Effectiveness of using ultrasound readings to predict carcass traits and sensory quality in young bulls

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Abstract

The objectives of this study were to develop ultrasound-derived prediction equations in young bulls before slaughter to estimate carcass traits and beef sensory parameters and to determine the optimum moment pre-slaughter to estimate the sensory quality of beef from young bulls. Ultrasound images were measured in twenty live young bulls at 50, 25 and 1 days prior to slaughter. Intramuscular fat content, longissimus lumborum muscle area, carcass fatness, texture and sensory analysis were measured by a trained panel after slaughter. Partial least square methodology was used to find the relationship between the ultrasound measurements and the dependent variables, such as carcass traits, Warner-Bratzler shear force and the sensory profile. Additionally, a stepwise procedure was used to select the most informative ultrasound variables in the prediction equations for carcass and beef sensory traits. The results indicate that ultrasound testing in the feedlot offers promising potential. Early ultrasound scan measurements were useful during fattening to predict intramuscular fat content (R^2=0.619, RMSE=0.44%, 50 days prior to slaughter), while ultrasound scans taken close to slaughter were useful to predict kidney fat content (R^2=0.717, RMSE=0.96%, 1 day prior to slaughter). However, the prediction of sensory beef attributes was only useful for fatty flavor (R^2=0.556, RMSE=0.47%) at 1 day pre-slaughter. Thus, the prediction of fat parameters using ultrasound measurements could constitute a valuable tool in the process of selecting beef quality traits in young bulls before slaughter.

Key words: Beef, carcass prediction, grading, palatability, ultrasound

1. Introduction

Using ultrasound technology has been reported previously in cattle breeding programs and for production purposes (Baker et al., 2006; Castilhos et al., 2018). The application of this technology, not only on carcasses at the slaughterhouse but also on live animals in farms, has already become a useful tool for beef producers, as it enables them to match selection decisions to market demands.

Current carcass grading systems rely on straightforward visual evaluation of carcass parameters. The European Beef Carcass Grading system is based on carcass conformation and fatness (OJEU, 2006), while in the USA, it is focused on carcass yield and intramuscular fat level, which are likely to have an impact on the sensory acceptance by consumers. In addition, until now, carcass classification methods have been performed after the animals are slaughtered. However, in order to slaughter animals at the optimum moment and to make an early pre-slaughter classification of the carcass and meat quality, methods to predict carcass characteristics and carcass quality are required.

Among the most widely-used techniques in live animals is ultrasound (Wall et al., 2004; Peña et al., 2014) combined with image analysis (Hwang et al., 1997; Cannell et al. 2002), which allows for the successful prediction of carcass parameters such as muscle area or back fat thickness (Hamlin et al. 1995a; 1995b). Nogalski et al. (2017) reported that both selected biometric ultrasound measurements (back fat and thickness of subcutaneous rump) and selected blood parameters, such as triglycerides, could be used to predict intramuscular fat content with satisfactory precision and accuracy. These techniques, which can be used to estimate beef marbling, can help producers to fulfill industry and consumer demands (Indurain et al., 2009).

The ultrasound technique has also been shown to be useful in determining the nutritional quality of beef. In fact, Indurain et al. (2006) reported that the ultrasound readings reflect the effect of fatness on fat composition (e.g. the fatty acid profile of subcutaneous fat). However, there is a lack of research to demonstrate the efficiency of ultrasound in estimating beef palatability.

Regarding beef production, Spain has a number of local breeds characterized by their high muscle growth and reduced fat content. These breeds include Pirenaica, which is well suited for beef production. Pirenaica is an early maturing beef breed, producing medium-muscled lean carcasses. It accounts for 1.1 % of the Spanish national cattle stock and it is the most abundant breed for beef production under the European Union's PGI Ternera de Navarra [Navarre Veal] Protected Geographical Indication (DOUE, 2004). Pirenaica breed cattle are raised in a semi-extensive system near the mountains in the Pyrenees (Northern Spain). The animals take advantage of the mountain pastures in spring and summer, while they remain in the valleys during the winter. Young bulls are weaned at six months and are fed in feedlots with concentrate and cereal straw until slaughter at approximately twelve months of age to obtained lean carcasses.

In this context, the main objective of this study was to develop ultrasound-derived prediction equations before slaughter to estimate carcass traits and beef sensory parameters in young bulls with lean carcasses.

2. Material and methods

2.1. Pre-harvest, handling, and harvesting procedure

A total of 20 yearling Pirenaica bulls under the Protected Geographical Indication Ternera de Navarra were used. The young bulls were reared in a semi-extensive system in the mountains until weaning. After weaning at approximately seven-eight months of age, they were fed on a concentrate in a local feedlot (85% barley, 10% soybean meal, 3% vegetal fat and 2% minerals and vitamins), and barley straw, both ad libitum. The chemical composition of the feeds corresponds to a medium-energy diet: Dry matter (88.2%); Crude protein (128.5g/kg DM); Crude fiber (48.4g/kg DM); Ash (53.7g/kg DM); Metabolizable energy (11.5 MJ/kg DM). The average feed consumption during the young bulls' growth period (four months) was 7.9 kg/day. The animals were considered as a representative sample of the market, and they are commonly slaughtered in the area. The bulls were raised according to the handling conditions in the Spanish rules and regulations for animal care (Directive 2010/63/EU) and slaughtered according to Spanish rules and regulations for animal care (Council Regulation EC 1099/2009). The research followed the official guidelines for the humane treatment, care and handling of animals.

2.2. *Ultrasound data collection*

The live animals were weighed and two cross-sectional ultrasound images (right and left side) between the 12th and 13th ribs were taken in the live animals at the feedlot, as described by Wall et al. (2004) and Bergen et al. (2005). The ultrasound images were measured on the skin after clipping the hair at 50, 25 and 1 days prior to slaughter, using Sonovet 600-real-time ultrasound equipment (Madison Co. Ltd. Korea) equipped with a linear probe (3.5 MHz, 120 x 20 mm). Mineral oil (Echoultragel; Pirrone & Co.SPA, Italy) at 20-25°C was used to ensure suitable acoustic contact between the probe and the skin.

The linear ultrasound measurements recorded on the live young bulls were: intramuscular fat content or marbling (IMF), longissimus muscle depth and dorsal fat thickness. The resulting two-dimensional images (longitudinal and transversal) were digitalized and stored. The young bulls were scanned twice and the images evaluated and interpreted by a laboratory technician at the Public University of Navarra, using the

Optimas 6.5 image analysis software (Media Cybernetics Inc., USA). The ultrasound measurements of longissimus muscle depth selected were longitudinal and transversal (UA_L and UA_T respectively), and dorsal fat thickness (UF_L), since this is a linear measurement. Gray levels of the longitudinal and transversal ($UIMF_L$ and $UIMF_T$, respectively), ultrasound images for intramuscular longissimus lumborum fat content was measured with the gray level scale in the software, with 0 black and 150 white.

2.3. *Carcass evaluation*

On the day of slaughter, the young bulls were transported a distance of approximately 20 km and harvested upon arrival using standard stunning and dressing methods in a licensed slaughterhouse according to Council Regulation EC Nº 1099/2009. The young bulls were slaughtered at 521±51.4 kg live weight and 351±15 days of age and yielded an average cold-carcass weight of 323±33.8 kg.

Immediately prior to cool storage for aging, the carcasses were graded by a licensed technician for fatness and conformation according to the EU beef grading system (DOUE, 2008). The SEUROP conformation scale (S superior; E excellent; U very good; R good; O fair; P poor) was transformed, with 1 = P- and 18 = S+. On the fat cover classification scale (5 very high; 4 high; 3 average; 2 slight; 1 low), the numerical transformation of standard grades were 1 = 1- and 15 = 5+. Kidney and pelvic fat (KPF) was collected from the carcasses and weighed by a trained technician. Carcass fat thickness (CFT) was measured at the 6th rib, taking 3/4 of the length ventrally over the longissimus muscle, with a chilled stainless-steel caliber (Renand, and Fisher, 1997). In addition, the longissimus lumborum area (CA) was measured with a grid.

Twenty-four hours post-mortem, the longissimus lumborum muscle was removed at the 6-13th rib level from the left carcass side, and was transported to the Meat Science

Laboratory at the Public University of Navarra (Pamplona-Spain), where the meat was aged in the dark for seven days at 2ºC. After ageing, the muscles were cut into steaks. One steak was cut for instrumental texture analysis (3.5cm thick) and another for sensory analysis (2.5cm thick) from the second lumbar vertebra. The steaks were then vacuum packed in polyamide/polyethylene pouches (Vaeseen Schoemarket Ind., Barcelona, Spain - film thickness of 120 µm and O^2 permeability of 1 cc/m^2/24 h, CO_2 permeability of 3 cc/m^2/24 h, and N_2 permeability of 0.5 cc/m^2/24 h measured at 5 ºC and 75% relative humidity; water vapor transmission rate 3 g/m^2/24 h at 28 ºC and 100% RH; vicat softening point of sealing 97ºC, and a dart drop strength of 1300g) and stored at -20ºC for subsequent analysis.

2.4. *Fat extraction*

At 24 h post-mortem, a steak from the longissimus lumborum muscle at the 12th rib level was used to determine the intramuscular fat content. The total lipids were extracted from each meat product with chloroform:methanol (2:1, v/v), according to the Folch et al. (1957) method. The results were expressed as total intramuscular fat content per 100g of fresh meat. Two replicates were taken per sample.

2.5. *Warner –Bratzler Shear Force*

The steaks for the Warner–Bratzler shear force (WBSF) test were thawed overnight at 4ºC. The beef fillet (3.5 cm thick) was cooked on a preheated sheet previously at 180ºC, turning the fillet every 4 minutes, until reaching an internal temperature of 70ºC (AMSA, 2016). The temperature inside the steak was controlled by temperature probes and a data acquirer (thermocouple probe TB 190 and data acquirer Almemo 5990-2 V5; Ahlborn mess-un Regelungstechnik GMBH, Holzkircken, Alemania). After cooling for two hours seven cores measuring 1 cm wide, 1 cm high and 3 cm long were removed from the cooked steaks parallel to the longitudinal axis of the muscle fibers. The Shear Force analysis was carried out using a TA-XT2i texture analyzer (Stable Micro Systems, Inc., Goodming, Surrey, UK). The maximum shear force (kg) was assessed with a Warner-Bratzler shearing device following the methodology proposed by Beltrán (2005). The samples were then tempered at room temperature. The analysis was reformed with a crosshead speed of 200 mm•min^{-1}, 50 kg load cell, 40 mm distance and a calibration weight of 10 kg. The full peak shear force was recorded and the maximum shear force was calculated in kg as the mean of the seven measurements.

2.6. Trained sensory panel

The sensory analysis was performed by a seven-member trained descriptive panel selected and trained as described by Cross et al. (1978) and Meilgaard et al. (1991). The members of the descriptive panel were selected and trained as described by the ISO 8586–2014 (International Organization for Standardization) to evaluate the beef samples. The definitions used for the different sensory attributes are listed in Gorraiz et al. (2000). Juiciness, hardness, characteristic beef flavor, liver flavor and fatty flavor were evaluated, using a 150 mm unstructured line scale, with a 10 mm mark from the left, representing "low intensity", and another mark 10 mm from the right, representing "high intensity".

