Regulación genética del acúmulo de grasa en porcino y su efecto sobre los caracteres de origen materno

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Universitat de Lleida

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Regulación genética del acúmulo de grasa en porcino y su efecto sobre los caracteres de origen materno

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*"Para los **sabios** las estrellas*
son problemas. Para mi
hombre de negocios
significaban oro"

El Principito, Antoine de Saint- Exúpery
1943

A mis padres
A mi marido
A mi hijo

Agraïments / Agradecimientos

Totes les histories tenen un inici i un final, i després de tants anys i tants bons records aquesta etapa arriba al seu fi. En aquestes breus línies vull agrair a cada una de les persones que durant el camí m'han ajudat i les quals he compartit una etapa molt important de la meva vida. Totes i cadascuna d'elles fan que aquest historia prengui sentit.

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Resumen

El contenido y la composición de la grasa intramuscular [GIM] influye en las propiedades organolépticas, tecnológicos y nutricionales de la carne de cerdo. En particular, un mayor índice de desaturación de la grasa se considera beneficioso para la salud del consumidor, dado que un exceso de grasa saturada incrementa el riesgo de enfermedades cardiovasculares. Esta tesis forma parte de una línea de investigación centrada en mejorar la calidad de la carne de cerdo mediante la selección por el contenido de GIM y la composición de ácidos grasos. Este trabajo se configura en cuatro estudios cuyo objetivo fue, por un lado, descubrir nuevos marcadores genéticos asociados al contenido de GIM y/o a su composición y, por otro, aportar nuevo conocimiento con respecto a su interacción con la nutrición y los caracteres maternales y reproductivos.

En el primer estudio se analizó la variación de la secuencia del gen diacilglicerol O-aciltransferasa-2 [*DGAT2*], enzima que interviene en el último paso de la síntesis de triglicéridos. Se observó que los cerdos portadores del alelo *DGAT2*-G (ss7315407085 G > A), localizado en el exón 9 del gen, expresaron más *DGAT2* en músculo y mayor proporción de ácidos grasos de cadena corta (C14:0 y C16:0 en lugar de C18:0), en especial de ácido palmitoleico. En su conjunto, los resultados obtenidos indican que DGAT2 muestra preferencia como sustrato por los ácidos grasos recién sintetizados, especialmente si son monoinsaturados. En el segundo estudio se investigó la interacción entre un polimorfismo en el gen de la estearoil-CoA desaturasa [*SCD*] (rs80912566 T>C), que afecta el índice de desaturación de la grasa, y el nivel de vitamina A en la dieta sobre el transcriptoma muscular y la GIM. La suplementación de vitamina A, a pesar de que cambió la expresión génica de muchas vías de transcripción, no afectó ni el contenido ni la composición de GIM. No obstante, el efecto de la vitamina A sobre el índice de saturación varió con el genotipo *SCD*, de tal manera que el genotipo *SCD*-CC, que es el que menos expresa el gen, fue el único en el que la vitamina A aumentó la desaturación. En el tercer y cuarto estudio se investigó el impacto de los genes receptor de la leptina [*LEPR*, rs709596309 C>T] y *SCD* sobre la capacidad maternal y reproductiva de una cerda. Se comprobó que el gen *LEPR* causa un efecto materno antagónico al directo sobre el peso del cerdo al final del engorde, lo que se explica porque el genotipo que es más graso y que más crece (*LEPR*-TT) es el que menos reservas moviliza durante la lactación de sus lechones. Además, las cerdas *LEPR*-TT tuvieron más tarde su primer parto y presentaron un menor tamaño de camada al nacimiento. Por el contrario, el genotipo *SCD* no causó efectos adversos en la reproducción de una cerda, excepto en el peso al destete de las primerizas portadoras del alelo *SCD*-T que se cubrieron a edades tempranas. Este efecto podría deberse a que el alelo *SCD*-T se asocia, por una parte, a un ligero retraso en el crecimiento corporal y, por otra, a camadas algo más numerosas. Por ello, se recomienda precaución en el manejo de las cerdas primerizas portadoras del alelo *SCD*-T, en especial cuando el alelo *LEPR*-T también segrega en la población.

Los resultados de esta tesis confirman que la deposición de grasa en porcino está sujeta a sutiles mecanismos de regulación genética, cuyos efectos pueden alcanzar también el comportamiento maternal y la reproducción de una cerda.

Resum

El contingut i la composició del greix intramuscular [GIM] influeix en les propietats organolèptiques, tecnològics i nutricionals de la carn de porc. En particular, un major índex de dessaturació del greix es considera beneficiós per a la salut del consumidor, atès que un excés de greix saturat incrementa el risc de malalties cardiovasculars. Aquesta tesi forma part d'una línia de recerca centrada a millorar la qualitat de la carn de porc mitjançant la selecció pel contingut de GIM i la composició d'àcids grassos. Aquest treball es configura de quatre estudis, l'objectiu dels quals va ser, per una banda, trobar nous marcadors genètics associats al contingut de GIM i/o a la seva composició i, de l'altra, aportar nous coneixements respecte a la seva interacció amb la nutrició i els caràcters maternals i reproductius .

En el primer estudi es va analitzar la variació de la seqüència del gen diacilglicerol O-aciltransferasa-2 [*DGAT2*], enzim que intervé en l'últim pas de la síntesi de triglicèrids. Es va observar que els porcs portadors de l'al·lel *DGAT2*-G (ss7315407085 G > A), localitzat a l'exó 9 del gen, van expressar més *DGAT2* en múscul i més proporció d'àcids grassos de cadena curta (C14:0 i C16:0 en lloc de C18:0), especialment d'àcid palmitoleic. En conjunt, els resultats obtinguts indiquen que DGAT2 mostra preferència com a substrat pels àcids grassos acabats de sintetitzar, especialment si són monoinsaturats. En el segon estudi es va investigar la interacció entre un polimorfisme al gen de l'estearoil-CoA desaturasa [*SCD*] (rs80912566 T>C), que afecta l'índex de dessaturació del greix, i el nivell de vitamina A a la dieta sobre el transcriptoma muscular i la GIM. La suplementació de vitamina A, malgrat que va canviar l'expressió gènica de moltes vies de transcripció, no va afectar ni al contingut ni a la composició del GIM. No obstant això, l'efecte de la vitamina A sobre l'índex de saturació va variar amb el genotip *SCD*, de manera que el genotip *SCD*-CC, que és el que menys expressa el gen, va ser l'únic en què la vitamina A va augmentar la dessaturació. Al tercer i quart estudi es va investigar l'impacte dels gens receptor de la leptina [*LEPR*, rs709596309 C>T] i *SCD* sobre la capacitat maternal i reproductiva d'una truja. Es va comprovar que el gen *LEPR* causa un efecte matern antagònic al directe sobre el pes del porc al final de l'engreixament, la qual cosa s'explica perquè el genotip que és més gras i que més creix (*LEPR*-TT) és el que menys reserves mobilitza durant la lactació dels seus garrins. A més, les truges *LEPR*-TT van tenir més tard el seu primer part i van presentar una menor grandària de garrinada al naixement. Per contra, el genotip *SCD* no va causar efectes adversos en la reproducció d'una truja, excepte en el pes al deslletament de les truges primerenques portadores de l'al·lel *SCD*-T que es van cobrir a edats primerenques. Aquest efecte podria ser degut al fet que l'al·lel *SCD*-T s'associa, d'una banda, a un lleuger retard en el creixement corporal i, de l'altra, a garrinades una mica més nombroses. Per això, es recomana precaució en el maneig de les truges primerenques portadores de l'al·lel *SCD*-T, especialment quan l'al·lel *LEPR*-T també segrega a la població.

Els resultats d'aquesta tesi confirmen que la deposició de greix en porcí està subjecta a subtils mecanismes de regulació genètica, els efectes de la qual poden afectar també el comportament maternal i la reproducció d'una truja.

Summary

Meat intramuscular fat content [IMF] and composition influence the organoleptic, technological and nutritional attributes of pork. In particular, improving fat desaturation rates is beneficial for the health of consumers because an excess of saturated fat increases the risk of cardiovascular diseases. This thesis is part of a research line focused on the genetic improvement of pork quality through selection for IMF and fatty acid composition. The present work is comprised of four studies whose aim was, on one hand, to discover new genetic markers associated with the IMF and/or its composition and, on the other hand, to contribute new knowledge regarding its interaction with nutrition and maternal and reproductive characteristics.

In the first study, it was analyzed the sequence variation of the diacylglycerol O-acyltransferase-2 [*DGAT2*] gene, an enzyme involved in the last step of triglyceride synthesis. It was observed that pigs carrying the *DGAT2*-G allele (ss7315407085 G> A), located in exon 9 of the gene, expressed more *DGAT2* in muscle and a higher proportion of short-chain fatty acids (C14:0 and C16:0 instead C18:0), especially palmitoleic acid. As a whole, the results obtained indicate that DGAT2 shows preference for newly synthesized fatty acids as a substrate, especially if they are monounsaturated. The second study investigated the interaction between a polymorphism in the stearoyl-CoA desaturase [*SCD*] gene (rs80912566 T> C), that affects the rate of fat desaturation, and the level of vitamin A in the diet on the transcriptome muscle and the IMF. Vitamin A supplementation, although it changed the gene expression of many transcription pathways, did not affect the IMF content or composition. However, the effect of vitamin A on the saturation index changed with the *SCD* genotype, in such a way that the *SCD*-CC genotype, which is the one with the lowest gene expression, was the only one in which vitamin A increased the desaturation. The third and fourth study investigated the impact of the leptin receptor [*LEPR*, rs709596309 C> T] and *SCD* genes on the maternal and reproductive capacity of a sow. The results showed that the *LEPR* gene causes an antagonistic maternal effect to the direct on pig w eight at the end of fattening, which is explained because the genotype that with higher fat and growth rates (*LEPR*-TT) is the one with lowest reserves mobilization during lactation of their piglets. Furthermore, *LEPR*-TT sows had their first parity later and had a smaller litter size at birth compared to other genotypes. In contrast, the *SCD* genotype did not cause adverse effects on the reproduction of a sow, except in the weaning weight of the gilts carrying the *SCD*-T allele that were covered at an early age. This effect could be due to the fact that the *SCD*-T allele is associated, on one hand, with a slight delay in body growth and, on the other hand, with more numerous litters. Therefore, caution is recommended in the management of gilts carrying the *SCD*-T allele, especially when the *LEPR*-T allele also segregates in the population.

The results from this thesis confirm that fat deposition in pigs is subject to subtle genetic regulation mechanisms, the effects of which can also affect maternal behavior and reproduction of a sow.

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Abreviaturas

BLUP	Best Lineal Unbiased Prediction
DE	Expresión diferencial
DEG	Genes diferencialmente expresados
DGAT2	Diacilglicerol O-aciltransferasa
GIM	Grasa intramuscular
GM	*Gluteus medius*
GWAS	Estudios de asociación de todo el genoma
LDL	Lipoproteínas de baja densidad
LEPR	Receptor de la leptina
LM	Músculo *longissimus*
MUFA	Grasas monoinsaturadas
NEFA	Ácidos grasos no esterificados
NGS	Secuenciación de nueva generación
PPARA	Receptores activados por proliferadores peroxisomales
PSE	Pálida, blanda y exudativa
PUFA	Grasas poliinsaturadas
QTL	Locus de carácter cuantitativo
RA	Ácido retinoico
RAR	Receptor del ácido retinoico
RXR	Receptor X retinol
SCD	Estearoil-CoA desaturasa
SFA	Grasas saturadas
SM	Músculo *semimembranosus*
SNP	Polimorfismo de nucleótido único

I- Introducción

1. Situación porcina actual

La carne aporta nutrientes valiosos para la salud, pero en estos últimos cuatro años (2018-2021) se está produciendo un descenso en el consumo de carne en los hogares españoles (-7% respecto al 2018) y a nivel mundial. Sin embargo, a diferencia de otras carnes, el consumo de la carne de cerdo está aumentando (+8,2% respecto al 2018); de hecho, es la segunda carne más consumida entre la población española. Una de las principales ventajas que presenta esta carne es que, en los establecimientos, tiendas y mercados se puede encontrar comercializada tanto en su estado fresco como congelado añadiendo, además, una amplia gama de productos elaborados.

Actualmente el consumo de carne fresca de cerdo en España se sitúa en 11 kg per cápita, mientras que la ingesta media de productos transformados (como embutidos, fiambre, etc.) se sitúa en 12,5 Kg per cápita (MAPAMA, 2020a). En la Figura 1 se presenta la tendencia en el consumo de carne de cerdo en el periodo 2018-2020 en la que se observa que el consumo de carne fresca de cerdo ha aumentado casi un 15% y el de productos transformados casi un 10%.

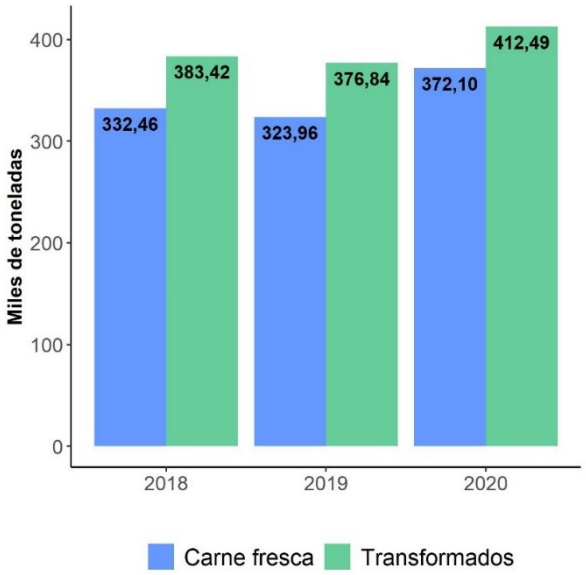

Figura 1. Evolución del consumo de carne de cerdo fresca y de productos transformados en España, en toneladas. Fuente: Panel de consumo MAPAMA.

Por este motivo, el sector porcino en España tiene una gran importancia en la economía del país. España es el tercer país con más censo porcino a nivel mundial, por detrás de China y Estados Unidos, y el principal productor a nivel de la Unión Europea (MAPAMA, 2020b) (Figura 2A). Dentro del territorio español, la producción porcina se distribuye principalmente en dos grandes regiones que producen alrededor del 50% del censo español total: Aragón (25,6%) y Cataluña (23,6%) (MAPAMA, 2020c) (Figura 2B). Este censo ha ido aumentando año tras año, lo que también se ha reflejado en el comercio exterior. En los últimos cinco años (2015-2020), las exportaciones se han incrementado un +67,8% de toneladas y las importaciones un +4,8%. Las exportaciones extracomunitarias son las que mayor importancia económica tienen, ya que representan un incremento de +217,4% de toneladas, a diferencia de las exportaciones dentro de la Unión Europea que en los últimos años (2015-2020), solo han incrementado un +1,6% (MAPAMA,

2020b). El principal destino de las exportaciones es China, con un incremento del +115% de toneladas respecto al año 2019. De hecho, España es el principal suministrador de carne de cerdo a este país asiático. Esto se debe principalmente a la epidemia de la Peste Porcina Africana que sufrió este país asiático el año 2018, que derivó en una caída del -21% de las reproductoras y del censo porcino debido a los sacrificios obligatorios. A causa de la crisis sanitaria vivida en China, el precio de la carne de cerdo en España ha experimentado un fuerte aumento durante el año 2019, alcanzando un máximo de 1,5 €/kg de cerdo respecto a 0,99 €/kg en el mismo periodo del año anterior (Mercolleida, 2020) (Figura 3). Las variaciones del precio de la carne de cerdo no solo dependen de la aparición de enfermedades porcinas que producen una disminución de la producción, sino también a la situación económica del país y del precio del pienso industrial o de las materias primas, porque la alimentación es el coste principal de las explotaciones (aproximadamente el 60%). Aun así, por su precio, la carne de cerdo es un tipo de carne asequible para la población.

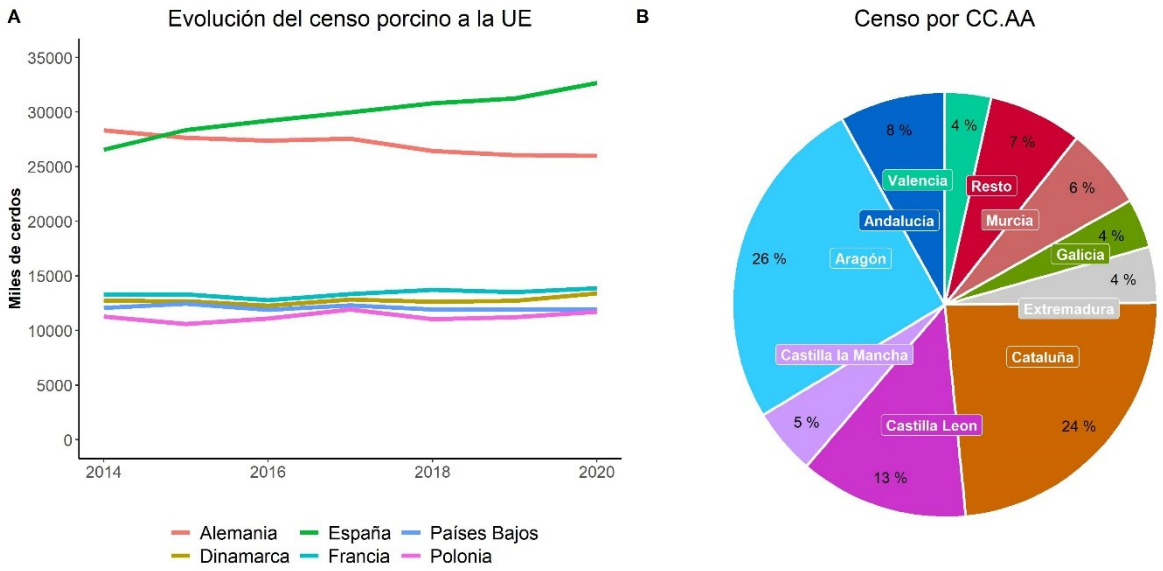

Figura 2. Censo porcino español. A. Evolución del censo porcino en la Unión Europea durante el periodo de 2014-2020. B. Censo porcino español en las principales comunidades autónomas productoras de cerdos en el año 2020. Fuente: Censo porcino MAPAMA.

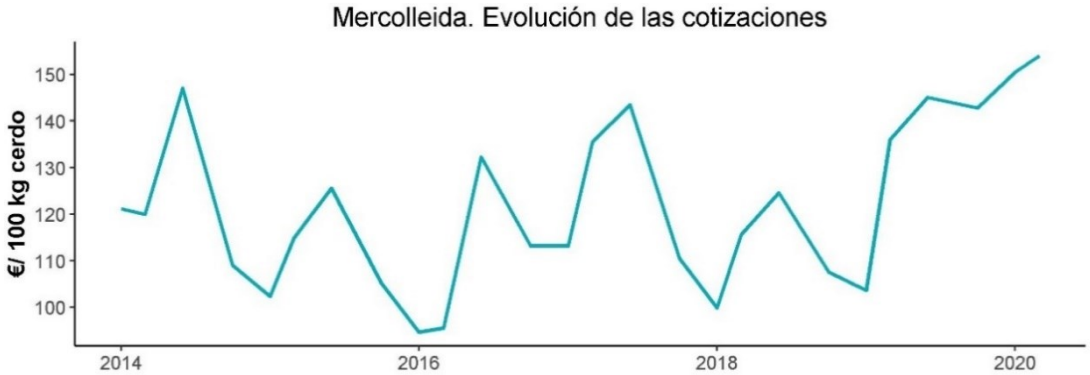

Figura 3. Evolución de las cotizaciones del precio de 100 kg de cerdo en Mercolleida durante el periodo 2014-2020. Fuente: Precio del cerdo en España, Mercolleida.

El tiempo de engorde necesario para obtener animales con el peso óptimo para el sacrificio es una gran determinante de los beneficios económicos de la industria porcina por su relación con el índice de conversión de los cerdos y, en consecuencia, el consumo de pienso. Por esta razón, el principal sistema productivo a nivel mundial es el intensivo dado que permite mayor control de los animales y la máxima producción a menor coste (mejor índice de conversión). Este sistema es el más utilizado en España y supone el 80% de las explotaciones totales, aunque en los últimos diez años ha sufrido cierto descenso (-1,6%) a favor del sistema extensivo (MAPAMA, 2020c). Aparte del tipo de sistema productivo, durante los últimos años el sector ha presentado una reestructuración, donde el número de explotaciones pequeñas se ha visto reducido drásticamente a favor de explotaciones de mayor tamaño (MAPAMA, 2020c).

No obstante, el éxito de un buen sistema de producción porcina depende de una serie de factores como son la sanidad, manejo/instalaciones, nutrición y genética. El abordaje de cada uno de estos factores permite realizar un seguimiento preciso del ciclo productivo y garantizar una correcta gestión económica. Es importante destacar que la genética utilizada tiene un impacto muy importante en la economía de la explotación, ya que no todas las líneas genéticas tienen el mismo potencial de crecimiento o reproductivo. La creación en los últimos 25 años de líneas especializadas (maternas y paternas) a través de la selección y su utilización mediante cruces ha significado una importante mejora de la prolificidad de las cerdas y la producción de un sistema más eficiente. Actualmente, en España las cerdas producen en media 14,6 nacidos vivos por camada y 12,4 destetados (BDporc, 2020). En cambio, en el año 2008 la media de lechones nacidos vivos se situaba en 11,2, de los cuales se destetaban 9,9 (BDporc, 2008). El tamaño de camada ha aumentado un 23% respecto al 2008, pero la mejora en prolificidad no se ha visto acompañada de una mejora en la supervivencia de los lechones hasta el destete debido a que los lechones nacen con menor peso y tienen más competencia para la obtención de alimento (Bidanel 2011).

Para concluir este apartado, cabe resaltar que para la industria porcina no solo es importante los valores productivos y reproductivos, sino también la calidad de la carne producida por el impacto que tiene sobre las características nutricionales y organolépticas lo cual supone un valor económico añadido al producto final.

2. Calidad de la carne y propiedades nutricionales

La carne se define según la FAO como "todas las partes de un animal aptas para el consumo humano de forma innocua". La carne está compuesta principalmente por proteínas de alta calidad, las cuales contienen todos los aminoácidos esenciales, además de grasas y ácidos grasos, minerales, vitaminas y carbohidratos en pequeñas proporciones (FAO, 2019). El consumo de carne es una importante fuente de nutrientes para la salud humana y su desarrollo. En una dieta equilibrada, aporta micronutrientes, como hierro y zinc, aminoácidos esenciales indispensables para el correcto desarrollo del metabolismo y es una importante fuente de energía y de proteína de calidad (FAO 1992; Biesalski et al., 2005; Godfray et al., 2018). Sin embargo, el consumo elevado de carne, en especial de carne roja y procesada, aumenta el riesgo de enfermedades cardiovasculares (Risk Factors Collaborators, 2015) y de ciertos cánceres, particularmente el cáncer de colon (Godfray et

al., 2018; González et al., 2020) debido a la presencia de componentes N-nitrosos e hidrocarburos aromáticos policíclicos que son clasificados como carcinógenos. El mecanismo de formación de estos componentes podría ser por la oxidación de los ácidos grasos poliinsaturados durante la maduración de la carne (IARC et al., 2018).

El término de calidad de la carne corresponde a un conjunto de propiedades y atributos que se aprecian cuando consumimos la carne o se somete a una transformación. Estas propiedades engloban diferentes aspectos que el consumidor final valora de forma subjetiva, y esta subjetividad reside en diferencias culturales y geográficas (p. ej., carnes oscuras altamente apreciadas en el mercado japonés) (Hartung et al., 2009; Listrat et al., 2016). Los atributos que definen la calidad de la carne influyen en definitiva en su valor económico (Figura 4).

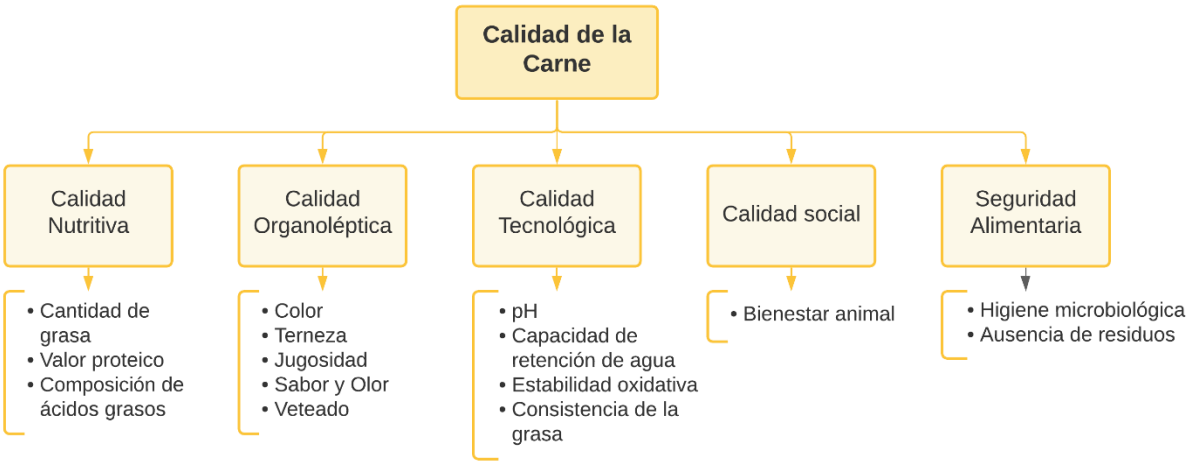

Figura 4. Atributos de la calidad de la carne.

2.1. Calidad nutritiva

La calidad nutricional de la carne engloba los atributos relacionados con la composición propia de la carne. Según el porcentaje de magro o la composición de ácidos grasos que presenta, la carne tendrá mayor o menor valor nutricional. Los lípidos presentes en la carne son un componente nutritivo fácilmente alterable, tanto en composición como en contenido, a través de la nutrición y de la genética del animal (Kausar et al., 2019; Alfaia et al., 2019; Juárez et al., 2021). La industria porcina ha ido mejorando estos dos atributos mediante la selección de los animales y se ha ido adaptando al gusto del consumidor.

La cantidad y la composición de la grasa intramuscular [GIM], a su vez de influir en la calidad nutricional, influyen en la calidad organoléptica (palatabilidad). Asimismo, el perfil de ácidos grasos presentes en el músculo puede tener un efecto beneficioso o perjudicial en la salud del consumidor. Una carne rica en ácidos grasos poliinsaturados [PUFA] omega-3, a diferencia de los ácidos omega-6, tiene un efecto beneficioso sobre la salud del consumidor (Ruxton et al., 2004). Los PUFA omega-3 son esenciales porque se obtienen básicamente de la dieta. Estos PUFA producen una reducción de los niveles de triglicéridos y de lipoproteínas de baja densidad [LDL] en sangre, y disminuyen la agregación plaquetaria y arritmias, de modo que una ingesta rica en omega-3 disminuye el riesgo de enfermedades cardiovasculares, entre otras enfermedades. En

cambio, una dieta rica en omega-6 tiene un efecto perjudicial sobre la salud. Por ejemplo, el ácido linoleico se transforma en acido araquidónico, el cual es el sustrato de moléculas proinflamatorias e incrementa la viscosidad de la sangre a consecuencia de la oxidación de las LDL, responsables de la formación de trombos (Simopoulos et al., 2008; Elagizi et al., 2018).

Asimismo, el exceso de grasas saturadas [SFA] incrementa el riesgo de enfermedades cardiovasculares, mientras que una dieta con grasas monoinsaturadas [MUFA] y PUFA disminuye la incidencia de estas enfermedades (Hu et al., 1997; Feingold, 2021). Este aumento del riesgo se debe a que el consumo de SFA está asociado a un incremento de las LDL y de los niveles de colesterol total en sangre, los cuales son factores de riesgo de enfermedades coronarias como la hipercolesterolemia (Griel et al., 2006; FAO, 2010; de Lorgeril y Salen, 2012). Estas LDL aumentan la viscosidad de la sangre porque se produce una agregación de los eritrocitos, a diferencia de las lipoproteínas de alta densidad que tienen un efecto contrario, disminuyendo la viscosidad, produciendo una relación inversa con el riesgo de enfermedades coronarias (Saleheen et al., 2015; Feingold, 2021).

2.2. Calidad organoléptica

Otro aspecto a valorar en la carne es su calidad organoléptica. Ésta es percibida por el consumidor en función del sabor, jugosidad, ternura y su color, los cuales son atributos plenamente subjetivos (FAO, 1992). Estos aspectos dependen principalmente de la composición de los ácidos grasos y de su oxidación, además de las proteínas principales del músculo (Wood et al., 2003; Guo y Greaser 2017).

Los cambios que se producen en el músculo *post mortem* generan una serie de cambios metabólicos y enzimáticos en las estructuras de las miofibrillas del músculo que afectan a la ternura del producto cárnico (Guo y Greaser, 2017). El contenido de GIM, a su vez, influye sobre varios parámetros relacionados con la calidad sensorial de la carne (veteado y palatabilidad). La palatabilidad de la carne se incrementa con el contenido de GIM: a mayor cantidad de GIM, mayor es la jugosidad de la carne porque la grasa, al ser masticada, estimula las glándulas salivares. Además, los lípidos tienen la capacidad de atrapar mayor humedad. Al mismo tiempo, con el contenido de GIM también se ve incrementada la ternura de la carne ya que las células adiposas que se encuentran entre las fibras musculares producen un ablandamiento de la estructura del músculo (Miller, 2002; Wood et al., 2003).

Otro atributo sensorial es el olor y sabor. Éstos se deben principalmente al estado de oxidación del producto cárnico. Un exceso de oxidación lipídica afecta negativamente la calidad cárnica pues produce un olor y un sabor rancio (Faustman et al., 2010). Asimismo, el olor sexual que producen los machos enteros también está valorado negativamente por los consumidores y por la industria porcina, pero este efecto indeseado se puede evitar en gran medida con la castración (química o quirúrgica) de los machos.

2.3. Calidad tecnológica

La capacidad de retención del agua es la capacidad de la carne para retener toda o parte del agua durante el proceso de oreo, maduración y/o cocción. Este aspecto es muy importante para determinar la calidad de la carne. Una carne con pérdidas de agua representa un coste económico para la industria cárnica. Además, la capacidad de retener el agua junto al alto contenido de GIM afecta de forma positiva a la jugosidad y ternura de la carne (Watanabe et al., 2018).

La variación del pH *post mortem* es uno de los factores más importante en relación a la calidad cárnica del porcino. Una caída drástica del pH mientras la canal está aún caliente produce la desnaturalización de las proteínas miofibrilares y esto se traduce a una reducción de la capacidad para retener el agua (Kim et al., 2016). Como resultado de este proceso se producen las carnes denominadas carnes PSE (pálida, blanda y exudativa, de las siglas en inglés) que no son aptas para el consumo (González-Rivas et al., 2020).

La pérdida de agua y el pH son dos atributos que están fuertemente relacionados. Tanto el pH como la capacidad de retener el agua se ven afectados por múltiples factores como es el estrés causado por el transporte o el sacrificio, la genética del animal (raza, genes mayores como el gen halotano o gen rendimiento *Napole*), el tipo de alimentación y la alteración de parámetros durante la cadena de procesado (Lammens et al., 2007; Hartung et al., 2009; González-Rivas et al., 2020). En consecuencia, estos factores predisponen a obtener carnes PSE con la correspondiente pérdida económica que representa.

2.4. Calidad social y seguridad alimentaria

La seguridad alimentaria debe estar garantizada en toda la cadena de producción hasta llegar al consumidor final. Los productos cárnicos deben almacenarse y procesarse adecuadamente con el fin de evitar el crecimiento microbiano y la presencia de residuos (antibióticos) no aptos para el consumo humano. La presencia de patógenos como la *Salmonella* o *Clostridium perfingens* pueden causar las denominadas toxiinfecciones alimentarias. La presencia de estos microrganismos se considera indeseable y los productos contaminados son eliminados de la cadena alimentaria (Pal et al., 2018).

En cuanto a la calidad social, el bienestar animal es un factor que también afecta a la calidad de la carne ya que unas buenas prácticas de manejo durante el transporte y sacrificio disminuyen el estrés de los animales antes del sacrificio y por consiguiente la generación de carnes PSE. Además, durante el engorde, el aumento de espacio entre cerdos mejora su bienestar animal y también los parámetros productivos (Nannoni et al., 2019).

A modo de conclusión de esta sección, la calidad de la carne depende de múltiples atributos que afectan a lo largo de toda la cadena de producción, pero la comprensión de estos atributos son claves para desarrollar estrategias que permitan maximizar la calidad de la carne durante su producción. El contenido de la GIM y la composición de los ácidos grasos son dos factores que contribuyen en gran parte a la mejora de la calidad tanto organolépticamente como nutricionalmente y que además, pueden ser mejorados a través de la genética y de la nutrición.

3. Metabolismo de los ácidos grasos

Los ácidos grasos son esenciales para la supervivencia de los seres vivos, dado que intervienen en funciones biológicas esenciales como el metabolismo de los lípidos, división celular e inflamación. Según la presencia y el número de dobles enlaces entre átomos de carbono, los ácidos grasos son clasificados en tres tipos; SFA, MUFA y PUFA.

Los SFA son ácidos grasos sin dobles enlaces y son altamente resistentes a la oxidación, a diferencia de los MUFA y PUFA. Los MUFA presentan un doble enlace simple y los PUFA presentan más de un doble enlace y son altamente susceptibles a la peroxidación (Stillwell, 2016). Los ácidos grasos más comunes de la carne de cerdo se presentan en la Tabla 1.

Tabla 1. Ácidos grasos más comunes, estructura y porcentaje aproximado en el músculo *gluteus medius* de cerdo.

Ácidos grasos insaturados		
Ácido Mirístico	C14:0	1,7%
Ácido Palmítico	C16:0	23,9%
Ácido Esteárico	C18:0	11,2%
Ácidos grasos monoinsaturados		
Ácido Palmitoleico	C16:1(n-7)	3,7%
Ácido Oleico	C18:1(n-9)	41,5%
Ácidos grasos polinsaturados		
Ácido Linoleico	C18:2(n-6)	10,7%
Ácido α-Linoleico	C18:3(n-3)	0,6%
Ácido Araquidónico	C20:4(n-6)	1,5%

Fuente: Wood et al., 2007.

La elongación y desaturación de los ácidos grasos es esencial para mantener la homeostasis de los lípidos. La alteración de estos procesos puede tener graves consecuencias en el metabolismo lipídico y procesos celulares. La elongación de los ácidos grasos implica la adición de dos átomos de carbono a un acil-CoA utilizando malonil-CoA como donador y NADPH como agente reductor. Este proceso se cataliza por enzimas conocidas como elongasas (Figura 5). En cambio, la desaturación es un proceso que se lleva a cabo con enzimas desaturasas, como la estearoil-CoA desaturasa [SCD] y las desaturasas de ácidos grasos. Estos enzimas son responsables de introducir un doble enlace en una posición específica de la cadena larga acil de ácidos grasos (Guillou et al., 2010).

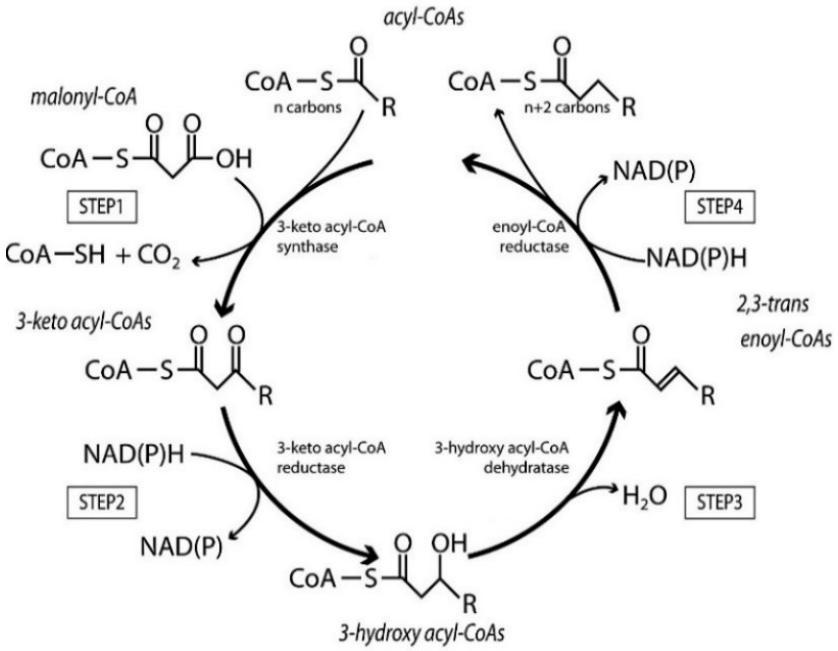

Figura 5. Ciclo de elongación de los ácidos grasos, formado por 4 fases; condensación de acil-CoA y malonil-CoA para formar 3-cetoacil-CoA, reducción para generar 3-hidroxiacil-CoA, deshidratación a trans-2-enoyl-CoA y reducción para formar el alargamiento de acil-CoA con dos átomos de carbono más largo que su precursor. Fuente: Denic y Weissman, 2007.

En situación de exceso energético, los ácidos grasos esenciales y no esenciales son depositados en forma de triglicéridos en gotas lipídicas del citoplasma de los tejidos adiposos y no adiposos. Estas gotas lipídicas son el principal reservorio de almacenamiento de energía y de precursores de los fosfolípidos (Coleman y Lee, 2004). Durante el metabolismo de formación de los triglicéridos hay múltiples enzimas que intervienen, como el diacilglicerol O-aciltransferasa [DGAT] que cataliza la esterificación de la molécula diacilglicerol para producir el triglicérido (Bhatt-Wessel et al., 2018). Esta enzima permite eliminar los ácidos grasos libres, que se consideran dañinos para la membrana, y los incorpora en triglicéridos no tóxicos, protegiendo a las células de su lipotoxicidad (Walterman et al., 2007). La disponibilidad de ácidos grasos determina la biosíntesis de los triglicéridos. Este proceso de almacenamiento y movilización está regulado por señales hormonales, nutricionales y transcripcionales que activan mecanismos reguladores a corto y largo plazo para el correcto metabolismo de los triglicéridos (Karantonis et al., 2009).

