UNIVERSIDAD D CÓRDOBA

ESTUDIO DE ASOCIACIÓN ENTRE LOS MARCADORES MOLECULARES DE SIETE GENES Y LA CALIDAD DE LA CARNE DE VACUNO PRODUCIDO EN LA DEHESA. ANÁLISIS DE SU IDONEIDAD PARA LA APLICACIÓN EN MEJORA

ASSOCIATION STUDY BETWEEN MOLECULAR MARKERS IN SEVEN GENES AND MEAT QUALITY OF BEEF PRODUCED IN THE DEHESA. SUITABILITY ASSESSMENT OF ITS APPLICATION IN ANIMAL BREEDING

TESIS DOCTORAL

CARMEN B. AVILÉS RAMÍREZ

2013

TITULO: *Estudio de asociación entre los marcadores moleculares de siete genes y la calidad de la carne de vacuno producido en la Dehesa. Análisis de su idoneidad para la aplicación en mejora*

AUTOR: *Carmen B. Avilés Ramírez*

Campus de Rabanales
Ctra. Nacional IV, Km. 396 A
14071 Córdoba

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MEMORIA DE TESIS DOCTORAL PRESENTADA POR

CARMEN B. AVILÉS RAMÍREZ

Tesis Doctoral con Mención Internacional
Doctoral Thesis with International Mention

DIRECTORES

Dr. Antonio Molina Alcalá Dr. Francisco Peña Blanco

Dra. Oliva Polvillo Polo

Córdoba, diciembre de 2013


MEJORA DE RAZAS AUTÓCTONAS Y GENÉTICA MOLECULAR
MERAGEM


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COVAP

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Fotografía vaca y ternero: Fernando Ortega
Maquetación cubiertas: Rosa Mª Morales Cid

TÍTULO DE LA TESIS: Estudio de asociación entre los marcadores moleculares de siete genes y la calidad de la carne de vacuno producido en la Dehesa. Análisis de su idoneidad para la aplicación en mejora

DOCTORANDO/A: Carmen B. Avilés Ramírez

INFORME RAZONADO DEL/DE LOS DIRECTOR/ES DE LA TESIS

(se hará mención a la evolución y desarrollo de la tesis, así como a trabajos y publicaciones derivados de la misma).

La Tesis Doctoral que se presenta se ha elaborado a modo de "compendio de publicaciones", al estar integrada por tres artículos publicados en revistas ISI, de gran impacto en la categoría *Agriculture, Dairy & Animal Science* y dos artículos que actualmente se encuentra en fase de revisión en otras dos revistas indexadas. Con los resultados de la presente Tesis se han propuesto una serie de marcadores para la constitución de una herramienta molecular de rutina que permita la mejora genética de la calidad de la carne de la población analizada, así como la contribución a la selección de animales para la creación de una línea de carne de calidad por parte de la industria de vacuno de carne del área de la Dehesa. La Tesis se ha estructurado en 3 capítulos:

Capítulo 1: Análisis preliminar de la distribución de frecuencias alélicas para una serie de marcadores detectados en el gen *CAPN1*. Estudio de asociación entre caracteres fenotípicos relativos a la terneza de la carne y los marcadores *CAPN1-316* y *UoG-CAST*. Este capítulo está integrado por dos trabajos:

- ✓ Avilés, C., P. J. Azor, L. Pannier, R. M. Hamill, A. Membrillo and A. Molina (2009). New single nucleotide polymorphisms in the μ-calpain gene in Spanish maternal beef breeds. Animal biotechnology 20(3): 161-164. Índice de impacto: 0,814 (2^{o} cuartil).

- ✓ Avilés, C., M. Juárez, F. Peña, V. Domenech, I. Clemente and A. Molina (2013). Association of single nucleotide polymorphisms in *CAPN1* and *CAST* genes with beef tenderness from Spanish commercial feedlots. Czech journal of animal science 58(10): 479-487. Índice de impacto: 0,922 (2^{o} cuartil).

Capítulo 2: Estudio de asociación desarrollado entre los marcadores *DGAT1-K232A*, *FABP4: g.7516G>C*, *LEP: g.73C>T*, *RORC: g.3290T>G* y *SCD1: g.878T>C* y la deposición de grasa intramuscular de la carne y el espesor de la grasa dorsal. Estudio de asociación entre el marcador *SCD1: G.878T>C* y la composición de ácidos grasos de la carne. Este capítulo está integrado por dos trabajos:

- ✓ Avilés, C., O. Polvillo, F. Peña, M. Juárez, A. L. Martínez and A. Molina (2013). Associations between *DGAT1*, *FABP4*, *LEP*, *RORC*, and *SCD1* gene polymorphisms and fat deposition in Spanish commercial beef. Journal of animal science 91(10): 4571-4577. Índice de impacto: 2,093 (1er cuartil).

- ✓ Avilés, C., O. Polvillo, F. Peña, A. Horcada, M. Juárez and A. Molina. Association study between a single nucleotide polymorphism in bovine *SCD1* gene with fatty acid composition in a Spanish commercial population. Animal biotechnology. Artículo actualmente en revisión.Índice de impacto: 0,882 (2^{o} cuartil).

Capítulo 3: Estudio de asociación entre los marcadores *CAPN1-316, UoG-CAST, DGAT1-K232A, FABP4: g.7516G>C, LEP: g.73C>T, RORC: g.3290T>G* y *SCD1: g.878T>C* y la calidad sensorial de la carne. Los resultados de este estudio se han publicado en el trabajo:

- ✓ Avilés C., Peña F., Polvillo O., Barahona M., Campo M. M., Sañudo C., Juárez M., Horcada A., Alcalde M. J., Molina A. Association between markers in candidate genes and organoleptic quality traits in beef cattle fed intensively. Livestock science. Artículo actualmente sometido.Índice de impacto: 1,249 (1er tercil).

Por la presente autorizamos la presentación y defensa de esta Tesis Doctoral bajo la modalidad de "compendio de publicaciones", para obtener el grado de Doctor Internacional.

Córdoba, 8 de noviembre de 2013
Firma de los directores

Antonio Molina Alcalá Francisco Peña Blanco Oliva Polvillo Polo

"Sabe esperar, aguarda que la marea fluya
- así en la costa un barco - sin que al partir te inquiete.
Todo lo que aguarda sabe que la victoria es suya;
porque la vida es larga y el arte es un juguete.

Y si la vida es corta
y no llega la mar a tu galera,
aguarda sin partir y siempre espera,
que el arte es largo y, además, no importa."

ANTONIO MACHADO

AGRADECIMIENTOS

En primer lugar tengo que agradecer a mis directores de tesis Antonio Molina, Francisco Peña y Oliva Polvillo que hayan depositado su confianza en mí, me hayan enseñado y me hayan ayudado tanto en los buenos, como en los malos momentos. Gracias por contar conmigo y darme la oportunidad de trabajar en lo que me gusta.

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A todos aquellos que me han hecho sentir como en casa cuando he estado en el extranjero: a Sara que me acogió y me enseñó a vivir en Dublín, a Manuel mi maestro Jedi a nivel investigador (lástima que no

a nivel deportivo) que hizo mis días en Lacombe mucho más gratos y a Rosa, mi compañera de piso, trabajo y fatigas varias en París.

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A Jesús, Manuela, Marta y Javi, qué bueno es encontrarse con buenas personas en la vida (¡y con tupperwares en el bolso al mediodía!).

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Concha por ser mi referente de vida y mi amiga y por estar siempre ahí. Gracias a los tres por ponerme donde estoy y por haberme ayudado a ser lo que soy.

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Y a Jesús, que me acompañas siempre (cerca y lejos), me aguantas y me apoyas. Gracias por tu paciencia, por hacerme la vida más fácil y por creer en mí.

ÍNDICE

ÍNDICE DE CONTENIDOS

LISTADO DE ABREVIATURAS UTILIZADAS

A	Adenina
A	Alanina
AA	Aminoácido
AOAC	*Association of Official Analytical Chemists*
BFT	Espesor de la grasa subcutánea
bp	Pares de bases
BTA	*Bos Taurus*
C	Citosina
CAPN1	μ-Calpaína
CAST	Calpastatina
CH	Genotipo predominantemente *Charolais*
CR	*Conventional ration*
DGAT1	Diacilglicerol O-aciltranferasa 1
DNA	Acido desoxirribonucleico
EDTA-K_3	Ácido etilen diamino tetra acético - sal tripotásica
EEC	Comunidad Económica Europea
EU	Unión Europea
F_1	Primera generación filial
FABP4	Proteína de unión a ácidos grasos 4
G	Guanina
GLM	*General linear model*
h	Hora
h^2	Heredabilidad
HI	*Health Index*
IA	Inseminación artificial
IMF	Infiltración grasa intramuscular
K	Lisina
kg	Kilogramo
kg/cm^2	Kilogramo/centímetro^2
L6	6ª Vértebra lumbar
LEP	Leptina
LI	Genotipo predominantemente *Limousin*
LM	*Longissimus muscle*
LSD	*Least square difference*
LSM	*Least square means*
m	Metro

m/s	Metro/segundo
MERAGEM	Mejora de razas y genética molecular
min	Minutos
ml	Mililitro
mm	Milímetro
mM	Milimolar
MUFA	Ácido graso monoinsaturado
ng	Nanogramo
ns	No significativo
^{o}C	Grados centígrados
PCR	Reacción en Cadena de la Polimerasa
PUFA	Ácido graso poliinsaturado
QTL	Locus con influencia sobre un carácter cuantitativo
RE	Genotipo predominantemente Retinta
RORC	Receptor huérfano asociado al RAR-γ
s	Segundo
SCAI	Servicio central de apoyo a la investigación
SCD1	Estearoil-coenzima A desaturasa 1
SD	Desviación estándar
SE	Error estándar
SFA	Ácido graso saturado
SFC	*Shear force in cooked meat*
SFR	*Shear force in raw meat*
SNP	Polimorfismo de un solo nucleótido
SW	Peso al sacrificio
t	Tendencia hacia la significación
T	Timina
T6	6ª Vértebra torácica
Taq	*Thermus aquaticus*
TMR	*Total mixed ration*
U	Unidad de actividad
UCO	Universidad de Córdoba
Yr	Año
α	Efecto medio de sustitución alélica
µl	Microlitro
µm	Micrometro

RESUMEN

RESUMEN

El objetivo principal de la presente Tesis Doctoral es comprobar la presencia de polimorfismos de bases individuales (SNP) en genes relacionados con la calidad de la carne en vacuno cuyo uso potencial en Selección Asistida por Marcadores pueda proporcionar un valor añadido a la carne de las principales razas ligadas al área Dehesa (Avileña Negra-Ibérica, Morucha, Retinta, y sus cruces con Limousin y Charolais) por su implicación en la expresión de la calidad instrumental y sensorial de la carne y analizar en los genotipos más extendidos (animales con mayor proporción de genes de raza Charolais, Limousin y Retinta), las posibles asociaciones entre los marcadores y dicha calidad.

Esta tesis doctoral se divide en tres capítulos que se corresponden con los tres tipos de caracteres asociados a los diferentes marcadores moleculares analizados: la terneza instrumental, la deposición grasa y perfil de ácidos grasos analizados de manera instrumental y la calidad de la carne evaluada mediante análisis sensorial. Cada uno de estos capítulos está integrado por artículos científicos incluidos en revistas ISI en los que se han publicado (o están en vías de publicación) los resultados obtenidos durante el desarrollo de la presente tesis.

En el primer capítulo, se aborda un análisis preliminar de la distribución de frecuencias alélicas para una serie de marcadores detectados en el gen *CAPN1* en tres razas bovinas autóctonas propias del área de la Dehesa: Retinta, Avileña Negra-Ibérica y Morucha. Posteriormente se analiza en una población comercial criada en la Dehesa y cebada en régimen intensivo, la asociación entre caracteres fenotípicos relativos a la terneza de la carne y dos polimorfismos: uno

de los marcadores previamente analizados, el *CAPN1-316*, y un marcador, el *UoG-CAST*, situado en el gen que codifica al inhibidor calpastatina. Los trabajos que integran el capítulo son:

- ✓ Avilés, C., P. J. Azor, L. Pannier, R. M. Hamill, A. Membrillo and A. Molina (2009). New single nucleotide polymorphisms in the μ-calpain gene in Spanish maternal beef breeds. Animal biotechnology 20(3): 161-164.
- ✓ Avilés, C., M. Juárez, F. Peña, V. Domenech, I. Clemente and A. Molina (2013). Association of single nucleotide polymorphisms in *CAPN1* and *CAST* genes with beef tenderness from Spanish commercial feedlots. Czech journal of animal science 58(10): 479-487.

En el segundo capítulo, se presenta un estudio de asociación desarrollado entre cinco marcadores asociados a la deposición de grasa *DGAT1-K232A*, *FABP4: g.7516G>C*, *LEP: g.73C>T*, *RORC: g.3290T>G* y *SCD1: g.878T>C* y la deposición de grasa intramuscular de la carne y con el espesor de la grasa dorsal de la canal de una población comercial criada y cebada en la Dehesa. Este capítulo también está integrado por el estudio de asociación entre el marcador *SCD1: g.878T>C* y la composición de ácidos grasos de la carne de la misma población. Los resultados han dado lugar a las siguientes publicaciones:

- ✓ Avilés, C., O. Polvillo, F. Peña, M. Juárez, A. L. Martínez and A. Molina (2013). Associations between *DGAT1*, *FABP4*, *LEP*, *RORC*, and *SCD1* gene polymorphisms and fat deposition in Spanish commercial beef. Journal of animal science 91(10): 4571-4577.

- ✓ Avilés, C., O. Polvillo, F. Peña, A. Horcada, M. Juárez and A. Molina. Association study between a single nucleotide polymorphism in bovine *SCD1* gene with fatty acid composition in a Spanish commercial population. Animal biotechnology. Artículo actualmente en revisión.

En el tercer capítulo de la tesis, se evalúan las asociaciones descritas en los dos capítulos anteriores con un tercer análisis en el que se emplean dos fuentes de información: la procedente de la evaluación sensorial llevada a cabo por un panel de jueces entrenados y por un panel de consumidores no entrenados. El trabajo que compone este último capítulo es el siguiente:

- ✓ Avilés C., Peña F., Polvillo O., Barahona M., Campo M. M., Sañudo C., Juárez M., Horcada A., Alcalde M. J., Molina A. Association between markers in candidate genes and organoleptic quality traits in beef cattle fed intensively. Livestock science. Artículo sometido.

Teniendo en cuenta todos los resultados obtenidos podemos recomendar, tanto por su asociación estadística como por la repetibilidad de sus efectos entre metodologías de detección, los marcadores *CAPN1-316*, el *DGAT1-K232A,* el *RORC: g.3290T>G* y el *SCD1: g.878T>C* para su inclusión en una herramienta molecular de rutina que permita la mejora genética de la calidad de la carne de la población analizada, así como la contribución a la selección de animales para la creación de una línea de carne de calidad por parte de la industria del vacuno de carne de la zona.

Las asociaciones detectadas en los marcadores *UoG-CAST*, *FABP4: g.7516G>C* y *LEP: g.73C>T* han sido débiles y/o no han mostrado la

repetibilidad entre técnicas de detección que presentan los marcadores anteriores, por lo que se propone profundizar en el análisis de estas últimas asociaciones para poder descartarlos o incluirlos definitivamente como indicadores del potencial genético para producir carne de calidad en la población estudiada.

SUMMARY

The main objective of the present doctoral thesis is to verify the presence of single nucleotide polymorphisms (SNP) in genes related to beef quality whose potential use in Marker Assisted Selection could provide an added value to the meat of the principal breeds linked to the Dehesa area (Avileña Negra-Ibérica, Morucha, Retinta, and their breed-crosses with Limousin and Charolais) due to their involvement in the expression of the instrumental and sensory meat quality and analyze in the genotypes more widespread (animals mainly Charolais, Limousin and Retinta) the possible associations between the markers and this quality.

This doctoral thesis is divided into three chapters that corresponded to the three types of characters associated with the different molecular markers analyzed: instrumental tenderness, fat deposit and fatty acid composition instrumentally analyzed and quality of meat evaluated by a sensory panel. Each chapter is integrated by scientific articles included in journals belonging to ISI where the results obtained during the development of the present thesis have been published (or are in publishing process).

In the first chapter, a preliminary analysis of the allelic frequency distribution for several markers detected in the *CAPN1* gene is developed in three autochthonous cattle breeds commonly reared in the Dehesa ecosystem. Later on, the association between phenotypic characters related to meat tenderness and two polymorphisms is analyzed in a commercial population reared in the Dehesa and fed intensively: one of the markers previously analyzed the *CAPN1-316*,

and a marker, the *UoG-CAST*, located in the gene that encodes the inhibitor calpastatina. The works that compose this chapter are:

- ✓ Avilés, C., P. J. Azor, L. Pannier, R. M. Hamill, A. Membrillo and A. Molina (2009). New single nucleotide polymorphisms in the μ-calpain gene in Spanish maternal beef breeds. Animal biotechnology 20(3): 161-164.
- ✓ Avilés, C., M. Juárez, F. Peña, V. Domenech, I. Clemente and A. Molina (2013). Association of single nucleotide polymorphisms in *CAPN1* and *CAST* genes with beef tenderness from Spanish commercial feedlots. Czech journal of animal science 58(10): 479-487.

In the second chapter, an association study developed between five markers linked to fat deposition (*DGAT1-K232A*, *FABP4: g.7516G>C*, *LEP: g.73C>T*, *RORC: g.3290T>G* y *SCD1: g.878T>C*) and the intramuscular fat deposition and the back fat thickness of the carcass is analyzed in a commercial population reared in the Dehesa and fed intensively. This chapter is also integrated by an association study between the marker *SCD1: g.878T>C* and the fatty acid profile of meat in the same population. The results led to the following publications:

- ✓ Avilés, C., O. Polvillo, F. Peña, M. Juárez, A. L. Martínez and A. Molina (2013). Associations between *DGAT1*, *FABP4*, *LEP*, *RORC*, and *SCD1* gene polymorphisms and fat deposition in Spanish commercial beef. Journal of animal science 91(10): 4571-4577.

- ✓ Avilés, C., O. Polvillo, F. Peña, A. Horcada, M. Juárez and A. Molina. Association study between a single nucleotide polymorphism in bovine *SCD1* gene with fatty acid composition in a Spanish commercial population. Animal biotechnology. Article under review.

In the third chapter of the thesis, the associations described in the two previous chapters are evaluated with a third analysis where two sources of information are used: the former coming from a sensory trained panel and the second one coming from a panel of non-trained consumers. This last chapter is composed by the following article:

- ✓ Avilés C., Peña F., Polvillo O., Barahona M., Campo M. M., Sañudo C., Juárez M., Horcada A., Alcalde M. J., Molina A. Association between markers in candidate genes and organoleptic quality traits in beef cattle fed intensively. Livestock science. Article submitted.

Keeping in mind the results obtained we can recommend due to their statistical association and the repeatability of their effects among detection methodologies the following markers: *CAPN1-316*, *DGAT1-K232A, RORC: g.3290T>G* and *SCD1: g.878T>C* to be included in a routinely molecular tool that allows the genetic improvement of the quality of meat in the population analyzed and the contribution to the selection of animals to create a meat quality line by the beef cattle industry of the Dehesa area.

The associations detected in markers *UoG-CAST*, *FABP4: g.7516G>C* and *LEP: g.73C>T* were low and/or did not show the repeatability among detection methodologies that presented the previous

markers, therefore we propose to deep in the analysis of these last associations to definitively discard o include the markers as indicators of the genetic potential to produce quality meat in the population studied.

INTRODUCCIÓN

La carne es un producto alimenticio de gran valor por ser fuente de la mayoría de los aminoácidos esenciales, minerales y vitaminas. Por este motivo la carne se considera esencial para el crecimiento y desarrollo óptimo del ser humano (HIGGS 2000).

En 2013 se prevé que la producción de carne mundial alcance 308,2 millones de toneladas de las cuales 68,1 millones son de vacuno (FAO 2013). A pesar de que el consumo anual per cápita en el mundo desarrollado apenas ha variado, en los países en desarrollo este consumo se ha duplicado desde 1980. Según las proyecciones de la ONU, la población mundial aumentará de los más de 7.000 millones de personas en la actualidad a 9.100 millones en 2050. Es decir, que dentro de 40 años habrá que alimentar a un 25% más de personas.

Por ello se espera que la demanda de alimentos continúe creciendo como resultado del incremento demográfico y de los ingresos. Este aumento procederá de los países en desarrollo que pasarán de un consumo actual de carne del 58% a un 72%. Para alcanzar a cubrir este consumo en países en desarrollo, las estrategias tradicionales de mejora genética utilizadas durante el pasado siglo en países desarrollados serán determinantes dado el considerable progreso que a partir de ellas se ha alcanzado en las producciones. Al mismo tiempo, en los países desarrollados resulta imprescindible continuar optimizando las estrategias de mejora en las especies ganaderas, ampliar la variabilidad en los caracteres objetivo de mejora para cubrir diferentes mercados y conservar los recursos zoogenéticos que se poseen actualmente porque si la diversidad genética es importante para cubrir las necesidades presentes, lo será aún más para las venideras.

Además, a día de hoy asistimos al crecimiento en importancia en los países de nuestro entorno de aspectos como la protección medioambiental y el cambio climático, la salud humana, la calidad distintiva de un producto o el bienestar animal que exigirán que se incluya una gama más amplia de criterios en los programas de selección (FAO 2010). En el caso del vacuno de raza autóctona, muy bien valorado en este sentido por estar bien adaptado al medio y constituir un medio de conservación medioambiental y desarrollo rural, los criterios de selección siguen en la actualidad, muy ligados a la mejora (cuantitativa) de la productividad. A pesar de ello, este tipo de razas difícilmente alcanzan los rendimientos obtenidos por las razas de aptitud cárnica mejoradas, y además para conseguir unos niveles de desarrollo muscular óptimos es necesario un mayor coste de producción con respecto a las razas de aptitud cárnica más especializadas dado su peor índice de transformación. En consecuencia, estas carnes suelen presentar un mayor precio de venta al público por lo que para competir en el mercado los productores deben apostar por la promoción de otros aspectos como su calidad, que supone un valor añadido que tiene que ser explotado al máximo.

Características como las organolépticas en un producto como la carne y en especial, en la de vacuno, juegan un papel fundamental en el concepto de calidad, y ésta además suele ir ligada a los distintivos de origen territorial o de elaboración del alimento. Y es que, a pesar de que la carne con marca de calidad posea unas propiedades más o menos homogéneas por el mero hecho de proceder de animales de una raza concreta, criados y engordados en un área geográfica específica o alimentados con una dieta particular, sigue presentado

una importante variabilidad que debe ser controlada para que las expectativas del consumidor se encuentren satisfechas contribuyendo así a su fidelización como cliente.

Sin embargo, mantener constante algo que depende de tantas variables como es el caso de la calidad organoléptica e instrumental de la carne de vacuno no es sencillo. Así, la predicción de la calidad sensorial de la carne a nivel de consumidor se ha convertido en un paradigma de vital importancia para el sector cárnico que busca seguir siendo competitivo en un mercado saturado de ofertas (HOCQUETTE *et al. In press*). El desarrollo de estándares con los que agrupar las canales y la carne como el MSA australiano, el USDA Beef Quality & Yield Grade americano, el JMGA japonés o el SEUROP europeo constituyen un esfuerzo para la clasificación del producto en función de su calidad (POLKINGHORNE and THOMPSON 2010), si bien son claramente insuficientes para determinar la calidad de la carne, especialmente el SEUROP que es el estándar bajo el que se rigen nuestras producciones. Así, siguen siendo necesarios métodos más exhaustivos y precoces (en cebadero e incluso en campo) que permitan predecir el potencial de un animal para producir una carne con una calidad sensorial e instrumental óptima. La cuantificación de los atributos en que se descomponen la calidad y la posterior recopilación de registros para utilizarlos en mejora clásica resultan costosas y difíciles de abordar dada la estructura del sistema productivo en el que es complicado mantener un grupo de individuos controlado durante la totalidad de la cadena productiva hasta la obtención del producto final. La genética molecular es un ejemplo de herramienta altamente cualificada para la mejora, debido a que salva

muchos de los inconvenientes anteriormente citados por las numerosas opciones que ofrece.

El final del siglo XX y principios del XXI ha traído consigo el conocimiento de la estructura y funcionalidad de los genomas y el desarrollo de técnicas de secuenciación (de fragmentos cortos primero y masiva después) que nos han permitido pasar de los sistemas tradicionales de selección a la integración de la información molecular entre las herramientas utilizadas en los programas de mejora animal, por ello en la actualidad hay una tendencia a la sustitución de las herramientas clásicas por la información genómica. Así por una parte, los marcadores moleculares han permitido la detección en los animales domésticos de numerosas regiones en el genoma con influencia sobre caracteres cuantitativos (QTL) de interés económico mediante barridos de microsatélites primero y chips de ADN después. Esta relación viene determinada por proximidad (desequilibrio de ligamiento) o porque el propio marcador constituye la mutación causal. Y por otra parte, muchos han sido los genes que se han asociado a caracteres cuantitativos económicamente importantes por su implicación en rutas metabólicas responsables de la expresión del carácter. A partir de estas dos estrategias se han desarrollado test capaces de predecir si un animal posee un potencial genético favorable para una determinada producción o no.

