Effect of the CAPN1 316 and 4751, CAST 282 and 2959, and LEP E2FB and E2JW markers upon the carcass and meat characteristics in Brahman livestock

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Abstract

Livestock meat production in Colombia is mostly based upon brahman breed specimens. Nonetheless, their meat has deficient-quality organoleptic characteristics since the alleles of the SNPs CAPN316, CAPN4751, CAST282, CAST2959, E2FB, and E2JW that favors them are found in very low amounts. In contrast, the most frequent alleles might contribute to their rusticity and adaptability to the lower tropic. This study assessed the effect of these markers upon the productive and meat quality characteristics in regards to the live weight, the sacrifice age, the weight and yielding of the hot carcass, the leg perimeter, the loin eye area, the ICTA carcass classification, the intramuscular fat, protein and moisture during the slaughter process, the pH, the water holding capacity, the Warner-Bratzler's shear force and the texture profile during the first, seventh and fourteenth day of maturation. Furthermore, the study wanted to identify whether these markers were associated with the muscle µ-calpain and calpastatin content. It was found that the CAPN4751, CAST282, and CAST2959 markers affect most of the productive and meat quality characteristics on brahman breed livestock that are raised and fattened under the lowland´s Colombian tropic conditions. Nevertheless, even though a significant amount of differences were found among the genotypes, these were of a low magnitude.

Introduction

The calpain-calpastatin complex is made of a group of cysteine proteases depending on the Ca^{2+}. The calpain 1 or the µ-calpaína and the calpain 2 or m-calpain (Goll et al., 2003; Pratiwi et al., 2016) and its inhibitor, the calpastatin (Campbell and Davies, 2012; Pratiwi et al., 2016) are the most studied (Casas et al., 2000; Page et al., 2002; Williams et al., 2009; Coria, Carranza and Palma, 2018). These proteins are involved in the renovation of the muscular tissue (Gerken et al., 1995), the growth regulation (Motter et al., 2013), and the performance of the animals in hostile environments (Cafe et al., 2010a), among others. On its behalf, leptin participates in the regulation of appetite and weight homeostasis, the corporeal composition, and the deposition of fat in the muscles. These functions are related to the production and meat quality characteristics of cattle (Cafe et al., 2010a; Motter et al., 2013; Allais et al., 2014; Calvo et al., 2014; Lee et al., 2014; Fernandes et al., 2020).

The µ-calpain is codified by the CAPN1 gene (GenBank: AH009246.3), which is mapped in the telomeric region of chromosome 29 of the Bos taurus (BTA29) (Smith et al., 2000); calpastatin is codified by the CAST gene (GenBank n.º AY008267), located in BTA7 (Barendse, 2002); and leptin is codified by the LEP gene (GenBank n.º U50365), located in BTA4 (Schenkel et al., 2005). These genes have single nucleotide polymorphisms (SNP) associated with some of the productive and meat quality characteristics: the C alleles of CAPN316 (AF252504:g.5709C > G) (Page et al., 2002), CAPN4751 (AH009246:g.6545C > T) (White et al., 2005) and CAST282 (AY008267:g.282C > G) (Schenkel et al., 2006); A of CAST2959 (AF159246:g.2959G > A) (Morris et al., 2006); and T of E2FB (AY138588:g305C > T) (Buchanan et al., 2002) and E2JW (AY138588:g252A > T) (Lagonigro et al., 2003) favor the expression of these characteristics in *Bos taurus* livestock.

Livestock meat production in Colombia is based upon Bos indicus animals (Novoa and Usaquén, 2010; Gómez et al., 2013), mostly from the brahman breed (Duitama et al., 2013, Martínez et al., 2016). This breed is a product of the crossing between taurine and indicine breeds. Nevertheless, their phenotypic (Rodríguez, 1993) and genetic (Meirelles et al., 1999) characteristics are indicine. It arrived in the country at the beginning of the last century (Rodríguez, 1993) and, due to its rusticity, it adapted to the lower tropic conditions. Thus, these animals have had productive advantages such as a greater weight gain in a short period and with the lowest amount of resources in comparison with taurine breeds (Flowers et al., 2018) under these same conditions. Although some authors question the organoleptic quality of the meat of brahman breed animals (Wheeler, Cundiff and Koch, 1994; Flowers et al., 2018), this product is highly demanded a sector of consumers in search of low-fat diets and with healthier fat acids profile (Flowers et al., 2018). Because of the aforementioned, this work's purpose is to determine the possible association of the CAPN316, CAPN4761, CAST282, CAST2959, E2JW, and E2FB SNPs with some productive and meat quality characteristics in the brahman breed livestock in commercial-extensive meat production systems in Colombia.

Materials And Methods

Study population

With the approval of the institutional committee for the use and care of animals (Cicua) of CES University (act number 5 of the 20th of August 2013, project 38), 410 males from the brahman breed from four livestock companies dedicated to the production of meat in improved-extensive production systems and rotational pasturage conditions were studied. Two of the companies are dedicated to full cycle production in the municipalities of Puerto Berrío (Antioquia) (n=102) and San Marcos (Sucre) (n=102), while the other two are located in Montería (n=103) and Pueblo Nuevo (Córdoba) (n=103) are dedicated to a fattening type of production.

Genotyping

The animals were genotyped for the CAPN316, CAPN4751, CAST282, CAST2959, E2FB, and E2JW SNPs through PCR-RFLP and PCR-HRM. The methodology were described in López-Rojas et al., (2017a, b) and in table 1 the allelic and genotypic frequencies among the general population are summarized.

Table 1
Allelic and genotypic frequencies of the studied markers.

SNP	Frequencies									
	Genotypes						Alleles			
CAPN316	CC	0,01	CG	0,06	GG	0,93	C	0,04	G	0,96
CAPN4751	CC	0,20	CT	0,14	TT	0,66	C	0,27	T	0,73
CAST282	CC	0,20	CG	0,31	GG	0,50	C	0,35	G	0,65
CAST2959	AA	0,52	AG	0,28	GG	0,20	A	0,66	G	0,34
E2FB	TT	0,07	CT	0,31	CC	0,62	T	0,22	C	0,78
E2JW	TT	0,00	AT	0,01	AA	0,99	T	0,01	A	0,99

Source: own elaboration.

Characteristics of the carcass

The animals were inspected before the slaughter in a commercial meat packing plant (Frigocolanta). The following aspects were registered: origin, live weight (LW, Kg), and age (calculated through the dental and bone development). These values were determined during the slaughter: the hot carcass weight (body of the bovine without head, legs, hands, skin, and entrails) (HCW, Kg), the leg perimeter (LP, cm), and the hot carcass yielding (HCY, %). During the butchering process, the following aspects were determined: the meat yieldings (MY, %), area of the loin eye (LEA, cm2) and the ICTA (Instituto colombiano de normas técnicas y certificación, 1997 [Colombian Institute for Technical Standards and certifications in English]) qualification for the carcass.

Chemical composition of the meat

In a 100g fragment of the *Longissimus dorsi* muscle, the following data were determined: the fat content (IMF, %) using the Soxhlet extraction method (Instituto colombiano de normas técnicas y certificación, 1973), the protein content (IMP, %) using the Kjeldahl method (Instituto colombiano de normas técnicas y certificación, 1999) and the moisture (IMM, %) through the thermogravimetric method at 103ºC (International Organization for Standardization, 1999) in the Bromatological and Chemical Analysis laboratory of the National University of Colombia, Medellin headquarters.

Quality characteristics of the meat

Three pieces of 300 g of the *Longissimus dorsi* muscle were vacuum sealed and matured at refrigeration temperature for 1 (D1), 7 (D7), and 14 (D14) days. After maturation, the samples were stored at -80 ºC. To perform the physical, chemical, and organoleptic analysis of the meat, the samples were defrosted to room temperature, taken out of the vacuum packing and put into polypropylene bags, and cooked until

they reached 70 ºC in average internal temperature with a thermometer with a thermocouple (HI93501, Hanna Instruments). After that, the samples were taken to room temperature to proceed to pH, water holding capacity (WHC, %), Warner and Bratzler's shear force (WBSF, KgF), and texture profile analysis (TPA) as follows:

The pH was determined using a pH meter for meat (Hanna Instruments, HI 99163). The WHC was determined by measuring the weight loss during the cooking (CL) of the meat samples with a 2,5 cm thickness, which were measured before (W1) and after (W2) cooking, CL = [(W1 – W2) / W1)] 100 (Bertram et al., 2003). The WBSF was measured in 1 x 1 x 2,5 cm (height x width x length) meat samples cut perpendicularly to the muscle fibers and using a Warner-Bratzler shear cell in a texturemeter (TA.XT2, Stable Micro Systems), in a 3,33 mm/s speed. The WBSF corresponds to the highest value in the time-force curve. The texture profile analysis was performed with a texturemeter (TA-XT2i, Stable Micro Systems) using 2,5 cm in diameter and 1,4 cm in height meat cylinders cut with a puncher. Each sample was submitted to two cycles of compression using a 75mm in diameter plate (SMSP/75) in a load cell of 50kg. With the information aforementioned the force-time graphs were built and these values were determined: hardness (kg), adhesivity (kg x s), chewiness (kh), cohesiviness, gumminess, resilience, and elasticity.

µ-calpain and the calpastatin quantification

The µ-calpain and the calpastatin content in the muscle through ELISA, using the ovine Calpain-1 catalytic subunit (CAPN1) and Bovine Calpastatin (CAST) ELISA kit (CUSABIO Life science, College Park) commercial cases, following the manufacturer’s instructions.

