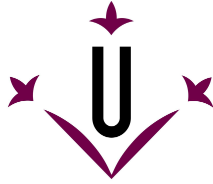

Universitat de Lleida

AGE- AND GENOTYPE-RELATED CHANGES IN INTRAMUSCULAR FAT CONTENT AND COMPOSITION IN PIGS USING LONGITUDINAL DATA

Lluís Bosch Puig

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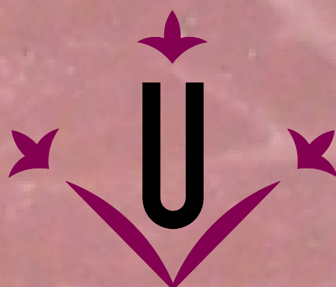

Universitat de Lleida

Departament de Producció Animal

AGE- AND GENOTYPE-RELATED CHANGES IN INTRAMUSCULAR FAT CONTENT AND COMPOSITION IN PIGS USING LONGITUDINAL DATA

Memoria presentada por

Lluís Bosch Puig

Para optar al **grado de Doctor**

Director: **Dr. Joan Estany Illa**

TESIS DOCTORAL

Lleida, Junio 2011

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El Dr. JOAN ESTANY ILLA, catedràtic de universitat del Departament de Producció Animal de la Universitat de Lleida,

Informa:

Que en Lluís Bosch i Puig ha realitzat sota la meva direcció el treball de recerca '*Age-and genotype-related changes in intramuscular fat content and composition in pigs using longitudinal data*'.

Aquest treball, dut a terme en el Departament de Producció Animal, s'ajusta als objectius previstos en el projecte de Tesi Doctoral i es presenta com un dels requisits per optar al grau de Doctor Enginyer Agrònom per la Universitat de Lleida.

Lleida, 4 de juliol de 2011

Dr. Joan Estany Illa

A la Mª Teresa, en Joan i en Lluís

Esta Tesis Doctoral se ha realizado en el Departamento de Producción Animal de la Universidad de Lleida.

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ABREVIACIONES

IMF:	Grasa intramuscular (Intramuscular fat).
FA:	Ácidos grasos (Fatty acids).
LM:	Músculo *longissimus dorsi.*
GC:	Cromatografía de gases licuados.
SX:	Análisis Soxhlet.
BE:	Biopsia de lomo precoz (antes de 30 días del sacrificio).
BL:	Biopsia tardía (los últimos 30 días antes del sacrificio).
BC:	Biopsia en canal.
GM:	Músculo *gluteus medius.*
PUFA:	Ácidos grasos poliinsaturados (Poliunsaturated fatty acids).
C18:2n-6	Ácido 9,12-octadecadienoico. Ácido linoléico.
C18:3n-3	Ácido 9,12,15-octadecatrienoico. Ácido linoléico.
C20:2n-6	Ácido 11-14-eicosadienóico.
C20:4n-6	Ácido 5, 8, 11,14-eicosatetranóico. Ácido araquidónico.
MUFA:	Ácidos grasos monoinsaturados (Monounsaturated fatty acids).
C16:1n-7	Ácido 9-hexadecenoico. Ácido palmitoléico.
C18:1n-9	Ácido 9-octadecenoico. Ácido oléico.
C20:1n-9	Ácido 9-eicosenóico.
SFA:	Ácidos grasos saturados (Saturated fatty acids).
C12:0	Ácido dodecanóico. Ácido láurico.
C14:0	Ácido tetradecanoico. Ácido mirístico.
C16:0	Ácido hexadecanoico. Ácido palmítico.
C18:0	Ácido octadecanoico. Ácido esteárico.
C20:0	Ácido eicosanóico.
n6:n3 ratio:	(C18:2+C20:2+C20:4)/C18:3
RTU:	Ultrassonidos en tiempo real.
MS:	Materia seca.

SUMMARY

This PhD is part of a line of research conducted in the Department of Animal Production of the Universitat de Lleida dedicated to the genetic improvement of pig meat quality, with particular reference to intramuscular fat content and composition. The PhD comprises four studies, with the first one focusing on the development of a method to jointly determine the content and composition of intramuscular fat from biopsies and small *post-mortem* samples and, in this way, to carry out studies with longitudinal data. It has been found that this particular methodology is useful and, in for intramuscular fat, small specimens of the target muscle are as informative as large samples of other muscles. In the second study the effect of age on the content and composition of the intramuscular and subcutaneous fat in the fattening period in Duroc pigs was investigated by an experiment using longitudinal data obtained following the methodology described above. It was concluded that a delay in the age of slaughter of the pig leads to an increase in intramuscular fat and oleic acid, although this comes at the cost of reducing the rate of lean growth. Moreover, it was proved that intramuscular and subcutaneous fat behaved differently in terms of fat accretion and composition and that the amount of fat itself affected composition. Whereas, for the intramuscular fat, values above the expected at a given age were because of increased monounsaturated fatty acid content, especially oleic acid, for the subcutaneous fat, they were due to the increased saturated fatty acid content. The final two studies considered whether allelic variation at the IGF-1 (insuline-like growth factor-1) and LEP (leptin) genes, as well as the concentration of IGF-1 and leptin in plasma, are associated to intramuscular fat content and composition and, if so, whether this is a function of age. It can be seen that the molecular polymorphisms studied are not neutral with regard to the content of intramuscular fat, but that their effects are not constant throughout the growing period. In this sense, both age and fatness can modify them.

RESUMEN

La presente Tesis Doctoral se emmarca en una línea de investigación del Departamento de Producción Animal de la Universidad de Lleida dedicada a la mejora genética de la calidad de la carne en porcino, en particular del contenido y la composición de la grasa intramuscular. La Tesis se compone de cuatro estudios, centrándose el primero de ellos en el desarrollo de un método para determinar el contenido y la composición de la grasa intramuscular a partir de biopsias y muestras *post-mortem* pequeñas con las que luego poder realizar estudios mediante diseños longitudinales. La metodología propuesta ha resultado útil, demostrándose que, especialmente para el contenido de grasa intramuscular, los especímenes pequeños del músculo objetivo son tan informativos como muestras grandes de otros músculos. En el segundo estudio se ha investigado mediante un experimento con datos longitudinales, obtenidos según la metodología descrita anteriormente, el efecto de la edad sobre el contenido y la composición de la grasa intramuscular y subcutánea durante el engorde de cerdos Duroc. Se concluye que un retraso en la edad de sacrificio comporta un aumento del contenido de grasa intramuscular y de ácido oleico, aunque ello se consigue a costa de disminuir la velocidad de crecimiento magro. Por otra parte, se demuestra que la grasa intramuscular y la grasa subcutánea tienen patrones distintos de crecimiento y composición y que la cantidad de grasa por sí misma influye en su composición. El que un cerdo sea más graso de lo esperado a una edad determinada es debido, en el caso de la grasa intramuscular, a que ha aumentado el contenido de grasa monoinsaturada, en especial de oleico, mientras que, en el de la subcutánea, a que se ha incrementado el de la saturada. En los dos últimos estudios se examina si la variación alélica en los genes IGF-1 (insulin-like growth factor-1) y LEP (leptina), así como la concentración de IGF-1 y leptina en plasma, se asocian con el contenido y la composición de la grasa intramuscular y, en caso de que así fuera, si tal asociación es función de la edad. Se constata que los polimorfismos moleculares estudiados no son neutrales respecto al contenido de grasa intramuscular, pero, también, que sus efectos no son constantes a lo largo del crecimiento. En este sentido, tanto la edad como el estado de engrasamiento pueden modificarlos.

RESUM

La present Tesi Doctoral s'emmarca en una línia d'investigació del Departament de Producció Animal de la Universitat de Lleida, dedicada a la millora genètica de la qualitat de la carn en bestiar porcí, en particular del contingut i composició del greix intramuscular. La Tesi es composa de quatre estudis, centrant-se el primer d'ells en el desenvolupament d'un mètode per a determinar el contingut i composició del greix intramuscular a partir de biòpsies i mostres *post-mortem* petites amb les que després es puguin portar a terme estudis en disseny longitudinal. La metodologia proposada ha resultat útil, demostrant-se que, especialment per al contingut de greix intramuscular, els espècimens petits del múscul objectiu són tan informatius com mostres grans d'altres músculs. En el segon estudi s'ha investigat, mitjançant un experiment amb dades longitudinals obtingudes segons la metodologia descrita anteriorment, l'efecte de l'edat sobre el contingut i composició del greix intramuscular i subcutani al llarg del cicle d'engreix en porcs de raça Duroc. Es conclou que un retard en l'edat de sacrifici implica un augment del contingut de greix intramuscular i d'àcid oleic, tot i que això s'aconsegueix a expenses de disminuir la velocitat de creixement magre. Per altra part es demostra que el greix intramuscular i el greix subcutani tenen comportaments diferents de creixement i composició i que la quantitat de greix per si mateix també influeix en la seva composició. El que un porc sigui més gras de l'esperat a una edat determinada és degut, en el cas del greix intramuscular, a que ha augmentat el contingut de greix monoinsaturat, en especial d'olèic, mentre que, en el del greix subcutani a que s'ha incrementat el contingut de saturat. En els dos últims estudis s'examina si la variació al·lèlica en els gens IGF-1 (insulin-like growth factor-1) i LEP (leptina), així com la concentració de IGF-1 i leptina en plasma, s'associen amb el contingut i la composició del greix intramuscular i, en cas que així fos, si aquesta associació és funció de l'edat. Es posa en evidència que els polimorfismes moleculars estudiats no són neutrals en relació al contingut de greix intramuscular, però també que els seus efectes no són constants al llarg del període de creixement. En aquest sentit, tant l'edat com l'estat d'engrassament poden modificar-los.

I. Introducción

Los programas de mejora genética porcina han dirigido tradicionalmente su atención a la mejora de los caracteres productivos, entre los que destacan los relacionados con la calidad de la canal, y, más recientemente, de los reproductivos. El éxito en la mejora de los caracteres productivos ha resultado en un aumento de la ganancia de peso diaria y del contenido magro (Huff-Lonergan et al., 2002), así como una disminución del índice de conversión (Webb, 1995). Sin embargo, debido a que la correlación genética entre el contenido magro de la canal y la calidad de la carne es desfavorable (Sellier, 1998), la mejora del porcentaje de magro ha traído consigo una disminución de algunos de los caracteres ligados a la calidad de la carne, en especial de la grasa infiltrada. No obstante, se ha mostrado que los valores de la correlación genética entre contenido magro y grasa infiltrada, aún siendo por lo general elevados, no lo son suficientemente como para no tener éxito en la mejora de ambos caracteres a la vez (Jeremiah, 1998; Solanes et al., 2009).

Por otra parte, las exigencias de los consumidores están cambiando; si bien el consumidor compra la carne atendiendo a su tamaño, forma, color, relación grasa/magro, textura y coste (McGill, 1981), también tiene en cuenta como factor de decisión la experiencia personal; así en la repetición de la compra se valoran factores como la jugosidad, la terneza y el flavor (Forrest, 1998). Una de las causas que se achacan a la no inclusión de la calidad de la carne en los programas de mejora genética es debida a la dificultad de definir el término calidad de la carne. Kaufmann et al. (1990), definen calidad en porcino como un término general que incluye una combinación de características que proporcionan un producto beneficioso, atractivo en apariencia y que es apetecible, nutritivo y palatable. Los parámetros más importantes relacionados con la calidad de la carne son, como cita Schinckel (2001), el color, la firmeza, la capacidad de retención de agua, el pH último, el marmoleo relacionado con la grasa intramuscular, la terneza y la palatabilidad (flavor y jugosidad). La tendencia actual del consumidor es la de pedir productos más palatables y de apariencia (Van Wijk et al., 2005), con un mínimo de grasa intramuscular, que se cifra en un contenido entre el 2-3% (Bejerholm y Barton-Gade,

1986; DeVol et al., 1988) en consumo en fresco y valores superiores para la producción de productos curados (Cilla et al., 2006; Ventanas et al., 2007).

Otro aspecto de importante interés en la cadena de producción porcina es el conocimiento de la composición de la grasa intramuscular, por su relación con la salud de los consumidores (Kouba et al., 2003; Alonso et al., 2009; Burgos et al., 2010). Varios estudios relacionan la composición y contenido de la grasa intramuscular con la salud cardiovascular (Rose, 1990; Ulbricht y Southgate, 1991; Azain, 2004), la obesidad (Switonski et al., 2010) y el riesgo de sufrir enfermedades cancerígenas (Terry et al., 2003; Larsson et al., 2004), por lo que es necesario disponer de métodos fiables para predecir la composición de la grasa intramuscular y su evolución.

Con el objetivo de mejorar la calidad de la carne existen diversos métodos, a través de los cuales se pueden predecir las características relativas al término calidad de la carne, tales como el color, la firmeza, la capacidad de retención de agua, el pH último, el marmoleo relacionado con la grasa intramuscular, la terneza, el flavor y la jugosidad. De estos métodos citados, pocos tienen la característica que puedan utilizarse en animales vivos, lo cual permitiría aprovechar los resultados obtenidos para la selección directa de futuros progenitores.

Los métodos que pueden utilizarse en la predicción de la calidad de la carne en vivo se pueden estructurar en cuatro diferentes grupos: 1. Obtención de biopsias in-vivo y posterior análisis de las muestras obtenidas; 2. Utilización de aparatos de ultrasonidos para la obtención de medidas de profundidad de grasa y lomo o posterior análisis de la imagen obtenida (RTU, real time ultrasound); 3. Utilización de indicadores fisiológicos con importancia en la evolución de la calidad de la carne; 4. Utilización de marcadores genéticos relacionados con la calidad de la carne.

La presente Tesis Doctoral se emmarca en una línea de investigación que se está llevando a cabo en el Departamento de Producción Animal de la Universitat de Lleida desde el año 2001, dedicada al estudio de la calidad de la carne en ganado

porcino, con especial interés en el contenido y composición de la grasa intramuscular. Desde su determinación en canal hasta la determinación *in-vivo* mediante las cuatro anteriores aproximaciones. Dentro de esta linea de investigación, la Tesis se ha centrado en el desarrollo de un método que permita determinar conjuntamente, el contenido y composición de la grasa intramuscular a partir de muestras pequeñas obtenidas en animales vivos, para de esta manera, poder llevar a término estudios con datos longitudinales, sobre la evolución del contenido y composición de la grasa intramuscular en animales vivos; lo que ha de permitir mejorar la eficiencia de este tipos de estudios respecto a la técnica de los sacrificios seriados. Con esta finalidad se han realizado los cuatro estudios que componen esta Tesis Doctoral; en el primero, se ha desarrollado el método para determinar simultáneamente el contenido y composición de la grasa intramuscular en biopsias y muestras *post-mortem*; en el segundo estudio se ha utilizado esta técnica para determinar la evolución del contenido y composición de la grasa intramuscular en una población de cerdos de raza Duroc, según edad y peso. Y finalmente, se ha utilizado la técnica desarrollada en el estudio 1 para a estudiar la variación alélica de dos genes potencialmente relacionados con la calidad de la carne - IGF1 (estudio 3) y LEP (estudio 4) – y su relación con el contenido y composición de la grasa intramuscular.

II. Revisión bibliográfica

II.1. CARACTERÍSTICAS DE LA CALIDAD DE LA CARNE PORCINA

II.1.1. Composición y calidad de la carne

Según Warris (2000), la composición aproximada de la carne de cerdo está formada por un 60% de agua, un 20% de proteina, un 15% de grasa, un 4% de minerales y un 1% de carbohidratos, de estos parámetros los más variables son el contenido en grasa y en agua.

No existe una definición estándar del término calidad de la carne, si bien una aproximación a su definición podría ser la realizada por Bray (1966) en la cual se expresan los distintos aspectos de este término *"Para algunos incluye aquellos factores asociados con rendimientos productivos, así como aquellos factores asociados a la palatabilidad. El científico define la calidad de la carne fresca como aquellos factores asociados a la palatabilidad de la carne fresca y de los productos curados y las pérdidas económicas durante el procesado y la distribución. El consumidor en el análisis final de la calidad de la carne porcina se refiere a la terneza, jugosidad y el flavor del producto cocinado. En las canales de cerdos jóvenes los factores más comúnmente asociados con estas características son el color, la textura y la firmeza del músculo y la cantidad de la grasa intramuscular (marmoleo)"*. Esta larga definición da dimensión a la complejidad del término y a los múltiples factores que la afectan.

Una definición más actualizada y sintética es la realizada por Hoffman (1994), la calidad de la carne se define como la totalidad de las propiedades y características de la carne que son importantes en relación a su valor nutritivo, sensorial, para la salud humana y para el procesamiento industrial.

Los aspectos que tienen una relación más importante con la calidad de la carne se pueden englobar en cuatro distintos bloques:

- **Calidad organoléptica**
- **Calidad tecnológica**
- **Calidad nutricional**
- **Calidad higiénica**

En relación a la **calidad organoléptica** los factores más importantes que la afectan son el aspecto visual, la terneza, la jugosidad y el flavor. En el aspecto visual el consumidor demanda una carne ni muy clara ni muy oscura, con un color homogéneo y poca presencia de grasa visible; si bien Fernandez et al. (1996) en un estudio realizado con consumidores, determinó que si bien la aceptación visual de los consumidores en relación a la grasa intramuscular era baja, después de la prueba de consumo, la carne mejor valorada fue la que tenía un contenido de grasa intramuscular situada entre el 2.5 y el 3.5%. La terneza se refiere a la facilidad con que la carne es cortada o masticada, y se relaciona con la cantidad y el grado de polimerización del colágeno muscular, así como con la estructura miofibrilar (Monin, 1983). La componente miofibrilar tiene relación principalmente con la evolución *post-mortem* del pH de la carne y de las reacciones proteolíticas que intervienen durante la maduración de la carne (Monin 1983; Valin 1988).

Diferentes trabajos han relacionado el efecto positivo (si bien en algunos estudios citado como poco importante), del contenido en grasa intramuscular con la terneza de la carne (Cromwell et al. 1978; DeVol et al. 1988; Touraille et al. 1989; Fernandez et al. 1996). En relación a la jugosidad (sensación de liberación de jugos durante la masticación), esta característica está relacionada con la cantidad de agua libre existente en la carne y a la secreción de saliva durante la masticación, estimulada principalmente por el contenido en grasa (Girard et al., 1988). La jugosidad está relacionada con la capacidad de retención de agua de la carne, influenciada por la evolución del pH *post-mortem*; Eikelenboom et al. (1996a) encontraron una correlación de 0.68 entre el pH último y la jugosidad de la carne. El contenido en grasa intramuscular tiene una correlación positiva con la jugosidad, oscilando entre 0.21 y 0.65 en distintos trabajos (Cromwell et al. 1978; Wood et al. 1996; DeVol et al. 1988; Touraille et al. 1989; Hodgson et al. 1991; Eikelenboom et al. 1996b). El flavor, que se relaciona con la percepción olfativa y gustativa, se

relaciona principalmente con el contenido en lípidos de la carne (DeVol et al. 1988, Touraille et al. 1989; Gandemer et al. 1990), situándose el nivel óptimo de grasa intramuscular entorno a 2.5-3.0% en el músculo *longissimus dorsi* (DeVol et al. 1988; Fernandez et al. 1996); dentro de la grasa intramuscular, un importante papel lo desempeñan los fosfolípidos (Mottram y Edwards, 1983). Olsson y Pickova (2005), determinaron que tanto el contenido de grasa intramuscular como su composición, tienen un importante efecto sobre algunos importantes caracteres relacionados con la calidad de la carne: capacidad de retención de agua, jugosidad y flavor. En relación al flavor, cabe destacar la importante afectación de la androsterona y del escatol en el caso de carne procedente de machos enteros y su relación con el olor sexual.

La **calidad tecnológica** está íntimamente ligada al proceso industrial de transformación, que se lleve a cabo sobre la carne por parte de la industria alimentaria, uno de los parámetros más importantes es la capacidad de retención de agua, si bien también cabe destacar el pH, la consistencia de la grasa y su estabilidad oxidativa.

La **calidad nutricional** de la carne está relacionada con las necesidades nutritivas de los humanos, y se ha ido perfilando a medida que los estudios médicos sobre requerimientos nutricionales en humanos han ido cambiando. El principal factor de controversia en este bloque es el contenido y composición de la grasa de la carne. Las recomendaciones nutricionales elaboradas por los organismos médicos han disminuido el consumo de grasas animales, principalmente el consumo de grasas saturadas, así como el aumento del contenido en grasa monoinsaturada y poliinsaturada (ácidos grasos ω-3). Estas recomendaciones favorecen el consumo de carne de porcino en relación a la carne de rumiantes, debido a su menor contenido en grasa intramuscular y a un menor contenido en grasa saturada en su composición respecto a los rumiantes, debido principalmente al hecho que los monogástricos no transforman las fuentes de grasa ingerida a diferencia de los rumiantes.

La **calidad higiénica** de la carne se relaciona con la ausencia de contaminantes en la carne, principalmente de microorganismos perjudiciales para la

salud animal y humana. Esta calidad se puede alterar a lo largo de toda la cadena alimentaria, teniendo especial repercusión el tratamiento *post-mortem*, siendo esencial evitar romper la cadena de frío de la carne. Otro parámetro relacionado con la calidad higiénica se refiere a la ausencia de residuos en la carne (antibióticos, pesticidas y metales pesados).

II.1.2. Contenido de grasa intramuscular

En el apartado anterior se ha hecho patente la importancia del contenido y composición de la grasa intramuscular en relación a la calidad de la carne, afectando a la calidad organoléptica, tecnológica y nutricional. Así pues el estudio de la grasa intramuscular es de gran importancia para la cadena productiva del porcino, tanto en la etapa de engorde, como en el posterior sacrificio y procesado de la carne hasta su consumo. No obstante, es necesario tener en cuenta su relación con el resto de depósitos grasos del organismo. Del contenido graso total de un animal se pueden distinguir cuatro tipos distintos de deposición: grasa subcutánea, grasa intermuscular, grasa interna asociada a órganos y grasa intramuscular. En la especie porcina la grasa subcutánea representa del 60 al 70% del total, la intermuscular del 20 al 35% y la grasa interna asociada a órganos del 10 al 15% del total (Kuhn et al., 1997; Kouba et al. 1999), siendo variable el contenido en grasa intramuscular.

A diferencia del ganado bovino, el depósito graso más abundante en el ganado porcino es la grasa subcutánea, variable sobre la cual los programas de mejora genética realizados los últimos años, han seleccionado en su contra a través de la disminución del espesor de la grasa dorsal. Esta selección ha propiciado como efecto asociado la disminución del contenido en grasa intramuscular, Hovenier et al. (1992) estimaron una correlación entre el contenido de grasa intramuscular y el espesor de grasa dorsal de 0.37, Suzuki et al. (2005) obtuvieron una correlación genética baja (0.28) entre el contenido en grasa intramuscular en el músculo *longissimus dorsi* y el espesor de grasa dorsal. Solanes et al. (2009) obtuvieron en una población de cerdos Duroc una correlación más alta (0.64) a 180 días de edad (IMF medida sobre el músculo *gluteus medius*), este valor permitiría modificar el

contenido de grasa intramuscular, sin aumentar el espesor de la grasa dorsal al seleccionar por peso vivo. Sellier (1998) concluyó que parte de la variación del contenido en grasa intramuscular es independiente del contenido de lípidos global de la canal, y por lo tanto se podría obtener una respuesta genética distinta en diferentes depósitos grasos de la canal. Reixach et al. (2009) concluyeron que existe variación genética asociada al espesor de grasa dorsal independiente del contenido de grasa intramuscular.

El contenido de grasa intramuscular hace referencia al total de lípidos asociados a las células presentes en un músculo o muestra de carne proveniente de un músculo. Dentro de estos lípidos se encuentran triglicéridos, fosfolípidos, monoglicéridos, diglicéridos, colesterol, ésteres de colesterol y ácidos grasos libres; siendo los componentes mayoritarios los triglicéridos y los fosfolípidos. Los fosfolípidos se encuentran principalmente en la membrana celular y su contenido es bastante constante, representando un contenido del 0.4-0.5% de grasa intramuscular en cerdos (Gerbens, 2004), si bien entre tipos diferentes de músculos su contenido varía, siendo menor en las fibras musculares blancas de tipo glicolítico y mayor en las fibras musculares rojas de tipo oxidativo (Leseigneur-Meynier y Gandemer, 1991, Tabla 1). Esta variación en el contenido de grasa intramuscular entre tipos de fibras, se debe principalmente a la variación en la acumulación de adipocitos entre los diferentes tipos de fibras (Kaufmann y Safani, 1967; Gondret et al., 1998). El incremento en grasa intramuscular se debe principalmente a un aumento de los triglicéridos, al ser los fosfolípidos más constantes, según se desprende de diferentes trabajos en cerdos (Cameron y Enser, 1991; Essen-Gustavsson et al., 1994; Fernandez et al., 1999). La variación del contenido en grasa intramuscular con la edad se debe principalmente a la hiperplasia y a la hipertrofia de los adipocitos (Hood y Allen, 1973).

Tabla 1. Composición de los lípidos de la grasa intramuscular en distintos tipos de músculos (Leseigneur-Meynier y Gandemer, 1991).

Músculo	Tipos de fibras musculares	g/100 g de músculo		
		Total lípidos	Triglicéridos	Fosfolípidos
longissimus dorsi	Glicolíticas	1.5	1.0	0.48
biceps femoris	Intermedias	1.4	0.8	0.63
psoas major	Intermedias	1.3	0.7	0.72
trapezius	Oxidativas	2.0	1.3	0.69
masseter	Oxidativas	1.8	0.9	0.86

II.1.3. Composición de la grasa intramuscular

La composición de la grasa intramuscular es de una gran importancia, tanto desde un punto de vista tecnológico, como desde un punto de vista nutricional y su relación con la salud de los consumidores (Wood et al., 2004). En los últimos años, el interés suscitado por los efectos beneficiosos de los ácidos grasos ω-3 en relación a la salud humana y los efectos negativos del elevado consumo de grasas saturadas, ha propiciado el estudio de la composición de la grasa intramuscular de los animales de abasto, así como de su modificación. Este hecho ha sido particularmente relevante en lo que se refiere a los ácidos grasos poliinsaturados, los cuales son los mayores componentes de los fosfolípidos intramusculares, Leseigneur-Meynier y Gandemer (1991) obtuvieron valores superiores al 30% de ácidos grasos poliinsaturados en los fosfolípidos, mientras que en los triglicéridos estos valores no superaron el 15% (Tabla 2), el porcentaje de ácidos grasos poliinsaturados en los fosfolípidos, fue mayor en los músculos con actividad oxidativa respecto a los músculos de actividad glicolítica. Van Laack y Spencer (1999) encontraron valores de ácidos grasos poliinsaturados superiores al 50% en los fosfolípidos de la grasa intramuscular de

distintos cruces porcinos, analizados para estudiar las diferencias genéticas en su composición. Así pues, los ácidos grasos deseados en la carne porcina, se encuentran principalmente formando parte de las membranas celulares y son proporcionalmente más estables en su composición (Enser, 1984; Cameron et al., 2000) que los triglicéridos, y al aumentar el contenido de grasa intramuscular su incremento es menor que en el caso de los triglicéridos.