La calidad instrumental y organoléptica de la carne constituye un ejemplo de carácter cuantitativo económicamente importante, ampliamente estudiado en numerosas poblaciones. A día de hoy se conocen genes asociados con caracteres como la terneza o la

infiltración grasa intramuscular y el efecto que sobre ellos tienen sus marcadores moleculares (alelos o variantes). Sin embargo es esencial la confirmación de dicho efecto en cada raza puesto que la asociación marcador-carácter es específica de población (HOCQUETTE *et al.* 2012).

El desarrollo de la presente tesis doctoral tiene lugar dentro del proyecto de investigación "TERNECO: Predicción in vivo de los parámetros de calidad de la carne de vacuno mediante técnicas ultrasonográficas y genómicas" que nace entre otras cosas, con el objetivo de ofrecer una herramienta al ganadero y al productor de carne de vacuno en la Dehesa que le permita predecir la calidad potencial de la carne que ofrece al consumidor. Cubriendo así mismo las necesidades del industrial con un instrumento para la clasificación de animales con características de calidad homogéneas y adecuadas a cada tipo de mercado. De esta forma tanto productor como industrial pueden disponer de una nueva fuente de información sobre el ganado o las canales para tomar decisiones de manejo, tipo de alimentación o momento de sacrificio. Esta herramienta además debía ser viable económicamente, rápida, no invasiva, precoz en la vida del animal y eficaz. Y todo ello sin gran consumo de recursos humanos ni de tiempo.

Para ello se propuso llevar a cabo en una muestra de animales criados en la Dehesa y cebados de manera intensiva un estudio de asociación entre marcadores moleculares en siete genes candidatos (*CAPN1*, *CAST*, *DGAT1*, *FABP4*, *LEP*, *RORC* y *SCD1*) previamente analizados en otras poblaciones de vacuno de carne, y los atributos que definen a la calidad de la carne, cuantificados tanto de manera objetiva como subjetivamente con la idea de confirmar y/o descartar

dicha asociación y determinar cuáles son los marcadores más convenientes para incluirlos en un futuro en una herramienta creada específicamente para nuestra población.

Nuestro grupo de investigación (MERAGEM) posee una dilatada experiencia en la mejora de la raza bovina Retinta a partir de metodologías de genética cuantitativa. Los objetivos del esquema de selección de la raza Retinta se han centrado en la mejora de la producción de carne manteniendo las buenas características reproductivas de la raza. Dado el fuerte impacto que ha tenido la genética molecular sobre los esquemas de selección en ganadería, de un tiempo a esta parte ha existido una inquietud por parte de la Asociación de Criadores y de la dirección técnica del programa de mejora por incluir marcadores relacionados principalmente con la calidad de la carne en la valoración y selección de sus reproductores. La raza Retinta y sus cruces con las razas Limousin y Charolais constituyen la población mayoritaria criada en el área de Dehesa del Valle de los Pedroches, este es uno de los principales núcleos de producción de carne de vacuno en España, por ello y por el interés mostrado por la Asociación de Criadores de esta raza autóctona, esta población ha tenido un peso específico en la presente tesis.

Por tanto, esta tesis doctoral se divide en tres capítulos que se corresponden con los tres tipos de caracteres asociados a los diferentes marcadores moleculares analizados: la terneza instrumental, la deposición grasa y perfil de ácidos grasos analizados de manera instrumental y la calidad de la carne evaluada mediante análisis sensorial. Cada uno de estos capítulos está integrado por artículos científicos incluidos en revistas ISI en los que se han

publicado (o están en vías de publicación) los resultados obtenidos durante el desarrollo de la presente tesis.

En el primer capítulo, se aborda un análisis preliminar de la distribución de frecuencias alélicas para una serie de marcadores detectados en el gen *CAPN1* en tres razas bovinas autóctonas propias del área de la Dehesa: Retinta, Avileña Negra-Ibérica y Morucha. Posteriormente se analiza en una población comercial criada en la Dehesa y cebada en régimen intensivo, la asociación entre caracteres fenotípicos relativos a la terneza de la carne y dos polimorfismos: uno de los marcadores previamente analizados, el *CAPN1-316*, y un marcador, el *UoG-CAST*, situado en el gen que codifica al inhibidor calpastatina. Los trabajos que integran el capítulo son:

- ✓ Avilés, C., P. J. Azor, L. Pannier, R. M. Hamill, A. Membrillo and A. Molina (2009). New single nucleotide polymorphisms in the μ-calpain gene in Spanish maternal beef breeds. Animal biotechnology 20(3): 161-164.
- ✓ Avilés, C., M. Juárez, F. Peña, V. Domenech, I. Clemente and A. Molina (2013). Association of single nucleotide polymorphisms in *CAPN1* and *CAST* genes with beef tenderness from Spanish commercial feedlots. Czech journal of animal science 58(10): 479-487.

En el segundo capítulo, se presenta un estudio de asociación desarrollado entre cinco marcadores asociados a la deposición de grasa (*DGAT1-K232A*, *FABP4: g.7516G>C*, *LEP: g.73C>T*, *RORC: g.3290T>G* y *SCD1: g.878T>C)* y la deposición de grasa intramuscular de la carne y con el espesor de la grasa dorsal de la canal de una población comercial criada y cebada en la Dehesa. Este capítulo

también está integrado por el estudio de asociación entre el marcador *SCD1: g.878T>C* y la composición de ácidos grasos de la carne de la misma población. Los resultados han dado lugar a las siguientes publicaciones:

- ✓ Avilés, C., O. Polvillo, F. Peña, M. Juárez, A. L. Martínez and A. Molina (2013). Associations between *DGAT1*, *FABP4*, *LEP*, *RORC*, and *SCD1* gene polymorphisms and fat deposition in Spanish commercial beef. Journal of animal science 91(10): 4571-4577.
- ✓ Avilés, C., O. Polvillo, F. Peña, A. Horcada, M. Juárez and A. Molina. Association study between a single nucleotide polymorphism in bovine *SCD1* gene with fatty acid composition in a Spanish commercial population. Animal biotechnology. Artículo actualmente en revisión.

En el tercer capítulo de la tesis, se evalúa las asociaciones descritas en los dos capítulos anteriores con un tercer análisis en el que se emplean dos fuentes de información: la procedente de la evaluación sensorial llevada a cabo por un panel de jueces entrenados y por un panel de consumidores no entrenados. El trabajo que compone este último capítulo es el siguiente:

- ✓ Avilés C., Peña F., Polvillo O., Barahona M., Campo M. M., Sañudo C., Juárez M., Horcada A., Alcalde M. J., Molina A. Association between markers in candidate genes and organoleptic quality traits in beef cattle fed intensively. Livestock science. Artículo actualmente sometido.

OBJETIVOS

El objetivo principal de la presente Tesis Doctoral es comprobar la presencia de polimorfismos de bases individuales (SNP) en genes relacionados con la calidad de la carne en vacuno cuyo uso potencial en Selección Asistida por Marcadores pueda proporcionar un valor añadido a la carne de las principales razas ligadas al área Dehesa (Avileña Negra-Ibérica, Morucha, Retinta, y sus cruces con Limousin y Charolais) por su implicación en la expresión de la calidad instrumental y sensorial de la carne y analizar en los genotipos más extendidos (animales con mayor proporción de genes de raza Charolais , Limousin y Retinta), las posibles asociaciones entre los marcadores y dicha calidad.

Los objetivos específicos planteados son los siguientes:

1. Detectar la variabilidad de marcadores SNP en genes relacionados con la calidad de la carne en las razas comúnmente criadas en la Dehesa.

2. Evaluar la existencia de asociación entre dos marcadores en los genes que codifican al enzima μ-calpaína (*CAPN1*) y su inhibidor, calpastatina (*CAST*) y la terneza de la carne cruda y cocinada madurada a diferentes tiempos y evaluada instrumentalmente en muestras de individuos con una base Charolais, Limousin y Retinta.

3. Evaluar en la población la existencia de asociación entre los genes que codifican a cinco proteínas implicadas en la deposición de grasa *DGAT1* (diacilglicerol O-aciltransferasa), *FABP4* (proteína de unión a ácidos grasos 4), *LEP* (leptina), *RORC* (receptor huérfano asociado al RAR-γ) y *SCD1* (estearoil-CoA desaturasa 1) y dos tipos de depósitos grasos, subcutáneo e

intramuscular, así como entre el último de los genes y la composición de ácidos grasos de la carne.

4. Valorar las asociaciones entre los genes de las siete proteínas citadas y las características sensoriales de la carne de la misma población con un panel de consumidores no entrenado y un panel de catadores entrenado.

5. Proponer un panel de marcadores a incluir en una herramienta molecular para predecir la calidad organoléptica e instrumental potencial de la carne de vacuno producida en la Dehesa.

CAPÍTULO I

CAPÍTULO I: ANÁLISIS PRELIMINAR DE LA DISTRIBUCIÓN DE FRECUENCIAS ALÉLICAS PARA UNA SERIE DE MARCADORES DETECTADOS EN EL GEN *CAPN1*. ESTUDIO DE ASOCIACIÓN ENTRE CARACTERES FENOTÍPICOS RELATIVOS A LA TERNEZA DE LA CARNE Y LOS MARCADORES *CAPN1-316* Y *UoG-CAST*.

✓ Avilés, C., P. J. Azor, L. Pannier, R. M. Hamill, A. Membrillo and A. Molina (2009). New single nucleotide polymorphisms in the μ-calpain gene in Spanish maternal beef breeds. Animal biotechnology 20 (3): 161-164.

✓ Avilés, C., M. Juárez, F. Peña, V. Domenech, I. Clemente and A. Molina (2013). Association of single nucleotide polymorphisms in *CAPN1* and *CAST* genes with beef tenderness from Spanish commercial feedlots. Czech journal of animal science 58 (10): 479-487.

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NEW SINGLE NUCLEOTIDE POLYMORPHISMS IN THE μ-CALPAIN GENE IN SPANISH MATERNAL BEEF BREEDS

C. Avilés[1], P. J. Azor[1], L. Pannier[2], R. M. Hamill[2], A. Membrillo[1], and A. Molina[1]

[1]*MERAGEM Research Group. Department of Genetics, Faculty of Veterinary Science, University of Córdoba, Córdoba, Spain*

[2]*Teagasc, Ashtown Food Research Centre, Ashtown, Dublin, Ireland*

Calpains play an important role in the postmortem tenderization process of meat and several SNP in the μ-calpain gene (CAPN1) have been reported to be associated with tenderness in beef cattle. Our objectives were to identify the previously reported CAPN1 331G>C SNP and to detect new polymorphisms in this gene in Spanish maternal beef breeds. A fragment (exon 8 to 10) of the bovine CAPN1 gene was sequenced and genotyped in a sample of the main Spanish maternal beef breeds including Retinta, Morucha, and Avileña Negra-Ibérica. These breeds are characterized for their high meat quality, their adaptation to adverse environmental conditions, and their good maternal aptitude. This adaptation makes it possible to rear these breeds in the south and west of Spain, where drought and feed shortages occur frequently. Six SNP in the μ-calpain gene were found, five of which (CAPN1 80C>T, 302C>G, 310G>A, 445C>T, 524A>C) have not been reported previously. Sequences obtained for these five newly found SNP were submitted to GenBank (Accessions EU386166 to EU386183).

Keywords: μ-Calpain; Beef tenderness; Single nucleotide polymorphism

Tenderness has been repeatedly reported as the most important quality attribute of meat (1). The micromolar calcium-activated neutral protease (*CAPN1*) gene encodes a cysteine protease, μ-calpain, which degrades myofibrillar proteins and appears to be the primary enzyme in the postmortem tenderization process (2). The identification of genetic markers in genes such as *CAPN1*, which are associated with beef tenderness variability, can help researchers gain more knowledge about the tenderization process and may help in the selection of animals to improve this trait. The *CAPN1* gene has been mapped to BTA 29 (3), and a number of SNP in this gene have been reported to be associated with meat tenderness in multiple populations (4, 5). This includes the *CAPN1 331G>C* SNP in exon 9, which has been

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Address correspondence to C. Avilés, MERAGEM Research Group, Department of Genetics, Faculty of Veterinary Science, University of Córdoba, 14071 Córdoba, Spain. E-mail: v92avrac@uco.es

associated with lower shear force values for homozygous CC animals (6). The objectives of this study were firstly to estimate the frequency of the *CAPN1 331G>C* SNP and secondly, to detect new polymorphisms in Spanish maternal beef breeds.

METHODS, RESULTS AND DISCUSSION

Seventy-five animals of Spanish maternal beef breeds were sampled. Numbers for each breed were as follows; Retinta (n = 25), Avileña N-I (n = 25) and Morucha (n = 25).

Genomic DNA was isolated from blood, hair, or muscle samples. DNA extracted from blood and hair samples was carried out using the salting out method (7) and a modified phenol-chloroform method (8), respectively. DNA extractions from muscle samples were carried out using the QIAmp DNA Mini Kit (Qiagen®, Germany) as per manufacturers' instructions.

Primers were designed using Primer3® software (http://fokker.wi.mit.edu/primer3/input.htm) to cover a fragment (exon 8–10) of the bovine *CAPN1* gene sequence (GeneID: 281661). New forward and reverse primer were as follow; (F: 5′-GGGTGAGGGTCCATGGAGGCTG-3′; R: 5′-GGTGTTCCAGTTGCG GAACCTCTGGCT-3′). PCR amplifications of the 669 bp fragment were carried out on all cattle samples in an Eppendorf thermocycler (Eppendorf® AG, Germany). Amplicons obtained were purified and visualized on 2% agarose electrophoresis gels. An automatic sequencer ABI 3730 (Applied Biosystems®, USA) was used for the sequencing reaction. Sequences were trimmed and aligned using Sequencher v.4.6 software (Gene Codes Corporation®, Ann Arbor, MI, USA 1991–2006). After alignment, polymorphic sites were determined and different genotypes assigned.

Allelic frequencies for each polymorphic locus were estimated for each breed in our sample. Furthermore, in order to make a comparison between allelic frequencies for the *CAPN1 331G>C* SNP in Spanish breeds, with frequencies from other breeds published in previous studies (6), a chi-squared maximum likelihood test was carried out with Statistica v.6.0 software (StatSoft, Inc.®, Tulsa, OK, USA) using groups of breeds based on their production purposes.

Six SNP in the μ-calpain gene, including the *CAPN1 331G>C* SNP, were found and five of them (*CAPN1 80C>T, 302C>G, 310G>A, 445C>T, 524A>C*) have not been reported previously. Chromosomal positions of the SNP are presented in Table 1 and sequences obtained were submitted to GenBank (Accessions EU386166 to EU386183). Four of the five new SNP occur in intronic sequences of the *CAPN1* gene. The *524A>C* polymorphism occurs in exon 10 and results in a conservative amino acid substitution from leucine to isoleucine. Furthermore, allelic frequencies for all loci of samples representing the 3 cattle breeds are presented in Table 1. Chi-squared maximum likelihood test for the *CAPN1 331G>C* SNP revealed that Spanish maternal beef breeds present a significantly higher frequency of the C allele compared to continental beef and European dual purpose breeds ($p < 0.01$) (Table 2).

Sequencing of the *CAPN1* gene in individuals, segregating alleles with phenotypic traits, has been used successfully in cattle to identify polymorphisms associated with divergent levels of tenderness (3, 9).

Table 1 Allele frequencies for the identified SNP in the *CAPN1* gene in the main Spanish maternal breeds (Accession number: EU386167)

	Location	Allele	Retinta	Avileña N-I	Morucha	Total
Intron 8	80	C	0.69	0.89	0.83	0.80
		T	0.31	0.11	0.17	0.20
	302	C	0.69	0.85	0.83	0.79
		G	0.31	0.15	0.17	0.21
	310	G	0.72	0.89	0.83	0.81
		A	0.28	0.11	0.17	0.19
Exon 9	331	G	0.71	0.89	0.83	0.81
		C	0.29	0.11	0.17	0.19
Intron 9	445	C	0.71	0.89	0.83	0.81
		T	0.29	0.11	0.17	0.19
Exon 10	524	A	0.83	1.00	1.00	0.94
		C	0.17	0.00	0.00	0.06

We sequenced a fragment of the *CAPN1* gene and identified 5 new SNP. According to previously published research, the C allele from the *CAPN1 331G>C* SNP, also known as the 316 marker, is associated with lower shear force values (6). The Spanish maternal beef breeds (Retinta, Avileña N-I, and Morucha) in our population presented a significantly higher frequency of the C allele ($p < 0.01$) compared to the continental beef and European dual purpose breeds. If this marker is associated with tenderness in Spanish breeds such as Retinta, Avileña N-I, and Morucha in the same way as the *Bos taurus* beef populations analyzed by other authors (6, 10), these breeds may present a high proportion of beef with low shear force. However, further efforts are required to analyze this and the novel SNP for

Table 2 Chi-squared maximum likelihood test for genic differentiation among Spanish breeds and other breeds for the identified SNP. (Data of Continental, European dual purpose and British traditional beef breeds is obtained from Ref. (10))

		CAPN1 331G>C SNP			Test M-L	
Breeds		Allele G	Allele C	Total	Chi-sq	p
Maternal Spanish cattle breeds[1]	no.	107	25	132		
	%	81.06	18.94			
Continental beef breeds[2]	no.	85	5	90		
	%	94.44	5.56			
	Total	192	30	222	11.411	0.0097**
European dual purpose breeds[3]	no.	70	4	74		
	%	94.59	5.41			
	Total	177	29	206	16.460	0.0091**
British traditional beef breeds[4]	no.	81	35	116		
	%	69.83	30.17			
	Total	188	60	248	27.31	0.00001***

[1]Retinta, Avileña N-I and Morucha breeds; [2]Charolais and Limousin breeds; [3]Simmental and Gelbvieh breeds; [4]Angus, Hereford and Red Angus breeds.

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

prospective associations with meat tenderness and other quality traits in Spanish maternal beef breeds. This will allow us to determine if these polymorphisms are potential markers for meat tenderness in the Spanish maternal beef breeds and will permit assessment of their suitability for subsequent inclusion in selection programs.

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Association of single nucleotide polymorphisms in *CAPN1* and *CAST* genes with beef tenderness from Spanish commercial feedlots

C. Avilés[1], M. Juárez[2], F. Peña[1], V. Domenech[1], I. Clemente[3], A. Molina[4]

[1]Departamento de Producción Animal, Campus de Rabanales, Universidad de Córdoba, Córdoba, Spain
[2]Agriculture and Agri-Food Canada, Lacombe Research Centre, Lacombe, Canada
[3]TEICA, Cortegana, Spain
[4]Grupo de Investigación MERAGEM, Departamento de Genética, Campus de Rabanales, Universidad de Córdoba, Córdoba, Spain

ABSTRACT: **F**requencies of two SNPs in the μ-calpain (*CAPN1*) and calpastatin (*CAST*) genes in local and foreign commercial cross-breeds used in south-western Spain (Charolais, Limousin, and Retinta) were evaluated and the association of these markers with texture analysis in animals fattened under different feedlot conditions was assessed. Marker frequencies were estimated in a 286 bull crossbred population and the *longissimus dorsi* muscles from subsequently selected 161 animals were used to measure Warner-Bratzler shear force in raw and cooked samples at three different ageing days (1, 7, and 21). Significant differences ($P \leq 0.05$) were found for shear force in raw and cooked meat samples for the three ageing days for the three crossbreeds analyzed. Significant associations were observed for raw meat for the Charolais between shear force and the *CAPN1* marker ($P = 0.019$), as well as between the *CAST* polymorphism and shear force ($P = 0.027$) in the Limousin. No associations were found between the markers and shear force in the Retinta ($P > 0.05$). In contrast, although these markers might be useful in particular selected populations due to their effect on objective texture parameters, no significant association ($P > 0.05$) was found for cooked meat in the sample of Spanish commercial crossbreeds used in this study. Further studies with a higher number of animals will be necessary to confirm these results.

Keywords: association studies; *Bos taurus*; calpain; calpastatin; meat tenderness; molecular markers; shear force; SNP

The dehesa of the south-western Iberian Peninsula is a "man-made" ecosystem characterized by a savannah-like physiognomy (Joffre et al., 1999). Breed crosses between local (Retinta) and foreign (mainly Charolais and Limousin) breeds are reared on these territories characterized by highly variable Mediterranean climate. The crossbreeds maintain the high adaptation capacity to natural conditions from the local breeds while they acquire a productive potential from the foreign breeds.

Production or handling factors, including breed, age, sex, feeding, and pre-slaughter management, can determine the potential quality of meat characteristics (Monsón et al., 2004). Inadequate tenderness is the most serious cause of consumers' dissatisfaction and any improvement in tenderness would increase the value of the final product (Brooks et al., 2000). In fact, decreasing of this variability is one of the main current objectives of the meat industry because of its concern to homogenize the products to suit different markets (Warner et al., 2010).

Ageing has been reported to be the most important factor influencing beef tenderness (Juárez et

Original Paper *Czech J. Anim. Sci., 58, 2013 (10): 479–487*

al., 2011). Warner-Bratzler shear force is one of the standard tools to quantify tenderness because it presents a negative correlation with initial tenderness (–0.61), amount of perceptible connective tissue (–0.49), and overall tenderness (–0.60) assessed by a trained sensory panel (Caine et al., 2003). Among the factors responsible for the postmortem meat tenderization during ageing, the calpain-calpastatin proteolytic system has been identified to play a key role (Koohmaraie and Geesink, 2006). Two enzymes responsible for this process are the micromolar calcium-activated neutral protease μ-calpain, and its inhibitor, calpastatin (Koohmaraie, 1996). The moderate heritability (h^2) of tenderness (0.14–0.47) (O'Connor et al., 1997; Dikeman et al., 2005; Wheeler et al., 2005; Boukha et al., 2011) represents a potential for improvement through animal selection in a breeding program. However, tenderness cannot be measured routinely in commercial conditions, so Marker Assisted Selection is considered as a good alternative to improve the trait.

Several single nucleotide polymorphisms (SNP) in the μ-calpain (*CAPN1*) and calpastatin (*CAST*) genes have been reported to be associated with tenderness in beef cattle. Two markers in the *CAPN1* and *CAST* genes previously reported (Page et al., 2002; Schenkel et al., 2006) take part in the composition of a commercial DNA test and have been validated by Van Eenennaam et al. (2007). The SNP referred to as *CAPN1* is located on BTA29. The marker, known as *CAPN1-316*, is a transversion from guanine to cytosine at position 5709 of the GenBank Accession No. AF252504. The SNP referred to as *CAST* (mapped to BTA7) is also a guanine to cytosine transversion at position 282 of the GenBank Accession No. AY008267. The *C* allele of both markers is associated with more tender meat.

The effects of the markers tend to be breed-specific and cannot be extended to all *Bos taurus* breeds (Allais et al., 2011) without a detailed analysis for each particular population. There are few association studies of markers related to meat tenderness in local beef breeds and their crosses with foreign breed populations reared in Spain (Avilés et al., 2007). Moreover, different feedlot production systems may also have a significant impact on the expression of those genes.

The aim of this study was to evaluate the frequencies of two SNPs in the *CAPN1* and *CAST* genes in local and foreign commercial crossbreeds used in south-western Spain and to assess the association of these markers with texture analysis in animals fattened under different feedlot conditions.

MATERIAL AND METHODS

Experimental design and sample collection

The experiment was developed in two replicates: year 1 (n = 137) and year 2 (n = 149). Two hundred eighty-six crossbred cows with a genetic basis of the main breed reared in the south-western Spain, the Retinta (RE), and a different level of Charolais (CH) or Limousine (LI) blood were mated to CH, LI, and RE purebred sires. The entire males of the F_1 population were originally selected from 20 different farms (in order to achieve the maximum variability of the population) based on morphological characteristics. The breed crosses and the number of individuals per cross were as follows: CH n = 98, LI n = 99, and RE n = 89. The animals were allocated to two different commercial diets (feedlot types): a mixture of concentrate, corn silage, straw, and beetroot pulp (type 1: 49 for CH, 49 for LI, and 51 for RE) and a conventional feeding diet of straw plus concentrate (type 2: 49 for CH, 50 for LI, and 38 for RE). The bulls were slaughtered when they reached approximately 550 kg of live weight (mean ± SD = 544 ± 35.3) in a commercial abattoir according to the Council Directive 93/119/EC (1993). Carcasses were chilled at 4°C for 24 h with a constant air velocity of 0.5 m/s and a relative humidity of 90%.

DNA extraction and genotyping

Blood samples (5 ml) were collected from the caudal vein of 286 entire males belonging to three crossbred types. Genomic DNA was isolated from aliquots of 200 μl of blood samples using a commercial kit (Dominion®, Dominion-MBL s. l., Cordoba, Spain) according to manufacturer's instructions. The quality and amount of DNA was measured using a NanoDrop® ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, USA). Genomic DNA of each animal was stored at –20°C until subjected to allelic discrimination assays.

