Universitat de Lleida

Biomarkers associated to fat content and composition in pigs

Rebeca Muñoz Forcada

Universitat de Lleida

Departament de Producció Animal

Biomarkers associated to fat content and composition in pigs

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Dr. Joan Estany and Dr. Marc Tor

DOCTORAL THESIS

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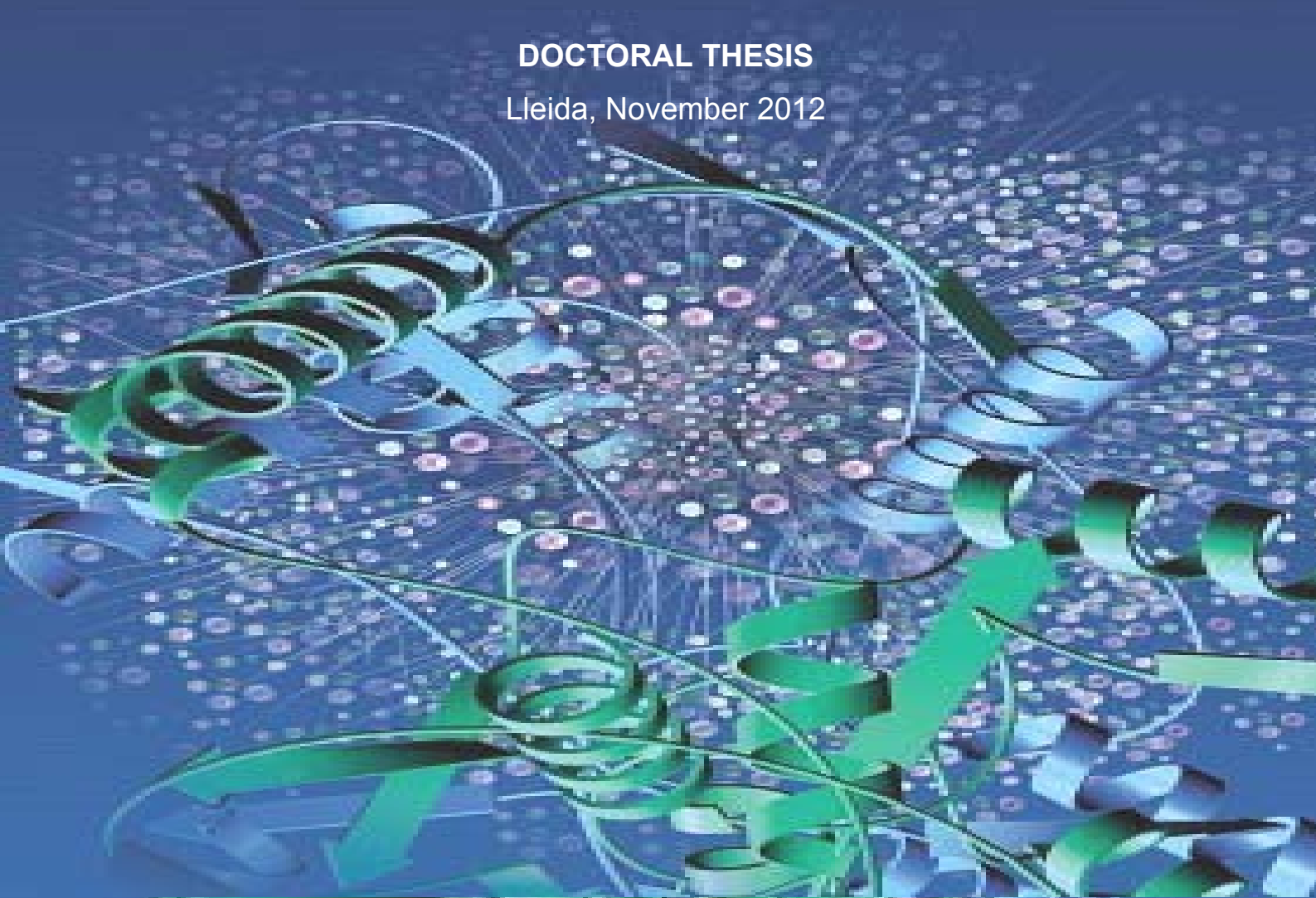

Universitat de Lleida
Departament de Producció Animal

Av. Alcalde Rovira Roure, 191
E-25198 LLEIDA (Catalunya)
Tel. +34 (9) 73 702557
FAX +34 (9) 73 702874

JOAN ESTANY ILLA, catedràtic de universitat del Departament de Producció Animal de la Universitat de Lleida, i MARC TOR NAUDI, professor titular del Departament de Producció Animal de la Universitat de Lleida

Informen:

Que la doctoranda Rebeca Muñoz Forcada ha realizat sota la seva direcció el treball de recerca '*Biomarkers associated to fat content and composition in pigs'*.

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 en Ciència i Tecnologia Agrària i Alimentària per la Universitat de Lleida.

Lleida, 4 de juliol de 2012

Dr. Joan Estany Illa

Dr. Marc Tor Naudi

PREFACE

This thesis is based on three studies, which will be referred to in the text as by roman numerals I, II, and III. The three studies have been carried out in the Department of Animal Production, University of Lleida, in the framework of the projects AGL2006-01243 and AGL2009-09779, both funded by the Spanish Ministry of Education and Innovation (MICINN). The third study was done as a collaborative project with the Department of Applied Sciences, University of the West of England, UK. During this time Rebeca Muñoz was the recipient of the MICINN fellowship BES-2007-16558.

The results in Chapter I and II have already led to the publications 'Fast determination of oleic acid in pork by flow injection analysis/mass spectrometry' by R. Muñoz, F. Vilaró, J. Eras, J. Estany, and M. Tor (*Rapid Communication in Mass Spectrometry, 2011, 25:1082-1088*), and 'Relationship between blood lipid indicators and fat content and composition in Duroc pigs', by R. Muñoz, M. Tor, and J. Estany (*Livestock Science, 2012, 148:95-102)*.

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SUMMARY

The present study is part of a line of research about the genetic improvement of pig meat quality. The goal of improvement is to obtain pigs with less subcutaneous backfat thickness, but maintaining an optimum of intramuscular fat (IMF) content, so as to produce meat of good quality at a minimum cost. The thesis comprises of three studies, with the aim at decreasing the cost of routinely IMF and acid oleic determinations, as well as investigating the biological mechanisms of fat deposition. In the first study, it was developed a high-throughput analytical method for oleic fatty acid quantification in pork using a flow injection analysis system based on electrospray ionization mass spectrometry (FIA/MS). The new method, which allows for a rapid quantification of oleic content in small pork samples, may be used as a suitable method for ranking pigs, carcasses, cuts or pork products by oleic content. The second study assesses the usefulness of serum lipid indicators as early biomarkers of IMF and subcutaneous fat content and composition. The serum concentration of lipid indicators, namely cholesterol, lipoproteins, tryglicerides, and insulin-like growth factor-I, display a very low correlation structure with fat content and composition. None of the serum lipid indicators investigated is recommended to be used as an early biomarker of fatness, even for the high-density lipoprotein, which was the one showing the most consistent relationship across age. The last study examined the effect of selection against backfat thickness at constant IMF on hepatic protein expression and fatty acid composition in liver, muscle, and subcutaneous fat. Moreover, the potential links between the hepatic enzyme expression and fatty acid composition in the three former tissues have been also investigated. Genetic selection for decreased backfat was accompanied by reduced expression of hepatic fatty acid synthase (FAS) and Δ6-desaturase (Δ6d). However, genetic selection had little influence on individual fatty acids, excepting the stearic/ palmitic ratio. There is evidence that hepatic FAS and Δ6d protein expression are positively related to subcutaneous saturated and polyunsaturated fatty acid content, respectively. It is concluded that hepatic protein expression pattern in pigs is affected by selection for decreased backfat thickness.

RESUMEN

El presente estudio es parte de una línea de investigación sobre la mejora genética de la calidad de carne en porcino. El objetivo de la mejora es obtener cerdos con menos espesor de grasa dorsal pero manteniendo unos niveles óptimos de grasa intramuscular (GIM) y así producir carne a bajo coste y de buena calidad. La tesis se compone de tres estudios que se realizaron con el fin de disminuir el coste analítico que supone la determinación rutinaria de la GIM y del ácido oleico, así cómo de investigar los mecanismos biológicos de la deposición de la grasa. En el primer estudio se desarrolló un método analítico de alto rendimiento para la cuantificación del ácido oleico en carne de cerdo usando un sistema de análisis por inyección de flujo basado en una espectrometría de masas con ionización por electrospray (FIA/MS). El nuevo método, el cual permite una cuantificación rápida del contenido en oleico en muestras pequeñas, podría ser adecuado para la clasificación de cerdos, canales, recortes ó productos de carne de cerdo por su contenido en oleico. El segundo estudio evalúa el uso de indicadores lipídicos en suero cómo biomarcadores de la composición y contenido de la GIM y la grasa subcutánea a edades tempranas. Las concentraciones de los indicadores lipídicos: colesterol, lipoproteínas, triglicéridos, y el factor de crecimiento insulínico tipo-I mostraron una correlación muy baja con el contenido y la composición grasa. Ninguno de los indicadores lipídicos investigados se recomiendan que se usen cómo un biomarcador precoz de la grasa, ni siquiera la lipoproteína de alta densidad que fue el biomarcador más consistente a lo largo de la edad. El último estudio examinó el efecto de la selección contra grasa dorsal a constante GIM sobre la expresión proteíca hepática, y la composición de ácidos grasos en hígado, músculo y grasa subcutánea. Además, los posibles vínculos entre la expresión de las enzimas hepáticas y la composición de ácidos grasos en los tres tejidos también se investigaron. La selección genética contra grasa dorsal fue asociada con una reducción en la expresión de la ácido graso sintasa (FAS) y la Δ6-desaturasa (Δ6d) hepáticas. Sin embargo, la selección genética influyó poco en los ácidos grasos individuales, exceptuando el ratio esteárico/palmítico. Hay evidencias de que la expresión proteíca de las enzimas hepáticas FAS y Δ6d está relacionada positivamente con el contenido de ácidos grasos saturados y poliinsaturados respectivamente en grasa subcutánea. Se concluye que el patrón de expresión proteíca hepática en cerdos es afectado por la selección contra grasa dorsal.