Statistical analysis

An association study was carried out to assess the effect of the CAPN316, CAPN4751, CAST282, CAST2959, E2FB, and E2JW markers upon the productive (LW, HCW, LP, HCY, MY, LEA and ICTA), meat quality characteristics (IMF, IMP, IMM, pH, WHC, WBSF, TAE) and the m-Calpain and Calpastatin contents according to the following model:

$$Y_{ijk} = m + F_i + GC_j + G_k + e_{ijk}$$

Where Y_{ijk} represents the variable that depends on the analysis, i.e, the productive or meat quality characteristics to be assessed; F_i is the effect of the livestock company, i number; GC_j is the fixed effect of the j- contemporary group that was composed by the monthly (March, July, and October) and yearly (2014 and 2015) slaughter effects; and G_k represents the fixed effect of the genotype for each gene.

Once the model was defined for determining the association among the polymorphisms of the genes and productive and meat quality variables, the study included each one of the markers in the association model, for which two different analyses were used. The first analysis included the genotypes as a fixed effect for determining the association among these and the answer variable. In the second analysis,

value numbers were assigned to the genotypes for transforming them into a co-variable and thus estimating the effect upon the allelic substitution as a regression coefficient between the variable and the genotype. Thus, number "0" corresponds to the highest frequency homozygous, "1" to the heterozygous, and "2" to the least frequent homozygous. When significance ($p < 0,05$) was found in a marker, a multiple means comparison was made through the lsmeans package in the R software.

Results And Discussion

Table 1 shows the allelic and genotypic frequencies of CAPN316, CAPN4751, *CAST282, CAST2959, E2FB and E2JW SNP*s genotyped by PCR-RFLP and PCR-HRM. Table 2 shows the least squares means of the studied Brahman population regarding the characteristics evaluated. The study evidenced an effect of the livestock and contemporary group upon most of the studied variables, Table 3 presents the markers that had significant effects ($p < 0,05$) upon the characteristics, the minimum square means for genotypes, the regression coefficients (β_1) of the allelic substitution effects of CAPN316, CAPN4751, CAST282, CAST2959, E2FB, and E2JW SNPs when they were significant ($p < 0,05$) upon the production and meat quality variables.

Table 2
Findings on productivity and quality in cattle meat production.

Variable	Day	Mean	SD	Min	Max	CV
Age	D0	31,55	6,0	19,5	47,8	19,06
LW	D0	435,41	40,0	357	652	9,18
HCW	D0	246,23	27,3	196	365	11,10
HCY	D0	56,41	1,91	52,26	60,50	3,39
MY	D0	38,42	1,40	35,14	41,99	3,65
LP	D0	79,52	6,50	72,87	101,90	8,17
ICTA	D0	4,08	0,72	2,00	5,00	17,66
LEA	D0	72,03	9,54	51,58	96,01	13,25
IMF	D0	3,02	1,71	0,60	8,55	56,68
IMM	D0	72,07	1,61	67,00	77,20	2,23
IMP	D0	22,41	0,76	20,40	25,40	3,39
pH	D1	5,81	0,13	5,52	6,12	2,18
	D7	5,93	0,10	5,68	6,18	1,70
	D14	5,86	0,11	5,64	6,14	1,80
WHC	D1	26,87	3,03	20,49	35,86	11,28
	D7	25,58	2,83	19,77	33,71	11,08
	D14	25,17	2,87	19,07	33,60	11,42
HAR	D1	5,71	1,21	2,57	8,98	21,13
	D7	5,02	1,26	2,03	9,54	25,17
	D14	4,83	1,28	2,20	8,77	26,50
ADH	D1	-1,62	0,85	-3,00	-0,17	-52,54
	D7	-1,95	0,96	-3,49	-0,20	-49,06
	D14	-2,19	1,04	-3,96	-0,26	-47,58

Adhesivity (ADH, kg x s), area of the loin eye (LEA, cm^2), water holding capability (WHC, %), carcass classification (ICTA), cohesiviness (COH), intramuscular humidity content (IMH, %), intramuscular fat (IMF, %) and intramuscular protein (IMP, %), age (age, months), after dressing (D0), days 1 (D1), 7 (D7) and 14 (D14) of maturation, hardness (HAR, kg), elasticity (ELA), Warner-Bratzler's shear force (WBSF, KgF), gumminess (GUM), chewiness (MAS, kg), leg perimeter (LP, cm), hot carcass weight (HCW, kg), live weight (LW, kg), hot carcass yielding (HCY, %), meat yielding (MY,%), resilience (RES).

Variable	Day	Mean	SD	Min	Max	CV
ELA	D1	0,62	0,07	0,46	0,76	10,74
	D7	0,64	0,05	0,40	0,77	8,54
	D14	0,65	0,05	0,51	0,85	7,55
COH	D1	0,54	0,05	0,44	0,76	8,67
	D7	0,53	0,04	0,34	0,75	7,97
	D14	0,52	0,03	0,44	0,62	5,29
GUM	D1	2,98	2,32	0,13	9,81	78,05
	D7	1,89	1,62	0,00	8,25	85,72
	D14	2,51	1,81	0,03	8,58	72,20
CHE	D1	1,91	1,50	0,08	8,93	78,40
	D7	1,18	0,98	0,00	5,35	82,94
	D14	1,56	1,07	0,00	6,59	68,48
RES	D1	0,29	0,02	0,23	0,35	7,94
	D7	0,28	0,02	0,22	0,33	7,51
	D14	0,29	0,02	0,24	0,34	6,48
WBSF	D1	6,82	1,42	2,13	9,56	20,81
	D7	6,44	1,47	2,21	10,81	22,91
	D14	5,63	0,65	3,34	7,53	11,59

Adhesivity (ADH, kg x s), area of the loin eye (LEA, cm^2), water holding capability (WHC, %), carcass classification (ICTA), cohesiviness (COH), intramuscular humidity content (IMH, %), intramuscular fat (IMF, %) and intramuscular protein (IMP, %), age (age, months), after dressing (D0), days 1 (D1), 7 (D7) and 14 (D14) of maturation, hardness (HAR, kg), elasticity (ELA), Warner-Bratzler's shear force (WBSF, KgF), gumminess (GUM), chewiness (MAS, kg), leg perimeter (LP, cm), hot carcass weight (HCW, kg), live weight (LW, kg), hot carcass yielding (HCY, %), meat yielding (MY,%), resilience (RES).

Table 3
Genotypes with association upon the carcass and meat quality in brahman livestock

SNPs	Variable	Day	P-value*	Adjusted means by genotype ± Standard deviation			Allelic substitution	
CAPN316				CC (n = 5)	CG (n = 25)	GG (n = 380)	β_1	P value**
	Capn1	D0	0,003	1,47 ± 0,11^{a}	1,76 ± 0,03^{b}	1,66 ± 0,01^{a}	0,04	0,12
	HAR	D1	0,010	5,42 ± 0,57^{ab}	5,24 ± 0,18^{a}	5,78 ± 0,05^{b}	-0,44	0,00
	CHE	D7	0,036	1,82 ± 0,39^{a}	0,94 ± 0,14^{a}	0,84 ± 0,04^{a}	0,24	0,04
	WBSF	D14	< 0,001	5,57 ± 0,27^{a}	5,16 ± 0,11^{a}	5,61 ± 0,03^{a}	-0,28	0,00
CAPN4751				CC (n = 82)	CT (n = 57)	TT (n = 271)		
	LW	D0	0,028	431,5 ± 2,62^{ab}	432,1 ± 2,70^{b}	425,8 ± 1,36^{a}	3,25	0,01
	CY	D0	0,001	55,94 ± 0,15^{a}	56,75 ± 0,16^{b}	56,34 ± 0,08^{b}	-0,16	0,03
	LEA	D0	0,005	72,30 ± 0,98^{a}	75,80 ± 1,04^{b}	72,00 ± 0,53^{a}	0,50	0,31
	Capn1	D0	< 0,01	1,85 ± 0,02^{c}	1,80 ± 0,02^{b}	1,61 ± 0,01^{a}	0,13	< 0,01
	HAR	D1	0,001	5,45 ± 0,10^{a}	5,82 ± 0,11^{b}	5,86 ± 0,06^{b}	-0,15	0,01
	ELA	D1	0,028	0,62 ± 0,01^{b}	0,60 ± 0,01^{a}	0,61 ± 0,00^{a}	0,01	0,03
	GUM	D1	0,000	2,52 ± 0,20^{a}	3,71 ± 0,20^{b}	2,48 ± 0,11^{a}	0,16	0,11

Adhesivity (ADH, kg x s), loin eye area (LEA, cm^2), water holding capacity (WHC, %), classification o the carcass (ICTA), regression coefficient (β_1), cohesiveness (COH), moisture content (IMM, %), intramuscular fat (IMF, %) and intramuscular protein (IMP, %), age (age, months), after slaughtering (D0) days 1 (D1), 7 (D7) and 14 (D14) of maturation, Hardness (HAR, kg), elasticity (ELA), Warner-Bratzler's shear force (WBSF, KgF), gumminess (GUM), chewiness (CHE, kg), muscle µ-calpain (Capn1) and calpastatin content (Cast), leg perimeter (LP, cm), weight of the hot carcass (HCW, kg), live weight (LW, kg), carcass yielding (CY, %), meat yielding (MY,%), resilience (RES), the letters mean statistic difference (a < b < c). P-value of the association model (*), P-value of the substitution model (**).