Amplification and genotyping of the *CAPN1* (SNP AF252504:g.5709G>C) and *CAST* (SNP AY008267:g.282G>C) genes was carried out using the 5' nuclease allelic discrimination assay.

Czech J. Anim. Sci., 58, 2013 (10): 479–487 *Original Paper*

Table 1. Primer and probe sequences (5' to 3') for each genotyped polymorphism

	CAPN1	*CAST*
Forward primer	gcagtgccgttttcctacag	ctgaatttggaaggaaggaattgca
Reverse primer	agctgctcccgcatgtaag	caattgtgagaatttaaattagtatgatttacatgtgaca
Probe 1[a]	tccacg**g**cgttcca	tttgggta**g**aaaattt
Probe 2	tccacg**c**cgttcca	ttgggta**c**aaaattt

[a]probe nucleotides in bold target the specific alternative alleles of a particular SNP

Samples were screened on an ABI PRISM® 7500 FAST Real Time system (Applied Biosystems, Foster City, USA) from the UCO-SCAI genomics facility. Primers and probes (with a different reporter dye on each probe) were designed (Table 1) by the manufacturer using the sequences previously analyzed by direct sequencing (see Avilés et al. (2009) for more details).

A panel of 17 microsatellites was used to assign each animal to its own population with the GeneClass (Version 1.0.02, xxxx) software. The probability of correct assignation was at least 75% with a maximum of 90% (mean = 81.8%). One hundred and sixty-one animals (54 for CH, 55 for LI, and 52 for RE) from the original 286 bull population were selected for subsequent meat quality analyses (animals wrongly assigned to the populations by morphological characteristics were then rejected).

Meat quality

In terms of the EEC Beef Carcass Classification Scheme, Commission Regulation (EU) No. 1249/08/EC (EU Commission Regulation, 2008), all carcasses fell within the class 2 for fatness and R+ to U– for conformation. The values of pH and temperature at the centre of the *longissimus dorsi* muscle were assessed at 20 min and 24 h post-mortem in order to detect carcasses with potential cold shortening risk (pH > 6.0 at temperatures < 10–12°C) or non-favourable ultimate pH (5.8–6.2). The *longissimus dorsi* muscle (between T6 and L6) was removed from the left carcass side 24 h post-mortem and sliced into 2 cm steaks. Three pairs of steaks, balanced by location, were vacuum packaged and stored at 4°C until they were subjected to shear force analysis after 1, 7, and 21 days of ageing.

Both steaks were used either as raw or cooked meat samples. Meat was cooked in a clamshell grill (PL. 4 model, Ascaso Factory, Barcelona, Spain) at 190°C until the internal temperature measured by HI98509 Checktemp®1C Pocket Thermometer (Hanna Instruments, Woonsocket, USA) reached 71°C. Six cores per steak (raw and cooked), with 1 cm^2 in cross section, were cut with muscle fibres parallel to the longitudinal axis of the sample. The cores were tested with a TA-XT2 instrument (Stable Micro Systems, Godalming, UK) using the Warner-Bratzler shearing device (crosshead speed 200 mm/m). The average shear force (for each steak amount of force necessary to completely cut the core) expressed in kg/cm^2 was reported as the average value for all evaluated cores.

Statistical methods

Genotype and allele frequencies were calculated for the genotyped sample set (n = 286). To assess the Hardy-Weinberg equilibrium, the total sample set and each crossbreed were analyzed using the exact probability test in GENEPOP (Raymond and Rousset, 1995a). Pairwise tests for genic differentiation (Raymond and Rousset, 1995b) were carried out to establish if the allelic frequencies were significantly different ($P \leq 0.05$) among breeds.

The data to compare the repeated shear force measurements for each genotype of the *CAPN1* and *CAST* genes across the 3 ageing days in raw and cooked meat were tested using a linear mixed effect model through the MIXED Procedure of SAS (Statistical Analysis System, Version 9.2, xxxx). An unstructured covariance structure was used assuming that the variability within ageing and the correlation among the three days are not homogeneous. The model was as follows:

$$y_{ijk} = \mu + G_i + A_j + F_k + (G \times A)_{ij} + (G \times F)_{ik} + (A \times F)_{jk} + \\ + (G \times A \times F)_{ijk} + \beta_{SW} + e_{ijk}$$

Separate analyses were carried out for each SNP and breed due to the significant differences ($P \leq 0.05$) of the breed × genotype interaction found in the previous model where the breed was included

as a fixed effect. For each marker, genotype (G_i), ageing day (A_j) and feedlot type (F_k) were included as fixed effects. Year was treated as a random effect. Second order interactions between effects were included. Slaughter weight was included as a linear covariate (β_{SW}). Mean separation was carried out using the Least Squares Means (LSM) option and accompanied by *F*-test protected LSD ($P \leq 0.05$). Quadratic contrasts were conducted to further explore the effect of genotype and ageing time on beef texture. A linear regression on the number of *C* alleles (0, 1, and 2) with the same models to calculate the genotype effects was used to estimate the average allele substitution effects.

RESULTS AND DISCUSSION

Allelic and genotypic distribution

The first objective of this study was to assess the frequencies of two SNPs in the *CAPN1* and *CAST* genes in local and foreign commercial crossbreeds commonly used by the beef industry in south-western Spain. These markers, which are included in commercial tests (such as GeneSTAR® Tenderness or Igenity® Tender-GENE), have been developed in previous works by examining populations that incorporate a wide variety of commercial crossbreeds.

Allele and genotype frequencies of each crossbreed are provided in Table 2. Significant deviations from the Hardy-Weinberg proportions were observed for the *CAPN1* marker ($P < 0.01$). However, no significant differences were found for the *CAST* marker ($P > 0.05$).

Similar allelic frequencies for the CH (*C* allele frequency = 0.36) and the LI (*C* allele frequency = 0.32) crossbreeds were found for the *CAPN1* marker. On the other hand, significant differences regarding the allelic frequencies of the RE crossbreed (*C* allele frequency = 0.64) were detected and pairwise tests for genic differentiation among crossbreeds confirmed that RE–LI ($P < 0.01$) and RE–CH ($P < 0.01$) for the *CAPN1* locus had significantly divergent allele frequencies. Page et al. (2004) estimated the *C* allele frequency in CH (0.05) and LI (0.08) breeds. However, Allais et al. (2011) found higher frequencies for the same allele, 0.09 in the CH and 0.27 in the LI purebred populations. Regarding the RE crossbreed, Avilés et al. (2009) estimated a frequency of 0.29 for the *C* allele.

The allelic distribution for the *CAST* marker in the three populations followed a different pattern. The *C* allele frequencies were 0.76 for the CH, 0.65 for the LI, and 0.67 for the RE crossbreeds. In the pairwise tests for genic differentiation among crossbreeds, the comparison between the LI and the CH breeds showed a significant difference of the allele frequencies for the *CAST* locus ($P = 0.035$), as well as a trend ($P = 0.092$) towards significance for RE–CH.

Schenkel et al. (2006) estimated the *C* allele frequencies in CH (0.69) and LI (0.73) crossbreeds. The small number of animals belonging to CH (8) and LI (28) breeds used by Schenkel et al. (2006) most likely contributed to the differences observed regarding the results here presented. For the RE crossbreed, the *C* allele frequency (0.67) was lower than that found in a preliminary study (0.91) (Avilés et al., 2007), where a population belonging to the Retinta's Herdbook was used. This variability might be attributed to the difference in the genotype (crossbred vs. purebred). Retinta breed is classified within the Red Convex Branch of cattle in the Iberian Peninsula and it has a high African influence (Pellecchia et al., 2007). This might explain the differences with crossbreeds from French origin beef cattle breeds.

Phenotypic traits

The value of pH measured 24 h post slaughter in the centre of the *longissimus dorsi* muscle was under 5.8 for all the carcasses. Significant dif-

Table 2. Genotype and allelic frequencies for the *CAPN1* and *CAST* loci for the three crossbred cattle populations genotyped

Breed	*CAPN1*					*CAST*				
	CC	*CG*	*GG*	*C*	*G*	*CC*	*CG*	*GG*	*C*	*G*
Charolais (n = 98)	0.26	0.21	0.53	0.36	0.64	0.56	0.39	0.05	0.76	0.24
Limousin (n = 99)	0.26	0.12	0.62	0.32	0.68	0.41	0.48	0.11	0.65	0.35
Retinta (n = 89)	0.59	0.11	0.30	0.64	0.36	0.43	0.49	0.08	0.67	0.33

Czech J. Anim. Sci., 58, 2013 (10): 479–487 *Original Paper*

Table 3. Probability of the *F*-test for genotype, ageing, and feedlot effects and interactions on shear force values for each cross-breed tested (*P*-values)

	Charolais		Limousin		Retinta	
	SFR	SFC	SFR	SFC	SFR	SFC
µ-Calpain marker						
CAPN1	4.01^{*}	0.36^{ns}	1.80^{ns}	2.38^{t}	0.33^{ns}	0.11^{ns}
Day	5.98^{**}	56.38^{***}	4.49^{*}	176.01^{***}	13.01^{***}	128.46^{***}
Feedlot	0.12^{ns}	7.11^{**}	0.98^{ns}	0.02^{ns}	1.47^{ns}	8.12^{**}
CAPN1 × day	0.99^{ns}	1.41^{ns}	1.39^{ns}	0.75^{ns}	2.01^{t}	0.14^{ns}
CAPN1 × feedlot	0.68^{ns}	0.36^{ns}	1.29^{ns}	1.86^{ns}	1.02^{ns}	1.87^{ns}
Day × feedlot	0.63^{ns}	3.85^{*}	1.82^{ns}	1.03^{ns}	0.04^{ns}	0.86^{ns}
CAPN1 × day × feedlot	0.23^{ns}	1.38^{ns}	0.05^{ns}	0.18^{ns}	1.68^{ns}	0.27^{ns}
Calpastatin marker						
CAST	2.58^{t}	1.58^{ns}	3.95^{*}	1.08^{ns}	0.70^{ns}	1.92^{t}
Day	10.22^{***}	37.80^{***}	3.43^{*}	66.17^{***}	9.42^{***}	92.63^{***}
Feedlot	2.89^{t}	18.97^{***}	1.97^{ns}	5.75^{*}	0.12^{ns}	3.20^{t}
CAST × day	2.28^{t}	0.52^{ns}	0.57^{ns}	0.84^{ns}	0.88^{ns}	0.56^{ns}
CAST × feedlot	0.08^{ns}	0.67^{ns}	3.10^{t}	1.29^{ns}	0.99^{ns}	0.94^{ns}
Day × feedlot	3.57^{*}	5.20^{**}	1.92^{t}	0.87^{ns}	0.43^{ns}	0.33^{ns}
CAST × day × feedlot	0.89^{ns}	0.38^{ns}	0.31^{ns}	0.92^{ns}	0.77^{ns}	0.68^{ns}

SFR = shear force in raw meat, SFC = shear force in cooked meat

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$;, $^{*}P < 0.1$, ns = nonsignificant differences

confidence level of predicted factors ($P \leq 0.05$)

ferences ($P \leq 0.05$) were found for shear force in raw (SFR) and cooked (SFC) meat among ageing days for the three crossbreeds analyzed (Table 3); no interaction ($P > 0.05$) was observed between ageing day and genotype for any of the markers.

Shear force values for raw and cooked meat from ageing days 1–21 in the three crossbred groups are given in Figure 1. The SFR did not present the same behaviour on the three populations. The CH and the RE crossbreeds showed higher values ($P \leq 0.05$) on day 1 compared to day 7, and kept the same shear force until day 21, meanwhile no significant differences ($P > 0.05$) were observed in SFR for the LI crossbreed between days 1, 7, and 21. However, the quadratic trend was more remarkable in SFR for the RE than for the CH and the LI crossbreeds. Lower SFR were observed than those reported by Christensen et al. (2011) for CH and LI breeds. No previous reports have been found about SFR in RE breed or crossbreeds.

The values observed for SFC in the present study were higher for the CH and lower for the LI crossbreeds than those reported by Christensen et al. (2011) for French CH and LI breed populations. A higher SFC was obtained for the RE crossbreed than that observed in a previous study (Sañudo et al., 1998). All crossbreeds started at a similar shear force level on ageing day 1 in cooked meat (10.2, 10.5, and 10.4 kg/cm^2 for CH, LI, and RE, respectively) but the decrease between days 1–16 was higher in the LI than in the CH and the RE breed types. However, from day 16 to day 21, although the SFC in the three crossbreeds continued decreasing ($P < 0.01$), it showed a quadratic trend for the LI crossbreed. Ageing time was shorter in the LI crossbreed than in the CH and the RE ones. Monsón et al. (2004) found LI breed was the most tender in the early ageing period comparing four different biotypes and recommended to consume the meat of this breed at short ageing times. Juárez et al. (2010) reported a quadratic trend of tenderness after extended ageing time due to, among other factors, moisture loss and its effect on cooking parameters. This tendency was also observed in the present study.

All the phenotypic differences among populations could be related to the genetic differences in the

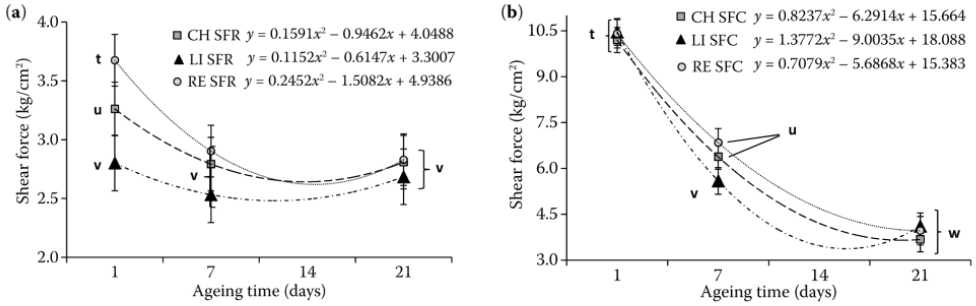

Figure 1. Shear force values for raw (**a**) and cooked (**b**) meat from ageing days 1–21 in the three crossbred groups

enzymatic activity of muscle and in its chemical-biological composition, differences in the intramuscular fat levels or differences in fibre typology, as reported by Sañudo et al. (2004). Shear force in raw beef has a positive relationship with total and insoluble collagen contents (Torrescano et al., 2003), in contrast to shear force in cooked meat, where the tenderization process is related to several aspects, among which myofibrillar degradation stands out (Uytterhaegen et al., 1994). Moreover, shear force differences among crossbreeds could be also explained by variability in the genotype composition and by the fact that purebred animals were not used in the present study.

Forage finishing of cattle has been shown to have negative consequences on meat tenderness. French et al. (2000) found that animals fed with a diet based on grass dry matter plus low levels of concentrate had lower Warner-Bratzler shear force than those fed with a silage plus concentrate diet, after 2 days of ageing. This difference was not found for ageing days 7 and 14.

CH and LI breeds are considered late-maturing. RE is an intermediate-maturing breed not as highly selected for muscular performance or carcass classification score as late-maturing breeds. These differences were reflected in fatness and leanness and hence, in meat characteristics. CH bulls were found to be heavier than LI ones. The differences in several other parameters related to fibre type, collagen contents, and enzymatic activities have also been reported (Jurie et al., 2005). This might explain why only meat from the CH crossbreed showed a different behaviour pattern when the diet changed. The interaction between ageing day and feedlot in the CH crossbreed showed significant differences ($P \leq 0.05$) for the effect of both markers on SFC and for the *CAST* SNP effect on SFR. Animals coming from feedlot 2 were more tender than those coming from feedlot 1 on day 1 (8.50 vs. 11.64 kg/cm^2; $P < 0.01$), however this difference became lower overtime and was not appreciable on days 7 and 21 ($P > 0.05$). The results in the present study showed the same pattern as those previously reported by other authors (French et al., 2000; Jurie et al., 2005). Changes in pH due to diet 24 h post slaughter might be behind the differences observed between feedlots.

Marker associations

Genotype Least Squares Means, standard errors, *P*-values, and average allele substitution effects of the *CAPN1* and *CAST* markers on SFR and SFC in the populations studied are reported in Table 4. The interaction between genotype and ageing times was not significant ($P > 0.05$).

In contrast to cooked meat, there are not previous association studies between raw meat and the *CAPN1* or *CAST* markers, since SFR has been used to detect collagen content variations. Nevertheless, significant associations ($P \leq 0.05$) were observed in the CH type between SFR and the *CAPN1* marker ($P = 0.019$) as well as between the *CAST* SNP and SFR ($P = 0.021$) in the LI crossbreed. The *CC* genotype of the *CAPN1* marker in the CH crossbreed as well as the *CAST* marker in the LI crossbreed were associated with more tender raw meat. The allele substitution effect for the trait estimated by the repeated measures analysis was −0.14 ± 0.06 for the CH crossbreed and *CAPN1* marker, and −0.11 ± 0.06 for the same trait between LI crossbreed and *CAST* marker. As expected, associations

Czech J. Anim. Sci., 58, 2013 (10): 479–487 *Original Paper*

Table 4. μ-Calpain and calpastatin SNP effect on shear force in raw and cooked meat of the three cross breeds

	Charolais		Limousin		Retinta	
	SFR	SFC	SFR	SFC	SFR	SFC
CAPN1						
CC	2.64 ± 0.30^{y}	6.72 ± 0.51	2.35 ± 0.18	6.57 ± 0.25	3.13 ± 0.16	6.94 ± 0.48
CG	3.10 ± 0.31^{x}	6.72 ± 0.59	2.34 ± 0.20	7.53 ± 0.39	3.28 ± 0.29	7.28 ± 0.64
GG	2.94 ± 0.29^{x}	6.98 ± 0.45	2.52 ± 0.17	6.81 ± 0.19	3.23 ± 0.20	6.83 ± 0.52
Average allele substitution effect ± SE (kg/cm$^{2)}$	−0.14 ± 0.06	−0.13 ± 0.20	−0.08 ± 0.05	−0.11 ± 016	−0.06 ± 0.09	0.03 ± 0.16
P-value	0.027	0.507	0.088	0.472	0.510	0.862
CAST						
CC	2.80 ± 0.29	6.68 ± 0.41	2.31 ± 0.15^{y}	6.70 ± 0.22	3.22 ± 0.18	6.60 ± 0.46
CG	3.05 ± 0.30	7.23 ± 0.45	2.57 ± 0.15^{x}	7.15 ± 0.22	3.19 ± 0.18	7.20 ± 0.47
GG	2.78 ± 0.47	7.44 ± 1.22	2.44 ± 0.18^{xy}	6.19 ± 0.39	2.82 ± 0.28	7.03 ± 0.60
Average allele substitution effect ± SE (kg/cm$^{2)}$	−0.19 ± 0.10	−0.51 ± 0.31	−0.11 ± 0.06	0.08 ± 0.20	0.14 ± 0.12	−0.35 ± 0.21
P-value	0.060	0.100	0.067	0.706	0.245	0.097

SFR = shear force in raw meat (Least Squares Means ± SE), SFC = shear force in cooked meat (Least Squares Means ± SE)
$^{x-y}$different letters within breed indicate significant differences among genotypes
confidence level of predicted factors ($P \leq 0.05$)

between shear force and markers dropped after cooking. Structural and distribution changes experimented by meat in factors such as collagen or water contents (Aalhus et al., 2009) after cooking diluted the effect of the myofibrillar component on the original texture characteristics. Warner-Bratzler shear force method can lose its ability to discriminate slight differences in shear force. Sensory panel or myofibrillar degradation index analyses could be more accurate to detect these associations with the *CAPN1* and *CAST* markers. No associations ($P > 0.05$) were found between raw meat and markers in the RE crossbreed.

No significant associations ($P > 0.05$) were found between SFC and the *CAPN1* marker, although there was a trend towards significance ($P = 0.076$) for the LI crossbreed, where *CC* genotype was related to more tender meat, as reported by Page et al. (2004). For the *CAST* SNP, no significant associations were observed between SFC and the marker although, in line with results obtained by Schenkel et al. (2006) and Van Eenennaam et al. (2007), the allele substitution effect in the RE crossbreed presented a trend towards significance ($P = 0.097$) with increased tenderness in beef from genotype *CC* vs. *CG* and *GG* (−0.60 and 0.43 kg/cm^2, respectively).

CONCLUSION

Significant associations were found between shear force and *CAPN1* and *CAST* markers in raw meat from commercial crossbreeds commonly used in south-western Spain. However, no significant associations were found between the *CAPN1* and cooked meat. Only the *CAPN1* marker in the LI crossbreed presented a trend towards significance for cooked meat. Consequently, samples were more homogeneous after cooking and associations disappeared. There are not strong associations between markers and shear force in the RE crossbreed probably because the RE purebred has not been highly selected and, as a result, its population is more heterogeneous in a wide range of different traits, such as tenderness. Moreover, the effects of marker tend to be breed-specific and these association studies have never used RE breed. Further studies with a higher number of animals will be necessary to confirm these results in Spanish commercial crossbreeds.

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Corresponding Author

Carmen Avilés, Universidad de Córdoba, Campus de Rabanales, Departamento de Producción Animal, Edificio de Producción Animal, 14071 Córdoba, Spain
Tel. +34 957 218 738, fax +34 957 218 707, e-mail: v92avrac@uco.es

CAPÍTULO II

CAPÍTULO II: ESTUDIO DE ASOCIACIÓN DESARROLLADO ENTRE LOS MARCADORES *DGAT1-K232A*, *FABP4: g.7516G>C*, *LEP: g.73C>T*, *RORC: g.3290T>G* Y *SCD1: g.878T>C*, LA DEPOSICIÓN DE GRASA INTRAMUSCULAR DE LA CARNE Y EL ESPESOR DE LA GRASA DORSAL. ESTUDIO DE ASOCIACIÓN ENTRE EL MARCADOR *SCD1: G.878T>C* Y LA COMPOSICIÓN DE ÁCIDOS GRASOS DE LA CARNE

- ✓ Avilés, C., O. Polvillo, F. Peña, M. Juárez, A. L. Martínez and A. Molina (2013). Associations between *DGAT1*, *FABP4*, *LEP*, *RORC*, and *SCD1* gene polymorphisms and fat deposition in Spanish commercial beef. Journal of animal science 91 (10): 4571-4577.

- ✓ Avilés, C., O. Polvillo, F. Peña, A. Horcada, M. Juárez and A. Molina. Association study between a single nucleotide polymorphism in bovine *SCD1* gene with fatty acid composition in a Spanish commercial population. Animal biotechnology. Artículo actualmente en revisión.

Associations between *DGAT1*, *FABP4*, *LEP*, *RORC*, and *SCD1* gene polymorphisms and fat deposition in Spanish commercial beef[1]

C. Avilés,*†[2] O. Polvillo,‡ F. Peña,*† M. Juárez,§ A. L. Martínez,† and A. Molina*

*MERAGEM Research Group, University of Cordoba, Campus de Rabanales, E-14071 Cordoba, Spain; †Department of Animal Production, University of Cordoba, Campus de Rabanales, E-14071 Cordoba, Spain; ‡CITIUS, Avda. Reina Mercedes, 4-B, E-41012 Sevilla, Spain; and §Lacombe Research Centre, Agriculture and Agri-Food Canada, 6000 C&E Trail, Lacombe, AB T4L 1W1, Canada

ABSTRACT: The objective of the present study was to assess the frequency distribution of markers in the diacylglycerol acyltransferase (*DGAT1*), fatty acid binding protein 4 (*FABP4*), leptin (*LEP*), retinoic acid receptor-related orphan receptor C (*RORC*), and stearoyl-CoA desaturase (*SCD1*) genes in a Spanish commercial crossbred population ($n = 286$) produced in southwest Spain. We have also evaluated the association of these 5 major SNP with backfat thickness (BFT) and intramuscular fat (IMF) to use them routinely in the industry (if the associations are confirmed) due to their ease of use. The *KK* genotype of the *DGAT1* gene was associated ($P = 0.046$) with the greatest BFT value. Bulls presenting the *GG* genotype for SNP in the *FABP4* gene showed greater values for the percentage of IMF ($P = 0.030$), which means an increase of 0.155% IMF per copy of the *G* allele of this marker ($P = 0.009$). A significant association was found between the *RORC: g.3290T > G* marker and the percentage of IMF. The *GG* genotype of the *RORC: g.3290T > G* marker showed the lowest IMF percentage ($P = 0.025$). The specific associations found in this study not only provide information about the involvement of these genes in the fat deposition at different levels in the southwestern Spain cattle population, but can also serve as a tool to improve certain meat quality attributes through Marker Assisted Selection. However, sensory studies are needed to explore further the usefulness of these genes in meat quality and the impact on the actual palatability of the beef.

Key words: association studies, backfat thickness, *Bos taurus*, intramuscular fat, molecular markers, single nucleotide polymorphisms

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INTRODUCTION

The inconsistent eating quality of beef is one of the most difficult challenges for consumer satisfaction (Aass et al., 2009). Fat depots have a strong influence on the expected palatability of beef (Jeremiah, 1996), and these are affected by many preslaughter factors, because they influence the transformation process of muscle into meat.

Very few traditional premortem methods for prediction of beef quality meet the requirements of the industry. Therefore, candidate genes have become a useful tool to predict these types of economically important traits. However, it is essential to have confirmation of the associations through independent studies before using the information provided by an association study (Barendse et al., 2010), as the association between a detected marker and the target trait appears to differ according to the breed (Hocquette et al., 2012).