RESUM

El present estudi és part d'una línia d'investigació sobre la millora genètica de la qualitat de carn en porcí. L'objectiu de la millora és obtenir porcs amb menys espessor de greix dorsal, mantenint alhora uns nivells òptims de greix intramuscular (GIM) i així produir carn de bona qualitat a baix cost. La tesi es compon de tres estudis que es van realitzar amb la finalitat de disminuir el cost analític que suposa la determinació rutinària de la GIM i de l'àcid oleic, així com d'investigar els mecanismes biològics de la deposició del greix. En el primer estudi, es va desenvolupar un mètode analític d'alt rendiment per a la quantificació de l'àcid oleic en carn de porc, mitjançant un sistema d'anàlisi per injecció de flux basat en una espectrometria de masses amb ionització per electrosprai (FIA/MS). El nou mètode, que permet una quantificació ràpida del contingut en àcid oleic en mostres petites, podria ser adequat per a la classificació de porcs, canals, peces carniceres o productes de carn de porc, pel seu contingut en àcid oleic. El segon estudi, avalua l'ús d'indicadors lipídics en sèrum com biomarcadors precoços de la composició i contingut del GIM i del greix subcutani. Les concentracions dels indicadors lipídics: colesterol, lipoproteïnes, triglicèrids i el factor de creixement insulínic tipus-I, van mostrar una correlació molt baixa amb el contingut i la composició grassa. Cap dels indicadors lipídics investigats es recomanen com un biomarcador precoç del greix, ni tan sols la lipoproteïna d'alta densitat que va ser el biomarcador més consistent amb l'edat. En l'últim estudi, es va examinar l'efecte de la selecció contra greix dorsal a GIM constant sobre el patró d'expressió proteica hepàtica i la composició d'àcids grassos en fetge, múscul i greix subcutani. L'estudi també va estudiar el possible vincle entre l'expressió dels enzims hepàtics i la composició d'àcids grassos en els tres teixits. Es va trobar una associació de la selecció genètica contra greix dorsal amb una reducció en l'expressió de l'àcid-gras-sintasa (FAS) i Δ6-desaturasa (Δ6d) hepàtiques. No obstant això, la selecció genètica, va influir poc en els àcids grassos individuals, exceptuant el ràtio esteàric/palmític. Hi ha evidències que l'expressió de la FAS i la Δ6d es va relacionar positivament, en greix subcutani, amb el contingut en àcids grassos saturats i poliinsaturats respectivament. Es conclou, que el patró d'expressió proteica hepàtica en porcs està afectat per la selecció contra greix dorsal.

INTRODUCTION

INTRODUCTION

1. Pig production and pork quality

1.1. The porcine sector

In Spain, pork has a great contribution to overall meat production; in fact Spain is the second largest pig meat producer in Europe and the fourth in the world. In 2010 pig meat production reached 3.4 millions of t, concentrating in six regions: Catalonia (41%), Castile-Leon (13%), Castile-La Mancha (9%), Andalusia (8%), Aragon (8%) and Murcia (8%).

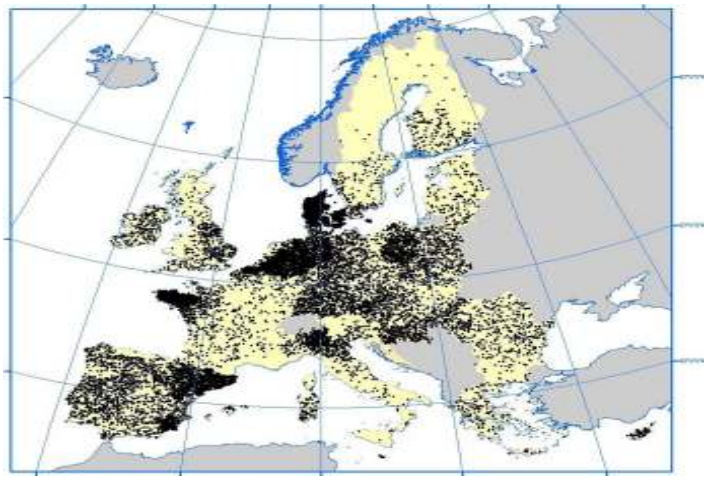

Figure 1: European sows populations by regions. *Source: Eurostat, European Comission*. *April 2011*.

Concerning pig meat in Europe, this continent represents a quarter of the total pork produced in the world and it is concentrated in the zone comprised from Denmark to Vlaams Gewest (Belgic), representing 30% of European sows. However, there are other important regions, such as Catalonia (Spain), Lombardy (Italy), Bretagne (France) and some areas of Central Poland and Northern Croatia. The whole of these countries account for more than 80% of the meat production and the pig population in Europe. In the intensive production systems, feed is about 60% of production cost of a pig farm and in spite of rise in cereals prices over the last years, the price of pig meat has increased

only slightly, causing a decrease of profitability in the porcine sector. Mercolleida is the main livestock market that fixes the reference prices for almost all of marketing pigs in Spain. The average price for the carcass for the year 2010 was 145.8 €/100 kg, whereas in Germany: the main producer country of Europe, was 144.5 €/100 kg (Eurostat. April 2011). The pork sector in Spain represented 34.2 % of the final livestock production and 12.4 % of the agrarian production in 2011. In addition, Spanish exportations of pig meat have increased during last years, reaching about 26% of national production. Fresh meat is exported in a high proportion to Europe and extra-communities countries, pointing out France, Russia and Hong Kong as the main destinies (DataComex. March 2011).

1.2. Structure of breeding programs in pigs

The structure of breeding programs is organized in a pyramidal form. At the top of the pyramid are the purebred or nucleus-breeding herds. Both purebred dam and sire lines are selected for particular characteristics. The dam lines are selected for an objective combining reproduction and production traits, mainly growth performance, feed efficiency and lean content, while the sire lines are only selected for production and meat quality traits, namely drip loss or intramuscular fat (IMF) content. The middle layer in the pyramid is made up of the multiplier herds, which produce the commercial crossbred sow using seedstock from two dam lines. In the bottom of the pyramide are the commercial units, where crossbred sows are mated to boars from sire lines to produce the fattening pigs (Sellier, 1976; Scheider et al., 1982). Large White and Landrace breeds have been mostly used for developing modern specialised dam lines due to their high prolificacy and good maternal behaviour. Common terminal sires are boars from Pietrain- or Duroc-based lines. Pietrain pigs are very lean but instead they show low levels of IMF. Contrarily, the Duroc-sired pigs have more fat tissue but also more IMF content. In Spain, particularly because of the great demand and popularity of dry-cured hams, whose production requires a minimum content of IMF, there are available Duroc lines selected for high IMF. Some of these lines are used as either purebred or crossbred with Iberian sows, with the objective of producing high-quality products at competitive production costs (Oliver et al., 1994).

1.3. Background of the problem

Pig selection over the last decades has improved animal production efficiency and carcass quality but only some aspects related to meat quality, basically those associated to defects concerning the pH (Van der Wal et al., 1988; Fujii et al., 1991; Lee and Choi, 1999). However, the increasing trend towards the production of leaner meat, which is negatively correlated to IMF (Solanes et al., 2009), has resulted in reduced IMF content and water holding capacity, both considered as traits that improve eating quality (Fernandez et al., 1999; Huff-Lonergan and Lonergan, 2005). As a result, selection for IMF limits the response perspectives for lean content. However, some studies reported that it should be possible to increase or maintain IMF without affecting lean growth (Warriss et al., 1990). The goal of the pig industry is not only to produce meat at the lowest cost but also to meet the consumer's preferences. During the last years, the consumer has paid more attention to meat quality, regarding both eating and nutritional aspects. Thus, nowadays the ongoing challenge for the pig industry is how to optimise efficiency (i.e. feed lean growth efficiency) with increased meat eating and nutritional quality.