Tabla 2. Composición de los ácidos grasos de la grasa intramuscular en distintos tipos de músculos (Leseigneur-Meynier y Gandemer, 1991).

Ácidos grasos	**Porcentaje total de ésteres metílicos presentes**				
	longissimus dorsi	*biceps femoris*	*psoas major*	*trapezius*	*masseter*
	Composición de los triglicéridos				
SFA	42.5	36.2	42.4	41.1	39.6
MUFA	50.3	49.0	46.8	47.4	49.4
PUFA	7.2	14.9	10.8	10.5	10.8
	Composición de los fosfolípidos				
SFA	35.5	35.4	36.0	35.0	35.8
MUFA	30.5	25.1	24.8	26.5	27.0
PUFA	33.7	39.7	38.8	36.6	36.6

A diferencia de los animales rumiantes, la composición de la grasa intramuscular del porcino está condicionada por la dieta consumida por los animales, así, dietas ricas en grasa poliinsaturada representan incremento del porcentaje de grasa poliinsaturada. Koch et al. (1968) y Brooks (1971) estudiaron el efecto de la dieta sobre la composición de la grasa en el porcino, obteniendo en ambos estudios un efecto marcado de la dieta sobre la composición final de la grasa, estudios posteriores han corroborado y profundizado en estas afirmaciones (Leskanich et al., 1997; Joo et al., 2002; Engel et al., 2001; Apple et al., 2008). Este hecho permite incrementar el contenido de ácidos grasos ω-3 en la carne porcina, si bien su

incremento se ha producido principalmente a partir de dietas de origen vegetal ricas en C18:3 y pobres en C20:5 y C22:6, los ácidos grasos ω-3 de mayor interés. En humanos la conversión de C18:3 a C20:5 es limitada, inferior al 8%, mientras que la conversión de C18:3 en C22:6 es aún más baja, inferior al 4% (Burdge y Calder, 2005). Kloareg et al. (2005) en un trabajo en cerdos, encontraron valores de conversión de C18:3 a C20:5, C22:5 y C22:6 superiores a un tercio, valores muy superiores a los encontrados en humanos, sugiriendo una mayor eficiencia de conversión en cerdos que en humanos, si bien Duran-Montgé et al. (2008) no apreciaron un incremento de C22:6 en la carne de cerdos a partir de dietas ricas en sus precursores; en el mismo trabajo encontraron que la composición de la grasa intramuscular no está tan afectada por la composición de la grasa de la dieta, como es el caso de otros depósitos grasos en el organismo, hecho este que dificultaría la modificación de la composición de la grasa intramuscular.

El interés en disponer de productos animales ricos en ácidos grasos ω-3, ha aumentado de forma importante en los últimos años, la relación del consumo de ácidos grasos ω-3 (principalmente C20:5 y C22:6) con la salud está bien documentada, sus efectos beneficiosos en relación a la protección contra el cáncer (Hardman, 2002), en la prevención de problemas coronarios (Wijendran y Hayes, 2004), en la función inmune (Miles y Calder, 1998) y su papel durante la gestación (Allen y Harris, 2001) hacen que en países con un elevado consumo de carnes y bajo consumo de pescado (principal contenedor de ácidos grasos poliinsaturados de cadena larga), sea de especial interés aumentar la concentración de estos ácidos grasos en los productos cárnicos, siendo lo más habitual enriquecer los productos cárnicos obtenidos a partir de animales monogástricos, con la inclusión de aceites vegetales ricos en ω-3, principalmente en C18:3, el cual, tal y como se ha citado en el parágrafo anterior, puede convertirse en C20:5 y C22:6 en el organismo animal en porcentajes variables. Otro parámetro de interés en relación al consumo de grasa y la salud humana es la relación ω-6/ω-3 especialmente alta en los países occidentales, superior a 15 y muy superior al valor máximo recomendado de 5, este índice se considera un factor de riesgo en las enfermedades coronarias (Simopoulos, 2001).

La composición de la grasa intramuscular tiene un efecto importante sobre la percepción de los atributos de la carne, Cameron y Enser (1991) encontraron que SFA y MUFA tiene una correlación positiva con el sabor de la carne, mientras que los PUFA tienen una correlación negativa. Campo et al. (2003) relacionaron la composición en ácidos grasos con la percepción del olor por parte de dos paneles entrenados, relacionando el ácido graso C18:1 con un olor aceitoso, el C18:2 con olor a aceite frito y el C18:3 con olor a pescado y a aceite de linaza. Además el mayor riesgo de oxidación de los ácidos grasos poliinsaturados, provoca mayores problemas de rancidez en carnes con elevado contenido en PUFA además de una grasa menos firme; Enser (1983) y Wood (1983) cifraron en un 15% el máximo de ácido linoléico en la grasa de la carne para tener una grasa firme y evitar problemas de oxidación.

II.2. MÉTODOS DE PREDICCIÓN DE LA CALIDAD DE LA CARNE IN-VIVO EN RELACIÓN CON LA GRASA INTRAMUSCULAR

II.2.4. Obtención de muestras *in-vivo*

La posibilidad de utilizar biopsias para la predicción *in vivo* de la calidad de la carne fue mencionada por Schmidt et al. (1971), en estos primeros experimentos se utilizaba una metodología de obtención de biopsias de complejidad considerable. Posteriormente Lundström et al. (1973) utilizaron un método de obtención de biopsias denominado "needle biopsy" (biopsia de aguja), con el cual conseguían muestras de grasa y de masa muscular, pero con el inconveniente de que la cantidad de muestra era insuficiente para realizar análisis de composición. Otro método utilizado para la obtención de biopsias *in vivo* ha sido el de disparo o "shot biopsy" (Schöberlein, 1976; Lahucky et al., 1987). Otros sistemas utilizados han sido el de muelle o "spring biopsy" (Kovac et al., 1992) y el de presión "press biopsy"

(Lahucky y Kovac, 1990). Actualmente el método de obtención de biopsias *in vivo* que parece más utilizado es el de muelle evolucionado o "spring loaded biopsy" (Lahucky et al., 1999).

La utilización de biopsias *in vivo* en porcino ha tenido diferentes finalidades, Villé et al. (1992) utilizaron un sistema de "needle biopsy" para la evaluación de la grasa intramuscular en lechones. En este experimento la obtención de la biopsia se realizaba en el músculo *biceps femoris*, y previamente a la inserción de la aguja se procedía a realizar una incisión con bisturí para facilitar la inyección; no aparecieron infecciones en los puntos de incisión, aunque fue necesaria la anestesia total. De los resultados descritos en el experimento, se concluye que el método es válido para la determinación de la grasa intramuscular.

Baas et al. (1998) evaluaron la utilización de biopsias ("press biopsy") como predictor de la calidad de la carne en cerdos vivos. De sus resultados, se concluye la necesidad de utilizar otros métodos combinados para mejorar la predicción de la grasa intramuscular, pues las correlaciones entre los análisis de biopsia *in vivo* y de muestras de la canal no fueron muy elevadas (0.39), argumentando que el principal causante de ello fue la falta de representatividad de la muestra, muy pequeña. Villé et al. (1997) en un experimento para evaluar dos métodos de ultrasonidos y uno de resonancia magnética, en relación a la predicción del contenido de grasa intramuscular, realizaron biopsias ("shot biopsy") a los animales a los 20 kg con anestesia total, a los 60 kg con anestesia local y a los 110 kg (*post-mortem*). El tamaño de la muestra obtenida con la biopsia fue de 500 mg a los 20 kg y de 1000 mg en las posteriores extracciones. Del experimento realizado se concluyó que el método de biopsia fue el más seguro de los cuatro analizados. Posteriormente Sánchez et al. (2002a) utilizaron el sistema de Spring Loaded Biopsy (Talmant et al., 1989) para la determinación del contenido en grasa intramuscular en el músculo *longissimus dorsi* a la altura de la última costilla; en este experimento una vez obtenida la muestra se procedía a la determinación de los lípidos en el músculo, mediante extracción por el método de Folch et al. (1957), transmetilándolos luego por el método de Morrison y Smith (1964). Posteriormente la cantidad de los diferentes ésteres metílicos se determinaba por cromatografía de gases obteniendo

finalmente el contenido en grasa intramuscular. Bee (2000) utilizó un método de shot biopsy en cerdas para determinar el efecto de dos tipos de alimentación en relación a la composición de ácidos grasos. Bellmann et al. (2003) llevaron a cabo diversas biopsias en vacuno a diferentes edades, para determinar la variación del contenido de grasa intramuscular en el músculo *Semitendinosus*, y Aburto y Azain (1996) determinaron el contenido y composición de la grasa a diferentes edades en porcino a partir de muestras de biopsia.

Una de las preocupaciones mayores que puede generar la utilización de biopsias en porcino, es la relativa al bienestar animal. En 1998, Geverink et al. llevaron a cabo un experimento para determinar el efecto de la biopsia ("shot biopsy") sobre el comportamiento, contenido en cortisol salival y frecuencia cardíaca en cerdos de sacrificio; de su estudio se concluye que la realización de biopsias a los animales *in vivo,* repercute en un incremento de la frecuencia cardíaca y la concentración de cortisol, siendo no aconsejable la utilización de este sistema en estudios donde sea necesario medir el estrés previo al sacrificio. En 1997, el Danske Slagterier empezó un estudio en el cual se realizaron alrededor de 2000 biopsias sin la utilización de anestesia alguna, de cada muestra de biopsia obtuvieron tres submuestras para determinar el pigmento, el glicógeno y el glicerol, como alternativa a la determinación del contenido en grasa intramuscular. Según este organismo la posibilidad de medir parámetros de calidad de la carne en animales vivos permitiría aumentar el progreso de la cría de animales un 30% respecto a los actuales esquemas de sacrificio de parientes (Danske Slagterier, 1999). Le Roy et al. (2000) utilizaron el método de shot biopsy también para la determinación in-vivo del contenido de glicógeno, glucosa-6-P, glucosa y lactato, con el objetivo de determinar el potencial glicolítico.

II.2.5. Equipos de ultrasonidos

La utilización de equipos de ultrasonidos para la determinación de la calidad de la canal en la industria porcina se inició en los años 50 con los trabajos de Claus (1956), Hazel y Kline (1959), Price et al. (1960ab) y Stouffer et al. (1961), en los

cuales se examinó la posibilidad de la utilización de equipos de ultrasonidos para la determinación de la profundidad de grasa dorsal y posteriormente del área del lomo. La aparición de estos equipos y su utilización, coincidió con el interés del sector porcino en la disminución de la grasa y el aumento de la masa muscular (Moeller, 2002).

Los primeros equipos utilizados eran los denominados A-mode (amplitud-profundidad), con los cuales se obtenía la profundidad de la grasa dorsal y del lomo. Uno de los problemas importantes detectados en relación a la utilización de los equipos de ultrasonido A-mode, era el relacionado con la habilidad del operador (Sather et al., 1986). Las correlaciones obtenidas por Price et al. (1960a), entre las medidas de grasa dorsal mediante ultrasonidos en vivo y ultrasonidos en canal, en relación a la grasa dorsal en canal eran en los dos casos de 0.89. Sather et al. (1986) en un estudio en el que comparó operario, aparato y punto de sondeo, detectaron que las medidas de grasa dorsal eran más exactas cuando existían dos capas de grasa subcutánea en lugar de tres; asimismo, detectaron una interacción importante aparato-operario, con lo cual la interpretación de los resultados variaba dependiendo del operario y del aparato utilizado. Posteriormente, la obtención de múltiples medidas a diferentes ángulos de incidencia con equipos A-mode, supuso la obtención de las primeras vistas de cortes transversales, con el objetivo de determinar el área del lomo (Price et al.,1960b; Stouffer et al., 1961). Sin embargo, esta determinación requiere de un tiempo largo de obtención, la inmovilidad del animal y la utilización de técnicos altamente cualificados (Moeller, 2002), con lo cual no se obtienen correlaciones muy altas, tan solo sobre 0.7 (Price et al. 1960b; Stouffer et al. 1961).

La introducción de los equipos de ultrasonidos B-mode (brillante) o Ultrasonidos en Tiempo Real (RTU en inglés) en 1984 (Stouffer y Liu, 1996) representó un salto cualitativo en la utilización de este tipo de equipos en la evaluación de animales vivos. Los primeros parámetros analizados con este tipo de aparato fueron la profundidad de la grasa dorsal y el área del lomo. A diferencia de los aparatos A-mode, los aparatos B-mode permiten la visualización de la imagen con la posibilidad de almacenar la imagen o realizar cálculos sobre la imagen congelada (Stouffer y Liu, 1996). Dion et al. (1996) compararon dos equipos de

ultrasonidos, un A-mode y otro B-mode (RTU) con el objeto de predecir el contenido en magro y el grado de marmoleo en porcino, las medidas de grasa dorsal se sobreestimaron y las de profundidad del lomo se subestimaron, ya que la calibración utilizada en el equipo RTU no pudo tener en cuenta la diferente velocidad de los ultrasonidos en la musculatura (1620 m/s) y la grasa (1480 m/s) y se supuso una velocidad media de 1550 m/s. Por otro lado en el equipo A-mode se calibró a 1480 m/s (corregiéndose posteriormente la profundidad del lomo con un factor del 10%); así pues los datos obtenidos con un equipo A-mode resultaron ser más exactos que en el equipo RTU debido a la dificultad de calibrar la velocidad de los ultrasonidos en los distintos tejidos. En el mismo estudio, y en relación a la predicción del grado de marmoleo se obtuvieron correlaciones muy bajas, llegando a la conclusión que, si bien los datos obtenidos no eran utilizables para la predicción *in vivo* a partir de ese trabajo, en posteriores trabajos se podría realizar de forma más precisa, mejorando la técnica.

Ragland et al. (1997) estudiaron la posibilidad de utilizar un equipo RTU en la predicción de la grasa intramuscular obtuviendo resultados prometedores, en más del 70% de los valores predecidos la clasificación fue correcta. Newcom et al. (2002) en un trabajo posterior, obtuvieron que la correlación entre las medidas de grasa intramuscular con RTU y las medidas de grasa intramuscular en canal fue de 0.60, si bien, en el modelo de predicción se utilizó el espesor de grasa dorsal además de cinco parámetros de las imágenes captadas. Posteriormente Schwab et al. (2009; 2010) utilizaron este último método para evaluar la eficacia de la selección por contenido de grasa intramuscular en una población de cerdos Duroc durante seis generaciones, obtuvieron una elevada correlación (0.86) entre el contenido de grasa intramuscular determinado sobre canal y el contenido de grasa intramuscular predecido a partir de las imágenes captadas mediante un equipo RTU (Aloka 500V SSD), haciendo patente la evolución de los equipos RTU y su potencial para predecir el contenido en grasa intramuscular.

En vacuno, la utilización de equipos de ultrasonidos en la predicción de profundidad de grasa dorsal, área del lomo, grasa intramuscular o el grado de marmoleo se ha utilizado para la generación de modelos en relación a la evolución de

estos parámetros (Brethour, 2000), encontrándose correlaciones entre los valores seriados de predicción de grasa intramuscular con ultrasonidos próximos a 0.85. Perkins et al. (1997) también en vacuno encontraron correlaciones de 0.69 entre valores de grasa intramuscular predecidos con ultrasonidos y determinados en canal; así Brethour (1994), Izquierdo (1996) y Herring et al. (1998) encontraron valores de correlación de 0.73, 0.60 y 0.61 respectivamente, mientras Greiner et al. (2003) de 0,89 y 0,86 entre la medidas de ultrasonidos en vivo y el contenido de grasa y el área del lomo.

Según Williams (2002) la utilización de medidas in-vivo, así como la utilización de datos propios del individuo (respecto a la utilización de la información de las parientes) significaría un ahorro próximo a 4500 $ en la selección de un futuro reproductor bovino, así como un ahorro de tiempo de uno a tres años.

El principal inconveniente de la utilización de la técnica de ultrasonidos en relación a la caracterización de la grasa intramuscular en animales vivos, es la imposibilidad de utilizar esta técnica para la determinación de la composición de la grasa, únicamente es utilizable para determinar el contenido de grasa intramuscular en animales vivos. A su favor cabe destacar el hecho de tratarse de una técnica no invasiva (al contrario que la técnica de utilización de biopsias) y más fácilmente aplicable en condiciones de granja.

II.2.6. Indicadores fisiológicos

Una posibilidad de utilizar determinaciones *in-vivo* para relacionarlas con la predicción de la calidad de la carne, se encontraría en el uso de indicadores fisiológicos tales como el potencial glicolítico (Larzul et al. 1998), los enzimas lipogénicos como el enzima málico (Bidanel et al. 2002), péptidos reguladores, entre los cuales destaca la leptina (Zhang et al. 1994, Vernon et al. 1999) y el factor IGF-1.

El enzima málico se relaciona con el contenido de grasa intramuscular (Mourot et al. 1999), en un estudio realizado por Bidanel et al. (2002) se detectó una

elevada correlación entre el enzima málico y el nivel de grasa intramuscular en cerdos de una población Large-White x Meishan. Bidanel et al. (2000) también detectaron una actividad basal de esta enzima en las razas más grasas. Otros enzimas estudiados han sido la acetil-CoA carboxilasa y el estearil-CoA desaturasa, Cánovas et al. (2009) encontraron una correlación positiva entre el contenido de grasa intramuscular y la enzima estearil-CoA desaturasa (r=0.48) si bien no se encontró correlación significativa con la enzima acetil-CoA carboxilasa.

La leptina, es una proteina secretada por los adipocitos, la cual se ha sugerido que tiene una función importante en la regulación del consumo de alimento en ratones y humanos (Ramsay et al. 1998) y con la distribución regional de la grasa (Xie et al. 1999). Asimismo, Bellmann et al. (2003) detectaron en terneros Charolais una correlación positiva entre el nivel de leptina y el contenido de grasa subcutánea. Houseknecht et al. (1998) determinaron que la leptina tiene un importante papel en la regulación de la grasa a largo plazo.

En lo que refiere al factor IGF-1, los resultados obtenidos en diferentes estudios difieren; mientras en porcino (Luxford et al. 1998) encontraron relaciones positivas entre la concentración plasmática de IGF-1 y el nivel de engrasamiento; en vacuno, Davis y Simmen (2000) encontraron una relación negativa. Ramsay et al. (1989) encontraron que el factor IGF-1 estimula la proliferación de adipocitos, y en las células del estroma vascular estimula la proliferación de preadipocitos y aumenta la diferenciación, incrementando el número de células por clúster. Frick et al. (2000) en un experimento con ratones observaron como el factor IGF-1 alteró el metabolismo del tejido adiposo, reduciendo la capacidad de metabolizar la glucosa.

El estudio de estos indicadores no permite la determinación de forma directa de la composición de la grasa intramuscular a la largo del ciclo de engorde, si bien si permite de forma indirecta el estudio del contenido y composición de la grasa intramuscular.

II.2.7. Marcadores genéticos

El uso de marcadores genéticos relacionados con la mejora de la calidad de la carne, es uno de los ejes con los que se está trabajando de forma más intensa en los últimos años, debido básicamente a los conocimientos adquiridos con relación a la secuenciación genética del genoma. Hasta el momento los genes más estudiados relacionados con la calidad de la carne son los genes Halotano (HAL) y Rendimiento Napole (RN), el primero relacionado con la obtención de carnes PSE y el segundo con un elevado contenido de glicógeno en la carne; la presencia de ambos genes se relaciona con un efecto claro sobre la calidad de la carne (Sellier, 1998). Sin embargo, muchos han sido en los últimos años los proyectos de investigación cuya finalidad era la de encontrar genes de expresión cuantitativa (QTLs), o marcadores genéticos relacionados con la calidad de la carne y otros caracteres de interés en la producción animal. La forma habitual de buscar estos QTL en el ganado porcino consiste en el examen del genoma, generalmente utilizando cruces F2 en lineas divergentes, como cerdo salvaje x Pietrain (Geldermann et al., 1996), Meishan x Pietrain (Geldermann et al., 1996), cerdo coreano x Landrace (Kim et al., 2005), Cerdo ibérico x Landrace (Clop et al., 2001), cerdo nativo brasileño x lineas comerciales (Lopes et al., 2002), Meishan x Large white (Bidanel et al., 2002), (Walling et al., 1998) o Meishan x Linea sintética (Campbell et al., 2003).

En la especie porcina se han identificado hasta el momento 6344 QTLs (Tabla 3) los cuales representan 593 caracteres diferentes, siendo los caracteres relacionados con la calidad de la carne los más importantes, tal y como se refleja en la Tabla 4.

Tabla 3. Número de QTLs identificados hasta el momento en la especie porcina (**http://www.genome.iastate.edu/cgi-bin/QTLdb/SS/browse**; mayo 2011).

Cromosoma	QTLs identificados	Cromosoma	QTLs identificados
X	273	9	201
Y	1	10	150
1	1391	11	119
2	565	12	162
3	218	13	229
4	569	14	221
5	232	15	176
6	546	16	108
7	704	17	103
8	286	18	96

Andersson et al. (1994) en un cruce de tercera generación entre cerdo salvaje y Large White encontraron un gen de expresión cuantitativa que explicaría un 20% de la varianza fenotípica para el contenido de la grasa dorsal y abdominal situado en el cromosoma 4. Janss et al. (1997), en un cruce con cerdos Meishan detectaron un gen recesivo con efecto sobre el contenido de grasa intramuscular, que explicaría porque los cerdos Meishan homocigotos negativos para este gen, tienen un 2,1% más de grasa intramuscular. En un estudio posterior (Sánchez et al., 2002b) se investigó la presencia de este gen en la población Duroc, encontrándose un efecto equivalente en esta raza.

Tabla 4. Caracteres más importantes analizados y número de QTLs relacionados en la especie porcina. (http://www.genome.iastate.edu/cgi-bin/QTLdb/index; mayo 2011).

Caracteres	Número de QTLs
Pérdidas por goteo	945
Espesor de grasa dorsal	158
Área del lomo	126
Longitud de la canal	122
Ganancia media diaria	82
Longitud vértebra cervical	80
Grasa dorsal décima costilla	76
Número de pezones	74
Grasa dorsal última costilla	73
Porcentaje de magro	65
Peso del jamón	64
Contenido de grasa intramuscular	63
PH 24 h *post-mortem* (lomo)	59
PH Longissmus Dorsi	54
Color L	52
Diámetro de los adipocitos	52

Por otro lado en el cromosoma 4 y en el cromosoma 6 respectivamente, se han encontrado los genes A-FABP y H-FABP dentro del grupo de los genes FABP ("Fatty Acid Binding Proteins") (Gerbens, 1997 y De Koning et al., 1998). Estos genes se relacionan con la grasa intramuscular, si bien dependiendo de los estudios, muestran efectos independientes respecto a la grasa dorsal (Gerbens, 2000) en un cruce con Meishan, o ligados a la grasa dorsal (Gerbens, 1999) en Duroc. En un estudio de Óvilo et al. (2001) se concluye que el gen A-FABP no tiene efectos relevantes sobre el contenido de grasa intramuscular, pero si podría afectar a su composición. Bidanel et al. (2002) encontraron una región situada en el cromosoma 7 con efectos significantes sobre la grasa intramuscular. Clop et al. (2003) detectaron

regiones del genoma porcino con efectos sobre la composición en ácidos grasos de la grasa, con resultados esperanzadores en los cromosomas 8 y 12. En un estudio más reciente, Cánovas et al. (2010) determinaron correlaciones significativas entre la expresión del gen HMGCR y el contenido y la composición de la grasa intramuscular.

Estos trabajos abren la posibilidad de utilizar los marcadores genéticos para mejorar la eficiencia de la producción porcina, disminuir costos y hacer posible la obtención de productos más saludables para los consumidores, así como hacer del cerdo un modelo biomédico más útil (Rothschild et al., 2007).

III. Objetivos

Los objectivos que se pretenden conseguir con la realización de esta tesis doctoral son los siguientes:

1. Desarrollar y evaluar un método para determinar simultáneamente el contenido y la composición de la grasa intramuscular en muestras de tamaño pequeño, tanto en animal vivo (biopsia) como en la canal (autopsia).

Posteriormente, con la metodología propuesta, obtener una serie de datos longitudinales del contenido y la composición de la grasa intramuscular, a fin de:

2. Determinar la evolución del contenido y la composición de la grasa intramuscular y subcutánea con la edad y el nivel de engrasamiento.

3. Examinar si la variación alélica en los genes IGF-1 (insulin-like growth factor-1) y LEP (leptina), así como la concentración de IGF-1 y leptina en plasma, se asocia con el contenido y la composición de la grasa intramuscular y si tal asociación es función de la edad.

IV. Material y Métodos General

IV.1. Material animal

Los datos utilizados para la realización de los estudios longitudinales incluídos en los trabajos de esta Tesis Doctoral se han basado en un conjunto experimental de cerdos Duroc. Este material animal estuvo compuesto por un total de 216 cerdos castrados de raza Duroc, los cuales fueron sometidos a un muestreo repetido para la obtención de muestras de músculo (de 2 a 7 muestras por cerdo). Los cerdos provenían de camadas producidas por 102 hembras y 36 verracos de la línea Duroc de la empresa Selección Batallé, y fueron criados hasta una edad de 220 días en tres lotes separados. El esquema de muestreo incluyó de una a dos biopsias del músculo *longissimus dorsi* (LM) tomadas al menos un mes antes del sacrificio (BE: biopsia precoz a 160 y 185 días); una biopsia de LM tomada dos semanas antes del sacrificio (BL: biopsia tardía a 210 días); una pequeña muestra del músculo LM que fue tomada *post-mortem* con el mismo aparato de obtención de biopsias, denominada biopsia de la canal (BC) y una muestra grande obtenida *post-mortem* de cada uno de los músculos LM y g*luteus medius* (GM).

Antes de recoger las muestras de BE y BL, los cerdos fueron pesados y se registró el espesor de la grasa dorsal y la profundidad de lomo a la altura de la última costilla a 5 cm de la línea media, con el aparato portátil de ultrasonidos Piglog 105 (SFK-Technology, Herlev, Dinamarca). Las biopsias fueron retiradas utilizando una cánula de 8 mm de diámetro insertada en un aparato de recogida de biopsias equipado con un resorte (PPB-U Biotech, Nitra, Slovakia) y fueron tomadas a 6 cm de profundidad en la misma localización donde fueron registrados los valores de grasa dorsal y profundidad de lomo por ultrasonidos, siguiendo el procedimiento descrito en Oksbjerg et al. (2004) convenientemente adaptado (apartado IV.2). Las muestras de músculo fueron recortadas para eliminar restos de piel y de grasa subcutánea, completamente congeladas en nitrógeno líquido, y almacenadas en tubos a -80ºC hasta su posterior análisis. Al final de la prueba, los cerdos fueron sacrificados en un matadero comercial equipado con un sistema de aturdimiento con dióxido de carbono (Butina ApS, Holbaek, Dinamarca), donde el espesor de grasa subcutánea y la profundidad del lomo a 6 cm de la línea media entre la tercera y

cuarta últimas costillas, fueron predecidos con el aparato de clasificación automática de canales Autofom (SFK-Technology, Herlev, Dinamarca). Después de enfriar las canales durante 24 horas a 2ºC, las muestras *post-mortem* BC, LM y GM fueron obtenidas del lado izquierdo de la canal. Los especimenes BC y LM fueron tomados en una muestra aleatoria de cada lote formada por 30 cerdos. La muestra de LM se obtuvo a partir de una sección de aproximadamente 1 kg de lomo, obtenida al nivel de la tercera y cuarta costilla. Las muestras fueron inmediatamente congeladas y almacenadas hasta su posterior utilización. La muestra de BC fue tomada y procesada de igual forma que las muestras de biopsia. Así, las muestras de BC fueron obtenidas con el mismo aparato de resorte de obtención de biopsias, en el mismo punto que en los animales vivos. Una muestra de alrededor de 50 g del músculo GM fue tomada de todos los cerdos sacrificados y fue procesada de igual forma que las muestras de LM. Todas las muestras fueron determinadas por duplicado por GC.