Some genes have been identified as potentially capable of predicting differences in the fat deposits of cattle: the *K232A* SNP on the diacylglycerol acyltransferase gene (***DGAT1***) and its effect on fat content (Thaller et al., 2003); the effect of the *g.7516G > C* SNP in the fatty acid binding protein 4 gene (***FABP4***) on fat deposits reported by Michal et al. (2006); the association of the transition *g.73C > T* of the leptin gene (***LEP***) with differences in carcass fat levels in cattle described by Buchanan et al. (2002); the effect of the *g.3290T > G* SNP in the retinoic acid receptor-related orphan receptor

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[2]Corresponding author: v92avrac@uco.es
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C (***RORC***) gene on fat deposits, confirmed by Barendse et al. (2010). In addition, the *C* allele of the *g.878T > C* SNP in the stearoyl-CoA desaturase (***SCD1***) enzyme reported by Taniguchi et al. (2004) was found to favor the production of intramuscular fat (Wu et al., 2011).

The objective of the present study was to assess the pattern of markers in 5 main candidate genes (*DGAT1*, *FABP4*, *LEP*, *RORC*, and *SCD1*) in the common commercial crossbreeds produced in southwest Spain and to evaluate the association of these SNP with fat depots.

MATERIALS AND METHODS

Procedures were approved by the Córdoba University Ethical Committee for animal experimentation and in accordance with the European Union normative for care and use of experimental animals (Directive 2010/63/EU on the protection of animals used for scientific purpose.).

Animals and Experiment Design

Crossbreeds between Retinta and Continental beef breeds (Charolais and Limousin) are common in the beef industry in the Spanish Dehesa to improve performance traits and carcass conformation. The Retinta breed consists of approximately 18,000 cows and 200 sires (with a maximum effective population size of 720 individuals estimated using the software ENDOG v. 4. 8 (www.ucm.es/info/prodanim/html/JP_Web.htm; Gutiérrez and Goyache, 2005) and a realized effective population size of 33.2 for the last generation (Gutiérrez et al., 2009) recorded in their Studbook, although on commercial farms (not belonging to the Studbook), the census may be as much as 5 times greater. The progeny of a sample of cows were monitored whose genetic basis was Retinta (at least 50% Retinta) with Charolais or Limousin and which were mated with Charolais, Limousin, and Retinta purebred sires from 20 different farms (to achieve the maximum variability of population). Two hundred eighty-six intact crossbred males were originally selected for their morphological characteristics (e.g., coat and skin color and muscularity) to obtain 3 batches of crossbred animals with the maximum of the Charolais, Limousin, and Retinta breed characteristics. The breed crosses and the number of individuals per cross were as follows: 1/2 Charolais × Charolais (**CH**; $n = 98$), 1/2 Limousin × Limousin (**LI**; $n = 99$), and 1/2 Retinta × Retinta (**RE**; $n = 89$). The experiment was performed in 2 replicas: yr 1 ($n = 137$) and yr 2 ($n = 149$). The animals were allocated to 2 different commercial diets (feedlot types): a mixture of concentrate, corn silage, straw, and beetroot pulp (**type 1**: 49 for CH; 49 for LI; and 51 for RE) and a conventional feeding diet of straw plus concentrate (**type 2**: 49 for CH; 50 for LI; and 38 for RE). Bulls were slaughtered when they reached approximately 550 kg BW (mean ± SD = 544 ± 35.3) in a commercial abattoir according to the European Union Council Directive 93/119/EC (EU Council, 1993). Carcasses were chilled at 4°C for 24 h with a constant air velocity of 0.5 m/s and a relative humidity of 90%.

DNA Extraction, Sequence Analysis, and Genotyping

Blood samples (5 mL) were collected from the caudal vein of the 286 bulls using the Vacutainer system. Samples were stored in 6–10 mg EDTA-K3 Vacutainer tubes (Vacutest-Kima, Italy) at 4 ºC until they were transported to the laboratory.

Genomic DNA was extracted from aliquots of whole blood (200 µL) per sample using the Dominion-MBL commercial kit (Córdoba, Spain). A NanoDrop 117 ND-1000 spectrophotometer (Thermo Fisher Scientific Inc., West Palm Beach, FL) was used to quantify the DNA concentration. To locate each SNP and to confirm their variability in the population, a random subsample of 30 animals was selected to amplify 5 genomic fragments of the analyzed genes: *DGAT1* (Genbank accession JQ897351-53), *FABP4* (Genbank accession KC660106-08), *LEP* (Genbank accession KC660109-14), *RORC* (Genbank accession KC660115-19), and *SCD1* (Genbank accession AY241932). The PCR reaction included 2.5 µL PCR buffer, 0.75 µL Mg Cl_2 (50 mM), 1.2 µL dNTP (4 mM), 2 µL of each primer (5 mM), 2 µL genomic DNA, and 0.1 Taq DNA polymerase (5U/µL; Biotools B&M Labs, Madrid, Spain) in a final volume of 25 µL (Table 1). The thermal profile consisted of a hot start step of 96°C for 3 min, 35 cycles of 96°C for 30 s, and 72°C for 45 s (hybridization-elongation step) and a final extension step of 72°C for 10 min. The PCR products were purified with SureClean (Bioline, Luckenwalde, Germany) and sequenced with ABI PRISM 3130 capillary electrophoresis equipment (Applied Biosystems, Foster City, CA). Amplicons were analyzed with the Sequencher v.4.1.4 software (Gene Code Corp., Ann Arbor, MI).

Once the SNP were located in the first 30 samples and the variability in the population confirmed, an automated method was sought: subsequent amplifications and genotyping of the genes of the remaining 256 animals were performed using the Taqman allelic discrimination methodology. Samples were screened on an ABI PRISM 7500 FAST Real Time system (Applied Biosystems) from the Central Research Support Services of the University of Córdoba (**UCO-SCAI**) genomics facility. Primers and probes (with a different reporter dye on each probe) were designed to identify each polymorphism using 20 ng of genomic DNA from each animal (Table 2).

Table 1. Summary of SNP, primer pairs designed to sequence the fragments, accession number and fragment length used to locate the SNP and check their variability in the population

Gene[1]	SNP	Forward primer	Reverse primer	Variation	Genbank accession	Amplicon length
DGAT1	A/K[2]	5'-tcccacagtgggctccgtgctg-3'	5'-gccaggcctgcctgctcaccttg-3'	115 to 116	JQ897351–53	477 bp
FABP4	G/C	5'-ggccaccaggctcctctgtccatg-3'	5'-ccaacccagggatcaaactggggt-3'	131	KC660106–08	493 bp
LEP	C/T	5'-gattccgccgcacctctccccag-3'	5'-ggctggcggaggaggctgtgcag-3	188	KC660109–14	424 bp
RORC	T/G	5'-cgccggggaggaaagactcctgc-3'	5'-gctgtgcccaccgactcaccgag-3'	566	KC660115–19	872 bp
SCD1	T/C	5'-cagaaaatttccttgcccatt-3'	5'-tgttgcttaactttcaagggttt-3'	10329	AY241932	552 bp

[1]DGAT1: diacylglycerol acyltransferase gene; FABP4: fatty acid binding protein 4 gene; LEP: leptin gene;RORC: retinoic acid receptor-related orphan receptor C gene; SCD1: stearoyl-CoA desaturase.

[2]The mutation named *K232A* refers to a dinucleotide polymorphism (*AA* vs. *GC*) that causes a modification of the protein [lysine (*K*) or alanine (*A*)] encoded at the AA 232.

Meat Analysis

A panel of 17 microsatellites was used to assign each animal to its own population with the GeneClass version 1.0.02 software (www1.montpellier.inra.fr/URLB/geneclass/geneclass.html). The probability of correct assignation was at least 75% with a maximum of 90% (mean = 81.8%). One hundred sixty-one individuals (54 for CH, 55 for LI, and 52 for RE) from the original 286 bull population were selected for subsequent meat quality analyses (animals wrongly assigned to the populations by morphological characteristics were rejected). The LM (between T6 and L6) was removed from the left carcass side at 24-h postmortem and sliced into 2-cm steaks. Backfat thickness (**BFT**) over the rib eye was measured in triplicate at a standardized location of the steak using a digital calliper (Mitutoyo, Kawasaki, Japan). The average BFT expressed in centimeters was reported as the average value for the 3 measurements recorded. Percentage of intramuscular fat (**IMF**) in the LM was determined on 2-d postmortem meat samples in triplicate using the Soxhlet extraction method according to the Association of Official Analytical Chemists (**AOAC;** AOAC, 1990) method. The Soxhlet method (ISO R-1443) was developed using a Foss Tecator AB Soxtec 2050 (FOSS Analytical, Hilleroed, Denmark).

Statistical Methods

Genotype and allele frequencies for each crossbreed were calculated. To assess the Hardy-Weinberg equilibrium, the population was analyzed using the exact probability test of Genepop (http://genepop.curtin.edu.au/; Raymond and Rousset, 1995b). Pairwise tests for genic differentiation (Raymond and Rousset, 1995a) were performed to establish if the allelic frequencies were significantly different ($P \leq 0.05$) among breeds.

The data to compare the 2 different fat deposits (BFT and IMF) for each genotype of the *DGAT1*, *FABP4*, *LEP*, *RORC*, and *SCD1* genes were tested using a linear mixed effect model through the MIXED procedure (SAS Inst. Inc., Cary, NC). Separate analyses were performed for each SNP.

The models were as follows:

$$y_{ijk} = \mu + Fi + DGAT1j + (F * DGAT1)ij + Rk + \beta SW + eij$$

$$yijk = \mu + Fi + FABP4j + (F * FABP4)ij + Rk + \beta SW + eij$$

$$yijk = \mu + Fi + LEPj + (F * LEP)ij + Rk + \beta SW + eij$$

$$yijk = \mu + Fi + RORCj + (F * RORC)ij + Rk + \beta SW + eij$$

$$yijk = \mu + Fi + SCD1j + (F * SCD1)ij + Rk + \beta SW + eij$$

The 3 populations were analyzed as a single metapopulation due to the lack of significance ($P > 0.05$) of the crossbreed × genotype interaction found in a previous model where the crossbreed was included as a fixed effect. For each model, μ is the general mean of the trait, the

Table 2. Primers and probes designs for the Taqman assays

Gene[1]	Forward primer	Reverse primer	Probe 1[2]	Probe 2[1]
DGAT1	cgcttgctcgtagctttgg	cgcggtaggtcaggttgtc	ttggccgcctt**a**cct	cgttggcctt**c**ttacct
FABP4	gggatcaaacctgcatctcttatgt	cataggtggcgttagcgatgaa	aaccccgctgccaat	aaaccccgctgccaat
LEP	ctttggccctatctgtcttacgt	tcttgatgagggttttggtgtca	ccatct**g**caaggtc	catc**g**gcaaggtc
RORC	cccttgaccctacaacaacca	ctgtgctcactttctcagtccatta	tggcagcct**a**atagg	tggcagcct**c**atagg
SCD1	ccccgagagaatattctggtttcc	ccactagacgtggtcttgct	cttaccc**g**cagctcc	acttaccc**a**cagctcc

[1]DGAT1: diacylglycerol acyltransferase gene; FABP4: fatty acid binding protein 4 gene; LEP: leptin gene;RORC: retinoic acid receptor-related orphan receptor C gene; SCD1: stearoyl-CoA desaturase.

[2]Probe nucleotides underlined target the specific alternative alleles of a particular SNP.

feedlot type ($\boldsymbol{F_j}$) and genotype of the marker (represented by the acronym of the gene) were included as fixed effects. Interaction between feedlot type × genotype was included. Year of replicate (***Rk***) was treated as a random effect. Slaughter weight was included as a linear covariate ($\beta\boldsymbol{SW}$). The percentage of phenotypic variability explained by each marker was estimated using the comparison of the adjustment of the previous model with and without the effect of the marker. Mean comparison was performed using the LSMEANS option using *F*-test protected LSD ($P \leq 0.05$). Quadratic contrasts were conducted to further explore the additive effect of the genotype on the traits. A linear regression on the number of hypothetic favorable alleles (0, 1, and 2) with models including feedlot type as a fixed factor and slaughter weight as a linear covariate was used to estimate the average allele substitution effects.

RESULTS AND DISCUSSION

Allelic and Genotypic Distribution

Table 3 shows the allelic and genotypic frequencies for the 286 bulls, with each polymorphism. The 3 populations were in Hardy-Weinberg equilibrium for the 5 markers. Pairwise tests for genic differentiation for the *DGAT1 K232A*, *LEP: g.73C > T*, and the *SCD1: g.878T > C* mutations indicate differences between Continental (CH and LI) and RE breed crosses; for the *FABP4: g.7516G > C* marker, however, the LI crossbreed presented different allelic frequency distribution regarding CH and RE crossbreeds; with respect to the *RORC: g.3290T > G* polymorphism, only CH and LI crossbreeds presented different frequency distributions.

In the present study, the frequency of the *K* allele of the *DGAT1 K232A* polymorphism was greater than that of the *A* allele for the 3 populations analyzed, similar to Renand et al. (2007) for CH and LI breeds and to Rodero et al. (2013) for endangered Spanish breeds. However, these results revealed differences in the allelic frequencies regarding the findings of Kaupe et al. (2004), who performed an exhaustive study of the marker with 38 different *B. taurus* and *B. indicus* breeds, where *K* was the predominant allele only in 5 breeds and none of the 5 was a Continental breed. In the same way, in the current analysis, *G* was the most common allele for the *FABP4: g.7516G > C* marker in the 3 populations, in contrast to Michal et al. (2006) who performed a study with a Wagyu × Limousin F_2 population and Pannier et al. (2010) who worked with Continental (including CH and LI breeds) dual-purpose and dairy cattle breeds.

On the other hand, as per Buchanan et al. (2002), who worked with 4 different European breeds, the present analysis of the *LEP: g.73C > T* SNP showed a heterogeneous frequency distribution where *C* was the most frequent allele for the Continental crosses (CH = 0.48 and LI = 0.60), unlike for the RE crossbreed, in which *T* was the most frequent allele with a value of 0.69.

Table 3. Genotypic and allelic frequencies for *DGAT1*, *FABP4*, *LEP*, *RORC*, and *SCD1* genes for the Spanish population tested

			Crossbreed[2]		
Gene[1]			CH (*n* = 98)	LI (*n* = 99)	RE (*n* = 89)
DGAT1	Alleles	A	0.18	0.17	0.39
		K	0.82	0.84	0.61
	Genotypes	AA	0.02	0.03	0.13
		AK	0.33	0.28	0.51
		KK	0.65	0.69	0.36
FABP4	Alleles	C	0.24	0.14	0.18
		G	0.76	0.86	0.82
	Genotypes	*CC*	0.06	0.01	0.04
		CG	0.35	0.25	0.27
		GG	0.59	0.74	0.69
LEP	Alleles	*C*	0.58	0.60	0.31
		T	0.42	0.40	0.69
	Genotypes	*CC*	0.36	0.39	0.13
		CT	0.44	0.40	0.35
		TT	0.20	0.20	0.52
RORC	Alleles	*G*	0.12	0.23	0.19
		T	0.88	0.77	0.81
	Genotypes	*GG*	0.00	0.03	0.04
		GT	0.23	0.39	0.29
		TT	0.77	0.58	0.66
SCD1	Alleles	*C*	0.61	0.58	0.31
		T	0.39	0.42	0.69
	Genotypes	*CC*	0.39	0.30	0.08
		CT	0.44	0.56	0.47
		TT	0.17	0.14	0.45

[1]DGAT1: diacylglycerol acyltransferase gene; FABP4: fatty acid binding protein 4 gene; LEP: leptin gene;RORC: retinoic acid receptor-related orphan receptor C gene; SCD1: stearoyl-CoA desaturase.

[2]CH = 1/2 Charolais × Charolais; LI = 1/2 Limousin × Limousin; RE = 1/2 Retinta × Retinta

Finally, the allelic frequency distributions of the *RORC: g.3290T > G* and *SCD1: g.878T > C* markers was similar to previous results from Barendse et al. (2007) who used Angus and Shorthorn breeds, Milanesi et al. (2008) who studied the *SCD1* marker in 11 Italian cattle breeds with different selective purposes, and Bartoň et al. (2010) who worked with Fleckvieh breed, with the sole exception of the RE crossbreed, in which the *C* allele for the *SCD1: g.878T > C* mutation presented a frequency greater than *T*. These dissimilarities in variability and allelic frequency confirm the importance of detailed analyses in each particular population, as allelic frequencies are highly variable even within breeds.

Meat Analyses

The averages and SD for the meat quality traits tested are presented in Table 4. To obtain the lean carcasses

Table 4. Slaughter weight and fat characteristics in commercial Spanish beef carcasses

Crossbreed[1]	Slaughter weight, kg	Backfat thickness, cm	Intramuscular fat, %
CH (n = 54)	569 ± 22.8	3.28 ± 1.32	1.74 ± 0.44
LI (n = 55)	553 ± 21.5	3.57 ± 1.43	1.77 ± 0.46
RE (n = 52)	508 ± 27.6	2.90 ± 1.20	1.67 ± 0.57
Overall population (n = 161)	544 ± 35.3	3.26 ± 1.34	1.73 ± 0.49

[1]CH = 1/2 Charolais × Charolais; LI = 1/2 Limousin × Limousin; RE = 1/2 Retinta × Retinta

demanded by the Spanish market, mainly RE bulls were slaughtered at lighter BW (508 kg ± 27.6) than the crossbreeds of CH (569 kg ± 22.8) and LI (553 kg ± 21.5). This is common practice in Spain because the RE crossbreed is considered an intermediate-maturing breed compared with CH and LI crossbreeds (late-maturing breeds). This practice, although useful in certain circumstances (e.g., to achieve better results in fat cover scores and to increase sales of carcasses to the retailer), can have a negative impact on the sensory traits of meat from the RE crossbreed (e.g., juiciness, flavor, or tenderness) compared with Continental crossbreeds. In fact, reduced values of BFT and IMF were observed (Table 4) for RE crossbreeds compared with CH and LI crosses.

Marker Analysis

Least square means, SE, and average allele substitution effects for BFT and IMF traits are shown in Table 5. There was a tendency toward the significance of the effect of the feedlot type on the traits ($P < 0.10$) and no significant differences ($P > 0.10$) were appreciated between the crossbreeds × genotype interactions in the model.

Unlike Fortes et al. (2009), who described a lack of association between the *DGAT1 K232A* polymorphism and BFT in a *B. indicus* population, a significant association ($P \leq 0.05$) was found in our sample between the marker and this trait, and the variation in the *DGAT1 K232A* SNP explained 0.24% of the phenotypic variation. Therefore, the *KK* genotype was associated ($P = 0.046$) with the greatest BFT value. A significant additive effect of the *K* allele of 0.455 cm ($P = 0.013$) was detected in the metapopulation analyzed. There were no differences ($P > 0.10$) among genotypes for IMF within the population analyzed. This result contrasts with the findings from Li et al. (2013), who reported a significant effect of the marker on the IMF in a Swedish beef breed population, but it is consistent with the conclusions from Renand et al. (2007) in French beef breeds (CH, LI, and Blonde d'Aquitaine) and Pannier et al. (2010) in a metapopulation of mixed-purpose *B. taurus* breeds among which CH and LI breeds were presented. As has been described previously (Yuan et al., 2013), discrepant results regarding the association of this SNP with different fat deposits have been published. This disparity highlights the fact that the effect of markers cannot be extended to all cattle populations.

Table 5. Least square means (± SE) for the genotype effects on fat deposits (BFT[1] and IMF[2]) of the crossbred cattle population of the SNP in *DGAT1*, *FABP4*, *LEP*, *RORC*, and *SCD1* genes

Gene[3,4]	Trait	Genotype			*P*-value	α[5]	*P*-value
DGAT1		AA	AK	KK			
	BFT	2.46 ± 0.46^{b}	2.98 ± 0.15^{b}	3.37 ± 0.12^{a}	0.046*	0.455 ± 0.165	0.013*
	IMF	1.64 ± 0.19^{a}	1.68 ± 0.07^{a}	1.75 ± 0.06^{a}	0.705^{ns}	0.055 ± 0.076	0.407^{ns}
FABP4		CC	CG	GG			
	BFT	3.58 ± 0.72^{a}	3.33 ± 0.14^{a}	3.47 ± 0.16^{a}	0.754^{ns}	0.055 ± 0.194	0.585^{ns}
	IMF	1.52 ± 0.20^{b}	1.64 ± 0.05^{ab}	1.83 ± 0.05^{a}	0.030*	0.155 ± 0.067	0.009**
LEP		CC	CT	TT			
	BFT	3.44 ± 0.19^{a}	3.11 ± 0.17^{a}	3.10 ± 0.18^{a}	0.342^{ns}	0.170 ± 0.137	0.207^{ns}
	IMF	1.72 ± 0.08^{a}	1.77 ± 0.07 ^{a}	1.67 ± 0.08^{a}	0.662^{ns}	0.025 ± 0.060	0.671^{ns}
RORC		GG	GT	TT			
	BFT	2.53 ± 0.93^{a}	3.44 ± 0.18^{a}	3.11 ± 0.12^{a}	0.250^{ns}	0.290 ± 0.203	0.260^{ns}
	IMF	1.10 ± 0.35^{c}	1.85 ± 0.07^{a}	1.66 ± 0.05^{b}	0.025*	0.280 ± 0.083	0.021*
SCD1		CC	CT	TT			
	BFT	3.37 ± 0.24^{a}	3.15 ± 0.16^{a}	3.23 ± 0.23^{a}	0.692^{ns}	0.070 ± 0.170	0.692^{ns}
	IMF	1.67 ± 0.09^{a}	1.72 ± 0.06^{a}	1.77 ± 0.09^{a}	0.457^{ns}	0.050 ± 0.064	0.457^{ns}

$^{a-c}$Different letters within row indicate significant differences among genotypes. Significance level: ns: > 0.1; t: < 0.1; *: < 0.05; **: < 0.01; ***: < 0.001.

[1]BFT = Backfat thickness (cm).

[2]IMF = Intramuscular fat of the LM (%).

[3]DGAT1: diacylglycerol acyltransferase gene; FABP4: fatty acid binding protein 4 gene; LEP: leptin gene;RORC: retinoic acid receptor-related orphan receptor C gene; SCD1: stearoyl-CoA desaturase.

[4]SNP Id: *DGAT1: K232A*; *FABP4: g.7516G > C*; *LEP: g.73C > T*; *RORC: g.3290T > G*; *SCD1: g.878T > C*.

[5]α: Average allele substitution effect ± SE.

No significant association ($P > 0.10$) was found between the *FABP4: g.7516G > C* marker and BFT in the population. However, a significant association of the marker with the percentage of the IMF was observed ($P = 0.030$) and the variation in the marker explained 0.22% of the variation of the trait. Bulls presenting the *GG* genotype for this SNP showed greater values for this trait, which means an increase of 0.155% IMF per copy of the *G* allele of this marker ($P = 0.009$). Diverse association studies are again contradictory. Different authors have reported both an effect on marbling score and BFT in a Wagyu × Limousin F_2 population (Michal et al., 2006) and lack of effect on both traits (IMF and BFT) in a *B. indicus* metapopulation (Curi et al., 2011) and on IMF in a *B. taurus* metapopulation (Pannier et al., 2010).

No significant differences were detected among genotypes for the *LEP: g.73C > T* mutation for both fat deposits in the study ($P > 0.10$), as reported by Pannier et al. (2009) in a study where almost one-half of the animals belonged to CH or LI breed, or by Barendse et al. (2005) who worked with a *B. taurus* and *B. indicus* metapopulation.

No differences ($P > 0.10$) in BFT were observed among *RORC: g.3290T > G* SNP genotypes. In contrast, a significant association was found between the *RORC: g.3290T > G* marker and the percentage of IMF, and the variation in the marker explained 0.76% of the phenotypic variability. The *GG* genotype of the *RORC: g.3290T > G* marker showed the lowest IMF percentage ($P = 0.025$). This result is consistent with the study performed by Barendse et al. (2010), who reported that the *T* allele of this SNP increased IMF. Nevertheless, this association could not be confirmed definitively because of the low frequency of the *GG* genotype. Further studies with a larger population would be necessary to confirm the additive effect of the *T* allele.

Finally, the differences on the genotype of the *SCD1: g.878T > C* marker did not influence BFT or IMF in the population tested in this work ($P > 0.10$). These results contrast with those published by Wu et al. (2011) in the Chinese Simmental breed.

In conclusion, fat content is a complex trait that has an important influence on meat quality. Despite differences in the frequency distribution of these 5 mutations across crossbreeds, they did not seem to be reflected in objective meat quality measurements. This may be due to other markers with a slight effect in the expression of the characters that have not been monitored in this study. In any case, it has been demonstrated that the effect of markers on fat deposits is breed specific and cannot be extended to all cattle populations. The specific associations found in this study not only provide information about the involvement of these genes in fat deposition at different levels in the southwest Spain cattle population, but can also serve as a tool to improve some meat quality attributes through marker-assisted selection.