SNPs	Variable	Day	P-value*	Adjusted means by genotype ± Standard deviation			Allelic substitution	
	WBSF	D7	0,001	6,28 ± 0,14^{a}	6,85 ± 0,15^{b}	6,84 ± 0,07^{b}	-0,16	0,04
	HAR	D7	0,001	4,47 ± 0,12^{a}	4,88 ± 0,13^{b}	4,92 ± 0,07^{b}	-0,22	0,00
	pH	D14	0,048	5,85 ± 0,01^{ab}	5,84 ± 0,01^{a}	5,87 ± 0,01^{b}	0,04	0,78
	WBSF	D14	< 0,001	5,38 ± 0,06^{a}	5,49 ± 0,06^{a}	5,67 ± 0,03^{b}	-0,13	0,00
	HAR	D14	< 0,001	4,18 ± 0,12^{a}	4,82 ± 0,13^{b}	4,76 ± 0,07^{b}	-0,25	0,00
CAST282				CC (n = 82)	CG (n = 123)	GG (n = 205)		
	CY	D0	0,023	56,08 ± 0,14^{a}	56,22 ± 0,12^{a}	56,59 ± 0,10^{b}	-0,19	0,02
	IMF	D0	0,000	2,10 ± 0,13^{a}	2,69 ± 0,10^{b}	2,62 ± 0,08^{b}	-0,21	0,01
	IMM	D0	0,004	72,57 ± 0,15^{b}	72,10 ± 0,12^{a}	72,02 ± 0,10^{a}	0,25	0,00
	Cast	D0	< 0,01	0,34 ± 0,01^{a}	0,45 ± 0,01^{b}	0,55 ± 0,00^{c}	-0,10	< 0,01
	pH	D1	0,000	5,84 ± 0,01^{b}	5,79 ± 0,01^{a}	5,81 ± 0,01^{a}	0,01	0,04
	WBSF	D1	0,021	6,82 ± 0,13^{a}	7,16 ± 0,11^{ab}	7,24 ± 0,09^{b}	-0,20	0,01
	HAR	D1	0,006	5,47 ± 0,10^{a}	5,76 ± 0,08^{b}	5,85 ± 0,07^{b}	-0,18	0,00
	WHC	D7	0,013	25,20 ± 0,23^{a}	25,80 ± 0,24^{b}	25,20 ± 0,12^{a}	0,07	0,58

Adhesivity (ADH, kg x s), loin eye area (LEA, cm^2), water holding capacity (WHC, %), classification o the carcass (ICTA), regression coefficient (β_1), cohesiveness (COH), moisture content (IMM, %), intramuscular fat (IMF, %) and intramuscular protein (IMP, %), age (age, months), after slaughtering (D0) days 1 (D1), 7 (D7) and 14 (D14) of maturation, Hardness (HAR, kg), elasticity (ELA), Warner-Bratzler's shear force (WBSF, KgF), gumminess (GUM), chewiness (CHE, kg), muscle µ-calpain (Capn1) and calpastatin content (Cast), leg perimeter (LP, cm), weight of the hot carcass (HCW, kg), live weight (LW, kg), carcass yielding (CY, %), meat yielding (MY,%), resilience (RES), the letters mean statistic difference (a < b < c). P-value of the association model (*), P-value of the substitution model (**).

SNPs	Variable	Day	P-value*	Adjusted means by genotype ± Standard deviation			Allelic substitution	
	HAR	D7	< 0,001	4,53 ± 0,11^{a}	4,66 ± 0,09^{b}	5,09 ± 0,07^{b}	-0,31	0,00
	WBSF	D14	< 0,001	5,40 ± 0,06^{a}	5,54 ± 0,05^{a}	5,70 ± 0,04^{b}	-0,15	0,00
	HAR	D14	0,035	4,47 ± 0,12^{a}	4,56 ± 0,09^{ab}	4,80 ± 0,08^{b}	-0,18	0,02
	ADH	D14	> 0,05	-2,26 ± 0,12^{a}	-2,34 ± 0,10^{a}	-2,10 ± 0,08^{a}	-0,10	0,01
CAST2959				AA (n = 213)	AG (n = 115)	GG (n = 82)		
	MY	D0	0,028	38,33 ± 0,06^{a}	38,57 ± 0,07^{b}	38,41 ± 0,09^{ab}	0,00	0,94
	LEA	D0	0,020	73,80 ± 0,59^{b}	72,20 ± 0,77^{ab}	71,00 ± 0,91^{a}	-1,10	0,03
	Cast	D0	< 0,01	0,46 ± 0,01^{a}	0,48 ± 0,01^{a}	0,56 ± 0,01^{b}	0,05	< 0,01
	pH	D1	> 0,05	5,80 ± 0,01^{a}	5,82 ± 0,01^{a}	5,83 ± 0,01^{a}	0,01	0,03
	WHC	D1	0,017	26,70 ± 0,14^{a}	27,00 ± 0,20^{ab}	27,40 ± 0,22^{b}	0,28	0,03
	WBSF	D1	0,008	7,02 ± 0,08^{a}	7,17 ± 0,10^{ab}	7,45 ± 0,12^{b}	0,21	0,00
	HAR	D1	< 0,001	5,60 ± 0,06^{a}	5,82 ± 0,09^{ab}	6,05 ± 0,10^{b}	0,23	0,00
	ADH	D1	0,038	-1,60 ± 0,06^{a}	-1,55 ± 0,08^{ab}	-1,32 ± 0,10^{b}	0,13	0,02
	ELA	D1	0,036	0,61 ± 0,00^{ab}	0,62 ± 0,01^{b}	0,60 ± 0,01^{a}	0,00	0,16

Adhesivity (ADH, kg x s), loin eye area (LEA, cm^2), water holding capacity (WHC, %), classification o the carcass (ICTA), regression coefficient (β_1), cohesiveness (COH), moisture content (IMM, %), intramuscular fat (IMF, %) and intramuscular protein (IMP, %), age (age, months), after slaughtering (D0) days 1 (D1), 7 (D7) and 14 (D14) of maturation, Hardness (HAR, kg), elasticity (ELA), Warner-Bratzler's shear force (WBSF, KgF), gumminess (GUM), chewiness (CHE, kg), muscle µ-calpain (Capn1) and calpastatin content (Cast), leg perimeter (LP, cm), weight of the hot carcass (HCW, kg), live weight (LW, kg), carcass yielding (CY, %), meat yielding (MY,%), resilience (RES), the letters mean statistic difference (a < b < c). P-value of the association model (*), P-value of the substitution model (**).

SNPs	Variable	Day	P-value*	Adjusted means by genotype ± Standard deviation			Allelic substitution	
	HAR	D7	< 0,001	4,77 ± 0,07^{a}	4,72 ± 0,09^{a}	5,30 ± 0,11^{b}	0,16	0,02
	WBSF	D14	< 0,001	5,50 ± 0,04^{a}	5,64 ± 0,05^{b}	5,84 ± 0,06^{c}	0,20	0,00
E2FB				CC (n = 254)	CT (n = 127)	TT (n = 29)		
	HCW	D0	0,012	239,9 ± 0,99^{a}	238,8 ± 1,42^{a}	248,5 ± 2,91^{b}	1,80	0,14
	CY	D0	0,031	56,39 ± 0,08^{ab}	56,18 ± 0,11^{a}	56,83 ± 0,24^{b}	0,02	0,88
	MY	D0	0,010	38,49 ± 0,05^{a}	38,24 ± 0,07^{a}	38,45 ± 0,16^{ab}	-0,14	0,03
	IMF	D0	< 0,01	2,48 ± 0,07^{a}	2,53 ± 0,10^{ab}	3,00 ± 0,22^{b}	0,16	0,07
	Cast	D0	0,011	0,49 ± 0,01^{ab}	0,47 ± 0,01^{a}	0,53 ± 0,01^{b}	0,00	0,95
	HAR	D1	0,073	5,82 ± 0,06^{b}	5,69 ± 0,08^{ab}	5,44 ± 0,17^{a}	-0,16	0,03
	pH	D7	0,028	5,94 ± 0,01^{b}	5,92 ± 0,01^{a}	5,92 ± 0,01^{ab}	-0,01	0,05
	HAR	D7	> 0,05	4,94 ± 0,07^{a}	4,73 ± 0,09^{a}	4,63 ± 0,19^{a}	-0,17	0,04
	WHC	D14	0,017	25,10 ± 0,16^{a}	25,70 ± 0,21^{b}	24,50 ± 0,46^{a}	0,08	0,67
	WBSF	D14	< 0,001	5,35 ± 0,03^{a}	5,50 ± 0,05^{a}	5,67 ± 0,10^{b}	-0,16	0,00
	COH	D14	0,036	0,52 ± 0,00^{a}	0,52 ± 0,00^{a}	0,53 ± 0.00^{a}	0,00	0,02

Adhesivity (ADH, kg x s), loin eye area (LEA, cm^2), water holding capacity (WHC, %), classification o the carcass (ICTA), regression coefficient (β_1), cohesiveness (COH), moisture content (IMM, %), intramuscular fat (IMF, %) and intramuscular protein (IMP, %), age (age, months), after slaughtering (D0) days 1 (D1), 7 (D7) and 14 (D14) of maturation, Hardness (HAR, kg), elasticity (ELA), Warner-Bratzler's shear force (WBSF, KgF), gumminess (GUM), chewiness (CHE, kg), muscle µ-calpain (Capn1) and calpastatin content (Cast), leg perimeter (LP, cm), weight of the hot carcass (HCW, kg), live weight (LW, kg), carcass yielding (CY, %), meat yielding (MY,%), resilience (RES), the letters mean statistic difference (a < b < c). P-value of the association model (*), P-value of the substitution model (**).

SNPs	Variable	Day	P-value*	Adjusted means by genotype ± Standard deviation			Allelic substitution	
	CHE	D14	0,011	1,36 ± 0,06[a]	1,60 ± 0,08[b]	1,65 ± 0,16[ab]	0,19	0,00

Adhesivity (ADH, kg x s), loin eye area (LEA, cm^2), water holding capacity (WHC, %), classification o the carcass (ICTA), regression coefficient (β_1), cohesiveness (COH), moisture content (IMM, %), intramuscular fat (IMF, %) and intramuscular protein (IMP, %), age (age, months), after slaughtering (D0) days 1 (D1), 7 (D7) and 14 (D14) of maturation, Hardness (HAR, kg), elasticity (ELA), Warner-Bratzler's shear force (WBSF, KgF), gumminess (GUM), chewiness (CHE, kg), muscle µ-calpain (Capn1) and calpastatin content (Cast), leg perimeter (LP, cm), weight of the hot carcass (HCW, kg), live weight (LW, kg), carcass yielding (CY, %), meat yielding (MY,%), resilience (RES), the letters mean statistic difference (a < b < c). P-value of the association model (*), P-value of the substitution model (**).