Durante los periodos de inanición se produce una movilización de grasa mediante la lipolisis de los triglicéridos almacenados en el tejido adiposo y su liberación al torrente sanguíneo. Este proceso produce los ácidos grasos no esterificados [NEFA] que ayudan a movilizar la energía hacia los órganos y tejidos a través del torrente sanguíneo (Duplus y Forest, 2002; Dunshea y D'Souza, 2003). Por lo tanto, la composición de NEFA en sangre pueden ser un marcador útil de la composición de ácidos grasos en el tejido adiposo (Hodson et al., 2008) aunque su utilidad práctica también ha sido cuestionada en trabajos más recientes (Tor et al., 2021).

El contenido y composición de los ácidos grasos, así como el depósito y metabolismo de los lípidos, están influenciados por una serie de factores, entre los que se incluyen la genética, la

nutrición, el sistema de producción, el sexo y edad de los animales, los cuales se presentan a continuación.

3.1. Genética

Las razas porcinas actuales han sido seleccionadas genéticamente en función de características de interés para la industria cárnica como es mejorar la eficiencia alimentaria, mejorar la eficiencia reproductiva (fertilidad y numero de lechones) y mejorar la calidad y la conformación de la canal (Li et al., 2013). Respecto a la calidad de la carne, los cerdos principalmente se han ido seleccionando o para obtener mayor porcentaje de magro y menor cantidad de grasa, incluida la GIM, o para obtener mayor cantidad y calidad de la GIM. La GIM se ve muy influida por la genética del cerdo. Dependiendo de la raza, el contenido de GIM es mayor (Ibéricos) o menor (Landrace) (Tabla 2).

Tabla 2. Características productivas de las razas Landrace, Pietrain, Duroc e Ibérico.

	Landrace	**Pietrain**	**Duroc**	**Ibérico**
Índice de conversión (Kg/Kg)	3,1	3,25	3,1	4,9
Rendimiento canal (%)	74,5	77	74	80
% de Magro	53	60	52	38
% de GIM en *Longissimus thoracis*	1,5[a]	1,8[b]	2,1[a]–2,5[b]	6,5[c]

Fuente: MAPAMA y [a]Zhang et al., 2018a, [b]Kim et al., 2020, [c]Bressan et al., 2016.

Para mejorar la calidad de la carne, se utilizan principalmente los cruces dirigidos entre líneas paternas. La raza Ibérica se caracteriza por su elevada calidad organoléptica ya que posee gran cantidad de GIM debido a su tendencia natural a depositar grasa. Otro ejemplo es la raza Duroc, al ser una raza con alto contenido de GIM en comparación con las otras razas magras, se ha utilizado ampliamente en cruces comerciales, con Landrace, Pietrain y Yorkshire, con el fin de mejorar la calidad de la carne de estas razas e incrementar el contenido de la GIM (Choi et al., 2016; Xue et al., 2018; Kim et al., 2020). En cambio, los cruces entre razas con menor porcentaje de GIM (p. ej. Landrace) son usadas para obtener carnes más magras (Kralik et al., 2006; Zhang et al., 2018a).

3.2. Alimentación

La alimentación nos permite mejorar la composición y la cantidad de ácidos grasos con una intervención directa sobre el animal. Hay múltiples estudios que indican que la dieta afecta a la composición de la GIM (Ayuso et al., 2015ª; Scollan et al., 2017; Palma-Granados et al., 2019). En general, se ha estudiado el efecto de la energía y de la proteína de la dieta sobre la GIM, y, más en particular, de la concentración de determinadas sustancias, como la vitamina A. Por otra parte, la alimentación de la cerda también puede afectar el contenido y la composición de la grasa de sus descendientes (ver también Sección 3.2.4).

3.2.1. Efecto de la energía de la dieta

Varios estudios demuestran que una dieta con alto contenido en energía, generalmente rica en PUFA, modifica la composición de ácidos grasos en los tejidos, aunque la GIM es menos susceptible a los tratamientos alimentarios comparado con otros tejidos como la grasa dorsal (Kouba et al., 2003; Duran-Montgé et al., 2007). Del mismo modo, también se puede incrementar el contenido de MUFA o SFA. Los estudios con una dieta suplementada en MUFA concluyen que se produce un aumento del contenido de MUFA en grasa dorsal, lo que resulta en un nivel más bajo de instauración y un efecto sobre la dureza del producto final (López-Bote et al., 2002). Otra manera de incorporar energía es mediante la adición en la dieta de glicerol o aceite de palma, ricos en SFA, durante el período de engorde del animal. Estos componentes alimentarios reducen el contenido de ácido linoleico, el cual permite incrementar la firmeza de la grasa. Este efecto es poco consistente, pues otros estudios no han encontrado ningún efecto sobre la composición de la GIM (Schieck et al., 2010; Suarez-Belloch et al., 2013; Segura et al., 2015). Esta variabilidad de resultados puede ser en base a la utilización de diferentes tipos genéticos, además de a las diferencias en el periodo en el que se aplica la modificación de la dieta.

Los cambios en la composición y cantidad de la GIM se deben a que, según el tipo de alimentación o suplementación, se produce una activación o inhibición de los enzimas lipogénicos y de los enzimas responsables de la desaturación y elongación de los ácidos grasos (Specht-Overholt et al., 1997; Kouba et al., 2003). La existencia de interacción entre dieta y tipo genético pueden deberse a la activación diferencial de estos enzimas en función de las variantes genéticas presentes en cada raza o línea.

3.2.2. Modificación de la proteína de la dieta

Una reducción del contenido de proteína en la dieta resulta en un incremento de GIM (Madeira et al., 2017), pero dependiendo de la cantidad de proteína reducida, puede producir efectos negativos sobre el crecimiento, el índice de conversión y la conformación de la canal del animal (mayor espesor de grasa dorsal) (Bunger et al., 2014).

La lisina y la leucina son dos aminoácidos esenciales en la dieta de los cerdos, de modo que una estrategia para aumentar la cantidad de GIM es modificando la cantidad de estos aminoácidos. Se ha demostrado que un déficit en lisina produce un efecto lipogénico (Katsumata et al., 2011; Palma-Granados et al., 2019). Niveles bajos de proteína activan las enzimas lipogénicas en el músculo y, en consecuencia, la síntesis *de novo* de los ácidos grasos en la GIM (Katsumata et al., 2011; Palma-Granados et al., 2019). Este tipo de dieta potencia la actividad de la enzima SCD en el músculo, ya que la expresión de *SCD* se correlaciona positivamente con el contenido de GIM y con la síntesis de MUFA, pero no en el tejido subcutáneo (Doran et al., 2006; Bessa et al., 2013).

En lugar de reducir la lisina, se puede aumentar el contenido de leucina, el cual produce un incremento de los niveles de GIM y en la firmeza del músculo. Esto se debe a que la leucina se transforma en acetil-Coa y entra en la ruta de síntesis de ácidos grasos (Hyun et al., 2007). No obstante, el principal problema es que un aumento de la leucina afecta negativamente sobre el

crecimiento y la canal, resultando en animales con pesos más bajos (Hyun et al., 2003). Sin embargo, si el incremento de la leucina se combina con una reducción de la lisina, no se penaliza el peso ni la calidad de la canal del animal, mientras que el contenido de GIM aumenta (Hyun et al., 2007; Katsumata et al., 2011). El efecto de la proteína sobre la composición de la GIM y sus efectos negativos sobre la canal dependen de los niveles y del tiempo de reducción-incremento del tratamiento y de la predisposición genética de la raza utilizada en relación a su capacidad de deposición de GIM y su potencial de crecimiento (Hyun et al., 2003; Bessa et al., 2013).

3.2.3. Efecto de la vitamina A

La vitamina A es un micronutriente esencial para el desarrollo de un individuo, ya que participa en múltiples procesos metabólicos que intervienen en el crecimiento, la reproducción, la diferenciación celular, la transformación de los lípidos, la regulación genética o la respuesta inmune (Al Tanoury, 2013). La vitamina A, de hecho, la forman un grupo de compuestos liposolubles que incluyen a retinoides preformados como el retinol y sus derivados, retinal y ácido retinoico, así como varios carotenoides que tienen función de provitamina A, en especial el beta-caroteno. La vitamina A se encuentra en múltiples alimentos, si bien en los de origen animal se presenta en forma de retinol o vitamina A mientras que en los vegetales lo hace como caroteno o provitamina A (Edem et al., 2009). Los animales convierten los carotenos en retinol y éste, por actividad oxidativa, se convierte en ácido retinoico.

El mecanismo de acción del ácido retinoico [RA], uno de los metabolitos activos de la vitamina A, es análogo al de las hormonas esteroides (estrógenos, progestágenos, glucocorticoides, 1,25-dihidroxivitamina D) y hormona tiroidea. Estas hormonas, al igual que el RA, se transportan a través de la circulación en asociación con una proteína transportadora y son solubles en la membrana plasmática de la célula. Sus receptores son intracelulares y actúan como potentes reguladores transcripcionales, regulando la expresión de genes principalmente a través de dos subfamilias de receptores nucleares: el receptor del ácido retinoico [RAR] y el receptor X retinol [RXR] (Figura 6) (Zhang et al., 2015). Además, el RA también modula la expresión génica a través de mecanismos epigenéticos, tanto a través de mecanismos de hipermetilación del ADN o de cambios en los patrones de acetilación de las histonas, según el gen (Bar-El Dadon & Reifen, 2015).

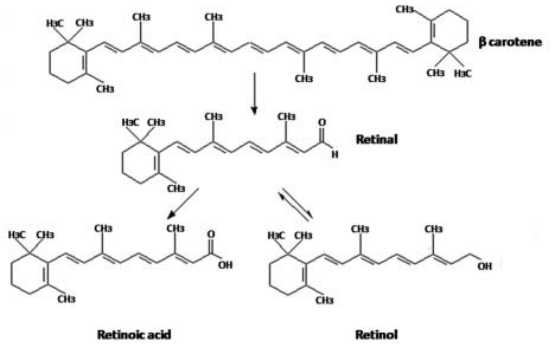

Figura 6. Estructura química de la vitamina A y sus derivados principales. Fuente: Bar-El Dadon & Reifen, 2015.

La vitamina puede inducir cambios en el fenotipo del animal, como en la composición y cantidad de la GIM, debido a cambios relacionados con el metabolismo de los adipocitos, puesto que el RA interacciona con la regulación de varios genes relacionados con la biosíntesis de ácidos grasos y proliferación y maduración de los adipocitos, como *RAR, RXR, SCD,* receptores activados por proliferadores peroxisomales [*PPARA*] y la sintasa de ácidos grasos, entre otros muchos genes (Wang et al., 2016; Zhao et al., 2016; Bar-El Dadon & Reifen, 2015).

En el cerdo, la restricción de vitamina A en la dieta puede incrementar el contenido de GIM (Ayuso et al., 2015a) y favorecer el depósito de grasas menos saturadas (+MUFA), tanto en músculo como en grasa dorsal (Olivares et al., 2009a; Ayuso et al., 2015a). El efecto de la restricción es más evidente cuanto antes se inicia, aunque depende también de la intensidad y su duración (Ayuso et al., 2015a; Ayuso et al., 2015b) (Tabla 3). Una modificación de la vitamina A en edades más tempranas podría ser una buena estrategia para modificar la GIM, pues en animales más jóvenes la adipogénesis es más activa y hay mayor proporción de preadipocitos (Wang et al., 2016). En cerdos, la restricción de vitamina al 75-80% del consumo de pienso *ad libitum* durante las 16-25 semanas de edad se asocia con un mayor depósito de GIM, aunque suele ir acompañado de un efecto negativo en el crecimiento y la calidad de la canal (D'Souza et al., 2003; Daza et al., 2007). Igualmente, la magnitud del efecto de la restricción de vitamina A también está sujeto a una fuerte componente genética, puesto que se observan diferencias en la respuesta al mismo tratamiento entre líneas genéticas (Tabla 3 y Olivares et al., 2009b). Sin embargo, hasta la fecha no se ha descrito ningún marcador molecular responsable de estas diferencias.

Tabla 3. Resumen de los experimentos publicados sobre el efecto de la suplementación/restricción de vitamina A en la dieta sobre el depósito de grasa y su composición en cerdo**.**

Referencia	Genética[1]	Vitamina A o precursores en la dieta		Duración experimento	Efecto de la dieta tratamiento sobre:	
		Dieta control	Dieta tratamiento		depósito de grasa	composición de la grasa
D'souza et al., 2003	LW x LD x DU	30 µg acetato de retinilo /kg	Sin suplemento	3 meses (edad: 60 a 160 d)	↑GIM	(sin datos)
Olivares et al., 2009a	DU x (LW X LD)	7.500 IU vit. A /kg	100.000 IU vit. A /kg	2 meses	no	↑C16:0, SFA y ↓ MUFA, MUFA/SFA en grasa dorsal. Tendencia similar en músculo
Olivares et al., 2009b	DU x (LW X LD)	0 IU vit. A /kg	100.000 IU vit. A /kg	2 meses	↑GIM	↑C18:0, SFA en músculo
	F2 (LW x LD)	0 IU vit. A /kg	100,000 IU vit. A /kg	2 meses	no	↑C18:0, SFA en músculo
Olivares et al., 2011	LW x (LW X LD)	13.000 IU vit. A /kg	1.300 IU vit. A /kg (dieta L)	3 meses	Dieta L: ↑ peso canal, ↑ GIM	Dieta L: ↓ MUFA, MUFA/SFA en grasa dorsal. Sin efecto en músculo
		13.000 IU vit. A/kg	0 IU vit. A/kg	1.5 meses		
Ayuso et al., 2015a	IB	10.000 IU vit. A /kg	0 IU vit. A /kg	4 – 7 meses	↑ GIM en semimembranoso	↑ MUFA, MUFA/SFA en grasa dorsal y músculo
Ayuso et al., 2015b	IB	10.000 IU vit. A /kg	0 IU vit. A /kg	2 – 9 meses	↑ lípidos neutros a los 9 meses	↑ MUFA, MUFA/SFA en músculo a los 9 meses
Henríquez-Rodríguez et al., 2017	DU	1.300 IU vit. A /kg (dieta L)	Dieta sin vit. A ni carotenos.	1 mes	↑ GIM, y la grasa dorsal y en hígado	↑ MUFA, MUFA/SFA

[1]LW – LargeWhite; LD – Landrace; DU – Duroc; IB – Ibérico

3.2.4. Modificación de la dieta de las madres

Una dieta rica en grasas durante la gestación y la lactación aporta a los lechones la energía y los ácidos grasos necesarios para la formación de los lípidos estructurales de los órganos y el almacenamiento de grasas. Estos lechones tienen un mayor número de células adiposas y a su vez más potencial de acumulación de grasa (Ajuwon et al., 2016). Por otro lado, una dieta rica en PUFA potencia el sistema inmunológico de los lechones y mejora la composición del calostro (Tanghe et al., 2014; Peng et al., 2019). Así, este tipo de suplemento en las madres beneficia a los lechones ya que también aumenta su supervivencia, desarrollo y crecimiento a través de una mayor disponibilidad de PUFA e inmunoglobulinas (Jin et al., 2017; Laws et al., 2018).

La nutrición de la madre no solo afecta directamente a la composición y cantidad de ácidos grasos en los lechones, sino que puede tener un efecto epigenético sobre ellos. La epigenética nutricional estudia los cambios que ejercen determinados nutrientes en el ADN/cromatina con capacidad para reprogramar rutas biológicas con repercusiones uni o multigeneracionales (Holliday, 2006; Bar-El Dadon & Reifen, 2017). Estos cambios son debidos principalmente a la metilación del ADN, a la modificación de las histonas y a la síntesis de microARN no codificantes (Goldberg et al., 2007; Niculescu y Lupu, 2011; Skinner, 2011). Así, hay estudios que demuestran que un déficit nutricional durante el periodo gestacional tiene efectos fenotípicos en las generaciones F1 y F2 debido a una alteración en la metilación del ADN que afecta la regulación y programación de los genes de los descendientes (Cooney et al., 2002). Como resultado, se producen alteraciones en el desarrollo y una mayor predisposición a enfermedades, entre ellas, las vasculares (Burdge et al., 2007; Remely et al., 2015; Zhang et al., 2018b; Zelko et al., 2019).

3.3. Sistema de producción

El sistema de producción o ambiente en el cual se crían los animales engloba otros factores como la alimentación y bienestar animal, de modo que la composición de GIM y calidad del producto final se ve alterada. El sistema de producción más extendido es el intensivo, donde la nutrición del animal es controlada, a diferencia del extensivo.

En rumiantes, el sistema extensivo (pastoreo) tiene efectos beneficiosos sobre el perfil de ácidos grasos. Se ha observado que estos animales tienen porcentajes de PUFA omega-3 más altos y una ratio PUFA/SFA también más alta (Petrič et al., 2005; Özcan et al., 2015). El perfil de ácidos grasos del músculo es diferente entre ambos sistemas a causa del tipo de alimentación que reciben en cada uno: el régimen intensivo recibe una alimentación a base de concentrados, el cual incrementa los SFA y MUFA respecto al sistema extensivo (Özcan et al., 2015).

Un ejemplo bien conocido de sistema extensivo en producción porcina, en vista a la calidad nutricional de su producto final, es la producción de cerdo ibérico en dehesas. Aunque es un sistema que se asocia a un crecimiento lento, seguramente a causa del ejercicio que realizan los animales y al bajo ritmo de crecimiento de esta raza, la composición de GIM se ve claramente mejorada (Tejerina et al., 2012; Velazco et al., 2013). Este tipo de sistema, igual que en otras especies, también incrementa el contenido de ácidos grasos insaturados con altas concentraciones de ácido

linoleico, ácido oleico y ácido linolénico resultando en una calidad nutricional distintiva de estos productos (Velazco et al., 2013). Este incremento en la desaturación de la grasa se debe a la reducción-oxidación de los lípidos a raíz de una dieta de finalización basada en bellotas y pastos con menor contenido de SFA (Andrés et al., 2001).

3.4. Sexo y edad

El contenido de GIM depende en gran medida del sexo del cerdo. Aunque algunos autores no detectaron ningún efecto del sexo sobre el contenido de GIM (Hamilton et al., 2000; Latorre et al., 2004), actualmente no hay duda que el sexo produce un cambio en el contenido y la composición de GIM (Bahelka et al., 2007; Latorre et al.,2009). En producción porcina encontramos, además del binomio macho-hembra, los animales castrados. La castración tanto de machos como de hembras es un factor de manejo para aumentar la GIM.

El uso de machos castrados para la producción de carne se inició antes de los años noventa principalmente para reducir el olor sexual y la agresividad de los machos enteros (American Veterinary Medical Association, 2013), pero también para aumentar la GIM, dado que los machos castrados depositan más grasa respecto a los enteros (Xue et al., 1997). Es más, se ha demostrado que los machos castrados crecen más rápido y tienen más contenido de GIM que las hembras, aunque el consumo de alimento también es mayor (Latorre et al., 2003; Correa et al., 2006). Este efecto en machos castrados se repite en las hembras castradas. Las hembras castradas presentan una mayor tasa de crecimiento, más espesor de grasa dorsal y diferencias en la composición de ácidos grasos, con una menor proporción de PUFA y mayor SFA respecto a las hembras enteras (Serrano et al., 2008; Peinado et al., 2011). Estas diferencias entre sexos se deben principalmente a los cambios hormonales (estrógeno y testosterona) causados por la castración y su efecto sobre el metabolismo de los lípidos y, por tanto, a la predisposición a la obesidad (Palmisano et al., 2018).

Asimismo, la edad de sacrifico es un factor muy importante a nivel económico que también influye en la cantidad y la composición de GIM. A más edad, los depósitos de ácidos grasos aumentan, pero este aumento no siempre es beneficioso en términos económicos, porque son animales que consumen más, tienen peor índice de conversión y depositan más SFA, tanto en la GIM como en la grasa dorsal (Lo Fiego et al., 2010). Además, el índice de magro también se ve afectado negativamente y, a su vez, la catalogación de las canales por el sistema SEUROP. En este sentido, los cerdos sacrificados a 210 días respecto a 160 días presentan mayor proporción de SFA y menor PUFA, siendo el ácido linoleico el ácido graso con mayor variación entre edades (Serrano et al., 2008; Bosch et al., 2012). No obstante, en la producción de jamón curado, el éxito de los programas de mejora sobre el contenido magro y calidad de la canal ha permitido reducir la edad de sacrificio sin afectar a la calidad de los productos, y en consecuencia reducir los costes de producción (Malgwi et al., 2021; Ortiz et al., 2021).

4. Estrategias genéticas para mejorar la calidad de la grasa

4.1. Estructura del sistema productivo

La industrialización de los años sesenta supuso un cambio en los sistemas de producción dada la necesidad de producir más animales para cubrir las necesidades de la población lo que derivó hacia un sistema de producción intensivo. Este tipo de sistema permite criar mayor número de cerdos en menos tiempo y por tanto obtener mayores beneficios. Al mismo tiempo, facilita controlar todo el sistema productivo, desde el nacimiento hasta el sacrificio (partos, alimentación, control de enfermedades, etc.), así como la implementación de programas de selección y mejora de los caracteres de mayor interés económico. Para beneficiarse de estos programas de selección, la producción porcina se estructura en forma de pirámide de producción (Figura 7), con un mínimo de tres estratos: el núcleo de selección, las granjas de multiplicación y la producción comercial.

El núcleo es la parte principal de los programas de selección, donde se encuentran los animales (F0) pertenecientes a las líneas maternas y paternas. Las líneas maternas se seleccionan principalmente (aunque no exclusivamente) por los caracteres reproductivos y las paternas por los caracteres productivos y de calidad de la carne, entre los que estaría, si fuera el caso, la GIM y su composición (Dekkers et al., 2011). En la fase de multiplicación (F1) se cruzan las líneas, preferentemente las maternas, para aprovechar la heterosis entre ellas y la diseminación de la mejora genética del núcleo. La heterosis es la mejora en el rendimiento de los descendientes cruzados respecto a los padres de raza pura, porque el cruce entre razas produce una reducción de loci deletéreos en favor de posiciones heterocigotas más favorables sobre la *fitness* (y la productividad) de los animales (Bates, 2010). Las cerdas cruzadas se destinan a las granjas de producción para cruzarse con un macho de la línea paterna para producir lechones (F2), que son los que se crían en condiciones comerciales hasta obtener el peso de sacrificio (generalmente 105-110 Kg) (Moeller, 2005; Dekkers et al., 2011).

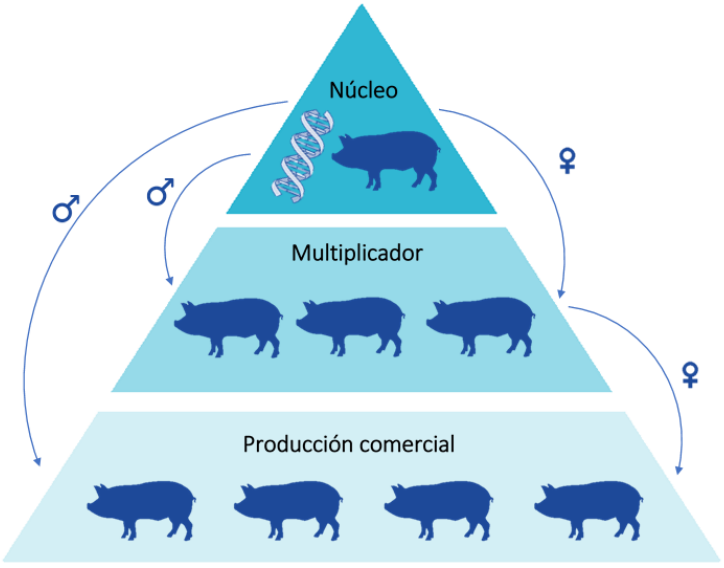

Figura 7. Pirámide de producción porcina, formada por las fases de selección, multiplicación y producción comercial.

Las razas más utilizadas como líneas maternas son las de base Landrace y Large White mientras que para las paternas lo son las de base Duroc y Pietrain. En particular, los machos Duroc son reconocidos como aquellos más adecuados para mejorar la calidad de la carne, por lo que son los más usados en esquemas destinados a la producción de productos elaborados (Lonergan et al., 2001; Zhang et al., 2018a). Por esta razón, suele utilizarse también en pureza y es la única raza autorizada en denominación en España (Real Decreto 4/2014, España) que se cruza con cerdos ibéricos para mejorar el bajo índice de crecimiento de éstos, y así producir productos de alta calidad.

4.2. La selección

La selección supone elegir a los mejores animales de cada generación para que sean los parentales de la generación siguiente. Los mejores animales son aquellos con mejor valor genético o de mejora. Como el valor genético no se conoce, se debe predecir. Al proceso de predicción del valor genético se le suele denominar evaluación genética. Durante los años ochenta, la evaluación genética de los reproductores se basaba principalmente en los datos fenotípicos propios. Posteriormente, ya en los noventa, con la aparición del BLUP (*Best Lineal Unbiased Prediction*, de las siglas en inglés) se incorporó en las evaluaciones la información genealógica, que podía incluir varias generaciones y que, además, permitía corregir de forma más precisa los fenotipos por el efecto de los factores ambientales conocidos (Hill, 2014). Este modelo matemático permite detectar los mejores animales dentro de una población y seleccionarlos como futuros reproductores. Por consiguiente, este modelo está ampliamente utilizado en los núcleos de selección.

Entrados los noventa, se empezaron a buscar, mediante el uso de herramientas biotecnológicas más precisas, genes (*loci*) que pudieran afectar a los caracteres (*trait*) de interés, mayoritariamente cuantitativos (*quantitative*), los llamados QTL (de las siglas en inglés). El objetivo era asistir al BLUP con la información aportada por los QTL. En general, los QTL son marcadores genéticos que permiten identificar una región genómica que está asociada a un efecto significativo sobre un carácter cuantitativo (Dekkers et al., 2004; Ollivier et al., 2009). Los marcadores genéticos son secuencias específicas e identificables de ADN. Estos marcadores revelan polimorfismos que permiten identificar genes candidatos para los caracteres estudiados. Los estudios realizados en esta época permitieron identificar gran cantidad de QTL a partir de mapas de baja densidad basados en paneles de marcadores microsatélites (Grindflek et al., 2001; Gao y Zhao, 2009; Bidanel, 2011). Los primeros estudios en la especie porcina para identificar QTL responsables del crecimiento y del engrasamiento se realizaron en una población experimental de cruce de jabalí x Large White (Andersson et al., 1994), mientras que los estudios para detectar caracteres reproductivos en hembras fueron realizados principalmente, aunque no exclusivamente, con cruces de Landrace x Large White (Bidanel, 2011). Utilizando estos mapas de baja densidad se han desarrollado estudios para detectar QTL asociados a la composición de la canal, GIM y caracteres reproductivos (Grindflek et al., 2001; Gao y Zhao, 2009). Para el tamaño de la camada, se han detectado QTL en 13 cromosomas distintos, entre ellos en el SSC6, donde encontramos el gen codificante para el receptor de la hormona leptina [*LEPR*] (Chen et al., 2004; Li et al., 2009). Por otra parte, el locus *LEPR* también está asociado como QTL para la grasa dorsal (Muñoz et al.,

2009; Ciobanu et al., 2011). No obstante, los estudios con QTL presentan la limitación de requerir un gran volumen de muestras y la falta de reproducibilidad.

Desde entonces, las nuevas tecnologías basadas en herramientas de genotipado masivo y secuenciación del genoma han permitido superar la baja resolución de los estudios realizados con microsatélites (Miles y Wayne, 2008; Verdugo et al., 2010). Para identificar un gen candidato es necesario encontrar variación en el genoma que explique las diferencias entre los animales para un determinado carácter, pero en muchas ocasiones hay un gran número de genes implicados con efectos pequeños, pero acumulativos, sobre el carácter (Tabor et al., 2002). Así, con el proyecto de secuenciación del genoma porcino se desarrollaron paneles de polimorfismos de nucleótido único (SNP, de las siglas en inglés). Los SNP son puntos de variación en el genoma que implican cambios de un solo nucleótido. Para detectar estos polimorfismos se recurre a comparar las secuencias específicas individuales con el genoma de referencia. El genoma de referencia porcino se inició en el año 2003 (Schook et al., 2005), y ha sido desde entonces mejorada hasta obtener la versión actual, Sscrofa 11.1 (Warr et al., 2020). En el último año, además, se han publicado la secuencia *de novo* de 9 razas (Meishan, Rongchang, Hampshire, Landrace, Piétrain, Bamei, Berkshire, Large White y Jinhuan) representativas de la diversidad fenotípica del cerdo euroasiático (Li et al., 2017) con el fin de hacer una descripción más precisa de la diversidad genética en esta especie.

Los SNPs son polimorfismos bialélicos muy comunes y fáciles de analizar mediante técnicas de genotipado. Inicialmente, se utilizaban protocolos basados en la PCR para secuenciar genes candidatos específicos y determinar los SNP y otros polimorfismos en esa región (Vignal et al., 2002) (Figura 8).

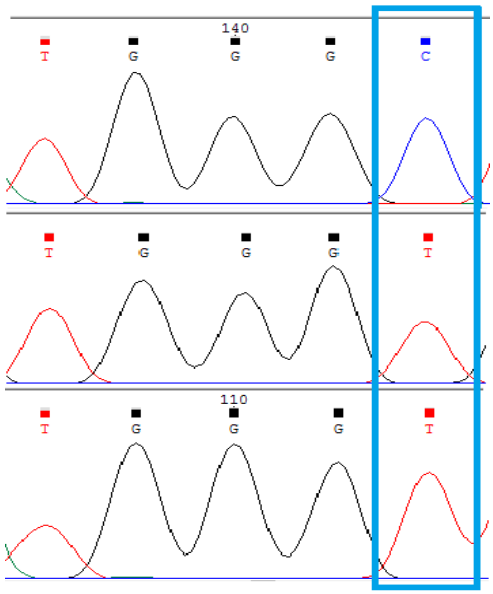

Figura 8. Detección de polimorfismos tipo SNP por alineamiento de secuencias de diferentes individuos.

Los nuevos SNP se analizaban entonces individualmente o en pequeños grupos para determinar su efecto sobre la productividad de poblaciones de cerdos. Se han estudiado miles de SNP en cerdos mediante esta aproximación, y más aún, se han identificado múltiples variantes que

contribuyen a explicar las variaciones del crecimiento y composición de la canal, y de la calidad de la carne (Tabla 4).

Tabla 4. Genes candidatos para caracteres relacionados con el crecimiento y composición de la canal, y la calidad de la carne.

	Gen candidato por carácter	**Acrónimo**	**Referencia**
Crecimiento y composición de la canal	Diacilglicerol O-aciltransferasa	*DGAT2*	Yin et al., 2012
	Hormona de crecimiento porcina	*GH*	Franco et al., 2005
	Hormona liberadora de la hormona de crecimiento	*GHRH*	Franco et al., 2005
	Factor de crecimiento insulínico-2	*IGF2*	Aslan et al., 2012
	Leptina	*LEP*	Peixoto et al., 2006
	Receptor de la hormona leptina	*LEPR*	Ovilo et al., 2005
	Receptor de la melanocortina-4	*MC4R*	Fan et al., 2009
	Miostatina	*MSTN*	Yu et al., 2007
	Fosfoenolpiruvato carboxiquinasa-1	*PCK1*	Latorre et al., 2016
	Perilipina	*PLIN*	Vykoukalová et al., 2009 Davoli et al., 2011
Calidad de la carne	Acetil-CoA carboxilasa α	*ACACA*	Muñoz et al., 2007
	Acil-CoA sintetasas de cadena larga	*ACSL4*	Mercadé et al., 2006
	Calpastatina	*CAST*	Nonneman et al., 2011
	Elongasa de ácidos grasos-6	*ELOVL6*	Corominas et al., 2013
	Proteínas ligadoras de ácidos grasos-3	*FABP3*	Gerbens et al., 1999
	Sintasa de ácidos grasos	*FASN*	Muñoz et al., 2007
	Desaturasa de ácidos grasos-2	*FADS2*	Renaville et al., 2013
	Polipéptido inhibidor gástrico	*GIP*	Muñoz et al., 2007
	Proteína de transferencia de triglicéridos microsomales	*MTTP*	Estellé et al., 2009
	Proteína quinasa AMPK-3	*PRKAG3*	Ryan et al., 2012
	Receptor de rianodina	*RYR1*	Fujii et al., 1991, Nicholas et al., 2011
	Estearoil-CoA desaturasa	*SCD*	Estany et al., 2014
	Titina	*TTN*	Wimmers et al., 2007

Un ejemplo de SNP clásico de crecimiento sería el gen receptor de la melanocortina 4, en el que se detectaron varios polimorfismos que producían cambios en la secuencia de aminoácidos de la proteína. El polimorfismo p.Arg236His está relacionado con la grasa dorsal y el crecimiento, mientras que p.Asp298Asn está asociado con la velocidad de crecimiento (Fan et al., 2009). En cambio, para la calidad de la carne, se encuentra una mutación en el gen del rendimiento *Napole* (Le Roy et al., 2000) que produce las llamadas carnes acidas, a causa de un mayor descenso del pH de la carne. Este fenómeno se ha observado sobre todo en la raza Hampshire y es debido a una

mutación *missense* en el gen *PRKAG3* (Scheffler y Gerrard, 2016). El gen del receptor de rianodina es un ejemplo claro de cómo los programas de selección han ido utilizando la información molecular para eliminar una mutación que producía carnes de baja calidad (blandas, pálidas y exudativas; en inglés PSE) y pérdidas económicas al final de la cadena de producción. Este gen está relacionado con la hipertermia maligna o síndrome del estrés porcino. En el cerdo, esta enfermedad hereditaria está causada por una mutación tipo SNP que produce un cambio de aminoácido (arginina → cistina). A raíz de este cambio, en situaciones de estrés, los cerdos padecen temblores musculares e hipertermia, rigidez muscular y acidosis metabólica, produciendo como resultado una carne PSE (Fujii et al., 1991; Nicholas et al., 2011).

Posteriormente, el desarrollo de las herramientas de genotipado masivo para miles de SNP han permitido emprender estudios de asociación a nivel genómico (GWAS, de las siglas en inglés). El GWAS permite asociar los fenotipos de interés con marcadores SNP representativos de todo el genoma (Goddard y Hayes, 2009; Bush y Moore, 2012). La disponibilidad de paneles de SNP de alta densidad en las especies domésticas ha facilitado la búsqueda de genes candidatos para los caracteres de los que se disponía de fenotipo. Estos paneles de marcadores se presentan mediante microarrays (también denominados microchips) de genotipado, que incluyen entre 10.000 y hasta 1.000.000 SNP en el caso de humanos.

La empresa *Illumina* fue de las primeras en comercializar chips de genotipado para porcino (Ramos et al., 2009). Actualmente ofrece un panel de 60K SNP con la tecnología BeadArray (PorcineSNP60 BeadChip v2). Más allá de este panel de alta densidad, se han propuesto paneles de SNP con baja densidad para reducir los costos de genotipado. La compañía *GeneSeek/Neogen* desarrolló así un panel de baja densidad denominado GGP Porcine LD Array que incluye 10.000 marcadores con una distancia media entre ellos de 250 kb. Además de esto, la empresa introdujo los HD SNP BeadChips, con mayor densidad (alrededor de 70.000 marcadores), incluyendo la mayoría del panel de *Illumina*. Por último, están también disponibles en el mercado paneles que contienen alrededor de 650K marcadores, que son comercializados por *Affymetrix* con la tecnología Axiom (Axiom_PigHDv1). Estos paneles incluyen todos los marcadores del panel *Illumina* y ha sido concebido para incluir también marcadores específicos de razas locales europeas y asiáticas.

En cerdos, los primeros resultados de GWAS fueron publicados por Duijvesteijn et al., (2010), donde detectaron 37 SNP en los cromosomas 1 y 6 que afectaban a los niveles de androsterona del tejido graso. A partir de ese momento, los estudios con GWAS para desvelar genes candidatos se han ido extendiendo hacia otras áreas como la calidad de la carne. Varios estudios de GWAS han corroborado el efecto del gen *SCD* sobre la calidad de la carne (Yang et al., 2013; Ros-Freixedes et al., 2016; Zhang et al., 2016a; Zhang et al., 2016b) pues el *SCD* es un gen candidato para la composición de grasa, dado que produce un incremento del ácido oleico en cerdos Duroc (Estany et al., 2014). Adicionalmente, Ros-Freixedes et al., (2016) describió la asociación entre los marcadores *SCD* y *LEPR* sobre el contenido de GIM y composición de ácidos grasos. De forma más reciente, en cerdos Duroc se detectaron 28 SNP significativos en 10 nuevos genes candidatos relacionados con la GIM. En su conjunto, estos estudios contribuyen a la

inclusión de un proceso de selección por marcadores en los programas de mejora (Ding et al., 2019).

Por último, la información genotípica de los chips de genotipado masivo también puede ser utilizada en las estrategias de selección genómica, propuesta en 2001 por Meuwissen y colaboradores. Esta metodología tiene por objetivo predecir el valor genético de los animales a partir de los genotipos de miles de marcadores. Los marcadores utilizados cubren todo el genoma sin necesidad de detectar ningún QTL previamente, pero asume que los marcadores se encuentran en desequilibro de ligamiento con los QTL (Meuwissen et al., 2001). Para iniciar la selección genómica, la población de referencia se fenotipa y se genotipa a fin de estimar los efectos de los marcadores en los individuos. Luego, esta información se utiliza para predecir el valor de mejora de los animales genotipados. Esta metodología permite un mayor progreso genético anual como ha sido demostrado en vacuno de leche (VanRaden, 2020). La contribución más importante en el caso del porcino es en mejorar la precisión de los índices de selección, particularmente entre hermanos (Mulder, 2016).

4.3. Otros enfoques biotecnológicos

Los avances en genética molecular actuales permiten identificar, comprender y cuantificar la expresión de genes y redes génicas responsables de los rasgos de la calidad de la carne y la canal, influyendo el mínimo posible en otros caracteres (Ciobanu et al., 2011). La obtención de la secuencia completa del genoma porcino ha resultado ser un recurso inmejorable para profundizar en la estructura genética de caracteres complejos, desarrollar y realizar estudios en el transcriptoma de esta especie e identificar regiones cromosómicas y genes relacionadas con fenotipos productivos.