La puesta a punto del método de realización de biopsias se ha llevado a cabo con el fin de determinar el contenido y composición de la grasa intramuscular, así como la composición de la grasa subcutánea. Todos los procedimientos experimentales llevados a cabo han sido aprobados por el Comité de Ética para la experimentación animal de la Universitat de Lleida (Acuerdo 2/01, Marzo 2001) y los procedimientos con animales y su cuidado se llevaron a cabo de acuerdo con la autorización AE2374 del Departamento de Agricultura, Ganadería y Pesca de la Generalitat de Catalunya. En un experimento previo se comprobó que no era necesario aplicar anestesia total (Bosch et al., 2003). Los aspectos prácticos de la técnica de obtención de biopsias se han publicado en Bosch et al. (2005) y se resumen a continuación.

IV.2. Método de obtención de biopsias

IV.2.8. Descripción del equipo “spring loaded biopsy”

Las biopsias se realizan con el equipo *“Spring loaded biopsy”* (PPB-U, Biotech, Nitra, Slovakia) utilizado anteriormente en diversos experimentos (Baas et

al., 1998; Ellis et al., 1998; Lahucky et al., 1999 y Hamilton et al., 2000). Este aparato consta de un muelle central recubierto de una camisa cilíndrica metálica; al final del muelle se dispone un eje central al cual se le acopla una cánula metálica formada por un cilindro metálico hueco en su interior (Figura 1), la cánula dispone de un extremo cortante con un mecanismo de cierre y corte (Figura 2). Todo el conjunto se coloca en el interior de un cilindro metálico, que actúa de calibrador de la profundidad de penetración y de cierre del mecanismo de la cánula. El conjunto una vez comprimido el muelle, impulsa la cánula a una velocidad superior a los 300 m/s (Kovac, comunicación personal) la cual atraviesa piel, grasa subcutánea y parte del músculo *longissimus dorsi*. Una vez cortada y recogida la muestra se procede a su extracción. Los diámetros de cánula utilizables son de 6 mm, 7 mm, y 8 mm.

Figura 1. Esquema del aparato *“Spring loaded biopsy”*.

Figura 2. Detalle cánula extracción de biopsias.

IV.2.9. Descripción de la técnica de obtención de biopsias

Para realizar la obtención de la biopsia en animales vivos de la forma más cómoda tanto para el animal como el operador, se debe inmovilizar al animal en una jaula, posteriormente se localiza la última costilla y se desplaza 5 cm de la columna vertebral, se aplica anestesia local en los alrededores del punto de inserción, mientras se espera a que la anestesia haga efecto, se procede a lavar con agua y jabón (con digluconato de clorhexidina al 4%) la zona elegida, se rasura una superficie de aproximadamente 5x5 cm. (Figura 3). Se utiliza un aparato de ultrasonidos estándar (Figura 4) para determinar el espesor de grasa dorsal y así decidir la profundidad de inserción de la biopsia. Se desinfecta la zona con solución de digluconato de clorhexidina al 5%, solución antiséptica. Una vez realizados estos pasos se procede a cargar y asegurar el "*Spring loaded biopsy*", a armar la cánula y montar la cánula en el aparato adecuando la profundidad. Se sitúa el "*Spring loaded biopsy*" sobre la zona rasurada y desinfectada, se quita el seguro y se dispara el aparato (Figura 5). Una vez disparada la cánula, se retira inmediatamente el aparato y se procede a desinfectar la zona con solución de digluconato de clorhexidina al 5%, se aplica aerosol tópico con clortetraciclina y finalmente aerosol tópico protector y coadyuvante de la cicatrización (Figura 6). Se aplica aerosol tópico protector y coadyuvante de la cicatrización 2 veces al día durante los siguientes 3 días a la realización de la biopsia, hasta su total cicatrización (Figura 7). No se observaron daños en el matadero; tal como determinó Villé et al. (1992). El tiempo mínimo entre la realización de la biopsia y el sacrificio del animal se estimó en 15 días, si bien se acepta un período de 15 a 21 días como correcto. La realización de biopsias 30 días previos al sacrificio de los animales, imposibilita la utilización del aerosol tópico con clortetraciclina con el fin de respetar el período de retirada del producto; en las pruebas llevadas a cabo, la no utilización de este producto no ha ocasionado infecciones locales salvo en contadas ocasiones (1-2% de los casos).

Una vez acabada el proceso sobre el animal se procede a armar y vaciar la cánula de su contenido; extraída la muestra de biopsia (Figura 8) se procede a su separación entre grasa subcutánea y músculo con ayuda de un bisturí, envasándose

cada fracción en un vial diferente y congelándose en nitrógeno líquido a –196ºC, conservándose posteriormente hasta su análisis en congelador a –80ºC. Después de vaciar la cánula se procede a realizar su limpieza y desinfección: se limpia la cánula con detergente desengrasante (se sumergen las cánulas durante 3-4 minutos), se frota con cepillo el interior de la cánula y se limpia a fondo teniendo especial cuidado con las cuchillas de la cánula, donde se deben quitar todos los restos existentes; se lava con agua limpia, agua destilada, se seca la cánula y finalmente se esteriliza con calor seco durante 20' a 180ºC. Finalmente se almacenan las cánulas en recipientes estériles (Para conservar las cánulas para períodos largos: una vez limpias se rocian con spray engrasante para proteger los mecanismos y evitar la corrosión; regularmente se procede al examen de las cánulas y si es preciso al afilado de estas).

Figura 3. Rasurado de la zona de incisión de la biopsia.

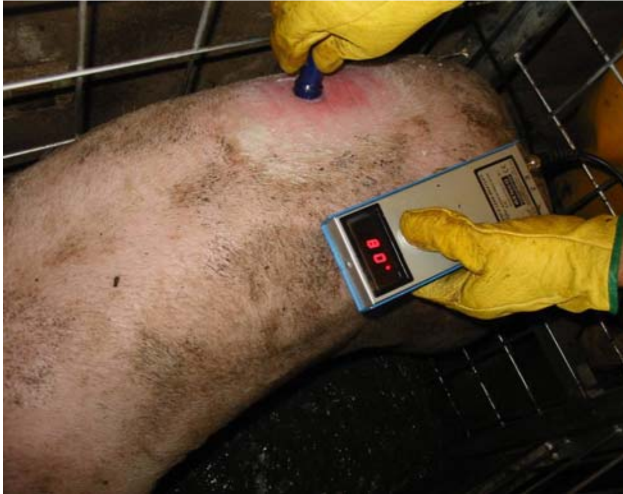

Figura 4. Determinación de la profundidad de la grasa dorsal con aparato de ultrasonidos.

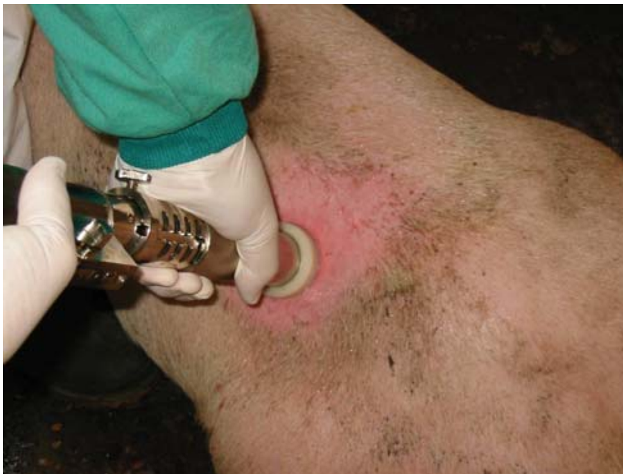

Figura 5. Realización biopsia.

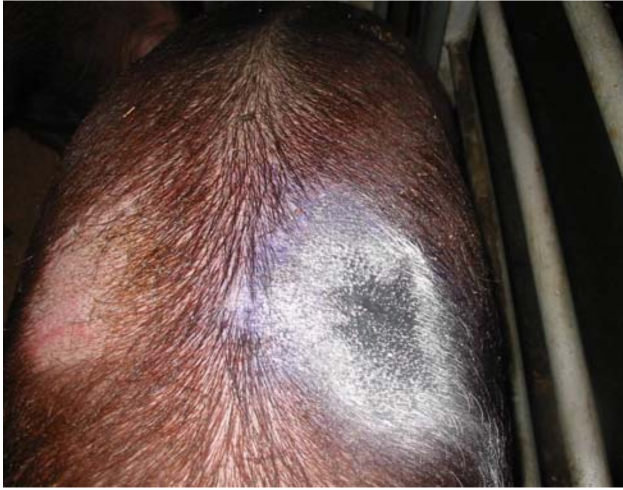

Figura 6. Estado de la zona después de la realización de la biopsia.

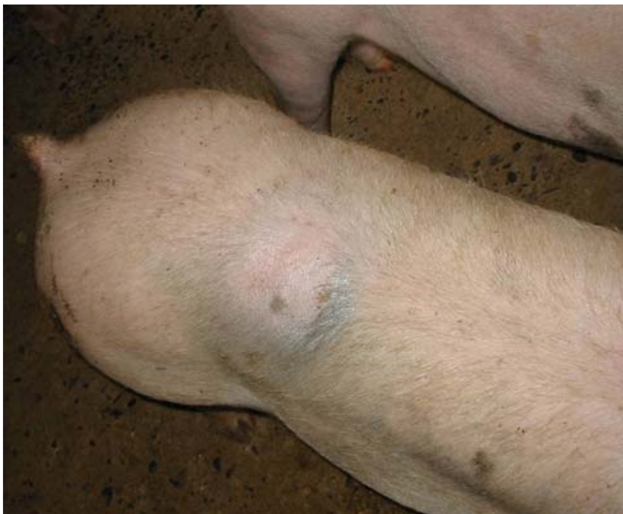

Figura 7. Estado de la zona de incisión 9 días después de realizar la biopsia.

Figura 8. Muestra extraída en la biopsia.

IV.3. Material necesario para la realización de las biopsias

El material necesario para la realización de biopsias *in-vivo* es el siguiente:

- Jaula inmovilización.
- Aparato de ultrasonidos estándar.
- Vaselina.
- *"Spring loaded biopsy"* BIOTECH PPB-U.
- Cánulas.
- Cargador de cánulas.
- Tubos de conservación de tejidos.
- Anestesia local.
- Jabón con digluconato de clorhexidina al 4%.
- Solución de digluconato de clorhexidina al 5%.
- Rasurador (cuchilla, hojas afeitar, navaja o afeitadora).
- Detergente, trapo y cubo.
- Detergente desengrasante, escobilla y cepillos para limpiar las cánulas.
- Esterilizador calor seco.

- Cajas metálicas esterilización con tapa.
- Aerosol tópico protector y coadyuvante de la cicatrización.
- Aerosol tópico con clortetraciclina.
- Tanque de nitrógeno líquido.
- Nevera y hielo.

IV.4. Determinación del contenido y la composición de la grasa intramuscular por cromatografía de gases.

Las muestras congeladas fueron extraídas del tanque de nitrógeno líquido o del congelador 12 horas antes de llevar a cabo los análisis de laboratorio. Las muestras de BE, BL y BC fueron directamente liofilizadas y posteriormente completamente homogeneizadas con la mezcla de arena con una varilla agitadora de vidrio. Debido a su pequeño tamaño, la materia seca de las muestras fue calculada como la diferencia de peso antes y después de liofilizarse, toda la muestra se utilizó para los análisis posteriores. Después de que las muestras de LM y GM fueron completamente descongeladas, las pérdidas de vacío por goteo fueron eliminadas, el músculo diseccionado, recortado de grasa subcutánea y intermuscular. Una pequeña cantidad de la muestra fue utilizada para determinar la materia seca mediante el secado durante 24 horas a 100-102ºC en un horno, mientras el resto de la muestra fue liofilizada y molturada utilizando un picador eléctrico. Una alícuota representativa de la fracción liofilizada y molturada se utilizó para la realización de los análisis químicos.

Los ésteres metílicos de los ácidos grasos fueron obtenidos directamente mediante transesterificación utilizando una solución de trifluoruro de boro al 20% en metanol (Rule, 1997). El análisis de los ésteres metílicos de los ácidos grasos se realizó por cromatografía de gases licados con una columna capilar de 30m x 0.25mm modelo SP2330 (Supelco, Tres Cantos, Madrid) y un detector de ionización de llama con helio como gas portador a 1 mL/min. El programa de control de temperatura del horno incrementó la temperatura de 150º a 225ºC a razón de 7ºC por

minuto, el inyector y detector tuvieron ambos una temperatura de 250ºC (Tor et al., 2005). La cuantificación se llevó a cabo a través de la normalización del área después de añadir en cada muestra 1, 2, 3-Tripentadecanoilglicerol como patrón interno. El valor del contenido de IMF fue calculado como la suma de cada ácido graso expresada como equivalente triglicérido (AOAC, 2000) sobre una base de tejido seco. La composición de los ácidos grasos del IMF fue calculada como el porcentaje de cada ácido graso en relación al total de ácidos grasos expresado como mg/g de ácido graso. La proporción de ácidos grasos poliinsaturados (PUFA) (C18:2n-6; C18:3n-3; C20:2n-6; y C20:4n-6), ácidos grasos monoinsaturados (MUFA) (C16:1n-7; C18:1n-9; y C20:1n-9) y ácidos grasos saturados (SFA) (C14:0; C16:0; C18:0; y C20:0) fue calculada.

V. Resultados y Discusión

ESTUDIO 1. ESTIMATING INTRAMUSCULAR FAT CONTENT AND FATTY ACID COMPOSITION IN LIVE AND *POST-MORTEM* SAMPLES IN PIGS.

Basado en el artículo de:

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Abstract

Repeated muscle sampling (n=732) on 216 pigs was performed to evaluate the effect of live sampling and sample size on intramuscular fat (IMF) content and fatty acid (FA) composition. The sampling scheme consisted of 1-3 biopsies of *longissimus dorsi* (LM), a small and a big *post-mortem* sample of LM, and a big *post-mortem* sample of *gluteus medius* (GM). IMF was determined by quantitative gas chromatography after direct transesterification. Data on LM were jointly analyzed using a mixed model on age with heterogeneous residual variances across sampling methods. Biopsies overestimated IMF and polyunsaturated FA content and underestimated monounsaturated FA content with decreasing sample size. Potential for bias and not sampling variance is the major limitation of using live samples for assessing changes in IMF with age. Small *post-mortem* samples of LM resulted as informative as big samples of GM for inferring on IMF content but not on IMF composition.

Key Words: **Fatty acid, Intramuscular Fat, Marbling, Muscle sampling, Swine.**

Introduction

Intramuscular fat (IMF) content and fatty acid (FA) composition are considered to play an important role in pig meat quality (Fernandez, Monin, Talmant, Mourot & Lebret, 1999; Wood et al., 2003), particularly in the acceptability of dry-cured products (Ruiz, García, Muriel, Andrés & Ventanas, 2002), and in the prevention of some human diseases (Williams, 2000). For this reason, in recent years more attention has been focused on studies assaying practical strategies aimed at their amelioration. Variations in the genetic type and diet, along with their interactions, were the factors mostly investigated (Wood et al., 2004; Cilla et al., 2006). The few experiments estimating the effect of age (Latorre, Medel, Fuenteaja, Lázaro & Mateos, 2003) or the weight at slaughter (Correa, Faucitano, Laforest, Rivest, Marcoux & Gariépy, 2006) on IMF traits relied on cross-sectional comparisons across age or weight groups. In such designs, pigs are serial slaughtered

at a fixed time and then response variables, which are measured once, one at a single time, compared across groups.

A more efficient design for estimating the effect of aging is assessing the within-subject change over time (Fitzmaurice, Laird & Ware, 2004). This only can be achieved by using longitudinal designs, which are based on the analysis of repeated measurements on the same subject over time. Such a recording scheme cannot be easily implemented for tissue composition traits unless biopsies are taken. Backfat biopsies have often been used in experiments testing for the effect of dietary treatments by age on backfat FA composition (Warnants, Oeckel & Boucqué, 1999; Pascual et al, 2005) but this approach has been rarely used for studying IMF traits (Sobocki, Fourtanier, Estany & Otal, 2006). Reasons for that include the difficulties in applying the conventional methods for fat determination to small specimens and the potential negative influences that small sampling may exert on the outcome. A primary concern is whether the statistical advantages of longitudinal analyses outweigh the expected increase in sampling error from small samples. The aim of this study was to assess a method for simultaneously determining the IMF content and FA composition in small samples and then to evaluate the effect of live sampling and sample size on IMF content and FA composition determinations. The use of biopsies and small samples for use in longitudinal studies and quality labeling concerning IMF traits is also discussed.

Materials and methods

Animals and experimental procedures

Two experiments were carried out in this study. Experiment 1 was designed to assess the method presented here for simultaneously estimating the IMF content and composition while Experiment 2 was undertaken to evaluate the effect of muscle sampling conditions on the determinations. The experimental procedures by means of which this study was conducted were approved by the Ethics Committee for Animal Experimentation of the University of Lleida (Agreement 2/01, March 2001).

Experiment 1 used 52 *m. longissimus dorsi* (LM) from three different pork quality types (Duroc at 220 days, n=24; Duroc at 185 days, n=15; and Duroc x Pietrain at 185 days, n=13). A section of around 1 kg was taken from the left loin of each carcass at the level of the third and fourth last ribs. The sample was immediately vacuum packaged and stored in deep freeze until required. IMF content was determined in duplicate both by ether extraction in a Soxhlet apparatus (SX) and by quantitative determination of the FA by gas chromatography (GC). SX was conducted according to the Association of Official Analytical Chemists regulations (AOAC, 2000) and GC is described in section 2.2.

In Experiment 2, a total of 216 Duroc barrows were subjected to repeated sampling for muscle specimens (from 2 to 7 per pig). The pigs were from litters produced by 102 sows and 36 boars from the Duroc line of Selección Batallé and were reared up to around 220 days of age in three separate batches. The muscle sampling scheme included 1 to 2 biopsies of LM taken at least one month before slaughter (BE: early biopsy); a biopsy of LM taken two weeks before slaughter (BL: late biopsy); a small *post-mortem* sample of LM taken with the biopsy needle, which, for simplicity, will be referred as carcass biopsy (BC); and a big *post-mortem* sample of both LM and *m. gluteus medius* (GM). The muscle sampling characteristics used in Experiment 2 by muscle sampling method are detailed in Table 1.

Before taking the BE and BL biopsies, the pigs were weighed and their backfat and loin-muscle depth at 5 cm of the midline at the position of the last rib ultrasonically recorded using the portable equipment Piglog 105 (SFK-Technology, Herlev, Denmark). Biopsies were removed using 8-mm cannula inserted into spring-loaded biopsy device (PPB-U Biotech, Nitra, Slovakia) and were taken 6 cm deep at the same location where ultrasonic backfat was measured following the procedures described in Oksbjerg, Henckel, Andersen, Pedersen & Nielsen (2004). Muscle samples were trimmed of fat and skin, snap frozen in liquid nitrogen, and stored in tubes at -80ºC until required for analyses. At the end of the trial pigs were slaughtered in a commercial slaughterhouse equipped with the carbon dioxide stunning system (Butina ApS, Holbaek, Denmark), where subcutaneous fat and loin thickness at 6 cm off the midline between the third and fourth last ribs were predicted

using the Autofom automatic carcass grading (SFK-Technology, Herlev, Denmark). After chilling for about 24 h at 2ºC, BC, LM and GM *post-mortem* samples were obtained from the left side of the carcass. BC and LM specimens were taken in a random sample of 30 pigs within each batch. LM was identically taken and processed as loin samples in Experiment 1 while BC was identically taken and processed as a biopsy. Thus, BC samples were removed with a spring-loaded biopsy device from the same anatomic site where BL samples were taken. A sample of around 50 g of GM was taken in all slaughtered pigs and was processed as LM. All samples in Experiment 2 were determined in duplicate by GC.

Determination of IMF content and composition by gas chromatography

Frozen samples were removed from the nitrogen tank or the freezer 12 h prior to laboratory analyses. BE, BL and BC samples were directly freeze-dried and thereafter thoroughly homogenized by mixing with sand using a glass stirring rod. Due to their small size, dry matter in biopsies was calculated as the weight difference before and after freeze-drying, and the whole sample used for subsequent analyses. After LM and GM samples were completed defrosted, vacuum drip losses were eliminated and the dissected muscle, trimmed of subcutaneous and intermuscular fat, was minced. A small quantity of the sample (Table 1) was used to determine dry matter by drying 24 h at 100 to 102ºC in air oven whereas the rest of the sample was freeze-dried and pulverized using an electric grinder. A representative aliquot from the pulverized freeze-dried muscle was used for chemical analyses.

FA methyl esters were directly obtained by transesterification using a solution of boron trifluoride 20% in methanol (Rule, 1997). Analysis of FA methyl esters were performed by GC with a 30m x 0.25mm capillary column SP2330 (Supelco, Tres Cantos, Madrid) and a flame ionization detector with helium as the carrier gas at 1 mL/min. The oven temperature program increased from 150 to 225ºC at 7ºC per min, and the injector and detector temperatures were both 250ºC (Tor, Estany, Francesch & Cubiló, 2005). The quantification was carried out through area normalization after adding into each sample 1, 2, 3-Tripentadecanoylglycerol as internal standard. IMF was calculated as the sum of each individual FA expressed as triglyceride equivalents (AOAC, 2000) on a dry tissue basis. IMF FA composition

was calculated as the percentage of each individual acid relative to total FA and expressed as mg/g FA. The proportion of polyunsaturated (PUFA) (C18:2n-6; C18:3n-3; C20:2n-6; and C20:4n-6), monounsaturated (MUFA) (C16:1n-7; C18:1n-9; and C20:1n-9) and saturated (SFA) (C14:0; C16:0; C18:0; and C20:0) fatty acid content were calculated.

Statistical analyses

Data from Experiment 1 were used for the comparison between GC and SX methods. The comparison was performed in terms of the mean difference, the correlation between GC and SX, and the regression of GC on SX. The precision of each method was calculated as the correlation between duplicates and as the relative standard deviation. Data from BE, BL, BC, and LM in Experiment 2 were used to assess the effect of the muscle sampling method. The analysis was conducted using a linear mixed model, in which fixed effects included the batch (1 to 3) and the sampling method of LM (BE, BL, BC, and LM), with quadratic polynomials on age and on sample weight by sampling method as covariates. The animal and the residual were the random effects. The animal effect was split into the sire, dam, pig, and pig by age effects. Residual effects were modeled to account for heterogeneous variance across sampling methods. A multivariate model including a muscle effect was used to assess the differences between LM and GM. Variances were estimated by restricted maximum likelihood and contrasted using a likelihood ratio test. Fixed effects were tested following the Kenward-Roger method and the differences between sampling methods by the Tukey test. The analyses were performed using SAS PROC MIXED (SAS Inst. Inc., Cary, NC). The correlation between sampling methods was calculated as the covariance between the corresponding animal effects divided by the square root of the appropriate total variances.

Table 1. Means (standard deviations) of muscle sample characteristics used in Experiment 2 by muscle sampling method[1]

	Method of muscle sampling[1]				
	Ante-mortem		*Post-mortem*		
Trait	BE	BL	BC	LM	GM
No of records	277	63	88	88	216
Age, days	174.9 (12.6)	207.9 (3.0)	221.2 (3.6)	221.3 (3.6)	221.9 (3.8)
Weight[2], kg	107.9 (13.8)	128.9 (11.7)	105.3 (9.6)	105.1 (9.5)	103.8 (10.7)
Backfat depth[3], mm	18.5 (3.9)	22.7 (4.5)	24.5 (2.9)	24.4 (2.9)	23.5 (3.5)
Loin depth[3], mm	43.9 (4.2)	46.0 (5.3)	44.1 (9.4)	44.1 (9.4)	43.3 (9.0)
Sample weight[4], mg	168.4 (61.0)	127.5 (55.8)	161.8 (57.7)	167.7 (45.4)	142.1 (26.4)
Dry matter, mg/g	281.5 (20.3)	288.2 (22.6)	272.2 (15.9)	282.1 (12.7)	305.7 (31.1)

[1] BE, biopsy taken at least one month before slaughter (1 to 2 per pig); BL, biopsy taken two weeks before slaughter; and BC, small *post-mortem* sample. Samples were obtained from the *m. longissimus dorsi* at 5 cm of the midline between the third and fourth last ribs using a spring-loaded device; LM (GM, representative aliquot of a well-mixed section of the *m. longissimus dorsi* (*m. gluteus medius*) removed from the carcass. Samples of LM were taken between the third and fourth last ribs.

[2] Live weight in BE and BL and carcass weight in BC, LM, and GM.

[3] Ultrasonically measured with Piglog 105© (in the live pig) or Autofom© (in the carcass).

[4] Dry matter weight of the biopsy (BE, BL, and BC) or the sampled aliquot (LM, and GM) used for chemical analyses.

Results and Discussion

Analytical methods

The results in Table 2 indicate that GC is technical feasible and precise, as well as highly correlated with SX (0.99). In the three sample groups, the relative standard deviation was always lower than 5%. Analytical determination of fat content has traditionally been done by gravimetric-based methods employing organic solvents for lipid extraction (Folch, Less & Sloane-Stanley, 1957; AOAC, 2000). Gravimetric methods may be unable to extract certain bounded lipids but instead they may extract some low polarity non-lipid compounds (Maxwell, 1987). A hydrolysis prior to solvent extraction gives rise to free all lipid compounds but also increases the non-fat extractable amount. A convenient way to overcome this problem is to redefine fat, in consistency with nutritional labeling requirements (FDA, 1995), as the sum of FA from a total lipid extract, expressed as triglycerides. This study has been conducted following these guidelines after in situ transesterification.