Therefore, it might be advisable to find other ways, such as sensory studies, to explore further the usefulness of these genes for meat quality prediction and their impact on the palatability of beef.

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Association study between a single nucleotide polymorphism in bovine SCD1 gene with fatty acid composition in a Spanish commercial population

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Title: Association study between a single nucleotide polymorphism in bovine *SCD1* gene with fatty acid composition in a Spanish commercial population

C. Avilés[a1], O. Polvillo[b], F. Peña[a], A. Horcada[c], M. Juárez[d], and A. Molina[a]

[a]MERAGEM research group. Veterinary Faculty. University of Cordoba, 14071, Cordoba, Spain; [b]CITIUS, Avda. Reina Mercedes, 4-B, 41012, Sevilla, Spain; [c]Department of Agroforestry Science. University of Sevilla. Spain; [d]Agriculture and Agri-Food Canada, Lacombe Research Centre, 6000 C&E Trail, T4L 1W1, Lacombe, Canada.

[1]Corresponding author: v92avrac@uco.es

Dpto. de Producción Animal. Campus de Rabanales. Universidad de Córdoba

14071, Córdoba (Spain). Phone nr. +0034 957 218 738

Abstract

Stearoyl-CoA desaturase gene (*SCD1*) is an enzyme responsible for the endogenous conversion of saturated fatty acid into mono-unsaturated fatty acids. The objective of this study was to assess the association of a single nucleotide polymorphism (SNP) in the *SCD1* gene with the fatty acid composition of beef intramuscular fat of a Spanish commercial bull population (n=155) finished with two different diets. The results suggested that the marker could be used as a candidate gene to obtain a healthier final product.

Keywords: association study; fatty acid composition; *Bos taurus*; molecular markers; SNP; stearoyl CoA-desaturase

Introduction

Fatty acid profile contribute importantly to various aspects of meat quality and are central to the nutritional value of meat (1). However, the concern about benefits and adverse effect of the dietary fat and fatty acid intake is a matter of current interest among the different population groups. Several molecular mechanisms regulating the synthesis and assembly of fatty acids are still scarcely studied but many authors agree that the putative atherogenic saturated fatty acids (SFA) are the C12:0, C14:0 and C16:0 (2). Stearoyl-CoA desaturase (*SCD1*), also known as Δ^9-desaturase, plays a key role in the conversion of SFA into mono-unsaturated fatty acids (MUFA). A single nucleotide polymorphism (SNP) in the position 878 of a DNA fragment of this gene (Accession # AB075020) has been published to be associated with beef fatty acid profile (3). Although the diet used in the finishing period of animals is crucial for intramuscular fat (IMF) of the meat, the genetic component of the animal is involved (to a lesser extent) in the final expression of this kind of traits. The

association of this marker previously described with the fatty acid composition of beef of the common cattle populations produced in the south-western Iberian Peninsula has not been so far described. The objective of the present study was to assess this association with the fatty acid composition IMF of beef of a Spanish commercial bull population finished with two different diets.

Material and methods

To carry out the experiment 155 bulls were allocated to two different feedlots with two different finishing diets: a Total Mixed Ration (TMR) composed by concentrate plus mineral-vitamins, maize silage and grass straw (n=73) and a Conventional Ration (CR) made of concentrate (including barley meal, maize meal, whole soybean, corn gluten feed, palm oil and mineral-vitamin) and grass straw provided separately (n=83). The animal management, blood sample collection and DNA analysis have been previously described (4). In order to determine the fatty acid composition, *longissimus dorsi* muscle (between the 6th thoracic and the 6th lumbar vertebra) was removed from the left carcass side at 24 h *post-mortem* and sliced into 2 cm thick steaks and trimmed of any external fat. Total fatty acids were extracted, methylated and analyzed by an adaptation of the method described by Aldai, et al. (5). Separation and quantification of the fatty acid methyl esters was carried out using a gas-chromatograph (GC, Agilent 6890N, Agilent Technologies Spain, S.L., Madrid, Spain) equipped with a flame ionization detector automatic sample injector HP 7683, and using a HP-88 J&W fused silica capillary column (100 m, 0.25 mm i.d.,0.2 µm film thickness, Agilent Technologies Spain, S.L., Madrid, Spain). Individual fatty acids were expressed as the percentage of total fatty acids identified and

grouped as follows: SFA, MUFA and polyunsaturated (PUFA). The C14, C16 and C18 indexes were calculated as follows: C14 index = C14:1 *cis*-9 / (C14:0 + C14:1 *cis*-9) × 100; C16 index = C16:1 *cis*-9 / (C16:0 + C16:1 *cis*-9) × 100; C18 index = C18:1 *cis*-9 / (C18:0 + C18:1 *cis*-9) × 100. Finally the MUFA/SFA ratio was estimated and also a Health index (HI) proposed by Li et al. (6) as HI = (total MUFA + total PUFA) / (4 × C14:0 + C16:0).

The counts of the genotype for each diet and overall population were calculated. To assess the Hardy Weinberg equilibrium, the population was analyzed using the exact probability test of Genepop (7). A Chi-squared test was carried out to establish if there were significant differences ($P < 0.05$) between the allelic and genotypic frequencies of each diet.

The data to compare the percentage of the main fatty acids in beef related to the activity of the enzyme, ratio and indexes for each genotype of the *SCD1* gene were tested using a general linear model through the GLM procedure of SAS (SAS Version 9.2, SAS Institute, Cary, NC, USA).

The statistic model was as follows:

$$y_{ij} = \mu + D_i + SCD1_j + (D * SCD1)_{ij} + \beta_{SW} + e_{ij}$$

where, y_{ij} = phenotypic observation, μ = general mean of the trait; D_j = fixed effect of the diet, $SCD1_j$ = fixed effect of the genotype of the marker, β_{SW} = linear effect of slaughter weight as covariate and e_{ij} = residual error. Interaction between diet × genotype was included. Mean comparison was carried out using the LSMEANS option using *F*-test protected LSD ($P < 0.05$). Quadratic contrasts were conducted to further explore the additive effect of the genotype on the traits. A linear regression on the number of hypothetic favourable alleles (0,

1, and 2) with models including diet as a fixed factor and slaughter weight as a linear covariate was used to estimate the average allele substitution effects. The contribution of the marker to the variation of the individual and groups of fatty acids observed was estimated as the reduction of the residual variance when the full model was compared with the reduced model without the effect of the *SCD1* genotype.

Results and discussion

As it was reported before, the beef content of fatty acids is highly influenced by the composition of the diet. In the present study, the difference between diets in the experiment affected the fatty acid composition of the meat in the population studied (Table 1). Significant effects of diet ($P < 0.05$) was detected in three individual fatty acids: C18:0, C16:1 *cis*-9 and C18:1 *trans*-1; two groups of fatty acid: SFA and MUFA; MUFA/SFA ratio and three indexes: C14, C16 and C18 index. The TMR was poorer in roughage and cereals than the CR; consequently the amount of unsaturated fatty acids of this second diet is higher than the quantity in the former (8). In ruminants, the biohydrogenation of unsaturated fatty acids performed by the microorganisms of the rumen is responsible for the variations in fatty acid composition of the IMF. However, the PUFA proportion of phospholipids is strictly controlled by an endogenous complex enzymatic system (9) where the *SCD1* is included. So the composition of fatty acids reveals the previous action of *SCD1* on some of the main saturated fatty acids stored in the fat depots such as C16:0 or C18:0 (10). In this study, the effect of the *SCD1* polymorphism was significant ($P < 0.05$) in the C14:1 *cis*-9, in the C14 index, in the MUFA/SFA ratio and in the HI. The contribution to the

variance of the effects ranged from 0.20% for the HI to 2.13 % for the C14 index. Moreover a tendency towards significance ($P < 0.1$) was also observed in the effect of the marker on the C14:0 and SFA.

No significant departures from Hardy–Weinberg equilibrium were identified for the *SCD1* SNP. The counts of the genotypes (*CC*, *CT* and *TT*) are in parenthesis in Table 2. The Chi-squared test did not indicate differences in the allelic or genotypic frequency distributions among diets and the overall population. The frequency distributions were not different to those previously reported by Avilés et al. (4) in a larger population of the common breeds reared in the South-west of the Iberian Peninsula.

Taniguchi et al. (3) firstly described the association of the *SCD1 c.878C>T* SNP with MUFA percentage and melting point in fat tissue of Japanese Black cattle. In our results, the CR provided to the animals, contributed to the accumulation of unsaturated fatty acids such as C16:1 *cis*-9, C18:1 *trans*-11 or the total amount of MUFA (Table 2) and this was reflected in the MUFA/SFA ratio, in contrast, levels of saturated fatty acids like C18:0 and total SFA were higher in animals fed with the TMR. Regarding to the effect of the *SCD1* marker, the *CC* genotype of the SNP was particularly associated to differences related to the C14 fatty acid, presenting higher values of C14:1 *cis*-9 ($P < 0.01$) and C14 index ($P < 0.001$). The approximate estimation of the *SCD1* activity made by this desaturation index suggested that in individuals with *CC* and *CT* genotype, the enzyme is more active compared with those with *TT* genotype. Moreover, this genotype seemed to be linked to lower amount of SFA ($P < 0.1$) and, as a consequence, higher MUFA/SFA ratio. These results are consistent with those previously reported by Bartoň et al. (11) in

Fleckvieh bulls, Li et al. (12) in two Spanish paternal breeds, Li et al. (6) in Canadian Angus and Charolais-based commercial crossbred beef steers and Ohsaki et al. (13) in Japanese Black cattle. Supporting this association, significant differences were also observed for the HI. The *CC* and *CT* genotypes presented higher values for the index than the *TT* genotype ($P < 0.05$) for individuals fed with the CR. This fact becomes important since the C14:0 acid was identified as the most atherogenic (14), with about four times cholesterol-raising potential of C16:0. The only significant interaction ($P < 0.05$) between diet and genotype was observed for C16:0 (Figure 1). Animals inheriting the *TT* genotype and fed with the CR presented higher levels ($P < 0.05$) of C16:0 regarding *CC* or *CT* genotype. This effect showed a different pattern when the animals were fed with the TMR. The C16:0 is the most abundant saturated fatty acid in meat and, together with C12:0 and C14:0, is considered unhealthy because of its atherogenic properties (2). So, this interaction suggests a management strategy based in the feeding scheme avoiding the CR for animals that inherit the *TT* genotype in order to obtain a healthier beef product. With respect to the C18:0, no significant effects of the marker on the fatty acid composition were found. This fatty acid is used to assess the nutritional implications of IMF on human diet through the desirable fatty acids estimation (MUFA+PUFA+C18:0) described by Huerta-Leidenz et al.(15). However, the SNP was not useful to estimate the amount of this favourable fatty acid.

To sum up we can conclude that, in spite of the population size, different genotypes of *SCD1 c.878C>T* SNP presented a demonstrated effect on the fatty acid composition of the IMF of beef in Spanish common cattle populations. The results suggested that this marker

could be used as a predictor of the nutritional quality of this meat to obtain a healthier final product.

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Table 1. F-test probability for diet, genotype effects and interactions on each trait tested in the Spanish commercial beef population (*P*-values)

	Diet	Marker	Feedlot × Marker
C14:0	$1.77^{n.s.}$	2.82^{t}	$1.20^{n.s.}$
C16:0	$0.06^{n.s.}$	$1.59^{n.s.}$	3.56*
C18:0	11.56***	$0.58^{n.s.}$	$0.06^{n.s.}$
C14:1 *cis*-9	$1.55^{n.s.}$	5.77**	$0.95^{n.s.}$
C16:1 *cis*-9	5.74*	$0.10^{n.s.}$	$0.92^{n.s.}$
C18:1 *cis*-9	3.74^{t}	$1.88^{n.s.}$	$1.50^{n.s.}$
C18:1 *trans*-11	16.45***	$0.00^{n.s.}$	$1.93^{n.s.}$
CLA *cis*-9, *trans*-11	$0.19^{n.s.}$	$0.95^{n.s.}$	$0.09^{n.s.}$
SFA	9.63**	3.02^{t}	$1.80^{n.s.}$
MUFA	7.12**	$1.79^{n.s.}$	$1.47^{n.s.}$
PUFA	$1.17^{n.s.}$	$0.21^{n.s.}$	$2.95^{n.s.}$
C14 index	13.29***	23.18***	$0.04^{n.s.}$
C16 index	7.93**	$0.02^{n.s.}$	$0.38^{n.s.}$
C18 index	7.91**	$1.11^{n.s.}$	$0.92^{n.s.}$
MUFA/SFA	11.12**	3.15*	$0.50^{n.s.}$
Health index	$1.97^{n.s.}$	3.27*	$2.44^{n.s.}$

Significance level: ns: $P > 0.1$; t: $P < 0.1$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$

Table 2. Least-square means of the marker and average allele substitution effect for each suggestive association in the Spanish commercial beef population tested

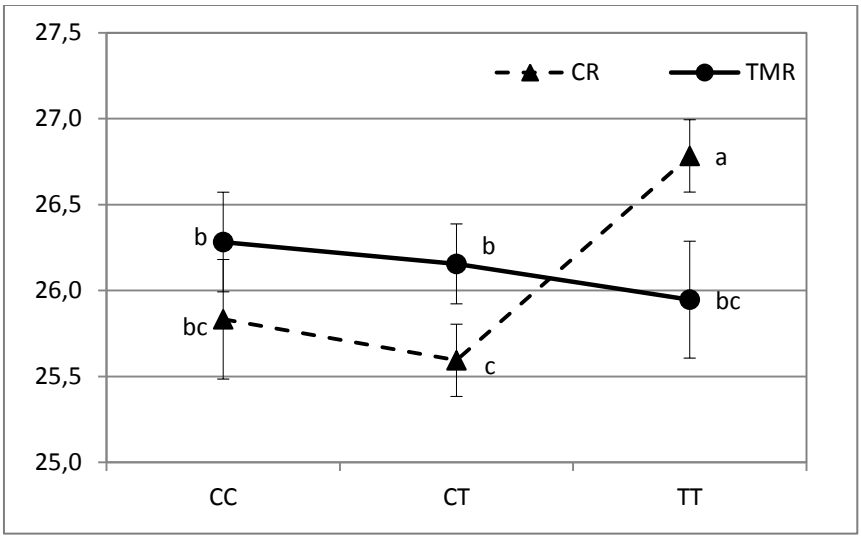
	TMR				CR			
	CC (23)	CT (34)	TT (16)	α[§]	CC (16)	CT (42)	TT (24)	α
C14:0	2.20 ± 0.12	2.21 ± 0.07	2.34 ± 0.10		2.14 ± 0.08^{b}	2.03 ± 0.06^{b}	2.33 ± 0.09^{a}	-0.09 ± 0.06^{t}
C16:0	26.20 ± 0.42	26.10 ± 0.25	26.05 ± 0.35		25.97 ± 0.21^{b}	25.61 ± 0.16^{b}	26.72 ± 0.24^{a}	-0.40 ± 0.17*
C18:0	18.96 ± 0.53	19.19 ± 0.31	19.20 ± 0.44		17.66 ± 0.33	18.203 ± 0.25	18.27 ± 0.37	
C14:1 *cis*-9	0.45 ± 0.03	0.42 ± 0.02	0.37 ± 0.02		0.50 ± 0.02^{a}	0.42 ± 0.02^{b}	0.40 ± 0.03^{b}	0.05 ± 0.02*
C16:1 *cis*-9	2.36 ± 0.15	2.40 ± 0.09	2.38 ± 0.12		2.65 ± 0.11	2.52 ± 0.08	2.67 ± 0.12	
C18:1 *cis*-9	28.31 ± 0.84	29.32 ± 0.50	27.64 ± 0.70		30.94 ± 0.75	29.71 ± 0.58	29.04 ± 0.86	
C18:1 *trans*-11	1.05 ± 0.08	1.15 ± 0.05	1.20 ± 0.06		1.46 ± 0.10	1.37 ± 0.08	1.32 ± 0.11	
SFA	49.66 ± 0.62	49.66 ± 0.37	49.87 ± 0.52		47.92 ± 0.37^{b}	48.18 ± 0.29^{b}	49.83 ± 0.42^{a}	-0.98 ± 0.30**
MUFA	33.48 ± 0.99	34.61 ± 0.59	32.80 ± 0.83		37.16 ± 0.85	35.48 ± 0.66	34.79 ± 0.97	
C14 index	16.73 ± 0.57^{a}	15.65 ± 0.34^{b}	13.54 ± 0.48^{c}	1.40 ± 0.51**	18.89 ± 0.54^{a}	17.09 ± 0.42^{b}	14.58 ± 0.62^{c}	2.22 ± 0.51***
C16 index	8.20 ± 0.42	8.34 ± 0.25	8.30 ± 0.35		9.21 ± 0.34	8.91 ± 0.25	9.06 ± 0.37	
C18 index	59.63 ± 1.24	60.36 ± 0.74	58.83 ± 1.04		63.55 ± 0.93	61.78 ± 0.72	61.21 ± 1.06	
MUFA/SFA	0.68 ± 0.02	0.70 ± 0.13	0.66 ± 0.02		0.78 ± 0.02^{a}	0.74 ± 0.02^{b}	0.70 ± 0.02^{c}	0.03 ± 0.01*
Health index	1.46 ± 0.05	1.46 ± 0.03	1.43 ± 0.04		1.52 ± 0.03^{a}	1.54 ± 0.02^{a}	1.39 ± 0.03^{b}	0.06 ± 0.02*

[§]α: Average allele substitution effect ± SE

Means bearing different superscript (in row) differ significantly ($P < 0.05$) or present a tendency towards signification ($P < 0.1$).

Significance level: ns: $P > 0.1$; t: $P < 0.1$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$

Figure 1. Interaction between *SCD1* genotype and diet for the C16:0 fatty acid



a-c Different letters within diet indicate significant differences among genotypes

CAPÍTULO III

CAPÍTULO III: ESTUDIO DE ASOCIACIÓN ENTRE LOS MARCADORES *CAPN1-316*, *UoG-CAST*, *DGAT1-K232A*, *FABP4: g.7516G>C*, LEP*: g.73C>T*, *RORC: g.3290T>G* Y *SCD1: g.878T>C* Y LA CALIDAD SENSORIAL DE LA CARNE

✓ Avilés C., Peña F., Polvillo O., Barahona M., Campo M. M., Sañudo C., Juárez M., Horcada A., Alcalde M. J., Molina A. Association between markers in candidate genes and organoleptic quality traits in beef cattle fed intensively. Livestock science. Artículo actualmente sometido.

Association between markers in candidate genes and organoleptic quality traits in beef fed intensively

Avilés C[a1]., Peña F[a]., Polvillo O.[b], Barahona M[c]., Campo M. M.[c], Sañudo C.[c], Juárez M.[d], Horcada A.[e], Alcalde M. J.[e], Molina A.[a]

[a]MERAGEM research group. Veterinary faculty. University of Cordoba, 14071, Cordoba, Spain; [b]CITIUS, Avda.Reina Mercedes, 4-B, 41012, Sevilla, Spain; [c]Animal Production and Food Science. Veterinary faculty. Zaragoza, Spain. [d]Agriculture and Agri-Food Canada, Lacombe Research Centre, 6000 C&E Trail, T4L 1W1, Lacombe, Canada. [e]Department of Agroforestry Science. University of Sevilla. Spain;

[1]Corresponding author: v92avrac@uco.es

Dpto. de Producción Animal. Campus de Rabanales. Universidad de Córdoba

14071, Córdoba (Spain). Phone nr. +0034 957 218 738

ABSTRACT

The aim of this study was to assess the associations between the SNPs in *CAPN1*, *CAST*, *DGAT1*, *FABP4*, *LEP*, *RORC* and *SCD1* genes and sensory meat quality in commercial genotypes intensively fed. This work concluded the analyses carried out in a common Spanish commercial sample of animals reared in the Dehesa ecosystem. To carry out the experiment 161 bulls were allocated to two different feedlots with two different finishing diets: a total mixed ration (unifeed diet) and a conventional ration (*ad libitum* diet). Steaks aged for 7 and 21 days were assessed by an untrained consumer panel and a trained sensory panel. Significant association and allelic substitution effect was observed for markers *CAPN1-316*, *DGAT1-K232A*, *RORC: g.3290T>G* and *SCD1: g.878T>C* on the different descriptors evaluated. There are not precedents of this kind of association studies in a Spanish commercial population. The study suggested that *CAPN1*, *DGAT1*, *RORC* and *SCD1* genes have a potential effect on the different measurements of sensory meat quality.

KEYWORDS

Acceptability; association studies; *Bos taurus*; consumers; molecular markers; trained panel; single nucleotide polymorphisms

INTRODUCTION

Biological characteristics of muscles, intramuscular fat and carcass adipose tissues, determine organoleptic and dietetic meat quality traits and, consequently, the quality of meat. The development of minimum invasive strategies like marker assisted selection to select and improve beef populations by economically important traits, such

as meat quality, constitutes a challenge for the industry nowadays, moreover taking into account the currently saturation of the markets to what consumers face off. Variability in meat sensory quality, especially in tenderness, flavour and overall liking depends in part on differences in the biological characteristics of skeletal muscles at slaughter (Hocquette et al., 2012). *Post-mortem* tenderization is caused by enzymatic degradation of key myofibrillar and associated proteins (Koohmaraie, 1996). μ-Calpain and calpastatin genes were previously hypothesized to be genetic factors influencing tenderization in beef. The single nucleotide polymorphism (SNP) known as *CAPN1-316* firstly associated to meat tenderness by Page et al. (2004) is located on the gene that encodes the μ-calpain protein. The SNP referred to as *UoG-CAST* was firstly described as tenderness predictor by Schenkel et al. (2006). Both polymorphisms constituted a guanine (*G*) to cytosine (*C*) transversion and, in both markers, were the *C* allele the one associated to more tender meat. The *K232A* SNP on the diacylglycerol acyltransferase gene (*DGAT1*) and its effect on fat content have been widely studied (Thaller et al., 2003; Pannier et al., 2010). Even more, Curi et al. (2011) firstly demonstrated a potential relationship between this SNP and back fat thickness (BFT) in beef cattle. Michal et al. (2006) reported the effect of the *g.7516G>C* mutation in the fatty acid binding protein 4 gene (*FABP4*) on the marbling score and the subcutaneous fat depth. Leptin, the hormone product of the obesity gene, regulates appetite and the energy metabolism. A cytosine (*C*) to thymine (*T*) transition in the exon 2 (*g.73C>T*) of the leptin gene (*LEP*) that encodes a change of an arginine to a cysteine was associated with differences in carcass fat levels in cattle (Buchanan et al., 2002). The retinoic acid receptor-

related orphan receptor C, whose gene is known as *RORC*, is a member of the steroid and thyroid hormone receptor superfamily and binds retinoic acid as well as thyroid hormone (Barendse et al., 2007). Barendse, Bunch et al. (2010) confirmed that the *T* allele of the *RORC* polymorphism increased the deposit of intramuscular fat (IMF). The stearoyl-CoA desaturase (*SCD1*) enzyme produces monounsaturated from saturated fatty acids introducing a double bond at the Δ^9-position of the saturated acids. The *SCD1:g.878T>C* mutation reported by Taniguchi et al. (2004) is located in the exon 5 of the *SCD1* gene. The *C* allele of this SNP was found to be favorable for the IMF of the loin (Wu et al., 2012). The association between a detected marker and the target trait appeared to be different according to breed (Hocquette et al., 2012). Previous association studies of these markers were described in commercial genotypes (breed-crosses) between Retinta (RE) and Continental beef breeds (Charolais (CH) and Limousin (LI)) (Avilés et al., 2013a; Avilés et al., 2013b; Avilés et al., *In press*). The aim of this study was to confirm these associations reported between the SNPs in *CAPN1*, *CAST*, *DGAT1*, *FABP4*, *LEP*, *RORC* and *SCD1* genes and objective and subjective meat quality traits in a commercial population produced intensively.

MATERIALS AND METHODS

Experimental design

To carry out the experiment 161 bulls from 3 different commercial genotypes were allocated to two different feedlots with two different finishing diets: an unifeed composed by concentrate plus mineral-vitamins, maize silage and grass straw, diet provided twice a day, and

a ration made of concentrate and wheat straw provided separately and both *ad libitum*. A detailed description of the population was presented in Avilés et al. (2013b). Bulls were slaughtered when they reached approximately 550 kg of live weight (mean ± SD = 544 ± 35.3 kg) in a commercial abattoir according to the EU Council Directive 93/119/EC (93/119/EC, 1993). Carcasses were chilled at 4 °C for 24 h with a constant air velocity of 0.5 m/s and a relative humidity of 90%.

Sensory analyses

To carry out the sensory analysis 2 portions of 10 cm each, from the *Longissimus* muscle (LM) of the left half of each carcass were extracted, vacuum packed and transported to the laboratory. The pieces were stored at 4ºC during two different periods of time: 7 and 21 days. After the different ageing times, two 2-cm thick steaks were obtained from each portion (four steaks per animal), and kept at -20ºC until the moment of the analysis.

