

Effect of the winter diet on meat quality traits of steers finished on mountain pasture with a barley supplement

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Abstract

The aim of this study was to evaluate the effect of two winter diets (WD) (100 F, *i.e.* 100% forage, 8.3 kg DM of lucerne hay + 0.3 kg DM of straw; and 65F:35C, *i.e.* 65% forage:35% concentrate, 5.4 kg DM lucerne hay + 0.3 kg DM straw + 3.0 kg DM barley), offered during 118 days on meat quality traits of 20-month old steers finished on mountain pasture supplemented with 4.1 kg DM barley d⁻¹. *Longissimus thoracis* intramuscular fat content and its fatty acid profile were determined (*in vivo*) after one month on pasture. The intramuscular fat content, fatty acid profile, texture (1, 8 and 15 days of ageing), colour (1, 2, and 8 days of oxygen exposure) and sensorial quality (8 and 15 days ageing) were evaluated *post-mortem* after 163 days on the finishing diet. Intramuscular fat content and fatty acid profile were affected by the WD *in vivo* ($p < 0.05$) but not *post-mortem*. Meat pH was not affected by the WD but the texture was affected by the interaction between the WD and the ageing time ($p < 0.001$), maximum stress decreased more rapidly in the 100F diet in the first 8 days of ageing. Meat colour was only affected by the oxygen-exposure time ($p < 0.001$). Panel test variables were not affected by the WD, but ageing time affected beef flavour intensity ($p < 0.05$). In conclusion, the winter diet affected intramuscular fat content after one month of grazing but had no major effects on *post-mortem* meat quality of pasture-finished steers.

Additional key words: ageing; beef; colour; fatty acids; management; sensory analysis; texture.

Resumen

Efecto de la dieta invernal sobre la calidad de la carne de cebones finalizados en pastos de montaña suplementados con cebada

El objetivo del estudio fue evaluar el efecto de dos dietas invernales (WD) (100F, es decir, 100% forraje, 8,3 kg MS heno de alfalfa + 0,3 kg MS paja; y 65F:35C, es decir, 65% forraje:35% concentrado, 5,4 kg MS heno de alfalfa + 0,3 kg MS paja + 3,0 kg MS cebada), ofrecidos durante 118 días sobre algunas variables de calidad de la carne de cebones sacrificados a los 20 meses de edad, finalizados en pastos de montaña y suplementados con cebada (4,1 kg MS d⁻¹). Se determinó el contenido en grasa intramuscular y el perfil de ácidos grasos del músculo *Longissimus dorsi* al mes de iniciarse el pastoreo (*in vivo*). Tras el sacrificio, se determinó el contenido en grasa intramuscular, el perfil de ácidos grasos, el pH, la textura instrumental (1, 8 y 15 días de maduración), el color (1, 2 y 8 días de exposición al oxígeno) y la calidad sensorial (8 y 15 días de maduración). La dieta invernal afectó al contenido en grasa intramuscular y a su composición después de un mes de pastoreo ($p < 0,05$) pero no al sacrificio. La dieta invernal no afectó al pH pero la textura se vio afectada por la interacción entre la dieta invernal y tiempo de maduración ($p < 0,001$). El color se vio afectado sólo por el tiempo de exposición al oxígeno ($p < 0,001$). La dieta invernal no afectó a las variables sensoriales ($p > 0,05$), pero la maduración afectó a la intensidad de flavor a vacuno ($p < 0,05$). En conclusión, la dieta invernal afectó a la cantidad de grasa intramuscular tras un mes de pastoreo pero no tuvo efectos importantes sobre la calidad *post-mortem* de la carne de los animales acabados en pastoreo.

Palabras clave adicionales: ácidos grasos; análisis sensorial; color; maduración; manejo; textura; vacuno.

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Abbreviations used: DM (dry matter); EU (Europe); GPA (Generalized Procrustes Analysis); MUFA (monounsaturated fatty acids); PUFA (polyunsaturated fatty acids); SFA (saturated fatty acids); WD (winter diet).