Primeramente, se desarrolló la tecnología microarrays que consiste en un chip que permite estudiar simultáneamente la expresión de múltiples genes conocidos. El primer microarray comercial de cerdos fue el *Operon Porcine AROS v1.0* (2003), con 10.665 sondas, y que evolucionó incrementando en número de sondas para obtener una mayor cobertura del transcriptoma, como, por ejemplo, el *Affymetrix Porcine Snowball Array* (2013), con 47.845 sondas (Pena et al., 2014). Sin embargo, debido a las limitaciones de los microarrays, como son la falta de reproducibilidad de los datos y la difícil interpretación de los resultados (Sedighi & Li, 2014), unido a la reducción de costes de las técnicas de secuenciación masiva, los microarrays han sido ampliamente substituidos por metodologías basadas en la secuenciación de nueva generación [NGS].

El desarrollo de métodos de alto rendimiento para la secuenciación del ADN ha proporcionado nuevas maneras de mapear, anotar y cuantificar el transcriptoma, además de descubrir nuevas mutaciones y/o variantes comunes asociadas a un fenotipo. Las principales limitaciones iniciales de las metodologías NGS, como eran la falta de *pipelines* bioinformáticos consensuados, los problemas de almacenamiento, análisis e interpretación de los datos y su elevado coste (Horner et al., 2009; El-Metwally et al., 2013; Kulski, 2016) han sido en parte superados en los últimos años por el trabajo conjunto de la comunidad científica y los avances en la capacidad de secuenciación y la calidad de secuencia de las nuevas plataformas (Kumar et al., 2019).

La tecnología RNA-Seq permite caracterizar el transcriptoma del individuo con mayor precisión y mejor información en comparación con la tecnología microarray. El método RNA-Seq permite la evaluación de los genes diferencialmente expresados [DEG] entre grupos en condiciones diferentes e identificar secuencias variantes de genes en regiones transcritas (isoformas) (Wang, 2009). El análisis RNA-Seq consta de tres fases: (a) la fragmentación del ARN y secuenciación de los fragmentos, (b) la cuantificación de los niveles de expresión de los genes y tránscritos, (c) y la identificación funcional de los genes (Figura 9). En primer lugar, se procede a la extracción del ARN de las muestras y fragmentarlas para poder obtener una librería de fragmentos con adaptadores que permiten realizar la secuenciación. Estas librerías son secuenciadas mediante diversas plataformas de secuenciación masiva (p. ej., las desarrolladas por *Ilumina* o *Ion-Torrent*). Cada una de ellas requiere de una librería específica. En segundo lugar, las lecturas obtenidas por secuenciación son alineadas y mapeadas frente al genoma de referencia. En este paso, con herramientas bioinformáticas se generan unos archivos que indican la posición de cada lectura dentro del genoma (cromosoma, gen y tránscrito correspondiente) y la calidad de la lectura. Posteriormente, mediante programas bioinformáticos (p. ej., DESeq2) se cuantifica el número de lecturas por cada gen y tránscrito, lo que permite saber la expresión diferencial [DE] que hay entre muestras o grupos. Una vez calculada la DE, se procede al análisis de todos los datos generados e identificar los DEG para hacer al análisis funcional de esos genes (Hung y Weng, 2017; Ji y Sadreyev, 2018).

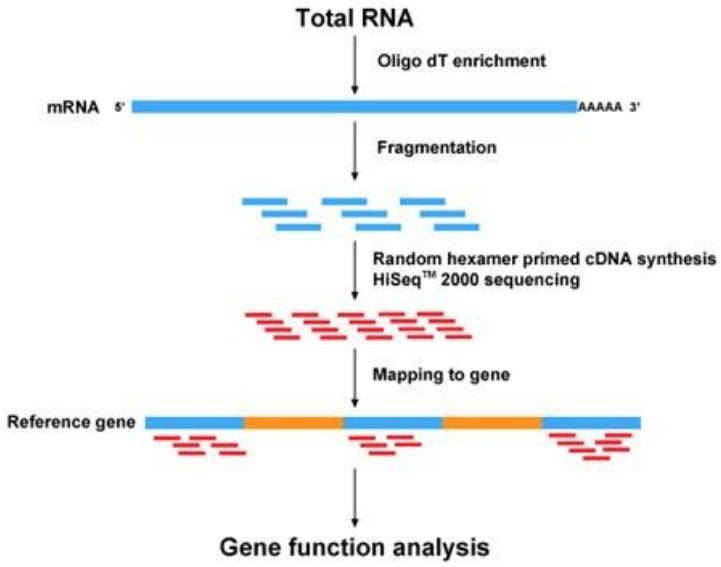

Figura 9. Representación del flujo de trabajo del análisis RNA-seq. Fuente: bgisequence.com.

Por otra parte, las técnicas DNA-seq tienen la capacidad de secuenciar gran cantidad de fragmentos de ADN de forma paralela lo cual permite detectar la heterogeneidad genética y/o detectar variantes de un gen concreto (Rubio et al., 2020). Su metodología es muy similar al RNA-seq y se basa en (a) la fragmentación del ADN y creación de librerías, (b) secuenciación de los fragmentos y (c) reconstrucción de la secuencia. Con las secuencias alineadas se procede a realizar la identificación de variantes (*variant calling*) mediante programas bioinformáticos (p. ej., GATK)

y posteriormente se procede a la priorización funcional de las variantes y el análisis de asociación con el fin de identificar los genes de interés.

Actualmente, las plataformas de secuenciación de *Ilumina* es la tecnología más utilizada. Ésta se basa en el uso de nucleótidos marcados fluorescentemente que, al unirse durante la secuenciación, generan una señal fluorescente que es captada por un escáner de alta resolución. La tecnología *Ilumina* genera fragmentos cortos (~150 nt) de secuencias que son ensamblados en tránscritos completos en el caso de las aproximaciones RNA-seq. Actualmente están en auge nuevas plataformas que secuencian los tránscritos enteros, sin fragmentar, generando secuencias largas e incluso la secuenciación directa del ARN sin requerir un paso de amplificación por PCR para introducir los adaptadores (Figura 10).

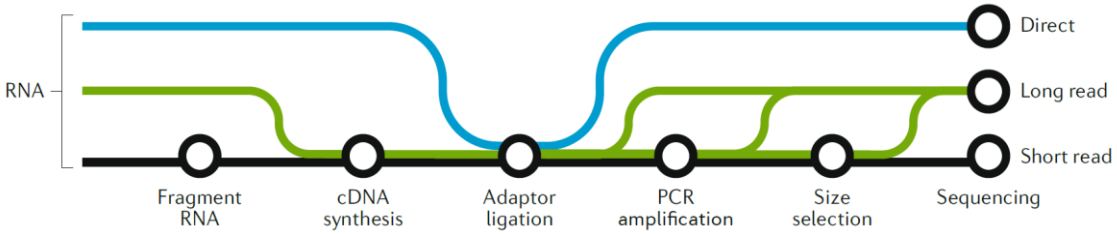

Figura 10. Comparación de los pasos requeridos en la preparación de librerías para metodologías de RNA-seq basado en secuencias cortas, largas o en la secuenciación directa del ARN. Fuente: Stark et al., 2019.

Las metodologías NGS se han aplicado de forma extensa en la especie porcina gracias a que se dispone de un genoma de referencia. Existen aplicaciones al estudio de la base genética relacionada con los caracteres productivos, reproductivos y de calidad de la carne, con el fin de detectar marcadores genéticos y mejorar la comprensión de las funciones de los genes (Samborski et al., 2013; Martínez-Montes et al., 2017; Gòdia et al., 2018; Piórkowska et al., 2018a; Piórkowska et al., 2018b). Asimismo, esta estrategia también ha sido utilizada para identificar genes responsables de enfermedades de interés productivo y comprender mejor su fisiopatología, como es el caso del síndrome reproductivo y respiratorio porcino (Lim et al., 2020; Dong et al., 2021).

La aplicación de estas metodologías tiene por finalidad encontrar marcadores genéticos que puedan ser útiles tanto para la selección de reproductores en los núcleos como para mejorar la comprensión de los procesos biológicos subyacentes a los caracteres de interés en porcino.

5. Antecedentes

Esta tesis doctoral se enmarca en una línea de investigación del *Grup de Millora Animal* de la Universidad de Lleida-Agrotecnio. La principal línea de estudio es la mejora genética de la calidad de la carne de cerdo. En el marco de esta línea de investigación, se han identificado diversos marcadores genéticos en cerdos de raza Duroc con efectos sobre el contenido y composición de la grasa (Estany et al., 2014; Ros-Freixedes et al., 2016; Gol et al., 2018). Dos de ellos ya se están utilizando rutinariamente en selección. El primero de ellos se localiza en el gen *SCD* (rs80912566 T>C) y afecta principalmente al contenido en ácidos grasos monoinsaturados, mientras que el segundo, que se encuentra en el gen *LEPR* (rs709596309 C>T), se asocia tanto con el contenido

graso de la canal, incluida la grasa intramuscular, como con la composición, aumentando el porcentaje de ácidos grasos saturados y disminuyendo el de ácidos grasos poliinsaturados. No obstante, se desconoce el efecto modulador que pueda ejercer la alimentación, en particular la vitamina A, sobre estos marcadores, así como los efectos colaterales que estos dos marcadores puedan tener en los caracteres reproductivos. Las investigaciones realizadas en esta tesis pretenden contribuir a resolver ambas cuestiones, a la vez que continúan la búsqueda de nuevos marcadores que ayuden a comprender mejor el metabolismo de deposición de la grasa y, en último término, a mejorar la calidad de la carne.

Los trabajos que se presentan en la tesis se engloban dentro del marco de los proyectos AGL2015-65846-R y RTI2018-101346-B-I00 financiados por el Ministerio de Ciencia e Innovación.

II- Objetivos

El objetivo principal de esta tesis es profundizar en el estudio de la regulación genética de la deposición de la grasa en porcino y de su relación con la capacidad materna de las cerdas. Para lograr este cometido se han planteado los siguientes cuatro objetivos específicos:

1. Investigar la existencia de variantes en la secuencia del gen diacilglicerol O-aciltransferasa-2 que puedan estar asociadas al contenido y la composición de la grasa (capítulo I).

2. Examinar la modulación que ejerce la vitamina A de la dieta sobre el efecto del gen de la estearoil-CoA desaturasa en el contenido y la composición de la grasa intramuscular (capítulo II).

3. Determinar si el gen del receptor de la leptina causa un efecto materno antagónico al directo (capítulo III).

4. Analizar los efectos del gen receptor de la leptina y del gen estearoil-CoA desaturasa sobre el rendimiento reproductivo de las cerdas (capítulos III y capítulo IV).

III- Material y métodos

1. Animales y registros

Los cerdos utilizados y muestreados en la presente tesis fueron criados y sacrificados en condiciones comerciales siguiendo las normativas y directrices de buenas prácticas de manejo, bienestar y protección animal para la ganadería, durante el transporte y sacrificio (Real Decreto 37/2014, España). De acuerdo con la directiva europea 2010/63/UE, el muestreo de los animales sacrificados no experimentales, siguiendo los procedimientos legales, no está dentro del alcance de la regulación europea sobre experimentación animal y no requiere autorización de proyecto específico. Los procedimientos fueron aprobados por el comité de ética de experimentación animal de la Universidad de Lleida (según el acuerdo de la CEEA 05/04 del 2015).

Los cerdos utilizados en el presente trabajo provinieron de una de las líneas Duroc de la empresa Selección Batallé (Riudarenes, Girona, España). En los experimentos de los capítulos I y IV se usaron 1.129 cerdos castrados, que se criaron en 15 lotes entre los años 2002 a 2017, mientras que en el experimento del capítulo II se utilizaron 108 machos castrados, que se engordaron en dos lotes durante los años 2015 y 2016, respectivamente. Además, durante los años 2017 y 2018 se criaron en 12 lotes un total de 413 cerdos. Todos estos cerdos fueron criados en condiciones comerciales y alimentados *ad libitum* hasta que se sacrificaron en torno a las 31 semanas de vida en un matadero comercial equipado con el sistema de aturdimiento de dióxido de carbono antes del sacrificio. En todos los experimentos se registró el peso de la canal y el espesor de grasa dorsal, que se midieron a 6 cm de la línea media entre la tercera y cuarta costilla mediante el equipo ultrasónico AutoFOM (SFK-Technology, Dinamarca). Después del sacrificio, se tomó una muestra de sangre y del músculo *gluteus medius* [GM], y, según el experimento, también de los músculos *longissimus* [LM] y *semimembranosus* [SM], así como de grasa subcutánea. Según el tipo de análisis, estas muestras se congelaron al momento y se conservaron a -80ºC o bien, una vez envasadas al vacío en el caso de las piezas grandes de músculo, se almacenaron a -20ºC.

En los capítulos III y IV se controlaron durante tres años y medio (2015-2019) un total de 1.246 cerdas (4.121 partos). Todas las cerdas se reprodujeron en la misma granja bajo condiciones estándar de manejo. De cada parto de estas cerdas se registró el número de lechones nacidos totales, nacidos vivos, nacidos muertos y destetados, además de la edad al parto y el peso de los lechones al destete. A 112 de estas cerdas, todas primerizas, se les extrajo una muestra de leche durante la primera semana de lactación tras inyección de oxitocina por vía intramuscular (20 UI de Hormonipra®, Hipra, España). Las muestras de leche fueron almacenadas a -40 ºC hasta su análisis. Para la detección en sangre de triglicéridos y NEFA se realizó la extracción de la sangre mediante venopunción a la yugular de 107 y 150 cerdos, respectivamente. El plasma fue separado mediante centrifugación y las muestras fueron almacenadas a -80 ºC hasta su análisis.

2. Determinación de la composición de ácidos grasos

El contenido de grasa intramuscular y la composición de ácidos grasos (Tabla 1) en el músculo se determinaron por duplicado a través de una alícuota representativa de una muestra liofilizada pulverizada mediante cromatografía de gases de acuerdo con Bosch et al., (2009). Los

esteres metílicos de ácidos grasos se obtuvieron por transesterificación usando una solución de trifluoruro de boro al 20% en metanol (Rule, 1997) y se determinó mediante cromatografía de gases usando una columna capilar SP2330 (30 m de 0,25 mm; Supelco Inc., Bellefonte, PA, USA) y un detector de ionización de llama con helio como gas portador. La presión de la columna utilizada fue de 172 kPa de forma constante y el programa utilizó un incremento de temperatura de 150 a 225 ºC a 7 ºC / min y las temperaturas del inyector y del detector fueron de 250 ºC.

La determinación del contenido de materia grasa de la leche se realizó por gravimetría siguiendo una modificación del método de Hara y Radin (1978) adaptado por Feng y Garnsworthy (2004). Todas las muestras se determinaron por duplicado y previamente la leche fue homogeneizada a 37 ºC/100 rpm durante 5 minutos. La extracción de la porción grasa se realizó mediante una solución de hexano:isopropanol (3:2 vol/vol) y sulfato de sodio (12%). Para determinar el contenido de lípidos se extrajo el hexano de la muestra en un rotoevaporador (30 min a 60 ºC) seguido de una fase de secado (60 min a 68 ºC) hasta peso constante. Para determinar la composición de ácidos grasos de la leche, se resuspendió el contenido de lípidos obtenidos previamente con una solución de trifluoruro de boro al 20% en metanol para obtener los ésteres metílicos de ácidos grasos por transesterificación. El análisis de los ésteres metílicos de ácidos grasos se realizó mediante cromatografía de gases con una columna capilar DB-23 PN (30 m x 0,25 mm, Agilent Technologies, Santa Clara, C, USA) y un detector de ionización de llama con helio. Las condiciones fueron las mismas que en el músculo.

Se procesaron los resultados obtenidos de músculo y leche con el programa Instrument Offline (Agilent ChemStation, Santa Clara, C, USA). La cuantificación se realizó mediante la normalización del área después de agregar en cada muestra un patrón interno, el 1,2,3-Tripentadecanoilglicerol en músculo y Tripentadecanoína en leche. La cantidad de cada ácido graso es expresado en valor absoluto (mg/g materia seca) y en porcentaje respecto al total de ácidos grasos. El contenido de grasa se calculó como la suma de los ácidos grasos expresados como triglicéridos equivalentes en tejido húmedo y seco.

Tabla 1. Perfil de ácidos grasos saturados, monoinsaturados y poliinsaturados determinados mediante cromatografía de gases (símbolo y nombre común).

Ácidos grasos saturados	
C14:0	Ácido mirístico
C16:0	Ácido palmítico
C18:0	Ácido esteárico
C20:0	Ácido araquídico

Ácidos grasos monoinsaturados	
C16:1(n-7)	Ácido palmitoleico
C18:1(n-7)	Ácido vaccénico
C18:1(n-9)	Ácido oleico
C20:1(n-9)	Ácido eicosanoico

Ácidos grasos poliinsaturados	
C18:2(n-6)	Ácido linoleico
C18:3(n-3)	Ácido α-linolénico
C20:2(n-6)	Ácido eicosadienoico
C20:3(n-6)	Ácido dihomo-gamma-linolénico
C20:4(n-6)	Ácido araquidónico

3. Extracción de ADN y genotipado

El ADN fue extraído de las muestras de músculo almacenadas a -80ºC usando un protocolo estándar de extracción de ADN (Green y Sambrook, 2017). Las muestras fueron lisadas en una solución de lisis con proteinasa K y el ADN fue purificado mediante una extracción de fenol: cloroformo, seguido de la precipitación del ADN con etanol. Finalmente, el ADN fue re-suspendido y almacenado con buffer TE. El ADN de la leche fue extraído mediante una resina de Chelex y proteinasa K basado en el protocolo de Amills (1997). La cuantificación y estimación de la calidad y la pureza del ADN genómico se realizó mediante un espectrofotómetro NanoDrop N-1000 (Thermo Fisher Scientific, Waltham, MA, USA), considerando una ratio A260/A180 de 1,8-2 como aceptable. La integridad fue testada por electroforesis en geles de agarosa al 1,2%.

Todas las muestras de ADN fueron genotipadas para los polimorfismos del gen *SCD* rs80912566 T>C en el cromosoma 14 y el gen *LEPR* rs709596309 C>T en el cromosoma 6. Además, se genotipó para el gen *DGAT2* ss7315407085 G>A en el cromosoma 9 (capítulo I).

El genotipo para los SNPs de *SCD, LEPR* y *DGAT2* fue determinado mediante un protocolo de High Resolution Melt (Luminaris Color HRM Master Mix, Thermo Fisher, Waltham, MA, USA) en un termociclador a tiempo real (QuantStudio 3 qPCR, Life Technologies, Waltham, MA, USA) usando 20 ng de ADN y 0,4 µM de cada *primer* en un volumen final de 5 µl (Tabla 2).

Las condiciones del termociclador fueron de 50 ºC durante 2 min / 95 ºC durante 10 min, 40 ciclos de 95 ºC durante 15 seg y 60 ºC durante 1 min, seguido de una curva de melting; 95 °C durante 15 seg, 60 °C durante 1 min, seguido de una rampa de 0,015 °C / seg de 60 hasta 95 °C. Para el análisis y genotipado se utilizó el software High Resolution Melt v3.1 (Applied Biosystems, Thermo Fisher, Waltham, MA, USA).

Tabla 2. *Primers* usados para determinar el genotipo de los polimorfismos de los genes *SCD, LEPR* y *DGAT2.*

Gen	***Primer***	**Secuencia 5' → 3'**	**Tm**	**Tamaño**
SCD	Forward	CAGCGAATAAAAGGGGTCAG	59,7 ºC	78 bp
	Reverse	CCAGGCTGGGTATTTAAAGG	58,6 ºC	
LEPR	Forward	CAGAGGACCTGAATTTTGGAG	58,8 ºC	94 bp
	Reverse	CATAAAAATCAGAAATACCTTCCAG	57,5 ºC	
DGAT2	Forward	TCAACCAGCACAAGACCAAG	59,9 ºC	58 bp
	Reverse	CAGTTCACCTCCAGGACCTC	59,7 ºC	

4. Extracción de ARN y expresión de genes

El ARN se aisló de muestras de músculo semimembranoso almacenadas a -80ºC con el reactivo TRI (Invitrogen, Carlsbad, C, USA) de acuerdo con el protocolo del fabricante, seguido de la precipitación del ARN con etanol. El ARN fue re-suspendido y almacenado con agua milli-

q. La pureza se evaluó mediante espectrofotometría con un NanoDrop N-1000 (Thermo Fisher Scientific, Waltham, MA, USA) y la cuantificación mediante un fluorímetro Qubit 3.0 (Thermo Fisher, Waltham, MA, USA). Además, la integridad fue testada mediante un gel de agarosa:formaldehido.

Para la transcripción inversa se usaron 1,5 µg de ARN total utilizando la enzima transcriptasa inversa SuperScript IV (Invitrogen, Carlsbad, C, USA) con 100 µM *random hexamers* a 23 ºC durante 10 minutos, 50 ºC durante 20 minutos y 80 ºC durante 10 minutos. El cADN se diluyó 1:30 en agua milli-Q.

La expresión del *DGAT2* se midió por PCR cuantitativa a tiempo real (qPCR) y se utilizaron dos genes de referencia (*B2M* y *RPL32*) para cuantificar y normalizar los datos de expresión (Tabla 3). El análisis de qPCR (QuantStudio 3 qPCR, Life Technologies, Waltham, MA, USA) se llevó a cabo por triplicado utilizando SYBR Green Supermix (Bio-Rad, Hercules, C, USA), 2 µM de cada *primer* y 3 µl de cADN diluido a 1:30 en un volumen final de 8 µl. Los parámetros de ciclo fueron de 50 ºC durante 2 min / 95 ºC durante 10 min, 40 ciclos de 95 ºC durante 15 seg y 60 ºC durante 1 min, seguido de una curva de melting. Para cuantificar y normalizar los datos de expresión se utilizó el método ΔΔCt contra la media geométrica de los dos genes de referencia.

Tabla 3. *Primers* usados para analizar la expresión de *DGAT2*.

Gen	*Primer*	Secuencia 5' → 3'	Tm	Tamaño
B2M	Forward	TCGGGCTGCTCTCACTGTCT	62,0 ºC	69 bp
	Reverse	GGCGTGAGTAAACCTGAACCTT	62,1 ºC	
RPL32	Forward	CACCAGTCAGACCGATATGTCAA	62,2 ºC	70 bp
	Reverse	CGCACCCTGTTGTCAATGC	63,7 ºC	
DGAT2	Forward	AGGACATTGACCTCTACCATGC	60,4 ºC	109 bp
	Reverse	CAGTTCACCTCCAGGACCTC	59,7 ºC	

5. Análisis estadísticos

El efecto de cada uno de los polimorfismos estudiados (*DGAT2, LEPR, SCD*), de la vitamina A de la dieta y de su interacción sobre los caracteres estudiados se realizó mediante un modelo lineal en el que se tuvo en cuenta la información familiar de la generación anterior. En los capítulos I y II los efectos se contrastaron mediante una prueba F y la diferencia entre los niveles de cada uno de ellos mediante la prueba de Tukey-HSD. El nivel de significación estadística se estableció en $P<0,05$. Estos análisis se realizaron utilizando el paquete estadístico JMP Pro14/15 (SAS Institute Inc, Cary, NC). En los capítulos III y IV los modelos se resolvieron en un entorno bayesiano, por lo que los efectos se contrastaron a partir de las probabilidades marginales posteriores. Se consideró que había una fuerte evidencia estadística de que dos genotipos producían un efecto diferente sobre un carácter cuando la probabilidad de que la diferencia entre el efecto de esos dos genotipos fuera mayor (o menor) que cero era de al menos el 95%. Estos análisis se hicieron con el programa TM (Legarra et al., 2008; http://genoweb.toulouse.inra.fr /~alegarra/tm_folder [deposited: 3 August 2011]).

IV- Capítulo I

Una variante en la secuencia del gen Diacilglicerol O-Aciltransferasa 2 (*DGAT2*) afecta al contenido de ácido palmitoleico en el músculo de cerdo

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A sequence variant in the diacylglycerol O-acyltransferase 2 gene influences palmitoleic acid content in pig muscle

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1. Introduction

Meat fat content and composition are important attributes that contribute to the nutritional quality and the consumer perception of meat products. Total fat dietary intake, particularly intake of saturated fatty acid [SFA], has been associated with obesity, circulating LDL-cholesterol and increased risk of coronary heart disease [1]. In line with this, public health recommendations advocate for low-fat diets and dietary substitution of SFA with monounsaturated fatty acids [MUFA] and polyunsaturated fatty acids [PUFA] [2-5]. On the other hand, although most consumers already prefer leaner meat products [6], intramuscular fat [IMF] content and fatty acid composition exert a great influence on meat organoleptic and technological properties [7, 8]. Increasing the knowledge of the genetic basis of the regulation of fat metabolism is key to improve the quality of meat products.

Triacylglycerols [TG] are the main stores of metabolic energy, serving as a reservoir of essential and non-essential fatty acids, and the precursors of phospholipids. In mammals, TG are primarily synthesized in the liver, intestine and adipose tissue [9]. Diacylglycerol O-acyltransferase [*DGAT1* and *DGAT2*] genes are involved in intestinal fat absorption and play a critical role in the synthesis of TG in the adipocytes [10, 11]. In particular, *DGAT1* and *DGAT2* encode membrane proteins that catalyse the formation of the ester bond between the hydroxyl group of 1,2-diacylglycerol and a long-chain fatty acyl-CoA (Fig. 1) [9, 12, 13], the final step in the production of TG in mammals [12 - 15]. Although DGAT1 and DGAT2 have similar functions in the adipocytes, DGAT2 has been found to be more critical in mice, where it is essential for post-natal survival and can as well compensate for disruptions of DGAT1 [16–18]. Recent findings indicate that DGAT2 is specifically engaged in *de novo* lipogenesis by favouring both the synthesis of TG from glycerol 3-phosphate and the incorporation of endogenous fatty acids into TG, particularly the MUFA that result from the desaturation of palmitate (C16:0) and stearate (C18:0) through the action of stearoyl-CoA desaturase [SCD] [19]. DGAT2 and SCD can localize in the endoplasmic reticulum membrane and this proximity may facilitate the provision of *de novo* SCD-mediated MUFA as substrate for DGAT2 to form TG.

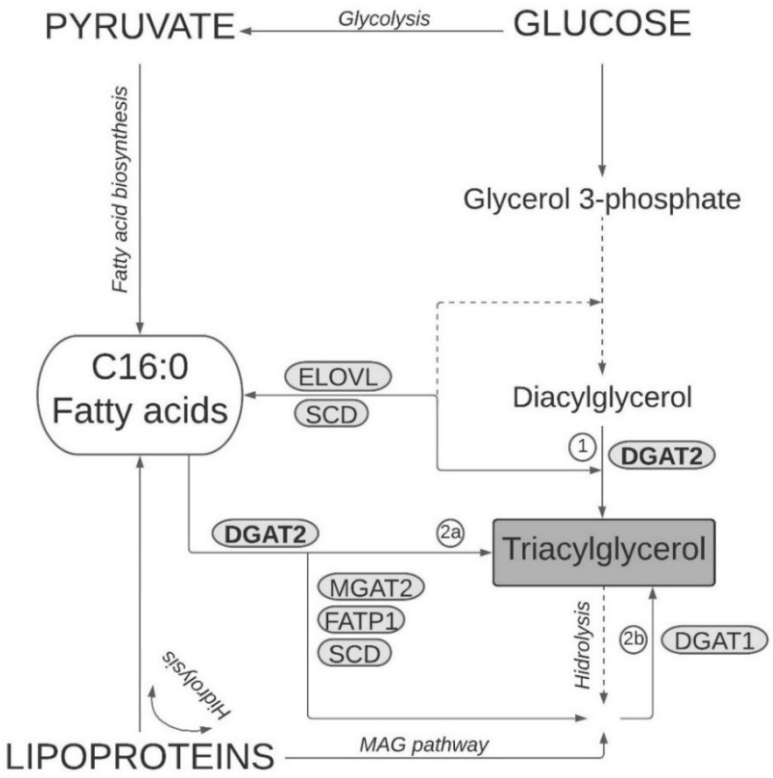

Figure 1. The role of DGAT2 in the synthesis pathway of triacylglycerol. Triacylglycerol is (1) synthesized de novo by sequential addition of fatty acyl moieties to a glycerol 3-phosphate (the G3P pathway) or (2) formed through the re-esterification of partial glycerides that results from either the hidrolysis of pre-existing triacylglycerol or via the monoacylglycerol (MAG) pathway. De novo transformation of diacylglycerol to triacylglycerol is catalyzed by DGAT2 (1) but re-esterification of partial glycerides is done by either DGAT2 (2a) or DGAT1 (2b). However, while DGAT1 specifically incorporates pre-formed fatty acids, DGAT2, which is able to form protein complexes with MGAT2, FATP1 and SCD, can use both endogenous and pre-formed fatty acids as substrates. DGAT1: diacylglycerol O-acyltransferase 1; DGAT2: diacylglycerol O-acyltransferase 2; ELOVL: fatty acid elongase; FATP1: fatty acid transport protein 1; MGAT2: monoacylglycerol acyltransferase 2; SCD: stearoyl-CoA desaturase.

Polymorphisms within *DGAT2* have the potential of affecting lipogenesis. Thus, *DGAT2* has been investigated as a candidate gene for fat-related traits such as obesity in humans [11, 20] and for lean content and muscle and backfat fatty acid composition in livestock species, particularly in pigs [14, 21, 22]. As a result of a genome-wide association study [GWAS] for fatty acid composition in Duroc pigs, we found that the region on SSC9 where *DGAT2* lies was associated with MUFA content. Here, using this Duroc line as a model, we investigate the role of *DGAT2* variants on fat metabolism in pigs. We first searched for sequence variants in the promoter, coding, and 3'-untranslated regions of porcine *DGAT2* and then validated their effect on fat content and composition using data and samples from a large biorepository [23]. Given that this line segregates for a functional variant in the *SCD* gene [24], potential interactions of *DGAT2* with *SCD* were also assessed.

2. Materials and methods

2.1 Ethics Statement

Pigs used in the study were raised and slaughtered in commercial units following applicable regulations and good practice guidelines on the protection of animals kept for farming purposes, during transport and slaughter (Royal Decree 37/2014, Spain) and tissue samples used were collected from these pigs at the slaughterhouse. In agreement with the European directive 2010/63/UE, tissue sampling from non-experimental animals slaughtered following legal procedures is not under the scope of European regulation on animal experimentation and does not require specific project authorization. The experimental protocol was approved by the Ethical Committee on Animal Experimentation of the University of Lleida. The study was carried out in compliance with the ARRIVE guidelines.

2.2 Animals and phenotypes

A total of 1,129 pigs from 158 sires and 559 dams of the same Duroc line were used in the experiment. Pigs were raised in 15 fattening batches between 2006 and 2016 following a similar protocol for data recording and tissue sampling [25]. In each batch, pigs were raised from 75 days of age until slaughter in the same farm under identical conditions. During this period, pigs had *ad libitum* access to commercial feed (Esporc, Riudarenes, Girona, Spain). At 206 (SD 8) days of age, all pigs were weighted and backfat [BT] and loin [LT] thickness were ultrasonically measured at 5 cm off the midline between the third and fourth last ribs using the portable equipment Piglog 105 (Frontmatec, Kolding, Denmark). Pigs were slaughtered at 211 (SD 9) days in a commercial slaughterhouse equipped with a carbon dioxide stunning system. Carcass composition traits were recorded, including carcass weight, BT and LT. Both BT and LT were measured at 6 cm off the midline between the third and fourth last ribs using an ultrasound automatic scanner (AutoFOM, SFK-Technology, Denmark). Immediately after slaughter, a sample of *semimembranosus* muscle (SM, n=40) and subcutaneous fat (SF, n=226) were collected, snap-frozen and stored at -80ºC. After chilling for about 24 h at 2 °C, a large sample of the muscles *gluteus medius* (GM, n=1093) and *longissimus thoracis* (LM, n=526) were collected, vacuum packaged, and stored at −20 °C until required. The IMF content in GM and LM, as well as the fatty acid composition in GM, LM and SF were determined in duplicate by quantitative gas chromatography [26]. The amount of each fatty acid was expressed as the percentage of each individual fatty acid relative to total fatty acid. The proportion of SFA (C14:0, C16:0, C18:0 and C20:0), MUFA (C16:1n-7, C18:1n-9, C18:1n-7 and C20:1n-9) and PUFA (C18:2n-6, C18:3n-3, C20:2n-6 and C20:4n-6) were calculated.

2.3 Genome-wide association study

In order to identify candidate genomic regions associated with fat-related traits, GWAS for each individual fatty acid were performed using a subset of 254 pigs. The genomic DNA of these pigs was isolated as described in [24] and used for single nucleotide polymorphism (SNP) genotyping with either the PorcineSNP60 v2 Genotyping BeadChip (n=138) or the GGP Porcine HD Array (n=116) porcine arrays (Illumina, San Diego, CA, USA). Those SNPs that displayed a

minor allele frequency below 0.10, a call rate below 0.95, or that could not be mapped to *Sus scrofa* reference genome (Sscrofa11.1) [27] were filtered out. A total of 36,000 SNPs remained after data quality control. For each trait, a GWAS was performed by fitting a linear mixed model using GEMMA [28], where phenotypes were adjusted for batch (11 levels) and IMF content as a covariate. The association of each SNP was tested using the Wald statistic considering the Bonferroni correction for multiple testing. Significance was set to a level of $P \leq 1.4 \cdot 10^{-6}$. Two regions were found to be associated with C16:1n-7, one on SSC9 and another on SSC14, the latter corresponding to a reported polymorphism in the *SCD* gene that is known to affect MUFA and particularly C16:1n-7 [24]. Candidate genes mapping within the SSC9 region were explored with Ensembl Genes Database using BioMart (https://www.ensembl.org/biomart/martview). Functional analyses like Gene Ontology and Reactome Pathway Enrichment Analysis were performed using Enrichr [29]. *DGAT2* was retrieved as the most promising candidate gene for C16:1n-7 on SSC9.

2.4 Sequence variation in *DGAT2*

Variant discovery in porcine *DGAT2* was examined by retrieving all sequence variants from the coding region, the 3'-untranslated region and 500 bp upstream on the proximal promoter of the gene (SSC9 10,031,627 to 10,068,464 bp) in a subset of 199 pigs of the same line with whole-genome sequencing data available. Sequenced pigs covered all representative sire families used in the experiment. DNA samples were submitted to Centre Nacional d'Anàlisi Genòmica (CNAG-CRG, Barcelona, Spain) for sequencing. Libraries were prepared and sequenced with paired-end reads with a NovaSeq 6000 instrument (Illumina) according to the manufacturer's protocol. Libraries were aligned to the Sscrofa11.1 [27] using the BWA-MEM algorithm [30]. The average realized sequencing coverage was 6.8x (SD=1.2x; min=4.4x; max=12.2x). Variants were identified following GATK HaplotypeCaller 3.8.0 software [31, 32]. The SNP ss7315407085 G>A in exon 9 (SNP5, 9:10,065,826; Table 1) was selected for further validation with the whole set of pigs.

2.5 Genotyping *DGAT2*

All pigs (*n*=1,129) used in the experiment were genotyped for SNP ss7315407085 in exon 9 of *DGAT2* using the primers described in Supplementary Table S1. Amplifications were performed by real-time PCR (QuantStudio3, Applied Biosystems, Thermo Scientific, Waltham, MA, USA) with High-Resolution Melt analysis (Luminaris Colour HRM Master Mix, Thermo Scientific) using 20 ng of genomic DNA and 0.4 µM of each primer in 5 µL final volume reaction. Thermocycling conditions were 50 °C 2 min, 95 °C 10 min, and 40 cycles of 95 °C 15 sec, 60 °C 1 min, followed by a high-resolution melting curve starting with a denaturation at 95 °C for 15 sec, annealing at 60 °C for 1 min and a slow ramp at 0.015 °C/sec up to 95 °C. High Resolution Melt software v3.1 (Applied Biosystems, Thermo Scientific) was used for melting data analysis and sample genotyping. All pigs were also genotyped for the *SCD* (rs80912566 T>C; on SSC14) and leptin receptor (rs709596309 C>T; on SSC6) SNPs following the protocols described in [24] and [33], respectively.

2.6 *DGAT2* expression

DGAT2 expression was measured by quantitative real-time PCR [qPCR] in the SM of 40 pigs from a single batch. RNA was isolated with TRI-Reagent according to the manufacturer's protocol and purity was assessed by spectrophotometry with a Nanodrop-1000. Total RNA (1.5 µg) was reverse-transcribed using SuperScript IV Reverse Transcriptase (Invitrogen, Thermo Scientific) with 100 µM random hexamers at 23 ºC for 10 min, 50 ºC for 20 min and 80 ºC for 10 min. The cDNA was diluted 1:30 in water. Real-time PCR assays were carried out in triplicate using SYBR Green Supermix (Bio-Rad, Hercules, CA, USA), 0.2 µM of each primer and 3 µl of diluted cDNA. The *B2M* and *RPL32* genes were used as reference genes to quantify and normalize the *DGAT2* expression data. Primers used for *DGAT2* expression (q*DGAT2*) are given in Supplementary Table S1.

2.7 Estimation of *DGAT2* tag-SNP effects

The effect of the *DGAT2* ss7315407085 G>A SNP genotypes on production traits (body weight, carcass weight, BT and LT) and IMF content and fatty acid composition was estimated using a mixed model that included the batch (15 levels) and the genotype for *DGAT2* (GG, AG and AA), *SCD* (TT, CT and CC) and leptin receptor (TT, CT and CC) as fixed effects and the sire and the dam as random effects. The leptin receptor polymorphism was included in the model because of its impact on fat content and fatty acid composition [33]. The slaughter age, for production traits, and IMF content, for fatty acids, were added as covariates. The additive and dominant effects of the *DGAT2* SNP were estimated by replacing the genotype effect with the covariates (1, 0, -1) and (0, 1, 0) for the GG, AG and AA genotypes, respectively. Gene expression was analysed only as a function of the *DGAT2* genotype. The effects of the genotype and covariates were tested using the F-statistic. Multiple pairwise comparisons among *DGAT2* genotypes were tested with the Tukey HSD test. Results are presented as least-square means ± standard error. All the analyses were performed using the statistical package JMP Pro 14 (SAS Institute Inc., Cary, NC).