The frozen steaks were thawed over night at 4ºC, and cooked on a grill to an internal temperature of 70ºC measured with a TB 190 probe (data logger Almemo 5990-2 V5; Ahlborn mess-un Regelungstechnik GMBH, Holzkircken, Germany). The steaks were then cut into 1.5x2x1.5cm cubes, which were wrapped in aluminum foil with an identification tag with a random three-digit code and kept in a heat-retaining container or waterless food warmer before serving. The tasting took place in the sensory testing room at the Meat Science Laboratory of the Public University of Navarra (Pamplona-Spain) under red lighting, with controlled temperature and humidity. The tasting hall meets UNE 87004 (1979) standard requirements for sensory trials. The panelists were provided with slices of apple and water to cleanse their palate between samples. Each panelist tasted five samples per session in a random order and each sample was assessed by all seven members of the panel.

2.7. Statistical Analyses

All the data were analyzed using SPSS software (SPSS V. 25.0, SPSS Inc. USA). A statistical descriptive analysis was carried out to describe the animal and carcass characteristics, ultrasound readings, shear force and sensory position and dispersion parameters. The effects on carcass traits of the moment when the ultrasound measurements were performed were analyzed using the repeated measures GLM procedure and a post hoc Fisher comparison of means test. Carcass weight was used as a linear covariate. Pearson correlation coefficients were calculated to assess the relationship between the ultrasound readings at three given points in the animals' life (50, 25 and 1 days prior to slaughter) and the corresponding carcass and beef measurements and sensory evaluation. A partial least square methodology was used to find the relationship between the dependent variables (carcass traits, Warner-Bratzler shear force and the sensory profile after 7 days of ageing) and the ultrasound measurements. Additionally, a stepwise procedure (forward selection of variables, significance criterion $P<0.05$) was used to select the most informative ultrasound variables in the prediction equations for carcass and beef sensory traits. Both the dependent variables and the explanatory variables were centered and reduced. The best equations for the prediction of carcass and sensory parameters using ultrasound measurements at three pre-slaughter times were chosen.

3. Results and discussion

3.1. Animal, carcass and beef sensory traits

After weaning at around seven months, the animals entered the feedlot with an average weight of 323.7 kg. At slaughter, their average weight was 521.5 kg and average age 350.8 days (Table 1). The average stay of the animals on the feedlot was 128.7 days, and the average daily weight gain during the finishing period was 1.5 kg/day.

Trait	Mean	SD	Minimum	Maximum
Productive measurements				
Start feedlot weight (kg)	323.7	36.55	243	395
LWS (kg)	521.5	51.41	445	610
Age at Slaughter (days)	350.8	15.05	307	366
Days on feedlot	128.7	34.16	52	174
ADG (kg/day)	1.5	0.22	1.1	1.9
Carcass measurements				
Carcass weight (kg)	323.5	33.80	264.5	377.5
Dressing %	62.4	2.97	57.7	66.8
Conformation score[a]	9.6	0.71	8	11
Fatness score[b]	4.4	0.51	4	5
KPF (% of total carcass)	1.4	0.50	0.7	2.3
CA (cm^2)	104.4	10.95	83.8	136.7

CFT (cm)	0.2	0.06	0.1	0.5
CIMF (%)	0.8	0.21	0.7	1.0
Instrumental measurements				
WBSF (kg/cm^2)	5.9	0.44	2.2	8.2
Sensory parameters[c]				
Juiciness	5.9	0.68	3.7	9.5
Hardness	7.7	0.24	5.1	10.0
Beef flavor	6.2	2.13	3.4	7.7
Liver flavor	3.8	0.01	2.5	5.2
Fatty flavor	2.8	0.71	1.7	3.7

SD: Standard deviation; LWS: live weight at slaughter; ADG: Average daily gain; Dressing %: Dressing percentage: carcass weight*100/live weight. [a]Conformation score: carcass conformation score by comparison with EU beef carcass standard grades. Numerical transformation of standard grades were 1 = P- and 18 = S+; [b]Fatness score: carcass fatness score by comparison with EU beef carcass standard grades where numerical transformation of standard grades were 1 = 1- and 15 = 5+; KPF: Percentage of kidney and pelvic fat to carcass weight; CA= carcass longissimus lumborum area at the 12th rib measured with a grid; CFT= carcass 12th-rib fat thickness measured with a caliber; CIMF = longissimus lumborum intramuscular fat content at the 12th rib; WBSF: Warner–Bratzler shear force test; [c]Sensory parameters measured by a trained panel using a 150-mm unstructured line scale.

Table 1 Descriptive statistics for productive, carcass and beef quality traits in young bulls.

According to Albertí et al. (2005), the carcass characteristics of the young bulls used in this experiment are within the standard range of continental carcasses on the European market (Table 1). These carcasses are described as light carcasses compared to the heavier British carcasses, as reported by Eriksson et al. (2002). The values of the longissimus lumborum area and fat thickness at the 12th rib in the Pirenaica breed (mean average 104.44 cm^2 and 0.23 cm, respectively) were in line with those reported by Peña et al. (2014) in the Retinta breed and other continental beef breeds (Charolais and Limousine). Moreover, the longissimus lumborum intramuscular fat content (average 0.85%) was in the range of light carcasses on the South European market. These findings agree with those of Piedrafita et al. (2003) and Albertí et al. (2008) in calves reared under the same production systems.

Table 1 shows the sensory attributes of the meat as determined by the trained panel. From these results, the meat from these young bulls could be described as moderately juicy and tender, with a not very intense beef flavor and a slight taste of liver. The assessments given by the trained panel were similar to those reported in previous studies (Beriain et al., 2016), which reported Pirenaica beef as having a medium degree of juiciness, tenderness, flavor and a reduced fat content.

3.2. Ultrasound measurements

Table 2 shows the measurements of the longissimus lumborum muscle area (UA_L and UA_T) and UF_L obtained by means of the scans on live animals 50, 25 and 1 days prior to slaughter. The scans were also used to measure gray level ($UIMF_L$ and $UIMF_T$) to determine the longissimus lumborum intramuscular fat content at the 12th rib (grey scale 0-150). The influence of the moment the ultrasound measurements were taken on differences was significant (P <0.05) for UA_T. In fact, the lowest value of UA_T was observed at 50 days ante-mortem, while after 25 days ante-mortem, no differences in UAT measurements were observed.

	50 days ante-mortem				25 days ante-mortem				1 day ante-mortem					
	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.	SEM	***P*-value**
UA_L (cm^2)	74.78	10.07	58.73	88.36	75.52	11.27	45.99	95.52	77.39	9.73	56.81	98.14	6.32	0.367
UA_T (cm^2)	86.28^a	13.24	63.41	104.16	93.62^b	13.12	72.84	117.47	94.88^b	14.36	76.15	127.30	8.52	0.008
UF_L (cm)	0.47	0.10	0.26	0.98	0.48	0.09	0.35	0.71	0.46	0.13	0.25	0.60	0.46	0.331
$UIMF_L$ (grey level)†	59.72^a	16,81	29.42	95.03	49.72^b	16,78	27.62	87.97	50.80^b	24,52	7.53	97.71	13.90	0.038
$UIMF_T$ (grey level)†	69.89^a	13,79	39.37	92.04	64.09^b	14.77	35.53	109.24	64.08^b	21,95	20.23	101.11	10.99	0.016

SD: Standard deviation; SEM: Standard error of the mean; UA_L: ultrasound of longissimus lumborum area at the 12th rib from longitudinal ultrasound measurement; UA_T: ultrasound of longissimus lumborum area at the 12th rib from transversal ultrasound measurements; UF_L = ultrasound of 12th fat thickness from longitudinal ultrasound measurement; $UIMF_L$: ultrasound of longissimus lumborum intramuscular fat content at the 12th rib from longitudinal ultrasound measurement; $UIMF_T$: ultrasound of longissimus lumborum intramuscular fat content at the 12th rib from transversal ultrasound measurement; ns: not significant; sig: significant; ^{a,b}: Marked differences are significant at $P < 0.05$ in the same line; †Grey level of ultrasound image scale 0-150.

Table 2 Least mean squares and fisher-test means for ultrasound data collected in young bulls at 50, 25- and 1-days ante-mortem.

The longissimus lumborum area at the 12th rib measured with a grid (104.44 cm^2; Table 1) was greater than that predicted by the in vivo ultrasound images (which ranged from 74.78 to 94.88cm^2; Table 2). This is in line with findings published by Bergen et al. (2005), who reported a tendency for the ultrasound measurements of the loin area to be lower than the final carcass values. In contrast, Greiner et al. (2003) stated that *in vivo* ultrasound measuring techniques can overestimate carcass loin areas by between 3.31 and 6.76 cm^2 in young bulls with low musculature. On the other hand, Smith et al. (1990) reported that the CA of bulls with a longissimus lumborum area around 104 cm^2 was generally underpredicted, whereas in cattle with a longissimus lumborum area lower than 85 cm^2, it was usually overpredicted. The fact that the cited authors performed their measurements on carcasses with a longissimus lumborum area of approximately 80 cm^2, while the Spanish cattle used in this study had bigger muscles and therefore a larger loin area (> 100 cm^2), might, at least partially, explain the greater differences found in the present study.

The UF_L means across the scanning time are presented in Table 2. The mean values across the scanning time (0.47 cm) were in the range of those reported by Peña et al. (2014) in a long scanning period from 212 days ante-mortem for continental breeds such as Retinta, Charolais and Limousine, reared under similar conditions to the Pirenaica breed. No variations in the average UF_L values between the first and the last scans were observed (*P*>0.05). The range of UFL values for carcass back fat thickness observed by ultrasound (from 0.46 to 0.48 cm; Table 2) was greater than carcass 12th-rib fat thickness measured with a caliber (0.23 cm; Table 1). These results are in agreement with findings by Bergen et al. (2005) who found that the *in vivo* ultrasound values were higher than carcass measurements when the differences were >0.5 cm. In fact, Peña et al. (2014) showed an overestimation of ±0.2 cm in 98.9% of the bulls' UF_L vs CFT in young bulls of continental breeds.

In contrast, Greiner et al. (2003) and Baker et al. (2006) found an underestimation of UF_L values in young bulls when the back fat thickness was greater (0.7 cm). According to Waldner al. (1992), an improvement in the accuracy of the relationship between ultrasound and carcass measurements during the animals' maturing period up to 16 months can be observed. Perkins et al. (1992a) stated that the accuracy of the ultrasound measurements of back fat thickness is highly dependent on the degree of rib fatness during the animals' growth. On the one hand, the misinterpretation of this accuracy could occur in fatter cattle, in contrast to the improved interpretation in lighter carcasses. In the present study, in 60% of the animals, the differences between the last UF_L measurement and CFT were < 0.2 cm. This accuracy is greater than that recorded by May et al. (2000) in fatter young bulls. This result may be explained because the animals used in the present research were lean and had a very thin layer of back fat (Perkins et al., 1992b; Charagu et al., 2000).

Ultrasound grey level for the three pre-harvest periods was used to compare intramuscular fat content in beef during the growth period (Table 2). A significant decrease in the grey level in longitudinal and transversal images (around 15%) was observed between the first and last scans. No significant differences in grey levels were observed ($P>0.05$) during the last scanning period (between 25 and 1-days ante-mortem). This finding was in agreement with Albrecht et al. (2006), who observed a relative decrease in intramuscular fat in young bulls during the fattening period up to the age of twelve months in Angus steers and a marked increase in intramuscular fat deposition from 12 to 14 months of age. However, Yang et al. (2006) affirmed that there was no clear relationship between intramuscular fat content and body weight in young bulls from lean breeds. In fact, Albrecht et al. (2006) reported that the connective tissue in the muscle is often present in strong cords which are not streaked with fat, and the highest UIM_F observed in the early scans of young bulls could be attributed to the relative abundance of connective tissue in the muscle, which is likely to be confused with fat during ultrasound image processing.