All the vacuum packed steaks were thawed previously to each session. They were kept in a refrigerator at 4º C during 24 h and, 2 h before the analysis, the steaks were taken out the cooler until they achieve an internal temperature of 15-17º C. The meat was cooked in a double plate grill SAMMIC at 200º C until the internal temperature reached 70º C. Then, the steaks were cut in 2 × 2 × 2 cm cubes and wrapped in aluminum foil coded with a random number of 3 digits and kept at 45º - 55º C until the moment of the analysis that took place immediately.

To develop the consumer analysis five hundred people were recruited (49.2% men and 50.8% women) ranging from 18 and 25 years of age (22.8%), 26 and 40 years of age (30.4%), 41 and 60 years of age (30.6%) and 61 and 75 years of age (16.2%). The experimental

design consisted of 50 sessions of 10 consumers each in laboratory conditions with white light. Each consumer was presented with ten samples, one per treatment, served and tasted in random order (Macfie et al., 1989). During the evaluation, tenderness acceptability, flavor acceptability and overall acceptability were assessed with hedonic scales structured in 9 levels. The intermediate level was removed forcing the consumer to choose the positive or negative side of the acceptability scale. Consumers were asked to eat a bit of bread and drink some mineral water at the beginning of the sensory evaluation and between samples in an attempt to taste each sample in the same palate conditions.

The trained sensory panel was made up by 9 members in individual cabins under controlled environmental conditions. Prior to the test, attributes to be assessed were defined in a training session. The panel evaluated tenderness, juiciness and beef flavour intensity on a 10-point semi-structured and continuous scale in which intensity ranged from very low (0) to very high (10). Evaluations were based on an incomplete blocks design for a balanced model (Cochran and Cox, 1978) with four samples per plate. Summarizing, 12 treatments were evaluated (3 commercial genotypes × 2 diets × 2 ageing times) along 30 sessions with 12 samples per session for a total of 30 judgments per treatment. For sensory descriptors measured in each type of analysis see Table 1.

Markers analysis

Blood samples (5mL) were collected from the caudal vein of the 161 bulls using the Vacutainer system following the procedures approved by the Córdoba University Ethical Committee for animal

experimentation and in accordance with the European Union normative for care and use of experimental animals.

Genomic DNA was extracted from aliquots of 200 µL per sample using the Dominion-MBL commercial kit (Córdoba, Spain). A NanoDrop 117 ND-1000 spectrophotometer (Thermo Fisher Scientific Inc., West Palm Beach, FL, USA) was used to quantify the DNA concentration. The procedure, primer designs and thermal profiles followed to amplify and genotype the animals for the 7 SNPs was described previously (Avilés et al., 2013a; Avilés et al., 2013b).

Statistical analysis

Genotype and allele frequencies for each commercial genotype were calculated. To assess the Hardy Weinberg equilibrium, the population was analyzed using the exact probability test of Genepop (Raymond and Rousset, 1995).

The data to compare the different sensory attributes for each genotype of the markers in the *CAPN1*, *CAST*, *DGAT1*, *FABP4*, *LEP*, *RORC* and *SCD1* genes were tested using a linear mixed effect model through the MIXED procedure of SAS (SAS Version 9.2, SAS Institute, Cary, NC, USA). Separate analyses were carried out for each SNP, type of panel (untrained and trained), ageing day in the case of markers linked to tenderness or finishing diet in the case of markers linked to fat deposition.

The models for markers in the *CAPN1* and *CAST* genes were as follows:

$y_{ijk} = \mu + F_i + P_j + G_k + \beta_{SW} + e_{ijkl}$

For each model, μ was the general mean of the trait; the ageing day (A_i), the consumer or trained panellist (C_j) and genotype of the

marker (G_l), were included as fixed effects. Slaughter weight nested by commercial genotype was included as a linear covariate (β_{SW}).

The models for markers in the *DGAT1*, *FABP4*, *LEP*, *RORC* and *SCD1* genes were as follows:

$y_{ijk} = \mu + A_i + P_j + G_k + \beta_{SW} + e_{ijkl}$

As in the previous model the three commercial populations were analyzed as a single metapopulation. The three commercial genotypes were analyzed as a single metapopulation due to the lack of significance ($P > 0.05$) of the commercial genotype × genotype interaction found in a previous model where the commercial genotype was included as a fixed effect. For each model, μ was the general mean of the trait; the feedlot type (F_i), the consumer or trained panellist (C_j) and genotype of the marker (G_l), were included as fixed effects. Slaughter weight nested by commercial genotype was included as a linear covariate (β_{SW}).

The percentage of phenotypic variability explained by each marker was estimated using the comparison of the adjustment of the previous models with and without the effect of the marker. Mean comparison was carried out using the LSMEANS option using *F*-test protected LSD ($P \leq 0.05$). Quadratic contrasts were conducted to further explore the additive effect of the genotype on the traits. A linear regression on the number of hypothetic favourable alleles (0, 1, and 2) with models including ageing time of feedlot type and the consumer or trained panellist as a fixed factor and slaughter weight as a linear covariate was used to estimate the average allele substitution effects. To avoid the declaration of false positive parameters when a large number of contrasts are analyzed, the Benjamini-Hochberg procedure (Benjamini and Hochberg, 1995) was

carried out to correct the corresponding p-values (with a False Discovery Rate of 5%). This methodology is an efficient way of controlling the False Discovery Rate in multiple testing, as it is more powerful than the classical Bonferroni correction (Thissen et al., 2002). Finally, a Principal Component Analysis (PCA) with a non-linear iterative partial least-squares (NIPALS) algorithm was performed to identify the effect of the SNPs and the origin of the meat (determined by the combination of the breed of the animal, the diet and the ageing time) on both sensory data: determined by consumers and by trained panel.

RESULTS AND DISCUSSION

Genotypic frequency distributions for each SNP are presented in Table 2. Although genotype frequencies were similar to those reported before in a larger population (Avilés et al., 2013a; Avilés et al., 2013b), significant deviation from the Hardy-Weinberg equilibrium proportions was also observed for the *LEP: g.73C>T* marker (the disequilibrium in *CAPN1-316* was already observed in the previous publication).

With respect to the markers linked to tenderness, least-square means per SNP and descriptor for the untrained consumer analysis are presented in Table 3. The *CAPN1-316* marker was highly significant for the 2 attributes assessed in both ageing times (7 and 21 days). The sensory scores for tenderness and overall acceptability improve when a copy of the *C* allele is present in the genotype of the animal showing an allelic substitution effect that range from +0.28 ± 0.07 to 0.12 ± 0.04 however, this effect was higher in steaks aged during 7 days than in those aged during 21 days. Moreover, in the

analysis performed by the trained panellists (Table 4), the significant association of the marker was only found with tenderness of steaks aged 7 days. The μ-calpain is autolyzed as ageing time increased, and only about 5-10% remains active after 14 days (Koohmaraie, 1996) and this could be the reason of the loss of association when ageing time was longer. Also, ageing time tends to homogenize meat characteristics, reducing differences between breeds or even between animals within the same breed (Monsón et al., 2005). The results agree with those obtained by Allais et al. (2011), in a population of Charolais breed that quantified the effect of the *G* allele in -0.25 RSD. The marker *UoG-CAST* gene was not found to be associated to any of the descriptors in none of the two ageing times in both analyses. However the results contrast with those obtained in the association study with shear force parameters in the same population (Avilés et al., 2013a). Considering the differences with the previous analysis that was carried out separately by commercial genotypes (and consequently with smaller sample sizes) and keeping in mind that the correlation between shear force and tenderness assessed by trained sensory panel for beef is highly variable depending of factors such as the muscle, the type of animal or the breed (Shackelford et al., 1995; Peachey et al., 2002) this dissimilarity was acceptable.

Regarding to the candidate genes associated to fat deposits in the untrained consumer analysis (Table 5) the separate analyses by finishing diet revealed that 4 SNPs presented significant allelic substitution effects only on the steaks of animals fed with the *ad libitum* diet: the *DGAT1-K232A* SNP showed an effect of +0.26±0.09 being those animals with the *GCGC* genotype more acceptable, the

RORC: g.3290T>G presented an effect on the flavor acceptability scores of +0.20±0.08 linked to the *T* allele and the *SCD1: g.878T>C* exerted an effect on both descriptors presenting the carriers of the *T* allele higher scores. This SNP was also associated to differences in the overall acceptability of the meat of animals fed with unifeed diet; however its allelic substitution effect was not significant. None of the last associations were described previously, so these SNPs may have a predictive value in relation to sensory properties of beef. With respect to the markers *FABP4: g.7516G>C* and *LEP: g.73C>T*, no significant allelic substitution effect was found, although the *CT* genotype of the marker *LEP: g.73C>T* was significant associated to higher scores for both flavour and overall acceptability in those animals fed with the unifeed diet. Table 6 showed least-square means per genotypes and descriptors for the trained panellist analysis. The associations in this analysis were nonexistent. Regarding to the former study of these markers and fat deposits carried out with the same population (Avilés et al., 2013b), the associations seemed consistent for the markers *DGAT1-K232A* and *RORC: g.3290T>G*. The modest genetic and phenotypic correlations found between back fat thickness and intramuscular fat and sensory traits (Jeremiah, 1996; Riley et al., 2003) could support these results. The effect of the marker *SCD1: g.878T>C* on the amount of saturated fatty acid and the saturation ability indexes (Avilés et al., *In press*) was also reflected on the effect of the genotypes on flavour acceptability and overall acceptability, in both studies the diet provided *ad libitum* was crucial to demonstrate the association because the effect of the marker diminished or disappeared with the unifeed diet.

Regarding to the PCA, only the significant markers were used to determine which of the main effects were closely related to the sensory characteristic of meat. One of the two components was significant in both untrained consumer and trained sensory panel analyses. As shown in Figure 1 and separated in a positive range by factor 1 (10% of total variance and 3.03 for the eigenvalue) the 3 sensory descriptors assessed by the untrained consumer panel were related to the *GG* genotype for *CAPN1-316* marker and *TT* genotype for *RORC: g.3290T>G* and *SCD1: g.878T>C* markers. Although commercial genotypes were not significantly affect the sensory descriptors, they were also grouped with the *ad libitum* diet for the CH genotype and the unifeed diet for the RE genotype, suggesting a higher effect of these genotypes on the traits evaluated. With respect to the analysis of the trained sensory panel, the first factor separated again the tenderness descriptor in a positive range (13% of total variance and 2.09 for the eigenvalue) together with the *CC* genotype of the *CAPN1-316* marker. Despite of the effect of the commercial genotype on none of the descriptors was significant, the meat of the RE genotype animals (whatever were the finishing diet or the ageing day) was closer to the tenderness descriptor than that of individuals with French genetic origin. These results suggested that the trained panel was more accurate separating the commercial genotype of the steaks.

Summarizing, the *CAPN1-316* was associated with tenderness and overall acceptability for each ageing time in the untrained consumer panel and with tenderness in meat aged during 7 days in the trained panel. The *DGAT1-K232A* presented an effect on the overall acceptability in the untrained consumer panel in samples of animal

fed with the diet supplied *ad libitum*. The marker *RORC: g.3290T>G* was associated to differences in flavour acceptability in the untrained consumer panel in samples of animal fed with the diet supplied *ad libitum*. And the *SCD1: g.878T>C* SNP presented an association with both parameters (flavour and overall acceptability) measured by the untrained sensory panel for the diet supplied *ad libitum* and with the overall acceptability for the unifeed diet. However, taking into account both analyses (trained and untrained) the *CAPN1-316* marker presented the greatest effect on the sensory quality of meat, and both trained panellists and untrained consumers rated steaks from animals with favorable alleles as more tender and overall acceptable and more tender respectively. However, this is the first time that these associations are studied in this population, so further investigations with a higher number of individuals and independent populations are recommended.

The association studies between molecular markers and sensory quality scores are not as widespread as those related to the technological or instrumental properties of meat. Moreover, in this type of studies, the origin of the consumers and their preferences plays a key role in the analysis (Oliver et al., 2006) and there are not precedents of this kind of association studies in a Spanish population.

This work concluded the analysis carried out in a common Spanish commercial sample reared in the Dehesa ecosystem and intensively fed. The study suggested that markers *CAPN1-316*, *DGAT1-K232A*, *RORC: g.3290T>G* and *SCD1: g.878T>C* have a potential effect on the different measurements of meat quality (objective and/or subjective), nevertheless we are aware of the limited size of our

sample and we strongly recommend to the industry further analysis in the population to obtain consistent conclusions.

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Table 1. Mean (±SD) of the sensory descriptors of meat from the population evaluated by a consumer and a trained sensory panel

Consumers			Trained panel		
Tenderness acceptability	Flavour acceptability	Overall acceptability	Tenderness	Juiciness	Flavour intensity
6.2 ± 2.00	6.6 ± 1.54	6.3 ± 1.76	5.4 ± 2.26	4.1 ± 2.06	5.0 ± 2.08

Table 2. Genotypic frequencies for the polymorphisms of the genes evaluated in the population tested.

Gene	Genotype	Frequency
CAPN1	*CC*	0.39
	CG	0.13
	GG	0.48
CAST	*CC*	0.51
	CG	0.42
	GG	0.07
DGAT1	*AAAA*	0.06
	GCAA	0.36
	GCGC	0.59
FABP4	*CC*	0.03
	CG	0.29
	GG	0.68
LEP	*CC*	0.32
	CT	0.36
	TT	0.33
RORC	*GG*	0.01
	GT	0.32
	TT	0.67
SCD1	*CC*	0.20
	CT	0.41
	TT	0.22

Table 3. Least square means (± SE) for the genotype effects of the SNP evaluated on sensory descriptors assessed by untrained consumers.

Gene	Trait	Genotype[1]			*P*-value[2]	α[3]	*P*-value
		0	*1*	*2*			
	7 ageing days						
CAPN1	Tenderness acceptability	6.0±0.09[a]	5.9±0.15[a]	5.5±0.10[b]	Sig.	0.28±0.07	Sig.
	Overall acceptability	6.3± 0.08[a]	6.2±0.13[a]	5.8±0.08[b]	Sig.	0.25±0.06	Sig.
CAST	Tenderness acceptability	5.9±0.08	5.7±0.08	5.9± 0.16	No Sig.	0.02±0.09	No Sig.
	Overall acceptability	6.1±0.07	6.0±0.07	6.4±0.14	No Sig.	-0.07±0.07	No Sig.
	21 ageing days						
CAPN1	Tenderness acceptability	6.6±0.07[b]	6.9± 0.11[a]	6.3±0.07[c]	Sig.	0.13±0.06	Sig.
	Overall acceptability	6.7±0.07[a]	6.9±0.11[a]	6.4±0.06[b]	Sig.	0.12±0.04	Sig.
CAST	Tenderness acceptability	6.6±0.07	6.5±0.07	6.7±0.14	No Sig.	0.01±0.07	No Sig.
	Overall acceptability	6.6±0.06	6.5±0.06	6.6±0.12	No Sig.	0.04±0.06	No Sig.

[1] Genotypes were coded as 0, 1, and 2, where 1 is always the heterozygote, 0 is the homozygote higher up the alphabet, and 2 is the homozygote lower down the alphabet.

[2] Significant results following Benjamini and Hochberg's FDR methodology (FDR=5%)

[3] Average allele substitution effect ± SE

a-b Different letters within row indicate significant differences among genotypes

Table 4. Least square means (± SE) for the genotype effects of the SNP evaluated on tenderness assessed by a trained panel.

Gene	Genotype[1]			*P*-value[2]	α[3]	*P*-value
	0	*1*	*2*			
	7 ageing days					
CAPN1	5.2± 0.09[a]	4.8±0.17[b]	4.7±0.08[b]	Sig.	0.18±0.06	Sig.
CAST	5.0±0.08	4.9±0.08	5.2±0.22	No Sig.	0.03±0.08	No Sig.
	21 ageing days					
CAPN1	6.0±0.09	5.7±0.17	5.7±0.08	No Sig.	0.01±0.06	No Sig.
CAST	5.8±0.08	5.7±0.08	6.1±0.21	No Sig.	0.05±0.08	No Sig.

[1] Genotypes were coded as 0, 1, and 2, where 1 is always the heterozygote, 0 is the homozygote higher up the alphabet, and 2 is the homozygote lower down the alphabet.

[2] Significant results following Benjamini and Hochberg's FDR methodology (FDR=5%)

[3] Average allele substitution effect ± SE

a-b Different letters within row indicate significant differences among genotypes

Table 5. Least square means (± SE) for the genotype effects of the SNP evaluated on sensory descriptors assessed by untrained consumers.

Gene	Trait	Genotype[1]			*P*-value[2]	α[3]	*P*-value
		0	*1*	*2*			
	ad libitum diet						
DGAT1	Flavour acceptability	6.5±0.14	6.6±0.08	6.7±0.06	No Sig.	0.15±0.08	No Sig.
	Overall acceptability	6.2±0.16[b]	6.4±0.09[b]	6.7±0.07[a]	Sig.	0.26±0.09	Sig.
FABP4	Flavour acceptability	7.0±0.34	6.6±0.08	6.7±0.04	No Sig.	0.05±0.08	No Sig.
	Overall acceptability	6.8±0.38	6.5±0.09	6.5±0.05	No Sig.	-0.01±0.09	No Sig.
LEP	Flavour acceptability	6.8±0.09	6.7±0.08	6.6±0.08	No Sig.	0.09±0.07	No Sig.
	Overall acceptability	6.7±0.10	6.5±0.09	6.4±0.09	No Sig.	0.13±0.08	No Sig.
RORC	Flavour acceptability	6.07±0.19[b]	6.6±0.09[a]	6.7±0.05[a]	Sig.	0.20±0.08	Sig.
	Overall acceptability	6.5±0.21	6.4±0.11	6.5±0.05	No Sig.	0.08±0.09	No Sig.
SCD1	Flavour acceptability	6.4±0.09[b]	6.7±0.06[a]	6.8±0.07[a]	Sig.	0.20±0.06	Sig.
	Overall acceptability	6.1±0.10[b]	6.7±0.07[a]	6.6±0.08[a]	Sig.	0.21±0.07	Sig.
	Unifeed diet						
DGAT1	Flavour acceptability	6.7±0.19	6.4±0.06	6.5±0.05	No Sig.	-0.01±0.07	No Sig.
	Overall acceptability	6.6± 0.22	6.1±0.07	6.2±0.06	No Sig.	-0.02±0.08	No Sig.
FABP4	Flavour acceptability	6.1±0.24	6.4±0.08	6.5±0.04	No Sig.	0.10±0.07	No Sig.

	Overall acceptability	5.7±0.28	6.2±0.09	6.2±0.05	No Sig.	0.08±0.08	No Sig.
LEP	Flavour acceptability	6.3±0.08[b]	6.6±0.05[a]	6.4±0.07[a]	Sig.	-0.09±0.05	No Sig.
	Overall acceptability	5.9±0.09[b]	6.4±0.06[a]	6.1±0.08[b]	Sig.	-0.09±0.06	No Sig.
RORC	Flavour acceptability	-	6.5±0.07	6.4±0.05	No Sig.	-0.05±0.09	No Sig.
	Overall acceptability	-	6.2±0.08	6.1±0.06	No Sig.	-0.05±0.11	No Sig.
SCD1	Flavour acceptability	6.6±0.09	6.4±0.06	6.5±0.08	No Sig.	-0.07±0.07	No Sig.
	Overall acceptability	6.4±0.10[a]	6.0± 0.06[b]	6.3±0.10[a]	Sig.	-0.03±0.08	No Sig.

[1] Genotypes were coded as 0, 1, and 2, where 1 is always the heterozygote, 0 is the homozygote higher up the alphabet, and 2 is the homozygote lower down the alphabet.

[2] Significant results following Benjamini and Hochberg's FDR methodology (FDR=5%)

[3] Average allele substitution effect ± SE

a-b Different letters within row indicate significant differences among genotypes

Table 6. Least square means (± SE) for the genotype effects of the SNP evaluated on sensory descriptors assessed by a trained panel.

Gene	Trait	Genotype[1]			*P*-value[2]	α[3]	*P*-value
		0	*1*	*2*			
	ad libitum diet						
DGAT1	Juiciness	4.5±0.17	4.1±0.09	4.0±0.07	N. sig.	0.20±0.09	N. sig.
	Flavour intensity	4.9±0.16	5.1±0.09	5.0±0.07	N. sig.	-0.02±0.09	N. sig.
FABP4	Juiciness	4.0±0.20	4.1±0.09	4.1±0.07	N. sig.	0.01±0.09	N. sig.
	Flavour intensity	5.2±0.19	4.9 ±0.09	5.0±0.06	N. sig.	-0.03±0.08	N. sig.
LEP	Juiciness	4.0 ±0.10	3.9±0.10	4.2 ±0.10	N. sig.	-0.06±0.08	N. sig.
	Flavour intensity	5.0±0.09	5.0±0.09	5.0±0.09	N. sig.	-0.01±0.07	N. sig.
RORC	Juiciness	3.2±0.31	4.2±0.10	4.0±0.06	N. sig.	0.04±0.10	N. sig.
	Flavour intensity	5.3±0.30	5.0±0.09	5.0±0.06	N. sig.	-0.08±0.09	N. sig.
SCD1	Juiciness	4.2±0.09	4.0±0.08	3.9±0.11	N. sig.	-0.18±0.07	N. sig.
	Flavour intensity	4.9±0.09	5.0±0.07	5.1±0.11	N. sig.	0.09±0.07	N. sig.
	Unifeed diet						
DGAT1	Juiciness	3.8±0.27	4.1±0.08	4.0±0.06	N. sig.	-0.04±0.09	N. sig.
	Flavour intensity	5.4±0.24	5.0±0.08	4.9±0.06	N. sig.	-0.18±0.08	N. sig.
FABP4	Juiciness	3.9±0.3	4.1±0.09	4.1±0.06	N. sig.	0.01±0.10	N. sig.
	Flavour intensity	4.6 ±0.29	4.8±0.08	5.0 ±0.06	N. sig.	0.20±0.09	N. sig.
LEP	Juiciness	4.1±0.10	4.0±0.07	4.1±0.09	N. sig.	-0.01±0.07	N. sig.
	Flavour intensity	4.9±0.10	4.9±0.07	5.0±0.08	N. sig.	-0.04±0.06	N. sig.
RORC	Juiciness	-	4.2±0.08	4.0±0.07	N. sig.	-0.18±0.10	N. sig.
	Flavour intensity	-	4.9±0.07	5.0±0.06	N. sig.	0.10±0.10	N. sig.

SCD1	Juiciness	4.1±0.12	4.0±0.07	4.1±0.10	N. sig.	-0.03±0.08	N. sig.
	Flavour intensity	4.9±0.11	4.9±0.07	5.0±0.09	N. sig.	0.05±0.07	N. sig.

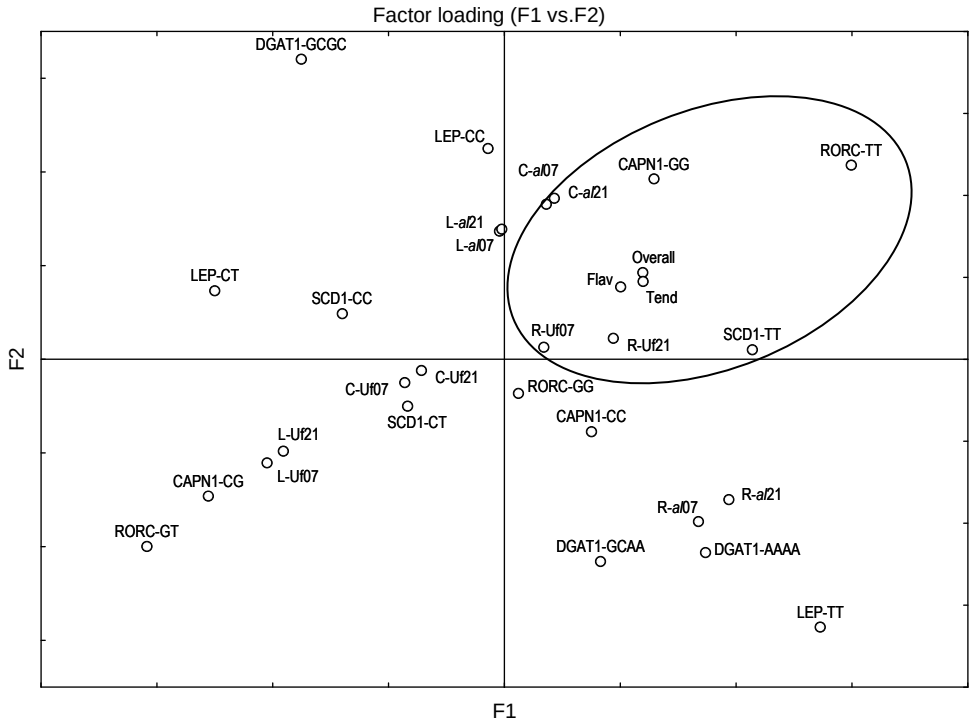
[1] Genotypes were coded as 0, 1, and 2, where 1 is always the heterozygote, 0 is the homozygote higher up the alphabet, and 2 is the homozygote lower down the alphabet.

