to AOAC (AOAC, 1990). The percentage of intramuscular fat (IMF) of the LD was determined by cold extraction with a mixture of chloroform–methanol 2:1 by volume according to a method adapted from Folch, Lees, and Stanley (1957). Extracts were dried and recorded weights were used to determine percentages.

Total collagen was estimated from the hydroxyproline (OH-Prol) content of 4 g of minced muscle as described by the Portuguese Norm (Norma Portuguesa, 1987). Briefly, individual samples were hydrolyzed in 3 M H_2SO_4, for 16 h at 105 °C, followed by colorimetric assay of OH-Prol in the hydrolysate. Collagen concentration was determined by the OH-Prol content, using the conversion factor 7.25, and expressed as mg of collagen per g of wet muscle (Cross, Carpentar, & Smith, 1973).

Collagen solubility was determined according to a procedure adapted from Hill (1966). Soluble (heat-labile) collagen was extracted from duplicate 5 g samples by heating in a water bath for 70 min at 77 °C in 0.25 strength Ringer's solution (Hill, 1966). Samples then were centrifuged twice at 3000 *g* for 30 min to separate supernatant from residue. The residue was dried at 105 °C and used to determine the OH-Prol content of heat-insoluble collagen as described for total collagen. Soluble collagen was determined by the difference between total collagen and heat-insoluble collagen. An average of two duplicates was used for each collagen fraction. Percentage of soluble collagen was calculated by dividing soluble collagen by total collagen.

2.4. Statistical analysis

Data for bull age, live weight, growth rate, muscle DM, ash, IMF and protein content were analysed by analysis of variance (ANOVA). Differences concerning fatness scores were tested by χ^2 analysis. Data of collagen content were subjected to factorial ANOVA including treatments and muscle as factors. When ANOVA detected significant differences within treatments or muscles, multiple comparisons were performed using Fisher's PLSD (post-hoc protected least-squares difference) test. Data expressed as percentage (collagen solubility) were previously transformed using the formula $P' = \arcsin \sqrt{P}$, P being the original value (Zar, 1996) but the results are presented in the original form for clarity.

3. Results and discussion

The chemical composition of the dietary components offered to the bulls is shown in Table 1 and the animal-related data from the experiment are summarised in Table 2. Animal age and body weight were not significantly different at the beginning of the finishing period. Bulls supplemented with 4 kg maize/day or 8 kg maize/day grew, respectively, 17 and 36% faster than bulls fed exclusively pasture, ($P<0.0001$). Growth performance of bulls was positively affected by the high dietary energy intake provided by maize supplementations at rates of 0.81 (4 kg maize/day) and 1.61 (8 kg maize/day) kg dry matter (DM)/100 kg initial live weight.

Ash and moisture contents of the muscles (LD, ST and SS) were not significantly different between treatments (data not shown for ST and SS muscles).

Intramuscular protein and fat contents determined only in the LD muscles are shown in Table 2. Energy supplementation of grazing animals did not influence total protein content, but increased significantly ($P<0.05$) intramuscular fat in the LD. The incorporation of maize in the diet also increased the fat cover and the fat content of the carcasses (as assessed by SEUROP fatness score). Intramuscular fat of *Longissimus dorsi* muscle increased 61% with 4 kg maize/head/day and 76% with 8 kg maize/head/day ($P<0.05$). However, there were no significant differences in intramuscular fat between animals

Table 2
Effect of finishing strategy on live weight gain, carcass fatness and chemical composition of *longissimus dorsi* (LD) muscle from Holstein bulls. Bulls were finished exclusively on pasture (control) or supplemented with two levels of ground maize (4 kg/head/day, 8 kg/head/day) for 85 days.

	Finishing strategy				
	Control	+4 kg day^{-1} maize	+8 kg day^{-1} maze	SEM	*P* value
No	11	11	11	–	–
Initial age (day)	478	493	487	18.3	NS
Age at slaughter (day)	563	578	572	18.3	NS
Initial live weight (kg)	422.8	421.5	424.6	15.89	NS
Final live weight (kg)	527.2	544.0	566.4	17.03	NS
Average daily gain (kg day^{-1})	1.228^{a}	1.441^{b}	1.667^{c}	0.0556	<0.0001
Fatness score[1]	1.38^{a}	1.90^{b}	2.00^{b}	–	0.0024
Subcutaneous fat depth (mm)[2]	0.75^{a}	2.89^{b}	2.66^{b}	0.402	0.0012
Longissimus dorsi (LD)					
DM (g/100 g)	24.66	25.26	25.21	1.377	NS
Ash (g/100 g)	1.067	1.00	1.00	0.016	NS
Total protein (g/100 g)	19,13	19.21	19.15	0.277	NS
Intramuscular fat (g/100 g)	1.84^{a}	2.96^{b}	3.24^{b}	0.332	0.014

a,b,c On each row, the values with different superscripts are significantly different ($P<0.05$).
[1]The fatness score was evaluated using a scale ranging from 1 (very low fat cover) to 5 (very high fat cover).
[2]Values refer to averaged original measurements taken at ½, ¼ and ¾ positions over the *longissimus* muscle.