3.3. Correlations between ultrasound measurements and carcass traits

The Pearson correlation coefficients between *in vivo* ultrasound readings (UA_L, UA_T, UF_L, $UIMF_L$ and $UIMF_T$) taken at 50, 25 and 1 days before slaughter and the values of carcass and beef parameters are presented in Tables 3 and 4, respectively. In overall terms, the ultrasound measurements taken in young bulls before slaughter correlated with some carcass parameters (Table 3), while few significant correlations with beef traits were observed (Table 4).

Ultrasound measurements^b	Ante-mortem scan day	Carcass weight	Conformation score^a	Fatness score^a	KPF^a	CA^a	CFT^a	CIMF^a
	50	0.452	0.490*	-0.228	-0.425*	0.473*	-0.117	-0.533*
UA_L	25	0.203	0.511*	-0.111	-0.477*	0.377	-0.420	-0.451*
	1	0.160	0.520*	-0.084	-0.464*	0.560*	-0.268	-0.491*
	50	0.386	0.485*	-0.174	-0.529*	0.445	-0.273	-0.616*
UA_T	25	0.459*	0.703*	-0.315	-0.635*	0.614*	-0.472*	-0.288
	1	0.511*	0.738*	-0.389	-0.574*	0.398	-0.558*	-0.139
	50	-0.223	-0.091	0.270	-0.054	-0.241	0.330	-0.012

UF$_L$	25	-0.372	-0.080	0.232	-0.153	0.019	0.068	0.215
	1	-0.074	-0.047	0.293	-0.116	0.434	-0.021	-0.417
UIMF$_L$	50	-0.193	-0.263	-0.042	0.247	-0.159	0.073	0.302
	25	-0.578*	-0.540*	0.575	0.444*	-0.111	-0.123	0.324
	1	-0.413	-0.435	0.420	0.529*	-0.263	0.328	0.538*
UIMF$_T$	50	0.029	0.037	-0.189	0.636*	0.010	-0.072	0.535*
	25	-0.536*	-0.441	0.439	0.556*	-0.319	0.107	0.468*
	1	-0.491*	-0.542*	0.383	0.718*	-0.394	0.457*	0.462*

[a]Text caption, see Table 1; [b]Text caption, see Table 2; *: $P < 0.05$.

Table 3 Pearson coefficients of correlation between carcass and ultrasound measurements at different scan days before slaughter.

Carcass weight was positively correlated with UA$_T$, but negatively with ultrasound measurements for fat traits (UF$_L$, UIMF$_L$ and UIMF$_T$) in most scans (Table 3). The significant correlations between a series of ultrasound carcass measurements and carcass weight were high, with values over 0.459. These findings were in agreement with those described by Peña et al. (2014) between slaughter weight and a series of ultrasound measurements in the muscle area (r=0.503), or the intramuscular fat percentage (r=-0.335) in young bulls of Retinta, Charolais and Limousine breeds.

In general, the correlations between ultrasound measurements for muscle traits (UA$_L$ and UA$_T$) were positively related to carcass conformation and CA measured in the carcass, while negative correlations were observed between UA$_L$ or UA$_T$ and fat traits of the carcass (KPF, CFT and CIMF). The strongest significant relationships (r = 0.485–0.738) were observed between the ultrasound measurements of longissimus lumborum area, at the three pre-harvest times, and the conformation score. In fact, the correlation tended to increase with the duration of the fattening phase. The role of longissimus muscle area for predicting carcass yield has proved to be controversial (Wall et al., 2004). Numerous studies have tested its usefulness in predicting cuttability; May et al. (2000) and Realini et al. (2001) correlated rib eye area both to sub-primal cut yields and the weights of the major primals (r-value between 0.64 and 0.45). However, Crews et al. (2002) and Wall et al. (2004) stated that the USDA yield grade is not heavily influenced by the longissimus lumborum area. Hodgson et al. (1992) and Tait et al. (2005) showed that the loin eye area had a low correlation coefficient with retail yield.

The UA_L readings at 50, 25 and 1-days ante-mortem correlated negatively with the percentage of KFT and CIMF. The highest correlations between muscular ultrasound scans (UA_L and UA_T) and intramuscular fat content (r=-0.533 and -0.616, respectively) were observed at early scanning times. Wall et al. (2004) obtained regression coefficients ranging between 0.44 and 0.42 for back fat and 0.47 for the degree of marbling estimates from UA_L. The cited authors found no significant improvement in estimates from scans taken closer to the day of slaughter, but it should be noted that the scans were taken between 2 and 3.5 months prior to animal slaughter. Renand and Fisher (1997) noted that if the back-fat estimates from ultrasound measurements for a population showed a standard deviation of more than 3 mm, the coefficients of correlation between the final and ultrasound values can reach 0.6, while for a SD of less than 2 mm, they barely reach 0.3.

The ultrasound longissimus muscle depth reading (UA_L and UA_T) also appears to be correlated, albeit negatively, with various carcass fatness measurements, such as the degree of marbling and the KPF score (Table 3), revealing an inverse relationship between muscular development and final fatness (Mendizabal et al., 1999; Alberti et al., 2005). The range of back fat thickness in Pirenaica calves (Table 1) was between 0.1 and 0.45 cm, with a mean value of 0.23 cm and a standard deviation of 0.08 cm, while 40% of the UF_L estimates deviated by more than 0.25cm from CFT.

The grey level value of a scan depends on tissue composition and therefore on the degree of marbling in the muscle. In general, positive correlations between ultrasound intramuscular fat content (UIMFL and UIMFT) and fat trait were observed in the carcasses. In fact, significant correlations at all scanning times were observed between UIMFT and carcass traits, such as KPF or CIMF. The correlation increased significantly between the first and last scans for UIMFT and KPF, while the correlation between UIMFT and CIMF decreased from the first to the last scans (Table 3). These results are not in agreement with Wall et al. (2004), who reported no changes in the correlations between marbling and ultrasound scans made 100 days before slaughter to preslaughter in fattened bulls from the Angus, Simmental, Red Angus and Charolais breeds.

3.4. Correlations between ultrasound measurements and beef quality traits

Table 4 shows the Pearson coefficients of correlation between the measurements obtained from the *in vivo* scans (UA_L, UA_T, UF_L, $UIMF_L$ and $UIMF_T$) taken 50, 25 and 1 days prior to slaughter, the Warner-Braztler shear force scores and the sensory parameters of the meat as evaluated by a trained panel. Fatty flavor was the only sensory parameter with significant coefficients of correlation with the ultrasound area (UA_L) and ultrasound intramuscular fat ($UIMF_T$) readings. Fatty flavor correlated negatively with the *in vivo* UA_L readings taken 50, 25 and 1 days prior to slaughter, and UA_T at 50 days ante-mortem (r=-0.616). Fatty flavor was correlated positively with $UIMF_T$ at the three measurement times and only with $UIMF_L$ at 1 day prior to slaughter (r=0.538; P<0.05). Correlations between fatty flavor and UA_L or $UIMF_T$ decreased constantly from 50 days before slaughter to the pre-slaughter scans.

Ultrasound measurement[a]	Ante-mortem scan day	WBSF	Juiciness	Hardness	Beef flavor	Liver flavor	Fatty flavor
UA_L	50	0.233	0.023	0.106	0.025	-0.337	-0.533*
	25	-0.247	0.081	-0.235	0.311	-0.225	-0.451*
	1	-0.096	-0.060	-0.175	0.105	0.060	-0.491*
UA_T	50	-0.002	0.101	-0.081	0.211	-0.186	-0.616*
	25	0.018	0.204	-0.176	0.046	0.036	-0.288
	1	0.034	-0.047	-0.050	0.107	-0.096	-0.139
UF_L	50	-0.308	-0.304	0.280	0.421	-0.324	-0.012
	25	-0.365	-0.031	-0.065	0.361	-0.062	0.215
	1	0.055	-0.253	-0.238	-0.130	-0.040	-0.417
$UIMF_L$	50	0.077	-0.131	0.328	-0.119	0.243	0.302
	25	-0.335	0.011	-0.236	-0.107	0.307	0.324
	1	0.041	-0.061	0.256	0.049	0.177	0.538*
$UIMF_T$	50	0.152	0.148	0.142	-0.161	0.361	0.535*
	25	-0.162	-0.009	-0.099	0.034	0.259	0.468*
	1	0.098	-0.057	0.186	0.090	0.260	0.462*

[a] Text caption, see Table 2; WBSF: Warner–Bratzler shear force test; *: $P < 0.05$.

Table 4 Pearson coefficients of correlation between beef quality traits and ultrasound measurements at different scanning days before slaughter.

3.5. *Prediction equations*

With the practical aim of saving time and costs, we identified the best moment for scanning to obtain an acceptable prediction. As regards the estimation of the beef parameters, the best significant equations ($P<0.05$ and $R^2>0.5$) to predict the percentage of kidney and pelvic fat, intramuscular fat content, carcass conformation, carcass fatness and the fatty flavor of the meat are shown in Table 5. The results of the regression equations included the scores obtained from the in vivo ultrasound scans taken 50, 25 and 1 days prior to slaughter. The best prediction equations for KPF and carcass fatness score were at 1 day prior to slaughter, while those for conformation carcass were obtained at 25 days prior to slaughter. The most favorable prediction of intramuscular fat content was 50 days prior to slaughter, since at this time the ultrasound measurement was more stable than that performed one day before the animals were slaughtered. This observation can be seen in Table 2 in which the ranges of dispersion between minimum and maximum observed in the gray levels in UIMFL and UIMFT were higher when the measurements were made one day before slaughtering of the calves than those observed when the ultrasound measurements were carried out 50 days before the animals were slaughtered. This finding suggests that better carcass parameter estimates result from scans taken in the last month prior to slaughter, but not necessarily the day before harvest.

Dependent Variables[*, a]	Best ante-mortem scan day[b]	R^2	*P*-value	RMSE	Best prediction equations[b]
KPF (%)	1	0.717	0.002	0.962	KPF (%) = $-5.27-4.16E^{-2}*UA_{L1}+4.80E^{-2}*UA_{T1}+0.60*UF_{L1}-2.91E^{-2}*UIMF_{L1}+0,11*UIMF_{T1}$
CIMF (%)	50	0.619	0.011	0.436	IMF (%) = $0.96-5.39E^{-3}*UA_{L50}-1.47E^{-2}*UA_{T50}+0.29*UF_{L50}-1.51E^{-2}*UIMF_{L50}+4.37E^{-2}*UIMF_{T50}$
Conformation score	25	0.738	0.001	0.556	Conformation score= $5.0-1.60E^{-2}*UA_{L25}+0.06*UA_{T25}+0.14*UF_{L25}-4.18E^{-2}*UIMF_{L25}+0.02*UIMF_{T25}$
Fatness score	1	0.550	0.031	0.395	Fatness score= $1.40+9.11E^{-3}*UA_{L1}-1.29E^{-3}*UA_{T1}+0.27*UF_{L1}+6.42E^{-3}*UIMF_{L1}+0.01*UIMF_{T1}$
Fatty flavor	1	0.556	0.029	0.470	Fatty flavor = $2.89-4.02E^{-2}*UA_{L1}+2.47E^{-2}*UA_{T1}-4.26*E^{-2}UF_{L1}+9.16E^{-3}*UIMF_{L1}+6.45E^{-3}*UIMF_{T1}$

*Only significant regression equations ($P<0.05$ and $R^2>0.5$) are shown.