The behavior of the SX and GC depended on the fat content of the sample (Table 2). GC showed lower values than SX in Duroc samples at 220 days (-9.82 mg/g dry matter, $p<0.01$) and higher in leaner crossbreds samples (+10.22 mg/g dry matter, $p<0.01$). No difference between methods was observed in Duroc at 185 days. A similar pattern was observed when comparing the methods within genetic type. Thus, GC was higher than SX in all crossbred samples but only in 5 of the Duroc samples at 220 days. In the three sample groups the regression of GC on SX was lower than unity (Table 2). The results in Duroc parallel those in Flickinger (1997), who concluded that gravimetric methods yielded a higher outcome than GC in a wide variety of food samples. It can be hypothesized that gravimetric approaches quantitate not only lipids but also components of low polarity. On the other hand, there are evidences indicating that, in non-fatty products, direct transesterification in one-step reaction is more efficient than gravimetric techniques requiring solvent extraction (Lepage & Roy, 1986; Eras, Ferran, Perpiña & Canela, 2004), particularly with regard to bounded lipids (Sattler, Puhl, Hayn, Kostner & Esterbauer, 1991). A

more complete recovery of bounded lipids, which are proportionally more abundant in lean meats, may therefore explain why GC was 16% more efficient than SX in crossbred loins.

Table 2. Comparison of quantitative gas chromatography after direct transesterification (GC) with conventional Soxhlet (SX) for the determination of intramuscular fat (IMF) content in the *m. longissimus dorsi*

		IMF (mg/g dry matter)				
Genetic type, age	n	GC	GC- SX	b[1]	r[1]	p[1]
Duroc, 220 days	24	161.3 ± 32.8	-9.82 ± 2.54 **	0.74 ± 0.03	0.99	0.98
Duroc, 185 days	15	112.8 ± 29.4	-1.58 ± 3.22 ns	0.73 ± 0.04	0.98	0.99
Duroc x Pietrain, 185 days	13	73.8 ± 18.7	10.22 ± 1.61**	0.78 ± 0.03	0.99	0.98
All	52	125.5 ± 46.3	-2.44 ± 1.90 ns	0.79 ± 0.01	0.99	0.99

[1]b: coefficient of regression of IMF by GC on IMF by SX; r: correlation between IMF by GC and IMF by SX; and p: precision of GC (correlation between replicates).
** $p<0.01$; ns, not significant ($p>0.05$).

Overall, the results indicate that GC tends to shrink the variance of the distribution of IMF content and therefore to reduce the differences among pigs. This does not affect the ranking but caution should be taken when comparing GC and SX values of samples greatly differing in IMF content. The main feature of GC is that it can be applied to small samples for simultaneously determining both IMF content and FA composition. Other methods, such as those based on gravimetric or infrared techniques, are difficult to adapt for use with biopsy samples (Villé, Maes, Schrijver, Spincemaille, Rombouts & Geers, 1995). Samples of at least 3 g are required for lipid extraction (AOAC, 2000) and much greater for predictions using near infrared reflectance spectroscopy (González-Martín, González-Pérez, Alvarez-García & González-Cabrera, 2005; Barlocco, Vadell, Ballesteros, Galietta, & Cozzolino,

2006). The glycerol concentration has been used for indirectly estimating IMF content in biopsies (Oksbjerg et al., 2004) but the results were not promising.

Muscle sampling and intramuscular fat content

The effect of the sampling method is given in Table 3. The animal variance for IMF content remained constant across sampling methods because the animal by age interaction was not significant. The difference between BC and LM is due to sampling small specimens while that between BE and BC can be interpreted as the effect of live sampling. The biopsy size was similar to reported in the literature (Geers, Decanniere, Villé, Hecke & Bosschaerts, 1995; Foury, Devillers, Sánchez, Griffon, Le Roy & Mormède, 2005).

IMF content did not differ between BC and LM and was unaffected by sample size in BC. On the contrary, BE was 33.0 mg/g ($p<0.01$) higher than BC for an average sample size of 160 mg, being this difference an inversely quadratic function of the biopsy size (Figure 1). These results prove that biopsies overestimate IMF content because they are taken from the live pig, not because they are small. Overestimated IMF content in biopsies can be attributed to contamination from subcutaneous backfat. As already noted by Villé et al. (1995), muscle biopsies obtained using current equipments are very sensitive to contamination by subcutaneous fat. That BC was unaffected by sample size might be due to the fact that BC was extracted from a cold carcass at a temperature in which both muscle and fat are more firm. The residual variance in BE and BC increased three-fold as compared to LM (Table 4). As small samples, BE and BC had higher sampling errors. The sampling variance associated to BE and BC was around one third of the total variance of IMF content.

Table 3. Least square means (± standard error) for intramuscular fat content (mg/g dry matter) and composition (mg/g of total fatty acids) in the *m. longissimus dorsi* by sampling method.

	Sampling method [1]			
Trait [2]	BE	BL	BC	LM
IMF	173.3 ± 8.6[a]	169.4 ± 8.5[a]	140.3 ± 5.8[b]	139.7 ± 4.6[b]
SFA	414.0 ± 7.5	410.4 ± 3.8	411.3 ± 2.0	416.2 ± 2.3
C14:0	16.5 ± 1.4[a]	16.5 ± 0.8[a]	13.5 ± 0.3[b]	12.4 ± 0.2[c]
C16:0	258.4 ± 4.8	257.0 ± 2.3	259.7 ± 1.2	260.6 ± 1.2
C18:0	135.9 ± 3.9[ab]	135.8 ± 2.1[a]	135.8 ± 1.4[a]	141.9 ± 1.4[b]
C20:0	1.6 ± 0.2[a]	1.5 ± 0.1[a]	2.4 ± 0.2 [b]	1.6 ± 0.1[a]
MUFA	447.0 ± 8.8[a]	462.6 ± 4.4[b]	478.6 ± 3.1[c]	477.4 ± 2.4[c]
C16:1	31.1 ± 2.4[a]	35.2 ± 1.2[ab]	37.7 ± 0.7[bc]	42.4 ± 0.7[d]
C18:1	405.0 ± 8.6[a]	418.2 ± 4.2[a]	433.0 ± 2.8[b]	427.1 ± 2.4[a]
C20:1	10.0 ± 0.5[a]	8.9 ± 0.3[b]	8.1 ± 0.2[c]	8.3 ± 0.1[c]
PUFA	138.4 ± 8.5[a]	126.4 ± 4.6[a]	111.1 ± 3.1[b]	105.3 ± 2.2[b]
C18:2	119.5 ± 7.3[a]	108.9 ± 4.0[a]	94.5 ± 2.5[b]	89.0 ± 1.9[c]
C18:3	8.3 ± 0.4[a]	6.5 ± 0.2[b]	4.3 ± 0.2[c]	3.9 ± 0.1[d]
C20:2	6.5 ± 0.4[a]	5.1 ± 0.2[b]	4.3 ± 0.1[c]	3.7 ± 0.1[d]
C20:4	7.5 ± 1.2[ab]	6.8 ± 0.6[a]	7.8 ± 0.4[ab]	8.6 ± 0.4[b]

[1] See footnote in Table 1 for abbreviations.

[2] IMF, intramuscular fat; SFA, saturated fatty acids (C14:0 + C16:0 + C18:0 + C20:0); MUFA, monounsaturated fatty acids (C16:1 + C18:1 + C20:1); PUFA, polyunsaturated fatty acids (C18:2 + C18:3 + C20:2 + C20:4). Means adjusted at 220 days of age and 160 mg dry matter of sample weight.

[a] Within a row means without a common superscript letter differ ($p<0.05$).

The sample size of biopsies decreased linearly with age (-0.9±0.2 mg dry matter/day), as a result of the negative relationship between backfat depth and sample size (-5.5±0.7 mg dry matter/mm, Figure 2). Accordingly, biopsies from fatter pigs are more prone to produce upward biased estimates of IMF content, as well as higher sampling variances. The difference between BL and BE accounts specifically for the effect of live sampling in fat pigs. Biopsies from fat pigs did not result in additional bias (Table 3) but increased the sampling variance (Table 4). The residual variance in BL more than doubled that in BE.

Table 4. Animal and residual variance by sampling method and correlation of each sampling method with intramuscular fat content (mg/g dry matter) and composition (mg/g of total fatty acids) in *m. longissimus dorsi*

	Variance [1]										
	Animal			Residual				Correlation with LM [2]			
Trait [3]	BE	BL	BC	BE	BL	BC	LM	BE	BL	BC	GM
IMF	1052	1052	1052	940	2089	884	306	0.64	0.51	0.65	0.56
SFA	339	257	243	105	101	45	141	0.62	0.67	0.73	0.86
C14:0	3.2	3.2	3.2	15.4	25.5	5.8	0.9	0.37	0.30	0.53	0.41
C16:0	117	70	64	43	29	24	39	0.53	0.64	0.67	0.81
C18:0	88	81	80	27	20	23	42	0.68	0.72	0.71	0.87
C20:0	0.1	0.1	0.1	0.5	0.2	1.5	0.4	0.08 [ns]	0.12 [ns]	0.05 [ns]	0.27
MUFA	252	267	288	146	92	223	59	0.66	0.78	0.68	0.86
C16:1	19	19	19	17	15	10	11	0.58	0.59	0.65	0.85
C18:1	173	205	228	136	62	203	123	0.56	0.70	0.59	0.81
C20:1	0.5	0.5	0.5	0.8	0.9	0.9	0.9	0.37	0.35	0.35	0.54
PUFA	354	285	285	155	208	256	6	0.71	0.74	0.72	0.88
C18:2	284	202	193	119	165	164	6	0.70	0.72	0.72	0.86
C18:3	0.7	0.3	0.2	1.5	0.9	0.9	0.2	0.32	0.33	0.32	0.54
C20:2	0.2	0.2	0.2	0.5	0.4	0.7	0.2	0.40	0.47	0.38	0.48
C20:4	4.4	5.3	6.0	1.4	2.5	6.1	6.9	0.55	0.56	0.58	0.68

[1] Animal variance calculated as the sum of the sire, dam, pig, and, if significant ($p<0.05$), the pig by age effects at the average age where samples were taken; residual variance for each sampling method; see footnote in Table 1 for abbreviations.

[2] Correlation values are all significant ($p<0.01$), except those marked with ns ($p>0.05$).

[3] See footnote in Table 3 for abbreviations.

The use of small samples for assessing IMF content has been criticized because of the high sampling errors (Baas, Christian & Rouse, 1998). However, they resulted to be, in terms of prediction of the IMF content in LM, as efficient as a big sample of GM. The correlation of BE and BC with LM, with values around 0.65, were higher than those of LM with GM (0.56) (Table 4). A similar correlation was obtained for predictions based on a model using both live real-time loin ultrasound images and ultrasound backfat measurements (Newcom, Baas & Lampe, 2002). Owing to depreciation costs, breeding or labeling decisions for IMF are often based on muscles other than LM, like GM (Solanes et al, 2009). The results evidenced that such decisions can be better based on using small specimens from the muscle itself rather than bigger samples of other muscles.

Muscle sampling and intramuscular fat composition

The sampling method affected MUFA and PUFA but not SFA (Table 3), the differences being mostly found between live and *post-mortem* sampling. Thus, BE showed greater levels of PUFA (27.3 mg/g FA, $p<0.01$) and lower of MUFA (31.6 mg/g FA, $p<0.01$) than BC, but BC did not differ from LM. In particular, BE samples had higher proportion of linoleic acid (25.0 mg/g FA, $p<0.01$) and lower of oleic acid (28.0 mg/g FA, $p<0.01$). Individual FA differences between BC and LM were lower than 6.0 mg/ g FA. The sample size affected IMF FA composition in biopsies, with a trend towards increasing MUFA and decreasing PUFA with biopsy size (Figure 1). This finding also indicates that muscle biopsies can be contaminated with subcutaneous fat. In the present study, subcutaneous fat had around 3-6% more PUFA and 2-4% less MUFA than IMF (not shown), a result which is in accordance with that either BE or BL had more PUFA and less MUFA than expected. The effect of sample size on IMF traits in biopsies can only be fully cancelled if unbiased estimates of the difference between BE or BL and LM, as control, are available (Table 3).

The animal variance for MUFA increased with age while it decreased for SFA and PUFA (Table 4). Heterogeneous residual variances across sampling methods for FA composition traits were observed, although the pattern they

displayed varied according to each individual FA. Thus, as compared to LM, the residual variance in BE was around 0.75-fold, 2.5-fold and 25-fold for SFA, MUFA and PUFA, respectively. The correlation of sampling methods with LM, as well as the correlation between GM and LM, tended to increase with FA content. Thus, for SFA; MUFA, and PUFA, and the most abundant FA, values were higher than 0.70 whereas, for minor acids, they did not exceed 0.35. The correlations of LM with GM were greater than those of LM with BE, BL, and BC. This correlation structure indicates that minor FA are more sensitive to sample size and that, for inferences on FA composition, big samples of muscles other than the target may perform better than small samples of the target muscle.

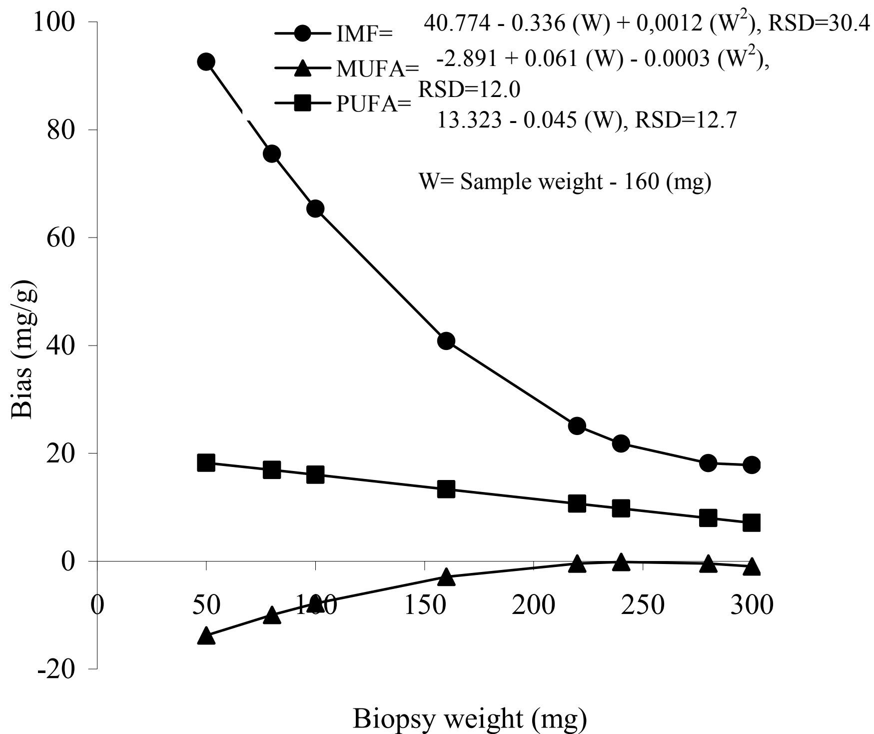

Figure 1. Bias in the estimation of intramuscular fat content (IMF, mg/g dry matter) and fatty acid composition (MUFA and PUFA: monounsaturated and polyunsaturated, mg/g of total fatty acids) of the *m. longissimus dorsi* muscle as a function of biopsy size.

Implications for aging studies

The estimation of effect of age on carcass traits in pigs is usually based on cross-sectional designs. In cross-sectional trials pigs are compared in a series of slaughter groups that happened to differ in age. Such comparisons are not estimates of the effect of aging, which is intrinsically a within-subject effect. Longitudinal analyses overcome this problem by directly assessing within subject changes, with the additional advantage of providing more powerful and efficient designs. However, obtaining repeated records of IMF traits across age requires the use of biopsies, which, as small samples, can be subjected to high sampling errors. It can be proved that a two repeated measures (BE and LM) design is more efficient than the corresponding serial slaughter design if the covariance between the paired records is greater than half of sampling variance associated to the biopsy. The results indicate that this condition is fulfilled for IMF content and for all individual FA except C20:0 and C18:3 whose correlation between BE and LM was lower than 0.32.

As compared to serial slaughter, a two-repeated sampling design for IMF content allows for reducing by half the number of IMF determinations and by quarter the number of pigs. A similar conclusion is reached if BE is substituted by BL for most of the FA but not for IMF content, where only a reduction of 12% is achieved. Using BC instead of LM can be viewed as a valuable alternative if LM is unaffordable to sample due to depreciation costs. Such design requires one-third of the pigs used in a serial-slaughter design. Therefore, potential for bias and not sampling variance is the major limitation of using live samples in longitudinal studies for IMF traits. The use of biopsies in animal experiments, as a source of animal welfare concern, needs to be weighed against the reduction in the number of experimental pigs. Results in this paper may help in addressing this question. Furthermore, they also evidenced that inferences on IMF traits are much more sensitive to sampling variation than to analytical inaccuracies.

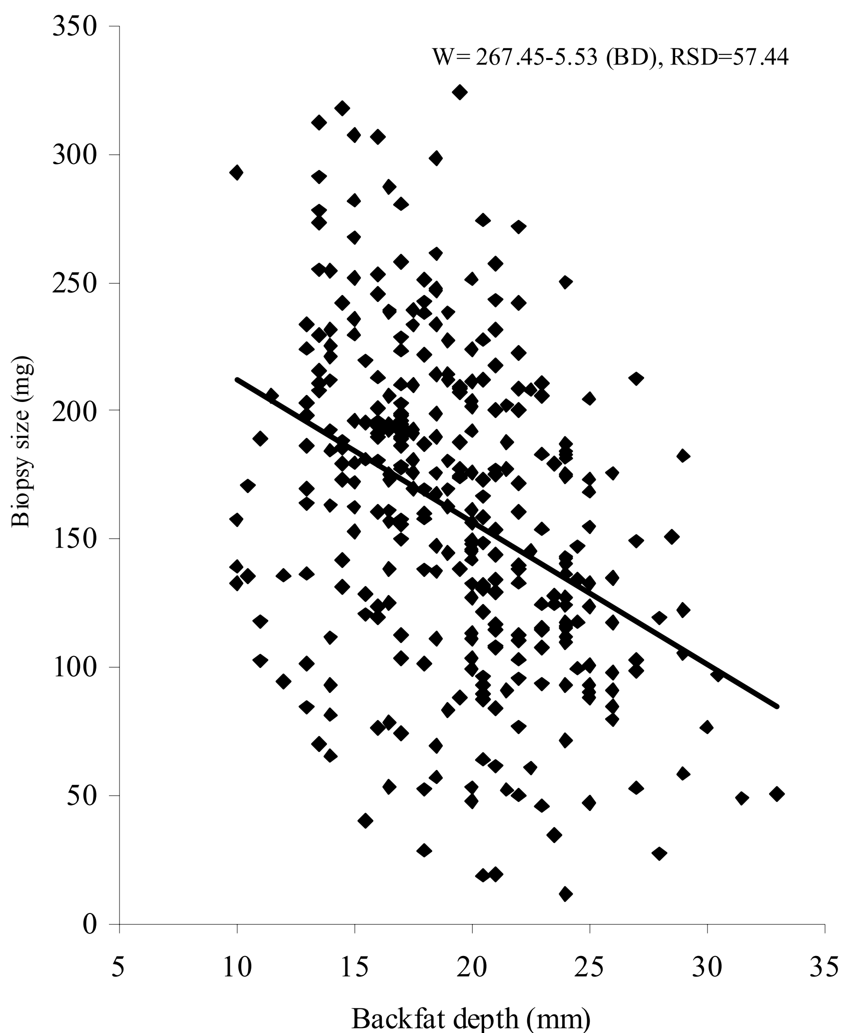

Figure 2. Biopsy size (W) of *m. longissimus dorsi* by backfat depth (BD)

Conclusion

Quantitative gas chromatography after direct transesterification is a useful method for simultaneously determining intramuscular fat content and composition in muscle samples weighing less than 1 g wet weight. There is a potential of overestimating IMF content and PUFA FA in biopsies, particularly in fat pigs.

However, their sampling variance, although high, is not increased enough to cancel the advantages of longitudinal over serial-slaughter designs. Therefore, bias rather than sampling variance is viewed as the major risk in developing longitudinal studies with biopsies. Small *post-mortem* specimens of the target muscle can result as informative as big samples of other muscles for inferring on IMF content but not on IMF composition.

Acknowledgements

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ESTUDIO 2. AGE-RELATED CHANGES IN INTRAMUSCULAR AND SUBCUTANEOUS FAT CONTENT AND FATTY ACID COMPOSITION IN GROWING PIGS USING LONGITUDINAL DATA

Basado en el artículo de:

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Enviado a Meat Science, 2011

Abstract

The evolution of intramuscular fat (IMF) and subcutaneous backfat (SB) content and fatty acid composition with age was investigated. A repeated sampling scheme (n=517) for *longissimus dorsi* (LM) and SB specimens was performed from 150 to 220 days on 216 purebred Duroc barrows. IMF content increased linearly with age at a rate of 0.05%/day, with major compositional changes occurring in C18:1 and C18.2, which, respectively, increased and decreased by around 4%. Backfat thickness increase rate declined with age, showing similar compositional changes to IMF but halved in magnitude. Fat content itself also influenced composition. However, whereas, for SB, a high content at a given age was because of increased SFA (C16:0 and C18:0), for IMF, it was due to increased MUFA (C18:1). A delay in age at slaughter will enhance IMF and C18:1 in relation to overall fatness but at expense of decreasing body and loin growth rate.

Key Words: **Fatty acid; Intramuscular fat; Oleic acid; Pigs.**

Introduction

There is an increasing concern in the pig industry for improving meat quality. Intramuscular fat (IMF) content and composition play an important role in both meat eating quality, particularly in the production of high quality dry-cured ham, and human health. It has been shown that IMF content, although within a range (Novakofsky, 1987), has a favorable effect on pork sensorial quality (Savell and Cross, 1988; DeVol et al., 1988; Ellis, Webb, Avery, & Brown, 1996; Fernandez, Monin, Talmant, Mourot, & Lebret, 1999) and that some specific fatty acids may have beneficial cardiovascular properties (Hartog, Verdouw, Komple, & Lamers, 1987; Schmidt, & Dyerberg, 1994; Williams, 2000). Several studies reported on the convenience, for human health, of decreasing dietary saturated fatty acids (SFA) and the polyunsaturated (PUFA) n-6:n-3 fatty acid ratio while increasing monounsaturated (MUFA) fatty acids (Wood and Enser, 1997; Williams, 2000).

Variations in the genetic type, diet, and gender have been the production factors mostly investigated with the purpose of improving IMF content and its fatty acid composition profile (Wood et al., 2004; Cilla et al., 2006). Increasing the age or weight at slaughter has also been studied as a strategy to enhance pork quality (García-Macías et al., 1996; Latorre, Medel, Fuenteaja, Lázaro, & Mateos, 2003; Correa et al, 2006; Latorre, Ripoll, García-Belenguer, & Ariño, 2009). However, to our knowledge, there has not been any formal intent to estimate the evolution of IMF content and composition, both in IMF and in subcutaneous backfat (SB), throughout the whole growing-finishing period. Assessing the age-related changes in fat content and composition, along with changes occurring in other economic traits, would be helpful in determining the optimal slaughter time-point for a given meat quality standard.

The experiments estimating the effect of age (Latorre et al., 2003) or the weight at slaughter (García-Macías et al., 1996; Correa et al., 2006) on IMF relied on cross-sectional comparisons across age or weight groups. In such designs, pigs are serial slaughtered at a fixed time and then response variables, which are measured once, one at a single time, compared across groups. A more efficient design for estimating the effect of aging is assessing the within-subject change over time (Fitzmaurice, Laird, & Ware, 2004). This only can be achieved by using longitudinal designs, which are based on the analysis of repeated measurements on the same subject over time. However, this recording scheme is not easy to implement for compositional traits. In a previous work,, Bosch, Tor, Reixach, & Estany (2009) developed a method for simultaneously determining fat content and composition in *ante*- and *post-mortem* small specimens that resulted to be advantageous in the design of longitudinal experiments for meat quality.

The aim of the present study was to use the method described in Bosch et al. (2009) to estimate the evolution of the IMF and SB content and fatty acid composition throughout the growing-finishing period using longitudinal data on these traits. Changes in compositional traits are discussed in terms of both age and fat accretion.

Materials and Methods

Animals and experimental procedures

The experimental procedures by means of which this study was conducted were approved by the Ethics Committee for Animal Experimentation of the University of Lleida (Agreement 2/01, March 2001) and all animal procedures and care performed in accordance with authorization AE2374 issued by the Catalan Ministry of Agriculture, Livestock, and Fishing, Spain.

A total of 216 Duroc barrows were subjected to repeated sampling for muscle and SB specimens (from 1 to 5 per pig, Table 1). Pigs were from purebred Duroc litters produced by 102 sows and 36 boars and raised up to slaughter in three separate batches in a commercial farm. They were allocated in pens of 12 individuals and were given *ad libitum* access to feed. A pelleted growing and finishing diet were given from 110 to 160 days and from 160 to 220 days, respectively (Table 2). The sampling scheme included 1-3 biopsies (at 160, 185, and 210 days of age; n=341) and a small (n=88) and big (n=88) *post-mortem* (220 days) samples of *m. longissimus dorsi* (LM) and SB. Before taking biopsies, pigs were weighed and their subcutaneous backfat (BT) and loin-muscle thickness at 5 cm of the midline between the third and fourth last ribs ultrasonically recorded using the portable equipment Piglog 105 (SFK-Technology, Herlev, Denmark). Age and live performance traits at control of the pigs used in the experiment are given in Table 1. Biopsies were taken at around 160 days (158.0, SD 7.0), 185 days (182.5, SD 4.3), and 210 days of age (207.9, SD 3.0), and were removed using 8-mm cannula inserted into spring-loaded biopsy device (PPB-U Biotech, Nitra, Slovakia). Biopsies were taken 5 cm deep at the same location where BT was measured and were extracted following the procedures described in Oksbjerg, Henckel, Andersen, Pedersen, & Nielsen (2004). All the necessary measures were taken to prevent animal discomfort during and after the process (Bosch, Tor, Villalba, Puigvert, & Estany, 2003). Muscle samples were trimmed of fat and skin and immediately frozen in liquid nitrogen until required for analyses. At the end of the trial pigs were slaughtered in a commercial slaughterhouse equipped with a carbon dioxide stunning system (Butina ApS,

Holbaek, Denmark), where BT and loin thickness at 6 cm off the midline between the third and fourth last ribs were predicted using the Autofom automatic carcass grading (SFK-Technology, Herlev, Denmark). The two post-mortem samples were taken after chilling for about 24 h at 2ºC in a random sample of around 30 pigs within each batch. The small sample was removed with the biopsy needle device from the same anatomic site where biopsies were taken and was identically processed as a biopsy. The big sample was obtained from a section of around 1 kg from the left loin at the level of the third and fourth last ribs. Then, the sample was immediately vacuum packaged and stored in deep freeze until required.

Table 1. Number of pigs and mean (SD) of on-farm performance traits by age at measurement.

	Age at measurement, days			
Trait [a]	160	185	210	220
No of pigs	86	191	64	88
Age, days	158.0 (7.0)	182.5 (4.3)	207.9 (3.0)	221.3 (3.6)
Weight , kg	95.4 (10.6)	113.5 (11.2)	129.5 (12.3)	105.1 (9.5)
Backfat thickness, mm	16.9 (3.8)	19.3 (3.7)	22.8 (4.5)	24.4 (2.9)
Loin thickness, mm	42.6 (4.1)	44.9 (4.1)	46.2 (5.4)	44.1 (9.4)

[a] Live weight and ultrasonic Piglog 105© backfat and loin thickness at 160, 185 and 210 days of age, and carcass weight and ultrasonic autofom© backfat and loin thickness at 220 days.