Effects Upon The Productive Characteristics Of The Meat

The effects of the CAPN4751 (LW, CY, and LEA), CAST282 (CY), CAST2959 (CY, LEA), and E2FB (HCW, CY, MY) SNPs were observed upon the assessed production characteristics. These results might have a relevant impact upon the meat productivity of the brahman breed livestock raised and grazed in the lower Colombian tropic conditions. Perhaps this is due to the high frequency of the alleles that favor these characteristics in the population studied (Table 1). The aforementioned is founded in the fact that these alleles have been related to a greater calpastatin expression, an inhibitor of the action of the µ-calpain (Goll et al., 2003). The µ-calpain participates in the renovation of the muscular tissue (Gerken et al., 1995) through a process with high energy requirements (Herd, Oddy, and Richardson, 2004) and, thus, the inhibition of the µ-calpain would favor the growth and deposition of fats in the animal (Motter et al., 2013). Given all of this, some authors have suggested that this enzymatic complex might be related with: i) the growth rate and the living weight of the animal (Howard, 2013, Motter et al., 2013); ii) the weight and yielding of the carcass (Cafe et al., 2010a; Howard, 2013); iii) the loin eye area (Casas et al., 2006; Motter et al., 2013; Allais et al., 2014; Calvo et al., 2014; Lee et al., 2014); the fat yielding (Schenkel et al., 2006; Motter et al., 2013); iv) dorsal fat thickness (Howard, 2013; Motter et al., 2013); and v) might be related to the adaptation capability of the *Bos indicus* specimens to adverse conditions (Cafe et al., 2010a).

It is important to highlight that even though there was not an observed association between the CAPN316 SNP upon the production characteristics of the animal alive and in carcass, it is known that the brahman breed specimens that are carriers of the CG and GG genotypes have better parameters in some of the values such as hump height (Casas et al., 2005), weight gains and hip fat (Cafe et al., 2010a). Besides, the Holstein (Ardicli et al., 2019), Simental (Ardicli et al., 2017) and Friesian (Ardicli et al., 2019) livestock breeds with these genotypes have a higher rate of food conversion and weight gain. Continuing, their LW, HCW, cold canal weight, LEA, and dorsal fat thickness are higher. In contrast, the carriers of the CC genotype had higher fat yielding, a smaller food conversion rate, and required longer times for achieving

400 kg. Added to this, there have been reports that claim that the low frequency of the C allele in the brahman livestock might be related to the adaptability to the tropic conditions (Cafe et al., 2010b).

Opposite to this is the LEP or obese gene, which codifies leptin. This proteic hormone participates in the regulation of food consumption and energetic equilibrium in the animal. Its exon number 2 have the E2FB and E2JW SNPs (Buchanan et al., 2002), which alleles are associated with fat deposition differences among bovines (Buchanan et al., 2002; Kononoff et al., 2005; Nkrumah et al., 2005; Schenkel et al., 2005). This defends the hypothesis that these markers might be useful for improving meat production in cattle. Thus, the low frequency of the favorable allele (T) in E2FB (0,22), and its practical absence in E2JW (0,001) (Table 1), might explain, partially, the reason why brahman breed animals present deficient carcass characteristics in comparison with those observed in taurine breeds. In these last, the T alleles are present in bigger proportions (Smith et al., 2009).

Moreover, even though there were no reports of the association of these markers with the LW —CY and MY—, there is evidence that supports that the T alleles in E2JW improve the food consumption (Lagonigro et al., 2003), and in E2FB favors the weight gain (da Silva et al., 2012). Both markers are related to the thickness of the dorsal fat (Buchanan et al., 2002; de Carvalho et al., 2012; da Silva et al., 2012; de Oliveira et al., 2013), fatty content, lean content (Schenkel et al., 2005) and LEA (da Silva et al., 2012), both in taurine and indicine breeds.

Effects Upon The Quality Attributes Of The Meat

Chemical composition of the meat. Characterizing the proximal composition of the meat is a powerful tool for decision making that allows productivity improvement, as well as knowing, underlive, and highlighting the attributes better valued by consumers (Mamani and Gallo, 2011; Faucitano et al., 2008). The chemical composition of the meat, represented mainly by the IMP, IMM, and IMF contents, has different functions in determining the meat quality, i.e., as a source of nutrients for human nutrition (McAfee et al., 2010; Lobato et al., 2014), which determines its acceptability (Mamani and Gallo, 2011, Lobato et al., 2014) and acts as a productivity indicator. This is made taking into account the efficiency and yielding achieved, both in the live animal as in its final products after slaughtering and butchering (Faucitano et al., 2008). Given the aforementioned, the effect of the CAPN, CAST, and LEP genes over the IMF, IMM, and IMP contents were assessed; the findings are compatible with what is stated in this study.

The animals that carry the CC genotype of the CAST282 marker had the highest IMM content and the lowest IMF (marbling). Even though there are no reports of this effect in brahman breeds, the study found a contrary relationship between these parameters (Cheng et al., 2015). Nonetheless, the water quantity in the muscle is not as relevant as its water holding capacity (WHC), given that this characteristic, alongside marbling, participate in the juiciness and tenderness sensation of the meat (Zhang et al., 2005; Pearce et al., 2011; Warner, 2014).

However, it is important to highlight that the animals that carry the GG genotype of CAST282 (Table 3) had the highest values in the WBSF (less tenderness). The aforesaid might be explained with a raise in the calpastatin levels in the animals with this genotype (Table 3). Under this circumstance, the proteolytic activity of the μ-calpain decreases. Consequently, the protein replacement processes of the muscle become slower. Thus, the energy requirements decrease (Herd, Oddy, and Richardson, 2004), and animal growth and muscle fact deposition are favored. This might explain the higher values in thickness and dorsal fat percentage in the Brangus breed specimens that are carriers of the GG genotype (Motter et al., 2013).

It must be highlighted that the IMF disposition (marbling) is a highly important attribute of meat quality, given how much it influences its texture, smell, and flavor. There is a direct correlation between the marbling grade and the tenderness sensitivity, while with the WBSF this relationship is inversely proportional. This last fact happens due to the fact that the IMF, located between the fibers, modifies its structure, which reduces its mechanical force and favors the tenderness of the bovine flesh (Nishimura, 2010).

The carriers of the TT genotype of E2FB have the highest IMF values. Something similar was found in animals from different taurine breeds with this genotype (Buchanan et al., 2002) and in animals of the Nellore breed that carry the CTAT haplotype of E2FB/E2JW. The results did not make evident an effect of the CAPN4751 SNP. Nevertheless, the bovine animals, carriers of the CC genotype, have the highest values (Chung, Shin, and Chung, 2014).

Among the population of the brahman breed from this study, there was no association between the studied markers with the protein contents in the muscle (IMP). This is a consistent result because bovine meat is considered an excellent source of protein. In general, its content is stable. Although there are reports of values that oscillate between 16 and 31% (Ferreira, 1999; Serra et al., 2004), the consensus is around 22% (Mamani and Gallo, 2011, Montoya, 2014; Wood, 2017). This has made that this content stopped becoming a preoccupation in the meat industry, which is made evident in the absence of works for raising its values. The interest has focused on characterizing the type of proteins and the efficiency of the biochemical context of the muscle for degrading them during maturation, cooking process, and mastication for obtaining quality products and providing the consumer with a better organoleptic perception at consumption (Bowker and Zhuang, 2013; Bowker, Eastridge, and Solomon, 2014).

pH. There is a pH descent in the postmortem period, from 7,0 to a value between 5,4 and 5,8 in ideal conditions (Lomiwes et al., 2014). If this does not happen, the meat develops deficient organoleptic and production characteristics (Grayson et al., 2016; Zhang et al., 2018) that affect color, smell, WHC (Muchenje et al., 2009), among others. Taking the aforementioned into account, both calpastatin as leptin participate in the regulation of the muscle's metabolism. Leptin stimulates glucose uptake and glycogen synthesis (Ceddia et al., 1998), while calpastatin participates in glycolysis regulation (Reardon et al., 2010). Consequently, they might affect the establishment of the pHu and the WHC.

Even though the CAST282 (D1), E2FB (D7), and CAPN4751 (D14) SNPs had an effect upon the meat's pH in different maturation times, there was a variation in the genotypes and the effect of the allelic substitution. Even so, among the studied population, the pH values were within the ranges considered ideal or intermediate (Córdoba et al., 2017). Hence, these effects might not have any practical utility over the organoleptic quality of the meat. There have been reports of the effect of the CAST282 SNP over the pHu. The highest pHu values were found in carriers of the GG genotype and not in CC carriers, as this study found. Even if there are no reports of association of the E2FB upon the pH, it was found that the carriers of the AT genotype of E2JW have the highest values (de Oliveira et al., 2013), which may be due to overdominance where the heterozygous present higher values on a given characteristic (Falconer and Mackay, 1996).

Water holding capability. The CAST2959 (D1), CAST282 (D7) and E2FB (D14) markers affected the WHC, which was assessed through the measurement of water loss during cooking in different meat maturation times (Table 3). Similar to the results obtained by Leal et al (2015) in brahman breed animals and its crossings with different breeds and in *Bos taurus* animals (Chung, Shin, and Chung, 2014, Kök S and Atalay, 2018), there were no effects of the CAPN316 and CAPN4751 SNPs. These results can be contrasted with the findings by Cafe et al. (2010b) in which the CG genotypes of CAPN316 and CC of CAPN4751 had the highest WHC values.