On the one hand, consumers consider that one of the most important attributes of meat is to have a good sensory experience during eating. Conversely, the price, safety and healthiness of the meat are the most critical aspects to take into consideration at the retail point (Issanchou, 1996; Brewer and Prestat, 2002; Dransfield, et al., 2005). The economic value of the carcass depends on several factors: national and international market conditions, consumer preferences, processors and retailers requirements, and economic traits in pork production (García-Macías et al., 1996). However, the tendency of the pork industry worldwide has focused on producing lean carcasses of good conformation (Hermesch, 2004), since these traits are the most profitable for producers and meat industry. In Spain, the carcass traits that are positively evaluated by the pork industry are: weight, killing out percent, conformation, carcass lean (measured indirectly through backfat and loin thickness), and pH. The gender of the pig is also taken into account, preferring barrows and sows (Urkijo et al., 2008). Both IMF and subcutaneous fat are important traits in manufacturing dry-cured products, particularly the ham. The

quality aspects of these fats play a key role in the maturation process and sensorial properties of the product and currently also for nutritional properties (Arnau et al., 2001).

A strategy to obtain the optimum IMF content without affecting negatively the production traits is selective lipid deposition. This can be achieved by modifying some management practices, particularly feeding practices, but also by genetic selection. Reixach et al. (2009) showed that pigs selected against backfat thickness at constrained IMF content had less backfat thickness and a similar amount of IMF content. This strategy, however, needs IMF to be routinely determined, and therefore to set up a cost-effective procedure of determination. Biomarkers associated to either subcutaneous fat or IMF would make feasible recording IMF and fat composition *in vivo* and contribute to make effective selective lipid deposition. In this respect, a better understanding of the biological mechanisms underlying fat deposition and partition may help in identifying biomarkers associated to specific patterns of fat content and composition and in preventing undesirable side effects, since traditional genetic selection acts as a black box technique without knowledge about the underlying physiological processes (Rauw et al., 1998).

1.4. Concept of meat quality

The concept of meat quality involves sensory, nutritive, hygienic and technological factors, and the level of importance of each aspect depends on requirements and acceptability of producers, industries and consumers. The fat content and composition of both fresh and processed meat products are important factors affecting eating quality and human health. However, many aspects valued by the consumer are difficult to measure in the final meat product and the level of contribution of IMF in meat quality is a controversial issue. Although most of reports consider IMF content has a favorable influence on sensory quality attributes of pork, particularly juiciness (Hodgson et al., 1991; Fernandez et al., 1999) and tenderness (Fortin et al., 2005), there have been reports where there was little or no association between IMF content and sensory quality attributes of meat (Lan et al., 1993; Blanchard et al., 2000).

Fatty acid composition of IMF has a considerable impact on eating quality and human health. Fatter pigs and with higher level of IMF were associated to more saturated (SFA) and monounsaturated fatty acids (MUFA) and less polyunsaturated fatty acids (PUFA) (Cameron and Enser, 1991). Saturated and MUFA are generally positively associated with eating quality traits, whereas that PUFA have a negative impact on pork flavour and are more susceptible to oxidation (Cameron et al., 2000). Pork is rich in essential nutrients: high quality protein, vitamins, iron and zinc, but also contains a considerable amount of cholesterol and fat, comprised mostly by SFA and MUFA (Verbeke et al., 1999; Valsta et al., 2005). Both high total fat and SFA intake has been related to increase the risk of cardiovascular diseases, whereas MUFA and PUFA intake seems to have cardioprotective effects (Mattson and Grundy, 1985; Williams, 2000; Laaksonen et al., 2005). Fat composition concerning healthy diet is taking more into consideration than total fat content, and this fact is corresponding with the latter nutritional recommendations: a P/S ratio between 0.6 and 0.7 and a n-6/n-3 ratio less than 4 (Webb and O'Neill, 2008). Fatty acids are important in their own right because of their effects on human health. It is important to select the production options that maximize both quality and healthiness of pork (Kouba et al., 2003; Wood et al., 2004).

2. Chemical methods in food lipid analysis in the last century

2.1. The first extraction procedures for lipids

There is a wide range of methods for fat analysis in food. The procedures comprise several steps, from preparation of sample and extraction of lipids with solvents to separation, detection and quantification of them with different techniques. Therefore, the combination of different possibilities, results in a large number of standardized methods available for researchers, who have the opportunity to choose the most appropriate for their aims (Firestone and Mossoba, 1997). First methods that were used for determination total lipid content in food are dated at the 19th century. The method described by Soxhlet (Soxhlet, 1879) is the most commonly used example for the extraction of lipids from foods. It is based on the extraction of oil and fat from solid material by repeating washing with an organic solvent, usually hexane or petroleum

ether, under reflux in a special glassware. The main disadvantages of this procedure are both the solvent-consuming and poor extraction of polar lipids.

Nowadays, extraction procedures for lipids from biological tissues are based on methods that have been developed in the fifties. In 1957, Folch described his classic and exhaustive lipid extraction procedure from animal tissues, particularly in meat, liver and brain. This procedure is one of the most used by lipidologists over the world. The method is based on the extraction of fat using a 2:1 chloroform-methanol mixture, and washing of the extract by addition of a saline solution. The polar mixture of solvents separates the extract into two phases, the lower consisting of chloroform-methanol-water, while the upper phase contains the non-lipid contaminants (Folch et al., 1957). Two years later, Bligh and Dyer (1959) developed a simple and rapid method for the extraction of lipids from frozen fish, using a minimum volume of solvent. However, this procedure underestimates the lipid content in samples containing more of than 2% lipid (Iverson et al., 2001). Several authors have examined other solvents to extract lipids from food samples as an alternative to chloroform-based methods. Gunnlaugsdottir and Ackman (1993) used the mix of reagents hexane/isopropanol and Dambergs (1956) acetone/water.

2.2. New and quicker extraction techniques

New techniques of extraction have been developed due to the need for faster lipid analyses in food. Among them, the most remarkable are the microwaves, supercritical fluids and the *in situ* synthesis of fatty acid methyl esters (FAME). Microwave technique has been used for extracting lipids from food samples in several studies (Ganzler et al., 1986; Paré et al., 1997; AOAC, 2006). Researchers developed an effective microwave extraction method, which consists on the irradiation of a mixture of sample and solvent several times. Particularly, partial extraction without solvents has been useful in studying fatty acid composition in adipose tissue in pigs (De Pedro et al., 1997; García-Olmo et al., 2002). The partial extraction has shown to be a rapid method that reduces solvent consumption volume. However, it is appropriate only for samples with high lipid content and a validation must be performed because fatty acid analyses can vary

according to the extraction method (Carpenter et al., 1993). Supercritical fluid extraction (SFE) is another fast alternative to conventional extraction methods. This method allows using less amount of solvent for extraction of fatty acids, which are exposed to the low temperature of the supercritical carbon dioxide fluid (Randolph, 1990). In addition, the selectivity of the supercritical fluid in dissolving lipids can be adjusted by the pressure and temperature extraction (Eller and King, 1996). No differences in fatty acids extracted in food products have been found when compared the SFE method with a reference extraction procedure (King et al., 1996; Berg et al., 1997). Particularly, Berg determined the lipid classes in meats and King the fatty acid composition in beef meat after lipid SFE.

2.3. Techniques for separation and isolation of lipid classes

The separation and isolation of the main lipid classes in biological extracts for subsequent use or analysis have been accomplished by preparative thin layer chromatography (TLC) and by solid-phase extraction (SPE). Thin layer chromatography technique consists of migration of samples along thin layer of adsorbent material under the influence of a solvent. The separation of lipids is achieved because compounds with higher affinity to stationary phase travel slowly while the others travel faster. Moreover, several studies showed that the separation of triglycerides (TG) from phospholipids could carry out by using combinations of two solvents (Macala et al., 1983; Kovács et al., 1986). The SPE is an alternative to TLC, which exploits different interactions between the chemical structure of analytes and chemical moieties at the surface of the solid phase, and so to achieve that the lipid fractions elute from the columns separately (Majewska et al., 2008). Kaluzny et al. (1985), was the first in developing a rapid method based on SPE technique, which got process up to ten lipid mixtures in one hour. Later, other authors have developed more methods according to the class of lipid to analyse (Agren et al., 1992; Christie, 1992). Nowadays, TLC is widely used in food analyses and the modern high-performance TLC (HPTLC) is an efficient method to quantify and detect analytes in food products. Some particular applications of TLC for the determination of lipids and fatty acids have been carried out (Sherma, 2000). Solid-phase extraction has been also used in food analysis, for instance to isolate and

determine cholesterol in animal fats and fractionate cis/trans FAME (Panagiotopoulou and Tsimidou, 2002). This technique involves creating a selective isolation of the compounds by serially altering the solid phase support, the solvent, or both. In a biphasic solid support /solvent system, a unique interaction exists between the compound to be isolated and the functional group of the solid phase. Compounds that are very diverse in chemical structure may differ greatly in their interactions with the moieties on the solid phase, whereas compounds that are similar in chemical nature may show only subtle but exploitable differences. Thus, by varying the solvent environment (pH, polarity, etc.) around the solid phase, or by changing the solid phase itself, compounds (in this case lipids) can be selective isolated with a high degree of purity and recovery.