Determination of IMF content and composition

Frozen samples were removed from the nitrogen tank or the freezer 12 h prior to laboratory analyses. Biopsies and small *post-mortem* specimens were directly freeze-dried and thereafter thoroughly homogenized by mixing with sand using a glass stirring rod. Due to their small size, dry matter in biopsies was calculated as the weight difference before and after freeze-drying, and then the whole sample used for

subsequent analyses. After post-mortem big samples were completed defrosted, vacuum drip losses were eliminated and muscle and subcutaneous fat were dissected out separately. Once minced, a small quantity of each was used to determine dry matter by drying 24 h at 100 to 102ºC in air oven whereas the rest of the sample was freeze-dried and pulverized using an electric grinder. A representative aliquot from the pulverized freeze-dried specimens was used for chemical analyses.

IMF content was determined by quantitative determination of the fatty acids by gas chromatography following the methodology described in Bosch et al. (2009). Fatty acid methyl esters of both IMF and SB were directly obtained by transesterification using a solution of boron trifluoride 20% in methanol (Rule, 1997). Analysis of fatty acid methyl esters were performed by gas chromatography with a capillary column SP2330 (Supelco, Tres Cantos, Madrid) and a flame ionization detector with helium as the carrier gas at 1 mL/min. The oven temperature program increased from 150 to 225ºC at 7ºC per min, and the injector and detector temperatures were both 250ºC (Tor, Estany, Francesch, & Cubiló, 2005). The quantification was carried out through area normalization after adding into each sample 1, 2, 3-Tripentadecanoylglycerol as internal standard. IMF was calculated as the sum of each individual fatty acid expressed as triglyceride equivalents (AOAC, 2000, Chap 39) on a dry tissue basis. IMF and SB fatty acid composition was calculated as the percentage of each individual fatty acid relative to total fatty acids, and expressed as mg/g fatty acid. The proportion of PUFA (C18:2n-6; C18:3n-3; C20:2n-6; and C20:4n-6), MUFA (C16:1n-7; C18:1n-9; and C20:1n-9) and SFA (C14:0; C16:0; C18:0; and C20:0) fatty acid contents were calculated. The n6:n3 ratio was calculated accordingly (C18:2n6-+C20:2n6+C20:4n6)/C18:3n3).

Table 2. Composition of the diets (g/kg).

Item	Growing	Finishing
Dry matter	893.2	886.1
Crude lipid	56.3	61.0
Crude protein	193.6	181.2
Ash	60.4	69.8
Crude Fiber	57.1	61.7
Nitrogen free extract	525.8	512.4
ME, MJ/kg	13.4	12.7
Fatty acids, mg/g fatty acid [a]		
C12:0, lauric	4.8	3.2
C14:0, myristic	18.3	16.2
C16:0, palmitic	220.8	229.8
C16:1, n-7 palmitoleic	22.6	23.6
C18:0, stearic	77.4	81.7
C18:1, n-9 oleic	301.1	294.7
C18:2, n-6 linoleic	327.7	311.7
C18:3, n-3 linolenic	15.5	19.7
C20:1, n-9 eicosenoic	3.7	6.8
C20:2, n-6 eicosadienoic	2.8	3.1
C20:4, n-6 arachidonic	1.1	1.3
SFA	321.3	330.9
MUFA	327.4	325.1
PUFA	347.1	353.8

[a]SFA, saturated fatty acids (C12:0 + C14:0 + C16:0 + C18:0). MUFA, monounsaturated fatty acids (16:1 + C18:1 + C20:1). PUFA, and polyunsaturated fatty acids (C18:2 + C18:3 + C20:2 + C20:4).

Statistical analysis

Data for IMF and SB traits were analyzed within tissue using a linear mixed model, in which fixed effects included the batch (1 to 3) and the type of sample (*ante-mortem*, *post-mortem*) with a quadratic polynomial on age. A quadratic polynomial on sample weight by type of sample was included as additional covariate for IMF-related traits (Bosch et al., 2009). The animal and the residual were the random effects. The animal effect was split into the sire, dam, pig, and pig by age effects. Residual effects were modeled to account for heterogeneous variance across sampling method (Bosch et al., 2009). IMF (BT) was added as a covariate for assessing the partial effect of IMF (BT) on fatty acid composition, respectively. In this analysis, a random pig by IMF (BT) effect was used in substitution of the pig by age effect. A multivariate model including the tissue (IMF, SB) and the batch and the age at control by tissue as fixed effects, with the pig as random effect, was used to assess fatty acid composition differences between IMF and SB at every control age. Variances were estimated by restricted maximum likelihood and contrasted using a likelihood ratio test. Age-related estimates are presented both as regression coefficients and *post-mortem* least-square means at control age. Fixed effects were tested following the Kenward-Roger approach and differences between control ages by the Tukey test. The analyses were performed using SAS PROC MIXED (SAS Inst. Inc., Cary, NC). The correlation between IMF and SB for each fatty acid was calculated as the covariance between the corresponding pig effects divided by the square root of the appropriate total variances

Results

Comparison of intramuscular and subcutaneous fatty acid composition

Fatty acid composition in LM and SB is given in Table 3. Results indicate that IMF had 6.1 % less PUFA than SB, which was achieved because MUFA and SFA increased by 4.1% and 2.0%, respectively. The MUFA/SFA and the MUFA/PUFA ratios were higher in IMF than in SB while the PUFA/SFA ratio was lower. Major individual changes occurred for oleic (C18:1) and linoleic (C18:2)

fatty acids, the first being higher in LM (+2.4%) and the second in SB (-5.5%). The n6:n3 ratio was 1.7-fold higher in LM than SB. The correlations between individual fatty acids in LM and SB, with values ranging from 0.14, for C18:3, to 0.69, for C18:0, confirmed that both tissues behave differently in relation to fat metabolism. Differences between tissues increased with age (see Tables 4 and 5) but were always consistent with those obtained at slaughter.

Table 3. Fatty acid composition in *m. longissimus dorsi* (LM) and subcutaneous backfat (SB) at slaughter (220 days).

Trait[c]	LM	SB	r [d]
SFA, mg/g FA	416.0 ± 2.6[a]	395.6 ± 2.8[b]	0.60
C16:0	260.5 ± 1.3[a]	245.6 ± 1.5[b]	0.44
C18:0	141.3 ± 1.7 [a]	135.1 ± 1.7[b]	0.69
MUFA, mg/g FA	480.0 ± 2.5[a]	438.9 ± 2.7[b]	0.44
C16:1	38.9 ± 0.5[a]	18.6 ± 0.4[b]	0.35
C18:1	432.3 ± 2.5[a]	408.5 ± 2.6[b]	0.38
PUFA, mg/g FA	104.0 ± 2.4[a]	165.4 ± 1.9[b]	0.30
C18:2	88.6 ± 2.0[a]	143.8 ± 1.7[b]	0.28
C18:3	3.7 ± 0.1[a]	11.3 ± 0.5[b]	0.14

[a,b] Columns with different superscripts differ significantly ($P<0.05$).
[c] See Table 2 for trait abbreviations.
[d] Correlation between LM and SB for each trait.

Intramuscular fat content and fatty acid composition by age

The evolution of IMF content and composition with age is given in Table 4. IMF content and MUFA increased from 160 to 220 days of age while PUFA decreased during the growing-finishing period. IMF content increased by 0.05% per day and PUFA (which showed a negative linear trend) and MUFA (which showed a positive quadratic trend), interchanged their compositional values by around 5%. Major changes occurred in C18:1 and C18.2, which, respectively, increased and decreased by around 4% in the studied period. SFA displayed minor changes

throughout the fattening period, with a maximum in the last half. In contrast with IMF and C18:1, on-farm performance traits (live weight, BT, and loin thickness) had a negative quadratic trend, indicating that a delay in age at slaughter may help to break the relationship between IMF and BT and enhance the C18:1 content. However, both changes are achieved at expense of decreasing body and loin growth rate. Aging showed a positive effect on the MUFA/SFA and MUFA/PUFA ratios but negative on the PUFA/SFA ratio. No difference was observed for the n6:n3 ratio with age. The design was powerful enough to find significant ($p<0.05$) individual variation for C16:0, C18:1, C18:2, and C18:3 deposition rates during the studied period. The correlations between individual values at 160 days and linear increase up to 220 days of age were all negative (-0.55, -0.80, and -0.93, for C18:2, C16:0, and C18:3, respectively), except for C18:1(0.06, $p>0.05$). This result indicates that C16:0, C18:2, and C18:3 deposition rates are lower in pigs having high levels of these fatty acids at 160 days but that the deposition rate for C18:1 is independent of the C18:1 value at 160 days. Unlike BT, no individual variation was found for the deposition rate of IMF content. No correlation was found between BT at 160 days and its linear increase up to 220 days of age (-0.02, $p>0.05$).

Table 4. Intramuscular fat content and fatty acid composition by age.

	Age (days)			Regression on age[e]	
Trait[d]	160	185	220	b_1	b_2
Live weight, kg	97.2 ± 0.9[a]	114.5 ± 0.9[b]	134.5 ± 1.0[c]	740.7 ± 20.2	-2.0 ± 0.4
Backfat thickness, mm	17.3 ± 0.2[a]	20.1 ± 0.2[b]	22.4 ± 0.4[c]	129.9 ± 7.8	-0.8 ± 0.2
Loin thickness, mm	42.9 ± 0.3[a]	44.7 ± 0.4[b]	44.8 ± 0.6[b]	103.8 ± 22.0	-1.3 ± 0.4
IMF, mg/g dry matter	107.1 ± 9.1[a]	120.3 ± 6.5[b]	138.6 ± 4.4[c]	525.2 ± 127.4	-
Dry matter, mg/g	268.8 ± 4.5[a]	274.3 ± 2.9[b]	281.9 ± 1.5[c]	218.6 ± 69.9	-
SFA, mg/g FA	416.9 ± 3.4[a]	421.8 ± 3.3[b]	412.6 ± 1.7[a]	385.7 ± 84.0	-7.6 ± 1.7
C16:0	259.7 ± 1.9[a]	263.5 ± 1.9[b]	260.1 ± 0.9[a]	253.1 ± 55.7	-4.1 ± 1.1
C18:0	145.9 ± 1.9[a]	145.7 ± 1.9[a]	137.3 ± 1.2[b]	91.2 ± 42.3	-3.9 ± 0.9
MUFA, mg/g FA	425.9 ± 3.7[a]	440.7 ± 3.8[b]	476.0 ± 2.3[c]	415.1 ± 101.7	7.0 ± 2.0
C16:1	33.0 ± 1.2[a]	35.7 ± 0.9[b]	39.5 ± 0.7[c]	109.6 ± 14.7	-
C18:1	389.3 ± 3.3[a]	399.3 ± 3.5[b]	428.2 ± 2.2[c]	225.0 ± 96.8	7.1 ± 1.9
PUFA, mg/g FA	150.3 ± 4.0[a]	132.4 ± 2.9[b]	107.3 ± 2.2[c]	-715.8 ± 53.9	-
C18:2	129.8 ± 3.5[a]	113.6 ± 2.6[b]	90.8 ± 2.0[c]	-650.4 ± 47.6	-
C18:3	6.5 ± 0.3[a]	5.5 ± 0.2[b]	4.1 ± 0.1[c]	-41.2 ± 4.0	-

[a,b,c] Columns with different superscripts differ significantly ($P<0.05$).
[d] Ultrasonic backfat and loin thickness measured with Piglog 105; IMF: intramuscular fat; see Table 2 for other trait abbreviations.
[e] b_1 (b_2): linear (quadratic) regression coefficient at 160 days (x1000). All coefficients are significant at $p<0.05$; ns: non-significant.

Subcutaneous fatty acid composition by age

Changes in SB composition by age are shown in Table 5. As for IMF, throughout the studied period subcutaneous MUFA increased in a similar amount to PUFA decreased, with SFA being rather stable. However, compositional age-related changes for SB were lower than for IMF. Increased values for MUFA in SB were lower than 2% and were mostly due to C18:1, while changes in PUFA did not reach 3%. The n6:n3 ratio increased 1.7-fold in SB from 160 to 220 days of age. Note that, despite SFA did not change from 160 to 220 days of age, C16:0 increased by 1.2% while C18:0 decreased by a similar amount. Evidence of individual variation for deposition rates in SB with age was only found for C18.1 ($p<0.05$).

Intramuscular fatty acid composition by intramuscular fat content

Fatty acid composition adjusted for age was modified as IMF increased (Table 6). Changes were in line with those observed with age (Table 4; i.e. a substitution of PUFA by MUFA) but lower in magnitude. Thus, a two-fold increase in IMF led to 1.3% increase in both MUFA and SFA and to 2.2% reduction in PUFA, a compositional change similar to that observed from 160 to 185 days. Increasing the IMF content reduced the n6:n3 ratio by around 14%. Major individual change was in C18:2, which decreased by 1.9%, a little higher than that observed in C18:1 (1.3%). However, no effect was found of IMF content on C18:0 and C18:3. Increased IMF content resulted in increased BT at the rate of 0.02 mm per mg/g dry matter. Both live weight and loin thickness were not affected by IMF content at fixed age. Deposition rates for C16:0, C18:3, and C18:2 were subjected to individual variation with IMF content, with negative correlations between individual values at 80 mg/g and linear increase with IMF (-0.54, -0.59, and -0.78, respectively, $p<0.05$).

Table 5. Subcutaneous backfat fatty acid composition by age.

	Age (days)			Regression on age[e]	
Trait[d]	160	185	220	b_1	b_2
SFA, mg/g FA	402.1 ± 7.3[a]	409.0 ± 6.8[b]	400.3 ± 2.0[a]	491.6 ± 147.7	-8.7 ± 3.2
C16:0	235.0 ± 3.3[a]	239.9 ± 2.1[b]	246.7 ± 1.0[c]	194.3 ± 50.4	-
C18:0	146.5 ± 3.1[a]	146.7 ± 3.0[a]	136.5 ± 1.3[b]	134.2 ± 61.0	-5.0 ± 1.4
MUFA, mg/g FA	418.4 ± 4.7[a]	425.9 ± 3.2[ab]	436.2 ± 1.9[b]	296.5 ± 73.5	-
C16:1	20.1 ± 1.2[a]	19.8 ± 1.2[a]	19.2 ± 0.4[a]	-15.5 ± 17.7 [ns]	-
C18:1	386.8 ± 4.5[a]	394.2 ± 3.0[b]	404.5 ± 1.8[c]	295.0 ± 69.7	-
PUFA, mg/g FA	189.9 ± 3.2[a]	178.9 ± 2.2[b]	163.3 ± 1.4[c]	-443.0 ± 44.7	-
C18:2	158.8 ± 3.0[a]	148.6 ± 2.9[b]	141.8 ± 1.2[c]	-496.5 ± 83.4	3.5 ± 1.6
C18:3	15.2 ± 0.9[a]	12.6 ± 0.8[b]	11.3 ± 0.2[c]	-129.5 ± 19.6	1.1 ± 0.4

[a,b,c] Columns with different superscripts differ significantly ($P<0.05$).
[d] See Table 2 for trait abbreviations.
[e] b_1 (b_2): linear (quadratic) regression coefficient at 160 days (x1000). All coefficients are significant at $p<0.05$; ns: non-significant.

Table 6. Intramuscular fatty acid composition adjusted for age by intramuscular fat content (IMF).

	IMF, mg/g dry matter		Regression on IMF[d]	
Trait[c]	80	160	b1	b2
Live weight, kg	114.8 ± 1.0[a]	115.3 ± 0.8[a]	6.3 ± 6.0[ns]	-
Backfat thickness, mm	18.2 ± 0.3[a]	19.8 ± 0.2[b]	20.0 ± 2.7	-
Loin thickness, mm	45.3 ± 0.5[a]	44.9 ± 0.3[a]	-4.1 ± 4.7[ns]	-
SFA, mg/g FA	413.4 ± 3.8[a]	426.2 ± 3.2[b]	160.4 ± 31.5	-0.3 ± 0.1
C16:0	258.0 ± 2.1[a]	264.8 ± 2.0[b]	114.9 ± 24.0	-0.4 ± 0.1
C18:0	148.3 ± 1.7[a]	148.4 ± 1.7[a]	0.8 ± 0.8[ns]	-
MUFA, mg/g FA	437.3 ± 3.7[a]	450.1 ± 3.5[b]	216.9 ± 39.0	-0.7 ± 0.1
C16:1	38.8 ± 0.9[a]	37.2 ± 0.9[b]	-20.2 ± 6.6	-
C18:1	392.3 ± 3.5[a]	405.6 ± 3.3[b]	220.7 ± 36.4	-0.7 ± 0.2
PUFA, mg/g FA	149.7 ± 3.0[a]	127.8 ± 2.4[b]	-336.4 ± 44.5	0.8 ± 0.2
C18:2	123.6 ± 2.5[a]	104.7 ± 1.9[b]	-305.6 ± 37.8	0.9 ± 0.2
C18:3	5.6 ± 0.2[a]	5.6 ± 0.2[a]	0.1 ± 1.5 [ns]	-

[a,b] Columns with different superscripts differ significantly ($P<0.05$).
[c] See Table 2 for trait abbreviations.
[d] Regression coefficients at 80 mg/g dry matter IMF and 185 days (x1000), b1 (b2): linear (quadratic) term. All coefficients are significant at $p<0.05$; ns: non-significant.

Subcutaneous fatty acid composition by backfat thickness

The effect of BT on SB fatty acid composition adjusted for age is shown in Table 7. SFA and PUFA content in SB were linearly affected by BT but not MUFA. SFA increased by 0.02% per each additional mm of BT, evenly distributed between C16:0 and C18:0. This increase in SFA is detracted from PUFA, namely from C18:2. This result indicates that SB accretion after 95 kg is mainly due to increased SFA synthesis, in opposition to IMF, where MUFA, particularly C18:1, were the fatty

acids most contributing to late fat deposition. The n6:n3 ratio was reduced by 1.2% per each additional mm in BT. Pigs showing higher values of BT resulted to be heavier and with fatter but thinner loins. Individual variation with BT was found for C16:0, C16:1, C18:1, and C18:2 ($p<0.05$) but only C18:2 at 14 mm was found to be correlated with the linear trend up to 26 mm ($r= -0.75$, $p<0.05$).

Table 7. Subcutaneous backfat composition adjusted for age by backfat thickness (BT).

	Backfat thickness, mm		Regression coefficient[d]
Trait [c]	14	26	b_1
Live weight, kg	109.7 ± 0.8[a]	119.9 ± 0.9[b]	8.5 ± 0.8
Loin thickness, mm	46.3± 0.5[a]	43.2 ± 0.7[b]	-2.5 ± 0.8
IMF, mg/g dry matter	103.0 ± 6.6[a]	144.6 ± 7.1[b]	34.7 ± 6.3
SFA, mg/g FA	396.9 ± 7.0[a]	418.7 ± 6.9[b]	18.2 ± 3.5
C16:0	243.1 ± 3.1[a]	251.6 ± 2.9[b]	7.1± 2.0
C18:0	139.8 ± 2.7[a]	148.6 ± 2.5[b]	7.3 ± 1.8
MUFA, mg/g FA	425.2 ± 3.4[a]	427.2 ± 3.9[a]	1.6 ± 3.3[ns]
C16:1	19.8 ± 0.8[a]	16.8 ± 0.7[b]	-2.5. ± 0.7
C18:1	372.8 ± 4.8[a]	379.3 ± 4.4[a]	5.4± 3.1[ns]
PUFA, mg/g FA	187.0 ± 2.4[a]	165.4 ± 2.5[b]	-18.1 ± 2.4
C18:2	171.8 ± 2.2[a]	152.5 ± 2.0[b]	-16.0 ± 2.2
C18:3	13.6 ± 0.5[a]	12.7 ± 0.4[b]	-0.4± 0.2

[a,b] Columns with different superscripts differ significantly ($P<0.05$).
[c] See Table 4 for trait abbreviations.
[d] Linear regression coefficients at 14 mm backfat thickness and 185 days (x10). All coefficients are significant at $p<0.05$; ns: non-significant.

Discussion

The present experiment was based on purebred Duroc barrows from a line selected for IMF, which were raised up to 32 weeks under commercial conditions used for intensive production of Spanish high quality dry-cured hams. Besides its practical interest, this setting has been chosen for the experiment because, being a potentially fat triggering factor combination, it provides a very appropriate scenario for assessing changes in IMF and SB with age. The use of longitudinal data on these traits has proved to be a good approach in assessing age-related changes in fat content and composition. It allowed for changes within subject effect, with the advantage of providing a more powerful design. Bosch et al. (2009) discussed the application of such designs to the estimation of the effect of age on IMF content and fatty acid composition, with the conclusion that, even in short series, longitudinal analyses are more efficient.

Results indicate that there exists a clear difference between LM and SB tissues, both in terms of fat accretion and fatty acid composition. IMF content in LM increased linearly with age, in contrast to SB, whose increase rate declined with age. The positive effect of age on IMF, although well-documented in the literature (Gerbens, 2004), has not always been found (Ellis et al., 1996; Beattie, Weatherup, Boss, & Walker, 1999; D'Souza et al., 2004; Lo Fiego, Macchioni, Minelli, & Santoro, 2010). A similar situation happens with SB, where the negative quadratic trend has been found in experiments in which pigs reached heavy weights (Wagner, Schinckel, Chen, Forrest, & Coe, 1999, up to 150 kg) but not in others (Gu, Schinckel, & Martin, 1992; Schwab, Baas, Stalder, & Mabry, 2007). These contradictory results evidence that, with regard to fat deposition, comparisons among experiments should take into account not only the genetic type, gender, or diet but also the age or weight range of the pigs used. In the present experiment, pigs were monitored for two months since 23 weeks of age, around 6 weeks more than in most of the trials. IMF resulted to be more monounsaturated and less polyunsaturated than SB. This different compositional pattern between both adipose tissues is consistent with results reported by other authors using either purebred (Estany, Villalba, Tor,

Cubiló, & Noguera, 2002; Landrace at 100 kg) or crossbred pigs (Teye et al., 2006a; Teye, Wood, Whittington, Stewart, & Sheard, 2006 b; three-way commercial crossbred at 100 kg; Ramirez, Morcuende, & Cava, 2007, in Iberian x Duroc crosses at 150 kg). Monziols, Bonneuau, Davenel, & Kouba (2006) conclude that there is a gradient of decreasing unsaturation from outer layer of SB to flare fat.

Fatty acid compositional changes with age were more relevant in IMF than in SB. Both in IMF and in SB increased MUFA (particularly, C18:1) and decreased PUFA (particularly, C18.2) values were observed with age, while SFA, rather stable against age, reached a maximum in both tissues at 185-190 days of age. The higher substitution rate of PUFA by MUFA in IMF than in SB led to increase compositional differences between both fat tissues with age. Thus, IMF is more monounsaturated than SB as age increases. Results in Lo Fiego et al. (2010) confirmed that in both adipose tissues there is an increase in MUFA and a decrease in PUFA from 6 to 8.5 months of age but not from this age onwards. However, these authors observed, although only in SB, an increase in SFA. However, in a previous experiment, Lo Fiego, Santoro, Macchioni, & De Leonibus, (2005), working with heavy pigs (150 to 175 kg), reported that SFA in SB increased with live weight but not MUFA, in agreement with Latorre, Ripoll, García-Belenguer, & Ariño, (2009), who encountered a similar result. This is in contrast with findings in Nürnberg, Wegner, & Ender (1998), who found a plateau for SFA in SB sampled at a similar age than in our experiment. Apple, Maxwell, Galloway, Hutchison, & Hamilton (2008), using composite samples, reported the same trend than here but with SFA reaching the maximum earlier.

A complementary approach that might help to clarify some of the former discrepancies is to assess fatty acid composition against the amount of fat. Cameron and Enser (1991) and Van Deckel, Casteels, Warnants, Van Damme, & Boucqué (1996), in accordance with our results, found that fatty acid composition depended on IMF content, with greater values of IMF involving increased SFA and MUFA and decreased PUFA. Nürnberg, Wegner, & Ender (1998) also reported that an increase in IMF leads to decrease PUFA, a result that could be attributed to the differing

relation between neutral lipids and phospholipids with amount of fat. Because neutral lipids over phospholipids ratio increases with IMF content, and phospholipids have a greater content of PUFA (Enser, 1984; Cameron et al., 2000), a reduction of PUFA with IMF content is expected. We found that the decrease in PUFA at a given age with IMF was due to C18:2 but not to C18:3, a result also obtained by Wood et al., (1996) and Cameron and Enser (1991). Similarly, Wood, Enser, Whittington, Moncrieff, & Kempster, (1989) investigated the effect of BT (from 8 to 16 mm) on SB fatty acid composition, encountering, as for IMF, that PUFA decreased and MUFA and SFA increased with fatness. However, many of the investigations carried out to assess the effect of age or weight on fatty acid composition do not disentangle between the effects of age and fat content. The results obtained here indicate that there is a combined effect of age and fat content in decreasing PUFA but it is mostly content that is influencing SFA. Results on MUFA differed by tissue, with MUFA, in IMF, being the result of both age and content while, in SB, being mostly due to aging and not to content. Therefore, increasing the slaughter age or weight at slaughter will result in pork chops with higher MUFA in IMF but not in SB, if BT is restrained.

To increase the slaughter age or weight has proved to be negative with regard to the PUFA/SFA ratio but not to the n6:n3 ratio. The PUFA/SFA ratio, which should be maintained above 45% according to the Department of Health of U.K. (1994), decreased with age, for both IMF (from 36.6% to 26.0%) and SB (from 46.2% to 41%). On the other hand, the n6:n3 ratio is recommended to be below 4 (Simopoulos, 1999). Although it was higher in IMF than in SB, with values around 25 and 10, respectively, in IMF there was no evidence of change with aging. Moreover, the n6:n3 ratio, both in IMF and SB, decreased with fatness at a given age. The reason for that is because C18:2 is reduced with the fat amount at a proportionally higher rate than C18:3. On the other hand, while undesirable SFA did not change with age and only slightly increased with fat amount, there was found a progressive increment of MUFA and C18:1, particularly in IMF, with age and fat content. The content in C18:1 is a valuable trait, in terms of both human nutrition (Grundy, 1986) and quality premium in dry-cured products. Previous work using the same Duroc line revealed that there is enough genetic variation for C18:1 in IMF at a fixed age (Reixach and Estany, 2010) to enable for scenarios where it can be improved without increasing BT. Moreover, results here indicate that there exists

individual variation in the fatty acid depositions rate, in particular for those associated to C16:0, C18:1, C18:2, and C18:3, with strong evidence that pigs with a high value of C16:0, C18:2, and C18:3 at an early age will thereafter display a low deposition rate for these fatty acids during the growing-finishing period. However, this is not the case for C18:1, where values at the beginning of the growing-finishing period are not expected to condition future deposition rates.