Introduction

In recent years, beef production and marketing have changed due to new consumer demands and European Policies. The consumer's image of meat quality does not consist only of sensory attributes or healthy aspects (Bello & Calvo, 2000; Dransfield *et al.*, 2005) but also depends on extrinsic cues (Bernués *et al.*, 2003). Accordingly, consumers have become increasingly interested in the origin of food, animal welfare and environmental protection (Grunert *et al.*, 2004). All these new consumer concerns have increased the demand for meat from animals reared under traditional grazing systems (Gil *et al.*, 2000). To meet these new consumer requirements, systems based on forage fed indoors or grazing can be an alternative to conventional beef production based on concentrate fed indoors. Furthermore, extensive systems satisfy EU policy requirements regarding the use of sustainable production methods, which bind livestock, population and environment (Bernués *et al.*, 2011). Therefore, extensive beef production could be an interesting system to produce high-quality red meat, using marginal mountain areas, contributing to the environmental conservation and fixing the population in rural areas. Nevertheless, beef production has to adapt to forage availability and, in dry mountain areas such as the Pyrenees, cattle have to be housed due to harsh climatic conditions (Casasús *et al.*, 2002). During this period different feeding strategies can be used bearing in mind that beef production systems need to be sustainable in economic terms. In order to achieve improved economic sustainability, the energy level of the diet during the winter housing period can be lowered to reduce feed costs (Blanco *et al.*, 2011) and take advantage of the forage resources available during the grazing period. However, to date, few studies have been carried out on the effect of winter diets on steers in Mediterranean dry mountain areas. It is feasible, from a technical point of view, to finish steers on pasture after receiving different diets during the winter period (Blanco *et al.*, 2012). Winter diet affects the growth paths, which, in turn can affect meat quality (Purchas *et al.*, 2002; Cassar-Malek *et al.*, 2004).

The aim of this study was to evaluate the effect of two different winter feeding strategies (winter diets), with different forage and barley contents followed by a grazing period, on the meat quality of steers. Their effect on animal performance and carcass quality has been presented elsewhere (Blanco *et al.*, 2012).

Material and methods

Animals and diets

Ten-month old Parda de Montaña steers ($n = 18$) were housed in a feedlot facility of CITA Research Station in Zaragoza ($41^{\circ}43'N$, $0^{\circ}48'W$, 225 m above sea level) and received lucerne (*Medicago sativa*) hay and barley (*Hordeum vulgare*) straw on an *ad libitum* basis and 0.5 kg DM barley for a 1-month transition period. Seven days after arrival, they were castrated by surgical removal of the testes using local anaesthesia and analgesia and local antibiotics and post-procedure analgesia. The recovery period lasted 20 days. Thereafter, steers were blocked by live weight and weight gain during lactation and randomly distributed into two treatment groups with different winter diet (WD). Animals were fed *ad libitum* two diets during the winter housing period (December to April, 118 d). The 100% forage-fed (100F) group received a diet of 97% lucerne hay and 3% straw, and had a daily intake of 8.3 kg DM of lucerne hay + 0.3 DM kg of straw. The 65% forage:35% concentrate (65F:35C) group was fed a diet of 62% lucerne hay, 3% straw and 35% barley, and a daily intake of 5.4 kg DM lucerne hay + 0.3 kg DM straw + 3.0 kg DM barley. In mid-April, the steers were transported to La Garcipollera Research Station in the Pyrenees ($42^{\circ}37'N$, $0^{\circ}30'W$, 945 m above sea level, 10-year average temperature and rainfall $10.9^{\circ}C$ and 999 mm, respectively), where they were finished on pastures until slaughter at the end of the grazing season (163 days), when they were 20 months old (live weight: 539 kg and 560 kg for 100F and 65F:35C groups, respectively). The steers were set-stocked on natural Pyrenean meadows, at a stocking rate of 6 steers ha^{-1} . The meadows were irrigated fortnightly during the summer. The pasture height was kept above 11 cm during the grazing period. The steers were supplemented daily on pasture with 4.1 kg DM barley from mid-June to slaughter (103 and 110 days for FC and F steers, respectively).

In vivo sampling: *Longissimus thoracis* biopsy

A biopsy sample of the *Longissimus thoracis* was obtained from each steer after 1 month of grazing (biopsy). This sampling schedule was applied in order to reduce the cumulative stress of transport, turnout to pasture and extra handling for surgical procedures. The