3. Results

The preliminary GWAS revealed two regions associated with C16:1n-7, one on SSC9 and another on SSC14 (Fig. 2). The region on SSC9 (9.8 Mb to 12.8 Mb) contained 18 significant SNPs ($P<1.4\cdot10^{-6}$). Candidate gene *DGAT2* mapped within this region, specifically at 10.0 to 10.1 Mb. This association was not identified in previous analyses in this Duroc line [32] and evidence for potential implications of sequence variation in *DGAT2* on fat content and composition in pigs is still very scarce [14, 21]. On the other hand, the region on SSC14 matched the variant in *SCD* described above [24]. No evidence of association was found between the genomic region of *DGAT1* (on SSC4) and fat content and fatty acid composition.

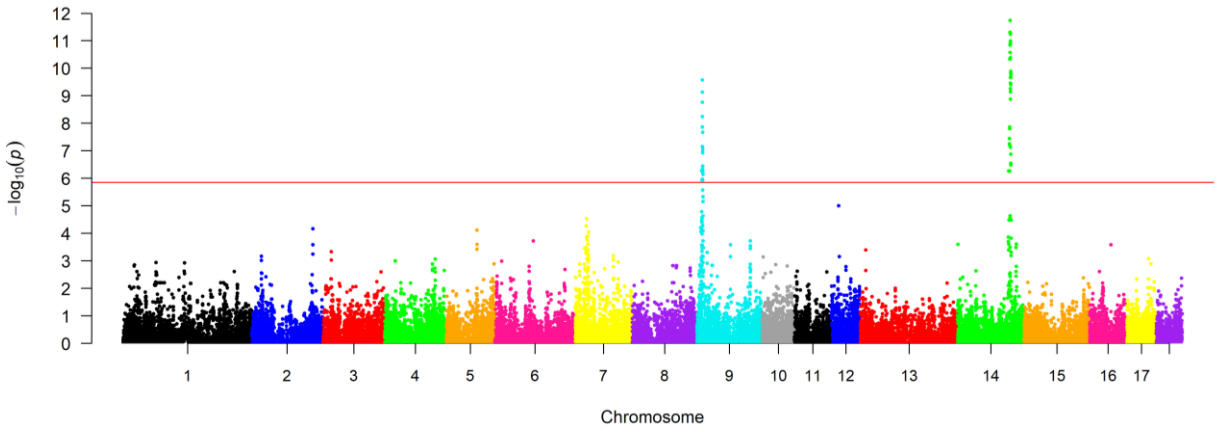

Figure 2. Genome-wide associations for palmitoleic acid content in the *gluteus medius* muscle. Different chromosomes are distinguished with different colours. The red line indicates the Bonferroni-corrected genome-wide significance threshold.

3.1 Sequence variation in *DGAT2*

Using whole-genome sequencing data from pigs of the same line, we identified a total of 33 sequence variants in *DGAT2*. Three of them were exonic, although synonymous (Table 1) and, from these, only SNP5 in exon 9 (ss7315407085) had a minor allele frequency greater than 0.25 (0.33). With 1,000 samples, which are around the current sample size of our biorepository, 0.25 is the minimum minor allele frequency required to detect ($P<0.05$) a difference of around 5% (0.5 SD) between genotypes for C16:1n-7 with a power of at least 80%. This SNP has been previously reported in other pig breeds and crossbreds [14, 21] and, in line with our results, the A allele was the minor allele in all genetic types, with a frequency that ranged from 0 to 0.32. The other 30 variants were located in the promoter region (2 SNPs) and in the 3'-UTR (21 SNPs and 7 indels). Using only the sequenced pigs, we confirmed that *DGAT2* exerted an additive influence on C16:1n-7 (Supplementary Table S2), with SNP5 and the sequence variants in the interval between INDEL13 and SNP22 presenting the most relevant associations. Linkage disequilibrium of all variants was analysed with Haploview 4.2 software (Supplementary Fig. S1), showing that SNP5 was in linkage disequilibrium with both INDEL13 ($r^2=0.51$) and SNP22 ($r^2=0.47$). In view of these results, we selected SNP5 (ss7315407085) as a tag variant of this haplotype for further validation.

Table 1. Position, alelles and location of the variants identified in the genomic region from SSC9 10,031,627 to 10,068,464 bp, which includes coding region, 3'-untranslated region and 500 bp upstream on the proximal promoter of the *DGAT2* gene.

Variant	Position (bp)	Location	*n*[1]	MAF[2]	Major/minor alleles
SNP1	10,031,901	Promoter	30	0.35	C/A
SNP2	10,032,155	Promoter	97	0.01	T/C
SNP3	10,054,197	Exon 3	183	0.09	C/T
SNP4	10,063,315	Exon 7	179	0.08	A/G
SNP5	10,065,826	Exon 9	120	0.28	G/A
SNP6	10,065,866	3'-UTR	119	0.23	G/A
SNP7	10,066,289	3'-UTR	138	0.49	G/C
SNP8	10,066,329	3'-UTR	142	0.30	C/T

SNP9	10,066,334	3'-UTR	128	0.45	C/T
INDEL10	10,066,364	3'-UTR	141	0.20	CA/C
SNP11	10,066,666	3'-UTR	113	0.18	G/A
INDEL12	10,066,708	3'-UTR	79	0.08	T/TC
INDEL13	10,066,711	3'-UTR	118	0.33	C/CA
INDEL14	10,066,728	3'-UTR	136	0.26	GGACCTGCTCTTCT/G
SNP15	10,066,750	3'-UTR	127	0.44	C/G
SNP16	10,066,770	3'-UTR	141	0.49	T/A
SNP17	10,066,775	3'-UTR	147	0.41	T/C
SNP18	10,066,885	3'-UTR	158	0.35	T/C
SNP19	10,066,886	3'-UTR	158	0.35	G/A
INDEL20	10,066,928	3'-UTR	157	0.36	CGA/C
SNP21	10,067,012	3'-UTR	156	0.34	A/G
SNP22	10,067,013	3'-UTR	155	0.31	C/T
SNP23	10,067,104	3'-UTR	159	0.03	G/A
INDEL24	10,067,277	3'-UTR	156	0.01	GC/G
SNP25	10,067,474	3'-UTR	144	0.50	A/G
SNP26	10,067,498	3'-UTR	153	0.38	C/A
SNP27	10,067,745	3'-UTR	152	0.28	T/G
SNP28	10,067,850	3'-UTR	158	0.02	G/A
SNP29	10,067,921	3'-UTR	157	0.28	A/G
SNP30	10,068,080	3'-UTR	156	0.31	T/C
SNP31	10,068,240	3'-UTR	156	0.35	A/G
SNP32	10,068,423	3'-UTR	116	0.41	T/C
INDEL33	10,068,438	3'-UTR	96	0.09	CA/C

[1] *n*: Number of animals genotyped

[2] MAF: Minor allele frequency

3.2 Validation of *DGAT2* tag SNP

The expression of *DGAT2* by ss7315407085 genotype is shown in Fig. 3. Despite the limited sample size, the results obtained indicate that the *DGAT2*-G show a favourable additive effect as compared to *DGAT2*-A on *DGAT2* mRNA expression in muscle (+0.65 ± 0.27, P<0.05). The *DGAT2*-GG pigs displayed around 1.5-fold increase in *DGAT2* expression compared to the *DGAT2*-AA pigs (+1.48 ± 0.58, P<0.05). Gene expression was measured in SM because it was the only muscle that could be sampled immediately after slaughter.

The effect of the *DGAT2* SNP on fatty acid composition was validated in GM (Table 2) and LM (Table 3) using the whole set of pigs in the experiment. In line with preliminary association results in Supplementary Table S2, the *DGAT2*-G allele exerted a positive additive effect on C16:1n-7 in both muscles (+0.12% ± 0.03, in GM, and +0.19% ± 0.03, in LM, P<0.0001). The effect of the *DGAT2*-G allele had also a positive impact on C14:0 (+0.04% ± 0.01, in GM, and +0.05% ± 0.01, in LM, P<0.01), C16:0 (+0.12% ± 0.06, in GM, P=0.05) and C18:1n-7 (+0.06% ± 0.02, in GM, P=0.01). However, the effect of the *DGAT2*-G allele was proportionally greater in

C16:1n-7 than in C16:0, as evidenced by subsequent changes in the C16:1n-7 to C16:0 ratio (+0.04 ± 0.01, in GM, and +0.07 ± 0.01, in LM, P<0.01, values x 10). In contrast, the *DGAT2*-G allele affected negatively C18:0 and C18:1n-9, particularly in LM (-0.13% ± 0.06, P< 0.05, and -0.40% ± 0.15, P<0.01, respectively).

The *DGAT2* SNP did not show dominance effects nor evidence for interaction with the *SCD* genotype for any of the fatty acids. The additive behaviour of the *DGAT2*-G allele was mantained across all *SCD* genotypes (Fig. 4). Thus, the maximum difference in C16:1n-7, which accounted for around 25% of the mean, occurred between the two extreme *DGAT2*/*SCD* haplotypes (from 4.05% ± 0.05, for GG/TT, to 3.23% ± 0.08, for AA/CC, P<0.0001). Despite this, the difference between *DGAT2*-GG and AA genotypes was greater for the *SCD*-CC genotype (+0.32% ± 0.09, P<0.001) than for the *SCD*-TT genotype (+0.15% ± 0.10, P= 0.13).

We did not find evidence of consistent effects of *DGAT2* on body weight or carcass fat content (Table 4) or on the fatty acid composition of SF (Supplementary Table S3) or liver (Supplementary Table S4). Only a minor effect on live body weight was observed (Table 4). Although *DGAT2*-GG pigs weighted 2.0 kg ± 0.7 (P<0.05) less than *DGAT2*-AG pigs, the G allele did not show a clear additive behaviour (-1.0 kg ± 0.6, P=0.08).

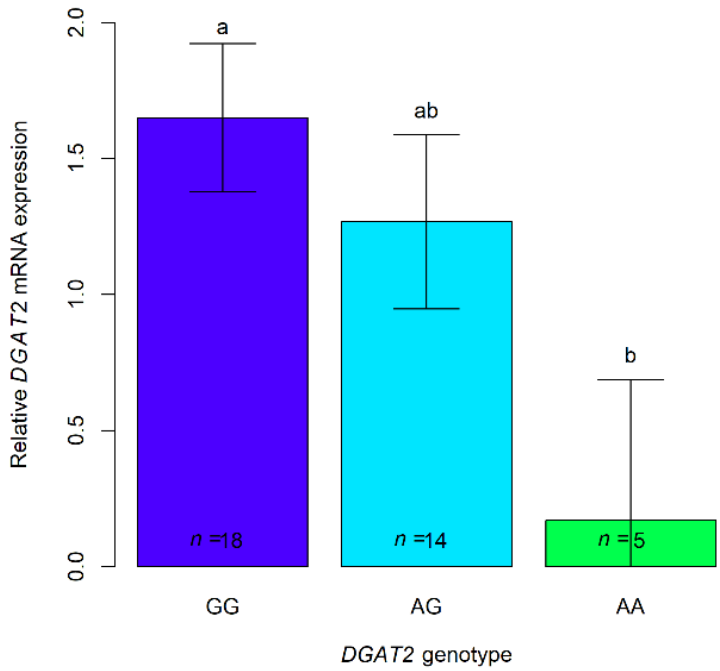

Figure 3. Relative expression of *DGAT2* across ss7315407085 genotypes. Numbers in boxes indicate the number of pigs used per genotype (*n*). Error bars represent standard errors. Columns lacking a common letter differ (P<0.05).

Table 2. Least square means and additive values (± standard error) for fatty acids content and fatty acid ratios in the *gluteus medius* muscle by *DGAT2* genotype.

	***DGAT2* genotype**			**Additive value[2]**	
Trait[1]	**GG (*n*=485)**	**AG (*n*=482)**	**AA (*n*=126)**	**a**	**P-value**
Fatty acid, %					
C14:0	1.65 ± 0.01[a]	1.61 ± 0.01[b]	1.57 ± 0.02[b]	0.04 ± 0.01	**<0.01**
C16:0	23.98 ± 0.06	23.94± 0.06	23.74 ± 0.11	0.12 ± 0.06	0.05
C18:0	11.35 ± 0.05[b]	11.51 ± 0.05[a]	11.51 ± 0.09[ab]	-0.08 ± 0.05	0.10
SFA	37.18 ± 0.10	37.27 ± 0.10	37.04 ± 0.18	0.07 ± 0.10	0.48
C16:1n-7	3.79 ± 0.03[a]	3.66 ± 0.03[b]	3.55 ± 0.05[c]	0.12 ± 0.03	**<0.0001**
C18:1n-7	4.79 ± 0.04[a]	4.74 ± 0.03[ab]	4.66 ± 0.05[b]	0.06 ± 0.02	**0.01**
C18:1n-9	41.01 ± 0.16	41.27 ± 0.14	41.25 ± .21	-0.12 ± 0.11	0.29
MUFA	49.52 ± 0.10	49.57 ± 0.10	49.59 ± 0.18	-0.03 ± 0.10	0.73
C18:2n-6	10.69 ± 0.07	10.62 ± 0.07	10.79 ± 0.12	-0.06 ± 0.07	0.40
C18:3n-3	0.60 ± 0.005	0.60 ± 0.005	0.61 ±0.01	-0.01 ± 0.01	0.23
PUFA	13.29 ± 0.09	13.16 ± 0.09	13.36 ± 0.15	-0.04 ± 0.08	0.66
Fatty acid ratio					
C16:1n-7/C16:0 (x10)	1.59 ± 0.01[a]	1.54 ± 0.01[b]	1.51 ± 0.02[b]	0.04 ± 0.01	**<0.01**
C16:1n-7/C18:1n-9 (x10)	0.81 ± 0.01[c]	0.84 ± 0.01[b]	0.89 ± 0.02[a]	0.04 ± 0.01	**<0.0001**
C18:1n-7/C18:0 (x10)	4.27 ± 0.06[a]	4.13 ±0.05[ab]	4.02 ± 0.08[b]	0.13 ± 0.04	**<0.01**
C18:1n-9/C18:0	3.66 ± 0.04	3.60 ± 0.03	3.55 ± 0.05	0.06 ± 0.03	0.06
(C16:1n-7+ C18:1n-7)/ C16:0 (x10)	3.39 ± 0.04[a]	3.30 ± 0.03[b]	3.21 ± 0.05[b]	0.09 ± 0.02	**<0.01**

[1] SFA: saturated fatty acids (C14:0+C16:0+C18:0+C20:0); MUFA: monounsaturated fatty acids (C16:1n-7+C18:1n-9+C18:1n-7+C20:1n-9); and PUFA: polyunsaturated fatty acids (C18:2n-6+C18:3n-3+C20:2n-6+C20:4n-6).

[2] Additive allele substitution of G for A

[a,b,c] Within trait, means with different superscripts differ significantly (P<0.05).

Bold font indicates statistical significance.

Table 3. Least square means and additive values (± standard errors) for fatty acids content and fatty acid ratios in *longissimus dorsi* by *DGAT2* genotype.

	***DGAT2* genotype**			**Additive value[2]**	
Trait [1]	**GG (*n*=218)**	**AG (*n*=238)**	**AA (*n*=70)**	**a**	**P-value**
Fatty acid, %					
C14:0	1.63 ± 0.02[a]	1.57 ± 0.01[b]	1.52 ± 0.02[b]	0.05 ± 0.01	**<0.0001**
C16:0	25.07 ± 0.08	25.03 ± 0.08	24.98 ± 0.12	0.05 ± 0.07	0.46
C18:0	12.13 ± 0.07	12.25 ± 0.06	12.38 ± 0.11	-0.13 ± 0.06	**0.04**
SFA	39.02 ± 0.13	39.04 ± 0.13	39.08 ± 0.2	-0.03 ± 0.12	0.80
C16:1n-7	4.04 ± 0.04[a]	3.88 ± 0.04[b]	3.67 ± 0.06[c]	0.19 ± 0.03	**<0.0001**
C18:1n-7	4.76 ± 0.04	4.76 ± 0.04	4.67 ± 0.06	0.05 ± 0.03	0.10
C18:1n-9	41.10 ± 0.22[b]	41.69 ± 0.20[a]	41.87 ± 0.29[a]	-0.40 ± 0.15	**<0.01**
MUFA	50.29 ± 0.14	50.38 ± 0.13	50.33 ± 0.21	-0.02 ± 0.12	0.84
C18:2n-6	8.32 ± 0.07	8.26 ± 0.07	8.29 ± 0.11	0.02 ± 0.06	0.78
C18:3n-3	0.37 ± 0.004	0.38 ± 0.004	0.38 ± 0.01	-0.00 ± 0.00	0.29
PUFA	10.69 ± 0.09	10.58 ± 0.09	10.60 ± 0.15	0.05 ± 0.08	0.57
Fatty acid ratio					
C16:1n-7/C16:0 (x10)	1.62 ± 0.01[a]	1.55 ± 0.01[b]	1.47 ± 0.02[c]	0.07 ± 0.01	**<0.0001**
C16:1n-7/C18:1n-9 (x10)	0.86 ± 0.02[c]	0.91 ± 0.02[b]	0.96 ± 0.02[a]	0.05 ± 0.01	**<0.0001**
C18:1n-7/C18:0 (x10)	4.06 ± 0.07	4.03 ± 0.06	3.91 ± 0.09	0.07 ± 0.05	0.17
C18:1n-9/C18:0	3.47 ± 0.05	3.51 ± 0.04	3.48 ± 0.06	-0.00 ± 0.04	0.93
(C16:1n-7+ C18:1n-7)/ C16:0 (x10)	3.40 ± 0.04	3.36 ± 0.04	3.27 ± 0.06	0.07 ± 0.03	**0.03**

[1] SFA: saturated fatty acids (C14:0+C16:0+C18:0+C20:0); MUFA: monounsaturated fatty acids (C16:1n-7+C18:1n-9+C18:1n-7+C20:1n-9); and PUFA: polyunsaturated fatty acids (C18:2n-6+C18:3n-3+C20:2n-6+C20:4n-6).

[2] Additive allele substitution of G for A

[a,b,c] Within trait, means with different superscripts differ significantly (P<0.05).

Bold font indicates statistical significance.

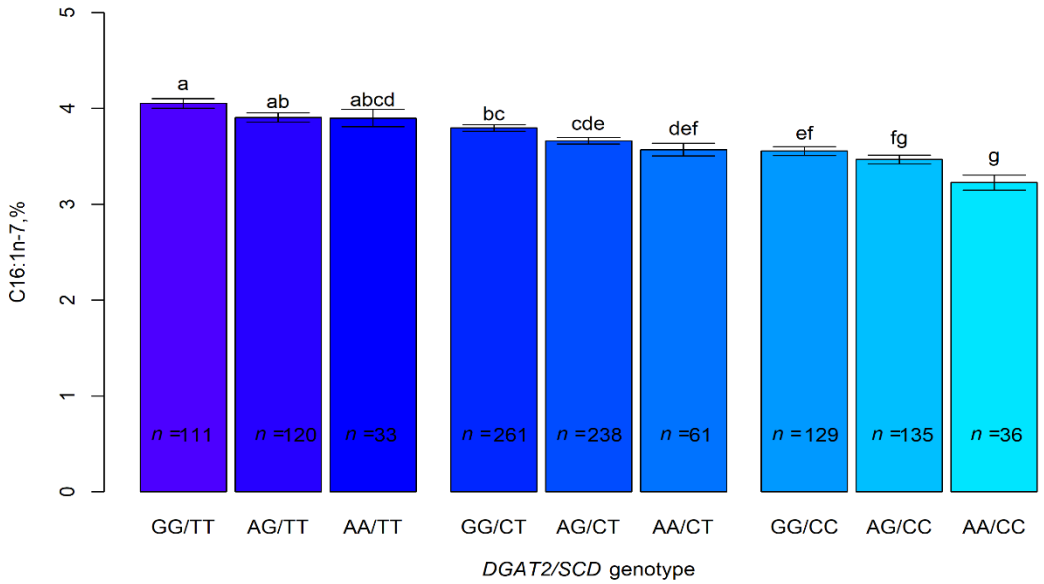

Figure 4. Effect of *DGAT2* by *SCD* genotype on palmitoleic acid (C16:1n-7) in muscle *gluteus medius*. Numbers in boxes indicate the number of pigs used per genotype (*n*). Error bars represent standard errors. Columns lacking a common letter differ (P<0.05).

Table 4. Least square means and additive values (± standard error) for body weight (BW), carcass weight (CW), backfat (BT) and loin (LT) thickness and intramuscular fat (IMF) content by *DGAT2* genotype.

	***DGAT2* genotype**			**Additive value[1]**	
Trait	**GG (*n*=496)**	**AG (*n*=488)**	**AA (*n*=129)**	**a**	**P-value**
Live measurements					
BW, kg	124.3 ± 0.6[b]	126.3 ± 0.6[a]	126.4 ± 1.1[ab]	-1.0 ± 0.6	0.08
BT, mm	21.9 ± 0.2	22.1 ± 0.2	22.4 ± 0.4	-0.3 ± 0.2	0.20
LT, mm	47.5 ± 0.3	47.1 ± 0.3	47.7 ± 0.5	-0.1 ± 0.3	0.83
Carcass measurements					
CW, kg	96.0 ± 0.5	97.4 ± 0.5	97.1 ± 0.9	-0.6 ± 0.5	0.24
BT, mm	25.6 ± 0.2	26.0 ± 0.2	26.4 ± 0.4	-0.4 ± 0.2	0.08
LT, mm	44.1 ± 0.4	43.8 ± 0.4	43.8 ± 0.7	0.1 ± 0.4	0.74
IMF content, % dry matter					
M. gluteus medius	18.1 ± 0.3	17.6 ± 0.3	18.1 ± 0.5	-0.02 ± 0.03	0.94
M. longissimus thoracis	13.3 ± 0.3	13.5 ± 0.3	13.0 ± 0.4	0.16 ± 0.23	0.48

[1]Additive allele substitution of G for A

[a,b] Within trait, means with different superscripts differ significantly (P<0.05).

4. Discussion

Understanding the regulation of fat metabolism is a milestone in the prevention and treatment of human lipid diseases, such as obesity, and in animal science. In this context, DGAT2 has received attention as the enzyme that catalyzes the final step in the synthesis of TG, the most common type of body fat. Here, we validated the effects of the ss7315407085 G>A SNP on fat content and fatty acid composition as a tag SNP for the haplotype in *DGAT2* that was identified following a preliminary GWAS and after mRNA expression and sequence variant association analyses. Using a larger sample, we confirmed that the minor allele A segregates in the studied Duroc line at a moderate frequency (0.33), and that it has a negative impact on C16:1n-7 content in muscle. The moderate minor allele frequency in the studied Duroc line is similar to estimates from other Duroc lines [14] and White and Asian breeds and crossbreds [14, 21, 22].

Our validation scheme evidenced a consistent effect of *DGAT2* SNP on C16:1n-7 in muscle that is independent of IMF content. A genetic variant in *SCD* with relevant effects on MUFA also segregates in this line [24]. The *DGAT2* SNP had a lower impact on C16:1n-7 than the *SCD* SNP variant, particularly in terms of genetic variance. While the additive value of *DGAT2* for C16:1n-7 was around half of that of *SCD*, the additive variance only reached 22% of that attributed to *SCD*. It has been shown that *SCD* co-expresses with *DGAT2* [34] and that their respective enzymes locate very close in the endoplasmic reticulum [11, 17], two findings that support the hypothesis that SCD is involved in the TG synthesis by providing a more accessible pool of MUFA through substrate channelling [12, 17]. This also would explain why cells overexpressing *DGAT2* show a greater proportion of MUFA and very particularly of C16:1n-7 [34]. Our results are in line with this hypothesis, because C16:1n-7 was the fatty acid most affected by the *DGAT2* SNP and the G allele, the one with higher *DGAT2* expression, was the one that led to accumulate more C16:1n-7. However, we did not find evidence for a positive interaction between *SCD* and *DGAT2* SNPs for C16:1n-7. Using RNAseq data from 40 other pigs of the same line, we were able to confirm that *DGAT2* genotype (GG: n=19, AG: n=14, AA: n=5) affected muscular *DGAT2* expression, but we did not detect differential expression of *SCD* by *DGAT2* genotype. In absence of interaction, the two genes mostly behaved in an allele-dose manner (Fig. 3).

The positive effect of the *DGAT2* G allele on C16:1n-7 was not observed on C18:1n-9. Zhang et al. [34] evidenced that the impact of DGAT2 on C16:1n-7 was proportionally greater as fatty acid composition turned more dependent on *de novo* fatty acids. For C18:1n-9, the same effect was only seen in mature adipocytes, especially when they were cultured in a fatty acid-rich medium. In line with these results, a plausible explanation to the differential trend between C16:1n-7 and C18:1n-9 could be that the most immediate (short-term) effect of DGAT2 is to alter fatty acid composition by selectively capturing as substrate the first available *de novo* fatty acids rather than to enhance the endogenous biosynthesis of longer-chain fatty acids. This may be the case here, since differences in *DGAT2* expression across genotypes were relatively small and no correlated responses in IMF content and *SCD* expression were observed. The favourable effect of the G allele on C14 and C16 fatty acids would reinforce this hypothesis. On the other hand, the impact of *de novo* fatty acids on final fatty acid composition is more apparent in C16:1n-7 and C18:1n-7 than in

C18:1n-9, which is proportionally more abundant in pig feed than C16:1n-7 and thus more likely to be influenced by the diet. Similarly, the effect of *DGAT2* on fatty acid composition was only observed in IMF, which is precisely the adipose tissue that develops later [35] and is less sensitive to dietary fat [36].

Changes in *DGAT2* expression are related to lipid accumulation. Results in human and murine cells indicate that DGAT2 promotes TG synthesis and storage in cytosolic lipid droplets [37] and accordingly *DGAT2* is expressed more abundantly in cells with greater *de novo* fatty acid synthesis [19]. This is in line with findings in pigs, in which *DGAT2* was most expressed in the liver of fatter breeds, where TG are expected to be more actively synthesized [38]. The expression of *DGAT2* was 2-fold higher in fatter than in leaner breeds and around 5-fold and 10-fold higher in liver than in subcutaneous fat and muscle, respectively. However, the mRNA expression of *DGAT2* was positively correlated with IMF content but not with BT. In view of these results, it would appear that the pigs carrying the *DGAT2* A allele, which down-regulates the expression of *DGAT2*, should be less fatty and, specifically, have less IMF content. Even though this association was reported in other studies [14, 21], we did not find evidence that *DGAT2* A entails a reduction in IMF content, BT or even fat content in liver. This may contradict biological expectations, but, in comparison with previous reports, validation here was performed using a much larger set of pigs from a single line so as to avoid breed or family biases. A 13-bp deletion in *DGAT2* 3'-UTR region has also been associated with increased *DGAT2* mRNA expression and BT [39]. This deletion allele showed higher transcriptional activity, most likely owing to a less stable 3'-UTR secondary structure. This indel was located at 905 bp downstream from the stop codon and matches INDEL14 (Table 1). In line with the results observed for SNP5, none of these polymorphisms including INDEL14 (r^2=0.57, Supplementary Fig. S1) affected BT in our Duroc line (Supplementary Table S2).

The results obtained are more appealing for lipid research than for pig breeding. In recent years, C16:1n-7 has received a lot of attention for its potential role as a lipokine, i.e. as a lipid hormone that acts in distant organs. Despite this, the effects of C16:1n-7 are still under debate. On the one hand, preclinical experiments with cell and rodent models show that C16:1n-7 supplementation has anti-inflammatory properties [40, 41] that protect against metabolic disorders. But, on the other hand, research in humans reported elevated blood levels of C16:1n-7 in patients with obesity and metabolic syndrome [42, 43]. Findings in pigs may contribute to disentangle the biological implications of C16:1n-7 as well as to understand the genetic regulation of fat metabolism.

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Supplementary Information

Table S1. Primers used for genotyping the polymorphism in exon 9 of the *DGAT2* gene and primers used to analyse its gene expression.

Primer	**Sequence 5' → 3'**	**Tm**	**PCR size**	**Protocol**
DGAT2 Fw	TCAACCAGCACAAGACCAAG	59.9	58bp	Genotyping
DGAT2 Rv	CAGTTCACCTCCAGGACCTC	59.7		
qDGAT2 Fw	AGGACATTGACCTCTACCATGC	60.4	109bp	Gene expression
qDGAT2 Rv	CAGTTCACCTCCAGGACCTC	59.7		

Table S2. Additive values (± standard errors) of sequence variants in the genomic region that harbours the *DGAT2* gene (Table 1) and associated P-values for palmitoleic acid (C16:1n7, % of fatty acids) and intramuscular fat (IMF, in % dry matter) in muscle *gluteus medius* and backfat thickness (BT) using data from 199 sequenced pigs.

	C16:1n-7, %		IMF, %		BT, mm	
Variant	**a**	**P-value**	**a**	**P-value**	**a**	**P-value**
SNP1	-0.12±0.10	0.23	-0.77±1.08	0.48	1.66±1.60	0.31
SNP2	0.32±0.48	0.51	9.65±4.26	0.03	10.19±5.84	0.09
SNP3	-0.06±0.09	0.49	-0.91±0.81	0.26	0.01±1.03	0.99
SNP4	0.03±.0.09	0.77	-1.20±0.87	0.17	-1.15±1.10	0.30
SNP5	0.14±0.06	**0.03**	-0.17±0.53	0.76	-0.12±0.78	0.88
SNP6	0.10±0.07	0.12	-0.57±0.57	0.32	-0.74±0.82	0.37
SNP7	-0.11±0.05	**0.02**	-0.56±0.42	0.18	0.09±0.53	0.87
SNP8	0.04±0.05	0.46	0.82±0.46	0.08	0.70±0.56	0.22
SNP9	0.08±0.05	0.07	0.73±0.45	0.11	0.55±0.58	0.35
INDEL10	0.09±0.06	0.13	-0.20±0.50	0.69	-0.32±0.62	0.60
SNP11	0.11±0.06	0.09	0.14±0.57	0.81	-0.11±0.70	0.88
INDEL12	-0.08±0.15	0.59	2.24±1.49	0.14	3.05±2.06	0.14
INDEL13	0.12±0.05	**0.01**	-0.72±0.45	0.11	0.06±0.61	0.92
INDEL14	0.13±0.05	**<0.01**	-0.44±0.46	0.34	0.11±0.58	0.85
SNP15	0.10±0.04	**0.02**	-0.35±0.43	0.43	0.53±0.57	0.36
SNP16	0.10±0.04	**0.02**	-0.03±0.41	0.95	0.11±0.51	0.82
SNP17	-0.11±0.04	**<0.01**	-0.03±0.43	0.95	-0.22±0.53	0.67
SNP18	-0.11±0.05	**0.03**	0.05±0.49	0.92	-0.23±0.59	0.70
SNP19	-0.11±0.06	**0.03**	0.05±0.49	0.92	-0.23±0.59	0.70
INDEL20	-0.10±0.05	0.06	-0.11±0.47	0.82	-0.24±0.58	0.69
SNP21	-0.14±0.05	**<0.01**	-0.34±0.50	0.49	0.05±0.59	0.94
SNP22	0.15±0.05	**<0.01**	0.15±0.45	0.75	-0.83±0.54	0.13
SNP23	0.23±0.15	0.12	1.42±1.37	0.30	2.92±1.67	0.08
INDEL24	0.12±0.32	0.72	-0.73±3.00	0.81	-3.88±3.66	0.29
SNP25	0.12±0.32	0.72	0.04±0.43	0.92	-0.44±0.54	0.41
SNP26	-0.06±0.05	0.24	-0.37±0.46	0.42	-0.34±0.57	0.55
SNP27	-0.02±0.05	0.64	0.08±0.49	0.87	1.10±0.60	0.07
SNP28	-0.02±0.16	0.92	-0.92±1.47	0.53	-0.11±1.79	0.95
SNP29	-0.04±0.05	0.50	-0.25±0.50	0.61	0.99±0.60	0.10
SNP30	-0.09±0.05	0.10	-0.25±0.51	0.62	0.06±0.62	0.92
SNP31	0.00±0.05	0.97	0.22±0.46	0.64	1.09±0.56	0.05
SNP32	0.05±0.05	0.30	0.06±0.48	0.91	1.26±0.60	0.04
INDEL33	0.10±0.10	0.31	-0.44±0.85	0.61	0.52±1.02	0.61

Bold font indicates statistical significance.

Table S3. Least square means and additive values (± standard error) for fatty acids and ratios in subcutaneus fat by *DGAT2* genotype.

	***DGAT2* genotype**			**Additive value[2]**	
Trait[1]	**GG (*n*=114)**	**AG (*n*=91)**	**AA (*n*=21)**	**a**	**P-value**
Fatty acid, %					
C14:0	1.46 ± 0.02	1.45 ± 0.02	1.41 ± 0.03	0.02 ± 0.01	0.14
C16:0	22.02 ± 0.28	22.16 ± 0.31	21.51 ± 0.43	0.26 ± 0.20	0.20
C18:0	11.23 ± 0.18	11.43 ± 0.20	11.14 ± 0.28	0.04 ± 0.13	0.73
SFA	34.90 ± 0.43	35.21 ± 0.48	34.44 ± 0.66	0.23 ± 0.30	0.46
C16:1n-7	2.21 ± 0.05	2.19 ± 0.06	2.21 ± 0.08	-0.00 ± 0.04	0.99
C18:1n-7	5.28 ± 0.13	5.40 ± 0.10	5.45 ± 0.25	-0.08 ± 0.14	0.54
C18:1n-9	37.28 ± 0.4[b]	38.39 ± 0.30[a]	37.36 ± 0.81[ab]	-0.04 ± 0.43	0.92
MUFA	47.05 ± 0.40	47.15 ± 0.42	47.65 ± 0.58	-0.30 ± 0.27	0.26
C18:2n-6	15.85 ± 0.30	15.37 ± 0.33	15.81 ± 0.46	0.02 ± 0.21	0.92
C18:3n-3	1.10 ± 0.04	1.11 ± 0.04	1.11 ± 0.06	-0.01 ± 0.03	0.74
PUFA	18.10 ± 0.34	17.61 ± 0.37	18.06 ± 0.52	0.02 ± 0.24	0.94
Fatty acid ratio					
C16:1n-7/C16:0 (x10)	1.05 ± 0.03	1.02 ± 0.04	1.05 ± 0.05	-0.00 ± 0.03	0.93
C16:1n-7/C18:1n-9 (x10)	0.54 ± 0.03	0.54 ± 0.03	0.56 ± 0.05	-0.00 ± 0.02	0.90
C18:1n-7/C18:0 (x10)	4.54 ± 0.19	4.65 ± 0.16	4.78 ± 0.36	-0.13 ± 0.19	0.53
C18:1n-9/C18:0	3.19 ± 0.09	3.28 ± 0.07	3.29 ± 0.16	-0.05 ± 0.09	0.58
(C16:1n-7+C18:1n-7)/ C16:0 (x10)	3.30 ± 0.09	3.36 ± 0.08	3.45 ± 0.18	-0.07 ± 0.09	0.44

[1] SFA: saturated fatty acids (C14:0+C16:0+C18:0+C20:0); MUFA: monounsaturated fatty acids (C16:1n-9+C18:1n-9+C18:1n-7+C20:1n-9); and PUFA: polyunsaturated fatty acids (C18:2n-6+C18:3n-3+C20:2n-6+C20:4n-6).

[2] Additive allele substitution of G for A

[a,b] Within trait, means with different superscripts differ significantly (P<0.05).

Table S4. Least square means and additive values (± standard error) for fatty acids and ratios in liver by *DGAT2* genotype.