Conclusions

The present research evidenced that IMF and SB behave differently in terms of fat accretion and age-related changes in fatty acid composition. IMF content increased linearly with age at a rate of 0.05%/day from 160 to 220 days, with major compositional changes occurring in C18:1 and C18.2, which, respectively, increased and decreased by around 4% in the studied period. BT increase rate declined with age, showing similar compositional changes to IMF but halved in magnitude. A delay in age at slaughter will enhance IMF and C18:1 in relation to overall fatness but at expense of decreasing body and loin growth rate. Fat content itself also determined fatty acid composition. However, IMF and SB behave differently at this respect. Whereas, for SB, values above the expected at a given age were because of increased SFA (C16:0 and C18:0), for IMF, they were due to increased MUFA (C18:1).

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ESTUDIO 3: ASSOCIATION OF A CA REPEAT POLYMORPHISM AT THE PROMOTER OF THE IGF1 GENE WITH CIRCULATING INSULIN-LIKE GROWTH FACTOR 1 CONCENTRATION, GROWTH AND FATNESS IN SWINE

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Abstract

Evidence is accumulating that intronic polymorphic CA-repeats may play a role in gene expression. In this work, we investigated if a polymorphic CA short tandem repeat (STR) located at the first intron of the IGF1 pig gene influences plasma IGF1 concentration in pigs as well as phenotypic variation at growth and fatness traits. We measured plasma IGF1 levels at 1-4 time points from 35 until 215 days of age in 340 performance-tested Landrace and Duroc pigs previously genotyped for the IGF1 STR. Data were analyzed within breed using a linear mixed model with a covariate on the number of CA repeats. At least five alleles were segregating in each breed, differing in 1-7 repeats. The results showed that, in each breed, circulating IGF1 at 160 days of age increased with the length of the shortest allele, accounting for an average trend of 4.38±1.28 ng/mL of IGF1 per additional repeat (P=0.001). Longer repeats were associated with early growth in Landrace boars (1.92 ± 0.92 kg at 160 days per CA, P=0.038) and with backfat thickness (-0.57±0.20 mm per CA, P=0.005) and lean content (7.52±3.00 g/kg per CA at 105 kg, P=0.013) adjusted for carcass weight in Duroc barrows, as expected from the effect of circulating IGF1 on these traits. The consistency of the results across populations supports the hypothesis that the length of the CA-repeats at intron 1 of the IGF1 gene is associated to circulating IGF1 levels, and that this effect is not neutral with respect to growth and fatness.

Key Words: **microsatellite; live weight; backfat; pigs**

Introduction

Insulin-like Growth Factor-1 (IGF1) is a mitogenic polypeptide that is responsible for a wide array of cellular actions (27, 42). Evidence suggests that circulating levels of IGF1 may play a role in several human chronic disorders (40) and in cancer (36) and aging (26). Studies undertaken in domestic animals have shown that serum IGF1 levels are correlated to a vast diversity of traits, such as growth and feed intake (7, 9, 16), body composition and meat quality (14, 15, 49), and reproduction efficiency (57). Quantitative genetic analyses in humans (25), mice

(5), pigs (7) and cattle (15) have shown that the levels of circulating IGF1 are under genetic control, with heritability estimates around 30%. This observation has been experimentally confirmed in mice (6), sheep (4), and cattle (35), where divergent selection upon IGF1 plasma concentration resulted in genetic divergence between lines.

The existence of genetic variation influencing IGF1 expression in conjunction with the extensive progress made in understanding the molecular biology of this hormone (43) have made the IGF1 gene a very suitable target for genetic manipulation (37) and an attractive candidate gene to search for DNA polymorphisms, both in humans (38) and in domestic species (2, 10, 21). Sequencing of the rat and human IGF1 genes revealed the presence of cytosine-adenosine $(CA)_n$ short tandem repeat (STR) in the promoter region (44). In humans evidence is accumulating that the length of this highly polymorphic $(CA)_n$ STR may be associated with circulating IGF1 concentration (41). There are also lines of evidence indicating that this polymorphism is associated with body weight and height characteristics (39, 54) and human diseases (12, 41, 53), although results were not always coincident (17, 51) and their molecular basis remains to be elucidated.

In the current work, we have analyzed a similar genetic model in a distantly related mammalian species. In pigs, a polymorphic $(CA)_n$ sequence repeat is located at the first intron of the IGF1 gene (31, 56), a region which often has an important regulatory role on gene transcription (18, 45). Moreover, there is evidence suggesting that the polymorphism of this STR might be linked to average daily gain (10). The purpose of our work was to examine the genetic variation at the IGF1 gene in commercial pigs and investigate whether the length of the intronic $(CA)_n$ sequence repeat is associated with circulating IGF1 and with growth and fatness traits. We have achieved this objective by measuring plasma IGF1 levels in performance-tested individuals with different IGF1-STR genotypes. Two pig breeds with completely different genetic origins and with distinct selective trajectories were used for assessing the consistency of the associations found across populations.

Materials and methods

The experimental procedures by means of which this study was conducted were approved by the Ethics Committee for Animal Experimentation of the University of Lleida and all animal procedures and care were performed in accordance with authorizations AE1170 (Landrace) and AE2374 (Duroc) issued by the Catalan Agency of Agriculture, Livestock, and Fishing, Spain.

Design of the experiments and protocols

The study consisted of two experiments, each one with 170 halothane gene free pigs, which were independently performed to validate the results across populations. Population characteristics and total number of records used in the analyses are given in Table 1.

Experiment 1 was based on three purebred Landrace (LN) dam lines of similar characteristics, all being selected for litter size and growth rate and against backfat depth. Lines A and B were from separate origins whereas line C was a synthetic line derived from A and B. Piglets used in the experiment were randomly chosen within line (42, 62 and 66, for lines A, B, and C respectively) from 92 litters sired by 25 boars. All pigs sampled from lines A and B were boars while those from line C were mostly gilts (52 gilts and 14 boars). Pigs from lines A and B were allocated randomly in four batches and raised until an age of 170 days, as were those from line C in a subsequent fifth batch. Experiment 2 used Duroc barrows (DU) from a closed dam line selected for the same traits as in the lines used in Experiment 1 plus intramuscular fat content. Pigs were randomly sampled from 104 litters sired by 36 boars, neutered during the first week of age, and raised in three batches until about 225 days. Throughout the experiment, from 30 days to the slaughter age, pigs were monitorized between 1 to 4 times (at around 35, 160, 185, and 215 days of age), and always at the reference age of 160 days (Table 1). In each sampling time about 10 mL of blood was obtained with a syringe and kept in tubes containing EDTA. Plasma was collected by centrifugation (3000 xg for 10 min) and stored at –40ºC until required for assay. Moreover, pigs were weighed and, from 90 kg onwards, their subcutaneous backfat depth was ultrasonically measured at 5 cm off the midline

between the third and fourth last ribs (A-mode equipment; Renco Corp, Minneapolis, MN). In Experiment 2, at the same location where backfat depth was taken, loin depth was also measured (Piglog A-mode equipment, SFK, Herlev, Denmark) and a biopsy of about 160 mg dry matter of the longissimus muscle, which was immediately stored at -80ºC, was taken using spring loaded biopsy equipment (Biotech PPB-U, Nitra, Slovakia). During the test period pigs from both breeds had ad libitum access to commercial diets. At the end of each experiment, pigs were slaughtered in commercial slaughterhouses, where the subcutaneous backfat and loin thickness at 6 cm off the mid line between the third and fourth last ribs were obtained with the SFK Fat-O-Meter (FOM) optical probe (Experiment 1) or the SFK AutoFOM ultrasound automatic scanning (Experiment 2) (SFK, Herlev, Denmark). The carcass lean content was estimated either from these two measurements (Fat-O-Meter) or on the basis of 35 measurement points (AutoFOM) by using the official approved equations (13, 23). After chilling for 24 h at 2ºC, each carcass was divided into primal cuts and the weight of untrimmed hams was registered. To determine the intramuscular fat content slices of about of 50 g from the semimembranosus (Experiment 1) or from the *gluteus medius* (Experiment 2) muscles were taken. Muscle samples were vacuum packaged in different bags and stored in a deep freeze. Intramuscular fat content was determined in duplicate by either ether extraction in a Soxhlet apparatus (3) or quantitative determination of the fatty acids by gas chromatography with capillary column (biopsy). In this latter case, fatty acid methyl esters were obtained using a solution of boron trifluoride 20% in methanol (46) and total fat was calculated as the sum of individual fatty acids expressed as triglyceride equivalents (3).

Table 1. Landrace and Duroc pig population characteristics for age of reference and total number of records used in the analyses

	Genetic type		
	Landrace		Duroc
	Boars (n=118)	Gilts (n= 52)	Barrows (n=168)
Age, d	158.6 ± 0.4	159.8 ± 0.7	155.8 ± 0.4
Live weight, kg	100.5 ± 0.9	94.8 ± 1.4	94.4 ± 0.8
Backfat depth, mm	11.7 ± 0.3	9.5 ± 0.4	16.5 ± 0.2
IGF1, ng/mL	156.4 ± 3.6	95.1 ± 4.2	89.9 ± 2.7
No. of Records			
Live weight	323	52	758
Backfat depth	160	52	627
IGF1	130	52	422

Values of variables are expressed as means ± SE.

Genotyping of the IGF1 intron 1 STR

Total DNA was isolated by from 300 mg muscle samples by incubating them in 5 ml lysis buffer (Tris 50 mM pH = 8, EDTA 20 mM and 5% SDS) and 50 µl proteinase K (10 mg/ml) at 37 ºC overnight. Approximately 0.5 mL of the lysate was phenol-chloroform extracted and ethanol precipitated. The resulting DNA pellet was resuspended in 50 µl ultrapure water and 2 µl RNase (10 mg/ml). The purification of genomic DNA from blood samples was performed as described in (55). A microsatellite located in the first intron of the IGF1 gene was used to type the

IGF1 gene (56). The PCR mixture contained 2.5 mM $MgCl_2$, 200 μM of each dNTP, 0.2 μM of each primer, 100 ng genomic DNA and 0.5 U AmpliTaq Gold (Roche) in a 10 μl final volume. Primer sequences were: Fw, 5'- GCT TGG ATG GAC CAT GTT G –3' (Tet labeled at 5'); Rev, 5'-CAT ATT TTT CTG CAT AAC TTG AAC CT–3'. The thermal profile was 94 ºC for 10 min, 27 cycles of 94 ºC for 15 sec, 52 ºC for 30 sec and 72 ºC for 1 min, and a final extension step of 72 ºC for 30 min. Samples were analyzed in an ABI Perkin Elmer 3100 capillary electrophoresis device.

Plasma IGF1 determination

Plasma IGF1 concentration was measured with a commercially available self-extraction ELISA kit by using an antibody raised against human IGF1 (Immunodiagnostic Systems Ltd, Boldon, UK). Plasma IGF1 was determined both in LN and DU from blood samples taken at sampling ages. Each sample was evaluated in a double assay. The inclusion of a serum control run in each assay indicated that the intra-assay and inter-assay coefficients of variation were lower than 5.3% and 18.8%, respectively. The lower limit of detection was 15.9 ng/mL.

Statistics

Plasma IGF1 concentration, live weight, and backfat thickness were analyzed within breed using a linear mixed model, in which fixed effects included the testing group and the IGF1 genotype with age as a covariate centered at the age of reference. The effect of age was modeled as a first-order polynomial except for complete repeated measurement analyses in DU, where a second-order polynomial was used. The covariance structure of the data was modeled by adding the sire and, if repeated measurements were available, the pig as random effects. Pigs of the same gender and line tested at the same time were considered as a testing group. The IGF1 genotype was defined according to the number of intronic $(CA)_n$ sequence repeats in each of the alleles of the IGF1 gene. The additive effect of each of the IGF1 alleles was based on a gene substitution model in which the effects of the different alleles were estimated by multiple regression analyses. The effect of the length of the polymorphism was estimated by substituting the genotype by either a covariate on the number of sequence repeats or length genotype classes. The same approach was

used for the analyses of plasma IGF1 concentration as a function of live weight or backfat thickness. Data for carcass traits were fitted to a model including the testing group and the genotype, as fixed effects, and sire, as the only random effect, with carcass weight as a covariate in substitution of age. The association between plasma IGF1 concentration and performance and carcass traits was analyzed using the same models but substituting the plasma IGF1 concentration at control ages for the IGF1 genotype. Within line and gender, as well as pooled estimates between breeds, were also obtained. Estimates are presented as the coefficients of regressions ± SE and least-square means ± SE. Significance testing between genotypes was done by a t-test pairwise comparison of the respective least square means and set at $P<0.05$. No transformation on dependent variables was performed as no consistent departures from normality according to the Shapiro-Wilk test were found. Data were analyzed with SAS (SAS Inst. Inc., Cary, NC) using MIXED procedures.

Results

IGF1 STR allele frequencies and genotypes

The studied IGF1 STR at intron 1 displayed a remarkable degree of polymorphism, with at least five alleles segregating in both LN and DU populations (Table 2). However, a different pattern of allele distribution was observed in each breed. The allele 199, which was the predominant in LN (frequency of 61.2%), was poorly represented in DU (frequency of 11.0%) and, viceversa, the allele 197, the most common in DU (frequency of 56.5%), only reached a frequency of 7.1% in LN. Alleles 195 and 203 were only found in LN while alleles 191 and 205 were specific of DU. No deviation from Hardy-Weinberg equilibrium was observed either in LN or DU ($P>0.05$) and no difference in the allele distribution was found among LN lines A, B, and C ($P>0.05$). The allele 199 was the predominant in the three LN lines and, with the exception of the allele 197, which was not present in pigs sampled from line A, all other alleles were found in each line. The average genotype was longer in LN than in DU (+0.54 CA repeats, $P=0.004$), being the difference mainly due to the length of the shortest allele in the genotype (+0.39 CA repeats, $P=0.007$). No significant difference for the length of the STR among LN lines was observed.

Table 2. Allele distribution of the IGF1 gene polymorphism in Landrace and Duroc populations

	Genetic type	
	Landrace	Duroc
No animals	170	168
No alleles	5	5
Allele 191	-	15
Allele 195	53	-
Allele 197	24	190
Allele 199	208	37
Allele 201	24	83
Allele 203	31	-
Allele 205	-	11
No Genotypes	14	13

Plasma IGF1 concentration, growth and fatness

Plasma IGF1 concentrations were within the expected range (7, 9, 49), with boars exhibiting 1.6-fold higher values than gilts (Table 1). However, plasma IGF1 level displayed a marked age-related pattern (Figure 1), showing a consistent increase from 35 to 160 days and a declining trend at older ages. Results in LN boars indicated that plasma IGF1 concentration at 160 days was more than 3-fold higher than at 35 days while results in DU barrows evidenced that from 160 to 185 days plasma IGF1 levels decreased by around 15%. Plasma IGF1 concentration at 35 days was uncorrelated with plasma IGF1 level at older ages, which, on the contrary, were

moderately correlated among them. The correlation between IGF1 plasma levels at 35 and 160 days was -0.08 (P=0.571) and -0.03 (P= 0.761), in LN boars and DU barrows, respectively, while the pooled intra-animal correlation between measurements taken at 160, 185, and 215 days in DU barrows was 0.35 (P<0.001).

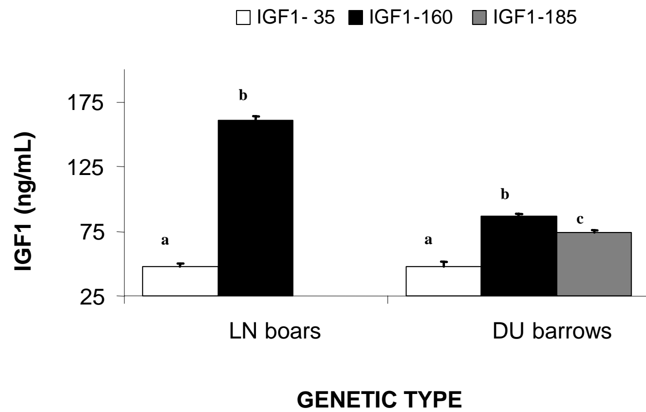

Figure 1. Plasma IGF1 concentration adjusted at 35 (IGF1-35), 160 (IGF1-160), and 185 (IGF1-185) days of age in Landrace (LN) boars and in Duroc (DU) barrows. Values are expressed as means per genetic type ± SE. Error bars denote SE. Statistical significance was performed using a t-test. Means lacking a common superscript within a breed differ. Plasma IGF1 concentration increased from 35 to 160 days (P<0.001) in the two breeds. In DU barrows plasma IGF1 concentration decreased from 160 to 185 days (P<0.001).

The association of plasma IGF1 concentration with live and carcass traits is presented in Table 3. Plasma IGF1 concentration was shown to be associated with live weight, although the strength of the relationship varied by genetic type and age. In DU barrows a positive association between circulating IGF1 and live weight was observed shortly after weaning (32±5 g per ng/mL, P<0.001, at 35 days), whereas in LN it was not significantly evidenced until commercial market weights (103±39 g per ng/mL, P=0.009, in boars at 160 days). On the contrary, no relationship between circulating IGF1 and live weight at 35 days was found in LN boars (2±8 g per ng/mL, P=0.850) and between circulating IGF1 and live weight at 160 days, neither in LN gilts (4±44 g per ng/mL, P=0.924) nor in DU barrows (30±25 g per ng/mL, P=0.231). Circulating IGF1 was not associated with backfat thickness (Table 3), which may be indicative that increased levels of circulating IGF1 improved growth performance by selectively altering body composition in favor of lean growth.

To test the hypothesis whether plasma IGF1 concentration affects body composition the effect of circulating IGF1 on backfat and loin thickness at fixed live weight was determined. Circulating IGF1 did not affect backfat thickness adjusted for live weight at 160 days in LN boars but results in DU barrows were consistent in showing that high levels of plasma IGF1 at ages approaching puberty were associated to higher lean content. Thus, backfat thickness adjusted for live weight decreased with the plasma IGF1 level (-15±8 μm per ng/mL at 160 days, P=0.057; and -22±10 μm per ng/mL at 185 days, P=0.022) while loin thickness adjusted for carcass weight (64±31 μm per ng/mL, P=0.039) and estimated lean content (34±15 mg/kg per ng/mL, P=0.026) increased. Moreover, intramuscular fat content was also affected by circulating IGF1 concentration. The IGF1 concentration at 185 days was negatively related with intramuscular fat content in both longissimus muscle adjusted for live weight at 185 days (-327 ± 160 μg/g MS per ng/mL, P=0.043) and in *gluteus medius* adjusted for carcass weight at slaughter (-544 ± 184 μg/g MS per ng/mL, P=0.004).

Table 3. Association between plasma IGF1 concentration and performance and carcass traits by genetic type and age

	Genetic type					
	Landrace boars		Duroc barrows			
	B160	P value	B160	P value	B185	P value
Live measurements at 160 days						
Live weight, kg	103 ± 39	**0.009**	30 ± 25	0.231	-10 ± 29	0.742
Backfat thickness, mm	7 ± 7	0.327	-9 ± 9	0.331	-17 ± 10	0.096
Loin thickness, mm	na	na	5 ± 11	0.671	4 ± 12	0.738
Carcass measurements						
Carcass weight, kg	90 ± 43	**0.040**	13 ± 30	0.666	51 ± 34	0.138
Backfat thickness, mm	na	na	- 18 ± 10	0.068	-15 ± 10	0.145
Loin thickness, mm	na	na	64 ± 31	**0.039**	25 ± 32	0.426
Lean content, g/kg	na	na	28 ± 15	0.065	34 ± 15	**0.026**
Weight of hams, kg	-1 ± 3	0.697	1 ± 5	0.845	6 ± 4	0.156
Intramuscular fat, mg/g dry matter	1 ± 197	0.997	-138 ± 207	0.507	-544 ± 184	**0.004**

B160 and B185 account for the change (x1000) in live (adjusted at 160 days of age) and carcass (adjusted at 80 kg and 105 kg carcass weight, in Landrace boars and Duroc barrows, respectively) measurements on plasma IGF1 concentration (ng/mL) at 160 days and at 185 days, respectively. Values are expressed as regression coefficients ± SE, with associated P-values. na: not available.

IGF1 STR genotypes and plasma IGF1 concentration

The multiple regression analyses revealed significant substitution effects between extreme alleles. Thus, in LN, the allele 203 showed a positive substitution effect for plasma IGF1 concentration at 160 days when compared to both the allele 195 (23.1±8.2 ng/mL, P=0.006) and the allele 197 (20.7±9.3 ng/mL, P=0.028). On the other hand, we found in DU barrows a positive additive effect when substituting the allele 201 by the allele 197 (10.0±4.8 ng/mL, P=0.043) on plasma IGF1 level at 35 days, as well as a negative effect of the allele 199 on circulating IGF1 level at 160 days and older ages (-8.6±4.2 ng/mL, P=0.040). The same effects were also seen after adjusting circulating IGF1 for live weight or backfat thickness. These results suggest that, at least in LN, there might be a positive relationship between the length of the IGF1 STR and circulating IGF1. To test this hypothesis specifically, plasma IGF1 concentration was modeled on the number of STR repeats by a repeatability model. The results of this analysis showed that circulating IGF1 at 160 days increased with the length of the shortest allele within each breed (Table 4), accounting for an average trend of 4.29±1.27 ng/mL of IGF1 per additional STR (P=0.001). This result was consistent with the observed trend in each of the LN lines (line A: 9.34±2.79, P=0.002; line B: 7.04±3.11, P=0.027; and line C: 3.35±3.63, P=0.359, ng/mL of IGF1 per additional STR). The positive effect that longer STR exerted on the plasma IGF1 level was more apparent in boars than in gilts, where it was not significantly evidenced, in accordance with the fact that LN gilts, as DU barrows, exhibited lower plasma IGF1 levels at 160 days (Table 1). A high (H) and a low (L) length genotype classes with respect to the length of the STR in the shortest allele were defined and compared using all the available IGF1 measurements (Figure 2). The genotypes with both alleles displaying a number of STR equal or greater than the allele 201 were allocated in class H whereas the rest were assigned to class L. In LN there were only three pigs with this genotype so that class H was extended to include pigs with the shortest allele being equal or greater than the allele 199. The class H displayed higher levels of plasma IGF1 at 160 days as compared with class L

(12.9±5.2, P=0.013, and 11.6±4.8, P=0.016, ng/mL, for LN boars and DU barrows, respectively).

Table 4. Plasma IGF1 concentration adjusted at 160 days of age by IGF1 genotype and genetic type

	IGF1 genotype (short allele)					
Genetic type	≤195	197	199	≥201	Trend	P (trend)
Landrace	119.6 ± 3.7^{a}	119.7 ± 7.8^{ab}	128.6 ± 2.9^{b}	166.4 ± 14.7^{c}	5.85 ± 1.99	**0.004**
Boars	149.0 ± 4.2^{a}	153.8 ± 15.6^{ab}	161.4 ± 3.5^{b}	194.0 ± 25.3^{ab}	6.62 ± 2.29	**0.005**
Gilts	95.0 ± 7.4^{a}	87.3 ± 9.6^{a}	93.8 ± 5.1^{a}	136.6 ± 19.3^{a}	4.16 ± 3.91	0.292
Duroc						
Barrows	81.6 ± 6.7^{a}	88.9 ± 2.9^{a}	76.7 ± 10.2^{a}	102.7 ± 6.1^{b}	3.62 ± 1.68	**0.032**
Pooled mean	99.9 ± 3.6^{a}	108.6 ± 3.1^{a}	107.2 ± 3.6^{a}	125.7 ± 5.5^{b}	4.38 ± 1.28	**0.001**

The genotype ≤195 equals to allele 191 in Duroc and to allele 195 in Landrace. In Landrace the genotype ≥201 includes alleles 201 and 203. Values are expressed as least-square means ± SE, in ng/mL. Landrace and pooled least-square means are adjusted for gender and breed, respectively. Within a row, means lacking a common superscript differ (P<0.05). Trend accounts for the increase in plasma IGF1 per copy of the CA repeat in the short allele of the IGF1 genotype and P(trend) for the associated P-value.

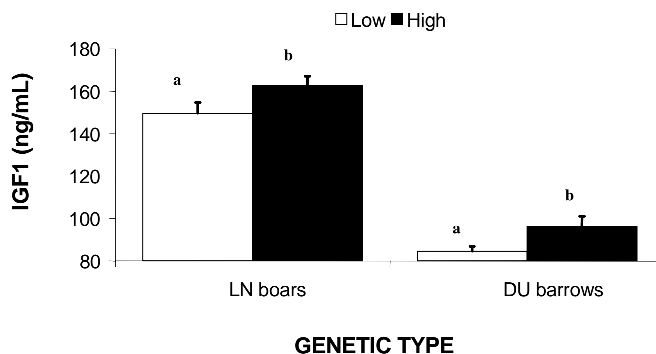

Figure 2. Plasma IGF1 concentration adjusted at 160 days of age by IGF1 genotype class in Landrace (LN) boars and in Duroc (DU) barrows. Low and High refer to the number of CA repeats in the shortest allele of the IGF1 genotype. Class High allocates the genotypes with the greatest number of repeats in the shortest allele in each breed (number of repeats equal or greater than allele 199, in LN boars (n=43 pigs), and equal or greater than allele 201, in DU barrows (n=16 pigs)). Class Low includes the rest of the genotypes (n=29 pigs, for LN boars, and n=151, for DU barrows). Values are expressed as means per genetic type ± SE. Error bars denote SE. Statistical significance was performed using a t-test. Within breed, means lacking a common superscript differ (P= 0.013, in LN boars; P=0.016, in DU barrows). In both breeds plasma IGF1 at 160 days increased as the length of the shortest allele increased.

IGF1 STR genotypes, growth and fatness

It was observed in LN a positive substitution effect of the allele 203 for the allele 195 for live weight (4.6±2.2 kg, P=0.035) and backfat thickness (0.8±0.4 mm, P=0.053) at 160 days, a feature which agrees with the positive trend of these traits with the length of the STR in the shortest allele (Table 5). On the contrary, the analyses conducted in DU barrows were not able to detect any significant effect of the IGF1 STR genotype on live weight and backfat thickness. The comparison of classes H and L for live weight and backfat thickness in LN boars and DU barrows is

depicted in Figure 3. The association pattern in LN gilts, although not significant, was consistent with the one observed in boars. Pigs in class H were heavier at 160 days than pigs in class L in LN boars (2.5±1.0 kg, P=0.015) but not in DU barrows. There was no significant difference between classes for backfat thickness at 160 days, neither in LN boars nor in DU barrows. The small increasing trend appearing in LN boars (0.4±0.3 mm, P=0.117) vanished after adjusting for live weight (0.2±0.3 mm, P=0.488).

In LN boars the substitution effect of the allele 203 for the allele 195 for the weight of the hams was -0.47±0.20 kg (P=0.024) while substituting it for the allele 197 led to increase the intramuscular fat content 75.8±22.4 mg/g dry matter (P=0.001). However, the length of the STR in the shortest allele was related to the weight of hams but not to the intramuscular fat content (Table 5). It was observed in DU barrows a negative substitution effect of the allele 201 for the allele 191 for carcass backfat thickness (-2.1±0.7 mm, P=0.005) adjusted for carcass weight, together with a positive effect for the carcass loin thickness (4.1±2.4 mm, P=0.086). In consequence, carcass lean content was higher for the allele 201 as compared to the allele 191 (23.9±11.3 g/kg, P=0.037). These results led in DU barrows to a negative association of carcass backfat thickness with the length of the STR in the shortest allele, as well as a positive relation with the loin thickness and the estimated lean content (Table 5). The comparison of classes H and L for carcass weight and estimated carcass lean content is depicted in Figure 4. Pigs in class H showed higher carcass weights than pigs in class L, both in LN boars and DU barrows (3.6±1.7 kg, P=0.036, and 6.5±2.8 kg, P=0.020, respectively) but, while in LN boars the carcass lean content was unchanged between classes, in DU barrows class H was leaner at constant carcass weight (+26.5±12.2 g/kg mm, P=0.032) than class L.