It is important to highlight that there has been documentation on the association of the CAST2959 SNP with the WHC in raw meat, which is assessed through the measurement of water loss by dripping. Even so, this technique assesses the meat yielding (Warner, 2014) and not its organoleptic characteristics, as is the case of the WHC in cooked meat (Yu et al., 2005).

Texture profile analysis and Warner-Bratzler's shear force. The texture is considered as the main attribute of meat. This attribute refers to the sensations perceived during mastication and its analysis focuses on characterizing the structure of the meat through sensitive perception (Cáceres, 2010). The study of texture is done through a sensory panel or with instrumental methods (Szczesniak, 1963), as the texture profile analysis (TPA) and the WBSF. TPA offers the possibility of assessing several characteristics of the meat with one single sample (Ruiz de Huidobro et al., 2001).

Because the TPA is not used regularly in the meat texture characterization (Ruiz de Huidobro et al., 2005), There is not enough available literature about the effect of molecular markers (Pinilla, 2014). Moreover, even though the WBSF only offers the possibility of characterizing the parameters of shear resistance, this is the most widespread methodology in academic and commercial contexts (Ruiz de Huidobro et al., 2005). There seems to be an adequate correlation between the hardness assessed through TPA and the WBSF (Onega, 2003). Nonetheless, these methodologies are focused on different components of the muscle. The WBSF defines the hardness due to the myofibrillar component (Möller, 1980), while the TPA focuses on the hardness related to the connective tissue (Lepetit and Culioli, 1994; Harper, 1999). Another aspect to bear in mind is that the texture in cooked meat is made of two main components: tenderness,

which explains the 64%; and juiciness, which represents 19%. Thus, the least juicy meats are considered less tender (Pinilla, 2014).

The hardness, adhesivity, elasticity, gumminess, and chewiness are the most used TPA parameters in the characterization of the meat quality (Ruiz de Huidobro et al., 2001); they have a significant correlation with the results obtained through a sensory panel (Ruiz de Huidobro et al., 2005). The studied molecular markers had a significant effect upon these characteristics as follows: CAPN4751 had an effect upon hardness (D1, D7, D14), elasticity (D1) and gumminess (D1); CAST282, upon Hardness (D1, D7, D14); CAST2959 upon hardness (D1, D7, D14), adhesivity (D1, D7) and elasticity (D1); and E2FB upon hardness (D1 and D7) and chewiness (D14). This contrasts with the findings of Pinilla (2014), where the CAST2959 SNP was found to have effects only in elasticity and extensibility, while CAST282 presented an effect upon hardness and extensibility. The CAPN4751 SNP did not have any effect upon any of the variables assessed by them. Thus, as observed in this study, the CAPN316 SNP had a very low frequency in the C allele, the reason why this marker was not included in the association analysis.

Despite the low frequencies of the CC genotype (0,04) and the C allele (0,01) of the CAPN316 SNP, the study found an effect of this marker upon the WBSF (D14). In this case, the lowest values were found among animals with the CG genotype. This result is similar to what is reported by different authors in brahman animals (Smith et al., 2009; Chung, Shin, and Chung, 2014; Rubio et al., 2016), Brangus (Corva et al., 2007), and among different taurine breeds (Page et al., 2002; Casas et al., 2006; Kök and Atalay, 2018), where the lowest values are found in animals carrying the C allele. The above contrasts with other findings in which the most tender meat, assessed with a sensory panel (Casas et al., 2005) and through WBSF (Cafe et al., 2010b), was found in brahman animals carrying the GG genotype.

Similarly, there was a significant effect of the CAPN4751 SNP. The best results were found in CC (D7) animals and carriers of the CC and CT (D14). This result coincides with the finding on animals of the brahman breeds (White et al., 2005; Smith et al., 2009; Cafe et al., 2010b; Rubio et al., 2016), Nellore (Pinto et al., 2010), in different taurine breeds (Casas et al., 2006, Alfaro et al., 2012, Chung, Shin and Chung, 2014) and its crossings with Nellore and Brahman (Corva et al., 2007, Curi et al., 2009), where the carriers of the C allele (CC, CT) present the lowest WBSF values.

The animals with a CC genotype in D1 and CG of the CAST282 SNP had the lowest WBSF values in D14, which is similar to the findings reported in the Nellore breed (Pinto et al., 2010) and in different breeds (Schenkel et al., 2006; Avilés et al., 2015; Kök and Atalay, 2018). An analogous result was found in the carriers of the AA genotype of the CAST2959 SNP in D1 and D14, which coincides with which was found in animals from different taurine breeds (Morris et al., 2006) and its crossings with brahman (Casas et al., 2006) and nellore (Curi et al., 2009) breeds.

The E2FB SNP was associated with this characteristic in D14. The lowest values were found among animals with the CC and CT genotypes, which are comparable to the findings reported in taurine livestock (Schenkel et al., 2005) and its crossings with indicine specimens (de Carvalho et al., 2012). Although these authors also reported an association between the E2JW SNP with this characteristic (Lagonigro et

al., 2003), this study did not find the said association due to the absence of the T allele, which has been reported as favorable.

The findings of this study and other authors adhere to the observation that these markers have an effect upon the WBSF in the brahman livestock meat (Smith et al., 2009; Casas et al., 2005, Cafe et al., 2010b; White et al., 2005). Nevertheless, other authors suggest that this association does not exist. For example, Pinilla (2014) did not evidence any association among the CAPN316, CAPN4751, CAST282, and CAST2929 SNPs and this variant of brahman animals and neither with its crossing with animals from other breeds.

Regardless of the aforementioned, these results must be analyzed bearing in mind the acceptability context of the meat by its degree of tenderness. Although an association is observed between these SNP with the WBSF (Table 3), the values of this characteristic are in the intermediate levels of tenderness (4,36 < WBSF > 5,37 kgF), tough (5,38 < WBSF > 6,38 KgF) and very tough (> 6,38), according to the scale proposed by Destefanis et al (2008). It must also be taken into account that the observed differences are smaller than 0,5 kgF, whence they are not perceptible by consumers untrained in sensory analysis (Miller et al., 1995; Huffman et al., 1996). Nonetheless, from a meat quality point of view, the existence of this difference is important, which when added to the effect of other genes might lead to important differences in the organoleptic qualities of the meat.

Effects upon the amount of calpastatin and µ-calpain in the muscle.

There was a significant effect of the CAST282, CAST2959, and E2FB SNPs upon the amount of calpastatin and by CAPN4751 upon the amount of µ-calpain. The carriers of the GG genotype of CAST282 had the highest values in the Cast. Something similar happened to the animals with GG genotype of CAST2959, where the change of an A allele for a G increased it by 0,04. Finally, higher values in the TT genotype in comparison with the CT were found in the E2FB marker, there was no difference between CT and CC. The change of a C for a T allele increased in 0,0004216 the amount of the Cast protein.

Upon the µ-calpain, there is a significant effect by CAPN4751. In this case, the CC genotype had the highest values; besides the change of a T allele for a C one increases the amount of this protein by 0,013. Equally, there were differences between GG and CG by CAPN316. Nonetheless, this result is not conclusive due to the unbalance of the samples that generate the low frequency of the C allele in this marker.

The µ-calpain enzyme content found were 3,6 times greater than those of the Cast one, which coincides with the findings of Saccà et al (2015) about the expression of the mRNAs of these genes. The effect of CAPN4751 upon the expression of the µ-calpain; as well as the effect of the CAST282 and CAST2959 SNPs upon the expression of calpastatin, is predictable. This happens due to the fact that these SNPs are located in CANP1 (intron 17) and CAST (intron 5 and region 3´UTR) regions, where there are sequences that regulate their expression. The results are consistent with the findings of Niciura et al. (2012), where

the animals carrying the GG genotype of CAST2959 had twice the amount of mRNAs of this gene as the carriers of the heterozygous genotype, both in taurine and indicine livestock. Besides, the CAPN316 SNP does not influence the CAPN1 expression, while the C allele of the CAPN4751 SNP does it in animals of the brahman and Angus breeds (Nattrass et al., 2014). Regarding the effect of the E2FB upon the expression of this protein, there are no relations reported about it in the available literature. The above is coherent with the effect of these markers upon some production and quality characteristics related to the levels of expression of these enzymes (Howard, 2013; Motter et al., 2013; Allais et al., 2014; Calvo et al., 2014; Lee et al., 2014). Nonetheless, these differences are small. For making them clearly evident, animals with more extreme tenderness-quality parameters must be used (Barendse, 2002).

Conclusions

The results confirm the effect of the C alleles of CAPN4751 and CAST282, A of CAST2959, and T of E2FB upon the variables related to the organoleptic quality of the meat. These alleles decrease the WBSF and Hardness and increase the IMF and WHC contents. Complementary, the A alleles of CAPN4751, G of CAST282, CAST2959, and C of E2FB are associated with production variables in the animal both alive and in carcass. The aforementioned suggests possible genetic differences between animals that have been selected with different approaches, i.e., among those oriented towards productivity concerning those oriented towards organoleptic quality.

It is necessary to assess the possible epistatic effects through a haplotypes analysis. Nevertheless, these markers have a great potential to improve the genetics in the Colombian livestock companies with brahman livestock according to the desired approach: oriented towards productivity or towards the organoleptic quality of the meat. Even though the study found that the CAPN316 SNP affects some of the variables, these results must be carefully analyzed due to the low frequency of the C allele. Finally, the E2JW marker is not useful in genetic improvement programs for the brahman breed given that its C allele is fixed in its population in this population with a frequency greater than 99%.

Declarations

Author contribution Material preparation, data collection, and analysis were performed by all authors. The first draft of the manuscript was written by Luis Ernesto López Rojas, and the other authors made their contributions to later versions of the paper.

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Availability of data and material The data sets generated and/or analyzed during the current study are available through the corresponding author upon reasonable request.