area around the 10th rib was shaved and disinfected (using 5% chlorhexidine gluconate solution). Biopsies were taken using an 8-mm cannula inserted into the spring-loaded biopsy device (PPB-U Biotech, Nitra, Slovakia). Afterwards, the area was treated with chlortetracycline (Aureomycin[®], Fort Dodge) and a protective healing spray (Aluspray[®], Neogen Corporation). Muscle samples were trimmed of fat and skin, snap-frozen in liquid nitrogen, and stored in tubes at -80°C until analysis. Fat content was quantified using the Ankom Procedure (AOCS Am 5-04) with an Ankom extractor (Model XT10, Ankom Technology, Madrid, Spain). Analyses of fatty acid methyl esters were performed by gas chromatography with a 30-metre capillary column SP2330 (Supelco, Tres Cantos, Madrid, Spain) and a flame ionisation detector with helium as the carrier gas at 1 mL min⁻¹. The oven temperature program increased from 150°C to 225°C at 7°C min⁻¹. Injector and detector temperatures were both 250°C (Tor *et al.*, 2005). Fatty acids were quantified by incorporating an internal standard, 1,2,3-tritridecanoylglycerol (tritridecanoin C13:0), in each sample (Rule, 1997). Proportions of polyunsaturated (PUFA), monounsaturated (MUFA), saturated (SFA) fatty acids and the PUFA:SFA ratio were obtained from individual fatty acid percentages.

Slaughter and meat sampling

Steers were slaughtered when they were 20 months old, in an EU-licensed abattoir according to commercial practice. Carcasses were kept at 4°C for 24 hours and the portion of loin between the 10th thoracic and 3rd lumbar vertebrae was excised and transported to the laboratory. Ultimate pH was measured at the 10th thoracic vertebra level with pH-meter equipped with a Crison 507 penetrating electrode (Crison Instruments S.A., Barcelona, Spain). Thereafter, the 10th thoracic vertebra was separated and the *Longissimus thoracis* muscle was excised and cut into two halves. One half was assigned to determine intramuscular fat content and the other half was minced and analyzed for intramuscular fatty acid. Both fat and fatty acid contents were quantified as previously described.

The *Longissimus thoracis* muscle from the 11th thoracic vertebra (3.5-cm thickness) was cut into three parts to measure colour parameters. The *Longissimus thoracis* muscle from the 12th and 13th and thoracic vertebrae and the *Longissimus lumborum* muscle from

the 1st lumbar vertebra (3.5-cm thickness) were used to determine texture. Finally, the *Longissimus lumborum* muscle from 2nd and 3rd lumbar vertebrae were sliced into 2-cm thick steaks and used in sensorial analysis.

Texture measurements

Samples for texture analysis were vacuum-packed and aged at 4°C for 1, 8 or 15 days for texture measurements. After ageing, samples were heated in a 75°C water-bath to an internal temperature of 70°C, monitored with a Jenway thermocouple provided with a probe. A minimum of 10 sub-samples of 10 × 10 mm² cross-section were obtained following longitudinal configuration (Lepetit & Culioli, 1994). Samples were sheared using an Instron 5543 fitted with a Warner-Bratzler device built-in on an Instron Universal testing machine (Model 5543). Maximum stress (maximum load per unit of cross-section, in N cm⁻²) was registered.

Muscle instrumental colour

Meat colour samples were placed in a polystyrene tray, wrapped with an oxygen permeable film without contact with the meat surface and kept in the dark at 4°C. Colour values were recorded on two locations randomly selected from the cranial surface of each piece to obtain a representative mean value, and colour was measured with a white surface below the samples. Muscle colour was measured after 1, 2 and 8 days of oxygen-exposure with a Minolta CM-2006d spectrophotometer (Konica Minolta Holdings, Inc, Osaka, Japan) in the CIELAB space (CIE, 1986) with a measured area diameter 8 mm, specular component included and 0% UV, standard illuminant D65 which simulates daylight (colour temperature 6,504 K), observer angle 10° and zero and white calibration. The lightness (L*), redness (a*) and yellowness (b*) were recorded, and hue angle (H°) and chroma (C*) indexes were calculated as $H^{\circ} = \tan^{-1}(b^*/a^*) \times 57.29$ expressed in degrees, and $C^* = (a^{*2} + b^{*2})^{0.5}$. Chroma is related to the quantity of pigments and high values represent a more vivid colour and denote lack of greyness (Miltenburg *et al.*, 1992). Hue is the attribute of a colour perception denoted by blue, green, yellow, red, purple, etc. (Wyszecki & Styles, 1982), and it is related to the state of pigments (Renerre, 1982).