2.4. Modern methods for lipid analyses

Nowadays, the use of gas chromatography (GC) and high-performance liquid chromatography (HPLC) to characterize fatty acid profiles of lipids in food samples after conversion to methyl ester is a routine practice of the laboratories (García-Olmo et al., 2002; Aldai et al., 2006). Fatty acid components need to be converted into FAME to improve their volatility, the chromatographic peak shapes and the accuracy of the method (Liu, 1994). Formation and subsequent extraction of FAME is normally accomplished in the presence of a catalyst dissolved in a reagent and a non-polar solvent. The majority of catalysts for theses reactions can be characterized as acidic (HCl, H_2SO_4 and BF_3) or alkaline ($NaOCH_3$, KOH and NaOH). Creation of the methyl esters can be realized in one extraction/methylation step, which is called direct methylation, or in multi steps. Direct methods have shown to be faster, less solvent-consuming and more efficient in fat extraction when compared to classical two step methods (Carrapiso and García, 2000; Carrapiso et al., 2000). Authors have developed different *in situ* derivatization strategies to approach better lipid determinations. Morrison and Smith (1964) were beginners in the use of boron fluoride-methanol for the preparation of fatty acid methyl esters; later Rule (1997) showed that methanolic boron trifluoride was also an effective methanolysis reagent in freeze-dried adipose tissue, muscle and liver samples. Other studies (Eras et al., 2004) transformed fatty acids in pentyl esters using chlorotrimethylsilane as a reagent in meat and vegetable samples and

isopropyl with boron fluoride-butanol in dairy samples (Iverson and Sheppard, 1986; Iverson and Sheppard, 1989).

Improvement in GC instrumentation has allowed determining fatty acid composition in a few minutes. Fast analyses are possible to perform by optimising column parameters and working conditions. Fast temperature programming rate, shorter and smaller diameter columns coated with thin films of high diffusivity stationary phase and carrier gas, play major roles in shortening gas chromatographic analysis (Korytár et al., 2002). Thus, fast GC provides more cost-effective determinations of compounds by reducing analysis time. Main applications of fast GC are analysis whose results are needed in a short time, e.g. product control, on-site environmental and industrial hygiene applications, and also in the case of the large sample series (Matisová and Dömötörová, 2003). Practical applications of fast GC have been developed in food analysis (Donato et al., 2007) and a wide range of compounds have been determined, e.g. pesticides, (Dömötörová and Matisová, 2008), oils in vegetables (David et al., 1999) and fats in animals. Two studies realized fast GC analysis in pork, particularly Mondello et al. (2004) quantified FAME in meat and Ficarra et al. (2010) in subcutaneous fat, where separation of fatty acids was carried in 3.6 min. In spite of the great advantages of high speed GC, several instrumental limitations have been found due to the minimum detectable amount of analytes decreases with the reduction of column internal diameter. This problem can be solved if larger sample volumes are injected using non-splitting injection techniques (Matisová et al., 2002). These limitations have been going over by suiting adequate instrumentation, until researches have achieved quick and reliable results with this technique. In the case of detectors, rapid flame ionization detectors have been widely used in fast GC applications. Moreover, mass spectrometer detectors have been also employed for fast GC applications because they provide very fast spectral acquisition rates. Mass analyzers combined to high-speed GC result in very quick analysis, but this methodology is characterized by the high cost. For this reason, if there is no need of high sample throughputs, other less expensive scanning systems (e.g. simple quadrupole) may be used for quantitative determinations (Mastovská and Lehotay, 2003).

Nowadays, the application of ionization techniques such as electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI), (Cai and Syage, 2006) have improved lipid analysis by HPLC/mass spectrometry (MS). Furthermore, the analysis can carry out without employing derivatization by methylation (Yang et al., 2007). Determination of lipid content for a wide range of foods, such as chocolate (Perret et al., 2004), vegetable oils and animal fats (Kerwin and Wiens, 1996; Kurata et al., 2005) have been carried out using liquid chromatography /MS technique.

Flow injection analysis (FIA) is a method that can be accopled to a large variety of detectors (mass spectrometer, sensors, spectrophotometer, pHmeter) without need to use a column. This method is based on samples are injected directly into a continuously moving stream of a carrier or reagent before reaching a detector (Ruzicka and Hansen, 1975). Flow injection analysis method is used in routinely automated analyses, which are suitable for a rapid quantification of analytes and processing large number of samples. This economic method only requires a few microliters of sample and has several applications such as, determination of nitrites in meat products, farmaceutical and agricultural analyses (Hlabangana et al., 2006; Ruiz-Capillas et al., 2007).

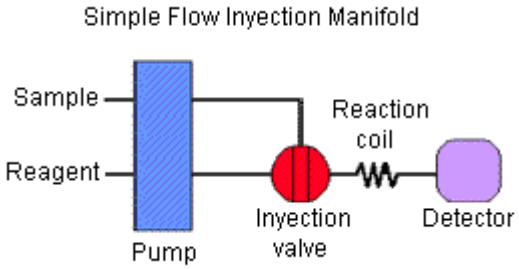

Figure 2: Basic components and principles of FIA

Finally, indirect methods, such as near infrared spectroscopy (NIRS) has became an attractive option to chemical methods for determination of the fatty acid composition in meat and subcutaneous fat (Hervás et al., 1994; Fernández-Cabanás et al., 2011). Near infrared spectroscopy consists of predicting the content of fatty acids, using equipments that have been previously calibrated with samples analysed by means of a reference method. Not only these methods carry out very rapid measurements on a great number

of samples but also allow determining fatty acid composition in meat with no treatment of the sample (González-Martín et al., 2005). However, this methodology is very sensitive to changes related to the instrument, the environment, preparation and matrix of sample, since these also affect the calibration of equipments (Fernández-Cabanás et al., 2007).

3. Lipid metabolism

3.1. Pig lipid metabolism

The main site of *de novo* fatty acid synthesis differs among species. For example in dogs, cats, pigs and ruminants fatty acid synthesis is centered in the adipose tissues, whereas in humans and rodents the liver is the primary site for *de novo* synthesis. These differences affect to overall pattern of lipid metabolism and role of regulatory factors. Furthermore, the principal carbon source used for fatty acid synthesis varies among different species (Bergen and Mersmann, 2005). In the case of pigs, glucose is the preferred substrate for *de novo* synthesis in adipose tissue, and acetate in the liver. This is due to complete absence of hepatic citrate cleavage enzyme, which is essential for producing acetyl-CoA from glucose (O´Hea and Leveille, 1969). Moreover, *de novo* lipid and lipoprotein synthesis are functionally and anatomically separated in pigs, since excess of glucose is used for *the novo* fatty acid synthesis, and subsequently TG storage in adipose tissue depots, whereas very low-density lipoproteins (VLDL) are synthesized in the liver. Thus, VLDL formation will depend on fatty acids available from adipose TG lipolysis (Dodson et al., 2010). Furhermore, intrahepatic fatty acid metabolism of pig has also specific characteristics, such as, a low rate of hepatic fatty acid oxidation, and a greater tendency towards esterificate acyls-CoA rather than oxidate them (Pégorier et al., 1983).

Fat deposition is the result of the formation and the degradation of TG (Bernlohr et al., 2002). Hormones, diet, genetic background and specific-tissue physiology influence on both anabolic and catabolic processes. Energy consumed in excess of that needed is stored as TG through the activity of acyl coenzyme A: diacylglycerol acyltransferase (DGAT) and glycerol-3-phosphate acyltransferase (GPAT) enzymes (Farese et al., 2000;

Gonzalez-Baró et al., 2007). In case of demand of energy, hormone-sensitive lipase (HSL) catalyses hydrolysis of intracellular TG to release fatty acids, which are oxidized to obtain energy (Saleh et al., 1999). As regard to breed, levels of synthesis and of catabolic genes are lower in leaner breeds than in Duroc pigs, which have tendency to accumulate more fat content (Reiter et al., 2007).

3.2. The development of fat depots

In pigs, the main fat depots are subcutaneous fats, representing 65% of total fat tissues, followed by the intermuscular fat, which associated with connective tissues represents 30% of the separable adipose tissues and lastly, the internal fats of organs with a 5% of total fat (Mourot and Hermier, 2001). Intramuscular fat is the amount of fat within muscles, whereas intermuscular fat is referred to the fat located between different muscles. Chemically, IMF is formed by phospholipids, TG, mono- and di-acylglycerols, cholesterol and free fatty acids (Gao and Zhao, 2009).

Development of each fat depot is mainly determined by the age of animal. During growth of pigs, subcutaneous adipose is the first tissue capable of being expanded, followed by intermuscular fat and the latest one is IMF. Increased carcass adipose tissue is mainly due to proliferation of adipocytes up to two months of age, later there is a combination of adipocyte hyperplasia and hypertrophy up to five months, coinciding with the maximum lipogenic enzymatic activity. Finally, after five months of age, adipose mass enlarges by increasing cell size (Anderson and Kauffman, 1973). It is known changes in adipocyte size during fat tissue development are accompanied by specific lipogenic gene expression and enzyme activities (Hood and Allen, 1973; Smith et al., 1999; Smith et al., 2006).

3.3. Biology of adipocyte

Previous studies showed that there are nutritional (Doran et al., 2006) and genetic (Gondret et al., 2001) factors determining the site of fatty acid synthesis, providing evidence of differential metabolic properties of intramuscular and non-muscular adipocytes (Gardan et al., 2006; Gondret et al., 2008). It has been shown that

subcutaneous adipose tissue has greater lipogenic capacity than IMF (Hood and Allen, 1977), and the response to diet manipulation shows differences between fat depots (Duran-Montgé et al., 2008).