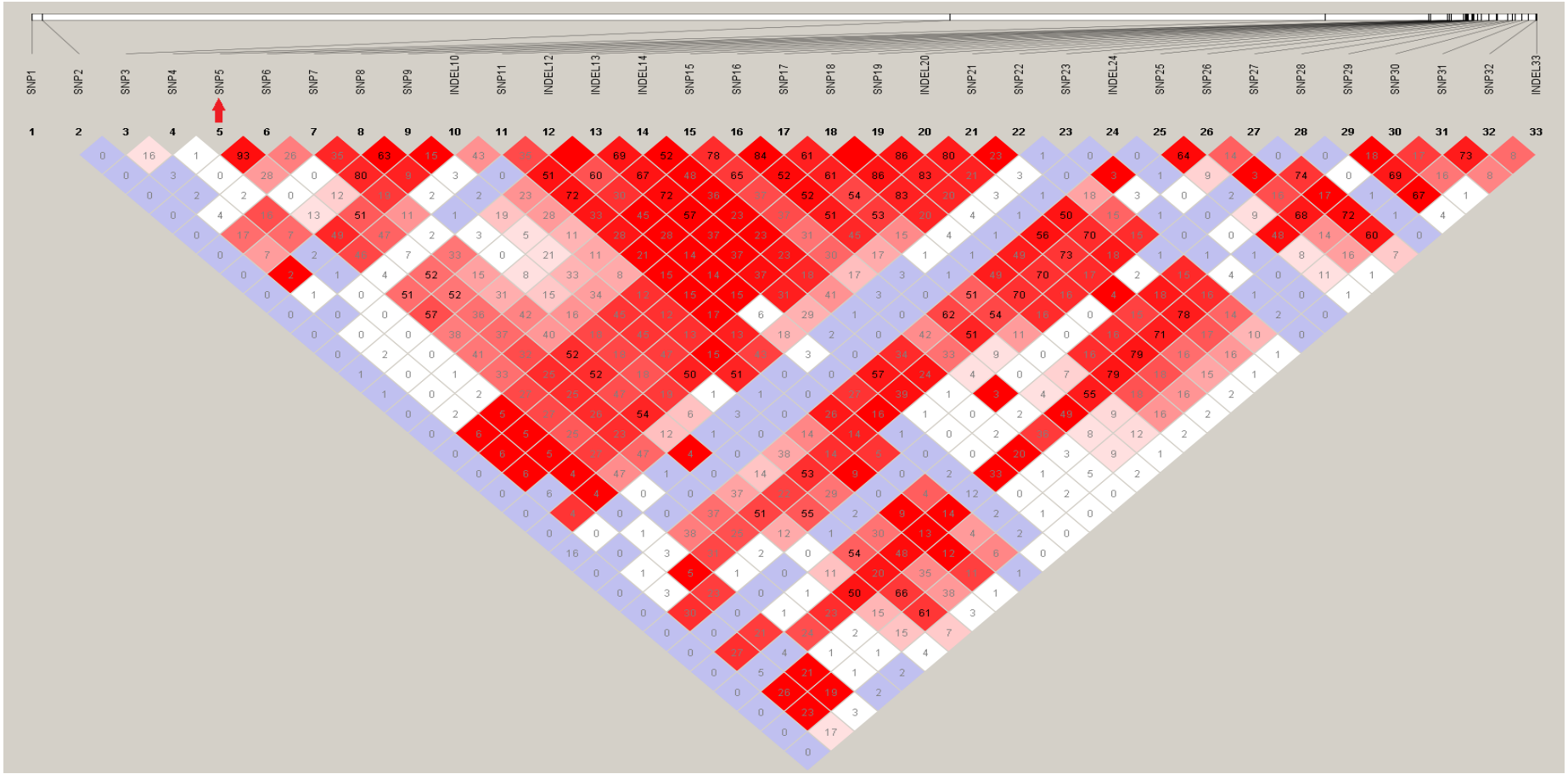
	***DGAT2* genotype**			**Additive value[2]**	
Trait[1]	**GG (*n*=76)**	**AG (*n*=65)**	**AA (*n*=16)**	**a**	**P-value**
Fat content, %DM	13.8 ± 0.3	14.0 ± 0.3	14.1 ± 0.6	-0.16 ± 0.31	0.61
Fatty acid, %					
C14:0	0.5 ± 0.03	0.55 ± 0.03	0.46 ± 0.06	0.02 ± 0.03	0.59
C16:0	17.60 ± 0.39	17.41 ± 0.38	16.98 ± 0.67	0.31 ± 0.35	0.37
C18:0	25.83 ± 0.6	24.46 ± 0.58	25.59 ± 0.96	0.12 ± 0.48	0.8
SFA	45.93 ± 0.73	44.39 ± 0.71	44.87 ± 1.13	0.53 ± 0.56	0.34
C16:1n-7	0.97 ± 0.04	0.99 ± 0.04	0.92 ± 0.07	0.03 ± 0.04	0.42
C18:1n-7	2.65 ± 0.08	2.64 ± 0.07	2.53 ± 0.14	0.06 ± 0.07	0.41
C18:1n-9	17.98 ± 0.64	18.03 ± 0.59	17.07 ± 1.08	0.46 ± 0.54	0.41
MUFA	21.5 ± 0.54	21.99 ± 0.53	20.86 ± 0.92	0.32 ± 0.47	0.49
C18:2n-6	18.42 ± 0.26	18.74 ± 0.25	18.88 ± 0.42	-0.23 ± 0.22	0.28
C18:3n-3	0.54 ± 0.02	0.55 ± 0.02	0.55 ± 0.03	-0.01 ± 0.02	0.74
PUFA	32.71 ± 0.95	33.69 ± 0.91	34.73 ± 1.55	-1.01 ± 0.79	0.2
Fatty acid ratio					
C16:1n-7/C16:0 (x10)	0.52 ± 0.02	0.54 ± 0.02	0.51 ± 0.03	0.01 ± 0.01	0.64
C16:1n-7/C18:1n-9 (x10)	0.57 ± 0.02	0.56 ± 0.02	0.53 ± 0.03	0.02 ± 0.01	0.15
C18:1n-7/C18:0 (x10)	1.11 ± 0.05	1.15 ± 0.05	1.04 ± 0.08	0.03 ± 0.04	0.46
C18:1n-9/C18:0	0.75 ± 0.04	0.78 ± 0.04	0.69 ± 0.07	0.03 ± 0.04	0.41
(C16:1n-7+ C18:1n-7)/ C16:0 (x10)	1.97 ± 0.04	2.02 ± 0.04	1.96 ± 0.07	0.00 ± 0.04	0.90

[1] SFA: saturated fatty acids (C14:0+C16:0+C18:0+C20:0); MUFA: monounsaturated fatty acids (C16:1n-9+C18:1n-9+C18:1n-7+C20:1n-9); and PUFA: polyunsaturated fatty acids (C18:2n-6+C18:3n-3+C20:2n-6+C20:4n-6).

[2] Additive allele substitution of G for A

[a,b] Within trait, means with different superscripts differ significantly (P<0.05).

Figure S1. Linkage disequilibrium structure for the *DGAT2* gene. Featured variants in Table 1 are given above the plot and the red arrow indicates the location of the tag SNP (ss7315407085) used for further validations. Pairwise linkage disequilibrium coefficients (r^2) are shown in each cell using the standard Haploview linkage disequilibrium colour scheme.



V- Capítulo II

Cambios en el transcriptoma debidos a la interacción entre la vitamina A y el genotipo *SCD* en cerdos Duroc

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Transcriptome shifts triggered by vitamin A and *SCD* genotype interaction in Duroc pigs

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1. Introduction

Intramuscular fat [IMF] content and its composition affect overall pork acceptability by influencing organoleptic attributes such as flavour, texture, and juiciness. Meat quality can be improved by direct husbandry practices, for instance through adapting the diet to meet production aims [1]. In this context, vitamin A restriction during the fattening period can improve IMF content and marbling score in beef cattle [reviewed in 2]. This effect is induced by retinoic acid, one of the bioactive compounds of the vitamin A family, which prompts two waves of effects over preadipocyte differentiation [3, 4]. On the one hand, it stimulates preadipocyte differentiation during the early commitment of embryonic stem cells into the adipocyte lineage but, at later stages, it has an inhibitory role over the terminal differentiation of preadipocytes. Thus, restriction of vitamin A or their precursors (β-carotenes) at early fattening stages (14-22 months of age in beef cattle) had a prominent effect over IMF content through enhanced hyperplasia of adipocytes [2].

In pigs, however, the effect of vitamin A restriction is controversial. The IMF of pigs fed a control feed (7,500 IU vitamin A /kg) did not differ from pigs supplemented with >10 times more vitamin A (100,000 IU/kg) [5]. Other authors have reported increases in IMF content with diets restricted in vitamin A [6 - 10], although the magnitude (and even the direction) of this effect depends on the genetic type of the pigs [11]. In contrast, the effect of vitamin A on IMF fatty acid [FA] composition is more robust across experiments. Vitamin A restriction promotes deposition of monounsaturated FAs [MUFA] and raises the desaturation index of fat [5, 7].

Intramuscular fat content and its composition are traits of moderate-to-high (range 0.26 to 0.86) heritability [12] and can therefore be improved through selection programs. As a result from a previous genome-wide association study [GWAS] using IMF content and composition data from muscle (*gluteus medius* and *longissimus thoracis*) and subcutaneous fat from a line of commercial Duroc pigs, we identified two genomic regions with prominent but distinct effects on IMF content (in SSC6) and on MUFA, notably oleic acid (in SSC14) [13]. These two regions co-located with the position of the leptin receptor [*LEPR*] and stearoyl-coA desaturase [*SCD*] genes, respectively. The *SCD* gene encodes a limiting enzyme in the biosynthesis of MUFA. In a previous work, we identified three linked SNP mutations in the promoter of this gene [14]. The haplotype H1, corresponding to the C-T-A combination of alleles, associated with a higher content of MUFA in IMF and subcutaneous fat regarding the alternative H2 haplotype (T-C-G). The middle SNP (rs80912566, C>T) changes a potential binding site for retinoid X receptors [RXR] [14]. Two families of the retinoic acid receptors (RAR and RXR) mediate signals in multiple physiological processes, including the modulation of genes involved in adipogenesis, mitochondria and lipid

metabolism, through their interaction with retinoic acid compounds [15]. However, on the other hand, no relationship has been described between the *LEPR* activity and vitamin A.

Given all the above, we have investigated here the effect of the interaction between dietary vitamin A content and the *SCD* genotype on IMF content and FA composition. A global transcriptome sequencing approach was used to characterise the changes in gene expression triggered by this interaction in pigs fed different levels of vitamin A supplement.

2. Material and Methods

2.1 Animals and Experimental Design

All pigs used in the study were raised and slaughtered in commercial units following applicable regulations and good practice guidelines on the protection of animals kept for farming purposes during transport and slaughter. All experimental procedures were approved by the Ethics Committee for Animal Experimentation of the University of Lleida (agreement CEEA 05-04/15). This study is reported in accordance with the ARRIVE guidelines.

In a first experiment to study the effect of the interaction between dietary vitamin A content and the *SCD* genotype on carcass and IMF content and FA composition, 108 Duroc barrows were reared in two batches and were maintained under the same rearing conditions with ad libitum access to feed (Additional file 1: Table S1). This population was generated by mating 59 sows with 28 boars and randomly choosing, on average, two barrows per litter. At 30 kg of live-weight, pigs were randomly assigned to two dietary treatments (Table 1 and Additional file 1: Table S2): a standard feed supplemented with vitamin A (VA+), or the same diet without supplementation (VA-).

Animals were slaughtered at 207 ± 7 days and 130 ± 11 kg of body weight in a commercial slaughterhouse equipped with a carbon dioxide stunning system. Measured body composition traits included carcass yield, loin thickness and backfat thickness between the third and fourth last ribs by using an ultrasound automatic scanner (AutoFOM, SFK-Technology, Denmark). The carcass lean percentage was estimated based on 35 measurements of AutoFOM points by using the official approved equation (Decision 2001/775/CE, 2001). Samples of semimembranosus muscle were collected immediately after slaughter and snap frozen in dry ice. After chilling for about 24 h at 4ºC, samples of the gluteus medius and longissimus thoracis were collected and stored at -80ºC until required.

In a second experiment to study changes in muscle transcriptome in the pigs from the first experiment, 40 pigs from the same batch were selected using a factorial design balanced for diet (VA+ and VA-) and *SCD* genotypes (TT and CC) (Additional file 1: Table S2).

Table 1. Retinyl acetate content in the feed used in this study.

Diet	Phase	Age	Retinyl acetate (mg/kg feed)
With vitamin A supplement (VA+)	STARTER	80-110 d	3.125
	GROWTH	110-160 d	3.580
	FINISHER	160-210 d	2.440
Without vitamin A supplement (VA-)	STARTER	80-110 d	0.275
	GROWTH	110-160 d	0.725
	FINISHER	160-210 d	0.395

2.2 Fatty acid analysis

Fatty acid content was analysed in a representative sample from pulverized freeze-dried muscle. For each sample, the IMF content and FA composition were determined in duplicate by gas chromatography [16]. Fatty acid methyl esters were obtained by transesterification using a solution of 20% boron trifluoride in methanol [17]. Methyl esters were determined by gas chromatography using a SP2330 capillary column (30 m by 0.25 mm; Supelco Inc., Bellefonte, PA). The quantification was performed through area normalization after adding into each sample 1,2,3-tripentadecanoylglycerol as an internal standard. The proportion of individual FA, saturated FA (SFA), polyunsaturated FA (PUFA) and MUFA were calculated as percentages relative to total FA content. Intramuscular fat content was predicted as the sum of each individual FA expressed as triglyceride equivalents [18].

2.3 Statistical analyses for carcass and fatty acid data

Data on carcass traits and FA composition were analysed using a linear model that included the rearing batch (2 levels), diet (VA+ and VA-), the *SCD* genotype (TT, TC, CC), and the interaction of diet by *SCD* genotype. The *LEPR* genotype was not included in the model as the number of pigs per genotypes was not balanced (there were very few *LEPR*-TT pigs). The age at slaughter was included as a covariate to analyse carcass traits and IMF content. To analyse FA composition, IMF content was used as a covariate. Multiple pairwise comparisons were performed with a Tukey test setting $P < 0.05$ as significance threshold. Analyses were performed using the statistical package JMP Pro 15 (SAS Institute Inc., Cary, NC).

2.4 DNA isolation and genotyping

Genomic DNA was isolated from muscle samples by incubation with a lysis buffer with proteinase K followed by phenol:chloroform purification using standard protocols [19]. The quantification and purity of DNA was determined in a Nanodrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). DNA integrity was tested by electrophoresis in agarose gels.

All samples were genotyped for *SCD* rs80912566 by real time qPCR (QuantStudio3, Applied Biosystems, Waltham, MA, USA) with High Resolution Melt analysis (Luminaris Color HRM Master Mix, Thermo Scientific, Waltham, MA, USA) as in [13].

2.5 RNA isolation and library construction

Total RNA was isolated from *m. semimembranosus* samples using TRI Reagent (Invitrogen, Thermo Scientific, Waltham, MA, USA) and Direct-zol™ RNA Miniprep Plus Kit (Zymo Research, BioSystems, CA, USA) according to the manufacturer's protocol. This muscle was selected because it was collected at slaughter and yielded RNA with the integrity needed for RNA-seq analysis. RNA integrity number (RIN) and purity were checked by a Bioanalyzer 2100 (Agilent Technologies, CA, USA). RIN was in the range 8.0-9.0.

The RNA samples were sequenced by Centre Nacional d'Anàlisi Genòmica (CNAG-CRG, Barcelona, Spain, http://www.cnag.crg.eu/). Libraries were prepared using the TruSeq SBS v-3HS kit (Ilumina, San Diego, CA) according to the manufacturer's protocol. Each library was paired-end sequenced (2 x 100bp) to 65M reads with phred quality score 80-90% in a Hi-Seq 2000 platform.

2.6 Analysis of RNA-seq transcriptomic data

Quality of the raw sequencing data was assessed with the fastp tool [20]. With this approach, in addition to the default parameters to filtering, reads with quality scores per base lower than 30, shorter than 36 bp or unpaired were removed. Filtered sequences were aligned to the *Sus scrofa* reference genome (Sscrofa11.1) with the STAR 2.5.4b tool [21]. Mapping statuses were analysed with qualimap and plotted with MultiQC v1.0 [22]. After the alignment, reads were counted with the Feature Counts v1.24.1 software [23] and differential expression (DE) analysis between *SCD* genotypes, diets, and their combined groups was performed with the approach implemented in DeSeq2 v.1.14.1 software [24]. Adjusted p-values (q-value < 0.10) and fold change (FC) > 1.2 (upregulated) or < 1/1.2 = 0.83 (downregulated) were set as the threshold for significantly different expression. Functional analysis of differentially expressed genes (DEG) was then subjected to gene ontology (GO) functional enrichment analysis, KEGG pathways and targets of transcription factor binding with Enrichr v1.0 software [25]. Visualisation of gene interactions was performed with Cytoscape 3.9.0 and the StringApp connector [26].

In total, an average of 62.4M (range 48.8-91.5M) raw reads per sample were generated. After filtering, an average of 58M of reads were retained for further analysis and 84.8% (range 77.4-86.8%) of the reads were uniquely mapped to the pig reference genome (Sscrofa11.1; Additional file 1: Table S3). Sequencing files are available from NCBI-GEO with access number GSE183909.

2.7 Validation of RNA-seq results by quantitative real-time PCR analysis

Quantitative real time PCR (qPCR) was used to validate ten relevant genes in the list of DEGs. Briefly, 2 µg of total RNA from the 40 pigs in the RNA-seq experiment were retrotranscribed using SuperScript IV retrotranscriptase (Invitrogen, Carlsbad, CA) with oligo-dT and random hexamers. Primers (Additional file 1, Table S4) were designed with the Primer Blast tool using the mRNA reference sequences provided in NCBI GENE, so that they will hybridise to all the transcripts described for each gene. Three reference genes (*HPRT*, *B2M* and *RPL32*), were included in this analysis. For each gene, a standard curve was generated by amplifying serial

dilutions of a control cDNA to check for linearity between initial template concentration and Ct values. Quantitative real-time PCR assays were carried out in triplicate in a QuantStudio3 device (Applied Biosystems, Waltham, MA, USA) in a final volume of 8 µl containing 1× Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific, Waltham, MA, USA), 200 nM of each primer and 3 µl cDNA template diluted 1:30 in water. The following thermal profile was used for all reactions: 10 min at 95°C, 40 cycles of 15 s at 95°C and 30 s at 60°C, followed by a slow ramp from 60 to 95°C to generate a dissociation curve to control the specificity of the amplified product. In order to quantify and normalise the expression data we used the ΔΔCt method [27] against the geometric mean of the three reference genes. For each gene, expression values between groups were compared with a t-test and differences were considered significant at $P<0.05$.

3. Results

3.1 Effect of vitamin A supplementation on carcass traits

Carcasses of pigs fed the VA- diet were on average 3.8 kg heavier than those on the VA+ diet ($P < 0.05$; Table 2). However, other carcass traits, including IMF content in three muscles, were not affected by the dietary vitamin A content ($P > 0.05$). On the other hand, pigs with the *SCD* TT genotype had more subcutaneous fat and less loin thickness than CC pigs, which resulted in a lower carcass lean content ($P < 0.05$). There was no significant interaction between the *SCD* genotype and the diet for the tested carcass traits (Table 2).

3.2 Effect of vitamin A supplementation on IMF composition

The FA composition in the IMF of the three muscles analysed was not affected by the vitamin A supplement. As described before [14], the rs80912566 TT genotype was associated with an increase in the C16:1, C18:1n9 and MUFA content at the expense of the C16:0, C18:0 and SFA, respectively (Table 3 and Additional file 1; Table S5), which led to increased desaturation indexes (C16:1/C16:0, C18:1n9/C18:0 and MUFA/SFA; Figure 1 and Additional file 2: Figure S1). There was a clear interaction between the *SCD* genotype and the dietary vitamin A content. This interaction was significant for most FAs related to the *SCD* desaturation pathway in the *m. gluteus medius* (Table 3 and Figure 1). The VA- diet tended to enlarge the compositional differences between the TT and CC genotypes. Conversely, in the pigs fed the VA+ diet, the differences between genotypes were smaller and sometimes not significant. Vitamin A restriction triggered differential FA compositional changes in TT and CC pigs ($P < 0.05$; Figure 1). The interaction between vitamin A and the *SCD* genotype was also detected in leaner muscles such as *m. longissimus thoracis* (C18:0, MUFA, C18:1n7/C18:0, C18:1n9/C18:0 and MUFA/SFA, at $P<0.05$) and *m. semimembranosus* (C18:0 at $P<0.05$ and SFA at $P<0.10$) (Additional file 1: Table S5 and Additional file 2: Figure S1). When the dietary vitamin A was restricted, CC pigs had more saturated FAs, while in TT pigs the FAs that result from SCD desaturation were only slightly affected by the diet. This trend was consistent in the three muscles analysed (Figure 1 and Additional file 2: Figure S1) and indicates that, overall, CC pigs were more sensitive to dietary vitamin A than TT pigs in increasing FA desaturation associated with SCD activity.

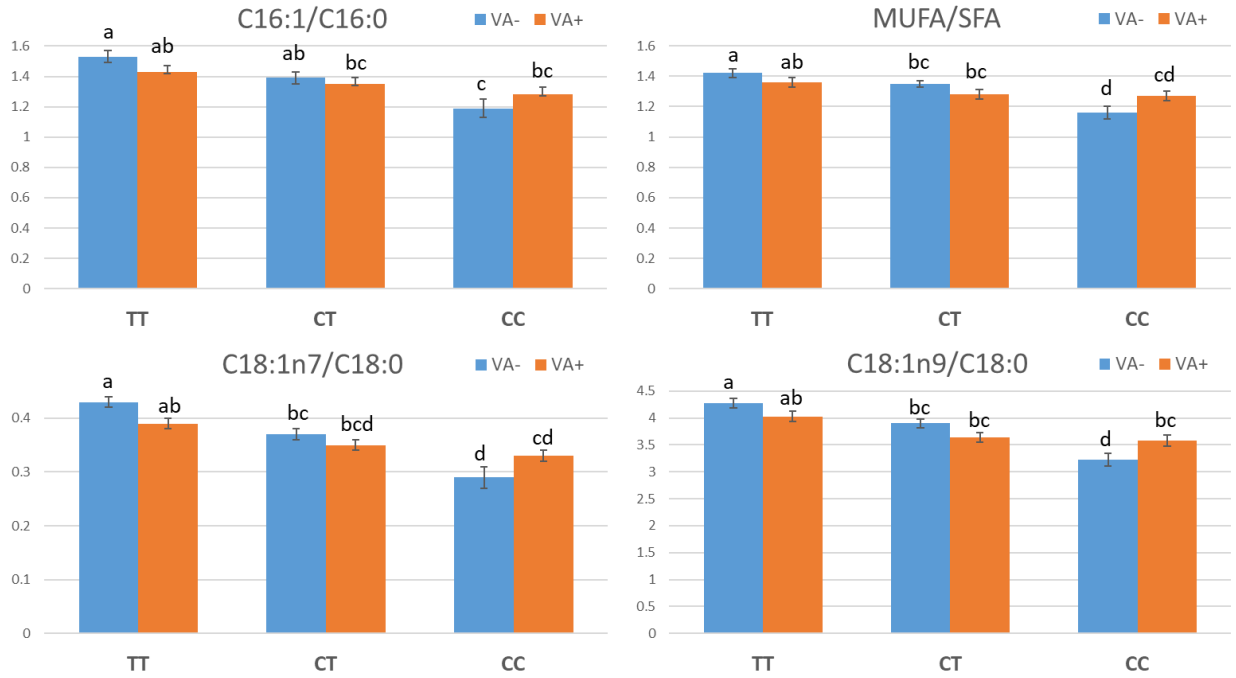

Figure 1. Effect of *SCD* genotype and diet on FA desaturation indexes. Diets with (VA+) and without (VA-) vitamin A supplement in the feed formulation. Within each panel, bars not connected with the same letter differ at P<0.05. Errors bars are SE.

Table 2. Least square means (±SE) for carcass traits and intramuscular fat content by diet and *SCD* rs80912566 genotype. Number of pigs per factor is indicated in parenthesis.

	Diet[2]		***SCD* rs80912566 genotype**			**P-value**		
Trait[1]	**VA-** (54)	**VA+** (55)	**TT** (38)	**TC** (44)	**CC** (27)	**Diet**	***SCD***	**Diet×*SCD***
Carcass weight, kg	98.97±1.40^{b}	95.17±1.33^{a}	97.84±1.51	96.68±1.35	96.69±1.81	0.03	n.s.	n.s.
BFT, mm	34.75±1.07	34.81±1.02	37.18±1.15^{a}	34.31±1.03^{ab}	32.85±1.38^{b}	n.s.	0.03	n.s.
Loin depth, mm	41.28±1.27	40.13±1.21	37.96±1.37^{a}	40.98±1.22^{ab}	43.17±1.64^{b}	n.s.	0.03	n.s.
Lean content, %	35.83±1.19	35.45±1.13	32.63±1.28^{b}	36.18±1.14a^{b}	38.09±1.54^{a}	n.s.	0.01	n.s.
IMF (GM), %	6.27±0.27	6.80±0.26	6.63±0.29	6.42±0.26	6.26±0.35	n.s.	n.s.	n.s.
IMF (LT), %	4.44±0.21	4.41±0.19	4.63±0.22	4.40±0.19	4.24±0.26	n.s.	n.s.	n.s.
IMF (SM), %	3.24±0.41	3.22±0.43	2.78±0.48	3.36±0.39	3.55±0.42	n.s.	n.s.	n.s.

[1]BFT – Backfat thickness; IMF – intramuscular fat content, expressed on a wet weight basis; GM – m. *gluteus medius*; LT – m. *longissimus thoracis*; SM – m. *semimembranosus*. Within each row and factor, means with different superscripts differ significantly (P<0.05). n.s. – not significant (P>0.05).

[2]Diets with (VA+) and without (VA-) vitamin A supplement in the feed formulation

Table 3. Least square means (±SE) for fatty acid composition in the intramuscular fat of m. *gluteus medius* by diet and *SCD* rs80912566 genotype. Number of pigs per factor is indicated in parenthesis.

	VA- diet[2]			VA+ diet			P-value		
	SCD genotype			*SCD* genotype					
Fatty acids[1]	**TT** (18)	**TC** (25)	**CC** (11)	**TT** (20)	**TC** (19)	**CC** (16)	**Diet**	***SCD***	**Diet×*SCD***
C16:0,%	24.88±0.28^{b}	25.22±0.24^{b}	26.57±0.37^{a}	25.27±0.28^{ab}	25.73±0.28^{ab}	25.26±0.32^{ab}	n.s.	0.03	<0.01
C16:1,%	3.80±0.09^{a}	3.51±0.08^{abc}	3.18±0.12^{bc}	3.61±0.09^{ab}	3.46±0.09^{abc}	3.21±0.10^{c}	n.s.	<0.0001	n.s.
C18:0,%	10.21±0.19^{d}	11.01±0.16^{bc}	12.51±0.25^{a}	10.74±0.19^{cd}	11.58±0.19^{b}	11.72±0.21^{a}	n.s.	<0.0001	<0.01
C18:1n7,%	4.33±0.06^{a}	4.09±0.05^{b}	3.66±0.08^{d}	4.16±0.06^{ab}	3.98±0.06^{bc}	3.79±0.07^{cd}	n.s.	<0.0001	0.05
C18:1n9,%	43.36±0.35^{a}	42.84±0.30^{ab}	40.55±0.47^{c}	42.95±0.35^{ab}	41.89±0.36^{bc}	41.63±0.40^{bc}	n.s.	<0.0001	0.02
SFA,%	37.09±0.44^{c}	38.14±0.38^{bc}	41.21±0.59^{a}	38.10±0.44^{bc}	39.43±0.45^{ab}	39.18±0.50^{ab}	n.s.	<0.0001	<0.01
MUFA,%	52.29±0.42^{a}	51.25±0.36^{ab}	48.15±0.56^{c}	51.53±0.42^{ab}	50.11±0.42^{bc}	49.40±0.47^{c}	n.s.	<0.0001	0.02
PUFA, %	10.62±0.25^{ab}	10.61±0.21^{ab}	10.65±0.33^{ab}	10.37±0.25^{ab}	10.47±0.25^{bc}	11.42±0.28^{a}	n.s.	0.03	n.s.

[1]Fatty acid traits presented as percentage of total fatty acids in the sample. SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids. Within each row, means with different superscripts differ significantly (P<0.05). n.s. – not significant (P>0.05).

[2]Diets with (VA+) and without (VA-) vitamin A supplement in the feed formulation

3.3 Differentially expressed genes between diet and *SCD* groups

The number of DEGs between pigs under different diet treatments or with different *SCD* genotypes was low (43 and 24, respectively), with no overlapping. However, the number of DEGs increased when dietary vitamin A by *SCD* genotype groups were studied (269 transcripts corresponding to 241 unique genes; Table 4). Only 28 of the 241 genes overlapped across the lists of DEGs in the four diet-by-genotype group comparisons (Figure 2). Most DEGs were classified as messenger RNA from protein coding genes (79%) (Table 4). The second most abundant RNA type was the long non-coding RNA (lncRNA) class, which accounted for 16% of total DEGs detected in this experiment. The full list of DEGs is shown in Additional file 1: Table S6, which includes 202 unique HGNC gene name identifiers (HUGO Gene Nomenclature Committee database).

The *SCD* gene was detected as a DEG when the two genotypes (TT vs CC) were compared (FC = 2.15) with boost effect in the diet with vitamin A supplementation (FC = 2.84) but not under the VA- diet (Additional file 1: Table S6). A group of seven genes (*COX15*, *ABCC2*, *CWF19L1*, *ENSSSCG00000048329*, *ENSSSCG00000049992*, *SEC31B* and *FBXW4*) located -500kb/+200kb from the *SCD* gene were also differentially expressed, which might represent a case of piggyback co-expression due to the close distance between genes [28].

Table 4. Number and classification of differentially expressed genes (n. DEG) by diet, *SCD* genotype and dietary vitamin A by *SCD* genotype groups.

Factor	Comparison	n. DEG	coding	lncRNA[1]	pseudogene	snoRNA	miRNA	MT tRNA
Diet	VA+ vs VA-	43	27	10		3	3	1
SCD	TT vs CC	24	17	7		1		
Diet in *SCD*_TT genotype	TT_VA+ vs TT_VA-	162	134	22	3	1	1	1
Diet in *SCD*_CC genotype	CC_VA+ vs CC_VA-	26	21	1	1	1	1	1
SCD genotype in VA+ diet	TT_VA+ vs CC_VA+	73	59	13		1		
SCD genotype in VA- diet	TT_VA- vs CC_VA-	8	7			1		

[1]lncRNA – long non-coding RNA; pseudogene – transcribed pseudogene; snoRNA – small nucleolar RNA; miRNA – microRNA, MT tRNA – mitochondrial transference RNA.

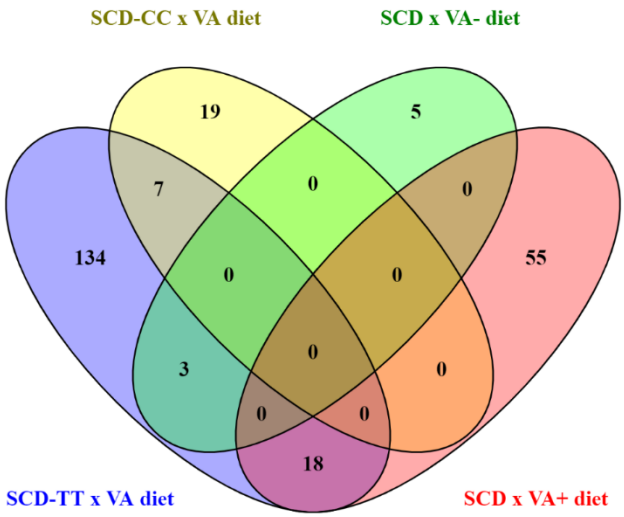

Figure 2. Venn diagram showing the number of overlapping differentially expressed genes between diets (VA+ and VA-) by rs80912566 *SCD* genotype and between *SCD* genotypes (*SCD*-TT and *SCD*-CC) by diet. Dietary treatment was the same but with (VA+) and without (VA-) vitamin A supplement in the feed formulation.

3.4 Functional analysis of DEG

The functional analysis of the full list of DEGs was based on GO terms, KEGG pathways and common transcription factors associated to each gene. Functional classification (Additional file 1: Table S7) revealed an overrepresentation of genes encoding proteins located in membrane-bounded vesicles (25 genes; P=0.007), lipid transport (10 genes; P=0.003) and connective tissue development (9 genes; P=0.002), amongst others. This latter group includes several genes with large fold-change values of differential expression, such as *TNMD* (tenomodulin) and *COMP* (cartilage oligomeric matrix protein), which were 131- and 52-times more expressed in TT fed the VA- diet respect to those fed the VA+ diet. *TNMD* encodes for a potent inhibitor of angiogenesis and *COMP* is a non-collagen extracellular matrix protein that triggers integrin signalling to the cell surface. Their expression has not been related to retinol pathways, so far. Visualisation of protein-to-protein and protein-to-DNA interactions based on the STRING database indicated three main networks of relationships (Additional file 2: Figure S2). The largest network included 20 genes membrane-bound vesicles (Additional file 2: Figure S3.A). Additionally, genes involved in the beta-oxidation of FA in peroxisomes also grouped together (*MVK*, *ACCA1*, *ECHDC2*, and peroxins *PEX6* and *PEX5L*) (Additional file 2: Figure S3.B).

The target transcription factor analysis identified ten transcription factors that can potentially regulate 104 of the 202 unique DEG gene name identifiers (Table 5). These 10 transcription factors, which include proteins activated by retinol (PPARG, PPARA, RARA, RXRA) or by sterols (SREBF1 and SREBF2), are known to interact among themselves in a competitive manner (reviewed in [29]; also [30 – 32]), which agrees with the partial overlap of regulated genes by transcription factor in Table 5. Functional classification within these 104 genes (Additional file 1: Table S8) revealed an overrepresentation of genes encoding proteins located in membrane-bounded vesicles (n=15; P=0.003), which had a prominent role in endocytosis (P=0.002), in the group of 40

genes regulated by SREBF1 and SREBF2 transcription factors. This classification partially overlapped with the genes regulated by the IRF2, HNF1A and HNF4A transcription factors. The genes regulated by the HNF1A and HNF4A transcription factors also included genes with activity related to lipid metabolic process (7 genes; P=0.02). On the other hand, genes regulated by RARA and RXRA nuclear receptors were involved in a variety of functions, which included immunity and inflammation responses (P=0.02), lipid binding (P=0.03) and apoptosis (P=0.03). Finally, the genes clustered under the regulation of PPARG and PPARA include genes with metal-binding (11 genes, P=0.03) and long-chain FA-modifying (2 genes, P=0.05) capacities.

Table 5. Regulators predicted for the set of annotated coding differentially expressed genes.

Transcription factor	n	Genes
HMGA1	13	*ACTC1*; *CGGBP1*; *CNST*; *FBXO33*; *IREB2*; *MPI*; *MTTP*; *NEGR1*; *PCDHGA6*; *PDE4D*; *PPM1D*; *PTX3*; *TBXAS1*
HNF1A	12	*ADPRH*; *AGK*; *COL17A1*; *ECE1*; *GPX2*; *ITGAL*; *NAV2*; *PYCARD*; *RNF122*; *STARD8*; *TBXAS1*; *TRAK2*
HNF4A	25	*ABCA1*; *AURKB*; *C12ORF49*; *CDK23*; *CGGBP1*; *CORO1A*; *CRYBB3*; *CYB561D1*; *ECE1*; *ECHDC2*; *GPX2*; *IMPAD1*; *IRX3*; *MTTP*; *NAV2*; *NECAP2*; *OTOGL*; *PPM1D*; *SAFB2*; *SCD*; *SFXN2*; *SLC9A8*; *STARD8*; *TIMP1*; *TRAK2*
IRF2	25	*ADPRH*; *ARHGEF37*; *COX15*; *DFFA*; *EIF1B*; *FGF10*; *FGG*; *GALR1*; *GPR35*; *HABP2*; *HNMT*; *IMPAD1*; *ITGAL*; *LIX1*; *MTTP*; *NEGR1*; *PEX6*; *PPM1D*; *PTX3*; *RGS9*; *RMND5B*; *SCD*; *SGSM1*; *TTPAL*; *ZFPL1*
PPARA	3	*BAZ1B*; *RARRES1*; *TIMP1*
PPARG	26	*ADCK5*; *AGK*; *C16ORF58*; *CNST*; *CORO1A*; *CYB561D1*; *ECE1*; *FUS*; *GPR68*; *HABP2*; *IP6K2*; *ITGAL*; *KANK3*; *KIAA0355*; *MYO3B*; *NECAP2*; *PDE4D*; *PITX2*; *PRMT3*; *PTX3*; *RARRES1*; *RYK*; *SCD*; *TMEM39B*; *VPS25*; *ZRANB1*
RARA	19	*ADCY10*; *AURKB*; *CORO1A*; *GPR68*; *HABP2*; *ITGAL*; *KANK3*; *KIAA0355*; *MTTP*; *MYO3B*; *NCF1*; *NECAP2*; *PRMT3*; *PTX3*; *RMND5B*; *SCD*; *SH2D3A*; *TMEM42*; *TTPAL*
RXRA	14	*ACTC1*; *BAZ1B*; *CORO1A*; *EXOSC1*; *FGF10*; *GPIHBP1*; *GSTO2*; *KIAA0355*; *PEX6*; *PRMT3*; *PYCARD*; *RARRES1*; *TMEM42*; *VPS25*
SREBF1	34	*AADAC*; *ABCA1*; *ACTC1*; *CACNA1I*; *CDK13*; *COL17A1*; *CORO1A*; *DFFA*; *DNER*; *ECE1*; *FGG*; *FUS*; *HK2*; *IP6K2*; *KANK3*; *LIX1*; *LRTM1*; *MORN4*; *MTTP*; *MVK*; *NAV2*; *NECAP2*; *PDE4D*; *PDE6D*; *PDZD8*; *PRMT3*; *RARRES1*; *RASA3*; *RGS9*; *SH2D3A*; *TIMP1*; *TMPRSS13*; *TNK2*; *ZFPL1*
SREBF2	10	*AADAC*; *ARHGEF37*; *IGFBP4*; *IP6K2*; *ITGB2*; *LZIC*; *NAV2*; *SLC12A9*; *TNK2*; *VPS25*

3.5 Quantitative PCR validation of RNA-seq results

Ten relevant genes were selected from the DEG list for the validation experiment based on the FC ratios and the functional annotation of the gene. Efficient qPCR assays were established for 8 out of the 10 selected genes. For all genes (Table 6), the fold-change ratios between groups were consistent in both assays. Expression differences between groups were significant for all genes tested (P<0.05) except for *ABCA1* and *MTTP*. In addition, in most cases the expression ratios were

lower in the qPCR experiment than in RNA-seq, which might be due to the higher background noise of the qPCR assay. Altogether, validation of RNA-seq data by qPCR showed a high correspondence between both analyses, confirming differential expression for 8 out of 10 group comparisons (Table 6).

Table 6. Validation of differentially expressed genes (DEG) by quantitative PCR.

DEG between	RNA-seq		qPCR	
	Gene	FC[1]	FC[1]	p-value
SCD genotypes (TT vs CC)	*SCD*	2.15	3.00	0.003
diets in *SCD*_TT pigs (TT_VA+ vs TT_VA-)	*ABCA1*	1.85	1.41	n.s.
	FGF10	5.30	1.95	0.03
	PPARA	0.63	0.78	0.05
diets in *SCD*_CC pigs (CC_VA+ vs CC_VA-)	*TNMD*	131.44	253.75	0.04
	OTOR	33.01	51.30	0.02
	CILP2	34.57	14.21	0.03
SCD genotypes under the VA+ diet (TT_VA+ vs CC_VA+)	*MTTP*	2.63	1.97	n.s.
	FGF10	6.55	2.08	0.03
	SCD	2.84	4.05	0.03

[1]FC, fold-change ratio between groups as indicated in the first column

4. Discussion

In the present study, carcasses from pigs fed the VA- diet were 3.8 kg heavier than those fed the VA+ diet. Despite of this, dietary vitamin A had no effect on carcass lean content, which agreed with previous studies in different pig lines fed with restricted or elevated vitamin A [5−11].