Code availability Not applicable.

Ethics approval The ethics committee for the care and use of animals (CICUA) of University CES has approved the project “Implementación y validación functional de ensayos moleculares para la determinación predictiva de la calidad organoléptica de la carne en bovinos” (act number 5 of the 20th of August 2013, project 38).

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

References

1. Alfaro, S., Rubio, L. M. S., Parra, M., Méndez, M. D., Pérez, L. C., Figueroa, S. F., Sánchez, E. A., Torrescano, G., Ríos, R. F. G., Braña, V. D., Sifuentes, A., Arellano, W., Macedo, R. E. F. y Jimenez, P. (2012). Genetic marker effects for quality traits in commercial beef from Mexico. *58th International Congress of Meat Sci and Technology*, Montreal, Canada
2. Allais, S., Levéziel, J. F., Hocquette, J. F., Rousset, S., Denoyelle, C., Journaux, L. and Renard, G. (2014). Fine mapping of quantitative trait loci underlying sensory meat quality traits in three French beef cattle breeds. Journal of Animal Science, 92, 4329–3241 doi:10.2527/jas2014-7868
3. Ardicli, S., Samli, H., Dincel, D., Soyudal, B. and Balci, F. (2017). Individual and combined effects of CAPN1, CAST, LEP, and GHR gene polymorphisms on carcass characteristics and meat quality in Holstein bulls. Archives Animal Breeding, 60, 303–313
4. Ardicli, S., Samli, H., Vatansever, B., Soyudal, B., Dincel, D. and Balci, F. (2019). Comprehensive assessment of candidate genes associated with fattening performance in Holstein–Friesian bulls. Archives Animal Breeding, 62, 9–32 https://doi.org/10.5194/aab-62-9-2019
5. Avilés, C., Peña, F., Polvillob, O., Barahona, M., Campoc, M. M., Sañudo, C., Juárez, M., Horcada, A., Alcalde, M. J. y Molina, A. (2015). Association between functional candidate genes and organoleptic meat traits in intensively fed beef. *Meat Science*,107, 33–38
6. Barendse, W. J. (2002). DNA markers for meat tenderness. International patent publication W0 02/064820.
7. Bowker, B. C. y Zhuang, H. (2013). Relationship between muscle exudate protein composition and broiler breast meat quality. Poultry Science, *92*, 1385–1392. http://dx.doi.org/10.3382/ps.2012-02806
8. Bowker, B. C., Eastridge, J. S. y Solomon, M. B. (2014). Measurement of muscle exudate protein composition as an indicator of beef tenderness. Journal of Food Science, *79*(7), 192–197. doi: 10.1111/1750-3841.12496
9. Buchanan, F. C., Fitzsimmons, C. J., Van Kessel, A. G., Thue, T. D., Winkelman-Sim, D. C. and Schmutz, S. M. (2002). Association of a missense mutation in the bovine leptin gene with carcass fat content and leptin mRNA levels. Genetics Selection Evolution,34(1), 105.

10. Cáceres, M. E. (2010). Comparación de las características organolépticas y fisicoquímicas de la carne bovina para consumo fresco en la zona céntrica de la ciudad de Taldil [Tesis de pregrado]. Universidad Nacional del Centro de la Provincia de Buenos Aires.
11. Cafe, L. M., McIntyre, B. L., Robinson, D. L., Geesink, G. H., Barendse, W. and Greenwood, P. L. (2010a). Production and processing studies on calpain-system gene markers for tenderness in Brahman cattle: 1. Growth, efficiency, temperament, and carcass characteristics. Journal of Animal Science,88, 3047–3058. doi:10.2527/jas.2009-2678
12. Cafe, L. M., McIntyre, B. L., Robinson, D. L., Geesink, G. H., Barendse, W., Greenwood, P. L., Pethick, D. W., Thompson, J. M and Greenwood, P. L. (2010b). Production and processing studies on calpain-system gene markers for tenderness in Brahman cattle: 2. Objective meat quality. Journal of Animal Science, 88, 3059–3069. doi:10.2527/jas.2009-2679
13. Calvo, J. H., Iguácel, L. P., Kirinus, J. K., Serrano, M., Ripoll, G., Casasús, I., Joy, M., Pérez-Velasco, L., Sarto, P., Albertí, P. and Blanco M. (2014). A new single nucleotide polymorphism in the calpastatin (CAST) gene associated with beef tenderness. Meat Science, 96, 775–782
14. Campbell, R. L. and Davies, P. L. (2012). Structure–function relationships in calpains. Biochemical Journal, 447, 335–351.
15. Casas, E., Shackelford, S. D., Keele, J. W., Stone, R. T., Kappes, S. M. and Koohmaraie, M. (2000). Quantitative trait loci affecting growth and carcass composition of cattle segregating alternate forms of myostatin. Journal of Animal Science, 78(3), 560–569.
16. Casas, E., White, S. N, Wheeler, T. L., Shackelford, S., Koohmaraie, M., Riley, D., Chase, C., Johnson, D. D. and Smith, T. P. L. (2006). Effects of calpastatin and mu-calpain markers in beef cattle on tenderness traits. Journal of Animal Science, 84(3), 520–525. https://doi.org/10.2527/2006.843520x
17. Casas, E., White, S. N., Riley, D. G., Smith, T. P. L., Brenneman, R. A. and Olson, T. A. (2005). Assessment of single nucleotide polymorphisms in genes residing on chromosomes 14 and 29 for association with carcass composition traits in Bos indicus cattle. Journal of Animal Science, 83(1), 13–19. https://doi.org/10.2527/2005.83113x
18. Ceddia, R. P., Willian, J. W. N., Lima, F. B., Carpineli, A. R. y Curi, R. (1998). Pivotal role of leptin in insulin effects. *The Brazilian Journal of Medical and Biological Research, 31*, 715–722.
19. Cheng, W., Cheng, J. H., Sun, D. W. and Pu, H. (2015). Marbling analysis for evaluating meat quality: methods and techniques. Comprehensive Reviews in Food Science and Food Safety, 14(5), 523–535.
20. Chung, H., Shin, S., & Chung, E. (2014). Effects of genetic variants for the bovine calpain gene on meat tenderness. *Molecular biology reports*, *41*(5), 2963–2970. Doi.10.1007/s11033-014-3152-3
21. Córdoba, C. P., Correa, G., Barahona, R. y Tarazona, A. (2017). Comportamiento de machos cebú en corrales presacrificio y su relación con el pH de la carne. Archivos de Zootecnia, *66* (256), 579–586.
22. Coria, M. S., Carranza, P. G. and Palma, G. A. (2018). Calpain System in meat tenderization: A molecular approach. Revista MVZ Córdoba, 23(1), 6523–6536 DOI:10.21897/rmvz.1247

23. Corva, P., Soria, L., Papaleo, J., Villarreal, E., Melucci, L., Mezzadra, C., Schor, A. y Motter, M. (2007). *Evaluation of genetic markers for beef tenderness in Brangus steers*. Sitio Argentino de Producción Animal. http://www.produccion-animal.com.ar/genetica_selección_cruzamientos/bovinos_de_carne/33-Corva-Calpaina.pdf
24. Curi, R. A., Chardulo, L. A. L., Mason, M. C., Arrigoni, M. D. B., Silveira, A. C. y De Oliveira H. N. (2009). Effect of single nucleotide polymorphisms of CAPN1 and CAST genes on meat traits in Nellore beef cattle (*Bos indicus*) and in their crosses with *Bos taurus*. *Animal Genetics, 40*(4), 456–462. https://doi.org/10.1111/j.1365-2052.2009.01859.x
25. da Silva, R. C., Ferraz, J. B. S., Meirelles, F. V., Eler, J. P., Balieiro, J. C. C., Cucco, D. C., Mattos, E. C., Rezende, F. M. and Silva, S. L. (2012). Association of single nucleotide polymorphisms in the bovine leptin and leptin receptor genes with growth and ultrasound carcass traits in Nellore cattle. Genetics and Molecular Research, 11(4), 3721–3728.
26. de Carvalho, T. D., Siquiera, F., Torres, J. R. A., Medeiros, S. R., Días, G. L., de Souza J. M. D. y Soares, C. O. (2012). Association of polymorphisms in the leptin and thyroglobulin genes with meat quality and carcass traits in beef cattle. *Revista Brasileira de Zootecnia, 41*(10), 2162–2168
27. de Oliveira, J. A., Cunha, C. M. D., Crispim, B. D. A, Seno, L. D. O., Fernandes, A. R. M., Nogueira, G. D. P. y Grisolia, A. B. (2013). Association of the leptin gene with carcass characteristics in Nellore cattle. *Animal Biotechnology*, *24*(3), 229–242.
28. Destefanis, G., Brugiapaglia, A., Barge, M. T. and Molin, E. D. (2008). Relationship between beef consumer tenderness perception and Warner–Bratzler shear force. Meat Science, 78, 153–156.
29. Duitama, O., González, L., García, D., Farah, M. and da Fonseca, R. (2013). Productividad acumulada y su relación genética con características reproductivas en hembras Brahman. Revista MVZ Córdoba, 18, 3658–3664.
30. Falconer D.S., Mackay, T.F.C. (1996). Introduction to quantitative genetics. Longman, Edinburgh, 4th Ed
31. Faucitano, L., Chouinard, P. Y., Fortin, J., Mandell, I., Lafrenière, C., Girard, C. and Berthiaume, R. (2008). Comparison of alternative beef production systems based on forage finishing or grain-forage diets with or without growth promotants: 2. Meat quality, fatty acid composition, and overall palatability. Journal of Animal Science, 86, 1678–1689. doi:10.2527/jas.2007-0756
32. Fernandes, J. S., Crispim, B. A., Seno, L. O., Aspilcueta, R. R. and Barufatti, A. (2020). Polymorphisms related to bovine leptin gene and association with productive and reproductive traits in Nellore heifers. Tropical Animal Science Journal, 43(1), 18–24. DOI: https://doi.org/10.5398/tasj.2020.43.1.18
33. Ferreira, F. (1999). Gordura da carne bovina e salud humana. I Parte. Pecuaria de Corte, *13*(4),146–150.
34. Flowers, S., Hamblen, H., Leal-Gutiérrez, J. D., Elzo, M. A., Johnson, D. A. and Mateescu, R. G. (2018). Fatty acid profile, mineral content, and palatability of beef from a multibreed Angus–Brahman population. Journal of Animal Science, 96, 4264–4275. doi: 10.1093/jas/sky300