Sensorial analysis

The sensorial analysis was carried out by a trained panel test. Samples were vacuum-packed and aged at 4°C for 8 or 15 days. Steaks were then frozen and stored at -18°C until analysis. On the day of evaluation, the vacuum-packed meat was thawed by immersing in tap water for 4 h until it reached an internal temperature of 17-19°C. It was then cooked wrapped in aluminium foil on a pre-heated double hot-plate grill at 200°C until an internal temperature of 70°C was reached. The meat was then cut into small portions, wrapped in coded aluminium foil and stored warm (60°C) until tasted. Samples were served to a trained (ISO-8586-1) seven-member panel (with panel members in individual booths) under red lighting to mask any differences in meat colour. A 2 × 2 factorial complete balanced design was used to evaluate the effect of the winter diet and ageing time. Panellists were asked to evaluate the following attributes on a 10-point scale: beef odour intensity, tenderness, juiciness, beef flavour intensity, liver flavour intensity and abnormal flavour intensity, with 1 as the lowest and 10 as the highest possible score for each attribute.

Statistical analysis

A statistical analysis was performed by SAS v.9.1. (SAS Inst. Inc., Cary, NC, USA). Before further analyses the normality of the residues of all the variables was tested with the Shapiro-Wilk test. The residues of meat

traits were normally distributed. Intramuscular fat content, instrumental texture and meat colour were analyzed using mixed models based on Kenward-Roger's adjusted degrees of freedom solution for repeated measurements including the winter diet, sampling date/time of oxygen exposure/ageing and its interaction as fixed effects, and animal as the random effect. The variance-covariance structure was chosen according to the Akaike Information criteria and the Schwarz Bayesian criteria. The panel test variables were analysed using mixed models with the winter diet, ageing time and their interaction as fixed effects and the panel member nested within the session and the animal as random effects. For all the abovementioned variables, least square means (LS Means) were estimated and differences were tested using a t-test. For all tests, the level of significance was set at 0.05. A Generalized Procrustes Analysis (GPA), which uses translation, rotation and isotropic scaling to minimize differences among panelists (Gower, 1975; Carlucci *et al.*, 1998) was performed using the statistics program XLStat 2006. Results are presented graphically in the form of a biplot that includes attributes and treatment.

Results

Intramuscular fat content and composition

Figure 1 shows the effect of the winter diet after one month of grazing and at slaughter on the intramuscular

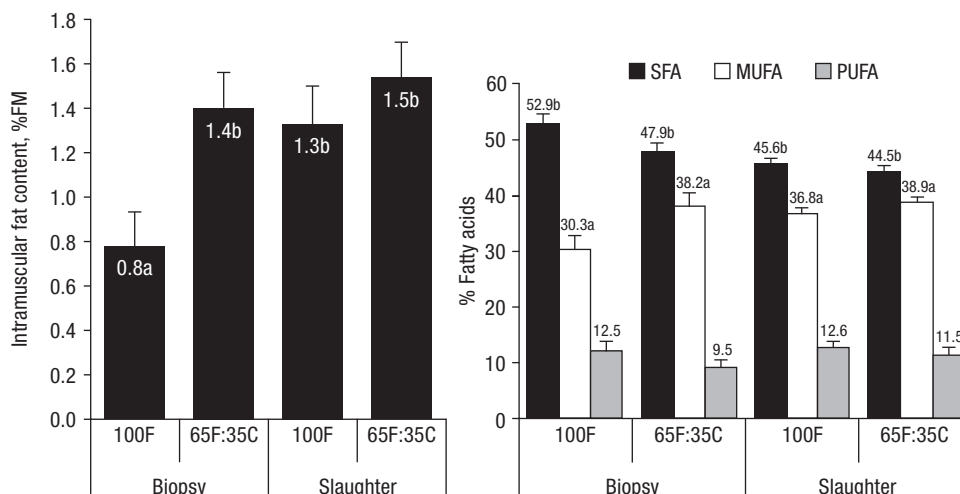


Figure 1. Effect of sampling time and winter diet (WD, 100F: 97% lucerne hay + 3% straw; 65F:35C: 62% lucerne hay + 3% straw + 35% barley) on intramuscular fat content expressed in % over fresh matter (FM) and on saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) content of the intramuscular fat of pasture-finished steers. a, b: within a parameter, means with different letters differ at $p < 0.05$.

fat content and the proportions of SFA, MUFA and PUFA. The winter diet affected intramuscular fat content after one month of grazing (biopsy), with steers fed the 65F:35C diet having greater intramuscular fat content than the steers fed 100F ($p < 0.05$), but it did not affect it at slaughter ($p > 0.05$). Moreover, intramuscular fat content of 100F steers increased during the grazing season ($p < 0.01$) while it did not change in 65F:35C steers ($p > 0.05$).