Regarding meat quality traits, we observed no differences in IMF between dietary vitamin A treatments in none of the three muscles analysed. The effect of dietary vitamin A on IMF deposition is particularly controversial in pigs. Several authors reported that a dietary reduction of vitamin A impacted the IMF content in *m. gluteus medius* [8], *m. longissimus thoracis* [9, 11] and *m. semimembranosus* [7] in pigs of different genetic lines, although the direction and magnitude of the changes were very variable. For instance, Olivares et al. [11] investigated the effect of supplementing the feed for 11 weeks with 100-fold the vitamin A daily recommendations of the National Research Council. This extra supplementation raised IMF content in Duroc-sired hybrid pigs but not in Large White × Landrace animals. In contrast, removing vitamin A supplements from the formulation also increased IMF content in Iberian [6] and Large White × Landrace [10] pigs. In a previous work with the same Duroc line used in this study, the complete removal of vitamin A from the diet the last 30 days of fattening also resulted in higher IMF content [8].

The experimental conditions, the duration of vitamin A restriction, age and genetic type are several factors that can explain the poor consistency of the results. Retinoic acid, an active metabolite of vitamin A, regulates the adipogenic differentiation of fibroblasts into adipocytes in intramuscular adipose tissue and, for this reason, dietary vitamin A could impact IMF deposition [4]. However, given the opposite effect of retinoic acid in early (positive) and late (negative)

differentiation of adipocytes [4], the time and duration of the dietary restriction might be critical to have relevant consequences in the pig.

As opposed to other experiments [5 – 9, 11], in our study the FA composition was not directly affected by the dietary vitamin A. In general, most studies have detected an increase in the desaturation index (MUFA/SFA) when dietary vitamin A was restricted. We detected this effect as an interaction between the diet and the *SCD* genotype of the pigs. In this sense, vitamin A supplementation promoted FA desaturation and increased C18:1n9 content at the expense of C18:0 in *SCD* CC pigs but not in TT and CT pigs (Figure 1). This trend was consistent on the three muscles analysed but was more evident in *gluteus medius*, probably due to the higher IMF content of this muscle and the larger number of pigs sampled for this muscle. The effect of the *SCD* genotype on MUFA content and FA desaturation indexes has been described before [14, 33, 34]. The TT pigs have more oleic acid (C18:1n9), MUFA and, consequently, C16:1/C16:0, C18:1n7/C18:0, C18:1n9/C18:0 and MUFA/SFA than CC pigs, which we observed in both diets, but the differences were more evident in pigs fed the vitamin A-restricted diet. This interaction reinforces the relationship between vitamin A and SCD activity, with the CC pigs being more sensitive to the effect of vitamin A. In a previous work, we described a genetic interaction between dietary vitamin A and the *SCD* genotype for liver levels of all-*trans*-retinol and all-*trans*-5-6-epoxy retinoic acid, the two most abundant forms of bioactive vitamin A [8]. The effect of the diet on the level of these two compounds was only evidenced in CC pigs, paralleling our current results on FA desaturation indexes. Note that the *SCD* rs80912566 polymorphism is not present in all pig breeds. The T variant was fixed in the Iberian, Piétrain and Landrace lines tested in [14] but is segregating in Large White lines [14, 35], which might partly explain the inconsistent results from previous studies.

Despite the small impact that the dietary vitamin A had on the phenotype of the pigs, we observed significant changes at the transcriptome level. The highest number of DEGs were detected when diets by *SCD* or *SCD* by diets groups were compared. Although most DEG were protein-coding genes, about 15% of DEGs represented transcripts from lncRNA genes, which are still not well annotated in pigs, and little is still know about their functional relevance. In fact, the highest differences in gene expression were mainly lncRNA genes. Other groups have described similar situations where dietary treatments have little effect in the phenotype but profound effects at the transcriptome levels (for instance [36] and [37] for carbohydrate and oleic-enriched diets in pigs, or [38] with pigs on different fasting periods).

The most striking result of our data is the high number of DEGs when the interaction between diets and *SCD* genotype was considered. On one hand, the vitamin A supplement changed the expression of 162 genes in TT pigs but only 26 genes in CC animals, with little overlap. On the other hand, the VA+ diet induced 73 DEG between pigs of opposite *SCD* genotypes in contrast with only 8 DEG when the pigs where fed the VA- diet. *SCD*-TT pigs have more MUFA and less SFA in muscle samples, which include adipocytes, myocytes, and fibroblasts. Oleic acid (C18:1n9) is a potent activator of several transcription cascades, including preadipocyte differentiation [39] and mammary gland FA biosynthesis [40, 41]. Targeted disruption of *SCD* has significant

consequences in the health of knockout mice, which are driven to general hypermetabolism and stimulation of FA beta-oxidation. Consequently, *SCD* knockout mice are protected from fat diet-induced obesity [42, 43]. In our study, the difference of *SCD* expression was 2.84-fold in pigs fed the VA+ diet but *SCD* expression did not differ between genotypes in pigs fed the VA- diet ([14] and Additional file 1: Table S6.A), which agrees with differences in liver *SCD* expression due to dietary vitamin A levels, reported in a previous experiment [8]. Overall, our animal material allows studying the long-term effect of higher MUFA content in muscle and fat tissues and its interaction with dietary retinoic precursors.

Retinol and retinoic acid, the biologically active compounds of vitamin A, mediate their function normally through specific retinoid receptors, which belong to the ligand-dependent transcription factors superfamily of nuclear receptors, although receptor-independent effects are also known [44]. Upon binding to retinoids, the nuclear receptors RXR, RAR, HNF4A can form heterodimers with ligand-mediated co-regulators PPAR and HNF1A or members of the NR2 nuclear repressors. Most of these transcription factors were identified as main regulators of 104 of the DEGs detected in this experiment. Retinol-mediated gene repression and transactivation have wide effects on cell proliferation, differentiation, cell adhesion, and apoptosis in different cell types, immunity, male and female reproduction, embryonic development, and barrier integrity [45]. This was also reflected in our data (Additional file 1: Tables S7 and S8), as many of these processes were included in the functional analysis of the DEGs.

Among the DEGs, it is interesting to highlight *ABCA1*, a cholesterol transporter involved in maintaining cholesterol homeostasis and lipid metabolism [46]. In pigs, *ABCA1* overexpression is directly associated with an increase in HDL levels [46, 47] and polymorphisms on this gene have been associated with atherosclerosis risk score [48]. *ABCA1* has been used as an epigenetic marker for evaluating meat quality in chicken [49] and it has been associated with beef tenderness and FA composition [50] but information in relation to pork quality is still lacking. A gene with a similar function is *MVK*, a mevalonate kinase that catalyses an early step in cholesterol biosynthesis and is associated with HDL cholesterol [51]. This SREBP2-responsive gene has been appointed as a candidate gene for fat deposition in broilers [52, 53]. Unfortunately, we were not able to validate the differential expression of these two genes by qPCR analysis. On the other hand, a group of four genes encoding extracellular matrix proteins (*COMP*, *CILP2*, *OTOR* and *TNMD*) were very highly expressed (30 to 130-fold) in CC pigs fed the VA+ diet with respect to the VA- diet. Three of these genes have been individually investigated in relation with adipocyte function (*CILP2* [54]) and differentiation (*TNMD* [55, 56]; *COMP* [57]) but not together. Several other genes are also well known in relation to adipose tissue differentiation, such as *FGF10* and *MTTP.* In chicken, muscle fibroblast growth factor 10 (*FGF10*) expression correlates with IMF content [58]. We were able to validate the higher expression of *FGF10* in TT pigs than in CC pigs fed the VA+ diet (6.55-fold by RNA-seq analysis and 2.08-fold by qPCR assay) and in the TT pigs fed the VA+ diet vs the VA- diet (5.30 vs 1.95-fold by RNA-seq and qPCR analysis, respectively). Microsomal triglyceride transfer protein (*MTTP*) is a protein essential to transport triglycerides from the endoplasmic reticulum membrane to lipid droplets [59]. Polymorphisms in this gene have been associated with

changes in FA composition in pork [60, 61] but there is so far no report on the effect on the expression of the gene on pork quality

Another interesting DEG is *PPARA*, which, as mentioned before, encodes a member of the retinol receptor family whose expression is inhibited by dietary vitamin A intake [62]. In agreement with this, in our study *PPARA* expression increased by 1.6-fold in TT pigs fed the VA- diet. The PPARα transcription factor promotes de novo lipogenesis, FA storage and improves glycogen synthesis [63]. In pigs, *PPARA* can be used as a genetic marker for overall adipose tissue accumulation, as polymorphisms in the 3' untranslated region (UTR) are associated with changes in backfat and IMF deposition [64]. The increase in *PPARA* expression agrees with the general observation that restricted dietary vitamin A promotes adipogenesis and fat deposition, although we were not able to capture this effect in the phenotype of our pigs.

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Supplementary Information

A.

B.

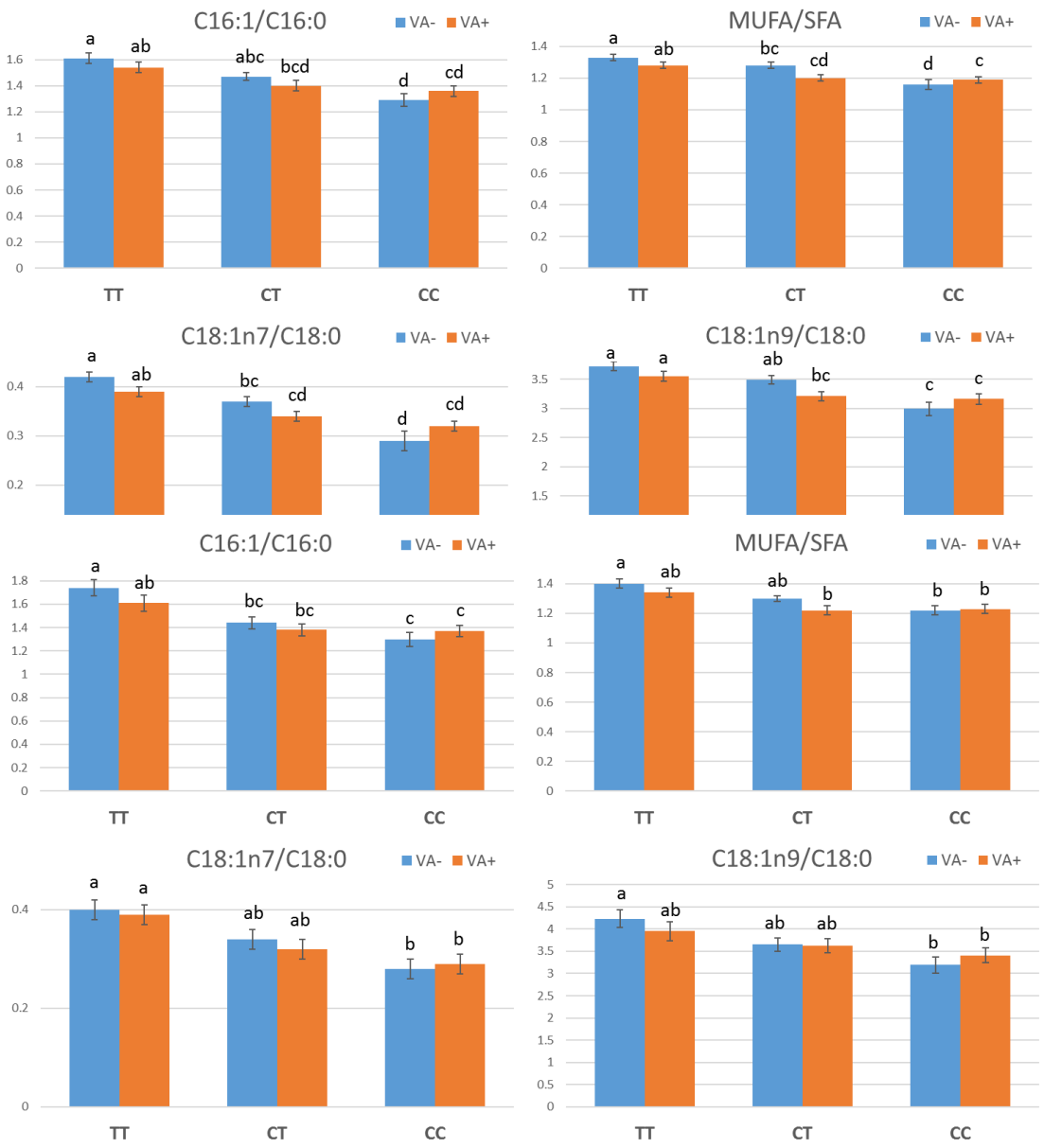

Figure S1. Effect of *SCD* rs80912566 genotype and diet on four fatty acid saturation indexes. A. m. *longissimus thoracis*. B. *m. semimembranosus*. Diets with (VA+) and without (VA-) vitamin A supplement in the feed formulation. Within each panel, bars not connected with the same letter differ at P<0.05. Errors bars are SE.

Figure S2. Gene network analysis of differentially expressed genes as determined by RNA Seq. The full list of 202 unique HGNC gene name identifiers was analysed using the STRING App of Cytoscape.

Please find the figure on the attached link:

https://docs.google.com/document/d/1ttCDRQPwfRzX37FSTAoryXjTLqlnTLlv/edit?usp=sharing&ouid=104868136500369812081&rtpof=true&sd=true

Figure S3. Close up look at the two main gene networks detected by the STRING App of Cytoscape. A. membrane-bound vesicle-related genes; B. Genes involved in fatty acid beta-oxidation in peroxisomes.

Please find the figure on the attached link:

https://docs.google.com/document/d/1ttCDRQPwfRzX37FSTAoryXjTLqlnTLlv/edit?usp=sharing&ouid=104868136500369812081&rtpof=true&sd=true

Table S1. Ingredients and nutrient content of the diet fed during the starting, growth and finishing period (as-fed basis).

	STARTER (80-110 d)	**GROWTH (110-160 d)**	**FINISH (160-210 d)**
Net Energy, kcal/kg	2300.00	2300.00	2400.00
Nutrient content			
Crude fat	4.02	4.15	5.55
Crude protein	14.50	14.65	13.46
Crude fibre	5.41	5.00	4.57
Neutral detergent fibre	15.44	15.50	15.45
Glucose	3.32	4.10	3.32
Starch	43.52	42.07	43.96
Main amino acids			
Total Lysine	0.97	0.94	0.86
Leucine	1.01	1.01	0.87
Methionine	0.29	0.29	0.25
Threonine	0.67	0.64	0.58
Fatty acids			
C16:0	0.63	0.67	0.87
C18:0	0.30	0.29	0.39
C16:1	0.06	0.06	0.12
C18:1	1.41	1.50	2.18
C18:2	1.19	1.20	1.24
SFA[1]	0.98	1.09	1.49
MUFA+PUFA[1]	2.78	2.88	3.71

[1]SFA - saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids

Table S2. Classification of pigs by *SCD* rs80912566 genotype and diet.

EXPERIMENT 1 (carcass traits and intramuscular fat composition study)	**diet**[1]	
***SCD* genotype**	**VA+**	**VA-**
TT	20	18
TC	18	25
CC	16	11
EXPERIMENT 2 (muscle transcriptomic study)	**diet**[1]	
***SCD* genotype**	**VA+**	**VA-**
TT	9	8
CC	13	10

[1]Diet with (VA+) or without (VA-) the Vitamin A supplement

Table S3. Summary of sequencing data of the 40 Duroc pigs used for RNA-seq transcriptome analysis.

Please find the table on the attached link:

https://docs.google.com/spreadsheets/d/1-4SRm6Pq_bU16pQsg_h7OVPyLBgoLVsw/edit?usp=sharing&ouid=104868136500369812081&rtpof=true&sd=true

Table S4. Primers used in the quantitative real-time PCR validation experiment of 10 differentially.

Gene	**Primer Fw (5'--> 3')**	**Tm**	**Primer Rv (5'--> 3')**	**Tm**	**PCR size**
TNMD	TGGGTGGTCCCTCAAGTGAA	61.7 ºC	CCTCTCTCATCCAACATGGGG	61.7 ºC	125 bp
ABCA1	GAAAACAGAACACCGCGGAC	60.6 ºC	CAGGGAAAACCCACCATACCTAA	60.5 ºC	146 bp
CILP2	GCACTGTGGCCACTAGCAAT	59.1 ºC	ACCAGGTAGGGCTTTTCCAA	58.5 ºC	184 bp
COMP	AGATAACGCTGGGTGCAGAC	58.1 ºC	GTCACACTCCATCACCGTGT	57.5 ºC	143 bp
MVK	GCCCTGGAGCATTACCTTGT	59.1 ºC	CTTCAGCATGCTCATCTGGC	59.9 ºC	196 bp
OTOR	TGGGCTGGAAGTGTTTATGG	58.6 ºC	GGACCAAGTTGCTGGGGAAA	62.1 ºC	76 bp
FGF10	GGAAGGTCAGCGGTACCAAG	59.6 ºC	TCTCCAGGATACTGTACGGG	55.5 ºC	51 bp
MTTP	TGCAAGCAAAATGGTCCGTC	62.0 ºC	CGAATGGGGACCACGTTCTAT	60.8 ºC	118 bp
PPARA	GGAAAGGCCAGCAATAACCC	60.4 ºC	GGCCATACACAGTGTCTCCA	57.6 ºC	60 bp
SCD	ATTGGGAGCTGTGGGTGAG	59.0 ºC	AAGTTGATGTGCCAGCGGTA	59.4 ºC	90 bp
HPRT1	AAGATGGTCAAGGTTGCAAGCT	59.9 ºC	ATTTCAAATCCAACAAAGTCTGGTCTA	59.5 ºC	85 bp
BM2	TCGGGCTGCTCTCACTGTCT	61.5 ºC	GGCGTGAGTAAACCTGAACC	57.4 ºC	69 bp
RPL32	CACCAGTCAGACCGATATGTCAA	59.9 ºC	CGCACCCTGTTGTCAATGC	61.6 ºC	70 bp

Table S5. Least square means (±SE) for fatty acid compositional traits in the intramuscular fat of *m. longissimus thoracis* and *m. semimembranosus*, by dietary treatment and *SCD* rs80912566 genotype. Number of pigs per factor is indicated in parenthesis.

	VA- diet			VA+ diet			Interaction diet*SCD
	SCD genotype			*SCD* genotype			
Fatty acid traits[1]	**TT**	**TC**	**CC**	**TT**	**TC**	**CC**	**P-value**
m. longissimus thoracis	(17)	(24)	(11)	(18)	(18)	(16)	
C16:0,%	25.96±0.24	26.03±0.20	26.74±0.31	26.21±0.24	26.69±0.23	26.69±0.26	0.06
C16:1,%	4.17±0.10^{a}	3.81±0.09^{abc}	3.44±0.13^{c}	4.03±0.10^{ab}	3.73±0.10^{bc}	3.64±0.11^{bc}	n.s.
C18:0,%	11.39±0.22^{c}	12.06±0.19^{bc}	13.58±0.28^{a}	11.83±0.22^{c}	12.75±0.21^{ab}	12.96±0.24^{a}	0.01
C18:1 n7,%	4.71±0.07^{a}	4.42±0.06^{b}	3.99±0.10^{c}	4.52±0.08^{ab}	4.23±0.07^{bc}	4.09±0.08^{c}	0.08
C18:1 n9,%	42.15±0.36^{a}	41.87±0.31^{ab}	40.46±0.46^{b}	41.78±0.36^{b}	40.69±0.35^{ab}	40.72±0.39^{b}	n.s.
SFA,%	39.18±0.43^{c}	39.94±0.37^{bc}	42.24±0.55^{a}	39.90±0.44^{a}	41.33±0.42^{ab}	41.57±0.47^{ab}	n.s.
MUFA,%	51.83±0.41^{a}	50.91±0.35^{ab}	48.67±0.53^{c}	51.14±0.42^{c}	49.44±0.40^{bc}	49.21±0.45^{c}	0.06
PUFA, %	8.99±0.23	9.15±0.20	9.09±0.31	8.96±0.24	9.23±0.23	9.21±0.26	0.05
m. semimembranosus	(8)	(16)	(10)	(8)	(13)	(13)	
C16:0,%	24.37±0.40	24.37±0.28	24.93±0.37	24.34±0.40	25.35±0.33	24.97±0.32	n.s.
C16:1,%	4.24±0.17^{a}	3.50±0.12^{bc}	3.25±0.16^{c}	3.92±0.17^{ab}	3.49±0.14^{bc}	3.41±0.14^{bc}	n.s.
C18:0,%	10.15±0.31^{c}	11.11±0.21^{bc}	12.57±0.28^{a}	10.29±0.31^{c}	11.62±0.25^{ab}	11.72±0.25^{ab}	0.02
C18:1 n7,%	8.81±2.09	5.46±1.46	3.07±1.91	2.99±2.10	3.16±1.72	3.05±1.68	n.s.
C18:1 n9,%	36.61±2.38	38.24±1.67	40.38±2.17	41.02±2.38	39.65±1.96	39.94±1.91	n.s.
SFA,%	36.05±0.68^{b}	36.92±0.47^{ab}	39.01±0.62^{a}	36.16±0.68^{b}	38.48±0.56^{ab}	38.24±0.54^{ab}	0.07
MUFA,%	50.36±1.00	47.89±0.70	47.38±0.91	48.63±1.00	47.01±0.82	47.07±0.80	n.s.
PUFA, %	13.59±1.23	15.19±0.86	13.61±1.12	15.21±1.23	14.52±1.01	14.70±0.98	n.s.

[1]Fatty acid traits presented as percentage of total fatty acids in the sample. SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids. Within each row, means with different superscripts differ significantly (P<0.05).

Table S6. Differentially expressed genes (DEG) between diets, *SCD* genotypes and their combination. A. DEG between *SCD* genotypes (TT vs CC). B. DEG between diets (VA+ vs VA-). C. DEG between diets in *SCD_TT* pigs (TT_VA+ vs TT_VA-). D. DEG between diets in *SCD_CC* pigs (CC_VA+ vs CC_VA-). E. DEG between *SCD* genotypes under the VA+ diet (TT_VA+ vs CC_VA+). F. DEG between *SCD* genotypes under the VA- diet (TT_VA- vs CC_VA-).

Please find the table on the attached link:

https://docs.google.com/spreadsheets/d/1-4SRm6Pq_bU16pQsg_h7OVPyLBgoLVsw/edit?usp=sharing&ouid=104868136500369812081&rtpof=true&sd=true

Table S7. Functional classification of full list of differentially-expressed genes. Gene Ontology clustering acording to DAVID enrichment analysis(https://david.ncifcrf.gov/).

Please find the table on the attached link:

https://docs.google.com/spreadsheets/d/1-4SRm6Pq_bU16pQsg_h7OVPyLBgoLVsw/edit?usp=sharing&ouid=104868136500369812081&rtpof=true&sd=true

Table S8. Functional classification of differentially-expressed genes regulated by common transcription factors by DAVID (https://david.ncifcrf.gov/).

Please find the table on the attached link:

https://docs.google.com/spreadsheets/d/1-4SRm6Pq_bU16pQsg_h7OVPyLBgoLVsw/edit?usp=sharing&ouid=104868136500369812081&rtpof=true&sd=true

VI- Capítulo III

Antagonismo entre efectos directos y maternos del gen receptor de la leptina sobre el peso corporal en porcino

El contenido de este capítulo ha sido publicado en PLoS One. 2021, 28;16(1):e0246198.

Doi: 10.1371/journal.pone.0246198.

Antagonistic maternal and direct effects of the leptin receptor gene on body weight in pigs

Emma Solé, Roger Ros-Freixedes, Marc Tor, Josep Reixach, Ramona N. Pena, Joan Estany

1. Introduction

Maternal effects occur when a mother influences her offspring beyond the direct effects of the genetic material that she transmitted to them. The importance of maternal influence on growth has been long known in livestock production. As already noted by Varro (1st century BCE) in his book on farming, a sow with piglets should be fed more bountifully because they will grow thin if she gives little milk [1]. Maternal effects on progeny growth can have a significant impact on offspring survival and therefore on the evolution of natural and domesticated populations [2]. Maternal effects can accelerate or impede the rate of response to selection of a trait, depending on whether their effect on the phenotype of the offspring is positive or negative [3]. Traits that result in maternal effects for growth traits, such as milk production, are themselves the result of the joint action of the maternal genotype and the environment, and thus they can also be subjected to genetic variation and selection. There are many examples of maternal environmental influences and how they can affect offspring development. One of the most well-known is precisely the favorable effect of the sow feeding status on piglet growth [4,5]. On the other hand, results indicate rather consistently a negative relationship between direct and maternal genetic effects, particularly in pigs [6,7]. However, the genetic underpinnings whereby this antagonism operates are unclear.

Feeding young piglets increases energy *demands* for females. During lactation, sows must simultaneously cope with self-maintenance and milk production, if not with their own growth [8]. Voluntary feed intake does not generally meet such surplus of energy demands [9, 10] and lactating sows have to resort to body reserves to support milk production. Since sow body weight and backfat thickness are indicators of the ability to mobilize body reserves [11], we hypothesize that variants in genes impacting these traits or body energy balance may be causative of maternal influences. Numerous experiments have been purposely designed to unravel the genetic architecture of body fat content and distribution in pigs, especially using F_2 crosses between divergent populations [12]. There is evidence for quantitative trait loci for fatness on chromosome 6 that map close to the leptin receptor [*LEPR*] gene in Iberian [13], Asian [14] and Duroc [15] breeds. Leptin is a hormone predominantly secreted by white adipocytes that it is known to regulate food intake and energy balance [16, 17]. Leptin deficiency causes excessive feed intake and energy savings and, consequently, greater body weight and fat mass. Defective leptin receptor expression produces similar obese phenotypes and hyperleptinemia, in an attempt to counteract the leptin resistance-like state triggered by the leptin receptor deficit [18]. A missense mutation (C>T) in exon 15 (exon 14 in former genome build versions) of the *LEPR* gene (rs709596309) has been identified as the possible causal polymorphism [13]. While fixed in Iberian, the T allele segregates in Duroc as full recessive, with TT pigs displaying increased serum leptin levels and overall fatness [19, 20]. Although there is less compelling evidence on the impact of this mutation on body weight, results from F_2 crosses indicate that the T allele may also boost growth [21].

Functional mutations in the porcine *LEPR* gene can be a useful model to investigate the genetic basis of the interaction between direct and maternal genetic effects on individual development. Here, we address the role of maternal effects on body growth that stem from the *LEPR* rs709596309 variant using a Duroc line where the T allele was segregating at an intermediate frequency [20]. In F_2 designs, direct and maternal effects are very difficult to disentangle due to no or very little variation in the genotype of the F_1 dam, as well as to between-breed linkage disequilibrium. Single gene analysis within segregating populations overcomes these shortfalls. More specifically, we first show that the *LEPR* rs709596309 variant is causative of a maternal effect in pigs that is at odds with the direct effect for piglet growth. Then, we demonstrate that this antagonism is due to a bias of TT pigs for self-maintenance rather than offspring investment. Based on these findings, we finally discuss evolutionary insights into the role and significance of *LEPR* as a transgenerational mechanism for energy allocation.

2. Material and methods

2.1 Animals, records and samples

All experimental pigs were from a purebred Duroc line mainly selected for an index including lean growth and intramuscular fat content [48] and primarily used for producing high-quality dry-cured products. Five independent experiments were conducted to examine maternal and individual influences (S4 Table). In the first experiment, twelve batches of barrows (n = 413) were raised under standard commercial conditions. At about 10 weeks of age pigs were moved to the fattening units, where they were allocated by sex in pens of 8 to 12 individuals and were given ad libitum access to commercial diets. Pigs raised at the same time and in the same farm were considered as one batch. All batches were slaughtered in the same abattoir at around 32 weeks of age (223 days, 11 SD), where carcass weight was recorded, and carcass backfat and loin thickness were ultrasonically measured with an automatic carcass grading equipment (AutoFOM, SFK-Technology, Denmark) at 6 cm off the midline between the third and fourth last ribs. Data used in the second experiment were obtained from a sow farm managed using standard practices, where gilts were monitored for oestrus at 6.5 months of age and then bred on their second detected oestrus. Sows were rebred on their first oestrus after weaning. The sow (n = 430) performance by parity including age at parity, number of piglets born alive, number of weaned piglets and litter weight at weaning (23 days, 2 SD) was recorded for 26 months (10 contemporary year-season farrowing batches). Creep feed was offered to litters from about 10 days after birth until weaning. In a random set of primiparous sows (n = 112), a 15-mL sample of milk was extracted at around the end of the first week of lactation (6 days, 3 SD) from anterior teats following intramuscular oxytocin injection (20 UI; Hormonipra, Spain). Milk samples were stored at -40ºC until analysis. In the third, fourth and fifth experiments, five additional batches of barrows, identically raised as in the first experiment, were used to examine feed intake (one batch, n = 20) and circulating triglycerides (two batches, n = 107) and free fatty acids (two batches, n = 150). In the first batch, feed intake from 70 days (1 SD) to 200 days (1 SD) was individually monitored in 10 full- or half-sibs pairs of different *LEPR* genotype (TT and C−) using an automatic feeding system (IVOG1, Insentec, Netherlands). Sib-pairs were allocated in pens with other 10 individuals. In the other four batches, blood samples

were collected using 8.5-mL serum (BD Vacutainer 1 SST™ II Advance, Franklin Lakes, NJ, USA) or plasma tubes (BD Vacutainer1 K2-EDTA, Franklin Lakes, NJ, USA) by jugular venipuncture at 184 days of age (4 SD) after 12-h fasting (for serum triglycerides) or at 175 days of age (6 SD) after 24-h (for plasma free fatty acids) fasting and were centrifuged (3,000 × g for 10 min at 4 ˚C). Harvested serum and plasma samples were stored at 4 ˚C and –80 ˚C, respectively, for subsequent analysis. Finally, we used genomic DNA from commercial genetic types and European wild boar specimens for monitoring allele segregation. All pigs used in the study were raised and slaughtered in commercial units following applicable regulations and good practice guidelines on the protection of animals kept for farming purposes, during transport and slaughter. The specific protocols for batches in the third series of experiments were approved by the Ethical Committee on Animal Experimentation of the University of Lleida (CEEA 08/01–12 and CEEA 05-04/15).

2.2 Genotyping

All sows and pigs used in the experiments were genotyped for *LEPR* (rs709596309; C>T; on SSC6) single nucleotide polymorphism. Genomic DNA was isolated from biological samples using a standard protocol. Quantification and purity of DNA was assessed by spectrophotometry with a NanoDrop N-1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and the integrity was tested by electrophoresis in agarose gels. The *LEPR* polymorphism was genotyped by real time qPCR (QuantStudio3, Applied Biosystems, Waltham, MA, USA) with High Resolution Melt analysis. Primers used for genotyping the region containing the target [19] are: forward 5'-CAGAGGACCTGAATTTTGGAG and reverse 5'-CATAAAAATCAGAAATACCTTCCAG. The PCR reaction was performed in a final volume of 5 µl including 1x Thermo Scientific™ Luminaris Color HRM qPCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA), 0.4 µM of each primer, and 20 ng of genomic DNA. Thermocycling conditions were 50 °C 2 min, 95 °C 10 min, and 40 cycles of 95 °C 15 sec, 60 °C 1 min, followed by a high-resolution melting curve starting with a denaturation at 95 °C for 15 sec, annealing at 60 °C for 1 min and a slow ramp at 0.015 °C/sec up to 95 °C. High Resolution Melt software v3.1 (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) was used for the melting data analysis and the genotyping of the samples.

2.3 Milk analysis

Milk fat content and fatty acid composition were determined in duplicate using the gravimetric solvent method of Hara and Radin [49] as adapted by Feng et al. [50] followed by gas chromatography [51]. Determinations were performed in duplicate. Milk samples were homogenized in a shaking water bath at 37 ºC, 100 rpm for 5 min, and 500-µl aliquots were extracted. A solution of hexane:isopropanol (3:2 vol/vol) was added into each aliquot and the mixture was stirred for 30 min at room temperature. Then, 2 mL of sodium sulfate solution (12%) were added in the mixture to separate hexane from isopropanol by centrifugation (5 min 3000 rpm) and the upper hexane layer was transferred to a 15 mL tube. The mixture was washed again with 2 mL hexane to recover the remaining lipid fraction and then placed into a rotary evaporator to

remove any exceeding hexane (for 30 min at 40 ºC). The lipid fraction was dried with nitrogen until constant weight to determine fat content. To determine fatty acid composition, the lipid content was resuspended using a solution of boron trifluoride 20% in methanol to obtain fatty acid methyl esters by transesterification. Fatty acid methyl esters were analysed by gas chromatography with a capillary column DB-23 PN (30 m x 0.25 mm, Agilent Technologies, Santa Clara, C, USA) and a flame ionization detector with helium as the carrier gas at 1 mL/min. The quantification was carried out through area normalization using tripentadecanoin (C15:0) as an internal standard. The amount of each fatty acid was expressed as the percentage of each individual fatty acid relative to total fatty acid (S2 Table).

2.4 Triglycerides and free fatty acid quantification

Serum triglyceride levels were measured enzymatically using a commercial kit (GPO-PAP colorimetric enzyme test, Olympus diagnostics, Clare, Ireland). Plasma free fatty acids were extracted following the method described by Hellmuth et al. [52] and quantified by the multiple reaction monitoring (MRM) approach using an ultra-high performance liquid chromatography (UHPLC) on an Acquity UPLC, HSS T3 column (2.1 × 150 mm; 1.8 µm particle size) coupled to a Xevo TQ-S mass spectrometer (Waters, Milford, MA, USA). Data were processed using QuanLynx® software, with palmitic fatty acid-d_{31} as internal standard. Total free fatty acid content in plasma was calculated as the sum of individual fatty acids the content of which was at least 0.4 µg/mL [53].

2.5 Models and distributions

The direct (pig) and maternal (sow) effects for carcass traits (weight, backfat thickness and loin thickness) due to the *LEPR* genotype were estimated independently for each trait. In matrix notation, the animal model was $\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e}$, where **y** is the vector of observations for a trait; **b**, **a** and **e** are the vectors of systematic (pig and sow *LEPR* genotype, batch and age at measurement as a covariate), polygenic and residual effects, respectively; and **X** and **Z** are the incidence matrices that relate **b** and **a** with **y**, respectively. The haplotype additive (a) and dominant (d) effects were tested replacing the genotype effect by the covariates a (TT: 1, CT: 0, CC: -1) and d (TT: 0, CT: 1, CC: 0). The traits were assumed to be conditionally normally distributed as $[\mathbf{y} \mid \mathbf{b}, \mathbf{a}, \mathbf{I}\sigma_e^2] \sim N(\mathbf{Xb} + \mathbf{Za}, \mathbf{I}\sigma_e^2)$, where σ_e^2 is the residual variance and **I** the appropriate identity matrix. The animal effects conditional on the additive genetic variance σ_a^2 were assumed multivariate normally distributed with mean zero and variance $\mathbf{A}\sigma_a^2$, where **A** was the numerator relationship matrix calculated from a two-generation pedigree. Other traits measured only once, either in the pig (feed intake, serum triglycerides and plasma free fatty acids) or in the sow (age at first parity and milk fat content), were analyzed with the same model but only including the genotype of the pig or the sow where applicable. Pigs in a given batch were contemporaneous raised and tested at the same time. Sow records with repeated measurements (number of piglets born alive, number of weaned piglets and litter weight at weaning) were analyzed with a repeatability model that accounts for the polygenic effect of the sow and with the sow *LEPR* genotype, the parity number (from 1 to 6) and

the batch as systematic effects. Litters in a given batch were born in the same year and season. The same distributions as above were assumed.

2.6 Inference

Statistical inferences for each of the above models were derived from the samples of the marginal posterior distribution using a Gibbs sampling Markov chain Monte Carlo algorithm with a chain of 500,000 iterations, where the first 100,000 were discarded and one sample out of 100 iterations retained. Software and source code is available (Legarra et al., 2008; http://genoweb.toulouse.inra.fr/~alegarra/tm_folder [deposited: 3 August 2011]). Flat priors were used for **b**. Convergence was tested using the Z-criterion of Geweke (1992) and visual inspection of convergence plots. Statistical evidence for the direct and maternal effects of the *LEPR* polymorphism was calculated as the marginal posterior probability of the difference between genotype estimates being greater or lower than zero. We considered that there was strong (suggestive) evidence of difference between the genotypes when the probability of that difference being greater or lower than zero was of at least 95% (90%).

3. Results

3.1 Direct and maternal effects on growth

In a first experiment, we proved that the *LEPR* gene is a source of maternal effects. We measured the carcass weight of 413 pigs from 199 sows and 18 sires that were individually traced from birth to slaughter. In line with previous results in this Duroc line [20], we grouped the CC and CT genotypes in a single class (C−) due to the recessive nature of the T allele, which was also evidenced in the set of pigs used here, particularly for carcass weight (S1 Table). The TT sows exerted a negative impact on the carcass weight of their offspring at the end of the growing period of similar extent to the positive direct effect of the TT genotype over each individual (Fig 1*A*). Thus, TT pigs from TT dams (n=62; mean=97.7 kg) were about as heavy as C– pigs from C– dams (n=207; mean=97.6 kg; difference: +0.1 kg; posterior probability of the difference being greater than zero: P(>0) = 0.53), but TT pigs from C– dams (n= 99; mean=100.5 kg) were around 5% heavier (+5.2 kg, P(>0) >0.99) than C– pigs from TT dams (n= 45; mean = 95.3 kg). In contrast, body composition was only influenced by *LEPR* direct effects, with TT pigs gaining more fat (Fig 1*B*) and less lean (Fig 1*C*) than C– pigs. Data collected in the next series of experiments enabled us to show that the thriftier behavior of the TT genotype is behind the antagonism between direct and maternal effects for body weight caused by the *LEPR* gene.