supplemented with the two levels of energy supplementation (4 and 8 kg/head/day of maize). As expected, carcasses and muscle (LD)from grass-finished bulls were characterized by their low fatness, (score 1.38), subcutaneous fat depth (0.75 mm) and intramuscular fat content (1.84%) less than reported in the literature (Cerdeño, Vieira, Serrano, & Mantecon, 2006; Padre et al., 2006; Thénard et al., 2006; Noci, French, Monahan, & Moloney, 2007) and certainly not well accepted by consumers as the lack of marbling is associated with a decrease in beef tenderness, juiciness and flavour (Moloney, Mooney, Kerry, & Troy, 2001; Scollan et al., 2006).

Total collagen content in each muscle (LD, ST and SS) was not affected by the increase in energy intake, as no differences ($P>0.05$) were found among treatments (Fig. 1). These results agree with Vestergaard et al. (2000), who showed that meat from Friesian bulls raised on pasture had markedly reduced intramuscular fat contents but unchanged collagen contents. Overall, total collagen content in the muscles did not differ from other studies on the same muscles in cattle of different breeds, ages and feeding management (Cranwell et al., 1996; Schnell et al., 1997; Renand et al., 2001; Monsón, Sañudo, & Sierra, 2004; Thénard et al., 2006). Whatever the diet, i.e. grass alone or supplemented with maize, LD had significantly lower ($P<0.001$) total collagen contents (6.24 mg/g) compared with ST (11.4 mg/g) and SS (14.8 mg/g), which is in accordance with earlier reports (Boccard et al., 1979; Shackelford et al., 1992; Listrat et al., 1999). Also, in animals fed exclusively on grass (control), collagen solubility was significantly ($P<0.001$) higher in the LD (24%) compared with the other muscles (18% and 16% in ST and SS, respectively). Similar trends were observed in the energy supplemented animals, which is indicative of the superior tenderness of this muscle compared with ST and SS muscles (Fig. 2). These results were not completely in accordance with others (Renand et al., 2001, Purslow, 2005) suggesting that total collagen content was related with tenderness differences among muscles, while soluble collagen was related to tenderness changes associated with advancing age. However, other reports have indicated that collagen solubility rather than the total amount of collagen was the main determinant of sensory characteristics (Cross et al., 1973; Hall & Hunt, 1982; Bailey, 1985; Crouse, Cross, & Seideman, 1985; Jeremiah, Dugan, Aalhus, & Gibson, 2003). Crouse et al. (1985) also reported that total intramuscular collagen content was not closely related to objective or subjective texture measurements, while percent soluble collagen was moderately related to sensory panel tenderness, perceived connective tissue and ease of fibre fragmentation.

In the present study, energy supplementation of grazing animals, significantly ($P<0.001$) increased the percentage of soluble (heat-labile) collagen (Fig. 2). Collagen solubility was

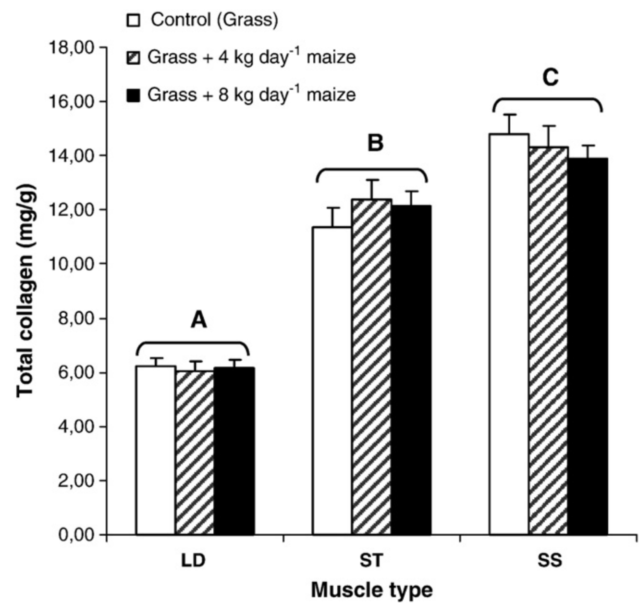

Fig. 1. Total collagen content in bovine *longissimus dorsi* (LD), *semitendinosus* (ST) and *supraspinatus* (SS) muscles of bulls fed grass only (unshaded bars) or grass supplemented with either 4 kg day^{-1} (hatched bars) or 8 kg day^{-1} (shaded bars). Values are means ± standard error of the means (11 animals per diet). Different capital letters (A,B,C) indicated significant ($P<0.001$) differences between muscle across all diets.