Table 5. Change in performance and carcass traits per additional copy of the CA repeat in the short allele of the IGF1 genotype

	Genetic type			
	Landrace boars		Duroc barrows	
	Trend	P value	Trend	P value
Live measurements at 160 days				
Body weight, kg	1.92 ± 0.92	**0.038**	0.10 ± 0.56	0.853
Backfat thickness, mm	0.28 ± 0.17	0.093	-0.15 ± 0.22	0.497
Loin thickness, mm	na	na	0.08 ± 0.23	0.724
Carcass measurements				
Carcass weight, kg	1.69 ± 0.88	0.059	0.74 ± 0.70	0.292
Backfat thickness, mm	0.40 ± 0.43	0.367	- 0.57 ± 0.20	**0.005**
Loin thickness, mm	-0.52 ± 0.60	0.394	1.24 ± 0.63	0.051
Lean content, g/kg	-3.85 ± 4.21	0.368	7.52 ± 3.00	**0.013**
Weight of hams, kg	-0.16 ± 0.07	**0.030**	0.03 ± 0.10	0.751
Intramuscular fat, mg/g dry matter	8.50 ± 6.24	0.177	1.88 ± 3.87	0.628

Trend accounts for the increases in live (adjusted at 160 days of age) and carcass (adjusted at 80 kg and 105 kg carcass weight, in Landrace boars and Duroc barrows, respectively) measurements per CA repeat in the shortest allele of the IGF1 genotype. Values are expressed as regression coefficients ± SE, with associated P-values. na: not available.

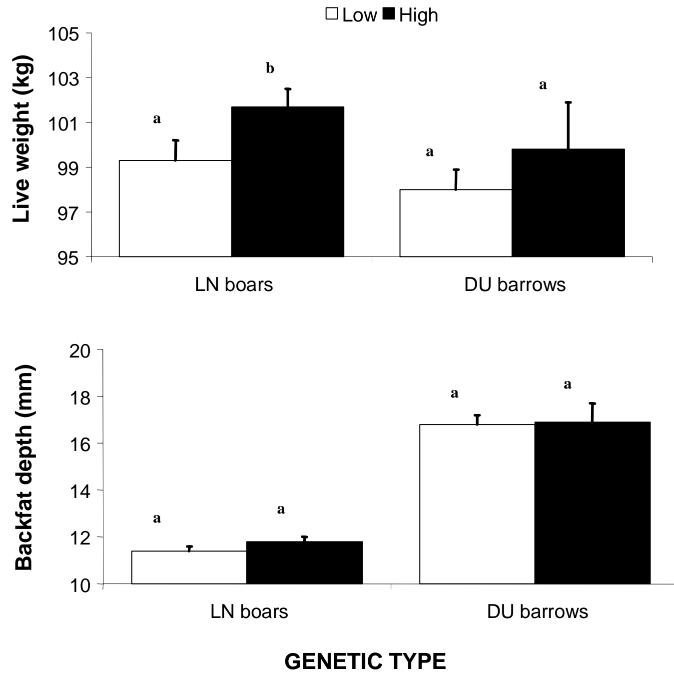

Figure 3. Live weight and ultrasonic backfat thickness adjusted at 160 days of age by IGF1 genotype in Landrace (LN) boars and in Duroc (DU) barrows. See legend of Figure 2 for definition of low and high IGF1 class genotypes, and statistical procedures used. Within breed, means lacking a common superscript differ. In LN boars the live weight at 160 days increased with the length of the shortest allele (P= 0.015) but not the backfat thickness (P=0.117), whereas in DU barrows both traits were unaffected by the length of the shortest allele (P=0.406 and P=0.914, for live weight and backfat thickness, respectively).

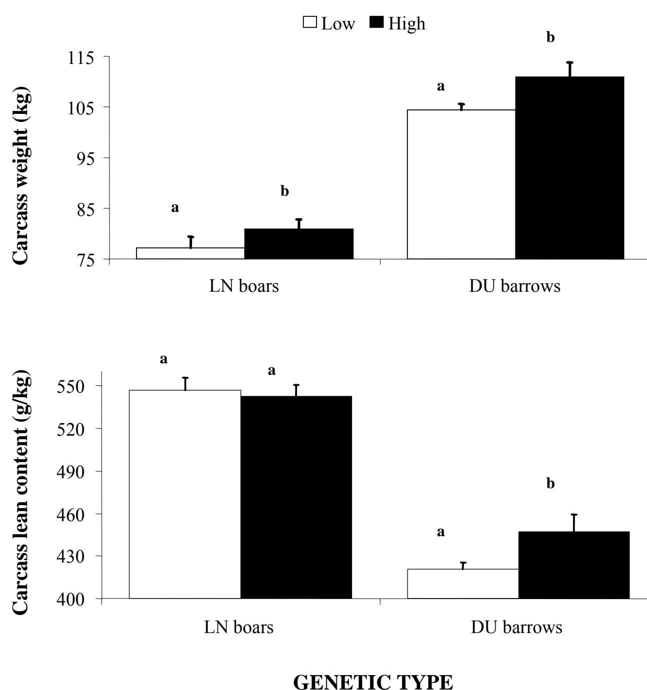

Figure 4. Carcass weight adjusted for age and estimated carcass lean content adjusted for carcass weight by IGF1 genotype in Landrace (LN) boars and in Duroc (DU) barrows. LN boars were slaughtered at 170 days (82 kg carcass weight) and DU barrows at 225 days (105 kg carcass weight). See legend of Figure 2 for definition of low and high IGF1 class genotypes, and statistical procedures used. Within breed, means lacking a common superscript differ. Carcass weight increased with the length of the shortest allele, both in LN boars (P=0.036) and in DU barrows (P=0.020) while estimated carcass lean content at constant carcass weight increased in DU barrows (P=0.032) but not in LN boars (P=0.587).

Discussion

Several lines of evidence from this study support the hypothesis that pigs bearing longer CA repeats at intron 1 of the IGF1 gene, specifically in the shortest allele, have greater circulating IGF1 levels at peripuberty. A plausible explanation of this genetic pattern is to assume that longer alleles have a much greater effect than the shorter ones, or that there is a non-linear increase of allelic effects with the length of the STR. Under this model, the slope of the regression of circulating IGF1 on STR number is expected to be more marked when calculated on the basis of the number of repeats in the shortest allele in a genotype than in the longest, as it is the case here. However, as regression estimates may be greatly influenced by sampling errors associated with extreme genotypes, usually less numerous, it is advisable to validate the results across populations. Our results were consistent across genetic groups in showing that the longer the short allele is the higher the concentration of circulating IGF1. However, the effect of the IGF1 genotypes on circulating IGF1 was only significantly evidenced at 160 days, with the differences more marked in boars than in gilts or barrows. This result can be explained in the light of the ontogeny of IGF1 and its differential pattern between genders. After weaning, circulating IGF1 concentration increases up to a peak at around 100-120 days before decreasing smoothly (8), with a higher and 2-3 weeks later peak in boars than in gilts or barrows (11, 34). Maximum variability in plasma IGF-1 concentration is expected to occur in boars at ages around the peak so therefore the measurement taken in boars at 160 days is where differences between genotypes, if exist, should be more likely detected.

Some authors have reported a relationship between the number of STR and plasma IGF1 levels in humans. The highly polymorphic $(CA)_n$ STR 1 kb upstream of the transcription site of IGF1, lying within the promoter region of IGF1, has been associated with serum IGF1 levels although with conflicting results. While some authors observed a positive relationship with the length of the STR (19, 41), others found that the relationship was negative (32, 53), null (1), or group-dependent (29, 30). Our findings are in line with seminal investigations reporting that the common allele, one of the shortest in the analyzed populations, was found to be associated with decreased IGF1 levels (19, 41) and not with those that suggest that there exists a

ceiling effect of IGF1 levels according to genotype or that a functional significance is limited to shorter repeats (12). It has been attempted to reconcile previous conflicting results, suggesting that there is a broader optimum for IGF1 gene-regulated transcriptional activity, with both shorter and longer alleles having lower circulating IGF1 levels (39). To the best of our knowledge, no similar results have been reported in pigs. The polymorphism we studied is located in another region, at intron 1, which has also been shown to have an important regulatory role on gene transcription (18, 45). On the other hand, there is increasing evidence that the number of intronic STR can cause quantitative changes in gene expression (22) or splicing efficiency (28), acting either as enhancers or repressors according to environmental challenges (48, 50). We can anticipate that the length of CA repeats at intron 1 of the IGF1 gene may have a functional significance, resulting in variable levels of IGF1 expression, although alternative explanations cannot be ruled out. Because circulating IGF1 levels are regulated by complex interactions among multiple loci (24) the existence of other DNA sequences in linkage disequilibrium with the IGF1 STR or affecting circulating IGF1 half-life cannot be discarded.

The IGF1 STR polymorphism has been found to be associated with live weight and carcass lean content, with longer STR showing higher live and carcass weights in LN boars while higher carcass weight and lean content in DU barrows. This result is in accordance with the effect that circulating IGF1 had on live weight and lean content. We have seen that, at early phases of growth, circulating levels of IGF1 were primarily related to growth, but as pigs approach to mature weight strongest associations were found with compositional traits. It is known that the relationship of circulating IGF1 with live weight decreases with age (52), as pigs become fatter. In consequence, a precocious fat deposition can lead to shorten the period in which the association of circulating IGF1 with live weight is more evident. DU barrows have less potential for protein deposition than LN boars, so it is not surprising that in DU barrows the STR IGF1 polymorphism is preferentially related with lean content while in LN boars with live weight. In DU barrows, the effect of the polymorphism on body weight only was significantly evidenced in the carcass, once the abdominal fat has been removed. Results on field trials carried out in pigs observed that juvenile blood IGF1 concentration was positively correlated with

backfat thickness but not with daily gain at the end of the performance test (7). This would suggest, in correspondence with our results, that high early circulating IGF1 may be indicative of pigs with precocious fat deposition. On the other hand, maintaining high levels of circulating IGF1 after puberty may help, as expected from the anabolic function of IGF-1, in promoting protein synthesis, with the desired consequence of increasing fat free mass while inhibiting fat accretion. In line with this, it has been shown that, in pigs expressing an IGF1 transgene, small elevations of circulating IGF1 increased lean content as pigs became older but did not have any major effect on growth performance (37). The effects of IGF1 on glucose uptake are different in skeletal muscle and adipose tissue. Prolonged treatment with IGF1 stimulates glycogen synthesis in the skeletal muscle, whereas lipogenesis is reduced in the adipose tissue via inhibition of insulin secretion (20).

There are some limitations to this study. The size of the experiments was sufficient for detecting relevant effects but a higher number of pigs would have given more validity to the research. Large samples are required to estimate the effect of rare individuals carrying extreme genotypes with precision. This was partially amended by using repeated measurements of IGF1 and compensated by looking for consistency across populations with different genetic backgrounds and selection trajectory, which are known to affect the relationship among circulating IGF1, live weight, and lean content at a given age (7, 9). Circulating IGF1 displayed a marked non-linear trend from shortly after weaning to peripuberty and it is easily influenced by recent feeding events (9, 52). It would have been desirable to have additional measurements to determine the IGF1 pattern, particularly around the peak and after enforced fasting. However, it was impossible to obtain these extra measurements because pigs belonged to commercial lines subjected to conventional management. Finally, not only endocrine but also autocrine effects play a relevant role in growth regulation. Circulating IGF1 is modulated by several blood binding proteins while differential expression of autocrine IGF1 may differ across muscles (33, 47). A proteomic approach involving more than one muscle would have provided a more complete picture of lean and adipose tissue growth regulation.

Two questions were addressed in this study: first, whether variation in the length of the intronic $(CA)_n$ STR in the IGF1 gene influences circulating IGF1 in pigs, and second, whether this polymorphism is associated with growth and fat traits. In summary, the consistency of the results across populations gives credit to the hypothesis that the STR polymorphism at intron 1 of the IGF1 is associated to growth and fatness, and that this effect is exerted through a modification of the IGF1 expression. Increased plasma IGF1 levels were evidenced in pigs displaying longer STR of the IGF1 gene and longer STR were associated with early growth and lean content at peripuberal ages. Lean content and intramuscular fat content are two important economic traits in pork production. Blood juvenile IGF1 level has been proposed as a biomarker for lean (7) or intramuscular fat content (49) in pigs. Our results indicate that age at measurement, especially if measured at early ages, is critical for using plasma IGF1 concentration as a method for biomarking pigs. The IGF1 STR polymorphism may be a valuable alternative. However, further studies are needed to determine whether circulating IGF1 could be useful for altering specifically either lean content or intramuscular fat, as the industry demands. In any case, the identification and follow up of extreme allelic variants may help to gain insight into the physiological mechanisms by which IGF1 regulates growth and fatness.

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Corrigendum

We would like to make an amendment to our article in which we describe a relationship between the length of one microsatellite located at the pig insulin-like growth factor 1 gene and plasma levels of this hormone. According to Winterø et al. [Assignment of the gene for porcine insulin-like growth factor 1 (IGF1) to chromosome 5 by linkage mapping. *Anim Genet* 25: 37–39, 1994], this microsatellite mapped to intron 1 of the pig IGF1 gene. In consequence, in our article we have described the location of this microsatellite as intronic. However, we have recently blasted the microsatellite primer sequences against the GenBank database, and, according to the anotation of sequences X64400 and X17638, this microsatellite in reality maps to the promoter of the pig IGF1 gene. This new mapping information gives more support to our hypothesis of a regulatory role for the IGF1 microsatellite on gene expression.

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ESTUDIO 4: AN AGE-DEPENDENT ASSOCIATION BETWEEN A LEPTIN C3469T SINGLE NUCLEOTIDE POLYMORPHISM AND INTRAMUSCULAR FAT CONTENT IN PIGS

Basado en el artículo de:

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Abstract

The objective of this work was to investigate whether the C3469T mutation at exon 3 of the pig leptin (*Lep*) gene is associated with fatness and if the magnitude and direction of these associations is consistent within age. Repeated measurements on body weight (BW), ultrasonic subcutaneous backfat (SB) and loin-muscle (LT) thickness, and intramuscular fat (IMF) content and composition were taken at 160, 180, 215 and 225 days of age on 162 Duroc barrows previously genotyped for the C3469T mutation. Plasma leptin concentration was determined at 180 d. A total of 505 records of IMF content and composition, which were determined both in live samples of *longissimus dorsi* muscle (LM) and in carcass samples of LM and *gluteus medius* muscle, were used in the analyses. The genotypic frequencies associated with the C3469T *Lep* mutation were 0.76, 0.23, and 0.01 for TT, TC, and CC genotypes, respectively. The effect of the *Lep* genotypes was assessed using a Bayesian approach based on a polynomial mixed model on age. No evidence was found for an association of the *Lep* genotypes neither with BW, SB thickness nor with plasma leptin concentration. The posterior mean of the difference TT-TC ranged, for LT, from 0.82 (160 d, posterior probability density (PP)>0 of 87%) to 1.98 mm (220 d, PP>0 of 96%) and, for IMF, from 0.96 (160 d, PP>0 of 84%) to -1.64 mg/g DM (220 d, PP>0 of 2%). The results suggest an age-dependent association of the C3469T *Lep* polymorphism with LT and IMF, which may be useful to explain some controversial results in the literature concerning the association of this polymorphism with IMF content.

Keywords: intramuscular fat, fatness traits, leptin, pig, polymorphism

Introduction

The leptin (*Lep*) gene has been proposed as a candidate gene for manipulating fatness traits because its product, the protein hormone leptin, which is mainly secreted by the adipose tissue, plays an important role in the long-term fat regulation (Houseknecht et al., 1998). Several *Lep* polymorphisms have been

investigated in a variety of porcine breeds, with no conclusive results with regard to their association with performance traits (for a review see Świtoński et al., 2003). It has been argued that the observed divergences among studies were due to the fact that the analyzed polymorphisms are not causal but in linkage disequilibrium with the functional variants (Świtoński et al., 2003). However, these discrepancies might be alternatively produced by comparing pigs at different stages of fat maturity. Fat deposition patterns might differ among genetic types and across fat tissues (Hauser et al., 1997). In consequence, the effect of the *Lep* genotypes on both the subcutaneous and the IMF fat content, as well as to their relationship, may change over time. This hypothesis is reinforced on the basis of the differential metabolism and hormonal expression profile between SB and IMF (Gardan et al., 2006). In the current work, we investigated the association between a silent C3469T substitution at exon 3 of the *Lep* gene with SB thickness and IMF content and fatty acids (FA) composition at different time-points throughout the finishing period with the aim of understanding if genotype effects are temporally modulated.

Materials and Methods

The experimental procedures by means of which this study was conducted were approved by the Ethics Committee for Animal Experimentation of the University of Lleida and all animal procedures and care were performed in accordance with authorization AE2374 issued by the Catalan Agency of Agriculture, Livestock, and Fishing, Spain.

A random sample of 162 Duroc pigs was taken from the male progeny of 35 boars and 101 sows (1 to 3 animals per litter). They were castrated during the first week of age and raised up in three batches until 225 d of age. Body weight and ultrasonic measurements of SB and *longissimus dorsi* (LM) thickness at the level of the last rib, 5 cm off the mid-line (PIGLOG 105, SFK-Technology, Herlev, Denmark), were recorded 3 times in each pig (around 160, 180, and 215 days of age). At this anatomic location and after weighing (around 1 to 3 times per pig), a muscle live sample of LM of about 160 mg DM was taken using a spring loaded biopsy equipment (Biotech PPB-U, Nitra, Slovakia). During the whole test period the

animals had ad libitum access to commercial diets. At the end of the experiment pigs were slaughtered in a commercial slaughterhouse, where SB and LM thickness at 6 cm off the midline between the third and the fourth last ribs were obtained using the SFK AutoFOM ultrasound automatic scanning equipment (SFK-Technology, Herlev, Denmark). After chilling for 24 h at 2 ºC, a sample of *gluteus* muscle (left ham) and LM were removed from each carcass. The details of the muscle sampling scheme used are given in Table 1. Live samples were trimmed of fat and skin and immediately frozen in liquid nitrogen while carcass muscle samples were vacuum packaged and stored in a deep freeze until required for analyses. A 10 mL blood sample was obtained at around 180 d with a syringe and kept in tubes containing EDTA. Plasma was collected by centrifugation (3000 g for 10 min) and stored at –40ºC until required for assay.

Intramuscular fat content was determined in duplicate by quantitative determination of the fatty acids by gas chromatography with a capillary column. Fatty acid methyl esters were obtained using a solution of boron trifluoride 20% in methanol (Rule, 1997) and total fat was calculated as the sum of individual fatty acids expressed as triglyceride equivalents (AOAC, 2000).

Leptin concentration in plasma was determined by using a leptin porcine ELISA kit (Diagnostic Systems Laboratories, Inc., Webster, TX, USA). All samples were evaluated in a double assay. Coefficient of variation between replicates was 7%.

Table 1. Number of IMF records (n), mean and standard deviation (SD) for performance and fat-related traits in a Duroc population

	longissimus dorsi				*gluteus medius*	
	Live sample		Carcass sample		Carcass sample	
	n= 274		n=83		n= 148	
Trait	Mean	SD	Mean	SD	Mean	SD
Age (d)	183.2	23.7	221.2	3.6	221.2	3.6
Weight (kg)[1]	111.3	17.8	105.1	9.5	104.0	10.0
Loin depth (mm)[2]	43.8	4.6	44.1	9.4	44.0	9.0
Backfat depth (mm)[2]	19.3	4.4	24.4	2.9	23.8	3.2
IMF (mg/g DM)[3]	14.1	5.0	14.9	3.4	19.7	5.7
SFA(%) [3]	41.5	2.0	41.5	2.3	40.1	2.2
MUFA(%) [3]	47.5	2.3	48.1	1.8	46.7	1.8
PUFA(%) [3]	11.0	2.7	10.4	1.7	13.2	2.2

[1] BW, for live sample, and carcass weight, for carcass samples.

[2] Ultrasonic measurements obtained using Piglog, for live sample, and Autofom, for carcass samples.

[3] Intramuscular fat (IMF) content and IMF composition in terms of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acid percentages.

Genomic DNA isolation from muscle and blood samples was performed as previously reported (Vidal et al., 2005; Estany et al., 2007). We genotyped a C3469T mutation at exon 3 of the pig *Lep* gene (Stratil et al., 1997). Primer sequences were: Lep-FW, 5'-CTG TCT CCT CCA AAC AGA GGG TCA-3', and Lep-REV, 5'-CAG CAG CCA GGG CTG AGG TCC A-3'. The PCR mixture contained 1.5 mM $MgCl_2$, 100 μM dNTP, 0.5 μM of each primer, 100 ng genomic DNA and 0.625 U Taq DNA polymerase (Ecogen SRL, Barcelona, Spain) in a final 25 μl volume. The thermal profile included 30 cycles of 94 ºC for 1 min, 69 ºC for 1 min and 72 ºC for 1 min. The 355 bp PCR product was digested with 5 U *Hinf*I for 4-5 hours and

electrophoresed in a 3% agarose gel. The *Hinf*I digestion of the *Lep* amplicon yielded two restriction patterns named as T (355 bp) and C (293 and 62 bp).
The difference between the leptin genotypes TT and TC on fat-related traits was assessed using a one-order polynomial on age with random regression coefficients model. The CC genotype was discarded from the analyses due to his low frequency (only 2 pigs out of 162). Fixed effects included the batch (from 1 to 3) by the type of sample (live sample of LM, carcass LM sample, and carcass *gluteus medius* sample) class, and the leptin genotype (TT or TC). Animal and residual effects were included as random effects.

The statistical analysis was based on Bayesian methods. The marginal posterior distribution of the difference between the leptin genotypes TT and TC at a given age was estimated by Gibbs sampling using GIBBS3F90 (Misztal, 2003). After visual assessment of convergence, 1,000 Gibbs samples were discarded as burn-in and 20,000 samples were used to estimate parameters of the marginal posterior distribution for the effect of TT-TC *Lep* genotypes. The probability *a posteriori* that the estimated effect was higher than zero (PP>0) was calculated as the tail area to the right of zero from the marginal posterior distribution. Values of PP>0 over 95% or below 5% indicate statistically positive or negative differences. To summarize the information contained in the posterior distribution, the highest posterior density region at 95% (HPD_{95}) has been calculated as the interval which contains a 95% of probability. Under the assumption of Gaussian marginal posterior distributions, the differences whose HPD_{95} does not include the zero value have a false discovery rate lower than 2.5%.

Results and Discussion

Genotype frequencies of the *Lep* SNP were TT: 0.76, TC: 0.23, CC: 0.01. These results are similar to those reported by Kennes et al. (2001), who found in Duroc, Landrace and Yorkshire that the C allele was much less frequent than the T allele. We did not find differences between TT and TC genotypes at any age for BW and SB thickness (PP>0 from 24 to 40%, Table 2), as well as for plasma leptin concentration at 180 d (+5.75 ng/mL, PP>0 of 54% for TC-TT). Our results for BW

and SB are in line with those reported by Kennes et al. (2001), Chen et al. (2004), and Szydlowski et al. (2004), who did not find evidence of an association of the C3469T polymorphism with both average daily gain and SB thickness in different pig breeds. However, results in the literature were not so conclusive, with some authors finding a favorable association of the TT genotypes with growth (Krĕnkowa et al., 1999; Kennes et al., 2001; De Oliveira et al., 2006) and others with the TC genotypes (Kulig et al., 2001; De Oliveira et al. 2006). The only study reporting significant associations with SB found that the TC individuals were fatter (De Oliveira et al, 2006). No results have been found in the literature concerning the association of this polymorphism with plasma leptin concentration.

Throughout the fattening period LM thickness was higher for the TT genotypes (Table 2), with differences ranging from 1, at 180 d, to 2 mm, at 220 d of age (PP>0 higher than a 95%). Opposite results were obtained by Kulig et al. (2001), who found that TC genotypes had more lean content. Szydlowski et al. (2004), working with a synthetic line, detected a population-specific association of the *Lep* genotypes with loin weight, which, however, was not consistently proved for other loin-related traits. Differences among *Lep* genotypes for IMF content changed with age (Table 2). At 160 d TT pigs had 0.96 mg/kg DM more IMF than TC pigs (PP>0 of 83.7 %) whereas TT pigs had 1.64 mg/kg DM less at 220 d (PP>0 of 98.4%). With regard to the average value, this means that the difference between TT-TC genotypes evolves from +5% at 160 d to -8% at 220 d. Szydlowski et al. (2004) observed a weak association between the C3469T polymorphism and IMF content but only in Polish Large White, one out of the three breeds they analyzed. In this breed, as in our case, the TT genotype had a 1.6% more IMF than the TC genotype at around 170 days of age. No relevant difference between genotypes was found for IMF FA composition at any age (PP>0 lower than 62%, Table 2), although the evolution of FA composition by genotype was consistent with the expected trend of SFA, MUFA, and PUFA with the IMF content change (Lawrence and Fowler, 1997).

Table 2. Estimated parameters for the marginal posterior distribution of the effect of leptin C3469T genotype (TT-TC) on fat-related traits classified by age.