Main function of adipocytes is to store TG and then in periods of food deprivation to oxidate them to obtain energy (Azain, 2004; Nafikov and Beitz, 2007). Adipogenesis comprises several physiological processes: proliferation and differentiation of the stem cells into preadipocytes and differentiation and expansion of immature cells into mature adipocytes. Firstly, preadipocyte differentiation requires activation of several transcription factors, including peroxisome proliferator-activated receptor-γ (PPAR-γ), CCAAT-enhancer binding protein-α (C/EBP-α) and the adipocyte determination and differentiation-dependent factor-1/sterol regulatory element-binding protein-1 (ADD-1/SREBP-1) (Gregoire et al., 1998; Kim et al., 1998a; Niemelä et al., 2008). These factors are required in the sequential activation of adipocyte-specific gene expression that determines the specific phenotype of adipocytes in each stage of differentiation. Several factors that also participate in adipocyte differentiation, such as growth factors and hormones have been identified. Among these are the insulin and the insulin like-growth factor-I (IGF-I), which play a combined role in stimulating fat cell formation (Ramsay et al., 1989; Suryawan et al., 1997; Ntambi and Kim, 2000). During the terminal phase of differentiation, adipocytes in culture markedly increase *de novo* lipogenesis, become sensitive to insulin, and increase the activity of enzymes responsible for TG synthesis including adenosine triphosphate (ATP), citrate lyase, malic enzyme, glycerol-3-phosphate dehydrogenase, fatty acid synthase (FAS) and stearoyl-CoA desaturase (SCD). Finally, the mature fat cells enlarge in size and store energy in the form of TG. Preadipocyte proliferation, differentiation and their interrelations are also controlled by other factors including paracrine factors, adipose tissue extracellular matrix and neural factors. Several paracrine factors secreted by enlarged fat cells can act in a paracrine manner by stimulating adipocyte proliferation locally. Among the factors implicated in regulating adipogenesis locally are IGF-I, transforming growth factor-β (TGF-β), tumor necrosis factor-α (TNF-α), basic fibroblast growth factor (bFGF), macrophage colony-stimulating factor (MCSF) and angiotensin II. In addition to these factors, some hormones are also implicated in adipocyte

differentiation, such as, thyroid hormones, and prostaglandines (Hausman et al., 2001; Niemelä et al., 2008). The influence of all these factors and hormones on adipose tissue expansion are modulated by neural inputs and circulating factors.

4. Lipogenic enzymes

4.1. Biosynthesis of fatty acids

Dietary fat and carbohydrate are digested, and the fatty acids and glucose are transported to liver, adipose tissue and muscle where will serve as substrates for fatty acid synthesis. Lipogenesis consists on the synthesis of palmitic fatty acid (C16:0) and the same that dietary fatty acids may be then converted into other fatty acids by elongation or desaturation. Thereby, both endogenous and dietary fat contribute to form the body fat in pig. All processes implicated in lipid metabolism are regulated by dietary and hormonal factors. One of the main regulatory factors is the insulin, which plays a central role in lipid synthesis by enhancing the ability of cells to take up glucose and up-regulating promoters of *de novo* synthesis enzymes, such as acetyl-CoA carboxylase (ACC) and FAS (Kim et al., 1998b; Tong, 2005; Postic and Girard, 2008). In lipogenic tissues, fatty acids are converted to acyl-CoA, and glucose is transformed to pyruvate in the glycolysis pathway. Pyruvate enters the Krebs cycle in the mitochondria and ends as citrate, converting to acetyl-CoA in the cytosol. Acyl-CoA derived from fatty acids, is also converted to acetyl-CoA through ß-oxidation in the mitochondria. Citosolic acetyl-CoA is utilized through FAS reactions to generate C16:0, which is utilized for the synthesis of TG and VLDL, and for obtaining energy by oxidizing through the citric acid cycle (Wakil and Abu-Elheiga, 2009).

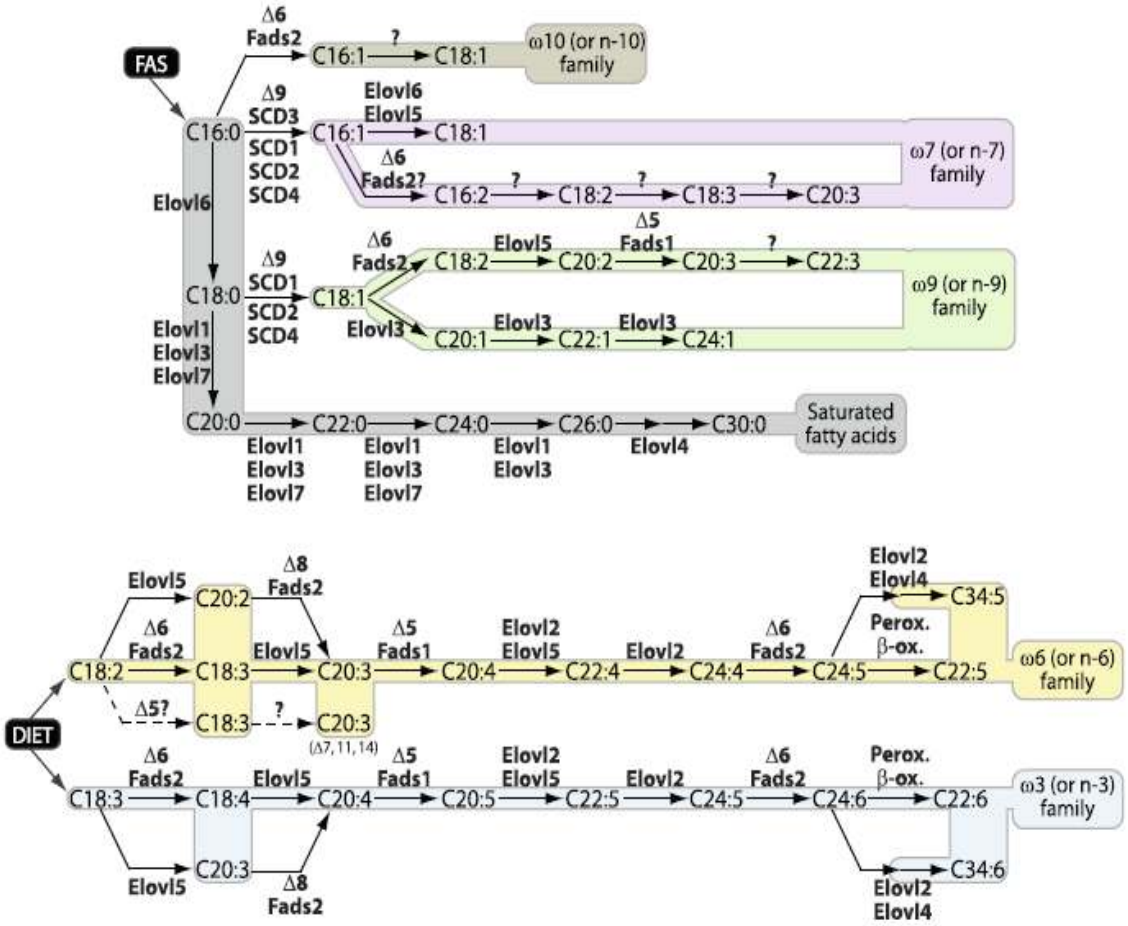

Figure 3: Biosynthesis of long-chain and very long-chain fatty acids in mammals. *Source: Guillou et al., 2010.*

4.2. Fatty acid synthase

Fatty acid synthase is a key lipogenic enzyme, which express in the major sites of fatty acid production in the body. This cytosolic and multifunctional enzyme is responsible for the synthesis of SFA, which is initiated by the elongation of the priming acetyl-CoA with two–carbon units donated from malonyl-CoA. This reaction is repeated in a cyclic manner producing even SFA containing up to 16 carbons (Wakil, 1989; Smith et al., 2003). The hepatic lipogenic enzyme FAS is negatively regulated by dietary PUFA by means of suppressing sterol regulatory element-binding protein-1 (SREBP-1) gene expression. Consequently, SREBP-1 nuclear content in hepatocytes is reduced and thereby FAS gene transcription is down-regulated (Jump and Clarke, 1999).

4.3. Stearoyl-CoA desaturase

Stearoyl-CoA desaturase is a membrane-bound enzyme localized in the endoplasmic reticulum, which is responsible for converting SFA into MUFA. Stearoyl-CoA desaturase catalyzes the introduction of the first cis-double bond in the delta9 position in the substrates palmitoyl-CoA, stearoyl-CoA and vaccenoyl-CoA for biosynthesis of oleic (C18:1), palmitoleic (C16:1), and conjugated linoleic fatty acids (CLA), respectively. Both C16:1 and C18:1 are the main fatty acids, which are incorporated into TG, cholesteryl esters, and membrane phospholipids (Ntambi and Miyazaki, 2004). Four isoforms have been well characterized in mice (Nakamura and Nara, 2004), and two isoforms in beefs, sheeps, pigs and humans (Zhang et al., 1999; Ren et al., 2004; Lengi and Corl, 2007). It has been shown SCD isoforms exhibit tissue-specific expression with SCD-1 expressing at high levels in the adipose tissue and liver in response to feeding a high-carbohydrate diet, whereas SCD-2, SCD-3, and SCD-4 have been found predominantly in brain, skin, and heart, respectively (Zheng et al., 2001; Heinemann and Ozols, 2003; Miyazaki et al., 2003). However, the novel SCD-5 isoform is expressed in brain of pigs, sheeps, chickens and humans (Wang et al., 2005b; Lengi and Corl, 2008). Stearoyl-CoA desaturase plays a key role in cholesterol and hepatic synthesis of TG and VLDL because is the rate-limiting enzyme in the cellular synthesis of MUFA (Miyazaki et al., 2000). Furthermore, synthesized MUFA by SCD contribute to the membrane fluidity, and it has been shown that modifications in the ratio of stearic acid to oleic acid have been related to various diseases including cardiovascular disease, obesity, insulin resistance and cancer (Roongta et al., 2011; Xueqing et al., 2011). Furthermore, endogenously synthesized MUFA, such as C16:1 and erucid fatty acid (C22:1) play a key role in whole-body lipid homeostasis by actuating like lipokines (Cao et al., 2008), or like crucial regulators of lipogenesis (Zadrevec et al., 2010). Stearoyl-CoA desaturase gene expression is regulated by dietary lipids (PUFA and cholesterol) and hormones (insulin) by means of transcription factors such as liver X receptor (LXR) and SREBP (Ntambi, 1999; Miyazaki and Ntambi, 2003). Dietary and genetic factors affect SCD expression in a tissue-specific fashion. Thus Doran et al. (2006) established activation of expression of the muscle but not subcutaneous fat SCD in response to reduced protein diet in pigs and Dance et al. (2009) showed breed-specific variations in

SCD expression and their catalysed products, namely CLA and MUFA in adipose tissue but not in *Semimembranosus* muscle.