[a] Text caption, see Table 1; [b] Text caption, see Table 2; RMSE: Root mead square error.

Table 5 Best prediction equations for young bull carcass characteristics and beef quality traits from ultrasound readings at 50, 25 and 1-days ante-mortem.

The regression coefficients (R^2) for the fat content in carcass and beef (KPF, CIMF and fatness score) were higher than 0.5 (R^2=0.717, 0.619 and 0.550 respectively) as reported also by Wall et al. (2004). The best prediction of the percentage of pelvic and kidney fat containing UA_L, UA_T, UF_L, $UIMF_L$ and $UIMF_T$ was observed at 1 day prior to slaughter. All independent variables accounted for 71.7% of the variation in KPF.

The R^2 values for the regression equations to estimate CIMF were in line with Aass et al. (2009) and Castilhos et al. (2018), who indicated that ultrasound constitute an accurate method for predicting in vivo intramuscular fat content in lean breeds. In fact, in this study, the R^2 values were higher (R^2= 0.619) than those reported by Chambaz et al. (2003) (R^2 = 0.420), but in agreement with the ones reported by Aass et al. (2009) (R^2 = 0.670) in lean beef. It can be therefore considered that in lean breeds, intramuscular fat can be predicted early in young bulls because intramuscular fat depot is a late-maturing tissue and changes cannot be expected at an early slaughter age.

With reference to sensorial variables, the best prediction equation for fatty flavor of beef (R^2=0.556; P=0.029) was obtained 1 day before slaughter. In accordance with Table 4, fatty flavor can be predicted from in vivo scans, using the combination of all the ultrasound measurements from the scan taken 1 day prior to slaughter, including a combination of UA_L, UA_T, UF_L, $UIMF_L$ and $UIMF_T$ measurements, which account for 56% of the variation in the fatty flavor of beef from young bulls.

In early scans, fat content could determine fatty flavor, while difficulties in predicting sensory parameters related to muscle development at different scanning times can be observed. A higher intramuscular fat content should increase oiliness, which plays a role in increased juiciness and causes meat to be perceived as more tender, as noted by Savell and Cross (1988). In addition, fat tissue is the main depot for the precursors of the volatile compounds produced when cooking beef, through Maillard's browning reaction and the oxidation of lipids (Mottram, 1998). The fat content in muscle correlates significantly with aroma intensity (Savell and Cross 1988), so that pleasant-tasting beef will tend to be fattier, with a higher degree of marbling (Berry et al., 1980). In fact, there is some controversy over the fat percentage in muscle required to achieve the optimal flavor intensity: Denoyelle (1995) defended a minimum of 4% of fat in muscle, whereas Savell and Cross (1988) lowered the percentage to 3%. However, Spanish beef is characterized by a low fatness level and marketed carcasses show less than 3-4% intramuscular fat in the longissimus lumborum muscle (Indurain et al., 2006). The animals used in the current study had a low percentage of intramuscular fat in the longissimus lumborum muscle (0.85 % of fresh meat) and, even at this low percentage, fatness would seem to have a positive impact on beef palatability. As in the estimation of the KPF and fatness scores, the best scans for predicting fatty flavor sensory quality are taken just prior to slaughter, although the best prediction of intramuscular fat could be propossed early.

Table 6 shows the best ultrasound variables to contribute to an acceptable prediction of carcass and beef parameters in young bulls from the Pirenaica breed. To predict KPF, the conformation and fatness score from two ultrasound measurement scans accounted for the highest variation, while for all the other parameters, only one ultrasound measurement accounted for the highest variation.

Dependent variables [*, a]	Independent variables[b]	R^2	*P*-value	RMSE
KPF (kg)	$UIMF_{T1}$; UF_{L1}	0.621	<0.001	1.010
CA (cm^2)	UA_{T25}	0.408	0.002	11.134
CFT (cm)	$UIMF_{T1}$	0.210	0.042	0.078
CIMF (%)	$-UA_{T50}$	0.306	0.011	0.519
Conformation score	UA_{T25}; $-UIMF_{L25}$	0.689	< 0.001	0.550

Fatness score	$UIMF_{L1}$; UF_{L1}	0.462	0.005	0.390
WBSF (kg/cm^2)	-UF_{L25}	0.225	0.035	1.394
Fatty flavor	$UIMF_{L1}$	0.279	0.017	0.529

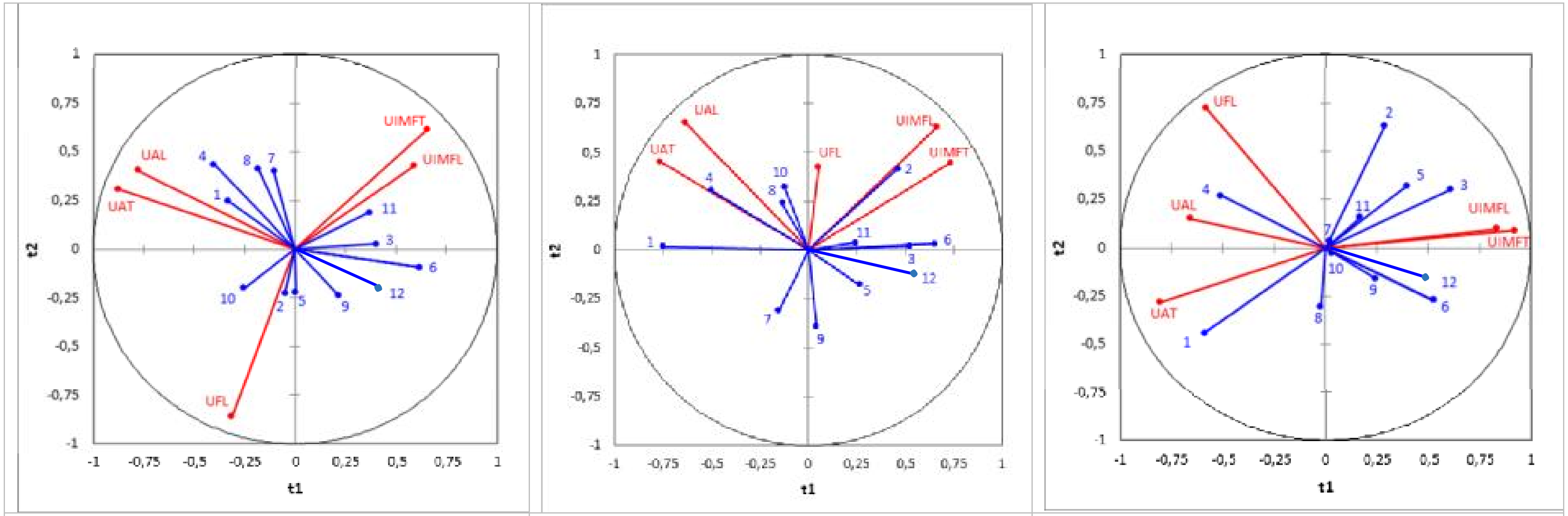
*Only significant variables (P<0.05) are showing.

[a]Text caption, see Table 1; [b]Text caption, see Table 2; Represented values scan at 50, 25 and 1-days before slaughter; RMSE: Root mead square error.

Table 6 Regression to predict young bull carcass and beef quality traits corresponding to the selected ultrasound measurements.

To predict KPF, CFT, carcass conformation, carcass fatness, WBSF and fatty flavor, ultrasound measurements from fat are the main requirements. However, to predict CA, CIMF and carcass conformation, what is needed is mainly ultrasound measurements from the muscular area (UA).

In general, the best ultrasound measurement to determinate fat traits (KPF, CFT, fatness score and fatty flavor) were determined close to slaughter (1 day pre-slaughter), while the best scan time to determine CA, conformation and intramuscular fat content was a longer time before slaughter. In KPF prediction, the contribution of measurements related to fat content at an early pre-slaughter time ($UIMF_T$ and UF_L) were positive (R^2=0.621; P<0.001). In CFT, the contribution of $UIMF_T$ prediction was positive too (R^2=0.210; P<0.042). In the case of carcass fatness prediction, $UIMF_L$ and UF_L both contributed positively (R^2=0.462; P<0.005). The contribution of $UIMF_L$ to predict fatty flavor in beef at 1 day pre-slaughter of the young bulls was positive (R^2=0.279; P<0.017) (Table 6). At 25 days pre-slaughter, the contribution of UAT was positive (R^2=0.408; P<0.002) to predict the longissimus lumborum area. To predict carcass conformation, UAT (positively) and UIMFL (negatively) both contributed to explaining the best prediction equation (R^2=0.689; P<0.001). As regards the prediction of WBSF, the UFL measurement at 25 days pre-slaughter contributed to explaining negatively 22.5% of the variation in beef tenderness. In intramuscular fat content prediction, the negative contribution of the UA_T measurement at 50 days pre-slaughter was the main factor ($R^2=0.306$; $P<0.011$). This measurement, which evaluates the animals’ muscle development, should be taken at 50 days prior to slaughter, when the animals are starting the fattening period to predict beef fat content. To provide a clearer picture of the data, Figure 1 shows the charts involving all the correlations between the components and explanatory and dependent variables at 50, 25 and 1-days ante mortem. Increasing the distance of the biplot points to the center of the graph indicates greater correlation between the components and explanatory and dependent variables.



50 day ante-mortem | 25 days ante-mortem | 1 day ante-mortem

1, Conformation score; 2, fatness score; 3, kidney and pelvic fat; 4, longissimus lumborum area; 5, carcass fat thickness; 6, intramuscular fat; 7, Warner–Bratzler shear force; 8, juiciness; 9, hardness; 10, beef flavor; 11, liver flavor; 12, fatty flavor.

Fig. 1. Biplot of the correlations between the explanatory and dependent variables with the first two components generated (t1 and t2) by the partial least squares (PLS) regression algorithm.

In general, the R^2 values for the best ultrasound measurements to determine the sensory parameters of beef were low (range R^2= 0.089 to 0.279; data not shown in table) in reference to carcass parameters (Figure 1). Only the significant variable regression to predict young bull sensorial quality traits corresponding to the selected ultrasound measurements for fatty flavor was observed.

4. Conclusions

While it is possible to use ultrasound to predict carcass traits in young bulls of lean breeds, ultrasound testing to predict pre-slaughter sensory beef parameters is not common. In order to predict intramuscular fat content in young bulls from lean cattle breeds, early ultrasound scans of the longissimus lumborum area measurement are recommended during fattening. However, to predict kidney and subcutaneous fat depots, ultrasound scans taken close to slaughter including intramuscular fat content and measurement of fat thickness are recommended. The only sensory variables which showed a relationship with the ultrasound measurements performed on the live animals at different times during the fattening of the young bulls were those related to the fat content in meat from young bulls of the Pirenaica breed. In this context, it is advisable to predict the fatty flavor of beef ultrasound scan of intramuscular fat measurement close to moment when the young bulls are slaughtered. However, further studies with other fatness bovine breeds would be needed to determine the validity of the use of ultrasound to predict the sensory fat parameters of bovine meat.