The winter diet affected the composition of the intramuscular fat content after one month of grazing, when 65F:35C steers had lower SFA ($p < 0.05$), higher MUFA ($p < 0.05$) and slightly lower PUFA ($p < 0.10$) than 100F steers, but not at slaughter ($p > 0.05$). From the biopsy to slaughter, SFA decreased ($p < 0.001$) and MUFA increased ($p < 0.01$) in 100F steers whereas the SFA tended to decrease ($p < 0.10$) and PUFA tended to increase ($p < 0.10$) in 65F:35C. Polyunsaturated fatty acids were affected neither by winter diet nor by the sampling point.

pH and meat texture

Data of pH and texture analysis are shown in Table 1. The pH was not affected by the winter diet ($p > 0.05$).

Winter diet did not affect the maximum stress in any time of ageing ($p > 0.05$) but as it was expected, ageing time did ($p < 0.01$). For 100F group, maximum stress was higher at 1 day of ageing and thereafter decreased, with no differences between 8 and 15 days. However, for the 65F:35C group maximum stress values decreased throughout the whole ageing period. Consequently, a significant interaction was found between winter diet and ageing time ($p < 0.001$).

Meat colour

Meat colour results are presented in Table 2. Meat colour variables were not affected by the winter diet ($p > 0.05$) but ageing time affected all the variables except L* ($p > 0.05$). Values for a*, b* and C* remained unchanged from day 1 to day 2 ($p > 0.05$) and decreased until day 8 ($p < 0.05$), whereas H⁰ values increased from day 1 to day 2 ($p < 0.05$), and decreased by day 8 ($p < 0.05$).

Panel test

Data from the panel test are shown in Table 3. The winter diet did not affect any of the studied variables. Ageing only affected beef flavour intensity, which increased as ageing time increased, whereas just a tendency was observed for tenderness ($p = 0.08$). Liver flavour intensity was affected by the interaction between the winter diet and ageing time ($p < 0.05$) for liver flavour intensity, it remained constant in 100F but it increased with ageing in 65F:35C.

Figure 2 shows the plot of the procrustes analysis. Factor 1 explained 50.42% of the variability and separated samples according to their ageing time, with a clear association between 8 days of ageing and abnormal flavour intensity. Factor 2 explained 27.31% of the variability and separated meat from 65F:35C aged during 15 days from the others. After 15 days of ageing, meat from 65F:35C steers was described as juicy, tender and with high intensity of beef flavour, whereas meat from 100F diet presented higher beef odour and higher liver flavour intensities.

Table 1. Effects of two winter diets (WD) on meat pH and effect of WD and ageing (A) time on the maximum stress (N cm⁻²) of pasture finished steers

	100F	65F:35C	SE	WD effect (p-value)
pH	5.6	5.7	0.04	0.32
Maximum stress (N cm ⁻²)				
1 day of ageing	91.3a	94.6a	0.34	0.74
8 days of ageing	63.6b	79.8b	3.45	
15 days of ageing	68.9b	58.1c	4.66	
Ageing effect (p-value)		0.001		WD*A < 0.001

¹ 100F: 97% lucerne hay + 3% straw; 65F:35C: 62% lucerne hay + 3% straw + 35% barley. a, b, c: within a winter diet, means with different letter differ at $p < 0.05$

Table 2. Effects of two winter diets (WD) and oxygen exposure time (T) on the *Longissimus dorsi* muscle colour of pasture finished steers

	100F			65F:35C			SE	<i>p</i> -value		
	1 day	2 days	8 days	1 day	2 days	8 days		WD	T	WD*T
L*	40.0	38.4	39.6	39.0	38.6	40.0	0.25	0.75	0.2	0.49
a*	14.9b	14.7b	12.3a	14.6b	14.8b	12.4a	0.19	0.91	0.001	0.88
b*	6.3b	6.9b	4.7a	6.8b	7.5b	4.4a	0.16	0.56	0.001	0.48
C*	16.2b	16.3b	13.2a	16.2b	16.6b	13.2a	0.22	0.86	0.001	0.93
H ⁰	22.8b	25.0c	20.9a	24.4b	26.7c	19.4a	0.43	0.78	0.001	0.25