4.4. Δ6-desaturase

Δ6-desaturase (Δ6d) catalyzes desaturation of both essential fatty acids, linoleic (C18:2) and α-linolenic (C18:3) for the production of long chain PUFA (Nakamura and Nara, 2004) such as arachidonic (C20:4) and docosahexanoic (C22:6) fatty acids, which are required for several physiological functions. The arachidonic fatty acid is stored forming membrane phospholipids, and consequently it is used for biosynthesis of eicosanoids involved in the mediation of cellular reactions (Jump and Clarke, 1999), and C22:6 is abundant in membranes of retinal and brain cells, which improves permeability of membranes and allows a better communication between cells (Lauritzen et al., 2001). Liver is an important site for biosynthesis of highly unsaturated fatty acids (Scott and Bazan, 1989) and for this reason, expression of hepatic Δ6d is high when compared to other lipogenic locations.

4.5. Very long-chain fatty acids enzymes

Fatty acids synthesized by desaturases or derived from the diet, can be further elongated and desaturated into very long-chain fatty acids (VLCFA) by distinct membrane-bound enzymes residing in the endoplasmic reticulum. Seven elongases (ELOVL) proteins have been identified and their regulation appears to be controlled by tissue-specific factors and at the transcriptional level. ELOVL1, ELOVL3, ELOVL6 and ELOVL7 catalyse the biosynthesis of very long-chain saturated fatty acids (VLC-SFA), and very long-chain monounsaturated fatty acids (VLC-MUFA), whereas ELOVL2, ELOVL4 and ELOVL5 the biosynthesis of very long-chain polyunsaturated fatty acids (VLC-PUFA) (Jakobsson et al., 2006; Guillou et al., 2010). Each of these enzymes realizes the elongation of its preferred substrates, with ELOVL3 controlling the synthesis of VLC-SFA and VLC-MUFA up to 24-carbon atoms, and ELOVL6 up to 18-carbon atoms. On the other hand, ELOVL1 catalyzes the formation of SFA containing as many as 26 carbons. ELOVL4 and ELOVL5 have been proposed to be involved in the elongation of PUFA previously synthesized by Δ6d and ELOVL2 in the elongation of

PUFA synthesized by delta 5-desaturase (Δ5d). Products catalysed by ELOVL3, and ELOVL6 are part a more complex system controlling cellular and whole lipid homeostasis (Matsuzaka et al., 2007; Zadrevec et al., 2010), whereas ELOVL1, ELOVL4 and ELOVL7 synthesize products, which are important components for optimal barrier function.

4.6. Regulation of lipogenic enzymes

A combination of multiple transcription factors such as SREBP-1c, peroxisome proliferator-activated receptor-α (PPAR-α), carbohydrate response element-binding protein (ChREBP), and LXR are involved in the regulation and control of desaturase gene expression. Dietary PUFA supress expression of Δ5d, Δ6d, and SCD in liver by means of reducing the active form of SREBP-1c, which is able to activate entire genes of fatty acid synthesis (Jump, 2004; Jump et al., 2005). Although it have been reported that there are similar pathways in the regulation of desaturases in mammalian (Nakamura and Nara, 2004), lipogenic enzymes contribute differently to IMF formation content in species, for example in cattle, a positive relationship was established between FAS, ACC, Δ6d and Δ5d protein expression and IMF content (Missotten et al., 2009), whereas in pigs, SCD protein was positively related to IMF (Doran et al., 2006; Cánovas et al., 2009a).

5. The physiology of lipoproteins metabolism

5.1. Structure and role of lipoproteins

Plasma lipoproteins were discovered at first time in 1929 by Michel Macheboeuf, who was able to isolate high-density lipoproteins (HDL) from horse serum. In next decades, other authors continued to further research into these particles (Brown and Goldstein, 1984; Fielding and Fielding, 1995). The major function of plasma lipoproteins is the transport of lipids from sites of absorption or production through the circulation to sites of utilization (Olson, 1998). The contribution of plasma lipoproteins in fat accretion is still little known in meat animals. Although it is generally thought that cholesterol and TG containing lipoproteins influence on rates of fatty acid synthesis in

tissues, the underlying mechanisms that regulate the role of lipoprotein metabolism in the adipose growth have still not elucidated in meat animals (Kris-Etherton and Etherton, 1982). Subsequent studies (Gallardo et al., 2008; Cánovas et al., 2010) investigated genetic aspects about serum lipid metabolism in pig and its association to fat deposition. These studies showed that some quantitative trait loci and metabolic pathways related to serum lipids and lipoproteins.

Lipoproteins, which circulate in plasma and lymph, are micellar particles with an inner core containing TG and cholesteryl esters, and an amphipathic outer surface containing phospholipids, unesterified cholesterol, and proteins. Lipoprotein particles are in a dynamic state of equilibrium due to transfer of components between lipoproteins are occurring continuously. Five classes of lipoproteins have been characterized: chylomicrons (CM), VLDL, low-density lipoproteins (LDL), HDL and intermediate density lipoproteins (IDL). These lipoproteins show differences in size, chemical composition and density, which allow separating them by density ultracentrifugation and electrophoretic mobility. In general rich-TG containing particles, such as VLDL and CM have lower density and are bigger than HDL, IDL and LDL, which contain more protein and less lipids. The proteins of the outer surface are called apoliproteins and are specific components of lipoproteins. There are at least eight apolipoproteins that differ in structure, function and distribution among lipoproteins, which are specialized lipid-binding proteins and play a key role in fat transporting. Apoproteins-A are primarily found in HDL, and apo-B in LDL. The apo-C is in all lipoproteins except LDL, and the E apopeptide is associated with VLDL, IDL and HDL (Mahley et al., 1984). The majority of lipoprotein apopeptides are synthesized in the liver or the intestine, but the apolipoprotein E, is synthesized in all cells, except the gut (Wu and Windmueller, 1979; Driscoll and Getz, 1984).

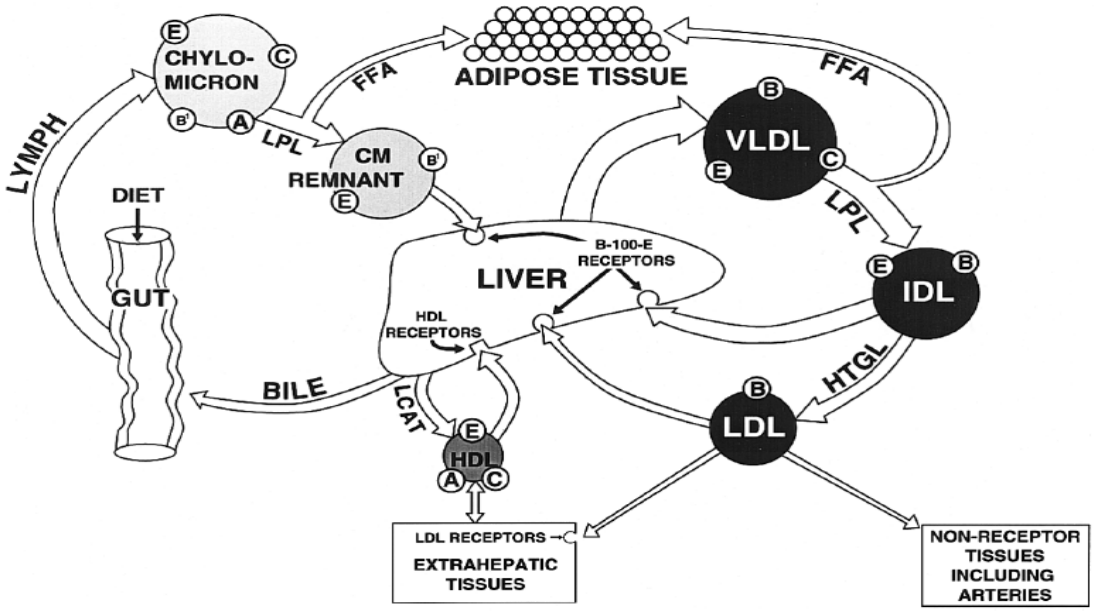

Figure 4: Metabolism of lipoproteins. Three plasma enzymes involved in lipoprotein metabolism include LPL (lipoprotein lipase), LCAT (lecithin-cholesterol acyltransferase) and HTGL (hepatic triglyceride lipase). The apopeptides contained in each lipoprotein are shown. *Source: Olson, 1998.*