3.2 Maternal influence

Energy intake that exceeds expenditure is the driver of weight gain. Until weaning, the limiting maternal resource for piglet growth is the sow milk production [22, 23]. In a second experiment, which involved 927 weaned litters from TT (n = 133) and C– (n = 337) sows, we demonstrated that TT sows produce less milk than C– sows by indirectly showing that their piglets were lighter at weaning (-150 g, posterior probability of the difference being lower than zero: P(<0) >0.99; mean weight of piglets from C− sows = 5.3 kg; Fig 2*A*). This decline could not be ascribed to differences

in litter size, either at birth (Fig 2*B*) or at weaning (Fig 2*C*), nor to different parity number. Primiparous sows, which produce less milk [24], were evenly distributed across genotypes (51.7% and 47.8% of total litters from TT and C– sows, respectively) and weights were adjusted for parity number. Moreover, piglets from primiparous TT sows were also lighter at weaning compared with those from primiparous C–sows (–132 g, P(<0) >0.99; mean weight of piglets from C− sows = 4.9 kg). The detrimental effect of TT sows on litter weight was likely underestimated, given that TT piglets, which are expected to grow more rapidly, were overrepresented in litters from TT sows compared to C– sows. In line with commercial practice, litter size was equalized by cross-fostering within 24 h of birth and creep feed was offered to all litters from 10 days after birth until weaning. Although genotype was not considered for adoptions, solely 31.5% of the litters received piglets (on average 2.4 piglets) from other litters and, therefore, cross-fostering only partially broke down the correlation between parent and offspring genotypes. On the other hand, creep feed has no effect on growth of early-weaned piglets [25, 26]. Most pigs are non-consumers and, besides, consumers are in fact lighter piglets [27]. We did not find enough evidence that TT sows had lower milk quality in terms of fat content (Fig 2*D*) and fatty acid composition (S2 Table). The negative maternal effect of TT sows on piglet growth arises from saving extra energy at the expense of reproduction. The later age at first parity of TT sows reinforced this assertion (+5.5 d, P(>0) = 0.99; mean of C– sows = 377.3 d; Fig 2*E*).

3.3 Individual influence

This thrifty behavior should be also expected for TT growing pigs. In a last series of experiments, we verified that TT growing pigs showed a more positive energy balance than C– pigs. They were not only more prone to eat more (+20.9 kg of feed, P(>0) = 0.90; mean of C– pigs = 333.4 kg; Fig 2*F*), as already observed in Duroc x Iberian crossbreds [28], but they also burnt off fewer calories. In two *ad hoc* trials we proved that, after fasting, TT pigs exhibited higher circulating levels of triglycerides (+57 μg/mL, P(>0) = 0.97; mean of C– pigs = 347 μg/mL; Fig 2*G*) and lower levels of total free fatty acids (–43 μg/mL, P(<0) >0.99; mean of C– pigs = 179 μg/mL; Fig 2*H*) compared to C– pigs. Even in energy-demanding scenarios, the metabolism of TT pigs favored fatty acid uptake over release.

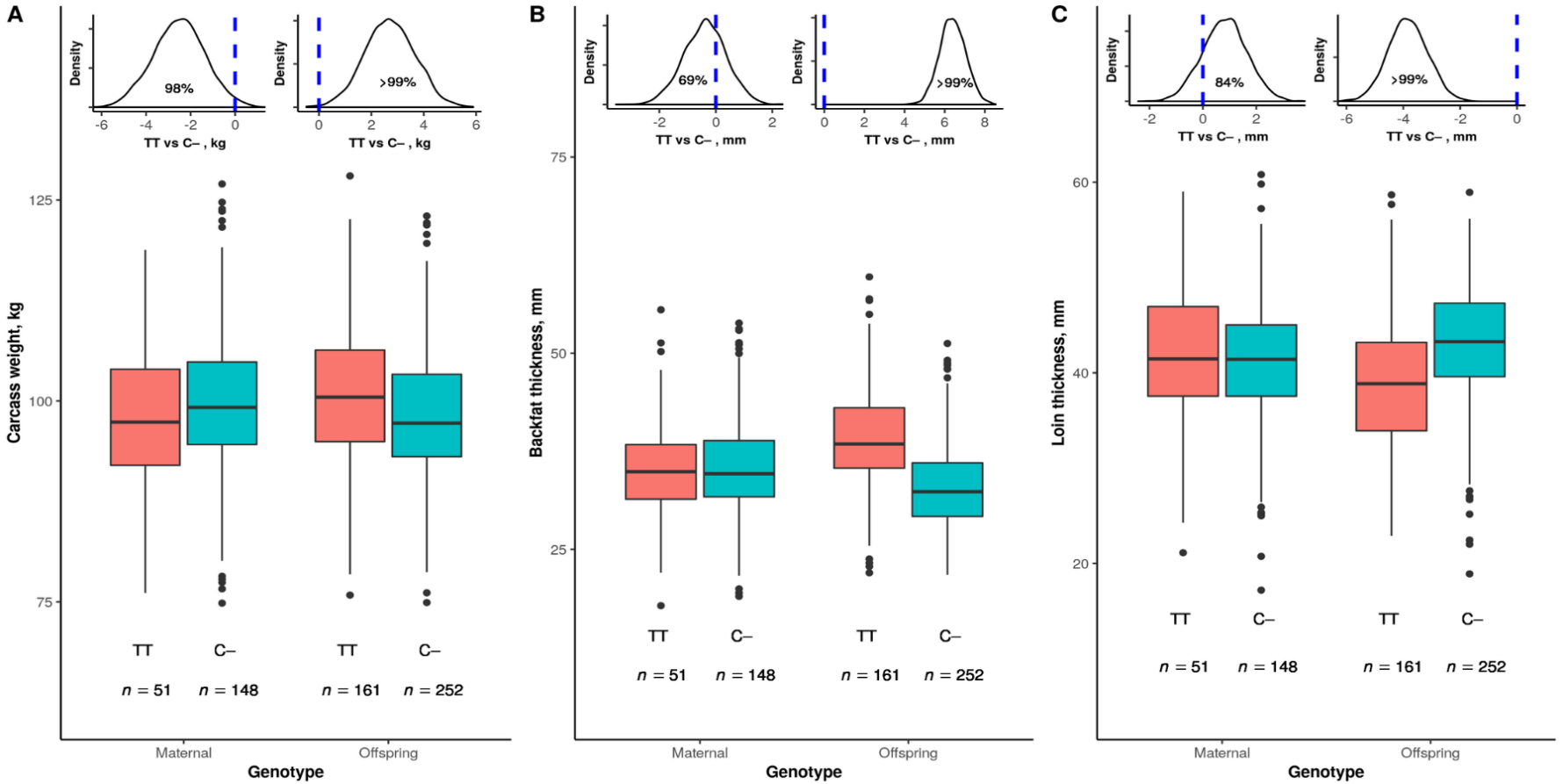

Figure 1. Antagonistic maternal and direct effect of LEPR gene on body weight at the end of the growing period. Boxplot distribution of carcass body weight (A), backfat thickness (B), and loin thickness (C) at 223 days of age by maternal (sow) and offspring (pig) LEPR genotype. Values represented are adjusted for systematic effects. For each trait, the marginal posterior distribution of the difference between TT and C– genotypes is depicted on the top of each panel, with the blue dotted line indicating the zero value (no difference) and the accompanying percentage standing for the posterior probability of TT being higher (area under of the curve at the right side of the line) or lower (area under of the curve at the left side of the line) than C–. LEPR genotypes were considered to differ if this probability value was ≥95%. Sample size (n) is given below each boxplot.

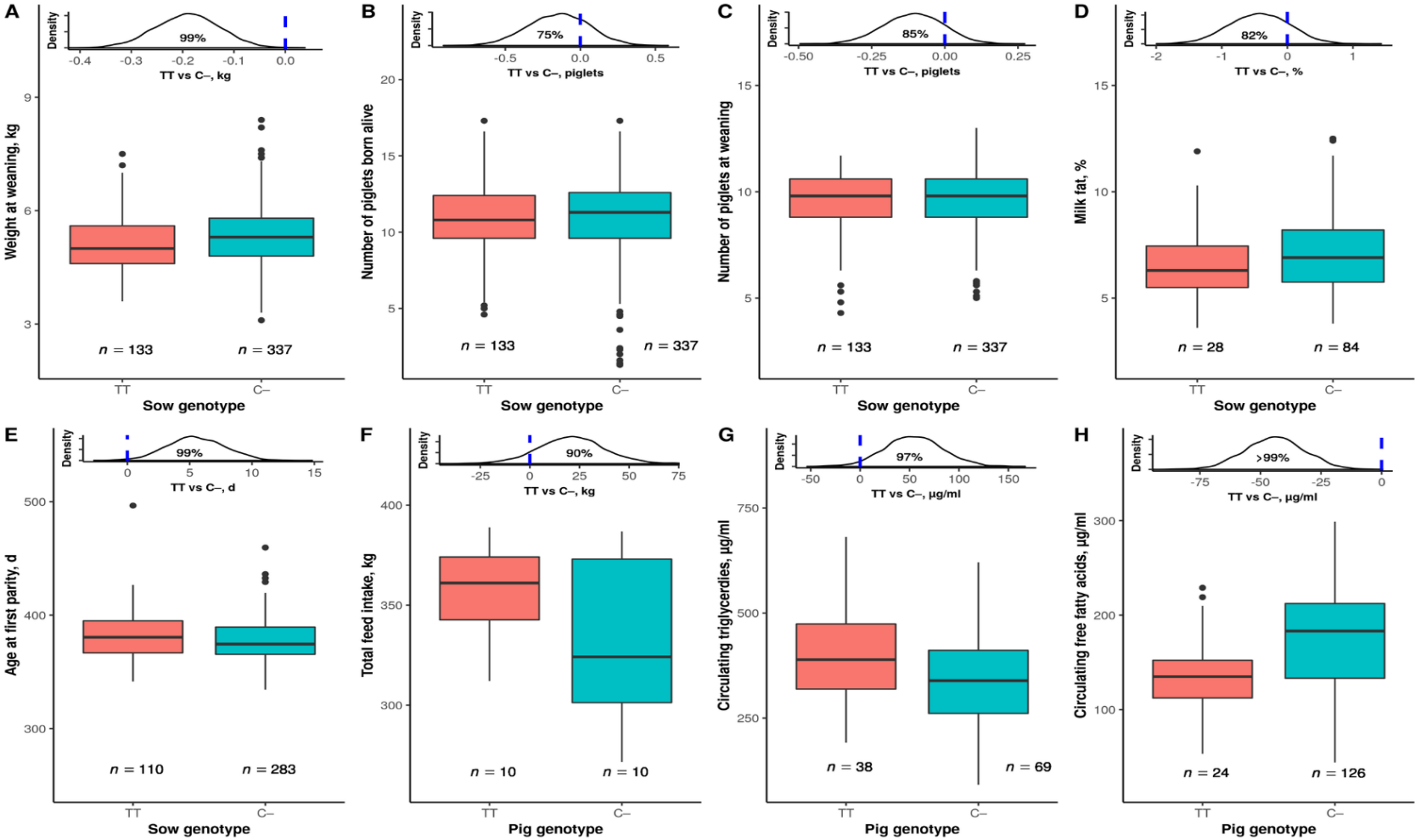

Figure 2. Effects of *LEPR* genotype on maternal and individual body energy traits. Boxplot distribution by the sow *LEPR* genotype for the average weight of piglets at weaning (*A*), number of piglets born alive (*B*) and at weaning (*C*) per litter, fat content in milk at day 6 of lactation (*D*), and age at first parity (*E*), as well as for total feed intake from 70 to 200 days of age (*F*) and blood circulating concentration of triglycerides after a 12-h fast (*G*) and total free fatty acids after a 24-h fast (*H*) in pigs of 184 and 175 days of age, respectively. Data displayed as in Fig. 1

4. Discussion

Direct and maternal sources of variation are usually examined using biometrical models [29, 30]. The model predominantly used postulates that the observed phenotype of an individual is the sum of a phenotypic direct effect due to the individual itself and a phenotypic maternal effect due to its dam [30, 31]. Direct and maternal phenotypes are accommodated into the model as the sum of additive genetic and environmental effects, which are individually fitted making use of available pedigree relationships [32, 33]. The implementation of this model is highly demanding, requiring sizeable datasets including dam-offspring pairs with records [34]. This population structure is difficult to obtain even in large commercial animal populations and thus, for simplicity, maternal effects have mostly been estimated assuming that dam and offspring are not environmentally correlated. Because this may lead to biased and inaccurate estimates, reported estimates of the genetic correlation between direct and maternal effects (often strongly negative) have been questioned, if not considered statistical artifacts [7, 31]. Despite the momentum of genomic discovery, there is still a lack of genetic support for a negative causal link between direct and maternal effects. In this study, using the *LEPR* gene as an example, we provide evidence of genes that simultaneously influence direct and maternal effects with opposite effects on body growth. Maternal effects may appear as a result of maternal imprinting [35], but this is inconsistent with the recessive inheritance of *LEPR* [19, 20] and with findings that point to paternal rather than maternal expression in the region around *LEPR* [14]. Furthermore, results on *LEPR* in particular allow drawing biological and evolutionary consequences.

Many environmental factors affect pre-weaning growth, such as maternal nutrition and feeding regime [36]. Diets for accelerated prepubertal growth rate decrease subsequent milk production as a result of impaired mammary development due to the higher energy demand for growth [37, 38]. In contrast, a high feed intake during gestation is beneficial for offspring performance [36]. However, this only applies if the sow is able to act as an energy buffer. Thus, heavier and fatter sows at parturition only produce heavier piglets at weaning as long as they are able to mobilize during lactation the energy surplus that they accumulated during gestation [11]. During lactation, the adipose tissue shifts towards greater net rates of lipolysis, thus elevating the concentration of circulating free fatty acids and glycerol in blood for use as energy substrates [39]. Furthermore, increased adipose tissue results in greater release and lower clearance of free fatty acids [40]. As a signal of available energy, leptin is expected to enhance lipolytic activity [41] and investment into reproduction [42]. However, TT pigs present the opposite pattern despite their increased levels of leptin relative to C– pigs [20], with less free fatty acids available in blood and impaired maternal environment. In line with individuals with hyperleptinemia due to defective leptin receptor signaling, the T allele attenuates but not inactivates [13] the function of leptin as a mediator between self-maintenance and offspring investment [42]. In this trade-off, the TT sows tip the balance towards self-maintenance, and by doing so, they indirectly outsource part of their reproductive success to the offspring. The absence of an antagonistic maternal effect for fat and lean mass would explain why direct effects are more easily detected in body composition [19,20] than in growth traits, for which direct effects only become entirely apparent in pigs raised by sows

with genotype other than theirs. Similarly, direct effects for body weight stand out at advanced ages as adipose tissue develops. Unlike nutrition-induced changes, the effects of *LEPR* are genetics-driven and, as such, have implications that extend over generations.

Growth and energy balance involve a set of traits potentially subjected to selection response and evolutionary change. As causal of both direct and maternal effects, *LEPR* benefits reproductive success by providing a system that allocates resources to the sow or the offspring according to whether selective pressure is stronger before or after weaning. In this way, *LEPR* contributes to uplift the population carrying capacity for a given environment. The fact that the T allele is fixed in the Iberian breed, which has traditionally been reared outdoors under limited and fluctuant feed resources [43], and present at a relative high frequency in the Duroc lines wherein selective breeding favored heavy and fat pigs [44] (like Duroc-line 1 in S3 Table), can be interpreted as proof-of-principle of the plasticity of the system. Weaning covers the transition period where piglets switch from feeding on sow milk to solid feed. In natural conditions, this is a gradual process that may last until up to 22 weeks of age [45]. Feed restrictions during this time prompt piglets to seek out food earlier to compensate for a lower energy intake from milk [26]. In this setting, the T allele, with a favorable direct effect for growth and fat accumulation, has a greater chance of increasing to a high frequency. Contrarily, in most commercial lines, where pigs are selected for lean efficiency under high feeding and management standards, the T allele, associated with decreased capacity for maternal ability and leanness, is no longer beneficial and therefore tends to be swept away (S3 Table). Similar mutations may exist in other pig breeds and species. Significantly, the *LEPR* promoter region shows great haplotypic diversity in wild boar [46], which can persist in very diverse habitats. Evidences of interaction between maternal and direct *LEPR* effects for body weight and fat mass have also been reported in humans [47]. The *LEPR* model provides a sensible biological mechanism for transgenerational energy allocation that could be extended with other genes.

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Supplementary Information

Table S1. Difference between *LEPR* (rs709596309 C>T) genotypes and additive (a) and dominant effects for investigated traits.

Trait	Genotype difference		Genetic effects	
	TT-CT	**CC-CT**	**a**	**d**
Direct effects				
Carcass weight, kg	3.0 (**1.00**)	0.2 (0.57)	1.9 (**1.00**)	-1.9 (**0.01**)
Backfat thickness, mm	6.0 (**1.00**)	-1.8 (**0.04**)	4.6 (**1.00**)	-4.1 (**0.00**)
Loin thickness, mm	-3.6 (**0.00**)	1.2 (0.87)	-2.8 (**0.00**)	2.4 (**1.00**)
Weight at weaning, g	-180 (**0.00**)	20 (0.59)	-110 (**0.00**)	100 (**0.96**)
No. of piglets born alive	-0.2 (0.23)	-0.1 (0.40)	-0.1 (0.31)	0.1 (0.73)
No. of piglets at weaning	-0.1 (0.26)	0.1 (0.66)	-0.1 (0.18)	0.02 (0.60)
Age at first parity, days	5.8 (**0.99**)	1.6 (0.71)	2.5 (**0.94**)	-4.13 (**0.02**)
Milk fat, %	-0.2 (0.33)	0.2 (0.62)	-0.2 (0.28)	0.1 (0.56)
Plasma triglycerides, µg/ml	53.3 (**0.95**)	-0.9 (0.49)	3 (**0.94**)	-3.2 (0.14)
Plasma free fatty acids, µg/ml	-53.6 (**0.00**)	-12.8 (0.11)	-13.0 (**0.02**)	21.2 (**0.98**)
Maternal effects				
Carcass weight, kg	-2.3 (**0.04**)	1.6 (0.85)	-2.0 (**0.01**)	0.84 (0.78)
Backfat thickness, mm	-0.8 (0.18)	-0.9 (0.20)	-0.1 (0.44)	0.5 (0.74)
Loin thickness, mm	1.0 (0.88)	0.6 (070)	0.4 (0.72)	-0.6 (0.21)

Mean of the estimated marginal posterior distribution of the difference between *LEPR-TT* and *LEPR-CC* genotypes with respect to the *LEPR-CT* genotype (TT-CT and CC-TT, respectively) and additive (a) and dominant (d) values for the *LEPR-T* allele (in parentheses, the probability of each value being greater than zero) for direct and maternal effects.

Table S2. Effects of *LEPR* (rs709596309) genotype on sow milk fatty acid composition.

Fatty acid, %	Mean	SD	Difference TT − C−	P>0
Capric acid (C10:0)	0.2	0.1	-0.03	0.12
Lauric acid (C12:0)	0.4	0.3	-0.03	0.27
Myristic acid (C14:0)	3.5	0.9	0.13	0.78
Myristoleic acid (C14:1n5)	0.2	0.1	-0.02	0.21
Palmitic acid (C16:0)	28.1	3.5	0.87	0.92
Palmitoleic acid (C16:1n7)	8.7	2.7	0.36	0.76
Stearic acid (C18:0)	4.2	0.8	-0.18	0.13
Vaccenic acid (C18:1n7)	2.5	0.5	-0.17	0.01
Oleic acid (C18:1n9)	33.9	5.6	-0.52	0.32
Linoleic acid (C18:2n6)	14.7	3.7	-0.48	0.23
α-linolenic acid (C18:3n3)	1.1	0.3	0.01	0.54
Eicosanoid acid (C20:1n9)	0.4	0.2	<0.01	0.49
Eicosadienoic acid (C20:2n6)	1.0	0.5	-0.06	0.20
Saturated fatty acids	36.6	4.0	0.75	0.85
Monounsaturated fatty acids	46.1	4.7	-0.32	0.36
Polyunsaturated fatty acids	17.3	3.4	-0.45	0.25

Mean of the estimated marginal posterior distribution of the difference between sow *LEPR* genotypes (TT, n=28, and C−, n=84) and probability of this difference being greater than zero (P>0) for fatty acid composition (% of total fatty acids) of sow milk. *LEPR* genotypes were considered to differ if this probability value was <0.05 (C− is higher than TT) or >0.95 (TT is higher than C−).

Table S3. Frequency of *LEPR* (rs709596309; C>T) genotypes and gene frequency of the allele T (q) in different pig breeds and crosses.

		***LEPR* genotype**			
Genetic type	**No. of pigs**	**CC**	**CT**	**TT**	**q**
Iberian	92	0	0	92	1.00
Duroc (line 1)	1135	345	539	251	0.46
Duroc (line 2)	25	13	10	2	0.28
Landrace	33	32	0	1	0.03
Pietrain	29	17	11	1	0.22
Landrace x Large White	142	122	20	0	0.07
Landrace x Duroc	12	9	3	0	0.12
Wild boar	7	7	0	0	0.00

Table S4. Summary of experimental data.

Experiment	Trait	No. of batches	No. of records by *LEPR* genotype		Mean	SD
			TT	C–		
1	Carcass weight, kg	12	161	252	99.4	9.9
	Backfat thickness, mm	12	161	252	35.4	7.9
	Loin thickness, mm	12	161	252	41.1	7.1
2	No. of sows	10	133	337	-	-
	No. of parities	10	248	679	-	-
	Weight at weaning, kg	10	248	679	5.2	0.9
	No. of piglets born alive	10	248	679	11.0	2.4
	No. of piglets at weaning	10	248	679	9.7	1.3
	Age at first parity, days	8	110	283	378.8	22.1
	Milk fat, %	3	28	84	7.0	2.8
3	Total feed intake, kg	1	10	10	345.5	35.8
4	Plasma triglycerides, µg/ml	2	38	69	367.0	139.5
5	Plasma free fatty acids, µg/ml	2	24	126	174.9	58.2

VII- Capítulo IV

Efecto del genotipo *SCD* sobre el tamaño de la camada y el peso al destete de los lechones

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The effect of the *SCD* genotype on litter size and weight at weaning

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1. Introduction

Sire lines used in crossbreeding schemes and purebred specialized lines for premium markets are often selected for meat quality traits. Since these traits are expensive to measure and cannot be recorded in vivo, the prediction of their genetic value is based on some form of marker-assisted or weighted genomic selection (Pena et al., 2016). One of these markers is a recessive missense mutation in the leptin receptor [*LEPR*] gene (Óvilo et al., 2005) that increases intramuscular fat content and saturated fatty acids and decreases polyunsaturated saturated fatty acids (Ros-Freixedes et al., 2016; Suárez-Mesa et al., 2021). We have recently shown that this *LEPR* polymorphism is also at the origin of a maternal effect that influences body weight, particularly at weaning (Solé et al., 2021a). Maternal effects, which include milk yield and other mothering abilities, are the main limiting genetic factor to improve piglet survival during the nursing period (Nguyen et al., 2021). Thus, the number and weight of weaned piglets per sow are crucial for reproductive success and, even for a sire line, reproductive success is important issue for the overall line efficiency.

Another genetic marker with a major effect on fat quality in pigs is a polymorphism in the promoter of the steatoryl-CoA desaturase [*SCD*] gene (Estany et al., 2014), which influences the monounsaturated fatty acids [MUFA] content in the adipose tissue. The SCD is the rate-limiting enzyme required for the biosynthesis of MUFA, catalyzing in particular the desaturation of palmitic acid (C16:0) and stearic acid (C18:0) to palmitoleic acid (C16:1n-7) and oleic acid (C18:1n-9), respectively. In connection with *SCD*, there is an exonic polymorphism in the diacylglycerol O-acyltransferase 2 [*DGAT2*] gene that specifically impacts C16:1n-7 (Solé et al., 2021b). The DGAT2 is the enzyme that, together with DGAT1 and SCD, intervenes in the final step of the biosynthesis of triglycerides. Although several studies in cattle associate *SCD* with fatty acid composition (Li et al., 2016; Gu et al., 2019) and *DGAT1* with milk yield and composition (Grisart et al., 2002; Tabaran et al., 2015), to our knowledge similar effects have not been investigated in pigs yet. The aim of this study was to evaluate potential side-effects of *SCD* on sow productivity and of *SCD* and *DGAT2* on milk fat content and fatty acid composition.

2. Material and methods

2.1 Animals and phenotypes

All pigs used in the study were raised and slaughtered in commercial units following applicable regulations and good practice guidelines on the protection of animals kept for farming purposes, during transport and slaughter. Reproductive data were obtained from a Duroc nucleus farm managed using standard practices, where gilts were monitored for oestrus at around 6.5 months of age and then bred on their second detected oestrus. Sows were rebred on their first

oestrus after weaning. The performance of 4,121 parities from 1,246 sows was monitored during three and a half years (15 contemporary year-season farrowing batches). Age at farrowing, number of piglets born alive, number of weaned piglets and litter weight at weaning (23 days, 2 SD) was recorded. In line with commercial practice, litter size was equalized by cross-fostering within 24 h of birth and creep feed was offered to litters from about 10 days after birth until weaning. In a random set of 99 primiparous sows from six batches, a 15-mL sample of milk was extracted at around the end of the first week of lactation (4.2 days, 1.6 SD) from anterior teats following intramuscular oxytocin injection (20 UI; Hormonipra, Spain). Milk samples were stored at -40ºC until analysis. Milk fat content and fatty acid composition were determined in duplicate using the gravimetric solvent method of Hara and Radin (1978) as adapted by Feng and Garnsworthy (2004) followed by quantitative gas chromatography (Bosch et al., 2009). The amount of each fatty acid was expressed as the percentage of each individual fatty acid relative to total fatty acids. The proportion of saturated (SFA: C10:0, C12:0, C14:0, C16:0, C18:0, C20:0), monounsaturated (MUFA: C14:1n-5, C16:1n-7, C18:1n-7, C18:1n-9, C20:1n-9) and polyunsaturated (PUFA: C18:2n-6, C18:3n-3, C18:3n-6, C20:2n-6) fatty acids were calculated. Complementarily, 1,070 barrows were raised in 15 batches under commercial conditions (Solé et al., 2021b) until 30 weeks of age (207 days, SD 8), at which time they were weighted and their backfat and loin thickness were ultrasonically measured at 5 cm off the midline at the position of the last rib (Renco, Minneapolis, USA). A few days later (211 days, 9 SD), all pigs in a batch were slaughtered at a time in an abattoir equipped with a carbon dioxide stunning system, where the carcass backfat and loin thickness at 6 cm off the midline between the third and fourth last ribs were predicted with an automatic carcass grading equipment (AutoFOM, Frontmatec Group, Denmark).

2.2 Genotyping

All sows and barrows used in the experiments were genotyped for the *SCD* (rs80912566 T>C, SSC14) single nucleotide polymorphism [SNP]. A subset of 732 sows plus all the barrows were also genotyped for the *LEPR* (rs709596309 C>T, SSC6) SNP. Sows with milk records were additionally genotyped for *DGAT2* (ss7315407085 G>A, SSC9). Genomic DNA was isolated from blood and ear notches (sows) and muscle (pigs) using standard protocols. Quantification and purity of DNA was assessed by spectrophotometry with a NanoDrop N-1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and the integrity was tested by electrophoresis in agarose gels. The three polymorphisms were genotyped by High-Resolution Melt analysis (Luminaris Colour HRM Master Mix, Thermo Scientific) in a real-time thermocycler (QuantStudio3, Applied Biosystems) using 20 ng of genomic DNA, 0.4 µM of each primer in final volume of 5 µL. Thermocycling conditions were 50 °C 2 min, 95 °C 10 min, and 40 cycles of 95 °C 15 sec, 60 °C 1 min, followed by a high-resolution melting curve starting with a denaturation at 95 °C for 15 sec, annealing at 60 °C for 1 min and a slow ramp at 0.015 °C/sec up to 95 °C. High Resolution Melt software v3.1 (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) was used for the melting data analysis and the genotyping of the samples. Primers used to genotype the *LEPR* rs709596309 and *DGAT2* ss7315407085 polymorphisms are described in Ros-Freixedes et al (2016) and Solé et al (2021b), respectively. The *SCD* rs80912566 SNP was genotyped with primers

5'- AGCGAATAAAAGGGGTCAGAGG-3' and 5'-TTAAAGGCTAGAGCTGGCAGTG-3', which amplify a 64 bp-long amplicon.

2.3 Statistical analyses

The effect of the *SCD* genotype on reproduction traits (number of total piglets born, born alive and weaned, and litter and average piglet weight at weaning) was estimated using a repeatability model that accounted for the effect of the sow and with the sow *SCD* (TT, CT and CC) and *LEPR* genotypes (TT and C−, which includes CC and CT), the parity number (from 1 to 8) and the batch (15 batches) as systematic effects. The duration of lactation was included as a covariate for weaning weight. In matrix notation, the model was $\mathbf{y} = \mathbf{Xb} + \mathbf{Zs} + \mathbf{e}$, where **y** is the vector of observations for a trait; **b**, **s** and **e** are the vectors of systematic, sow and residual effects, respectively; and **X** and **Z** are the incidence matrices that relate **b** and **s** with **y**, respectively. The traits were assumed to be conditionally normally distributed as $[\mathbf{y} \mid \mathbf{b}, \mathbf{s}, \mathbf{I}\sigma e2] \sim N(\mathbf{Xb} + \mathbf{Zs}, \mathbf{I}\sigma e2)$, where σe2 is the residual variance and **I** the appropriate identity matrix. The sow effects conditional on the sow variance σs2 were assumed multivariate normally distributed with mean zero and variance **I**σs2. The sow and the parity number were removed from the model when only data on first parities were used. A simple animal model was used for milk (fat content and fatty acid composition) and production (body weight and backfat and loin thickness) traits. The model for milk traits included the batch (6 batches), the sow *SCD*, *LEPR* and *DGAT2* (GG, AG and AA) genotypes and the duration of lactation and the milk fat content (for fatty acids) as covariates, whereas the model for production traits included the batch (15 batches), the pig *SCD* and *LEPR* genotype and the age at measurement as a covariate.

Genotype effects were estimated in a Bayesian setting with the TM software (Legarra et al., 2008; http://genoweb.toulouse.inra.fr/~alegarra/tm_folder [deposited: 3 August 2011]). Statistical inferences for each of the above models were derived from the samples of the marginal posterior distribution using a Gibbs sampling Markov chain Monte Carlo algorithm with a chain of 500,000 iterations, where the first 100,000 were discarded and one sample out of 100 iterations retained. Flat priors were used for **b** and variance components. Convergence was tested using the Z-criterion of Geweke (1992) and visual inspection of convergence plots. Statistical evidence for the effects of the *LEPR* polymorphism was calculated as the marginal posterior probability of the difference between genotype estimates being greater ($P_{[>0]}$) or lower ($P_{[<0]}$) than zero. We considered that there was strong (suggestive) evidence of difference between the genotypes when the probability of that difference being greater or lower than zero was of at least 0.95 (0.90).

3. Results and discussion

The *SCD* SNP did not have a negative impact on litter size and weight at weaning, rather the opposite (Table 1). Using all parities, the results indicate that the *SCD*-T allele, the one that enhances MUFA, increased litter size at birth (*SCD*-TT sows had 0.33 piglets born alive more per litter than *SCD*-CT sows, P[>0]=0.99). The *SCD*-T allele also decreased the number of stillborn piglets (*SCD*-TT sows had 0.16 stillborn piglets less per litter than *SCD*-CT sows, P[<0]=0.98), with no effect on the weight of piglets at weaning. A similar trend was observed if litter size was

not adjusted for the *LEPR* SNP, thereby using all sows genotyped for the *SCD* SNP (593, 603 and 50 sows for *SCD*-TT, CT and CC, respectively, accounting for 4,121 parities). With this set of data, *SCD*-TT sows had 0.14 piglets born alive more (P[>0]=0.92) and 0.09 stillborn piglets less (P[<0]=0.95) per litter. Moreover, *SCD* genotypes did not differ for the age at the first farrowing and reproduction rate (the average number of parities in two years was 3.97, for *SCD*-CT sows, and 3.85, for *SCD*-TT sows). Taken as a whole, these results would confirm that the *SCD* SNP is not expected to entail adverse consequences in the reproductive lifetime of a breeding sow. This contrasts with the negative effects of *LEPR*-T on reproduction of homozygous sows. In line with previous findings (Solé et al., 2021a), results obtained here indicate that *LEPR*-TT sows, as compared with *LEPR*-C– sows, showed a delay in the age at first farrowing (5.7 days, P[>0]=0.99) and a reduction in the litter size at birth (-0.23 piglets born alive per litter, P[<0]=0.94). To our knowledge, no studies so far have investigated the effects of *SCD* on reproduction in pigs. However, recent research in cattle indicates that enhanced *SCD* activity can be beneficial for oocyte development. Thus, Aardema et al (2017) observed that the desaturation of C18:0 into C18:1n-9 via *SCD* in cumulus cells would protect the oocyte against toxicity by saturated free fatty acids. On the other hand, Asadollahpour et al., (2014) reported a preliminary evidence that a SNP in the bovine *SCD* gene could be associated with pregnancy length, dry days and open days.

The differences between *SCD* genotypes for litter size in the first farrowing were in line with the results obtained using all parities (Table 1). However, a further look at the effects in the first farrowing reveals that the *SCD* SNP is not neutral with respect to weaning weight. In particular, piglets from the first farrowing of *SCD*-TT sows weighted 126 g less at weaning (P[<0]=0.97) than those from *SCD*-CT sows. The negative impact of the *SCD*-TT sows on weaning weight was maintained after removing the *LEPR* genotype from the model (-137 g, P[<0] =0.98). This effect could be attributed to differences in litter size at birth, but not exclusively. First, because *SCD*-TT sows only weaned lighter piglets at first parity while they had larger litters in all their parities and, second, because it was still detected after adjusting for the number of total piglets born and age at farrowing. Moreover, as litter size was equalized during lactation (Table 1), the effect of increased litter size on piglet weight at weaning reduced to 22 g less per additional piglet born, P[<0] >0.99). Cross-fostering was done regardless of the genotype, so it is most likely that more piglets from *SCD*-TT sows, with larger litters at birth, have been transferred to *SCD*-CT litters than vice versa. If so, the negative effect of primiparous *SCD*-TT sows on weaning weight could have been even greater than the estimated here.

The negative effect of primiparous *SCD*-TT sows on weaning weight, although smaller in magnitude, was also detected using all available sows, i.e. also including those that were genotyped for *SCD* but not for *LEPR* (piglets from *SCD*-TT sows weighted 89 g less at weaning than piglets from *SCD*-CT sows, $P_{[<0]}$=0.98). Contrarily, it vanished when only the litters that were not genotyped for *LEPR* (205 from *SCD*-TT sows and 182 *SCD*-CT sows) were analysed (-10 g, $P_{[<0]}$=0.57). This finding, given that most of these litters were from the earliest five batches, suggests that *SCD-TT* gilts in these batches were favoured by some temporary effect. Interestingly, the average age at first farrowing in the first five batches occurred 19 days later than in the other

of batches (396 days vs 377 days), which in practice could mean that skipping a heat may be beneficial for the *SCD*-TT gilts. All in all, these results indicate that, despite the lack of unfavourable effects of the *SCD* SNP over the sow lifetime performance, caution should be taken in the management of *SCD*-T carrier gilts, particularly if the *SCD*-TT genotype combines with the *LEPR*-TT genotype (Fig.1). Findings here confirm that *LEPR*-TT sows produce lighter pigs than *LEPR*-C– sows, both in the first (-154 g, $P_{[<0]}>0.99$) and in all parities (-126 g, $P_{[<0]}>0.01$). They also show that the effect of the *SCD* SNP is independent of the *LEPR* genotype. The detrimental effect of primiparous *SCD*-TT sows on weaning weight as compared to that of *SCD*-C– sows (CT and CC sows were included in a single group due to the limited number of CC sows) was maintained between *LEPR* genotypes (-125 g, $P_{[<0]}=0.87$, if *LEPR* is TT, and -126 g, $P_{[<0]}=0.96$, if *LEPR* is C–, Fig. 1). As a result, the effect of one of the genotypes adds to the other and thus the piglets in litters from primiparous *SCD*-TT/*LEPR*-TT sows weighted 277 g less at weaning ($P_{[<0]}>0.99$) than piglets in litters from *SCD*-C–/*LEPR*-C– sows.

We have seen that the unfavourable effect of *LEPR*-TT sows on weaning weight can be attributed to a lower capacity of these sows to mobilize body reserves (Solé et al., 2021a), which may lead to impaired milk production. However, this does not seem the case for the *SCD*-T allele, whose potential negative effect on weaning weight is restricted to the first farrowing. Moreover, there is no evidence that the *SCD* genotype has a relevant impact on circulating free fatty acids after fasting (Tor et al., 2021), the source from which energy reserves are mobilized to organs and tissues. Alternatively, we have investigated whether this effect could be explained by either body condition at puberty or milk quality. From examining production data, we found that *SCD*-TT pigs presented lower body condition at the end of fattening (Table 2), with 0.62 mm less backfat thickness ($P_{[<0]}=0.96$) and 0.82 mm less loin thickness ($P_{[<0]}=0.98$) than contemporaneous *SCD*-CT pigs. Also, although only using data from 28 *SCD*-TT and 35 *SCD*-C– barrows that were individually monitored using automatic feeders, we found evidence that in this period the *SCD*-TT pigs consumed less feed than the *SCD*-C– pigs (18.6 kg less from 70 d to 200 d of age, $P_{[<0]}= 0.99$). On the other hand, minor changes in milk content and composition were observed in lactating primiparous sows (Table 3). Thus, as compared to *SCD*-C– sows, the milk of *SCD*-TT sows tended to have less fat (0.73%, $P_{[>0]}=0.10$) but more MUFA, especially for the C18:1n-9/C18:0 ratio, which increased with the T allele content (Table 3). In contrast, neither the *DGAT2* (Table 3) nor the *LEPR* (Solé et al., 2021a) SNP substantially altered milk characteristics. In particular, we did not observe, as in muscle, that the milk of *DGAT2-GG* sows was richer in C16:1n-7 (*DGAT2*-GG sows had 0.05% less C16:1n-7 than *DGAT2*-AA sows, $P_{[<0]}=0.47$) nor had higher values of the ratio of C16:1n-7 to C16:0 (the value of the C16:1n-7 to C16:0 ratio was 0.06 (x10) lower in *DGAT2*-GG sows than in *DGAT2*-AA sows, $P_{[<0]}=0.37$). Milk fatty acid profile tends to reflect the maternal diet composition during gestation (Laws et al., 2009). However, fatty acids in milk also originate from de novo synthesis in the mammary gland or, particularly in case of negative energy balance, from fatty acids released from body fat stores. The fact that the effect of the *SCD*-T allele as a desaturation enhancer can be detected in milk could be interpreted as an indirect evidence that dietary fatty acids are less prevalent in the milk of sows carrying this allele. Thus, while differences

between *SCD* genotypes for milk fat content could be attributed to differential accumulation of body reserves prior to the first lactation, the differences in milk fatty acid composition can be explained by a differential desaturase activity in the mammary gland, in line with results in dairy cows, where the *SCD* gene has been associated with the MUFA content in milk (Mele et al., 2007; Li et al., 2016).