	Leptin genotype effect (TT-TC)					
Trait	Age	Mean	Median	PSD[2]	PP>0[3]	HPD_{95}[4]
BW, kg	160	-0.98	-1.00	1.59	0.262	-4.1 ; 2.2
	180	-0.83	-0.84	1.65	0.304	-4.1 ; 2.4
	200	-0.67	-0.68	1.79	0.350	-4.2 ; 2.8
	220	-0.51	-0.53	1.99	0.396	-4.4 ; 3.4
Loin thickness, mm	160	0.82	0.82	0.72	0.874	-0.6 ; 2.2
	180	1.21	1.21	0.62	0.975	0.0 ; 2.4
	200	1.60	1.60	0.80	0.977	0.0 ; 3.2
	220	1.98	1.99	1.14	0.958	-0.3 ; 4.3
Backfat thickness, mm	160	-0.38	-0.38	0.55	0.243	-1.5 ; 0.7
	180	-0.34	-0.34	0.52	0.255	-1.4 ; 0.7
	200	-0.30	-0.29	0.54	0.288	-1.4 ; 0.8
	220	-0.26	-0.25	0.61	0.337	-1.4 ; 0.9
IMF, mg/g DM [1]	160	0.96	0.96	0.98	0.837	-1.0 ; 2.9
	180	0.09	0.09	0.75	0.550	-1.4 ; 1.6
	200	-0.78	-0.78	0.66	0.117	-2.1 ; 0.5
	220	-1.64	-1.65	0.75	0.016	-3.1 ; -0.2
SFA, mg/g FA [1]	160	0.14	0.48	0.14	0.617	-0.8 ; 1.1
	180	0.02	0.38	0.02	0.522	-0.7 ; 0.8
	200	-0.10	0.33	-0.10	0.380	-0.8 ; 0.6
	220	-0.22	0.36	-0.22	0.265	-0.9 ; 0.5
MUFA, mg/g FA [1]	160	-0.13	-0.13	0.49	0.393	-1.1 ; 0.8
	180	-0.05	-0.05	0.38	0.446	-0.8 ; 0.7
	200	0.03	0.03	0.34	0.536	-0.6 ; 0.7
	220	0.11	0.10	0.41	0.603	-0.7 ; 0.9
PUFA, mg/g FA [1]	160	-0.14	-0.14	0.50	0.781	-1.1 ; 0.8
	180	-0.02	-0.02	0.41	0.957	-0.8 ; 0.8
	200	0.10	0.10	0.36	0.790	-0.6 ; 0.8
	220	0.21	0.21	0.37	0.565	-0.5 ; 0.9

[1] Intramuscular fat (IMF) content and composition in terms of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids

[2]Posterior standard deviation

[3]Posterior Probability density of a value higher than zero

[4]Highest posterior density region at 95%

The results obtained in pigs with regard to the association between the C3469T *Lep* polymorphism and fat-related traits are contradictory in terms of both magnitude and direction (see Świtoński et al. 2003 for a review). Divergence among studies, as well as to the experimental design and the statistical methods used, are usually attributed to population specific linkage disequilibrium of the polymorphisms with causal mutations. Our findings highlight that genotype effects are not constant throughout the growing period and therefore estimated effects can be influenced by age of measurement and/or the fattening state. Using measurements taken at different time-points throughout the fattening period, we prove that the association of the C3469T *Lep* polymorphism with some traits increases with age (i.e. LM thickness) or even changes to the opposite direction (i.e. IMF content). This result may help to explain contradictory results from studies using either pigs tested at different ages or pigs tested at the same age but which are from genetic types differing in their fat deposition pattern. We have found a differential association of the C3469T *Lep* polymorphism with SB and IMF. It is known that there is also a differential expression of genes and biochemical constituents, including leptin, in adipocytes from different tissues (Harper and Pethick, 2004). In particular, Gardan et al. (2006) found that the *Lep* mRNA levels differed between IMF adipocytes at 160 and 210 d of age but not in SB adipocytes. It can be then hypothesized that the C3469T polymorphism is associated with a differential expression of leptin across fat tissues and throughout time, either because it has a regulatory role or because it is linked with another mutation affecting gene expression.

Conclusions

This study provides evidence that the associations between gene polymorphisms and fatness vary with age and fat tissue. Although this circumstance makes more difficult the identification of causal mutations, it can help for acting at specific ages or tissues. Thus, the fact that, at late ages, the C3469T *Lep* polymorphism may be preferentially associated with intramuscular fat content but not to subcutaneous fat can be useful for selecting them independently of each other.

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VI. Discusión General

La tendencia de los programas de mejora genética llevada a cabo durante las últimas décadas ha mejorado la eficiencia en el crecimiento de los cerdos, disminuyendo el espesor de grasa dorsal y aumentando el porcentaje de magro. Esta mejora del crecimiento y el aumento del contenido magro ha afectado negativamente caracteres relacionados con la calidad de la carne (Lonergan et al., 2001). Por otro lado, en los últimos años ha aumentado el interés de los consumidores en disponer de productos de mayor calidad y más saludables. Este hecho ha modificado los programas de mejora genética en la última década, para poner mayor énfasis en la mejora de los caracteres ligados a la calidad de la carne. Tal y como se ha citado en el inicio de esta Tesis Doctoral, la definición de calidad de la carne es compleja y engloba varios caracteres relacionados, de estos caracteres, el contenido en grasa intramuscular tiene un importante papel (Fernandez et al., 1996), el cual se amplifica si se tiene en cuenta su composición en ácidos grasos, por sus implicaciones en la salud del consumidor final.

El primer objetivo de esta Tesis Doctoral, ha sido el de desarrollar un método para determinar conjuntamente el contenido y la composición de la grasa intramuscular, a partir de biopsias y muestras pequeñas *post-mortem*. El desarrollo de una técnica de estas características, tiene la ventaja de permitir el estudio de estos caracteres en cerdos vivos, pudiendo determinar la evolución a lo largo del engorde del contenido y la composición de la grasa intramuscular.

La metodología de obtención de muestras *in-vivo* mediante biopsias sobre el músculo *longissimus dorsi* desarrollada en el estudio 1, es un método viable tanto para la determinación *in-vivo* del contenido de grasa intramuscular, como para la determinación de su composición; si bien cabe tener en cuenta algunos factores limitantes debidos al propio método. El principal problema de su aplicación, está relacionado con la posible contaminación de grasa subcutánea sobre la muestra de músculo, este hecho es especialmente problemático cuando se realizan mediciones en cerdos con un elevado espesor de grasa dorsal. Villé et al. (1995) señalaron la gran sensibilidad a la contaminación de las muestras de músculo por grasa subcutánea. Los cerdos utilizados en estos estudios han sido mayoritariamente de la raza Duroc,

con un elevado espesor de grasa dorsal, que osciló entre 16.9 mm de media a 160 días de edad y 24.4 mm de media a la edad de sacrificio, con valores límites cercanos a 33 mm; estos importantes espesores implican dos dificultades; la primera es un mayor riesgo de contaminación de la gasa subcutánea sobre la muestra de músculo, y la segunda es una menor muestra de músculo al no poder penetrar lo suficiente la cánula en el músculo. La relación negativa encontrada entre profundidad de grasa dorsal y tamaño de muestra vendría a confirmar estas hipótesis. Esta relación además se ha incrementado con la edad, aumentando la correlación negativa entre ambos valores.

La realización de la biopsia sobre canal (BC) elimina la sobreestimación del contenido de IMF debido a las diferentes condiciones de realización de la biopsia (canal fría), si bien esta técnica no permite predecir *in-vivo* el contenido de IMF, sí permite determinar el contenido de IMF sobre canal sin requerir de una gran muestra de músculo, con la consiguiente ventaja al disminuir la depreciación de la canal.

Otro inconveniente en la utilización de la metodología expuesta es el pequeño tamaño de muestra obtenido para su posterior análisis. Este pequeño tamaño de muestra comporta un mayor error de muestreo (Baas et al., 1998); si bien en este trabajo se ha podido demostrar que, en relación con la determinación del contenido de IMF del músculo LM, muestras pequeñas del propio músculo han resultado ser al menos tan eficientes, como muestras grandes de otros músculos (GM), consiguiendo correlaciones similares a las obtenidas por Newcom et al. (2002) con la utilización de equipos de RTU.

En relación con la composición de la grasa intramuscular el método analizado tiene como factor limitante la sensibilidad a la contaminación con grasa dorsal. En este caso el mayor contenido en PUFA de la grasa dorsal puede provocar una valoración al alza de la composición en PUFA respecto al resto de ácidos grasos; si bien las correlaciones entre los valores BE y LM fueron para SFA, MUFA y PUFA superiores a 0.62, incrementándose este valor para BL. Otro inconveniente del método para la predicción de la composición de la grasa intramuscular radica en la dificultad de analizar los ácidos grasos menos abundantes, principalmente el C20:0.

Teniendo en cuenta las limitaciones comentadas de la metodología estudiada, se puede afirmar que, para la predicción del contenido de IMF a partir de animales vivos, la utilización de muestras pequeñas obtenidas a partir de biopsias *in-vivo* del mismo músculo objetivo, es al menos tan eficiente como la utilización de muestras grandes de otros músculos (GM). Para corroborar esta afirmación se ha llevado a cabo una tabla sobre la pérdida estimada de la respuesta fenotípica para un nivel determinado de intensidad de selección. Esta tabla ha sido calculada con la siguiente función:

$$P= 100*(y_{LM} - y_{A})/y_{LM}$$

donde:

P: pérdida estimada de la respuesta fenotípica para un nivel determinado de intensidad de selección.

y_{LM}: para selección direccional, el valor de la media fenotípica de los valores más altos del ránking de candidatos (para selección estabilizante, la media de los candidatos que están más cerca del valor medio) seleccionados de acuerdo al valor LM.

$y_{A:}$ la media fenotípica de los valores más altos del ránking de candidatos (los candidatos que están más cerca del valor medio) seleccionados a partir de otro método alternativo de determinación pero calculados utilizando su valor LM.

A partir de este cálculo se ha realizado la Tabla 1, en la cual se muestra para dos niveles de intensidad de selección, las pérdidas de valor fenotípico estimadas en el caso de selección direccional (mayor contenido en IMF) y en el caso de selección estabilizante (contenido medio de IMF). Asimismo se ha separado la variable BE en los dos distintos controles, BE160 (biopsia en vivo a 160 días de edad) y BE185 (biopsia en vivo a 185 días de edad), utilizando también los valores BE y BL sin corregir y corregidos por el modelo en función de la edad y el valor cuadrático de la edad.

Se puede observar como, en caso de practicar selección direccional a favor de LM con una intensidad del 5%, es mejor utilizar como criterio BE160, BE185 y BL

que GM. Para una presión de selección del 25%, es indistinto utilizar BE160, BE185 o GM como criterio de selección.

En caso de practicar selección estabilizante, fijado entorno al valor medio de IMF en LM, con una presión de selección del 5%, GM y BE185 son equivalentes, pero, con una presión del 25%, BE160 y BL son mejores que GM. Tan sólo BE185 resulta ligeramente peor que GM. En general, pues, las pérdidas fenotípicas de BE respecto a LM se sitúan entre el 8.8 y el 18.6%, en la selección direccional, siendo algo mayores, entre 12.6 y el 20.4%, para la estabilizante.

Tabla 1. Pérdida fenotípica esperada en IMF cuando la selección se basa en valores obtenidos por otro criterio diferente del objetivo de selección.

Criterio de selección[2]	Objectivo de selección[1]							
	Direccional				Estabilizando			
	LM		GM		LM		GM	
	5%	25%	5%	25%	5%	25%	5%	25%
BE160	8.8	11.3	23.7	19.0	20.4	12.6	37.1	22.4
BE185	18.6	9.4	33.6	18.1	13.6	16.8	44.8	31.2
BL	16.7	16.3	35.4	26.9	22.1	13.8	10.7	19.5
BC	4.5	9.9	20.1	16.5	15.5	14.7	11.5	6.5
LM	-	-	25.3	17.5	-	-	9.9	16.9
GM	28.0	9.6	-	-	12.6	15.9	-	-

[1]Objetivo de selección incrementando (direccional) o manteniendo (estabilizando) el contenido en grasa intramuscular en el músculo *longissimus dorsi* (LM) o en el músculo *gluteus medius* (GM). Presión de selección fijada al 5 o 25%. Valores expresados en porcentaje.

[2]Contenido de grasa intramuscular estimado utilizando BE160 (BE185; BL y BC) una muestra de músculo LM tomada a 160d (185d; 210d; y sobre la canal a 220 d) utilizando biopsias o LM (GM), una alícuota representativa de un muestra homogeneizada de LM (GM) obtenida de la canal.

La metodología expuesta en el estudio 1 ha permitido analizar la evolución del contenido y composición de la grasa intramuscular, tal y como se describe en el estudio 2. A diferencia de otros trabajos previos, ello se ha conseguido a partir de medidas longitudinales, hasta un total de 3 mediciones *in-vivo* y 2 mediciones *post-mortem* sobre el músculo *longissimus dorsi,* sin necesidad de realizar sacrificios seriados. Esta técnica permite estimar individualmente la evolución del contenido y la composición de IMF y, en consecuencia, la variabilidad de las respectivas curvas de deposición. El sesgo por contaminación de grasa subcutánea que se comete al utilizar biopsias, se corrige mediante los valores obtenidos en canal.

Los elevados pesos a los cuales se han sacrificado los cerdos, han propiciado que los parámetros de evolución de contenido de grasa intramuscular determinados no han resultado similares a los obtenidos en anteriores estudios. En el tramo de edad analizado, el contenido en IMF ha aumentado de forma lineal, si bien en anteriores trabajos (Ellis et al., 1996, D'Souza, 2004; Realini et al., 2010) realizados en rangos de edad (y peso) inferiores, este incremento de IMF no se ha hallado, Lo Fiego et al. (2010), en un trabajo en el que se evaluaba el contenido y composición de la grasa intramuscular a diferentes edades al sacrificio, apreciaron un aumento del contenido en IMF al aumentar la edad de sacrificio de 6 a 9.5 meses, si bien este incrementó no resultó significativo, resultados equivalentes obtuvieron Virgili et al. (2003) en edades al sacrificio de 8 y 10 meses respectivamente.

En la comparación de la composición de la grasa subcutánea con la grasa intramuscular, se han hallado diferencias importantes, el porcentaje de SFA y MUFA es superior en la grasa intramuscular respecto a la grasa subcutánea, mostrando el porcentaje de PUFA una relación inversa; estos resultados encontrados están en consonancia a los expuestos en otros trabajos (Estany et al., 2002; Lo Fiego et al., 2010), Teye et al. (2006a) y Teye et al. (2006b) encontraron un porcentaje de PUFA superior en la grasa dorsal respecto a IMF, mientras que en el trabajo de Enser et al. (1996) el porcentaje de PUFA fue menor en la grasa dorsal. Las diferencias menores entre tejidos se han hallado en los ácidos grasos saturados C16:0 y C18:0, mostrándose la mayores diferencias en el ácido oleico (C18:1) y el ácido linoleico (C18:2). Las mayores correlaciones entre tejidos se han hallado en los ácidos grasos

saturados. En relación al cociente ω-6/ω-3, el ratio ha resultado menor en la grasa subcutánea que en la grasa intramuscular.

La evolución de los SFA de la grasa intramuscular en función de la edad ha seguido una tendencia cuadrática negativa, detectándose el mayor porcentaje de SFA en la grasa intramuscular a los 185 días, para posteriormente disminuir a valores equivalentes a los 160 días; esta evolución fue detectada también por Apple et al. (2009) en un rango de pesos de 28.1 kg a 113.6 kg, el mayor porcentaje de SFA se obtuvo a los 68.1 kg de peso en cualquiera de las dietas experimentales que utilizaron (un total de 4 dietas distintas). La evolución de los MUFA fue lineal y positiva, incrementándose de forma significativa en el periodo analizado, Apple et al. (2009) en el rango estudiado encontraron un aumento de los MUFA al aumentar el peso al sacrificio en todas las dietas, excepto en la dieta rica en PUFA. Dentro de los MUFA, la variación más importante se debe al ácido oleico, el cual tiene una evolución cuadrática positiva dentro del periodo analizado. Finalmente y en relación a los PUFA su composición disminuyó linealmente con la edad. Estas tendencias son similares a las encontradas en anteriores estudios (Kouba et al., 2003; Virgili et al., 2003; Lo Fiego et al., 2010), si bien al utilizar dietas ricas en PUFA los resultados son variables, Kouba et al. (2003) en una dieta rica en aceite de lino determinaron que el porcentaje de PUFA disminuía, mientras Apple et al. (2009) en una dieta rica en aceite de soja lograron mantener el porcentaje de PUFA. Estos estudios indican la importancia de las fuentes de grasa suministradas en la dieta sobre la deposición de grasa en el organismo animal.

La evolución de la composición de la grasa está sujeta a la composición de la grasa de la dieta, tal como se puede comprobar en distintos trabajos (Kouba et al., 2003, Wood et al., 2004 y Apple et al., 2009). En el experimento descrito en el estudio 2 se utilizaron dos dietas durante la fase de estudio. Las dos dietas eran similares, pero con alguna variación composicional en algún ácido graso relevante, como, por ejemplo, en C18:2, menos presente (sobre un 1.5%) en la dieta de finalización que en la de crecimiento. Las dietas utilizadas en el estudio fueron las dietas comerciales habituales de la empresa propietaria de los animales, dietas formuladas para conseguir una calidad de la canal y de la carne adecuada a los

estándares de la empresa. No obstante, las variaciones en composición de las dietas fueron inferiores al 1% para SFA, MUFA y PUFA.

La edad ha mostrado un efecto importante sobre la composición de la grasa dorsal. En general, las tendencias han sido similares a las encontradas en la grasa intramuscular, si bien con una oscilación menor. Así, los SFA alcanzan un punto máximo alrededor de los 185 días, mientras que los MUFA aumentan y los PUFA disminuyen con la edad, como en el caso de IMF. Así en trabajos previos, Kouba et al. (2003) y Raj et al. (2010) determinaron que el incremento de peso implica un aumento de SFA y MUFA así como una disminución de PUFA, tanto en grasa subcutánea como en IMF, la evolución encontrada en estos trabajos coincide con el estudio 2, excepto en el caso de SFA.

La técnica desarrollada ha permitido también el estudio de la evolución de la composición de la grasa intramuscular en función de su contenido. El aumento del contenido de IMF ha comportado un aumento los SFA y MUFA y una disminución de los PUFA, obteniéndose unos resultados similares a los publicados por Cameron y Enser (1991) y Van Oeckel et al. (1996). Esta evolución conviene entenderla en el contexto de los componentes de la grasa intramuscular; los fosfolípidos, con mayor porcentaje de PUFA (Wood et al., 2004), son más constantes en IMF, mientras que los lípidos neutros, de presencia más variable, tienen un porcentaje menor en PUFA. Así, al aumentar el contenido de IMF en el músculo, se produce un mayor aumento de lípidos neutros, con la consiguiente disminución de PUFA. Este diferente comportamiento de los fosfolípidos respecto a los triglicéridos se puede comprobar en la Tabla 2, extraída del estudio de Wood et al. (2004), en el que se comparan cuatro razas porcinas. En este trabajo no se encontraron diferencias en el contenido de fosfolípidos y sí en el de lípidos totales y neutros, lo que refleja que el aumento total de lípidos se realiza a costa de aumentar los lípidos neutros.

Tabla 2. Composición en lípidos neutros, fosfolípidos y contenido total de lípidos del músculo *Longissimus* y composición del total de lípidos en cuatro razas porcinas (Wood et al., 2004, extraído de Wood et al., 2008).

	Berkshire	Duroc	Large White	Tamworth
Espesor de grasa dorsal (mm)	15[b]	9[a]	8[a]	15[b]
Fosfolípidos[d]	0.39[a]	0.42[a]	0.38[a]	0.38[a]
Lípidos neutros[d]	1.67[b]	1.35[b]	0.60[a]	0.82[a]
Total lípidos[d]	2.05[b]	1.77[b]	0.97[a]	1.20[a]

[a-c-]en una fila, con diferentes superíndices indican diferencias significativas (P<0.05).
[d] g/100g músculo.

En el segundo estudio de la Tesis se ha analizado también la composición de la grasa dorsal en función del espesor de la grasa dorsal. El aumento del espesor de grasa dorsal ha implicado un aumento de SFA y una disminución de PUFA, manteniéndose el porcentaje de MUFA estable en el periodo analizado. Wood et al. (1989), con un rango de espesor de grasa dorsal situado entre 8 a 16 mm (inferior al analizado en este estudio), determinaron un aumento de SFA y MUFA a costa de una disminución en el porcentaje de PUFA.

La técnica desarrollada en el estudio 1 se ha aplicado a dos estudios de asociación genética con marcadores moleculares. En el estudio 3 se ha investigado si un microsatélite polimórfico Citosina-Adenosina localizado en el promotor del gen IGF1 influencia el nivel circulante de IGF1, así como su relación con variaciones fenotípicas en el crecimiento y caracteres de engrasamiento, entre ello, el contenido de grasa intramuscular. Al disponer de varias mediciones en animal vivo a lo largo del engorde, se ha podido contrastar el efecto del marcador sobre el nivel circulante de IGF1 y los caracteres productivos a distintas edades. La asociación longitudinal del gen IGF1, así como de su concentración plasmática, con el contenido y composición de la grasa intramuscular se ha hecho en cerdos Duroc (experimento 2), ya que sólo en estos animales se dispuso de biopsias seriadas. Se ha observado una correlación negativa entre el contenido de IMF en el músculo *longissimus dorsi* a una edad de 185 días y el nivel de IGF1 circulante. Al comparar el contenido de IMF en

canal en el músculo *gluteus medius* la correlación ha sido equivalente. Asimismo, en cerdos Duroc, se ha detectado una asociación positiva entre IGF1 circulante y contenido magro a edades cercanas a la pubertad, así como un mayor espesor del lomo. En lo que refiere al espesor de grasa dorsal, el aumento de los niveles de IGF1 hizo decrecerlo. Los resultados encontrados evidencian la hipótesis de que el microsatélite polimórfico estudiado, está asociado al crecimiento y engrasamiento, y que este efecto se ejerce a través de una modificación de la expresión de IGF1. El aumento de los niveles plasmáticos de IGF1 se evidenció en cerdos con el microsatélite del gen IGF1 más largo, y la mayor longitud del microsatélite se asoció a crecimiento precoz y contenido magro a edades peripuberales.

El nivel de IGF1 en sangre a edades tempranas se ha propuesto como un marcador para el tejido magro (Bunter et al., 2005), o para el contenido de IMF (Suzuki et al., 2004). Los resultados hallados en el estudio 3 indican que la edad de medida puede resultar crítica para su utilización como criterio de selección. En este sentido, el microsatélite estudiado es una alternativa a considerar. Son necesarios más estudios para determinar si el nivel circulante de IGF1 o el polimorfismo estudiado puede ser útil para alterar el contenido de IMF o del contenido magro.

Finalmente, en el estudio 4 se ha investigado si la relación de la mutación del exón 3 del gen de la leptina en porcino, está asociada al engrasamiento. Las frecuencias detectadas del gen fueron similares a las analizada por Kennes et al. (2001) en cerdos Duroc, Landrace y Yorkshire. Al igual que en la bibliografía consultada (Kennes et al., 2001, Chen et al., 2004 y Szydlowski et al., 2004), no se ha encontrado diferencia en el genotipo TT y el TC en relación al peso ni al espesor de grasa dorsal. Las diferencias entre los genotipos LEP para el contenido de IMF cambiaron con la edad. A 160 días los cerdos con el genotipo TT tuvieron alrededor de 1 mg/kg en materia seca más de IMF que los TC, mientras que a los 220 días éstos tuvieron 1.6 mg/kg menos de IMF que los de genotipo TC .En relación al valor medio esto significa que la diferencia entre genotipos evoluciona de +5% a 160 días hasta -8% a 220 días. Este experimento demuestra que los efectos de un genotipo no son constantes a lo largo del período de crecimiento.

Los estudios descritos en esta Tesis Doctoral se han basado en datos longitudinales obtenidos mediante la toma previa de biopsias de músculo y grasa. Esta técnica, en tanto que invasiva, puede afectar el bienestar animal; pero, en tanto que más potente, puede reducir el número de animales experimentales y, en consecuencia el número de sacrificios seriados. A modo de ejemplo, en el caso del diseño de un esquema de muestreo con dos muestras repetidas para el contenido de IMF (carácter lineal), el número de animales se reduciría a una cuarta parte y el número de determinaciones de IMF a la mitad. Utilizando BC en lugar de LM se podría considerar como una alternativa útil si la obtención de LM no está al alcance, debido a los costos de depreciación. A nivel ético, por tanto, se puede discutir cuál de los dos diseños tiene un impacto más negativo sobre el bienestar de los animales. Los resultados presentados en esta Tesis pueden ayudar a tomar una decisión.

VII. Conclusiones

Las conclusiones de esta tesis son:

I. En relación con el primer objetivo:

I.1. La cromatografía de gases cuantitativa con transesterificación directa es un método útil para determinar simultáneamente el contenido y la composición de la grasa intramuscular en muestras de tamaño inferior a un gramo de peso fresco.

I.2. El contenido de grasa intramuscular y el de ácidos grasos poliinsaturados puede sobrestimarse en biopsias extraídas en cerdos grasos.

I.3. La determinación del contenido y la composición de la grasa intramuscular a partir de muestras pequeñas está sujeta a un alto error de muestreo. Sin embargo, para el contenido de grasa intramuscular, especímenes pequeños del músculo objetivo son tan informativos como muestras grandes de otros músculos.

I.4. La varianza muestral, aunque alta, no lo es tanto como para eliminar la ventaja de un diseño longitudinal respecto a un diseño con sacrificios seriados. Así, el sesgo, más que la varianza muestral, es el factor limitante para desarrollar estudios longitudinales a partir de biopsias.

II. En relación con el segundo objetivo:

II.1. La grasa intramuscular y la grasa subcutánea presentan un patrón distinto de crecimiento cuantitativo y composicional. La grasa intramuscular es menos poliinsaturada que la grasa subcutánea y tiene una mayor proporción de ácido oleico.

II.2. El contenido en grasa intramuscular aumenta linealmente con la edad, a razón de 0.05% por día entre los 160 y los 220 días de edad, mientras que la velocidad de deposición de la grasa subcutánea declina con la edad.

II.3. El contenido en ácidos grasos poliinsaturados decrece con la edad y el de los ácidos grasos monoinsaturados aumenta, manteniéndose el contenido de

ácidos grasos saturados relativamente estable a lo largo del engorde, tanto en la grasa intramuscular como en la subcutánea.

II.4. El ácido oleico y el linoleico son los dos ácidos grasos que experimentan un mayor cambio durante el engorde. El contenido en ácido oleico aumenta con la edad en una proporción similar a la que disminuye el ácido linoleico.

II.5. Un retraso en la edad de sacrificio comporta un aumento del contenido de grasa intramuscular y de ácido oleico, aunque ello se consigue en detrimento de la velocidad de crecimiento magro.

II.6. La cantidad de grasa influye en la composición. Un contenido de grasa superior al esperado según la edad implica, en la grasa intramuscular, un mayor contenido de grasa monoinsaturada, en particular de ácido oleico, mientras que, en el caso de la grasa subcutánea, un mayor contendio de ácidos grasos saturados.

II.7. Se han encontrado evidencias de que los cerdos con un alto contenido de ácido palmítico, ácido linoleico y ácido linolénico al inicio del engorde, luego, durante el engorde, los depositan a menor ritmo.

III. En relación con el tercer objetivo:

III.1. Existe una relación positiva entre la longitud de la repetición CA en la región del promotor del gen IGF-I y el nivel circulante de IGF-I.

III.2. El nivel circulante de IGF-I a edades próximas a la pubertad se relaciona positivamente con el contenido magro y negativamente con el contenido de grasa intramuscular.

III.3. Se ha evidenciado que la asociación entre el polimorfismo C3469T en el exón 3 del gen de la leptina y el contenido de grasa intramuscular depende de la edad.

III.4. A edades tardías el polimorfismo C3469T se asocia más al contenido de grasa intramuscular que al de subcutánea. A las 30 semanas de edad, el genotipo TC presenta más grasa intramuscular que el TT, aunque no de subcutánea.

III.5. Los experimentos realizados, todos ellos basados en un diseño de tipo longitudinal, demuestran que los efectos de un genotipo no son constantes a lo

largo del período de crecimiento y que, en este sentido, tanto la edad como el estado de engrasamiento pueden modificarlos.

VIII. Referencias

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