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Effectiveness of using ultrasound readings to predict carcass traits and sensory quality in young bulls

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Abstract

The objectives of this study were to develop ultrasound-derived prediction equations in young bulls before slaughter to estimate carcass traits and beef sensory parameters and to determine the optimum moment pre-slaughter to estimate the sensory quality of beef from young bulls. Ultrasound images were measured in twenty live young bulls at 50, 25 and 1 days prior to slaughter. Intramuscular fat content, longissimus lumborum muscle area, carcass fatness, texture and sensory analysis were measured by a trained panel after slaughter. Partial least square methodology was used to find the relationship between the ultrasound measurements and the dependent variables, such as carcass traits, Warner-Bratzler shear force and the sensory profile. Additionally, a stepwise procedure was used to select the most informative ultrasound variables in the prediction equations for carcass and beef sensory traits. The results indicate that ultrasound testing in the feedlot offers promising potential. Early ultrasound scan measurements were useful during fattening to predict intramuscular fat content (R^2=0.619, RMSE=0.44%, 50 days prior to slaughter), while ultrasound scans taken close to slaughter were useful to predict kidney fat content (R^2=0.717, RMSE=0.96%, 1 day prior to slaughter). However, the prediction of sensory beef attributes was only useful for fatty flavor (R^2=0.556, RMSE=0.47%) at 1 day pre-slaughter. Thus, the prediction of fat parameters using ultrasound measurements could constitute a valuable tool in the process of selecting beef quality traits in young bulls before slaughter.

Key words: Beef, carcass prediction, grading, palatability, ultrasound

1. Introduction

Using ultrasound technology has been reported previously in cattle breeding programs and for production purposes (Baker et al., 2006; Castilhos et al., 2018). The application of this technology, not only on carcasses at the slaughterhouse but also on live animals in farms, has already become a useful tool for beef producers, as it enables them to match selection decisions to market demands.

Current carcass grading systems rely on straightforward visual evaluation of carcass parameters. The European Beef Carcass Grading system is based on carcass conformation and fatness (OJEU, 2006), while in the USA, it is focused on carcass yield and intramuscular fat level, which are likely to have an impact on the sensory acceptance by consumers. In addition, until now, carcass classification methods have been performed after the animals are slaughtered. However, in order to slaughter animals at the optimum moment and to make an early pre-slaughter classification of the carcass and meat quality, methods to predict carcass characteristics and carcass quality are required.

Among the most widely-used techniques in live animals is ultrasound (Wall et al., 2004; Peña et al., 2014) combined with image analysis (Hwang et al., 1997; Cannell et al. 2002), which allows for the successful prediction of carcass parameters such as muscle area or back fat thickness (Hamlin et al. 1995a; 1995b). Nogalski et al. (2017) reported that both selected biometric ultrasound measurements (back fat and thickness of subcutaneous rump) and selected blood parameters, such as triglycerides, could be used to predict intramuscular fat content with satisfactory precision and accuracy. These techniques, which can be used to estimate beef marbling, can help producers to fulfill industry and consumer demands (Indurain et al., 2009).

The ultrasound technique has also been shown to be useful in determining the nutritional quality of beef. In fact, Indurain et al. (2006) reported that the ultrasound readings reflect the effect of fatness on fat composition (e.g. the fatty acid profile of subcutaneous fat). However, there is a lack of research to demonstrate the efficiency of ultrasound in estimating beef palatability.

Regarding beef production, Spain has a number of local breeds characterized by their high muscle growth and reduced fat content. These breeds include Pirenaica, which is well suited for beef production. Pirenaica is an early maturing beef breed, producing medium-muscled lean carcasses. It accounts for 1.1 % of the Spanish national cattle stock and it is the most abundant breed for beef production under the European Union's PGI Ternera de Navarra [Navarre Veal] Protected Geographical Indication (DOUE, 2004). Pirenaica breed cattle are raised in a semi-extensive system near the mountains in the Pyrenees (Northern Spain). The animals take advantage of the mountain pastures in spring and summer, while they remain in the valleys during the winter. Young bulls are weaned at six months and are fed in feedlots with concentrate and cereal straw until slaughter at approximately twelve months of age to obtained lean carcasses.

In this context, the main objective of this study was to develop ultrasound-derived prediction equations before slaughter to estimate carcass traits and beef sensory parameters in young bulls with lean carcasses.

2. Material and methods

2.1. Pre-harvest, handling, and harvesting procedure

A total of 20 yearling Pirenaica bulls under the Protected Geographical Indication Ternera de Navarra were used. The young bulls were reared in a semi-extensive system in the mountains until weaning. After weaning at approximately seven-eight months of age, they were fed on a concentrate in a local feedlot (85% barley, 10% soybean meal, 3% vegetal fat and 2% minerals and vitamins), and barley straw, both ad libitum. The chemical composition of the feeds corresponds to a medium-energy diet: Dry matter (88.2%); Crude protein (128.5g/kg DM); Crude fiber (48.4g/kg DM); Ash (53.7g/kg DM); Metabolizable energy (11.5 MJ/kg DM). The average feed consumption during the young bulls' growth period (four months) was 7.9 kg/day. The animals were considered as a representative sample of the market, and they are commonly slaughtered in the area. The bulls were raised according to the handling conditions in the Spanish rules and regulations for animal care (Directive 2010/63/EU) and slaughtered according to Spanish rules and regulations for animal care (Council Regulation EC 1099/2009). The research followed the official guidelines for the humane treatment, care and handling of animals.

2.2. *Ultrasound data collection*

The live animals were weighed and two cross-sectional ultrasound images (right and left side) between the 12th and 13th ribs were taken in the live animals at the feedlot, as described by Wall et al. (2004) and Bergen et al. (2005). The ultrasound images were measured on the skin after clipping the hair at 50, 25 and 1 days prior to slaughter, using Sonovet 600-real-time ultrasound equipment (Madison Co. Ltd. Korea) equipped with a linear probe (3.5 MHz, 120 x 20 mm). Mineral oil (Echoultragel; Pirrone & Co.SPA, Italy) at 20-25°C was used to ensure suitable acoustic contact between the probe and the skin.

The linear ultrasound measurements recorded on the live young bulls were: intramuscular fat content or marbling (IMF), longissimus muscle depth and dorsal fat thickness. The resulting two-dimensional images (longitudinal and transversal) were digitalized and stored. The young bulls were scanned twice and the images evaluated and interpreted by a laboratory technician at the Public University of Navarra, using the

Optimas 6.5 image analysis software (Media Cybernetics Inc., USA). The ultrasound measurements of longissimus muscle depth selected were longitudinal and transversal (UA_L and UA_T respectively), and dorsal fat thickness (UF_L), since this is a linear measurement. Gray levels of the longitudinal and transversal ($UIMF_L$ and $UIMF_T$, respectively), ultrasound images for intramuscular longissimus lumborum fat content was measured with the gray level scale in the software, with 0 black and 150 white.

2.3. *Carcass evaluation*

On the day of slaughter, the young bulls were transported a distance of approximately 20 km and harvested upon arrival using standard stunning and dressing methods in a licensed slaughterhouse according to Council Regulation EC Nº 1099/2009. The young bulls were slaughtered at 521±51.4 kg live weight and 351±15 days of age and yielded an average cold-carcass weight of 323±33.8 kg.

Immediately prior to cool storage for aging, the carcasses were graded by a licensed technician for fatness and conformation according to the EU beef grading system (DOUE, 2008). The SEUROP conformation scale (S superior; E excellent; U very good; R good; O fair; P poor) was transformed, with 1 = P- and 18 = S+. On the fat cover classification scale (5 very high; 4 high; 3 average; 2 slight; 1 low), the numerical transformation of standard grades were 1 = 1- and 15 = 5+. Kidney and pelvic fat (KPF) was collected from the carcasses and weighed by a trained technician. Carcass fat thickness (CFT) was measured at the 6th rib, taking 3/4 of the length ventrally over the longissimus muscle, with a chilled stainless-steel caliber (Renand, and Fisher, 1997). In addition, the longissimus lumborum area (CA) was measured with a grid.

Twenty-four hours post-mortem, the longissimus lumborum muscle was removed at the 6-13th rib level from the left carcass side, and was transported to the Meat Science

Laboratory at the Public University of Navarra (Pamplona-Spain), where the meat was aged in the dark for seven days at 2ºC. After ageing, the muscles were cut into steaks. One steak was cut for instrumental texture analysis (3.5cm thick) and another for sensory analysis (2.5cm thick) from the second lumbar vertebra. The steaks were then vacuum packed in polyamide/polyethylene pouches (Vaeseen Schoemarket Ind., Barcelona, Spain - film thickness of 120 µm and O^2 permeability of 1 cc/m^2/24 h, CO_2 permeability of 3 cc/m^2/24 h, and N_2 permeability of 0.5 cc/m^2/24 h measured at 5 ºC and 75% relative humidity; water vapor transmission rate 3 g/m^2/24 h at 28 ºC and 100% RH; vicat softening point of sealing 97ºC, and a dart drop strength of 1300g) and stored at -20ºC for subsequent analysis.

2.4. Fat extraction

At 24 h post-mortem, a steak from the longissimus lumborum muscle at the 12th rib level was used to determine the intramuscular fat content. The total lipids were extracted from each meat product with chloroform:methanol (2:1, v/v), according to the Folch et al. (1957) method. The results were expressed as total intramuscular fat content per 100g of fresh meat. Two replicates were taken per sample.

2.5. Warner –Bratzler Shear Force

The steaks for the Warner–Bratzler shear force (WBSF) test were thawed overnight at 4ºC. The beef fillet (3.5 cm thick) was cooked on a preheated sheet previously at 180ºC, turning the fillet every 4 minutes, until reaching an internal temperature of 70ºC (AMSA, 2016). The temperature inside the steak was controlled by temperature probes and a data acquirer (thermocouple probe TB 190 and data acquirer Almemo 5990-2 V5; Ahlborn mess-un Regelungstechnik GMBH, Holzkircken, Alemania). After cooling for two hours seven cores measuring 1 cm wide, 1 cm high and 3 cm long were removed from the cooked steaks parallel to the longitudinal axis of the muscle fibers. The Shear Force analysis was carried out using a TA-XT2i texture analyzer (Stable Micro Systems, Inc., Goodming, Surrey, UK). The maximum shear force (kg) was assessed with a Warner-Bratzler shearing device following the methodology proposed by Beltrán (2005). The samples were then tempered at room temperature. The analysis was reformed with a crosshead speed of 200 mm•min^{-1}, 50 kg load cell, 40 mm distance and a calibration weight of 10 kg. The full peak shear force was recorded and the maximum shear force was calculated in kg as the mean of the seven measurements.

2.6. Trained sensory panel

The sensory analysis was performed by a seven-member trained descriptive panel selected and trained as described by Cross et al. (1978) and Meilgaard et al. (1991). The members of the descriptive panel were selected and trained as described by the ISO 8586–2014 (International Organization for Standardization) to evaluate the beef samples. The definitions used for the different sensory attributes are listed in Gorraiz et al. (2000). Juiciness, hardness, characteristic beef flavor, liver flavor and fatty flavor were evaluated, using a 150 mm unstructured line scale, with a 10 mm mark from the left, representing "low intensity", and another mark 10 mm from the right, representing "high intensity".