100F: 97% lucerne hay + 3% straw; 65F:35C: 62% lucerne hay + 3% straw + 35% barley. a, b, c: within a winter diet, means with different letter differ at $p < 0.05$

Table 3. Effects of two winter diets (WD) and ageing time (A) on the sensory variables of meat of pasture finished steers studied by a panel test

	100F		65F:35C		SE	<i>p</i> -value		
	8 days	15 days	8 days	15 days		WD	A	WD*A
Beef odour intensity (1-10)	4.9	5.0	5.1	5.2	0.30	0.27	0.48	0.88
Tenderness (1-10)	4.3	4.9	4.4	4.8	0.41	0.96	0.08	0.73
Juiciness (1-10)	4.5	4.8	4.5	4.7	0.31	0.85	0.34	0.99
Beef flavour intensity (1-10)	5.3 a	5.7 b	5.4 a	5.8 b	0.32	0.51	0.02	0.70
Liver flavour intensity (1-10)	3.1 ab	3.2 ab	3.0 a	3.8 b	0.43	0.51	0.11	0.03
Abnormal flavours intensity (1-10)	3.7	3.4	3.4	4.5	0.66	0.53	0.46	0.20

100F: 97% lucerne hay + 3% straw; 65F:35C: 62% lucerne hay + 3% straw + 35% barley. a, b, c: within a winter diet, means with different letter differ at $p < 0.05$.

Discussion

Intramuscular fat content and composition

Cerdeño *et al.* (1999) found intramuscular fat contents of 1.0-1.7% in extensively-reared animals, which are similar to those found in the present study. Different feeding regimes cause large differences in intramuscular fat content with, in general, concentrate fed animals having greater intramuscular fat content than grass-fed cattle (Vestergaard *et al.*, 2000; French *et al.*, 2001; Sami *et al.*, 2004; Moloney *et al.*, 2011). In the current experiment, energy intake of 65C:35F steers was greater than that of 100F steers during the winter housing period which enhanced fat deposition in the former, assessed by subcutaneous fat thickness (Blanco *et al.*, 2012). Differences in intramuscular fat content found at the biopsy had disappeared after 5.5 months of grazing, which agrees with results of Blanco *et al.* (2012) in terms of subcutaneous fat thickness. This reflects a similar nutritional status achieved after a relatively long finishing period, that minimizes the

influence of the previous winter diet. Other studies indicate that the degree of fatness after a period of divergent growth rate influenced the composition of weight gains thereafter (McCurdy *et al.*, 2010). Therefore we hypothesize that due to a different degree of maturity at turnout to pasture, a different composition of gain may have occurred in the pasture finishing period, which could explain the lack of difference in intramuscular fat content at slaughter.

There is a lack of consensus in the literature regarding the effects of diet on fatty acid profile. Nuernberg *et al.* (2005) found no differences between diets in total SFA and MFA fatty acids, but an effect on total PUFA, being higher in grass-based systems than in concentrate-based systems. However, French *et al.* (2000) demonstrated that the PUFA/SFA ratio increased linearly when forage intake increased and Varela *et al.* (2004) found differences between diets (pasture vs. corn silage) on some particular fatty acids, but not on the sums of fatty acids groups. These disagreements show that type of feed influences the profile of fatty acids. Differences found in the current study in MUFA

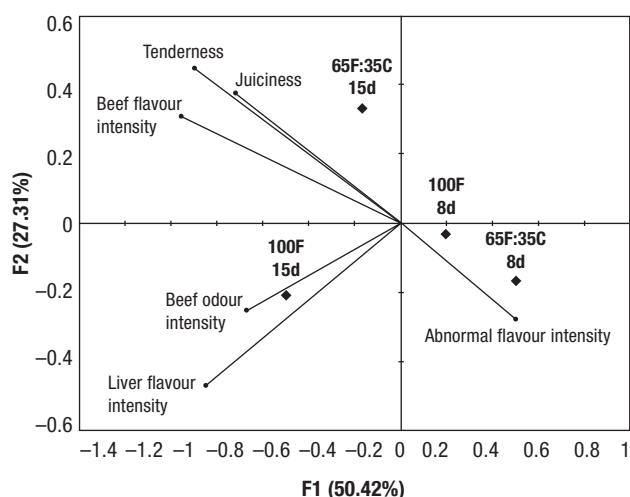


Figure 2. Procrustes analysis biplot with sensory panel traits, according to two winter diets (100F: 97% lucerne hay + 3% straw; 65F:35C: 62% lucerne hay + 3% straw + 35% barley) and ageing time of meat of pasture finished steers.