The lipoproteins mainly implicated in TG transport are CM and VLDL, whereas LDL and HDL are responsible for the cholesterol transport. Chylomicrons are involved in transporting TG derived from diet after ingestion of food, whereas synthesized TG in liver are packaged into VLDL (endogenous synthesis) to be distributed to peripheral tissues such as adipose tissue, heart and skeletal muscle. Lipoprotein lipase (LPL) attached to the surface of capillary endothelium hydrolyzes TG packed into VLDL and CM to release fatty acids into the circulation, which are consequently taken up locally by fat cells and reesterified into TG. On the other hand, hormone-sensitive lipase (HSL) catalyzes the release of fatty acids from adipose tissues to so, consequently these fatty acids enter the plasma and supply lipid fuel for all tissues. Chylomicron remnants are recognized by the apoprotein E receptor on hepatocytes and metabolized in liver whereas VLDL remnants are transformed to IDL. Some of these IDL will be transformed to LDL by plasma hepatic triglyceride lipase (HTGL) and other will be catabolized in the liver (Kris-Etherton and Etherton, 1982; Daniels et al., 2009).

Low-density lipoproteins play a central role in distributing cholesterol to cells that require it. Cholesterol-carrying LDL bind to a specific high affinity receptor on the

plasma membrane of target cells and then cholesterol is uptaken by cellular endocytosis. Once LDL deliver cholesterol to peripheral cells, LDL will return to the liver via receptor-mediated uptake, and further will be degradated and their cholesterol released for be used in lipid metabolism (Brown and Goldstein, 1984).

High-density lipoproteins are synthesized by the liver and intestine and they are responsible for removing of cholesterol from extrahepatic cells. It is carried out through following pathway: once HDL bind to the plasma membrane cellular, the lecithin-cholesterol acyltransferase (LCAT), an enzyme associated with HDL, catalyzes the esterification of cholesterol provided by cells to cholesteryl ester, and consequently this is incorporated into the core of HDL. Finally, rich-cholesterol HDL deliver the cholesterol back to the liver, where they will be catabolized (reverse cholesterol transport) (Fielding and Fielding, 1995).

5.2. Characteristics of swine lipoproteins

The differences in the site of fatty acid synthesis among species influence on pattern of lipid metabolism including, activity of lipogenic enzymes, metabolism of lipoproteins, and fat deposition (Coppo et al., 2003; Bergen and Mersmann, 2005). Several studies have characterized swine lipoproteins and determined their plasmatic concentration. Etherton and Kris-Etherton, 1980 realized a study that reported that swine VLDL transported approximately 80-90% of the TG found in plasma. Furthermore, in pigs there is a low synthesis of LDL from catabolism of VLDL (Birchbauer et al., 1992) and HDL-cholesterol esters are transferred unidirectionally to only LDL-cholesterol esters, despite the absence of plasma cholesteryl ester transfer activity (Terpstra et al., 1993). Furthermore, it has been shown that levels of lipoproteins in plasma follow an age-related pattern, suggesting age also affects concentration of lipoproteins in addition to intrinsic characteristics of species (Mersmann et al., 1982).

Several authors have found positive relationships between levels of lipoproteins (HDL and VLDL) and cholesterol in plasma with economic traits in pigs, such as fatness, and weight (Taylor et al., 1992; Rauw et al., 2007; Gallardo et al., 2008). It is interesting to note all these studies coincide with the fact that cholesterol traits were

related to body weight and backfat thickness. Particularly, in Gallardo et al. (2008) and Rauw et al. (2007), body weight and backfat thickness were moderately related to total cholesterol (TC), HDL and LDL at 6 months of age, and in the case of Taylor et al. (1992), TC and LDL were correlated with fat deposition at 30 and 90 kg. Moreover, moderate estimates for heritability of serum lipid traits confirm that these traits could be altered by selection (Pond et al., 1986; Pond et al., 1997; Casellas et al., 2010). In fact, genetically high and low plasma cholesterol pigs were developed by Young et al. (1993) and Pond et al. (1997) after three and seven generations of selection, respectively. These latter authors found that pigs selected for low cholesterol had lower TC, HDL, TG and body weight than those selected for high cholesterol.

6. Biology of IGF-I and its relation to performance traits

6.1. Components and biological action of somatotropic axis

Somatotropic axis consisting of growth hormone (GH), insulin-like growth factors (IGF-I and IGF-II), their specific receptors and binding proteins, play an important role in the regulation of body mass and adipose tissue development. However, some hormones and dietary factors are also involved in controlling lipid metabolism via modulation of GH and IGF-I synthesis (Renaville et al., 2002). Growth hormone is a polypeptide synthesized mainly by pituitary gland in a pursatile manner and exerts its actions through binding to specific receptors on the cell surface. These receptors are highly expressed in liver but also in other tissues. Growth hormone may act directly on tissues or by *via* the insulin-like growth factors (IGF-I and IGF-II), which are released from tissues in response to GH (Louveau and Gondret, 2004). Although the function and production of IGF-I is dependent on GH, it is not known if IGF-I can act independently (Ohlsson et al., 2009). The insulin-like growth factor-I is a polypeptide chain, whose amino acid sequence is highly conserved across species. The insulin-like growth factor-I is synthesized locally in several tissues and in the liver, and has the biological property of acting in both endocrine and autocrine/paracrine manner (Breier, 1999). The GH-stimulated IGF-I is mainly produced by the liver, which is considered the main source of circulating IGF-I. Growth hormone and IGF-I exert growth-promoting activity, but also play a key role in glucose and lipid metabolism (Wabitsch et al., 1995). The effects of

IGF-I are opposite to those GH, whereas GH exerts lipolitic actions and decreases lipogenesis, IGF-I promotes insulin action in several tissues including adipose tissue (Le Roith et al., 2001). However, IGF-I at low levels acts as a lipolitic factor (Lewis et al., 1988), but when there are enough IGF-I amounts to activate insulin receptor, IGF-I would exert an antilipolytic action (Schuartz and Goodman, 1976; Shimizu et al., 1986). There are two types of IGF receptors located on cell surfaces, which differ in their amino acid sequence, secondary structure and affinity for substrates (Louveau and Gondret, 2004). The type I IGF receptor is very similar to the insulin receptor and has high affinity for IGF-I and little for insulin and IGF-II, whereas the type II IGF receptor shows a very weak affinity for IGF-I and no affinity for insulin (Rechler and Nissley, 1985; Shimizu et al., 1986).

6.2. The carrier proteins (IGFBPs)

Most IGFs are transported in extracellular tissues bound to a family of binding proteins (IGFBPs). These carrier proteins regulate the biological actions of IGF-I by extending their half-life in the circulation, modulating the affinities for their IGF receptors and enhancing or inhibiting IGF activity (Kostecká and Blahovec, 2002). Six binding proteins (IGFBP-1 to IGFBP-6) have been characterized forming a 50 kDa binary or 150-200 kDa trinary complex with the acid-labile subunit protein. The majority of circulating IGFs are bound to the trinary IGFBP-3 complexes, approximately a quart part of the IGFs are associated with the other IGFBPs forming binary complexes and a little amount of the IGFs are found in the circulation in free form (Rajaram et al., 1997). One of the main functions of circulating IGFBPs is to establish and maintain a large circulating pool of IGFs. However, the most important biological action of these IGFBPs is to target IGF activity by acting as specific-tissues carriers and binding to specific IGFBPs cell surfaces receptors (Hossner et al., 1997).

6.3. Circulating IGF-I levels

Circulating IGF-I levels are affected by nutritional alterations and physiological development. Furthermore, environmental and genetic factors are also taking part into the complex metabolism of somatotropic axis. The main direct determinants of

circulating IGF-I levels are growth hormone, insulin, thyroid hormones and activities of IGFBPs. Other indirect factors are aging, body fat and sex hormones (Rosen and Pollak, 1999). In general, serum IGF-I levels reach the maximum level at puberty and decline with age. Breed and sex or castration are important factors that have effect on serum IGF-I concentration. Several studies have shown barrows and females have less serum IGF-I level than entire males at the same age (Louveau et al., 1991; Louveau and Bonneau, 1996). It is known GH-IGF-I somototropic axis is influenced by dietary factors. Nutritional alterations, such as fasting and protein restriction are associated with a decrease in circulating IGF-I concentrations. These variations in IGF-I levels as consequence of dietary treatments indicate that systemic/local IGF actions are implicated in nutritional physiology. Furthermore, several studies postulate that GH-IGF-I system play a key role in the homeostatic regulation of cellular growth responses to protein, nutrient and energy restriction (Simmen et al., 1998). Particularly, it is known that GH orchestrates biological mechanisms related to nutrient partitioning between adipose tissue and skeletal muscle by means of modulation of tissue responsiveness to insulin and lipogenic enzyme activity, resulting in redirecting available energy used for lipogenesis to protein synthesis (Etherton, 2000). Furthermore, one of the biological actions of IGF-I at cellular level is to enhance differentiation in preadipocytes and induce expression of lipogenic enzymes and fatty acid binding proteins (FABP) (Ramsay et al., 1989; Blake and Clarke, 1990), to that adipocytes can subsequently accumulate triacylglicerol droplets and become mature cells (Smith, 1988). These evidences support to the idea IGF-I system could act as a possible connector between nutrition and adipose tissue growth and metabolism (Wabitsch et al., 1995).