The frozen steaks were thawed over night at 4ºC, and cooked on a grill to an internal temperature of 70ºC measured with a TB 190 probe (data logger Almemo 5990-2 V5; Ahlborn mess-un Regelungstechnik GMBH, Holzkircken, Germany). The steaks were then cut into 1.5x2x1.5cm cubes, which were wrapped in aluminum foil with an identification tag with a random three-digit code and kept in a heat-retaining container or waterless food warmer before serving. The tasting took place in the sensory testing room at the Meat Science Laboratory of the Public University of Navarra (Pamplona-Spain) under red lighting, with controlled temperature and humidity. The tasting hall meets UNE 87004 (1979) standard requirements for sensory trials. The panelists were provided with slices of apple and water to cleanse their palate between samples. Each panelist tasted five samples per session in a random order and each sample was assessed by all seven members of the panel.

2.7. Statistical Analyses

All the data were analyzed using SPSS software (SPSS V. 25.0, SPSS Inc. USA). A statistical descriptive analysis was carried out to describe the animal and carcass characteristics, ultrasound readings, shear force and sensory position and dispersion parameters. The effects on carcass traits of the moment when the ultrasound measurements were performed were analyzed using the repeated measures GLM procedure and a post hoc Fisher comparison of means test. Carcass weight was used as a linear covariate. Pearson correlation coefficients were calculated to assess the relationship between the ultrasound readings at three given points in the animals' life (50, 25 and 1 days prior to slaughter) and the corresponding carcass and beef measurements and sensory evaluation. A partial least square methodology was used to find the relationship between the dependent variables (carcass traits, Warner-Bratzler shear force and the sensory profile after 7 days of ageing) and the ultrasound measurements. Additionally, a stepwise procedure (forward selection of variables, significance criterion $P<0.05$) was used to select the most informative ultrasound variables in the prediction equations for carcass and beef sensory traits. Both the dependent variables and the explanatory variables were centered and reduced. The best equations for the prediction of carcass and sensory parameters using ultrasound measurements at three pre-slaughter times were chosen.

3. Results and discussion

3.1. Animal, carcass and beef sensory traits

After weaning at around seven months, the animals entered the feedlot with an average weight of 323.7 kg. At slaughter, their average weight was 521.5 kg and average age 350.8 days (Table 1). The average stay of the animals on the feedlot was 128.7 days, and the average daily weight gain during the finishing period was 1.5 kg/day.

Trait	Mean	SD	Minimum	Maximum
Productive measurements				
Start feedlot weight (kg)	323.7	36.55	243	395
LWS (kg)	521.5	51.41	445	610
Age at Slaughter (days)	350.8	15.05	307	366
Days on feedlot	128.7	34.16	52	174
ADG (kg/day)	1.5	0.22	1.1	1.9
Carcass measurements				
Carcass weight (kg)	323.5	33.80	264.5	377.5
Dressing %	62.4	2.97	57.7	66.8
Conformation score[a]	9.6	0.71	8	11
Fatness score[b]	4.4	0.51	4	5
KPF (% of total carcass)	1.4	0.50	0.7	2.3
CA (cm^2)	104.4	10.95	83.8	136.7

CFT (cm)	0.2	0.06	0.1	0.5
CIMF (%)	0.8	0.21	0.7	1.0
Instrumental measurements				
WBSF (kg/cm^2)	5.9	0.44	2.2	8.2
Sensory parameters[c]				
Juiciness	5.9	0.68	3.7	9.5
Hardness	7.7	0.24	5.1	10.0
Beef flavor	6.2	2.13	3.4	7.7
Liver flavor	3.8	0.01	2.5	5.2
Fatty flavor	2.8	0.71	1.7	3.7

SD: Standard deviation; LWS: live weight at slaughter; ADG: Average daily gain; Dressing %: Dressing percentage: carcass weight*100/live weight. [a]Conformation score: carcass conformation score by comparison with EU beef carcass standard grades. Numerical transformation of standard grades were 1 = P- and 18 = S+; [b]Fatness score: carcass fatness score by comparison with EU beef carcass standard grades where numerical transformation of standard grades were 1 = 1- and 15 = 5+; KPF: Percentage of kidney and pelvic fat to carcass weight; CA= carcass longissimus lumborum area at the 12th rib measured with a grid; CFT= carcass 12th-rib fat thickness measured with a caliber; CIMF = longissimus lumborum intramuscular fat content at the 12th rib; WBSF: Warner–Bratzler shear force test; [c]Sensory parameters measured by a trained panel using a 150-mm unstructured line scale.

Table 1 Descriptive statistics for productive, carcass and beef quality traits in young bulls.

According to Albertí et al. (2005), the carcass characteristics of the young bulls used in this experiment are within the standard range of continental carcasses on the European market (Table 1). These carcasses are described as light carcasses compared to the heavier British carcasses, as reported by Eriksson et al. (2002). The values of the longissimus lumborum area and fat thickness at the 12th rib in the Pirenaica breed (mean average 104.44 cm^2 and 0.23 cm, respectively) were in line with those reported by Peña et al. (2014) in the Retinta breed and other continental beef breeds (Charolais and Limousine). Moreover, the longissimus lumborum intramuscular fat content (average 0.85%) was in the range of light carcasses on the South European market. These findings agree with those of Piedrafita et al. (2003) and Albertí et al. (2008) in calves reared under the same production systems.

Table 1 shows the sensory attributes of the meat as determined by the trained panel. From these results, the meat from these young bulls could be described as moderately juicy and tender, with a not very intense beef flavor and a slight taste of liver. The assessments given by the trained panel were similar to those reported in previous studies (Beriain et al., 2016), which reported Pirenaica beef as having a medium degree of juiciness, tenderness, flavor and a reduced fat content.

3.2. Ultrasound measurements

Table 2 shows the measurements of the longissimus lumborum muscle area (UA_L and UA_T) and UF_L obtained by means of the scans on live animals 50, 25 and 1 days prior to slaughter. The scans were also used to measure gray level ($UIMF_L$ and $UIMF_T$) to determine the longissimus lumborum intramuscular fat content at the 12th rib (grey scale 0-150). The influence of the moment the ultrasound measurements were taken on differences was significant (P <0.05) for UA_T. In fact, the lowest value of UA_T was observed at 50 days ante-mortem, while after 25 days ante-mortem, no differences in UAT measurements were observed.

	50 days ante-mortem				25 days ante-mortem				1 day ante-mortem					
	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.	SEM	*P*-value
UA_L (cm^2)	74.78	10.07	58.73	88.36	75.52	11.27	45.99	95.52	77.39	9.73	56.81	98.14	6.32	0.367
UA_T (cm^2)	86.28^{a}	13.24	63.41	104.16	93.62^{b}	13.12	72.84	117.47	94.88^{b}	14.36	76.15	127.30	8.52	0.008
UF_L (cm)	0.47	0.10	0.26	0.98	0.48	0.09	0.35	0.71	0.46	0.13	0.25	0.60	0.46	0.331
$UIMF_L$ (grey level)†	59.72^{a}	16,81	29.42	95.03	49.72^{b}	16,78	27.62	87.97	50.80^{b}	24,52	7.53	97.71	13.90	0.038
$UIMF_T$ (grey level)†	69.89^{a}	13,79	39.37	92.04	64.09^{b}	14.77	35.53	109.24	64.08^{b}	21,95	20.23	101.11	10.99	0.016

SD: Standard deviation; SEM: Standard error of the mean; UA_L: ultrasound of longissimus lumborum area at the 12th rib from longitudinal ultrasound measurement; UA_T: ultrasound of longissimus lumborum area at the 12th rib from transversal ultrasound measurements; UF_L = ultrasound of 12th fat thickness from longitudinal ultrasound measurement; $UIMF_L$: ultrasound of longissimus lumborum intramuscular fat content at the 12th rib from longitudinal ultrasound measurement; $UIMF_T$: ultrasound of longissimus lumborum intramuscular fat content at the 12th rib from transversal ultrasound measurement; ns: not significant; sig: significant; ^{a,b}: Marked differences are significant at P <0.05 in the same line; †Grey level of ultrasound image scale 0-150.

Table 2 Least mean squares and fisher-test means for ultrasound data collected in young bulls at 50, 25- and 1-days ante-mortem.

The longissimus lumborum area at the 12th rib measured with a grid (104.44 cm^2; Table 1) was greater than that predicted by the in vivo ultrasound images (which ranged from 74.78 to 94.88cm^2; Table 2). This is in line with findings published by Bergen et al. (2005), who reported a tendency for the ultrasound measurements of the loin area to be lower than the final carcass values. In contrast, Greiner et al. (2003) stated that *in vivo* ultrasound measuring techniques can overestimate carcass loin areas by between 3.31 and 6.76 cm^2 in young bulls with low musculature. On the other hand, Smith et al. (1990) reported that the CA of bulls with a longissimus lumborum area around 104 cm^2 was generally underpredicted, whereas in cattle with a longissimus lumborum area lower than 85 cm^2, it was usually overpredicted. The fact that the cited authors performed their measurements on carcasses with a longissimus lumborum area of approximately 80 cm^2, while the Spanish cattle used in this study had bigger muscles and therefore a larger loin area (> 100 cm^2), might, at least partially, explain the greater differences found in the present study.

The UF_L means across the scanning time are presented in Table 2. The mean values across the scanning time (0.47 cm) were in the range of those reported by Peña et al. (2014) in a long scanning period from 212 days ante-mortem for continental breeds such as Retinta, Charolais and Limousine, reared under similar conditions to the Pirenaica breed. No variations in the average UF_L values between the first and the last scans were observed (*P*>0.05). The range of UFL values for carcass back fat thickness observed by ultrasound (from 0.46 to 0.48 cm; Table 2) was greater than carcass 12th-rib fat thickness measured with a caliber (0.23 cm; Table 1). These results are in agreement with findings by Bergen et al. (2005) who found that the *in vivo* ultrasound values were higher than carcass measurements when the differences were >0.5 cm. In fact, Peña et al. (2014) showed an overestimation of ±0.2 cm in 98.9% of the bulls' UF_L vs CFT in young bulls of continental breeds.

In contrast, Greiner et al. (2003) and Baker et al. (2006) found an underestimation of UF_L values in young bulls when the back fat thickness was greater (0.7 cm). According to Waldner al. (1992), an improvement in the accuracy of the relationship between ultrasound and carcass measurements during the animals' maturing period up to 16 months can be observed. Perkins et al. (1992a) stated that the accuracy of the ultrasound measurements of back fat thickness is highly dependent on the degree of rib fatness during the animals' growth. On the one hand, the misinterpretation of this accuracy could occur in fatter cattle, in contrast to the improved interpretation in lighter carcasses. In the present study, in 60% of the animals, the differences between the last UF_L measurement and CFT were < 0.2 cm. This accuracy is greater than that recorded by May et al. (2000) in fatter young bulls. This result may be explained because the animals used in the present research were lean and had a very thin layer of back fat (Perkins et al., 1992b; Charagu et al., 2000).

Ultrasound grey level for the three pre-harvest periods was used to compare intramuscular fat content in beef during the growth period (Table 2). A significant decrease in the grey level in longitudinal and transversal images (around 15%) was observed between the first and last scans. No significant differences in grey levels were observed ($P>0.05$) during the last scanning period (between 25 and 1-days ante-mortem). This finding was in agreement with Albrecht et al. (2006), who observed a relative decrease in intramuscular fat in young bulls during the fattening period up to the age of twelve months in Angus steers and a marked increase in intramuscular fat deposition from 12 to 14 months of age. However, Yang et al. (2006) affirmed that there was no clear relationship between intramuscular fat content and body weight in young bulls from lean breeds. In fact, Albrecht et al. (2006) reported that the connective tissue in the muscle is often present in strong cords which are not streaked with fat, and the highest UIM_F observed in the early scans of young bulls could be attributed to the relative abundance of connective tissue in the muscle, which is likely to be confused with fat during ultrasound image processing.