between the two groups of steers could be related to differences in barley intake level during winter period, and differences found in PUFA could be related to forage intake variations in the same period.

pH and meat texture

Our pH values were within normal ranges for beef cattle (Campo, 1999; Alberti *et al.*, 2002; Sami *et al.*, 2004). The lack of effect of the WD on pH is in agreement with most studies (Sami *et al.*, 2004; Varela *et al.*, 2004; Cerdeño *et al.*, 2006; Duckett *et al.*, 2007; Minchin *et al.*, 2010; Moloney *et al.*, 2011).

Maximum stress was not affected by the winter diet but was influenced by ageing time. Several authors have shown the absence of diet effect on texture (McCaughey & Cliplef, 1996; French *et al.*, 2000; Sami *et al.*, 2004; Cerdeño *et al.*, 2006; Duckett *et al.*, 2007; Minchin *et al.*, 2010). Alberti *et al.* (1991) reported that the influence of the diet on the meat tenderness is small if the energy and protein levels are similar. According to the total carotenoids content in serum, which are related to forage intake (Serrano *et al.*, 2006), in the current study both groups of steers had similar forage intake during the grazing period (Blanco *et al.*, 2012). As the amounts of concentrates provided on pasture were equal for both treatments, it can be assumed that energy and protein intake were similar in both groups, which would explain the lack of differences in texture.

Regarding the effect of ageing time on maximum stress, most authors have shown that ageing time has a strong effect on shear force, which decreases as ageing time increases (Campo, 1999; French *et al.*, 2000; Sinclair *et al.*, 2001; Macie, 2002). Varela *et al.* (2004) showed that diet could affect meat texture at early ageing times, but as ageing time increased, the diet effect disappeared. In the present study, shear force decreased with ageing time, but there was an interaction between winter diet and ageing, because in 65F:35C diet, the tenderisation occurred over both weeks whereas in the 100F diet the majority of tenderisation occurred in the first week. Campo *et al.* (1997), Sañudo *et al.* (2003) and Braghieri *et al.* (2005) have reported that the main tenderisation process was in the first seven days of ageing but Macie (2002), working with Parda de Montaña young bulls slaughtered at 550 kg, concluded that in this breed, the tenderisation process is over by 7 days of ageing. Several factors affect ageing and in fact, the optimum ageing period varies with the age of the animal, fat content, collagen content, and feeding system (Jurie *et al.*, 1998). In the current study, the age at slaughter and intramuscular fat content at slaughter were similar in both groups of steers but the growth paths were different (Blanco *et al.*, 2012). Thus, it could be possible that the winter diet affected collagen content or collagen solubility, since it affected the growth pattern of the steers. Hence, 65F:35C steers showed an ADG of 1.08 kg d⁻¹ during the winter period and 0.73 kg d⁻¹ during grazing, whereas 100F steers presented weight gains of 0.78 kg d⁻¹ and 0.76 kg d⁻¹ in the winter and grazing periods, respectively (Blanco *et al.*, 2012). It was demonstrated that ADG influenced collagen content and solubility (Hall & Hunt, 1982; Damergi *et al.*, 1998) so it could be hypothesized that the contribution of collagen to shear force would be less important in 100F than in 65F:35C, at least in the first week. Nonetheless, the fact that texture values at one day of ageing did not differ between groups could indicate that some other factors may influence the ageing process and hence, it is reasonable to suppose that there could be some differences between the winter diets in the proteolytic enzymes responsible of the tenderisation. Although some authors have indicated no evidence of ageing effect on mechanical strength of connective tissue (Harris *et al.*, 1992), certain enzymatic systems degrade it (Dutson *et al.*, 1980; Wu *et al.*, 1981). Consequently, it could be theorized that this enzymatic system could affect the matrix of the connective tissue in different way

depending on the winter diet, which could explain the decrease of maximum stress values observed during the second week of ageing in 65F:35C group. Unfortunately, the relationships among growth rates, tenderness and the calpain proteolytic system remain unclear (Sazili *et al.*, 2003; Therkildsen *et al.*, 2008).