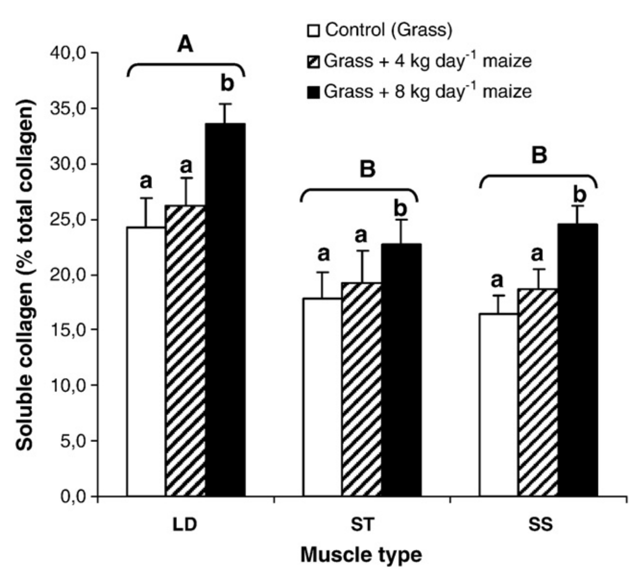

Fig. 2. Soluble collagen (% of total collagen) in bovine *longissimus dorsi* (LD), *semitendinosus* (ST) and *supraspinatus* (SS) muscles of bulls fed grass only (unshaded bars) or grass supplemented with either 4 kg day^{-1} (hatched bars) or 8 kg day^{-1} (shaded bars). Values are means ± standard error of the means (11 animals per diet). Different lower case letters (a,b) indicated significant ($P<0.005$) differences between diets within muscle types, while different capital letters (A,B) indicate significant ($P<0.001$) differences between muscles across all diets.

significantly higher in animals supplemented with 8 kg/head/day of maize (34%, 23% and 25% in LD, ST and SS, respectively), compared with animals supplemented with 4 kg/head/day of maize (26% in LD and 19% in both ST and SS muscles) and animals fed on grass exclusively (24%, 18% and 17% in LD, ST and SS, respectively). This increase in the solubility of collagen might be due to the increase in growth rate as a consequence of energy supplementation, which might have affected protein turnover allowing less collagen cross-linking and thus more tender beef (Aberle, Reeves, Judge, Hunsley, & Perry, 1981; McCormick, 1994). Miller et al. (1987) and Cranwell et al. (1996) also found that feeding mature cows a high-concentrate diet increased soluble collagen content in the *longissimus* muscle compared to that in non-concentrate fed cows. Similarly, Sami et al. (2004) comparing four diets based on different proportions of maize silage and concentrate formulated to allow different growth rates, found no differences in collagen content but higher collagen solubilities in animals with higher daily weight gains. However, other studies failed to show such effects regarding diet, growth rate and collagen content and solubility (Maltin et al., 1998; Serrano et al., 2007).

In all muscles (LD, SS and ST), independently of initial collagen content and characteristics, the increase of collagen solubility was associated with higher energy intake by the animals. Neither shear force nor sensory tests were investigated but the higher intramuscular fat contents and higher collagen solubility may be associated with increased tenderness in the muscles. In situations where toughness mainly depends on connective tissue, soluble collagen has been recognized to be an important factor with regard to meat tenderness with correlations between soluble collagen and tenderness approaching unity in the absence of other factors (Lepetit, 2007). Also, Cranwell et al. (1996) found an increase of 63% in overall tenderness associated with an increase of 22% in soluble collagen. In the present study, the experimental conditions were identical across treatments, except for the variable under study (i.e. the amount of maize/energy ingested) and the results clearly showed an effect of the level of energy supplementation on collagen solubility, an increase of approximately 40% in the group finished with 8 kg of maize per day compared to the controls suggesting that finishing the cattle with 8 kg of maize per day would result in an increase in overall tenderness of the meat.

Further research is required to obtain better knowledge of the biological mechanisms which control the *in vivo* make-up of muscle characteristics, including collagen and myofibrillar protein turnover, the ultimate objective being to control these mechanisms since they partly determine meat quality.

4. Conclusions

Energy supplementation for a short finishing period of young bulls previously raised exclusively on pasture had no effect on total muscle collagen, but produced meat with higher collagen solubility due to a higher proportion of neo-formed collagen, which is less cross-linked. Therefore, a finishing period for grazing bulls with 8 kg/head/day of ground maize could enhance meat tenderness by increasing intramuscular fat and soluble collagen. Further research is needed to evaluate the effect of replacement of grass with maize on the dietetic/nutritional quality of the meat.

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