3.3. *Correlations between ultrasound measurements and carcass traits*

The Pearson correlation coefficients between *in vivo* ultrasound readings (UA_L, UA_T, UF_L, $UIMF_L$ and $UIMF_T$) taken at 50, 25 and 1 days before slaughter and the values of carcass and beef parameters are presented in Tables 3 and 4, respectively. In overall terms, the ultrasound measurements taken in young bulls before slaughter correlated with some carcass parameters (Table 3), while few significant correlations with beef traits were observed (Table 4).

Ultrasound measurements[b]	Ante-mortem scan day	Carcass weight	Conformation score[a]	Fatness score[a]	KPF[a]	CA[a]	CFT[a]	CIMF[a]
UA_L	50	0.452	0.490*	-0.228	-0.425*	0.473*	-0.117	-0.533*
	25	0.203	0.511*	-0.111	-0.477*	0.377	-0.420	-0.451*
	1	0.160	0.520*	-0.084	-0.464*	0.560*	-0.268	-0.491*
UA_T	50	0.386	0.485*	-0.174	-0.529*	0.445	-0.273	-0.616*
	25	0.459*	0.703*	-0.315	-0.635*	0.614*	-0.472*	-0.288
	1	0.511*	0.738*	-0.389	-0.574*	0.398	-0.558*	-0.139
	50	-0.223	-0.091	0.270	-0.054	-0.241	0.330	-0.012

UF$_L$	25	-0.372	-0.080	0.232	-0.153	0.019	0.068	0.215
	1	-0.074	-0.047	0.293	-0.116	0.434	-0.021	-0.417
UIMF$_L$	50	-0.193	-0.263	-0.042	0.247	-0.159	0.073	0.302
	25	-0.578*	-0.540*	0.575	0.444*	-0.111	-0.123	0.324
	1	-0.413	-0.435	0.420	0.529*	-0.263	0.328	0.538*
UIMF$_T$	50	0.029	0.037	-0.189	0.636*	0.010	-0.072	0.535*
	25	-0.536*	-0.441	0.439	0.556*	-0.319	0.107	0.468*
	1	-0.491*	-0.542*	0.383	0.718*	-0.394	0.457*	0.462*

[a]Text caption, see Table 1; [b]Text caption, see Table 2; *: $P < 0.05$.

Table 3 Pearson coefficients of correlation between carcass and ultrasound measurements at different scan days before slaughter.

Carcass weight was positively correlated with UA$_T$, but negatively with ultrasound measurements for fat traits (UF$_L$, UIMF$_L$ and UIMF$_T$) in most scans (Table 3). The significant correlations between a series of ultrasound carcass measurements and carcass weight were high, with values over 0.459. These findings were in agreement with those described by Peña et al. (2014) between slaughter weight and a series of ultrasound measurements in the muscle area (r=0.503), or the intramuscular fat percentage (r=-0.335) in young bulls of Retinta, Charolais and Limousine breeds.

In general, the correlations between ultrasound measurements for muscle traits (UA$_L$ and UA$_T$) were positively related to carcass conformation and CA measured in the carcass, while negative correlations were observed between UA$_L$ or UA$_T$ and fat traits of the carcass (KPF, CFT and CIMF). The strongest significant relationships (r = 0.485–0.738) were observed between the ultrasound measurements of longissimus lumborum area, at the three pre-harvest times, and the conformation score. In fact, the correlation tended to increase with the duration of the fattening phase. The role of longissimus muscle area for predicting carcass yield has proved to be controversial (Wall et al., 2004). Numerous studies have tested its usefulness in predicting cuttability; May et al. (2000) and Realini et al. (2001) correlated rib eye area both to sub-primal cut yields and the weights of the major primals (r-value between 0.64 and 0.45). However, Crews et al. (2002) and Wall et al. (2004) stated that the USDA yield grade is not heavily influenced by the longissimus lumborum area. Hodgson et al. (1992) and Tait et al. (2005) showed that the loin eye area had a low correlation coefficient with retail yield.

The UA_L readings at 50, 25 and 1-days ante-mortem correlated negatively with the percentage of KFT and CIMF. The highest correlations between muscular ultrasound scans (UA_L and UA_T) and intramuscular fat content (r=-0.533 and -0.616, respectively) were observed at early scanning times. Wall et al. (2004) obtained regression coefficients ranging between 0.44 and 0.42 for back fat and 0.47 for the degree of marbling estimates from UA_L. The cited authors found no significant improvement in estimates from scans taken closer to the day of slaughter, but it should be noted that the scans were taken between 2 and 3.5 months prior to animal slaughter. Renand and Fisher (1997) noted that if the back-fat estimates from ultrasound measurements for a population showed a standard deviation of more than 3 mm, the coefficients of correlation between the final and ultrasound values can reach 0.6, while for a SD of less than 2 mm, they barely reach 0.3.

The ultrasound longissimus muscle depth reading (UA_L and UA_T) also appears to be correlated, albeit negatively, with various carcass fatness measurements, such as the degree of marbling and the KPF score (Table 3), revealing an inverse relationship between muscular development and final fatness (Mendizabal et al., 1999; Alberti et al., 2005). The range of back fat thickness in Pirenaica calves (Table 1) was between 0.1 and 0.45 cm, with a mean value of 0.23 cm and a standard deviation of 0.08 cm, while 40% of the UF_L estimates deviated by more than 0.25cm from CFT.

The grey level value of a scan depends on tissue composition and therefore on the degree of marbling in the muscle. In general, positive correlations between ultrasound intramuscular fat content (UIMFL and UIMFT) and fat trait were observed in the carcasses. In fact, significant correlations at all scanning times were observed between UIMFT and carcass traits, such as KPF or CIMF. The correlation increased significantly between the first and last scans for UIMFT and KPF, while the correlation between UIMFT and CIMF decreased from the first to the last scans (Table 3). These results are not in agreement with Wall et al. (2004), who reported no changes in the correlations between marbling and ultrasound scans made 100 days before slaughter to preslaughter in fattened bulls from the Angus, Simmental, Red Angus and Charolais breeds.

3.4. *Correlations between ultrasound measurements and beef quality traits*

Table 4 shows the Pearson coefficients of correlation between the measurements obtained from the *in vivo* scans (UA_L, UA_T, UF_L, $UIMF_L$ and $UIMF_T$) taken 50, 25 and 1 days prior to slaughter, the Warner-Braztler shear force scores and the sensory parameters of the meat as evaluated by a trained panel. Fatty flavor was the only sensory parameter with significant coefficients of correlation with the ultrasound area (UA_L) and ultrasound intramuscular fat ($UIMF_T$) readings. Fatty flavor correlated negatively with the *in vivo* UA_L readings taken 50, 25 and 1 days prior to slaughter, and UA_T at 50 days ante-mortem (r=-0.616). Fatty flavor was correlated positively with $UIMF_T$ at the three measurement times and only with $UIMF_L$ at 1 day prior to slaughter

($r=0.538$; $P<0.05$). Correlations between fatty flavor and UA_L or $UIMF_T$ decreased constantly from 50 days before slaughter to the pre-slaughter scans.

Ultrasound measurement[a]	Ante-mortem scan day	WBSF	Juiciness	Hardness	Beef flavor	Liver flavor	Fatty flavor
UA_L	50	0.233	0.023	0.106	0.025	-0.337	-0.533*
	25	-0.247	0.081	-0.235	0.311	-0.225	-0.451*
	1	-0.096	-0.060	-0.175	0.105	0.060	-0.491*
UA_T	50	-0.002	0.101	-0.081	0.211	-0.186	-0.616*
	25	0.018	0.204	-0.176	0.046	0.036	-0.288
	1	0.034	-0.047	-0.050	0.107	-0.096	-0.139
UF_L	50	-0.308	-0.304	0.280	0.421	-0.324	-0.012
	25	-0.365	-0.031	-0.065	0.361	-0.062	0.215
	1	0.055	-0.253	-0.238	-0.130	-0.040	-0.417
$UIMF_L$	50	0.077	-0.131	0.328	-0.119	0.243	0.302
	25	-0.335	0.011	-0.236	-0.107	0.307	0.324
	1	0.041	-0.061	0.256	0.049	0.177	0.538*
$UIMF_T$	50	0.152	0.148	0.142	-0.161	0.361	0.535*
	25	-0.162	-0.009	-0.099	0.034	0.259	0.468*
	1	0.098	-0.057	0.186	0.090	0.260	0.462*

[a] Text caption, see Table 2; WBSF: Warner–Bratzler shear force test; *: $P < 0.05$.

Table 4 Pearson coefficients of correlation between beef quality traits and ultrasound measurements at different scanning days before slaughter.

3.5. Prediction equations

With the practical aim of saving time and costs, we identified the best moment for scanning to obtain an acceptable prediction. As regards the estimation of the beef parameters, the best significant equations ($P<0.05$ and $R^2>0.5$) to predict the percentage of kidney and pelvic fat, intramuscular fat content, carcass conformation, carcass fatness and the fatty flavor of the meat are shown in Table 5. The results of the regression equations included the scores obtained from the in vivo ultrasound scans taken 50, 25 and 1 days prior to slaughter. The best prediction equations for KPF and carcass fatness score were at 1 day prior to slaughter, while those for conformation carcass were obtained at 25 days prior to slaughter. The most favorable prediction of intramuscular fat content was 50 days prior to slaughter, since at this time the ultrasound measurement was more stable than that performed one day before the animals were slaughtered. This observation can be seen in Table 2 in which the ranges of dispersion between minimum and maximum observed in the gray levels in UIMFL and UIMFT were higher when the measurements were made one day before slaughtering of the calves than those observed when the ultrasound measurements were carried out 50 days before the animals were slaughtered. This finding suggests that better carcass parameter estimates result from scans taken in the last month prior to slaughter, but not necessarily the day before harvest.

Dependent Variables$^{*, a}$	Best ante-mortem scan day^{b}	R^2	*P*-value	RMSE	Best prediction equations^{b}
KPF (%)	1	0.717	0.002	0.962	KPF (%) = $-5.27-4.16E^{-2}*UA_{L1}+4.80E^{-2}*UA_{T1}+0.60*UF_{L1}-2.91E^{-2}*UIMF_{L1}+0,11*UIMF_{T1}$
CIMF (%)	50	0.619	0.011	0.436	IMF (%) = $0.96-5.39E^{-3}*UA_{L50}-1.47E^{-2}*UA_{T50}+0.29*UF_{L50}-1.51E^{-2}*UIMF_{L50}+4.37E^{-2}*UIMF_{T50}$
Conformation score	25	0.738	0.001	0.556	Conformation score= $5.0-1.60E^{-2}*UA_{L25}+0.06*UA_{T25}+0.14*UF_{L25}-4.18E^{-2}*UIMF_{L25}+0.02*UIMF_{T25}$
Fatness score	1	0.550	0.031	0.395	Fatness score= $1.40+9.11E^{-3}*UA_{L1}-1.29E^{-3}*UA_{T1}+0.27*UF_{L1}+6.42E^{-3}*UIMF_{L1}+0.01*UIMF_{T1}$
Fatty flavor	1	0.556	0.029	0.470	Fatty flavor = $2.89-4.02E^{-2}*UA_{L1}+2.47E^{-2}*UA_{T1}-4.26*E^{-2}UF_{L1}+9.16E^{-3}*UIMF_{L1}+6.45E^{-3}*UIMF_{T1}$

*Only significant regression equations ($P<0.05$ and $R^2>0.5$) are shown.