Meat colour

As in the current experiment, Minchin *et al.* (2010) found no differences in meat colour between cows fed different diets in winter and finished on pasture. Similarly, Duckett *et al.* (2007) reported that the growth rate during the winter period had no effect on colour but finishing diet influenced it. In the current study, both groups of steers grazed with supplementation during the 5.5-month-long finishing period, which would explain the absence of differences in meat colour associated to the previously fed winter diet.

Regarding ageing time effect, it can be observed that lightness remained fairly constant over time of oxygen exposure ($p > 0.05$), in accordance with Resconi (2007) and Beriain *et al.* (2009) and contrary to Joseph & Connolly (1977) or McDougall (1982) who reported that modifications of protein structures over time increase L^* . The meat colour was more stable throughout the time of oxygen exposure than that observed by Ripoll *et al.* (2013) in concentrate-fed animals. This can be explained because colour stability of meat depends on both storage temperature-time and presence of antioxidants (Boles *et al.*, 2005). There is a positive effect of the grass diet on meat colour stability because of the accumulation of lipid-soluble antioxidants and reduced intramuscular fat in relation to concentrate-fed cattle (O'Sullivan *et al.*, 2003) and Muramoto *et al.* (2003) found that dietary β -carotene supplementation lengthened the colour life of Japanese Black cattle.

Panel test

The high variability of the data (17.6% on average) agrees with other studies (Campo *et al.*, 1997; Chambaz *et al.*, 2003). Campo *et al.* (1999) working with Parda de Montaña breed described a variability of 15.4% for beef odour, 20.0% for tenderness, 17.5% for juiciness, 13.8% for beef flavour and 24.6% for liver flavour, that is, 17.9% on average.

The nonappearance of a diet effect on sensory properties has been reported by several authors (McCaughey & Cliplef, 1996; French *et al.*, 2000; Sami *et al.*, 2004; Cerdeño *et al.*, 2006). As in the current experiment, Duckett *et al.* (2007) showed that the stocking growth rate did not affect the sensory variables but they found differences between finishing diet, which suggest that the amount of lipids influenced sensory perceptions. Additionally, Muir *et al.* (1998) concluded that if cattle were finished to carcass weight or same degree of fatness there were no feeding-type effects on sensory properties.

The effect of ageing on sensory characteristics has been widely demonstrated (Dransfield *et al.*, 1991; Koochmarai, 1996; Campo, 1999), but surprisingly this effect was only statistically significant for beef flavour and only a tendency ($p = 0.08$) was observed for tenderness. The interaction found between the winter diet and ageing time in texture could explain this lack of significance for tenderness. In 100F steers there were no differences in maximum stress between 8 and 15 days, hence it can be assumed that meat was fully aged at 8 days post-mortem, whereas in the 65F:35C steers the tenderisation extended throughout the ageing period. For 100F steers, maximum stress values decreased 30% between 1 and 8 days of ageing ($p < 0.001$), but did not decrease between 8 and 15 days ($p > 0.05$). However, for the 65F:35C steers, maximum stress decreased by 16% between 1 and 8 days of ageing ($p < 0.001$) and by 27% between 8 and 15 days of ageing ($p < 0.001$). Therefore, from the current results it could be suggested that the threshold for detecting tenderness differences between samples should to exceed a minimum value that seems to be above 20 N (or 20% over the initial value) in the current experiment. Several studies have been carried out with consumers to establish threshold values of shear force which lead a noticeable difference in sensory perception. According to Miller *et al.* (1995) or Huffman *et al.* (1996) a change of 1 kg (9.81 N) or more is necessary to find a difference between sensory properties of steaks, whereas Destefanis *et al.* (2008) reported that 1 kg of difference was too restrictive. However, most of the authors agree to set a value around 45 N as a threshold between meat perceived as tender or tough (Shackelford *et al.*, 1991; Miller *et al.*, 2001; Destefanis *et al.*, 2008). In the current results, all samples presented shear force values above 45 N, which could explain the lack of significance in the panel test.

In these experimental conditions, it can be concluded that the winter diet had no effect on instrumental meat quality and sensory traits. Therefore, it is feasible to choose the most adequate feeding level for each particular situation (based on availability of natural sources, price of feedstuffs and management expertise for example) without any effect on the overall perception of beef quality. Ageing had a slight effect on meat quality characteristics and the tenderisation rate depended on the feeding strategy. Further analysis would be desirable in order to establish the optimum ageing period for this meat.

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