35. Gerken, C. L., Tatum, J. D., Morgan, J. B., Smith, G. C. (1995). Use of genetically identical (clone) steers to determine the effects of estrogenic and androgenic implants on beef quality and palatability characteristics. Journal of Animal Science, 73(11), 3317–3324. https://doi.org/10.2527/1995.73113317x
36. Goll, D. E., Thompson, V. F., Li, H., Wei, W., Cong, J. (2003). The calpain system. Physiological Reviews. 83(3), 731–801. https://doi.org/10.1152/physrev.00029.2002
37. Gómez, Y. M., Fernández, M., Rivera, D., Gómez, G., Bernal, J. E. (2013). Genetic characterization of Colombian Brahman cattle using microsatellites markers. Russian Journal Genetics; 49, 737–745. https://link.springer.com/article/10.1134/S1022795413070041
38. Grayson, A. L., Shackelford, S. D., King, D. A., McKeith, R. O., Miller, R. K., Wheeler, T. L. (2016). Effect of degree of dark cutting on tenderness and sensory attributes of beef. Journal of Animal Science, *94*(6), 2583–2591. https://doi.org/10.2527/jas.2016-0388
39. Harper, G. S. (1999). Trends in skeletal muscle biology and the underlive of Hardness in beef. Australian Journal of Agricultural Research, 50(7),1105 – 1129. https://doi.org/10.1071/AR98191
40. Herd, R. M., Oddy, V. H., Richardson, E. C. (2004). Biological basis for variation in residual feed intake in beef cattle. Review of potential mechanisms. Australian Journal of Experimental Agriculture, 44(5), 423–430. https://doi.org/10.1071/EA02221
41. Howard, T. (2013). Evaluation of 54 years of Louisiana bull testing, and SNP affecting growth and performance of yearling bulls on a forage performance bull test [tesis de maestría, Universidad Estatal de Luisiana]. LSU Master's Theses. https://digitalcommons.lsu.edu/gradschool_theses/2521
42. Huffman, K. L., Miller, M. F., Hoover, L. C., Wu, C. K., Brittin, H. C., Ramsey, C. B. (1996). Effect of beef tenderness on consumer satisfaction with steaks consumed in the home and restaurant. Journal of Animal Science, 74(1), 91–97. https://doi.org/10.2527/1996.74191x
43. Kök, S., Atalay, S. (2018). The Use of various SNPs in CAST and CAPN1 genes to determine the meat tenderness in turkish grey cattle. Kafkas Universitesi Veteriner Fakultesi Dergisi, *24*(1), 1–8. Doi: 10.9775/kvfd.2017.17617
44. Kononoff, P. J., Deobald, H. M., Stewart, E. L., Laycock, A. D., Marquess, F. L. (2005). The effect of a leptin single nucleotide polymorphism on quality grade, yield grade, and carcass weight of beef cattle. Journal of Animal Science, 83(4), 927–32. https://doi.org/10.2527/2005.834927x
45. Lagonigro, R., Wiener, P., Pilla, F., Woolliams, J. A., Williams, J. L. (2003). A new mutation in the coding region of the bovine leptin gene associated with feed intake. Animal Genetics, 34(5), 371–4. https://doi.org/10.1046/j.1365-2052.2003.01028.x
46. Leal, J. D., Jiménez, L. M., Ariza, M., Manrique, C., López, J., Martínez, C., Pinilla, Y., Castro, S., García, N., Bedoya, C., Jiménez, A. (2015). Polimorfismos de los genes CAPN1, CAST, DES, PRKAG3 y RYR1 asociados a la capacidad de retención de agua en crudo y cocinado en carne de bovino en cruces Bos indicus y Bos taurus en Colombia. Archivos de Zootecnia *64*(245), 29–35. https://www.uco.es/ucopress/az/index.php/az/article/view/371

47. Lee, S. H., Kim, S. C., Chai, H. H., Cho, S. H., Kim, H. C., Lim, D., Choi, B. H., Dang, C. G., Sharma, A., Gondro, C., Yang, B. S., Hong, S. K. (2014). Mutations in calpastatin and μ-calpain are associated with meat tenderness, flavor and juiciness in Hanwoo (Korean cattle): molecular modeling of the effects of substitutions in the calpastatin/μ-calpain complex. Meat Science 96(4), 1501–1508. https://doi.org/10.1016/j.meatsci.2013.11.026
48. Lepetit, J., Culioli, J. (1994). Mechanical properties of meat. Meat Science,36(1–2), 203–237. https://doi.org/10.1016/0309-1740(94)90042-6
49. Lobato, J. F. P., Freitas, A. K., Devincenzi, T., Cardoso, L. L., Tarouco, J. U., Vieira, R. M., Dillenburg, D. R., Castro, I. (2014). Brazilian beef produced on pastures: Sustainable and healthy. Meat Science 98(3), 336–345. https://doi.org/10.1016/j.meatsci.2014.06.022
50. Lomiwes, D., Farouk, M. M., Wu, G., Young, O. A. (2014). The development of meat tenderness is likely to be compartmentalised by ultimate pH. Meat Science, *96*(1), 646–651. https://doi.org/10.1016/j.meatsci.2013.08.022
51. López-Rojas, L. E., Patiño L, López, A., Zuluaga, J. J. (2017a). Genotyping of SNPs associated with meat tenderness: comparison of two PCR-based methods. Genetics and Molecular Research 16(2). Doi: 10.4238/gmr16029635
52. López-Rojas, L. E., Patiño, L., López, A., Zuluaga, J. J. (2017b).Variabilidad genética en seis SNPs de los genes CAPN1, CAST y LEP de toros brahman en ganaderías del trópico bajo colombiano. Revista CES Medicina, Veterinaria y Zootecnia, 12(2), 88–102. http://dx.doi.org/10.21615/cesmvz.12.2.2
53. Mamani, L. W., Gallo, C. (2011). Composición química y calidad instrumental de carne de bovino, llama (lama glama) y caballo bajo un sistema de crianza extensiva. Revista de Investigaciones Veterinarias del Perú 22(4), 301–311. http://www.scielo.org.pe/scielo.php?script=sci_arttext&pid=S1609-91172011000400003
54. Martínez, R. A., Dassonneville, R., Bejarano, D., Jimenez, A., Even, G., Mészáros, G., Sölkner, J. (2016). Direct and maternal genetic effects on growth, reproduction, and ultrasound traits in zebu Brahman cattle in Colombia. Journal of Animal Science, 94(7), 2761–2769. Doi:10.2527/jas2016-0453
55. McAfee, A. J., McSorley, E. M., Cuskelly, G. J., Moss, B. W., Wallace, J. M. W., Bonham, M. P., Fearon, A. M. (2010). Red meat consumption: An overview of the risks and benefits. Meat Science, 84(1), 1–13. https://doi.org/10.1016/j.meatsci.2009.08.029
56. Meirelles, F. V., Rosa, A. J. M., Lobo, R.B., García, J.M., Smith, L. C., Duarte, F. A. M. (1999). Is the zebu really *Bos indicus*? Genetics and Molecular Biology, *22*(4), 543–546. https://doi.org/10.1590/S1415-47571999000400013
57. Miller, M. F., Hoover, L. C., Cook, K. D., Guerra, A. L., Huffman, K. L., Tinney, K. S., Ramsey, C. B., Brittin, H. C., Huffman, L. M. (1995). Consumer acceptability of beef steak tenderness in the home and restaurant. Journal of Food Science, 60, 963–965. https://doi.org/10.1111/j.1365-2621.1995.tb06271.x
58. Möller, A. (1980). Analysis of Warner-Bratzler shear pattern with regard to myofibrillar and connective tissue components of tenderness. Meat Science, 5(4), 247–260. https://doi.org/10.1016/0309-