[a]Text caption, see Table 1; [b]Text caption, see Table 2; RMSE: Root mead square error.

Table 5 Best prediction equations for young bull carcass characteristics and beef quality traits from ultrasound readings at 50, 25 and 1-days ante-mortem.

The regression coefficients (R^2) for the fat content in carcass and beef (KPF, CIMF and fatness score) were higher than 0.5 (R^2=0.717, 0.619 and 0.550 respectively) as reported also by Wall et al. (2004). The best prediction of the percentage of pelvic and kidney fat containing UA_L, UA_T, UF_L, $UIMF_L$ and $UIMF_T$ was observed at 1 day prior to slaughter. All independent variables accounted for 71.7% of the variation in KPF.

The R^2 values for the regression equations to estimate CIMF were in line with Aass et al. (2009) and Castilhos et al. (2018), who indicated that ultrasound constitute an accurate method for predicting in vivo intramuscular fat content in lean breeds. In fact, in this study, the R^2 values were higher (R^2= 0.619) than those reported by Chambaz et al. (2003) (R^2 = 0.420), but in agreement with the ones reported by Aass et al. (2009) (R^2 = 0.670) in lean beef. It can be therefore considered that in lean breeds, intramuscular fat can be predicted early in young bulls because intramuscular fat depot is a late-maturing tissue and changes cannot be expected at an early slaughter age.

With reference to sensorial variables, the best prediction equation for fatty flavor of beef (R^2=0.556; P=0.029) was obtained 1 day before slaughter. In accordance with Table 4, fatty flavor can be predicted from in vivo scans, using the combination of all the ultrasound measurements from the scan taken 1 day prior to slaughter, including a combination of UA_L, UA_T, UF_L, $UIMF_L$ and $UIMF_T$ measurements, which account for 56% of the variation in the fatty flavor of beef from young bulls.

In early scans, fat content could determine fatty flavor, while difficulties in predicting sensory parameters related to muscle development at different scanning times can be observed. A higher intramuscular fat content should increase oiliness, which plays a role in increased juiciness and causes meat to be perceived as more tender, as noted by Savell and Cross (1988). In addition, fat tissue is the main depot for the precursors of the volatile compounds produced when cooking beef, through Maillard's browning reaction and the oxidation of lipids (Mottram, 1998). The fat content in muscle correlates significantly with aroma intensity (Savell and Cross 1988), so that pleasant-tasting beef will tend to be fattier, with a higher degree of marbling (Berry et al., 1980). In fact, there is some controversy over the fat percentage in muscle required to achieve the optimal flavor intensity: Denoyelle (1995) defended a minimum of 4% of fat in muscle, whereas Savell and Cross (1988) lowered the percentage to 3%. However, Spanish beef is characterized by a low fatness level and marketed carcasses show less than 3-4% intramuscular fat in the longissimus lumborum muscle (Indurain et al., 2006). The animals used in the current study had a low percentage of intramuscular fat in the longissimus lumborum muscle (0.85 % of fresh meat) and, even at this low percentage, fatness would seem to have a positive impact on beef palatability. As in the estimation of the KPF and fatness scores, the best scans for predicting fatty flavor sensory quality are taken just prior to slaughter, although the best prediction of intramuscular fat could be propossed early.

Table 6 shows the best ultrasound variables to contribute to an acceptable prediction of carcass and beef parameters in young bulls from the Pirenaica breed. To predict KPF, the conformation and fatness score from two ultrasound measurement scans accounted for the highest variation, while for all the other parameters, only one ultrasound measurement accounted for the highest variation.

Dependent variables [*, a]	Independent variables[b]	R^2	*P*-value	RMSE
KPF (kg)	$UIMF_{T1}$; UF_{L1}	0.621	<0.001	1.010
CA (cm^2)	UA_{T25}	0.408	0.002	11.134
CFT (cm)	$UIMF_{T1}$	0.210	0.042	0.078
CIMF (%)	$-UA_{T50}$	0.306	0.011	0.519
Conformation score	UA_{T25}; $-UIMF_{L25}$	0.689	< 0.001	0.550

Fatness score	$UIMF_{L1}$; UF_{L1}	0.462	0.005	0.390
WBSF (kg/cm^2)	-UF_{L25}	0.225	0.035	1.394
Fatty flavor	$UIMF_{L1}$	0.279	0.017	0.529

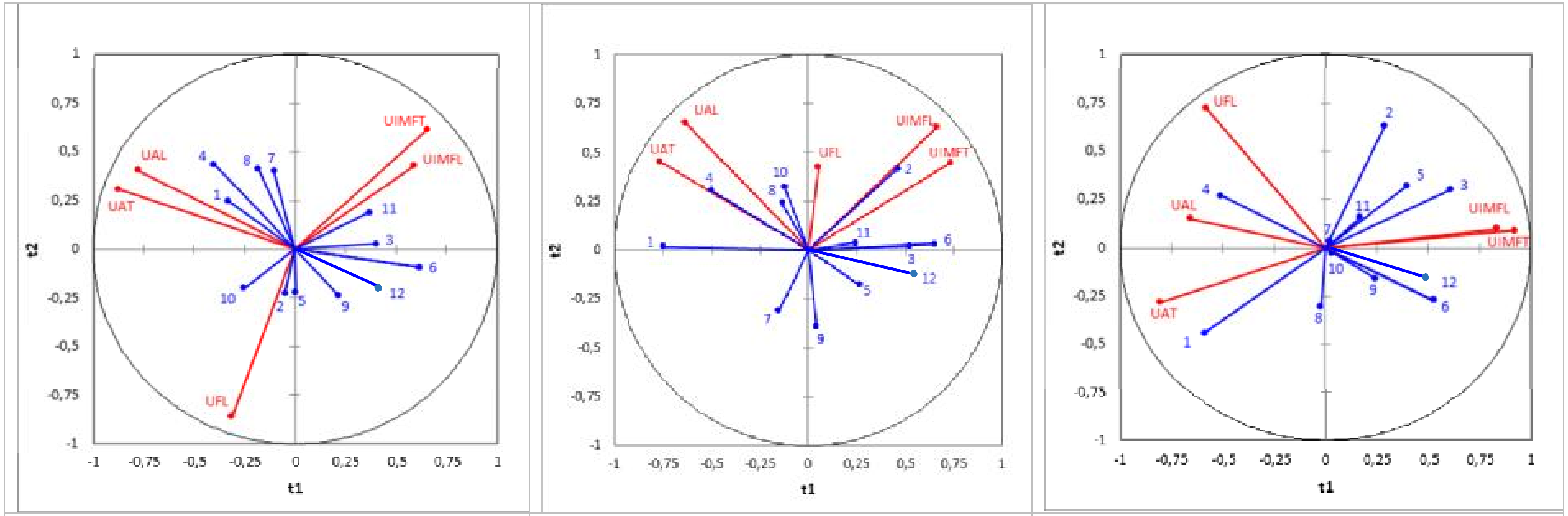
*Only significant variables ($P<0.05$) are showing.

^{a}Text caption, see Table 1; ^{b}Text caption, see Table 2; Represented values scan at 50, 25 and 1-days before slaughter; RMSE: Root mead square error.

Table 6 Regression to predict young bull carcass and beef quality traits corresponding to the selected ultrasound measurements.

To predict KPF, CFT, carcass conformation, carcass fatness, WBSF and fatty flavor, ultrasound measurements from fat are the main requirements. However, to predict CA, CIMF and carcass conformation, what is needed is mainly ultrasound measurements from the muscular area (UA).

In general, the best ultrasound measurement to determinate fat traits (KPF, CFT, fatness score and fatty flavor) were determined close to slaughter (1 day pre-slaughter), while the best scan time to determine CA, conformation and intramuscular fat content was a longer time before slaughter. In KPF prediction, the contribution of measurements related to fat content at an early pre-slaughter time ($UIMF_T$ and UF_L) were positive (R^2=0.621; $P<0.001$). In CFT, the contribution of $UIMF_T$ prediction was positive too (R^2=0.210; $P<0.042$). In the case of carcass fatness prediction, $UIMF_L$ and UF_L both contributed positively (R^2=0.462; $P<0.005$). The contribution of $UIMF_L$ to predict fatty flavor in beef at 1 day pre-slaughter of the young bulls was positive (R^2=0.279; $P<0.017$) (Table 6). At 25 days pre-slaughter, the contribution of UAT was positive (R^2=0.408; $P<0.002$) to predict the longissimus lumborum area. To predict carcass conformation, UAT (positively) and UIMFL (negatively) both contributed to explaining the best prediction equation (R^2=0.689; $P<0.001$). As regards the prediction of WBSF, the UFL measurement at 25 days pre-slaughter contributed to explaining negatively 22.5% of the variation in beef tenderness. In intramuscular fat content prediction, the negative contribution of the UA_T measurement at 50 days pre-slaughter was the main factor ($R^2=0.306$; $P<0.011$). This measurement, which evaluates the animals' muscle development, should be taken at 50 days prior to slaughter, when the animals are starting the fattening period to predict beef fat content. To provide a clearer picture of the data, Figure 1 shows the charts involving all the correlations between the components and explanatory and dependent variables at 50, 25 and 1-days ante mortem. Increasing the distance of the biplot points to the center of the graph indicates greater correlation between the components and explanatory and dependent variables.



50 day ante-mortem | 25 days ante-mortem | 1 day ante-mortem

1, Conformation score; 2, fatness score; 3, kidney and pelvic fat; 4, longissimus lumborum area; 5, carcass fat thickness; 6, intramuscular fat; 7, Warner–Bratzler shear force; 8, juiciness; 9, hardness; 10, beef flavor; 11, liver flavor; 12, fatty flavor.

Fig. 1. Biplot of the correlations between the explanatory and dependent variables with the first two components generated (t1 and t2) by the partial least squares (PLS) regression algorithm.

In general, the R^2 values for the best ultrasound measurements to determine the sensory parameters of beef were low (range R^2= 0.089 to 0.279; data not shown in table) in reference to carcass parameters (Figure 1). Only the significant variable regression to predict young bull sensorial quality traits corresponding to the selected ultrasound measurements for fatty flavor was observed.

4. Conclusions

While it is possible to use ultrasound to predict carcass traits in young bulls of lean breeds, ultrasound testing to predict pre-slaughter sensory beef parameters is not common. In order to predict intramuscular fat content in young bulls from lean cattle breeds, early ultrasound scans of the longissimus lumborum area measurement are recommended during fattening. However, to predict kidney and subcutaneous fat depots, ultrasound scans taken close to slaughter including intramuscular fat content and measurement of fat thickness are recommended. The only sensory variables which showed a relationship with the ultrasound measurements performed on the live animals at different times during the fattening of the young bulls were those related to the fat content in meat from young bulls of the Pirenaica breed. In this context, it is advisable to predict the fatty flavor of beef ultrasound scan of intramuscular fat measurement close to moment when the young bulls are slaughtered. However, further studies with other fatness bovine breeds would be needed to determine the validity of the use of ultrasound to predict the sensory fat parameters of bovine meat.

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