[2] Significant results following Benjamini and Hochberg's FDR methodology (FDR=5%)

[3] Average allele substitution effect ± SE

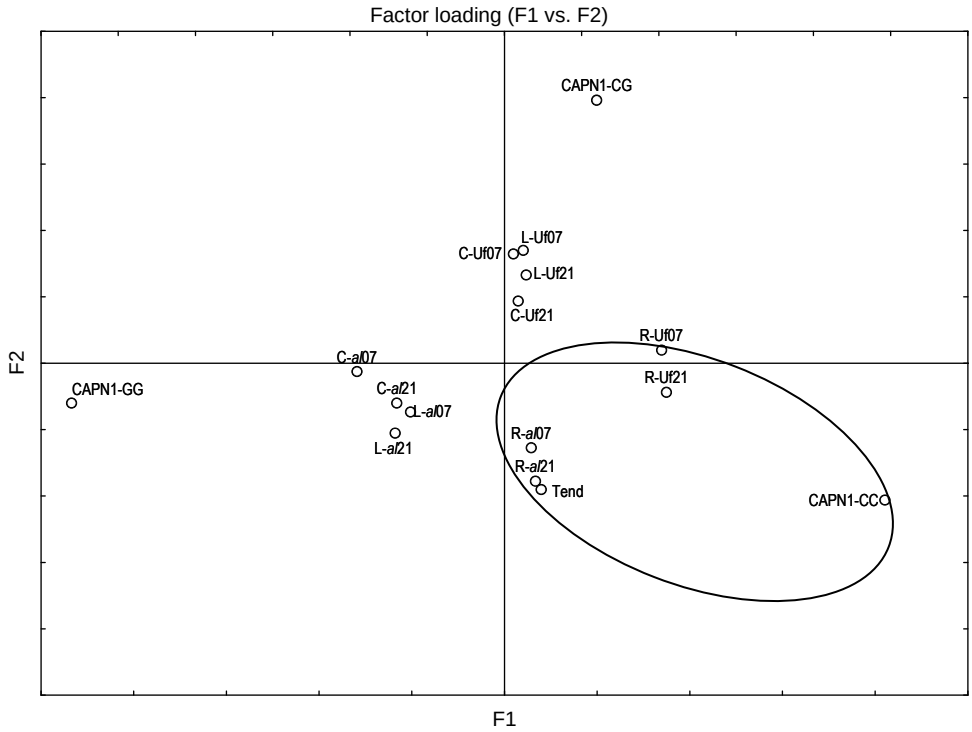
a-b Different letters within row indicate significant differences among genotypes

Figure 1. Results of the PCA analysis measured within the three meat quality descriptors measured by the consumer sensory panel. Factor coordinates are shown.



Sensory descriptors: Tend: Tenderness acceptability; Flav: Flavour acceptability; Overall: Overall acceptability. Markers genotypes: CAPN1-cc, CAPN1-cg and CAPN1-gg: 3 genotypes of the *CAPN1* marker; CAST-cc, CAST-cg and CAST-gg: 3 genotypes of the *CAST* marker; LEP-cc, LEP-ct and LEP-tt: 3 genotypes of the *LEP* marker; RORC-gg, RORC-gt and RORC-tt: 3 genotypes of the *RORC* marker. Beef groups by breed, ageing and diet: C-*al*07, C-*al*21, C-Uf07, C-Uf21, L-*al*07, L-*al*21, L-Uf07, L-Uf21, R-*al*07, R-*al*21, R-Uf07 and R-Uf21; where C: Charolais genotype; L: Limousin genotype; R: Retinta genotype; *al*: *ad libitum* diet; Uf: Unifeed diet; 07: 7 ageing days; 21: 21 ageing days.

Figure 2. Results of the PCA analysis measured within the meat tenderness descriptor measured by the trained sensory panel. Factor coordinates are shown.



Sensory descriptor: Tend: Tenderness. Marker genotypes: CAPN1-CC, CAPN1-CG and CAPN1-GG: 3 genotypes of the *CAPN1* marker. Beef groups by breed, ageing and diet: C-al07, C-al21, C-Uf07, C-Uf21, L-al07, L-al21, L-Uf07, L-Uf21, R-al07, R-al21, R-Uf07 and R-Uf21; where C: Charolais genotype; L: Limousin genotype; R: Retinta genotype; al: ad libitum diet; Uf: Unifeed diet; 07: 7 ageing days; 21: 21 ageing days.

DISCUSIÓN GENERAL

Según un estudio reciente elaborado por el Observatorio del Consumo y la Cadena Agroalimentaria sobre los hábitos de compra de la carne de vacuno entre los consumidores españoles, los factores más importantes a la hora de comprar son la conservación y el aspecto de la pieza ascendiendo a un 37,7% el porcentaje de encuestados que dice desconocer el origen de la misma (CRUZ 2013). A pesar de ello, existen determinados nichos de mercado que demandan productos con indicadores de calidad y que valoran de manera muy positiva que el alimento esté ligado a un territorio, que provenga de una determinada raza en el caso de productos de origen animal y se haya elaborado siguiendo un determinado procedimiento o de una manera sostenible. Estos consumidores identifican la marca de calidad con unos atributos determinados que esperan encontrar cuando compran el producto que ya conocen. Controlar la presencia de esos atributos constituye un reto para el productor de carne de vacuno que necesita herramientas que le permitan predecir la expresión de aspectos como los organolépticos. Para ello la genética ofrece diferentes alternativas aunque las más recomendables científicamente no son siempre las más factibles de llevar a la práctica. Ya que, a pesar de que hoy en día existen estrategias alternativas al uso de genes candidatos que poseen una gran precisión, como es el caso de los microarrays de ADN, su puesta en marcha es mucho más costosa y además implican una fase posterior de análisis de los marcadores seleccionados por lo que también su desarrollo se dilata más en el tiempo. Por ello para el desarrollo de este estudio se optó por el uso de marcadores en genes candidatos detectados previamente en otras poblaciones y la comprobación de su efecto en nuestra muestra de animales.

En esta sección se presenta un resumen discutido de los resultados más relevantes obtenidos en esta memoria de tesis doctoral, los cuales responden a los objetivos planteados en la sección inicial de la misma.

Objetivo específico 1. Detectar la variabilidad de marcadores SNP en genes relacionados con la calidad de la carne en las razas comúnmente criadas en la Dehesa.

Con los resultados de la aproximación inicial realizada con el gen *CAPN1* en el primer trabajo llevado a cabo en esta tesis se comprobó que la distribución de frecuencias de dicho gen (el primero de los siete genes que componen el presente estudio de asociación) en las razas autóctonas ligadas a la Dehesa era diferente a las previamente descritas en otras razas (Tabla 2 del artículo AVILÉS *et al.* (2009)). Es más, en este primer análisis se concluyó que, en el caso de que la asociación en las razas autóctonas fuera la misma que en las razas de aptitud cárnica mejorada, la situación de partida de las primeras para este gen, sería muy positiva dada la alta frecuencia de presentación del alelo favorable (*C*) para el carácter terneza con respecto al resto de razas. Sin embargo es conocido que la asociación marcador-carácter es específica de raza (ASLAN *et al.* 2007; HOCQUETTE *et al.* 2012) por lo que estos resultados preliminares dieron pie al planteamiento de un segundo estudio más ambicioso en el que se evaluó la asociación de éste y de otros genes ligados tanto a terneza como a deposición grasa con la calidad de la carne cuantificada de diferentes formas. Dado que el proyecto se planteó para dar respuesta a las necesidades de la industria del área más meridional de la Dehesa, la población objetivo se centró en los tipos genéticos

mayoritarios en esta localización geográfica es decir, en animales con predominio, (aunque generalmente no criados en pureza), de genotipo Charolais, Limousin y Retinta. De aquí en adelante nos referiremos a ellos como CH para el genotipo Charolais, LI para el genotipo Limousin y RE para el genotipo Retinta.

Objetivo específico 2. Evaluar la existencia de asociación entre dos marcadores en los genes que codifican al enzima μ-calpaína (*CAPN1*) y su inhibidor, calpastatina (*CAST*) y la terneza de la carne cruda y cocinada madurada a diferentes tiempos y evaluada instrumentalmente en muestras de individuos con una base Charolais, Limousin y Retinta.

En este apartado se evaluó en primer lugar la distribución de frecuencias alélicas y genotípicas de los marcadores *CAPN1-316* y *UoG-CAST*. Los resultados para el gen *CAPN1* variaron con respecto a los obtenidos en análisis preliminar en la única raza en común, la Retinta, convirtiéndose el alelo *C* en el mayoritario en este nuevo análisis (Tabla 2 del artículo AVILÉS *et al.* (2013a)). Esto puede deberse a que la muestra del primer estudio procedió de una población de animales inscritos en el Libro Genealógico de la raza mientras que la muestra del segundo estudio lo hizo de una población comercial por lo que la base genética de los animales no fue la misma y en consecuencia tampoco lo fue la distribución de frecuencias del marcador. En los otros dos tipos genéticos (CH y LI) la distribución de frecuencias de este marcador fue similar entre ellas pero diferente a las previamente descritas (PAGE *et al.* 2004; ALLAIS *et al.* 2011). Con respecto al marcador en el gen *CAST* nos encontramos con unos resultados más homogéneos para las tres razas si los comparamos

con el anterior marcador (tabla 2 del artículo AVILÉS *et al.* (2013a)). Sin embargo, la información de los animales con mayor proporción de genes de origen francés contrasta con la publicada por SCHENKEL *et al.* (2006) para las mismas razas. Los motivos de estas diferencias pueden ser por una parte, el pequeño tamaño de la población en esta última publicación, pero también el hecho de que son poblaciones distintas, tanto la raza Charolais como la Limousin son consideradas razas integradas en España debido al largo periodo de tiempo que llevan criándose en nuestro país, esto ha hecho que se establezcan diferencias a nivel genético tanto con la población original como con las criadas en otros países.

A pesar de los distintos tamaños muestrales, en ambos marcadores se encontraron diferencias no solo entre razas, sino también dentro de ellas al compararlos con los resultados de trabajos similares en razas Charolais, Limousin y Retinta y aunque los individuos de nuestro estudio procedieron de una población comercial, su pureza racial estimada a partir del porcentaje de adscripción giró en torno al 80%, por lo su genética habría de ser muy similar a la de la raza criada en pureza. Este resultado por tanto confirma que la distribución de frecuencias depende no solo de raza sino también de la población dentro de raza, que será tanto más diferente en función de la distancia genética que exista entre dichas poblaciones.

Para evaluar la asociación de los genes con la textura instrumental de la carne, se midieron dos variables: resistencia al corte de la carne cruda y resistencia al corte de carne cocinada. No existen en la literatura antecedentes en la detección de asociaciones entre marcadores en los genes *CAPN1* y *CAST* y la resistencia al corte de la

carne cruda. Esta variable está correlacionada positivamente con el contenido de colágeno total e insoluble de la carne (TORRESCANO *et al.* 2003). Nuestros resultados mostraron una asociación significativa entre dicha variable y el marcador *CAPN1-316* en el tipo CH y entre el mismo carácter y el marcador *UoG-CAST* en el tipo LI (tabla 3 del artículo AVILÉS *et al.* (2013a)) estando el genotipo *CC* en ambos casos asociado a una menor resistencia al corte (α = -0.14 ± 0.06 kg/cm^2 y -0.11 ± 0.06 kg/cm^2 respectivamente). Éstos resultados sugieren que podría existir, al menos en los genotipos CH y LI, una relación indirecta entre los marcadores en genes asociados con la proteólisis miofibrilar (*CAPN1* y *CAST*) y el contenido de colágeno. Dada la correlación negativa entre la terneza y el contenido de colágeno (RENAND *et al.* 2001), los anteriores marcadores podrían servir para predecir la terneza de la carne. En el caso de la carne de raza Retinta la existencia de esta asociación no resulta imprescindible, debido a su bajo contenido de colágeno total y a que éste además manifiesta una elevada solubilidad (SAÑUDO *et al.* 1998; CHRISTENSEN *et al.* 2011).

Por otra parte, la resistencia al corte de la carne cocinada (por encima de 60°C) está asociada al componente miofibrilar de la misma (PURSLOW 2005). En nuestros resultados se observó una tendencia hacia la significación entre dicha resistencia y el marcador *CAPN1-316* en el tipo LI y entre el mismo carácter y el marcador *UoG-CAST* en el tipo RE siendo igualmente el genotipo *CC* el asociado a una menor resistencia al corte en ambos casos (tabla 4 del artículo AVILÉS *et al.* (2013a)) a pesar del pequeño tamaño de nuestra muestra. La asociación entre la textura instrumental de la carne cocinada y los marcadores analizados en esta sección ha sido ampliamente demostrada (PAGE *et al.* 2004; CASAS *et al.* 2005; WHITE *et al.* 2005;

MORRIS *et al.* 2006; SCHENKEL *et al.* 2006; VAN EENENNAAM *et al.* 2007; SMITH *et al.* 2009; ALLAIS *et al.* 2011). Es más, recientemente se ha llevado a cabo un estudio de asociación de genoma completo para detectar QTLs con influencia sobre la resistencia al corte (MCCLURE *et al.* 2012) en el que se estimó que la variación en los genes *CAPN1* y *CAST* explica el 1,02 y el 1,85% respectivamente de la variación fenotípica para la textura instrumental.

Por lo tanto en este primer estudio de asociación se detectaron indicios de dicha asociación entre la textura instrumental y los dos marcadores. A pesar de ser necesaria una confirmación con un mayor número de muestras para poder sacar una conclusión firme, ambos marcadores se mantuvieron en el listado de marcadores susceptibles de ser incluidos en nuestra herramienta molecular para predecir la calidad instrumental de la carne.

Objetivo específico 3. Evaluar en la población la existencia de asociación entre los genes que codifican a cinco proteínas implicadas en la deposición de grasa como son el *DGAT1* (diacilglicerol O-aciltransferasa), *FABP4* (proteína de unión a ácidos grasos 4), *LEP* (leptina), *RORC* (receptor huérfano asociado al RAR-γ) y *SCD1* (estearoil-CoA desaturasa 1) y dos tipos de depósitos grasos, subcutáneo e intramuscular, así como entre el último de los genes y la composición de ácidos grasos de la carne.

Como en el apartado anterior, en primer lugar se evaluó la distribución de frecuencias alélicas y genotípicas de cinco marcadores localizados en los genes *DGAT1*, *FABP4*, *LEP*, *RORC* y *SCD1*. La distribución de frecuencias alélicas del marcador *DGAT1-K232A* en nuestra población en estudio tiene cierta homogeneidad: los alelos

AA son mayoritarios en los 3 tipos genéticos (tabla 3 del artículo AVILÉS *et al.* (2013b)), siendo las frecuencias similares a las descritas por RENAND *et al.* (2007) para los genotipos de origen francés y sin embargo muy diferentes a las presentadas por AVILÉS *et al.* (2007) para los individuos del tipo RE. Esto puede deberse, como en el caso del gen *CAPN1*, al pequeño tamaño de la muestra usada en el artículo previo (n=15) y al diferente nivel de pureza genética de los individuos existente entre ambos estudios.

La distribución que presentan las frecuencias alélicas del marcador *FABP4: g.7516G>C* en las tres poblaciones estudiadas es similar, siendo el alelo más abundante el *G* en todos los casos (tabla 3 del artículo AVILÉS *et al.* (2013b)). Esta distribución difiere de la presentada por MICHAL *et al.* (2006), que analizó el cruce de razas Wagyu × Limousin, y de la publicada por PANNIER *et al.* (2010) con una población compuesta por individuos de razas Charolais y Limousin entre otras. Para el SNP localizado en el gen *LEP*, la frecuencia de presentación del alelo *C* fue mayoritaria para los tipos CH y LI (0,58 y 0,60 respectivamente) estas frecuencias fueron similares a las encontradas por PANNIER *et al.* (2009) para las mismas razas y por BUCHANAN *et al.* (2002) para razas continentales. En cambio, para el tipo RE el alelo mayoritario fue el *T* al igual que en las razas de origen inglés utilizadas en los dos artículos previamente citados.

La distribución de frecuencias alélicas del marcador *RORC: g.3290T>G* en nuestra población fue relativamente homogénea y similar a la descrita por BARENDSE *et al.* (2007) para una población de razas de origen inglés. Sin embargo, la distribución de frecuencias del marcador en el gen *SCD1: g.878T>C* fue más heterogénea que en el

gen anterior: en los cruces de origen francés el alelo C fue el mayoritario, al igual que en estudios previos (MILANESI *et al.* 2008; BARTOŇ *et al.* 2010) para razas continentales y en cambio en el tipo RE el alelo mayoritario fue el *T*.

Una vez más la variedad de resultados en las distribuciones de frecuencias de presentación no hace sino confirmar que éstas dependen de las poblaciones analizadas, así como de la distancia genética de las poblaciones con respecto a la población ancestral en la que surgió la mutación. Este es uno de los motivos por los que en selección asistida por marcadores (al igual que en la genómica) es necesario, además de confirmar la existencia de asociaciones, realizar periódicamente actualizaciones en el valor de las frecuencias alélicas a la hora de establecer la estrategia más adecuada para la mejora de los caracteres asociados. El genoma de las poblaciones se va modificando conforme las generaciones se suceden y las asociaciones marcador-fenotipo de interés evolucionan con él.

Para evaluar la asociación de los genes con las diferencias en la deposición de grasa, se midieron dos caracteres de gran importancia en el caso del vacuno, la infiltración grasa intramuscular y el espesor de la grasa subcutánea.

La asociación del marcador *DGAT1-K232A* con caracteres vinculados a la deposición grasa es muy controvertida porque en la multitud de trabajos publicados se han encontrado resultados muy dispares (THALLER *et al.* 2003; RENAND *et al.* 2007; FORTES *et al.* 2009; PANNIER *et al.* 2010; SOUZA *et al.* 2010; WU *et al.* 2011; LI *et al.* 2013). En nuestro estudio se encontró una asociación significativa entre este marcador y el carácter espesor de la grasa dorsal estando el genotipo *KK* ligado

a una mayor deposición grasa (Tabla 5 del artículo AVILÉS *et al.* (2013b)). Por lo que respecta al porcentaje de grasa intramuscular, no se encontró asociación significativa con el SNP en cuestión. Estos resultados están en consonancia con los presentados por PANNIER *et al.* (2010) y RENAND *et al.* (2007) que utilizaron individuos de razas Charolais y Limousin. Sin embargo los resultados contrastan con los de THALLER *et al.* (2003), en vacuno de leche, y los de LI *et al.* (2013) con una población compuesta por razas Aberdeen Angus, Charolais, Hereford, Limousin y Simmental donde solamente dos de las tres variantes genotípicas habían segregado.

En el caso del gen *FABP4* no se encontró efecto significativo sobre el carácter espesor de la grasa dorsal (Tabla 5 del artículo AVILÉS *et al.* (2013b)), al igual que en el trabajo publicado por CURI *et al.* (2011). Por el contrario, el porcentaje de grasa intramuscular sí pareció influenciado de manera significativa por el marcador *FABP4: g.7516G>C* y como en el trabajo de MICHAL *et al.* (2006), los individuos portadores del genotipo *GG* mostraron mayor porcentaje de grasa intramuscular.

Las variaciones alélicas del marcador *LEP: g.73C>T* no parecen estar asociadas a diferencias en ninguno de los dos depósitos grasos analizados en nuestra población. Este resultado coincide con varios estudios previamente publicados (BARENDSE *et al.* 2005; FORTES *et al.* 2009; PANNIER *et al.* 2009).

Con respecto al marcador *RORC: g.3290T>G*, se ha demostrado una asociación significativa del SNP con el porcentaje de grasa intramuscular (Tabla 5 del artículo AVILÉS *et al.* (2013b). Por lo tanto, estos resultados han servido para probar en una nueva población el

efecto del SNP que BARENDSE *et al.* (2010) asociaron al marmoreo en razas británicas y derivados del *Bos indicus* y que apenas se ha explorado en el resto de razas.

Finalmente, el marcador *SCD1: g.878T>C* no se ha asociado a ninguna de las dos variables analizadas en la población estudiada, al igual que ocurrió en el caso del gen de la leptina. Estos resultados coinciden con los de LI *et al.* (2013) en razas cárnicas de origen francés e inglés así como en razas de doble aptitud.

Dado que la población estaba constituida por individuos de distinta precocidad en la deposición de grasa, estos fueron sacrificados a diferentes edades para compensar las diferencias en el nivel de engrasamiento por lo que el efecto de la raza no resultó significativo en el modelo utilizado para evaluar la asociación de los marcadores con los depósitos grasos. Así en los tres marcadores podemos comprobar como el tipo comercial con mayor espesor de grasa subcutánea e infiltración grasa (LI), es a su vez el que presenta mayor frecuencia del genotipo *KK* para el marcador *DGAT1-K232A*, del alelo *G* para el marcador *FABP4: g.7516G>C* y del genotipo *GT* para el marcador *RORC: g.3290T>G*.

Con todo ello, los polimorfismos localizados en los genes *DGAT1*, *FABP4* y *RORC* son los marcadores recomendables para predecir deposición grasa, ya que además su efecto es complementario: el marcador del gen *DGAT1-K232A* se asoció a espesor de la grasa subcutánea y los dos últimos a diferencias en la deposición de grasa intramuscular, con lo que se podría dirigir la selección de individuos con grasa de cobertura óptima sin sacrificar la infiltración grasa intramuscular deseable.

En el caso del marcador *SCD1: g.878T>C*, existen bastantes más estudios de asociación en la literatura con el perfil de ácidos grasos que con la deposición grasa, ya que en la composición de ácidos grasos es donde se valora de forma más precisa si el marcador tiene algún efecto en la funcionalidad de la Δ9-desaturasa.

Este estudio de asociación se realizó a dos niveles debido a que la alimentación tiene un efecto determinante en la expresión final de este tipo de carácter (perfil de ácidos grasos) y a que en el diseño experimental nuestra población se dividió en dos grupos según la dieta suministrada a los animales: una mezcla unifeed rica en energía y una ración convencional suministrada *ad libitum*. El efecto del marcador fue observado especialmente en los animales alimentados con dieta convencional. En este caso, en los individuos portadores del genotipo *CC* y *CT* la enzima desaturasa resultó ser más activa que en los portadores del genotipo *TT* (su acción fue especialmente significativa sobre los niveles del C14:0, C14:1 cis-9 y el índice C14, así como en la cantidad total de SFA y en la relación MUFA/SFA). Estos resultados fueron consistentes con los publicados anteriormente por diferentes autores (OHSAKI *et al.* 2009; BARTOŇ *et al.* 2010; LI *et al.* 2010; LI *et al.* 2012). Los ácidos grasos más abundantes en la fracción lipídica muscular son los saturados, siendo los principales de mayor a menor concentración: Palmítico (C16:0), Esteárico (C18:0) y Mirístico (C14:0) y los monoinsaturados (Oléico (C18:1) seguido del Palmitoléico (C16:1) (ENSER *et al.* 1996). La composición final de ácidos grasos de la grasa intramuscular depende de la acción de, entre otras, la Δ9-desaturasa sobre los principales ácidos grasos saturados que componen este depósito (KIM and NTAMBI 1999). Por ello encontrar un predictor de la composición de ácidos grasos de la

carne como el marcador *SCD1: g.878T>C* es crucial ya que ésta determina no sólo su valor nutritivo sino también varios aspectos de su calidad organoléptica como el flavor (WARREN *et al.* 2008). En cualquier caso, será fundamental a la hora de usar el marcador como predictor de calidad tener en cuenta el hecho de que las asociaciones son significativas con un tipo de dieta y no con la otra. Esto puede deberse a que los fosfolípidos sobre los que actúa la Δ9-desaturasa predominan en los lípidos de los forrajes (MORAND-FEHR and TRAN 2001) como los que forman parte de la dieta convencional. Esta diferencia en el efecto en función de la dieta es un factor que cobra importancia en la toma de decisiones de manejo de los animales portadores de genotipos favorables porque, de poco servirá haber hecho una selección previa de los individuos si posteriormente se les proporciona una dieta en la que el efecto del marcador se diluya.

Por tanto, recomendamos mantener el marcador *SCD1: g.878T>C* entre los marcadores propuestos para el diseño de nuestra herramienta genómica de predicción de calidad, ya no sólo organoléptica e instrumental, sino también nutricional.

Objetivo específico 4. Valorar las asociaciones entre los genes de las siete proteínas citadas y las características sensoriales de la carne de la misma población con un panel de consumidores no entrenado y un panel de catadores entrenado.

Este último análisis de asociación ha servido para completar el análisis de asociación de los siete marcadores analizados con parámetros de calidad organoléptica de la carne medida mediante la evaluación por parte de un panel no entrenado de consumidores y panel entrenado de catadores. En el caso de los parámetros relativos

a la terneza el análisis de asociación se realizó entre éstos y los genes *CAPN1* y *CAST* teniendo en cuenta los tiempos de maduración y en el caso de los parámetros vinculados con la deposición grasa, el estudio se llevó a cabo entre los genes *DGAT1*, *FABP4*, *LEP*, *RORC* y *SCD1* y los descriptores más relacionados con la misma teniendo en cuenta el tipo de dieta con la que finalizaron el cebo los animales.