Table 1. Mean (standard deviation) of the marginal posterior distribution of the difference between *SCD* and *LEPR* genotypes for litter size and weight in all parities and in the first parity.

	SCD			***LEPR***
Trait	**Mean TT**	**Mean TT-CT**	**Mean TT-CC**	**Mean TT-C–**
All parities				
No. of sows	331 (TT)	357 (CT)	44 (CC)	
No. of parities	904	1,101	135	
No. of total piglets born	12.1	0.25 (0.15) [b]	0.20 (0.30)	-0.21 (0.15) [c]
No. of piglets born alive	11.4	0.33 (0.14) [a]	0.26 (0.28)	-0.23 (0.14) [c]
No. of piglets stillborn	0.7	-0.16 (0.08) [b]	-0.08 (0.16)	-0.10 (0.08)
No. of weaned piglets	9.6	0.00 (0.07)	0.07 (0.15)	-0.07 (0.08)
Litter weight at weaning, kg	52.3	-0.16 (0.57)	-0.08 (1.20)	-1.73 (0.59) [a]
Piglet weight at weaning, g	5,430	-5 (41)	-23 (85)	-126 (43) [a]
First parity				
No. of sows	290 (TT)	298 (CT)	34 (CC)	
Age at farrowing, days	376.8	1.2 (1.7)	-2.9 (3.7)	5.7 (1.8) [a]
No. of total piglets born	11.3	0.21 (0.20)	0.66 (0.43) [b]	0.02 (0.21)
No. of piglets born alive	10.6	0.23 (0.20)	0.74 (0.43) [b]	-0.08 (0.21)
No. of piglets stillborn	0.6	-0.18 (0.16)	0.09 (0.28)	-0.18 (0.15)
No. of weaned piglets	9.5	-0.00 (0.12)	0.22 (0.26)	-0.07 (0.13)
Litter weight at weaning, kg	44.8	-1.30 (0.82) [c]	-0.38 (1.78)	-2.02 (0.85) [a]
Piglet weight at weaning, g	4,761	-126 (63) [c]	-128 (137)	-155 (65) [a]

[a,b,c] Posterior probability of the difference between genotypes being greater (if positive) or lower (if negative) than zero is 0.99 ([a]), 0.95 ([b]) and 0.90 ([c]).

Table 2. Mean (standard deviation) of the marginal posterior distribution of the difference between *SCD* genotypes for live and carcass weight and fatness.

	***SCD* genotype**		
Trait	**Mean TT**	**Mean TT-CT**	**Mean TT-CC**
Live measurements (207 d of age)			
No. of pigs	184 (TT)	447 (CT)	254 (CC)
Body weight, kg	123.7	-1.01 (0.98)	-0.65 (1.11)
Backfat thickness, mm	21.2	-0.62 (0.36) [b]	-0.27 (0.41)
Loin thickness, mm	47.2	-0.82 (0.41) [b]	-1.01 (0.46) [a]
Carcass measurements (211 d of age)			
No. of pigs	253 (TT)	532 (CT)	285 (CC)
Carcass weight, kg	95.3	-0.87 (0.74)	-0.57 (0.85)
Backfat thickness, mm	26.1	-0.16 (0.35)	0.38 (0.40)
Loin thickness, mm	44.4	-0.25 (0.60)	-1.30 (0.68)

[a,b] Posterior probability of the difference between genotypes being greater (if positive) or lower (if negative) than zero is 0.99 ([a]) and 0.95 ([b]).

Table 3. Mean (standard deviation) of the marginal posterior distribution of the difference between *SCD* and *DGAT2* genotypes for for fat content and fatty acid composition in milk.

	***SCD* genotype**			***DGAT2* genotype**	
Trait [1]	**Mean $_{TT}$**	**Mean $_{TT-CT}$**	**Mean $_{TT-CC}$**	**Mean $_{GG-GA}$**	**Mean $_{GG-AA}$**
No. of sows	39 (TT)	50 (CT)	10 (CC)	50 (GA)	18 (AA)
Milk fat, % DM[A]	6.82	-0.63 (0.59)	-1.50 (0.91) [b]	-0.15 (0.50)	-0.32 (0.63)
Fatty acid, % FA[B]					
C14:0	3.1	0.14 (0.19)	-0.15 (0.29)	-0.11 (0.17)	0.05 (0.21)
C16:0	26.7	0.11 (0.75)	-1.31 (1.16)	0.20 (0.67)	0.22 (0.84)
C18:0	4.3	-0.28 (0.22) [c]	-0.36 (0.33)	0.20 (0.19)	0.10 (0.23)
SFA	34.9	-0.08 (0.86)	-1.91 (1.32) [c]	0.24 (0.74)	0.36 (0.92)
C16:1n-7	8.5	1.21 (0.67) [b]	0.21 (1.03)	-0.05 (0.57)	-0.05 (0.71)
C18:1n-9	35.4	-0.75 (1.32)	3.28 (2.05) [b]	-0.13 (1.19)	1.03 (1.48)
C18:1n-7	2.7	0.17 (0.09) [b]	0.27 (0.14) [b]	0.06 (0.08)	0.13 (0.10) [c]
MUFA	47.5	0.44 (1.05)	3.61 (1.63) [a]	-0.14 (0.89)	1.29 (1.10)
C18:2n-6	15.5	-0.34 (0.75)	-1.41 (1.17)	-0.12 (0.66)	-1.53 (0.81) [b]
C18:3n-3	1.1	-0.02 (0.06)	-0.11 (0.09)	-0.01 (0.05)	-0.12 (0.06) [b]
PUFA	17.7	-0.42 (0.83)	-1.73 (1.29) [c]	-0.10 (0.72)	-1.74 (0.87) [b]
Fatty acid ratio					
C16:1n-7/C16:0 (x10)	3.1	0.42 (0.19) [a]	0.17 (0.29)	-0.06 (0.17)	-0.06 (0.21)
C18:1n-9/C18:0	8.5	0.46 (0.35) [c]	1.49 (0.54) [a]	-0.39 (0.30)	0.02 (0.37)
(C16:1n-7+C18:1n-7) /C16:0 (x10)	4.2	0.42 (0.17) [a]	0.28 (0.26)	-0.03 (0.15)	0.05 (0.19)
MUFA/SFA	1.4	0.01 (0.06)	0.19 (0.09) [b]	0.00 (0.05)	0.04 (0.06)

[A] DM: dry matter;[B]SFA: saturated fatty acids (C10:0 + C12:0 + C14:0 + C16:0 + C18:0 + C20:0); MUFA: monounsaturated fatty acids (C14:1n-5 + C16:1n-7 + C18:1n-9 + C18:1n-7 + C20:1n-9); PUFA: polyunsaturated fatty acids (C18:2n-6 + C18:3n-3 + C18:3n-6 + C20:2n-6). Only fatty acids with a pertentage greater than 1% are given.

[a,b,c] Posterior probability of the difference between genotypes being greater (if positive) or lower (if negative) than zero is 0.99 ([a]), 0.95 ([b]) and 0.90 ([c]).

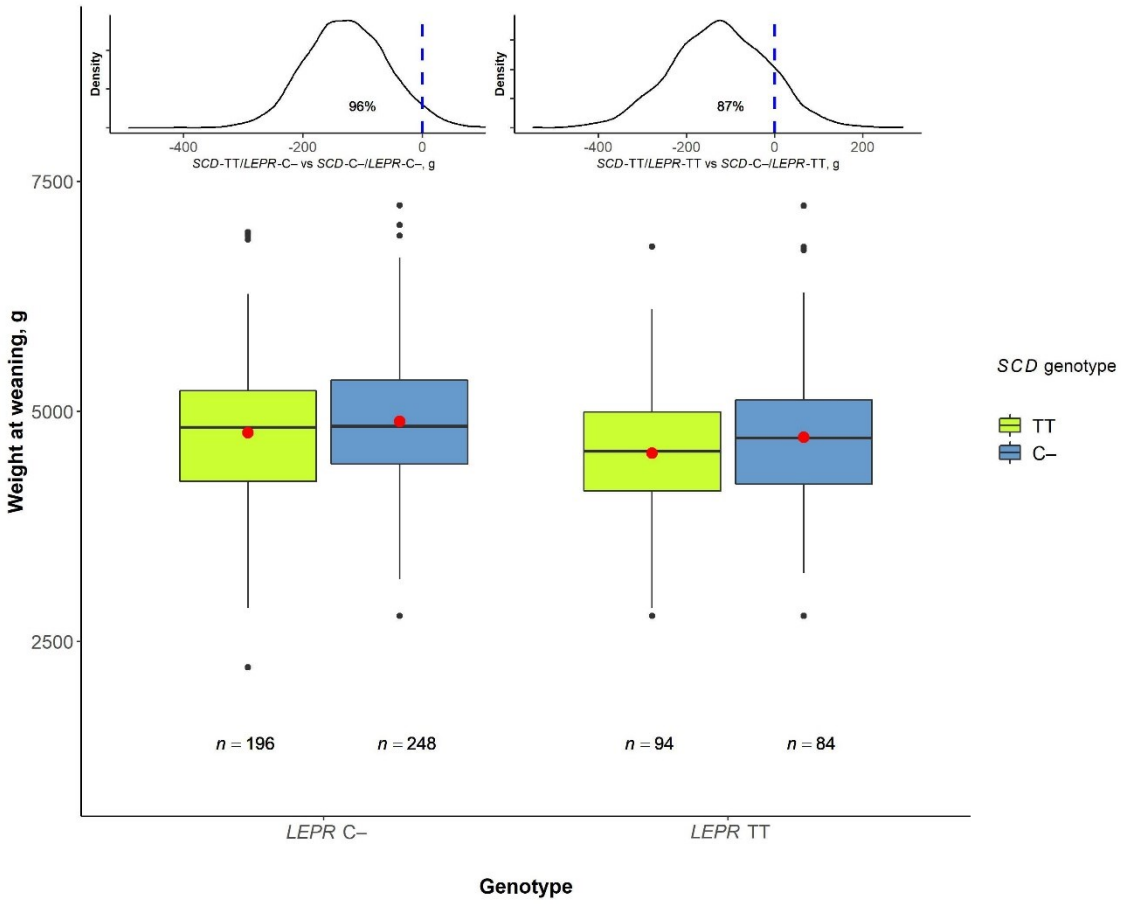

Figure 1. Distribution of weight at weaning at first farrowing by *SCD* and *LEPR* genotype. Boxplot distribution of weight at weaning at first farrowing by *SCD* and *LEPR* genotype, with the red point indicating the mean value. Values represented are adjusted for systematic effects. The marginal posterior distribution of the difference between genotypes is depicted on the top of each panel, with the blue dotted line indicating the zero value (no difference) and the accompanying percentage standing for the posterior probability of the difference being lower (area under of the curve at the left side of the line) than zero. Sample size (*n*) is given below each boxplot.

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VIII- Discusión general

En las últimas décadas los programas de selección han enfatizado la selección de aquellos caracteres que inciden directamente en el margen económico de las explotaciones, como son el crecimiento, el porcentaje de magro, el índice de conversión y, más recientemente, el tamaño de camada. No obstante, el incremento de la demanda de productos de mayor calidad por parte del consumidor y las recomendaciones nutricionales de reducir la ingesta de SFA y sustituirlas por MUFA y PUFA ha llevado a algunas empresas de selección a incluir la calidad de la carne entre los objetivos de selección. En esta tesis se ha combinado el estudio de genes candidatos con las metodologías NGS con la finalidad de identificar y comprender mejor los mecanismos biológicos que explican la deposición de la grasa, especialmente en relación con algunos marcadores asociados a la mejorar de la calidad de la carne en Duroc.

La calidad de la carne es un término complejo, ya que engloba atributos relacionados con la calidad organoléptica, nutricional y sensorial (Wood et al., 2008). Uno de los caracteres que más se asocia a la calidad de la carne es la GIM, por su relación con la capacidad de retención de agua, la jugosidad y el aroma. Por este motivo, tanto su cantidad como su composición son factores de estudio en los grupos de investigación genética porcina. Aunque su valor puede variar según la población y su historial de selección, tanto el contenido de GIM como su composición en ácidos grasos son caracteres de moderada a alta heredabilidad (0,45-0,54; Ros-Freixedes et al., 2012; Cabling et al., 2015), por lo que es de esperar que respondan bien a la selección. Por ello, resulta necesario considerar también la respuesta correlacionada en otros caracteres de interés, pues la correlación de GIM con estos caracteres, sean reproductivos, productivos o de calidad de la carne puede que no sea favorable. La identificación de marcadores genéticos relacionados con el contenido y/o la composición de GIM puede contribuir a tanto a mejorar la respuesta a la selección como a conocer sus limitaciones. Se sabe que hay alelos en los genes *SCD* y *LEPR* que influyen sobre el contenido y la composición de GIM, pero se desconoce hasta qué punto su efecto interacciona con la dieta y la reproducción. Los trabajos de esta tesis abordan estos dos aspectos, con la finalidad de entender mejor los mecanismos reguladores de la deposición de grasa y de las consecuencias prácticas de su utilización en un programa de mejora.

En el capítulo I se recurrió a la realización de un GWAS para dar respuesta a la identificación de nuevos genes candidatos relacionados con la GIM. Los resultados mostraron dos regiones genómicas asociadas con el ácido C16:1(n-7), una en SSC14 y otra en SSC9. La región en SSC14 corresponde con la localización del gen *SCD*, donde ya se ha descrito un SNP (rs80912566 T>C) asociado con el grado de insaturación de la grasa de cerdo (Estany et al., 2014). En la región del SSC9 mapea el gen *DGAT2*. La enzima DGAT2 presenta interés de estudio en genética animal debido a su función clave en el metabolismo de los triglicéridos en mamíferos y procesos de formación de ácidos grasos (Harris et al., 2011; Wurie et al., 2012). Esta enzima se localiza en la membrana del retículo endoplasmático junto a la enzima SCD (Figura 1) (Man et al., 2006). La proximidad entre ambas enzimas les permite forma complejos para sintetizas triglicéridos a partir de los ácidos grasos, especialmente de MUFA resultantes de la desaturación del C16:0 y C18:0 (Wurie et al., 2012).

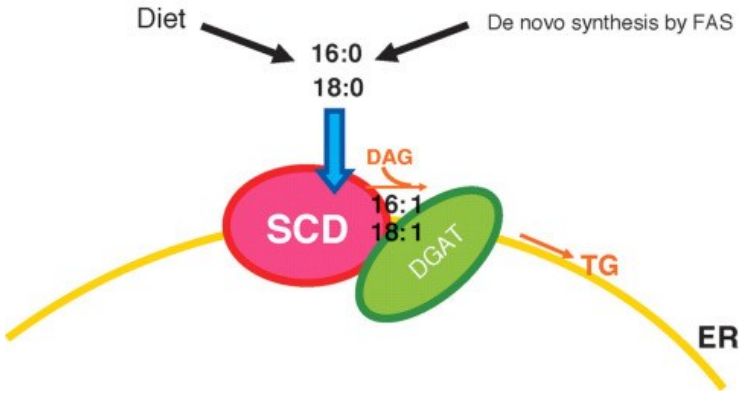

Figura 1. Representación de la síntesis de triglicéridos (TG) a partir de los MUFA sintetizados endógenamente desde SCD a DGAT2. El ácido palmítico (C16:0) y esteárico (C18:0) de la dieta o de la síntesis de ácidos grasos de *novo* son desaturados por la SCD y canalizados a DGAT para la síntesis de triglicéridos en el retículo endoplasmático. Fuente: Man et al., 2006.

Con los resultados obtenidos mediante el GWAS y la implicación del *DGAT2* en la lipogénesis, se procedió a estudiar la secuencia de este gen y su asociación con el gen *SCD* con el fin de analizar su potencial uso en los programas de selección. Entre todos los polimorfismos detectados el SNP ss7315407085 G>A fue seleccionado a causa de su localización exónica y su frecuencia génica intermedia (0,33 del alelo menos común) para determinar su efecto sobre los caracteres productivos y composición de ácidos grasos. Aunque este marcador no se asoció a ningún carácter productivo ni a la GIM, sí se observó que afectaba la composición de los ácidos grasos, aumentando la proporción de los de cadena más corta, especialmente el C16:1(n-7), lo que podría explicarse por una mayor preferencia de este enzima por los primeros ácidos grasos disponibles sintetizados de *novo*. Se ha demostrado que el gen *SCD* y *DGAT2* se coexpresan, lo que sugiere que la funcionalidad de ambos genes está estrechamente relacionada (Zhang et al., 2014). Indirectamente, ello indicaría que *SCD* está involucrado en la síntesis de triglicéridos y explicaría el hecho de que el genotipo que más expresa *DGAT2* en músculo impacte más en C16:1(n-7) que en C16:0.

Las diferencias entre los genotipos *DGAT2* no fueron relevantes en términos productivos y de calidad de la carne, pero la implicación metabólica del *DGAT2* es interesante para entender la regulación genética del metabolismo de los ácidos grasos, ya que proporciona evidencias de que el C16:1(n-7) es el sustrato principal de la enzima DGAT2. Los beneficios del ácido C16:1(n-7) sobre la salud humana han sido puestos de relieve en los últimos años. Hay estudios que demuestran que el C16:1(n-7) promueve efectos antiinflamatorios y una reducción de los efectos nocivos que causan los SFA (de Souza et al., 2018), con consecuencias sobre la epidemiologia de ciertas enfermedades. Por ejemplo, un estudio de metaanálisis mostró que niveles altos en sangre de C16:1(n-7) reducen el desarrollo de diabetes tipo 2, lo cual es de interés en epidemiologia humana (Guillocheau et al., 2020). En este sentido, aunque el impacto del marcador *DGAT2* ss7315407085 sobre C16:1(n-7) es pequeño, en especial en relación con el marcador *SCD*, su efecto lo hace atractivo para incluirlo en un programa de mejora que tenga por objetivo mejorar la calidad nutricional de la carne.

Como se ha citado anteriormente, los caracteres de calidad de la carne tienen una heredabilidad moderada, por lo que todavía hay una parte ambiental que los afectan. Entre los efectos ambientales, la nutrición incide de manera directa sobre el crecimiento, el contenido graso y la composición de la grasa. La vitamina A tiene múltiples funciones fisiológicas, pero en los últimos años, dado el efecto que presenta sobre el metabolismo de los lípidos, diversos estudios han valorado su utilización en la dieta para mejorar la calidad de la carne de cerdo y, en particular, la GIM (Al Tanoury, 2013). El metabolito activo de la vitamina A es el RA, que influye en los procesos de adipogénesis a través de la activación de los receptores nucleares RXR y RAR (Wang et al., 2016). Estos son factores de transcripción que regulan la proliferación celular y la oxidación de los lípidos y modulan la activación génica en múltiples procesos fisiológicos como el metabolismo energético (Zhang et al., 2015; Wang et al., 2016). Por ejemplo, el RA induce cambios en los niveles de expresión de la enzima SCD, lo cual afecta a la desaturación de los lípidos (Daniel et al., 2004). En este contexto, en el cerdo se ha descrito un haplotipo (H1) de tres mutaciones tipo SNP (C-T-A) en el promotor del gen *SCD* que está asociado con un mayor contenido de MUFA en la GIM y en la grasa subcutánea respecto al haplotipo alternativo (H2) (T-C-G) (Estany et al., 2014). El segundo de los tres SNPs (rs80912566 T>C) modifica una secuencia de unión potencial para los receptores del retinol (RXR y RAR). En la anotación más reciente del genoma porcino, la posición de este SNP se sitúa en el promotor central (Smale y Kadonaga, 2003), en posición 5' (tránscritos ENSSSCT00000077474.1 y ENSSSCT00000011546.5) o 3' (tránscrito (ENSSSCT00000074035.1) del inicio de transcripción del gen *SCD*. En este último caso, su anotación coincide también con la región 5' no codificante del tránscrito.

Para comprender mejor la interacción entre la vitamina A y el gen *SCD* y su efecto sobre la adipogénesis, en el capítulo II se presenta un estudio que tiene como finalidad investigar los cambios que se producen en el transcriptoma y en la composición de ácidos grasos en el músculo entre cerdos alimentados sin suplementación de vitamina A y con suplementación de vitamina A y su interacción con el genotipo *SCD*. Los resultados del estudio muestran que una dieta sin suplemento de vitamina A no modifica ni el contenido ni la composición de la GIM, pero sí que produce cambios relevantes a nivel del transcriptoma, ya que un elevado número de genes, tanto genes codificantes como no codificantes, resultan diferencialmente expresados (43 DEG) entre ambas dietas. Estos resultados se deben a que el RA de la vitamina A es un potente agente capaz de inducir alteraciones en la transcripción de los genes y puede producir cambios en el fenotipo (Bar-El Dadon & Reifen, 2015). Ahora bien, cuando se analiza la interacción entre la Vitamina A y el gen *SCD* se observa que el número de DEG es mucho mayor, sobre todo en los animales con genotipo *SCD*-TT respecto los *SCD-CC* (162 en TT y 26 en CC DEG). Los cerdos con un genotipo TT presentan mayor contenido de oleico. Este ácido graso induce importantes cambios en la diferenciación de los adipocitos por medio de la activación de varias cascadas de transcripción, como la PPARA, que induce la lipogénesis (Wang et al., 2019).

Aunque los resultados no mostraron ningún efecto de la restricción de la vitamina A sobre la composición de la GIM, sí que se vio claramente que existía una interacción entre la vitamina A y el *SCD*. Los cerdos *SCD*-TT/CT alimentados sin suplementación de vitamina A presentaron

mayores ratios de desaturación, mayor proporción de MUFA y de C18:1(n-9) que los alimentados con una dieta suplementada con vitamina A. En cambio, en los cerdos *SCD*-CC se observó la tendencia opuesta, es decir, que la suplementación con vitamina A aumentó la desaturación de la GIM. La interacción entre el genotipo *SCD* y la dieta fue consistente entre los tres músculos analizados. Estos resultados refuerzan la hipótesis de la acción de la vitamina A sobre la enzima SCD, probablemente mediante la activación de los receptores RXR y RAR de forma diferente según el genotipo *SCD.*

En los últimos años la modificación de vitamina A en la dieta ha sido objeto de numerosos estudios en cerdo, sin un claro consenso sobre los resultados obtenidos. Algunos estudios muestran claramente que la restricción de vitamina A modifica la GIM y la composición de ácidos grasos (Ayuso et al., 2015a; Ayuso et al., 2015b; Henríquez-Rodríguez et al., 2017), mientras que otros estudios no observaron estas diferencias (Olivares et al., 2009b). La variabilidad en los resultados puede deberse a diferencias entre estudios en cuanto al tipo genético utilizado, edad de los animales o la duración y nivel de restricción/suplementación. Además, cabe destacar que la variante *SCD* rs80912566 no segrega en todas las razas, como en Ibérico (donde está fijado el alelo T) o segrega muy poco, como en Piétrain y Landrace (Estany et al., 2014). Los resultados de la presente tesis apuntan a que, en líneas donde domine el genotipo *SCD*-TT, la restricción de la vitamina A en la dieta puede ser una buena estrategia para mejorar el índice de desaturación de los ácidos grasos sin afectar al contenido de la GIM.

A diferencia de *SCD* rs80912566*,* el alelo T del polimorfismo rs709596309C>T del gen *LEPR* favorece el incremento de la GIM. Sin embargo, el aumento de la GIM va acompañado de un mayor engrasamiento del conjunto del animal, como se deprende del aumento del espesor de grasa subcutánea. Además, el alelo T favorece el depósito de SFA tanto en la GIM como en la grasa subcutánea (Henriquez-Rodriguez et al., 2016), impactando negativamente sobre la calidad nutricional de la carne. En un programa de mejora, la selección combinada de los alelos *SCD*-T y *LEPR*-C podría ser una buena estrategia para incrementar la proporción de MUFA/SFA (Henriquez-Rodriguez et al., 2016). No obstante, no se ha comprobado si estas dos variantes tienen un impacto en los caracteres maternales.

Diversos autores han estimado en porcino una correlación genética negativa entre efectos directos y maternos para caracteres reproductivos como la mortalidad al nacimiento (Ibáñez-Escriche et al., 2009; Nguyen et al., 2021) y para caracteres de crecimiento como el peso de los lechones (Alves et al., 2018; Nguyen et al., 2021). Aunque el fundamento genético de tal antagonismo no está claro, es plausible que, en algunos casos, como es en el peso de los lechones al destete, no sea independiente del estado corporal de la cerda. Es necesario conocer la relación entre caracteres reproductivos, de producción y de calidad de la carne para el buen diseño de un programa de selección genética. Las líneas genéticas paternas están seleccionadas para caracteres de crecimiento debido a que la selección combinada de crecimiento y tamaño de camada puede llevar a disminuir la supervivencia de los lechones (Holm et al., 2004; Heuß et al., 2019; Nguyen et al., 2021). Esta relación negativa se debe a que una selección por crecimiento lleva a que la cerda destine más recursos a su crecimiento que al desarrollo de la camada, produciendo camadas grandes

menos desarrolladas (Holm et al., 2004; Heuß et al., 2019). Asimismo, el tamaño de camada está relacionado con el peso al nacimiento. Un aumento de la prolificidad puede conllevar una falta de espacio intrauterino y resultar en un retraso del crecimiento de los lechones (Wu et al., 2006; Alves et al., 2018). Las variaciones en el peso al nacimiento o al destete terminan, por otra parte, afectando el ritmo de crecimiento de los animales y la calidad de la carne (Vázquez-Gómez et al., 2018a). Los lechones con bajo peso al nacimiento presentan al final de su vida productiva peor conformación de la canal, menor contenido de GIM, más SFA y MUFA, menos PUFA, y una peor tasa de supervivencia durante la lactación (Vázquez-Gómez et al., 2018b; Heuß et al., 2019). Los lechones con menor peso ingieren menos nutrientes de la leche debido a la competitividad para conseguir alimento, lo que provoca una menor ingestión del calostro, que es fundamental para la supervivencia de los lechones, y un crecimiento más lento durante las fases de lactación y transición. En contrapartida, se ha observado que durante el cebo estos cerdos suelen presentan un crecimiento compensatorio al tener acceso a alimentación *ad libitum,* lo que les permite acortar la diferencia de peso, aunque no lo suficiente para llegar al peso óptimo de matadero (Vázquez-Gómez et al., 2018a,b). Así, los lechones de menos peso al nacimiento tienden a llegar al matadero con menor peso vivo y peso canal, lo que afecta también a la conformación y composición de la canal (Berard et al., 2008; Berard et al., 2010; Vázquez-Gómez et al., 2018a,b; Zhang et al., 2018c).

Los efectos maternos debidos a la capacidad lechera de la cerda y su condición corporal en gestación son el factor limitante para mejorar la supervivencia de los lechones durante la lactación debido a su efecto sobre el desarrollo y el crecimiento de los lechones. Aunque el estudio de Holm et al., (2004) sugiere que la selección por el espesor de grasa dorsal no influye en los caracteres reproductivos, otros estudios (Houde et al., 2010; Flisar et al., 2012; Roongsitthichai y Tummaruk, 2014) destacan la importancia del espesor de grasa dorsal de la cerda sobre el rendimiento de los lechones debido a la movilización de lípidos que proporciona. Una cerda más pesada presenta lechones más pesados al nacer y al destete. Sin embargo, un mayor engrasamiento durante la gestación se asocia con más muertes al nacimiento y menor número de destetados (Zhou et al., 2018; Lavery et al., 2019; Nguyen et al., 2021). El número y el peso de los lechones destetados son cruciales para el éxito reproductivo y eficiencia de una línea genética. Pese a que los caracteres de origen materno han sido ampliamente estudiados en porcino, todavía no existen ejemplos de genes que expliquen las razones del antagonismo entre efectos directos y maternos ni del efecto de los marcadores de calidad de la carne sobre los caracteres reproductivos. En esta línea, en los capítulos III y IV se han analizado los efectos de las variantes estudiadas en los genes *SCD* y *LEPR* sobre los caracteres reproductivos y el efecto materno que ejercen sobre el crecimiento y composición corporal de los cerdos descendientes.

El gen *LEPR* no solo afecta la deposición y composición de la grasa en cerdos (Óvilo et al., 2005; Ros-Freixedes et al., 2016) sino que también puede tener un papel importante en la reproducción, ya que la leptina interviene en la implantación embrionaria y en la regulación del crecimiento fetal durante la gestación (González-Añover et al., 2011). En el capítulo III se determinó que el polimorfismo rs709596309 C>T gen *LEPR* no afecta al tamaño de camada, ni al nacimiento ni al destete. Por el contrario, esta variante presenta un efecto materno sobre el peso,

desde el destete al sacrificio. Las cerdas *LEPR*-CC o *LEPR*-CT (C−) favorecen el peso de sus lechones, pero son los cerdos *LEPR*-TT los que crecen más durante el engorde. Así, los cerdos *LEPR*-TT de madres *LEPR*-C− pesaron un 5% más al sacrificio que los cerdos *LEPR*-C− de madres *LEPR*-TT, mientras que los cerdos *LEPR*-TT de madres *LEPR*-TT y viceversa no presentaron diferencias entre ellos. Para explicar biológicamente mejor esta relación negativa entre efecto directo y materno, se realizaron cuatro experimentos en los que se analizaron las diferencias entre los genotipos *LEPR* para la composición de la leche, el consumo individual de pienso durante el engorde, y la concentración plasmática de triglicéridos y de ácidos grasos libres después de un ayuno prolongado. Los cerdos *LEPR*-TT presentaron mayor consumo de pienso y más triglicéridos en sangre, pero menos ácidos grasos libres en sangre tras un ayuno. Estos resultados indican que el genotipo *LEPR*-TT se asocia con cerdos que acumulan más grasa, no solo porque ingieren más energía, sino porque además la movilizan menos. Así, el efecto materno negativo de las madres *LEPR*-TT sobre el peso puede explicarse porque las madres *LEPR*-TT producen menos leche al movilizar menos recursos energéticos durante la lactación. Esta hipótesis se refuerza con un análisis complementario de un grupo de 65 madres primerizas donde se midió el espesor de la grasa dorsal al final de la lactación (26 días, 4 SD) mediante el equipo ultrasónico AutoFOM (SFK-Technology, Dinamarca). Para el análisis estadístico se utilizó un modelo lineal que incluyó el efecto de año-estación (11 niveles), genotipo *LEPR* (TT y C–), *SCD* (CC, CT y TT) y *DGAT2* (AA, AG y GG), como efectos fijos, y el número de nacidos totales y edad de la madre como covariable. Los resultados muestran que las madres *LEPR*-TT tienen 2,3 mm más de espesor de grasa dorsal al final de la lactación que las madres *LEPR*-C– (P<0,01) (Figura 2). Aunque no se disponía de la grasa dorsal al inicio de la lactación estos resultados pueden interpretarse como que las cerdas *LEPR*-TT no movilizan todas las reservas de las que disponen. Además, ésta menor movilización de los recursos genera un retraso en la edad de primer parto por parte de las madres *LEPR*-TT, de forma que se produce una disminución de la eficiencia reproductiva de la cerda.

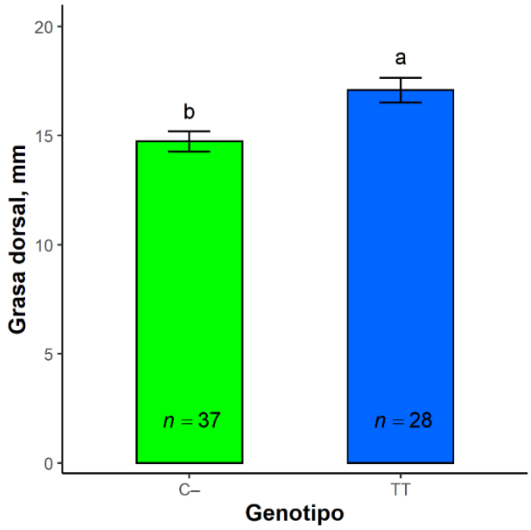

Figura 2. Medias mínimo-cuadráticas del espesor de grasa dorsal de una cerda al final del destete según genotipo *LEPR*. Las barras de error representan los errores estándar. Las medias con diferentes superíndices difieren significativamente (P <0,01).

La hipótesis del genotipo ahorrador (en inglés, *thrifty genotype*) fue propuesta por James Neel en 1962 (Neel, 1962) como parte de una teoría evolutiva. Esta hipótesis establece que los individuos capaces de almacenar más energía extra en periodos de escasez de alimentos habrían tenido una ventaja evolutiva. Esta adaptación, en hembras reproductivas, produce un efecto materno sobre el desarrollo de los lactantes (González-Bulnes et al., 2016). La mutación rs709596309C>T del gen *LEPR* contribuye a este fenómeno ahorrador, que también puede interpretarse como un mecanismo transgeneracional de distribución de los recursos, en el que éstos se asignan a la madre o a la descendencia en función de si la presión de selección es mayor antes o después del destete. Desde el punto de vista de la producción porcina, la selección a favor del alelo T para aumentar el contenido de GIM debe valorarse bien, incluso en mercados donde este carácter sea muy apreciado, pues, a pesar de que aumenta la velocidad de crecimiento, ello se obtiene a costa de más consumo de pienso, de canales más grasas y de un manejo reproductivo más exigente.

Siguiendo la línea del gen *LEPR*, se realizó un estudio similar con el otro marcador de calidad de la carne, el gen *SCD.* Se ha visto que el contenido de MUFA y PUFA del calostro y de la leche está relacionado con el peso de los lechones y la tasa de mortalidad hasta el destete y que la ingesta de calostro rico en PUFA proporciona funciones inmunológicas beneficiosas para la supervivencia de los lechones (Skrzypczak et al., 2015; Heuß et al., 2019). En bovino, este gen se ha asociado con la duración de la gestación y la composición de ácidos grasos de la leche (Asadollahpour et al., 2014; Kęsek-Woźniak et al., 2020). Estas asociaciones refuerzan la necesidad de investigar la relación del gen *SCD* con los caracteres reproductivos y de aquellos susceptibles de ser afectados por la madre. A diferencia del marcador *LEPR*, no se observó que el marcador *SCD* sea causante de un efecto materno en el peso al sacrificio ni que tenga consecuencias negativas sobre la vida reproductiva de la cerda, excepto en el peso al destete de las primerizas y la edad de primer parto. Los lechones de las cerdas primerizas *SCD*-TT pesaron menos al destete que los de las cerdas *SCD*-CT, además las madres presentaron un ligero retraso de la edad de primer parto. En este sentido, en un programa de selección donde la calidad de la carne sea el objetivo principal de la línea es aconsejable tener especial cuidado en el manejo de cerdas primerizas portadoras del alelo *SCD*-T, especialmente cuando se combina con el genotipo *LEPR*-TT. Las causas de este efecto requieren mayor investigación, pero una posible hipótesis podría ser que las madres *SCD*-TT tienen camadas algo más numerosas y un crecimiento corporal más tardío. Quizás por ello las primerizas *SCD*-TT tienden a producir una leche menos grasa, aunque con más contenido de MUFA, sobre todo en el ratio C18:1(n-9)/C18:0. En bovino, el efecto de este gen en la composición de ácidos grasos de la leche puede explicarse por una mayor expresión del gen durante la lactación, y por tanto mayor actividad desaturasa (Suárez-Vega et al., 2015). En cerdos, hay muy pocos estudios del efecto del genotipo de la madre sobre la modificación de los ácidos grasos de la leche. Los resultados obtenidos confirmarían que el genotipo *SCD* afecta la composición de la leche materna, a diferencia de los genes *LEPR* y *DGAT2* que no alteran su composición.

En su conjunto, los resultados expuestos en esta tesis amplían el conocimiento genético sobre el metabolismo lipídico en porcino, en tanto que delimitan los efectos del gen *DGAT2,* muestran la

existencia de interacciones entre genes y nutrientes, y ponen en evidencia que los genes relacionados con la deposición de grasa pueden tener implicaciones reproductivas.

IX- Conclusiones

Las conclusiones de esta tesis se alinean con los objetivos específicos planteados:

1. El gen diacilglicerol O-aciltransferasa-2 presenta variaciones en su secuencia que influyen en la expresión génica y en el perfil de ácidos grasos del músculo, de tal manera que la variante que aumenta la expresión génica favorece la deposición de ácidos grasos de cadena más corta, lo que sugiere que la enzima DGAT2 prefiere como sustrato los ácidos grasos que primero se sintetizan. Aunque *DGAT2* y *SCD* actúan de forma aditiva, el hecho de que la sobreexpresión de *DGAT2* impacte más en ácido palmitoleico que en el palmítico indicaría que *DGAT2* y *SCD* están estrechamente relacionados.

2. El nivel de vitamina A en la dieta durante el engorde provoca cambios relevantes en el perfil de expresión génica en el músculo, pero no afecta ni el contenido ni la composición de la grasa intramuscular. Sin embargo, el efecto de la vitamina A sobre la composición de los ácidos grasos es diferente según el genotipo *SCD*, de tal manera que sólo en los cerdos de genotipo *SCD*-CC la vitamina A aumenta la desaturación.

3. La mutación (rs709596309 C>T) en el gen *LEPR* provoca un efecto materno antagónico al efecto directo en el crecimiento de los lechones. Los lechones criados por madres *LEPR*-TT pesan de menos al final del engorde lo que aproximadamente pesan de más los cerdos *LEPR*-TT. Este antagonismo se explica porque el genotipo *LEPR*-TT, más graso, prioriza la acumulación a la movilización de reservas y, de este modo, su propio mantenimiento al de la descendencia.

4. Las cerdas *LEPR*-TT tienen más tarde su primer parto, menos lechones por parto y sus lechones pesan menos al destete. Por el contrario, el genotipo *SCD* (rs80912566 T>C) no presenta efectos adversos en el rendimiento reproductivo de las cerdas. Aun así, debe vigilarse el manejo de las primerizas portadoras del alelo *SCD*-T, particularmente si el alelo *LEPR*-T segrega en la población, ya que una pronta cubrición de estas cerdas afecta negativamente el peso de sus lechones al destete. Una hipótesis que explica este efecto es que las cerdas portadoras del alelo *SCD*-T llegan a la primera cubrición con peor condición corporal y a la vez tienen camadas algo más numerosas.

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