1740(81)90015-2

59. Montoya, R. (2014). *Caracterización de algunas variables de calidad de carne en bovinos manejados bajo diferentes condiciones de producción en el trópico colombiano* [tesis de maestría, Universidad Nacional de Colombia]. Repositorio Institucional UNAL. https://repositorio.unal.edu.co/handle/unal/54351
60. Morris, C. A., Cullen, N. G., Hickey, S. M., Dobbie, P. M., Veenvliet, B. A., Manley, T. R., Pitchford, W. S., Kruk, Z. A., Bottema, C. D. K., Wilson, T. (2006). Genotypic effects of calpain 1 and calpastatin on the tenderness of cooked M. longissimus dorsi steaks from Jersey × Limousin, Angus and Hereford-cross cattle. Animal Genetics 37, 411–414. https://doi.org/10.1111/j.1365-2052.2006.01483.x
61. Motter, M. M., Corva, P. M., Marrube, G., Miquel, M. C., Papaleo, J., Villarreal, E. L., Melucci, M. L., Mezzadra, C. A., Schor, A., Soria, L. A. (2013). Asociación de dos marcadores del gen de la calpastatina con variables productivas de novillos Brángus engordados en pasturas. Revista Argentina de Producción Animal 33(1), 21–29. https://ppct.caicyt.gov.ar/index.php/rapa/article/view/3563
62. Muchenje, V., Dzama, K., Chimonyo, M., Strydom, P. E., Hugo, A., Raats, J. G. (2009). Some biochemical aspects pertaining to beef eating quality and consumer health: A review. Food Chemistry, *112*(2), 279–289. https://doi.org/10.1016/j.foodchem.2008.05.103
63. Nattrass, G. S., Cafe, L. M., McIntyre, B. L., Gardner, G. E., McGilchrist, P., Robinson, D. L., Greenwood, P. L. (2014). A post-transcriptional mechanism regulates calpastatin expression in bovine skeletal muscle. Journal of Animal Science, 92(2), 443–455. https://doi.org/10.2527/jas.2013-6978
64. Niciura, S. C. M., Ibelli, A. M. G., Gouveia, G. V., Gromboni, J. G. G., Rocha, M. I. P., de Souza, M. M., de Almeida Regitano, L. C. (2012). Polymorphism and parent-of-origin *Meat* Nielsen, R., Slatkin, M. (2013). *An introduction to population genetics: theory and* effects on gene expression of CAST, leptin and DGAT1 in cattle. *Science*, *90* https://doi.org/10.1016/j.meatsci.2011.08.00
65. Nishimura, T. (2010). The role of intramuscular connective tissue in meat texture. Animal Science Journal, 81, 21–27. DOI: 10.1111/j.1740-0929.2009.00696.x
66. Nkrumah, J. D., Li, C., Yu, J., Hansen, C., Keisler, D. H., Moore, S. S. (2005). Polymorphisms in the bovine leptin promoter associated with serum leptin concentration, growth, feed intake, feeding behavior, and measures of carcass merit. Journal of Animal Science, 83, 20–8. doi:10.2527/2005.83120x
67. Novoa, M. A., Usaquen, W. (2010). Population genetic analysis of the Brahman cattle (Bos indicus) in Colombia with microsatellite markers. Journal of Animal Breeding and Genetics, 127(2): 161–8. Doi: 10.1111/j.1439-0388.2009.00811.x.
68. Onega, M. (2003). Evaluación de la calidad de carnes frescas [tesis de doctorado, Universidad Complutense de Madrid]. Repositorio Institucional UCM. https://eprints.ucm.es/id/eprint/5138/
69. Page, B. T., Casas, E., Heaton, M. P., Cullen, N. G., Hyndman, D. L., Morris, C. A., Smith, T. P. L. (2002). Evaluation of single-nucleotide polymorphisms in CAPN1 for association with meat tenderness in cattle. Journal of Animal Science, 80, 3077–3085. https://doi.org/10.2527/2002.80123077x

70. Pearce, K. L., Rosenvold, K., Andersen, H. J., Hopkins, D. L. (2011). Water distribution and mobility in meat during the conversion of muscle to meat and ageing and the impacts on fresh meat quality attributes – A review. Meat Science, 89(2), 111–124. https://doi.org/10.1016/j.meatsci.2011.04.007
71. Pinilla, Y. C. (2014). Efecto de SNPs de genes candidatos asociados a textura de la carne en bovinos Bos indicus y sus cruces [tesis de Maestría, Universidad Nacional de Colombia]. Repositorio Institucional UNAL. https://repositorio.unal.edu.co/handle/unal/52166
72. Pinto, L. F. B., Ferraz, J. B. S., Meirelles, F. V., Eler, J. P., Rezende, F. M., Carvalho, M. E. (2010). Association of SNPs on CAPN 1 and CAST genes with tenderness in Nellore cattle. Genetics and Molecular Research, *9*(3), 1431–1442. https://doi.org/10.4238/vol9-3gmr881
73. Pratiwi, N., Maskur, M., Priyanto, R., Jakaria, K. (2016). Novel SNP of calpain-1 (CAPN1) gene and its association with carcass and meat characteristics traits in Bali cattle. Journal of Indonesian Tropical Animal Agriculture 41(3), 109–116. Doi: 10.14710/jitaa.41.3.109-116
74. Reardon, W., Mullen, A. M., Sweeney, T., Hamill, R. M. (2010). Association of polymorphisms in candidate genes with colour, water-holding capacity, and composition traits in bovine M. longissimus and M. semimembranosus. Meat Science, *86*(2): 270–275. https://doi.org/10.1016/j.meatsci.2010.04.013
75. Rodríguez, J. M. (1993). Razas Bovinas en Colombia. Universidad Nacional de Colombia.
76. Rubio, M. S., Alfaro, S., Sifuentes, A., Parra, G., Braña, D., Méndez, R. D., Pérez, C., Rios, F., Sánchez, A., Torrescano, G., Figueroa, F. (2016). Meat tenderness genetic and genomic variation sources in commercial beef cattle. Journal Food Quality, *39*(2), 150–156. https://onlinelibrary.wiley.com/doi/epdf/10.1111/jfq.12185
77. Ruiz de Huidobro, F. R., Miguel, E., Blázquez,. B, Onega, E. (2005). A comparison between two methods (Warner–Bratzler and texture profile analysis) for testing either raw meat or cooked meat. Meat Science, 69(3), 527–536. Doi: 10.1016/j.meatsci.2004.09.008
78. Ruiz de Huidobro, F., Cañeque, V., Lauzurica, S., Velasco, S., Pérez, C., Onega, E. (2001). Sensory characterization of meat texture in sucking lambs. Methodology. Investigación Agraria: Producción y Sanidad Animales, 16(2), 223–234. https://dialnet.unirioja.es/servlet/articulo?codigo=112386
79. Saccà, E., Corazzin, M., Pizzutti, N., Lippe, G., Piasentier, E. (2015). Early postmortem expression of genes related to tenderization in two I talian S immental young bulls' skeletal muscles differing in contractile type. Animal Science Journal, 86(12), 992–999. https://doi.org/10.1111/asj.12386
80. Schenkel, F. S., Miller, S. P., Jiang, Z., Mandell, I. B., Ye, X., Li, H., Wilton, J. W. (2006). Association of a single nucleotide polymorphism in the calpastatin gene with carcass and meat quality traits of beef cattle. Journal of Animal Science, 84(2), 291–299. https://doi.org/10.2527/2006.842291x
81. Schenkel, F. S., Miller, S. P., Ye, X., Moore, S. S., Nkrumah, J. D., Li, C. (2005). Association of single nucleotide polymorphisms in the leptin gene with carcass and meat quality traits of beef cattle. Journal of Animal Science, 83(9): 2009–20. Doi:10.2527/2005.8392009x
82. Serra, X., Gil, M., Gispert, M., Guerrero, L., Oliver, M. A. (2004). Characterization of Young bulls of the Bruna Perineus cattle breed (selected from old Brown swiss) in relation to carcass. Meat Science,

66(2), 425–436. https://doi.org/10.1016/S0309- 1740(03)00131-1

83. Smith, B. L., Lu, C. P., Bremer, J. R. A. (2009). High-resolution melting analysis (HRMA): a highly sensitive inexpensive genotyping alternative for population studies. Molecular Ecology Resources, 1–4. Doi: 10.1111/j.1755-0998.2009.02726.x
84. Smith, T. P. L., Casas, E., Rexroad, C. E., Kappes, S. M., Keele, J. W. (2000). Bovine CAPN1 maps to a region of BTA29 containing a quantitative trait locus for meat tenderness. Journal of Animal Science, 78(10), 2589–2594. https://doi.org/10.2527/2000.78102589x
85. Szczesniak, A. S. (1963). Objective Measurements of Food Texture. Journal of Food Science, 28(4), 410–420.https://doi.org/10.1111/j.1365-2621.1963.tb00219.x
86. Warner, R. (2014). Measurement of meat quality: I measurements of water-holding capacity and color: objective and subjective. En C. Devine, M. Dikeman (eds.), Encyclopedia of Meat Sciences (2.ª ed.). Academic Press.
87. Wheeler, T. L., Cundiff, L. V., Koch, R. M. (1994). Effect of marbling degree on beef palatability in Bos taurus and Bos indicus cattle. Journal of Animal Science, 72, 3145–3151. https://www.ncbi.nlm.nih.gov/pubm ed/7759364
88. White, S. N., Casas, E., Wheeler, T. L., Shackelford, S. D., Koohmaraie, M., Riley, D. G., Smith, T. P. (2005). A new single nucleotide polymorphism in CAPN1 extends the current tenderness marker test to include cattle of Bos indicus, Bos taurus, and crossbred descent. Journal of Animal Science, 83(9), 2001–8. https://doi.org/10.2527/2005.8392001x
89. Williams, J. L., Dunner, S., Valentini, A., Mazza, R., Amarger, V., Checa, M. L., Levéziel, H. (2009). Discovery, characterization and validation of single nucleotide polymorphisms within 206 bovine genes that may be considered as candidate genes for beef production and quality. Animal Genetics, 40(4), 486–491. https://doi.org/10.1111/j.1365-2052.2009.01874.x
90. Wood, J. D. (2017). Meat Composition and Nutritional Value. En F. Toldrá (ed.), *Lawrie's Meat Science*. Woodhead Publishing.
91. Yu, L. H., Lee, E. S., Jeong, J. Y., Paik, H. D., Choi, J. H., Kim, C. J. (2005). Effects of thawing temperature on the physicochemical properties of pre-rigor frozen chicken breast and leg muscles. Meat Science, 71(2), 375–382. https://doi.org/10.1016/j.meatsci.2005.04.020
92. Zhang, S. X., Farouk, M. M., Young, O. A., Wieliczko, K. J., Podmore, C. (2005). Functional stability of frozen normal and high pH beef. Meat Science, 69, 765–772. https://doi.org/10.1016/j.meatsci.2004.11.009
93. Zhang, Y., Qin, L., Mao, Y., Hopkins, D. L., Han, G., Zhu, L., Luo, X. (2018). Carbon monoxide packaging shows the same color improvement for dark cutting beef as high oxygen packaging. Meat Science, *137*, 153–159. https://doi.org/10.1016/j.meatsci.2017.11.016