De todos ellos, el marcador *CAPN1-316* presentó una asociación significativa tanto en carne evaluada por el panel de consumidores como en el entrenado. Siendo en todos los casos el genotipo *GG* el peor valorado. Los efectos medios de sustitución alélica para el panel de consumidores oscilaron entre +0,28 ± 0,07 y 0,13 ± 0,06 puntos en la nota de aceptabilidad de la terneza y +0,25 ± 0,06 y 0,12 ± 0,04 puntos en la nota de aceptación general. En el caso del panel entrenado de catadores el efecto medio de sustitución alélica para el carácter terneza de la carne madurada 7 días fue de +0,18 ± 0,06 puntos. No se encontró ninguna asociación entre el marcador situado en el gen *CAST* y los parámetros vinculados a la terneza. Con respecto a los parámetros relacionados con los depósitos grasos, los marcadores de los genes *DGAT1*, *RORC* y *SCD1* se asociaron de manera significativa a diferencias sensoriales en la carne de animales cebados con la dieta convencional suministrada *ad libitum* evaluada por el panel de consumidores. Si bien se observó una asociación significativa de los marcadores *LEP: g.73C>T* y *SCD1: g.878T>C* con las diferencias en la carne de animales cebados con dieta unifeed, su efecto medio de sustitución alélica no resultó significativo. Finalmente, no se encontraron asociaciones ni efectos significativos de los marcadores vinculados a la infiltración grasa sobre los

caracteres sensoriales evaluados por el panel entrenado de catadores.

Por lo tanto, los marcadores propuestos para incluir en la herramienta molecular por su asociación con la calidad sensorial son: el situado en el gen *CAPN1* por su claro efecto sobre la terneza y la aceptación general y los localizados en los genes *DGAT1*, *RORC* y *SCD1* por su efecto en la aceptación general en el primer caso, en la aceptación del flavor en el segundo y en ambos, flavor y aceptación general en el tercero de los marcadores.

Objetivo específico 5. Proponer un panel de marcadores a incluir en una herramienta molecular para predecir la calidad organoléptica e instrumental potencial de la carne de vacuno producida en la Dehesa.

Tras los análisis de las distintas asociaciones en cada gen y las recomendaciones obtenidas tras cada estudio, los marcadores moleculares aconsejados para ser incluidos en una herramienta molecular por su efecto significativo sobre la calidad de la carne, por la repetibilidad de dichos efectos entre metodologías de detección y por tanto, por su relación con el potencial para producir carne de calidad son:

- ✓ *CAPN1-316* por su efecto sobre la textura instrumental de la carne cruda, la aceptación general y de la terneza cuantificadas por un panel de consumidores no entrenado y la terneza evaluada por un panel sensorial entrenado.

- ✓ *DGAT1-K232A* por su efecto sobre el espesor de la grasa subcutánea y la aceptación general cuantificada por un panel de consumidores no entrenado.
- ✓ *RORC: g.3290T>G* por su efecto sobre la infiltración grasa intramuscular y la aceptación del flavour cuantificada por un panel de consumidores no entrenado.
- ✓ *SCD1: g.878T>C* por su efecto sobre el perfil de ácidos grasos, la aceptación general y del flavour cuantificadas por un panel de consumidores no entrenado.

Si bien se han encontrado asociaciones en los marcadores *UoG-CAST*, *FABP4: g.7516G>C* y *LEP: g.73C>T,* éstas han sido débiles y/o no han mostrado la repetibilidad entre técnicas de detección que presentan los cuatro marcadores recomendados. Por ello se propone profundizar en el análisis de estas últimas asociaciones para poder descartarlos o incluirlos definitivamente como indicadores del potencial genético para producir carne de calidad en la población estudiada.

Así mismo se propone el uso de la herramienta a dos niveles:

- A nivel de la industria de producción cárnica (cebadero y matadero) que explota esta población. A partir del uso del test de marcadores a la entrada de los animales en cebadero, éstos se podrían dirigir hacia un manejo estándar (alimentación, momento óptimo del sacrificio, etc.) o hacia una línea orientada a la producción de carne de máxima calidad (alimentación más cuidada, elección del momento del sacrificio óptimo mediante análisis de ultrasonidos, mayor tiempo de maduración de la carne, etc.) en función de su potencial para

producir dicha carne de calidad. Esto supondría un valor añadido que ofrecer a grandes superficies, minoristas y consumidores a la hora de vender el producto, ya que permitiría obtener una carne con las máximas probabilidades de poseer una calidad superior (incluso bajo el paraguas de una marca distintiva de calidad). De esta forma el industrial podría rentabilizar este tipo de producción ofertando una carne con un precio de venta al público superior que compensara la mayor inversión realizada a cambio de una garantía de calidad. Todo ello teniendo en cuenta el nicho al que orienta el producto, así si por ejemplo si el propósito del productor fuese ofertar un producto dietéticamente saludable tendría que seleccionar aquellos animales con genotipo *CC* o *CT* para el marcador *SCD1: g.878T>C*, pero si su intención fuese vender un producto con buenas notas sensoriales, el genotipo de elección para el marcador *SCD1: g.878T>C* sería el *TT*.

- A nivel de programa de mejora de la raza Retinta (raza más extendida en el área de la Dehesa). Utilizándola como criterio para seleccionar reproductores con características óptimas heredables. Con el uso de este test de marcadores moleculares se impulsaría el desarrollo de la Selección Asistida por Marcadores, a partir de la cual se aumentarían y diversificarían los objetivos de selección de esta población para adaptarlos a las demandas del mercado. Desde 2008 se viene incluyendo información genotípica para los marcadores *CAPN1-316, CAPN1-530* y *UoG-CAST* en los catálogos de sementales de la raza Retinta. Sin embargo esta información sólo se ofrece en algunos de los sementales con prueba de descendencia superada. La herramienta genómica está optimizada para un mayor número de marcadores cuya asociación está probada en el genotipo Retinto y su detección automatizada. Por ello podría incluirse en los análisis de

rutina a realizar en los machos sometidos a prueba de testaje que serán en muchos casos candidatos a la extracción de semen para IA o futuros sementales en prueba de descendencia, ampliando así la información disponible de este tipo de animales. Así si tenemos en cuenta que el mercado español demanda a día de hoy carne poco infiltrada y que los animales participantes en prueba de testaje son evaluados a partir de un índice sintético en el que se tiene en cuenta el crecimiento, para realizar una elección de reproductores óptima, habría que seleccionar aquellos con velocidad de crecimiento rápida (la infiltración grasa intramuscular es un depósito tardío) evitando los genotipos *GG* en el marcador *FABP4: g.7516G>C* y *GT* en el marcador *RORC: g.3290T>G*.

CONCLUSIONES

Conclusiones del CAPÍTULO I

1. Se ha detectado por primera vez en vacuno una asociación estadísticamente significativa entre la textura instrumental de la carne cruda (resistencia al corte) y los marcadores moleculares *CAPN1-316* y *UoG-CAST* al menos en los tipos Charolais y Limousin.

Czech Journal of Animal Science 58 (10): 479-487

2. De la misma forma se han detectado asociaciones (con tendencia hacia la significación) entre el marcador *CAPN1-316* en el tipo Limousin y la textura instrumental de la carne cocinada y entre la misma variable y el marcador *UoG-CAST* en el tipo Retinto.

Czech Journal of Animal Science 58 (10): 479-487

3. Se han encontrado diferencias estadísticamente significativas en la distribución de frecuencias alélicas y genotípicas para el marcador asociado a la terneza de la carne *CAPN1-316* en las poblaciones en estudio. Esta distribución condiciona la estrategia de utilización en una posible mejora de la terneza de la carne en estas poblaciones.

Animal Biotechnology. 20 (3): 161-164

Conclusiones del CAPÍTULO II

4. Según nuestros resultados la combinación de marcadores *DGAT1-K232A, FABP4: g.7516G>C* y *RORC: g.3290T>G* puede utilizarse para llevar a cabo una selección de individuos con deposición grasa deseable discriminando entre tipos de depósitos (subcutáneo e intramuscular).

Journal of Animal Science 91 (10): 4571-4577

5. El marcador *SCD1:g.878T>C* presenta un efecto significativo sobre la composición de ácidos grasos de la carne, en el índice de saturación del ácido mirístico (C14:0) considerado poco saludable por sus propiedades aterogénicas y en el denominado "índice de alimentación saludable". Según nuestros resultados esta asociación depende en gran medida del tipo de dieta administrada a los animales, siendo más patente en aquella con mayor contenido de fosfolípidos.

Animal Biotechnology. Artículo en revisión

Conclusiones del CAPÍTULO III

6. Según nuestros resultados los marcadores *CAPN1-316*, *DGAT1-K232A*, *RORC: g.3290T>G* y *SCD1: g.878T>C* ejercen su efecto sobre diferentes descriptores de la calidad sensorial evaluada de manera subjetiva tanto por un panel de catadores entrenados (marcador *CAPN1-316*) como por un panel de consumidores (marcadores *CAPN1-316*, *DGAT1-K232A*, *RORC: g.3290T>G* y *SCD1: g.878T>C*).

Livestock Science. Artículo sometido

Conclusiones generales

7. Teniendo en cuenta todos los resultados obtenidos podemos recomendar, tanto por su asociación estadística como por la repetibilidad de sus efectos entre metodologías de detección, los marcadores *CAPN1-316*, el *DGAT1-K232A*, el *RORC: g.3290T>G* y el *SCD1: g.878T>C*. para su inclusión en una herramienta molecular de rutina que permita la mejora genética de la calidad de la carne de la población analizada, así como la contribución a la selección de animales para la creación de una línea de carne de calidad por parte de la industria del vacuno de carne de la zona.

8. Las asociaciones detectadas en los marcadores *UoG-CAST, FABP4: g.7516G>C* y *LEP: g.73C>T* han sido débiles y/o no han mostrado la repetibilidad entre técnicas de detección que presentan los marcadores anteriores, por lo que se propone profundizar en el análisis de estas últimas asociaciones para poder descartarlos o incluirlos definitivamente como indicadores del potencial genético para producir carne de calidad en la población estudiada.

Conclusions CHAPTER I

1. This is the first time that an association statistically significant has been found between the instrumental texture of raw meat (shear force) and the molecular markers *CAPN1-316* y *UoG-CAST* at least in Limousin and Charolais commercial types.

Czech Journal of Animal Science 58 (10): 479-487

2. It has been detected associations (with a tendency towards significance) between the marker *CAPN1-316* in the Limousin genotype and the instrumental texture of the cooked meat as well as between the same variable and the marker *UoG-CAST* in the Retinto genotype.

Czech Journal of Animal Science 58 (10): 479-487

3. It has been found differences statistically significant in the allelic and genotypic frequencies distribution for the marker linked to tenderness (*CAPN1-316*) in the population studied. This distribution determines the strategy of a feasible improvement of meat tenderness in these populations.

Animal Biotechnology. 20 (3): 161-164

Conclusions CHAPTER II

4. According to our results, the combination of markers *DGAT1-K232A*, *FABP4: g.7516G>C* and *RORC: g.3290T>G* can be used to carry out a selection of the individuals with desirable fat deposition distinguishing between fat depots (subcutaneous and intramuscular).

Journal of Animal Science 91 (10): 4571-4577

5. The marker *SCD1:g.878T>C* present a significant effect on the fatty acid profile of meat, in the saturation index of the myristic acid (C14:0) considered unhealthy due to its atherogenic properties and in the known as "health index". According to our results, this association greatly depends on the diet supplied to the animals, being more evident in the diet with the highest phospholipid content.

Animal Biotechnology. Article under review

Conclusions CHAPTER III

6. According to our results the markers *CAPN1-316*, *DGAT1-K232A*, *RORC: g.3290T>G* and *SCD1: g.878T>C* exert their effect on different descriptors of sensory quality assessed in a subjective way by a trained panel (marker *CAPN1-316*) and by an untrained panel (markers *CAPN1-316*, *DGAT1-K232A*, *RORC: g.3290T>G* y *SCD1: g.878T>C*).

Livestock Science. Article submitted

General conclusions

7. Keeping in mind the results obtained we can recommend due to their statistic association and the repeatability of their effects among detection methodologies the following markers: *CAPN1-316*, el *DGAT1-K232A*, el *RORC: g.3290T>G* y el *SCD1: g.878T>C* to be included in a routinely molecular tool that allows the genetic improvement of the quality of meat in the population analyzed and the contribution to the selection of animals to create a meat quality line by the beef cattle industry of the Dehesa area.

8. The associations detected in markers *UoG-CAST*, *FABP4: g.7516G>C* y *LEP: g.73C>T* were low and/or did not show the repeatability among detection methodologies that presented the previous markers, therefore we propose to deep in the analysis of these last associations to definitively discard o include the markers as indicators of the genetic potential to produce quality meat in the population studied.

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LISTADO DE PUBLICACIONES

En esta sección se presenta un listado en el que se recogen todas las publicaciones a las que ha dado lugar esta Tesis Doctoral, a nivel nacional e internacional.

PUBLICACIONES EN REVISTAS ISI

AUTORES: C. Avilés, F. Peña, O. Polvillo, M. Barahona, M. M. Campo, C. Sañudo, M. Juárez, A. Horcada, M. J. Alcalde, A. Molina
TÍTULO: Association between markers in candidate genes and organoleptic quality traits in beef reared intensively
REFERENCIA: Livestock Science. Artículo sometido.
ISSN: 1871-1413
IF= 1,249 (2 ^{o} cuartil) 16/54 en Agriculture Dairy & Animal Science

AUTORES: C. Avilés, O. Polvillo, F. Peña, A. Horcada, M. Juárez, A. Molina
TÍTULO: Association study between a single nucleotide polymorphism in bovine *SCD1* gene with fatty acid composition in a Spanish commercial population
REFERENCIA: Animal Biotechnology. Artículo en revisión.
ISSN: 1049-5398
IF= 0,882 (2^{o} cuartil) 26/54 en Agriculture Dairy & Animal Science

AUTORES: C. Avilés, O. Polvillo, F. Peña, M. Juárez, A. L. Martínez, A. Molina
TÍTULO: Associations between *DGAT1*, *FABP4*, *LEP*, *RORC*, and *SCD1* gene polymorphisms and fat deposition in Spanish commercial beef
REFERENCIA: Journal of Animal Science 2013. 91(10) 4571-4577
ISSN: 0021-8812
DOI: 10.2527/jas.2013-6402
IF= 2,093 (1er cuartil) 5/54 en Agriculture Dairy & Animal Science

AUTORES: C. Avilés, M. Juárez, F. Peña, V. Domenech, I. Clemente, A. Molina
TÍTULO: Association of single nucleotide polymorphisms in *CAPN1* and *CAST* genes with beef tenderness from Spanish commercial feedlots
REFERENCIA: Czech Journal of Animal Science 2013. 58 (10) 479–487
ISSN: 1212-1819
IF= 0,922 (2^{o} cuartil) 24/54 en Agriculture Dairy & Animal Science

AUTORES: C. Avilés, P.J. Azor, L. Pannier, R. Hamill, A. Membrillo, A. Molina
TÍTULO: New single nucleotide polymorphisms in the μ-calpain gene in Spanish maternal beef breeds
REFERENCIA: Animal Biotechnology 2009. 20 (3)161 - 164
ISSN: 1049-5398
DOI: 10.1080/10495390902876115
IF= 0,814 (2º cuartil) 22/50 en Agriculture Dairy & Animal Science

OTRAS PUBLICACIONES EN REVISTAS ISI
AUTORES: E. Rodero, A. González, C. Avilés, M. Luque
TÍTULO: Conservation of endangered Spanish cattle breeds using markers of candidate genes for meat quality
REFERENCIA: Animal Biotechnology 2013. 24 (1) 15-24
ISSN: 1049-5398
DOI: 10.1080/10495398.2012.737394
IF= 0,882 (2º cuartil) 26/54 en Agriculture Dairy & Animal Science

OTRAS PUBLICACIONES en revistas de divulgación
AUTORES: C. Avilés, F. Peña, A. Molina, M. Barahona, M.M. Campo, C. Sañudo
TÍTULO: Asociación entre polimorfismos en los genes *FABP4*, *LEP* y *RORC* y la aceptabilidad del sabor de la carne en la Raza Retinta
REFERENCIA: FEAGAS 2011. 37: 95-99
ISSN: 1887-4177

AUTORES: C. Avilés, P.J. Azor, J.A. Pérez, F. Álvarez, I. Fernández, A. Molina
TÍTULO: Estudio de caracteres fenotípicos y genotípicos en calidad de la carne de vacuno Retinto
REFERENCIA: Revista ACRE 2008. 145: 35-37.
ISSN: 1889-2078

AUTORES: C. Avilés, P.J. Azor, F. Álvarez, I. Fernández, J.A. Pérez, A. Membrillo, G. Dorado, A. Molina
TÍTULO: Determinación de SNPs en el gen de la *DGAT1*: Primeros resultados en razas bovinas maternales
REFERENCIA: FEAGAS 2007. 32: 97-99
ISSN: 1887-4177

AUTORES: Avilés C., Azor P.J., Álvarez F., Fernández I., Pérez J.A., Molina A.
TÍTULO: Los genes de la µ-calpaína y la calpastatina bovina y su vinculación al carácter terneza de la carne en la raza Retinta
REFERENCIA: Revista ACRE 2007. 144: 17-21
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AUTORES: C. Avilés, P.J. Azor, F. Álvarez, I. Fernández, J.A. Pérez, A. Membrillo, G. Dorado, A. Molina
TÍTULO: Polimorfismos de la µ-calpaína y la calpastatina bovina en las razas de la dehesa
REFERENCIA: FEAGAS 2007. 31: 82-89
ISSN: 1887-4177

CONGRESOS INTERNACIONALES
AUTORES: C. Avilés, F. Peña, M. Barahona, M.M. Campo, C. Sañudo, A. Molina
TÍTULO: Effect of markers in *FABP4*, *LEP* and *RORC* genes on taste in Spanish South-western beef cattle
CONGRESO: 63rd Annual Meeting of the European Association for Animal Production
ENTIDAD ORGANIZADORA: EAAP
PUBLICACIÓN (ISSN/ISBN): Book of Abstracts of the 63th Annual Meeting of the European Association for Animal Production (978-90-8686-206-1)
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TIPO DE PARTICIPACIÓN: Póster (Premio mejor póster de la sección de vacuno)
LUGAR DE CELEBRACIÓN: Bratislava - Eslovaquia
FECHA: 27 al 31 de agosto de 2012

AUTORES: C. Avilés, F. Peña, M. Barahona, M.M. Campo, C. Sañudo, A. Molina
TÍTULO: Association of *CAPN1* and *CAST* markers with technological and sensory traits in Spanish beef cattle
CONGRESO: 63rd Annual Meeting of the European Association for Animal Production
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PUBLICACIÓN (ISSN/ISBN): Book of Abstracts of the 63th Annual Meeting of the European Association for Animal Production (978-90-8686-206-1)
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TIPO DE PARTICIPACIÓN: Póster (Premio mejor póster de la sección de vacuno)
LUGAR DE CELEBRACIÓN: Bratislava - Eslovaquia
FECHA: 27 al 31 de agosto de 2012

AUTORES: C. Avilés, P.J. Azor, J.A. Pérez, F. Álvarez, I. Fernández, A. Molina
TÍTULO: Estudio de la variabilidad de los genes *CAPN1* y *CAST* y su relación con la terneza de la carne en razas bovinas autóctonas españolas
CONGRESO: VIII Congreso de la Federación Iberoamericana de razas criollas y autóctonas
ENTIDAD ORGANIZADORA: FIRC
PUBLICACIÓN (ISSN/ISBN): Libro de actas del VIII Congreso de la Federación Iberoamericana de razas criollas y autóctonas
VOLUMEN/PÁGINAS: 34
TIPO DE PARTICIPACIÓN: Oral
LUGAR DE CELEBRACIÓN: Valdivia - Chile
FECHA: 5 al 7 de diciembre de 2008

AUTORES: C. Avilés, P.J. Azor, G. Dorado, A. Molina
TÍTULO: Sequence analysis of μ -calpain, a candidate gene for meat tenderness, in Spanish beef cattle breeds
CONGRESO: 38th Annual research conference food, nutrition and consumer sciences
ENTIDAD ORGANIZADORA: UCC
PUBLICACIÓN (ISSN/ISBN): Book of Abstracts of the 38th Annual research conference food, nutrition and consumer sciences
VOLUMEN/PÁGINAS: 5
TIPO DE PARTICIPACIÓN: Oral
LUGAR DE CELEBRACIÓN: Cork - Irlanda
FECHA: 4 de septiembre de 2008

AUTORES: C. Avilés, M. Juárez, P.J. Azor, P. Pajuelo, O. Polvillo, F. Álvarez, J.A. Pérez, I. Fernández, A. Molina
TÍTULO: Association of single nucleotide polymorphisms in the μ-calpain and calpastatin gene with proteases activities in Retinta beef cattle: Preliminary results
CONGRESO: 53rd International Congress of Meat Science and Technology
ENTIDAD ORGANIZADORA: ICOMST

PUBLICACIÓN (ISSN/ISBN): Book of Abstracts of the 53rd International Congress of Meat Science and Technology
VOLUMEN/PÁGINAS: 195-196
TIPO DE PARTICIPACIÓN: Póster
LUGAR DE CELEBRACIÓN: Pekín - China
FECHA: 5 de agosto de 2007

CONGRESOS NACIONALES
AUTORES: C. Avilés, M. Juárez, F. Peña, A. Molina
TÍTULO: Marcadores, proteasas y terneza de la carne de vacuno de la Dehesa
CONGRESO: VII Congreso nacional ciencia y tecnología de los alimentos
ENTIDAD ORGANIZADORA: Conferencia de decanos de centros donde se imparten titulaciones de CYTA
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VOLUMEN/PÁGINAS: O-19
TIPO DE PARTICIPACIÓN: Oral
LUGAR DE CELEBRACIÓN: Córdoba
FECHA: 12 al 14 de junio de 2013

AUTORES: C. Avilés, F. Peña, M. Barahona, M.M. Campo, A. Molina
TÍTULO: Evaluación del efecto de tres polimorfismos de una sola base en los genes *FABP4*, *LEP* y *RORC* sobre parámetros sensoriales de la carne de Raza Retinta
CONGRESO: IV Congreso Nacional de la Carne de Vacuno
ENTIDAD ORGANIZADORA: INVAC
PUBLICACIÓN (ISSN/ISBN): Libro de actas del IV Congreso Nacional de la Carne de Vacuno
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TIPO DE PARTICIPACIÓN: Póster
LUGAR DE CELEBRACIÓN: Madrid
FECHA: 21 y 22 de junio de 2012

AUTORES: C. Avilés, M. Juárez, O. Polvillo, F. Álvarez, I. Fernández, J.A. Pérez, I. Clemente, A. Molina
TÍTULO: Asociación entre SNPs y caracteres fenotípicos relacionados con la calidad de la carne en la raza Retinta: primeros resultados
CONGRESO: III Congreso Nacional de la Carne de Vacuno
ENTIDAD ORGANIZADORA: INVAC

PUBLICACIÓN (ISSN/ISBN): Libro de actas del III Congreso Nacional de la Carne de Vacuno
TIPO DE PARTICIPACIÓN: Oral
LUGAR DE CELEBRACIÓN: Ávila
FECHA: 19 al 21 de noviembre de 2008

AUTORES: C. Avilés, P.J. Azor, A. Membrillo, G. Dorado, F. Álvarez, I. Fernández, J.A. Pérez, A. Molina
TÍTULO: Detección de single nucleotide polymorphisms en genes relacionados con la terneza de la carne (*CAPN1* y *CAST*) en las razas bovinas autóctonas españolas
CONGRESO: IV Jornadas Ibéricas de Razas Autóctonas y sus Productos Tradicionales
ENTIDAD ORGANIZADORA: Universidad de Sevilla, Escuela Superior Agraria de Élvas
PUBLICACIÓN (ISSN/ISBN): Libro de actas de las IV Jornadas Ibéricas de Razas Autóctonas y sus Productos Tradicionales (SE-6431-2007)
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TIPO DE PARTICIPACIÓN: Oral
LUGAR DE CELEBRACIÓN: Sevilla
FECHA: 30 de noviembre y 1 de diciembre de 2007

AUTORES: C. Avilés, P.J. Azor, A. Membrillo, G. Dorado, F. Álvarez, I. Fernández, J.A. Pérez, A. Molina
TÍTULO: Búsqueda de single nucleotide polymorphisms en el gen *DGAT1* (diacilglicerol o-acil transferasa 1) vinculado a la infiltración grasa: resultados preliminares en razas bovinas autóctonas
CONGRESO: IV Jornadas Ibéricas de Razas Autóctonas y sus Productos Tradicionales
ENTIDAD ORGANIZADORA: Universidad de Sevilla, Escuela Superior Agraria de Élvas
PUBLICACIÓN (ISSN/ISBN): Libro de actas de las IV Jornadas Ibéricas de Razas Autóctonas y sus Productos Tradicionales (SE-6431-2007)
VOLUMEN/PÁGINAS: 129-134
TIPO DE PARTICIPACIÓN: Oral
LUGAR DE CELEBRACIÓN: Sevilla
FECHA: 30 de noviembre y 1 de diciembre de 2007