6.4. Relationships between IGF-I and production traits

Several studies (Estany et al., 2007) showed that there are heritable determinants of the circulating IGF-I phenotype. Furthermore, IGF-I is moderately heritable and genetically correlated with growth and fatness (Lamberson et al., 1995; Lahti et al., 2001). However, correlations between serum IGF-I level and performance traits have a marked age-related pattern (Louveau et al., 1991; Carter et al., 2002) and for this condition previous studies have reported contradictory relationships. Whereas Luxford

(1998), Cameron et al. (2003) and Bunter et al. (2005) estimated positive genetic and phenotypic correlations between backfat and circulating IGF-I, Suzuki et al. (2004) and Estany et al. (2007) did not find any relationship between these two traits. In spite of fact that IGF-I level shows high variability with age, several authors have postulated that juvenile IGF-I concentration could be used as an early physiological indicator of performance traits measured latter in life (Owens et al., 2000). It would have some advantageous aspects, such as the easy IGF-I measurements in blood and a more early predictability of candidate boars for selection (Bunter et al., 2005). These affirmations have been based on the positive genetic correlations found between IGF-I concentration measured in juvenile pigs and backfat depth at later ages in several studies (Luxford, 1998; Hermesch, 2001). Moreover, it is in accordance with phenotypic correlations found in some studies (Bunter et al., 2005) and genetic studies carried out by Estany et al. (2007), which presented that animals with lower juvenile IGF-I concentrations are leaner in later ages.

7. Strategies for manipulation of IMF

7.1. Repartition of fat deposition

The increasing level of IMF is associated with improved consumer acceptance of meat, but the overfeeding of livestock lead to fattening also other fat depots, which is a waste of resources. If animal scientists knew the adipocyte biology and regulation, fat repartition could be manipulated by feeding and genetic strategies (Hausman et al., 2009). The underlying biological processes that regulate the partition of energy for different activities are little known. Energy is required for many biological processes, between them muscle growth and fat accretion. Protein deposition requires both energy and amino acids and the relation between both is lineal until it reaches an upper limit. It means further increase in energy intake has no effect on protein accretion, and therefore all the excess of energy goes into fat accretion (Pettigrew and Esnaola, 2001). Rauw (2009), explained the principles of the theory of resource allocation and their applications to farm animal production. This theory is based on the availability of the energy and its partition into vital activities and development of structures. Concerning aspects of animal production, selection of animals towards genotypes with high

production efficiency may involve that great amount of energy is allocated into growth, leaving little resources for defending against pathogens, reproduction or other physiological functions. Genetic selection without an understanding of the underlying physiological processes could produce side effects. For this reason, the knowledge of implications of selection in other biological processes is very important to avoid these negative effects on animal development (Rauw et al., 1998).

7.2. Dietary manipulation

Meat fatty acid composition is influenced by genetic, dietary and physiological factors (Nürnberg et al., 1998). These factors are able to modulate deposition of body fat and several studies have reported, it is possible to manipulate fat depots separately (Doran et al., 2006; Solanes et al., 2009). Furthermore, it has been shown that fat deposition can be altered by controlling level of energy in diet. In general, restriction of energy intake results in a decrease in IMF content (Wood et al., 1996) and the reduction of both dietary protein and energy result in favor of IMF accretion (Karlsson et al., 1993). Some explanations to this last finding, is that body protein deposition takes place at higher energetic cost (Knap, 1999) and further research has shown insulin IGF-I signaling pathway and SCD enzyme are involved in muscle energy metabolism (Da Costa, 2004). Diet is one important factor in modifying IMF composition and content. A few dietary manipulations have been carried out to increase IMF content, maintaining backfat thickness. Thus in Doran et al. (2006), IMF level increased in response to feeding a reduced protein diet, and in Pettigrew and Esnaola's (2001) review, IMF content increased and subcutaneous fat reduced by means of diets supplemented with CLA. Other nutritional strategies to enhance IMF content are the reduction of vitamin A in the diet (Olivares et al., 2011) and tallow supplementation of diets (Duran-Montgé et al., 2008).

7.3. Genetic manipulation

Concerning genetic manipulation means, Reixach et al. (2008) demonstrated that selection against backfat thickness at constant IMF in pigs could be a useful strategy for reducing backfat thickness without affecting IMF levels. The process of selective

breeding is based on variation existing for any character in a population. Part of this variation is due to the animals possess different genes and therefore it allows selecting better animals to improve a breed. However, variability and heritability in a trait are not sufficient to include them into a genetic selection program, the effects that selection in one trait has on the other traits of interest are also determinant factors (Owens et al., 2000). Estimated breeding values (EBV) are a useful tool available to compare animals' genetic merits in a population. These values are estimated through the animal's and its relatives' performance (full-sibs, half-sibs, sire, dam, etc.) (Keele et al., 1988). Mixed model best linear unbiased prediction (BLUP) is a genetic evaluation method utilized widely in breeding companies to maximize the accuracy of the resulting EBV (Chen et al., 2003; Dekkers, 2004). As regard to meat quality traits, IMF content and composition is moderately to highly heritable (around 0.50, Sellier, 1998; Suzuki et al., 2005) and it presents an important genetic variation both between and within breed (Oliver et al., 1994; Cilla et al., 2006). Furthermore, several studies have estimated some genetic correlations of IMF with production and quality traits in pigs. Generally IMF has been positively and moderately to highly correlated to backfat thickness and body weight and negatively to loin area (Suzuki et al., 2005; Solanes et al., 2009). However, it has been demonstrated there is a part of the genetic variability in IMF that is independent of carcass fatness (De Smet et al., 2004). On the other hand, several studies have estimated genetic parameters for individual fatty acids in IMF, enzymatic indices and serum lipid traits in pigs, obtaining moderate estimates for heritability, except in some fatty acids, such as, stearic (C18:0), C16:0 and C18:1 (Ntawubizi et al., 2009; Reixach et al., 2009; Casellas et al., 2010) and plasma triglycerides (Pond et al., 1986), which were highly heritable. Moreover, high genetic correlations among fatty acid composition and meat production and quality traits have been informed. In particular, C18:0 is positively correlated to IMF and subcutaneous backfat and negatively to loin area, whereas C16:0 is negatively correlated to daily gain (Suzuki et al., 2006). Therefore, IMF content is a trait that could be included into a genetic selection program without affecting the traditional goals of industry. In fact, selection against backfat at constant IMF could implement if phenotypic data of IMF or indirect markers were recorded.

GENERAL OBJECTIVES

GENERAL OBJECTIVES

The present study is part of a line of research about the genetic improvement of pig meat quality, with particular reference to IMF content and composition. The final goal of this line is to give new inputs for genetically obtain pigs with increased lean content but maintaining optimum IMF levels or, equivalently, to produce good quality meat at minimum cost. Thus, the three studies in this thesis focused on decreasing the cost of the IMF determination and investigating the biological mechanisms of fat deposition.

The main objectives of this thesis were:

1. To develop a high-throughput analytical method for oleic fatty acid quantification in pork using a flow injection analysis system based on electrospray ionization mass spectrometry.

2. To assess whether serum lipid indicators may be used as biomarkers for early prediction of IMF and subcutaneous fat content and composition.

3. To investigate the effect of the selection against backfat at constant IMF on lipogenic protein expression and fatty acid profiles.

CHAPTER I

Determination of oleic acid in pork

ABSTRACT

In some Mediterranean products such as olive oil or ham, oleic acid is the most abundant component of the total fat. Due to the large volume of trade in these products, it may be necessary to analyze oleic fatty acids in high numbers of samples in short periods of time. However, using classic lipid analysis techniques, it is not always possible to cope with these high demands. To solve this problem, a high-throughput analytical method for oleic fatty acid quantification in pork is presented. The purpose of the method is to avoid liquid chromatography processes using a flow injection analysis (FIA) system based on electrospray ionization mass spectrometry. The use of pentadecanoic fatty acid as an internal standard overcame matrix effects. The oleic FIA technique could be used as a suitable method for discriminating carcass samples for selection and labeling by oleic acid content when large numbers of pork samples must be processed in a short period of time.

1. Introduction

Throughout the last decade, dietary fat intake has received increasing interest because of its effect on human health. Presently, the relationship between dietary fat and coronary heart disease and cancer are not definitive. However, there is evidence on the modification effects of total, saturated, monounsaturated and polyunsaturated fats on cardiovascular morbidity and mortality (Hooper et al., 2001). Thus, the control of dietary fat intake and analysis of fat composition have become preventative strategies for some chronic diseases. The Dietary Guidelines for Americans, 2005 recommends that people consume less than 10% of their total daily calories from saturated fatty acids.

For these reasons, the screening of lipid composition arises as an important area of interest in food analysis and the fatty acid profile is now a frequently requested piece of information, both by food producers and consumers. In some cases, this situation makes necessary the analysis of a high number of samples in short periods of time. Using classic lipid analysis techniques, however, it is not always possible to cope with these demands. Particularly in animal production, fatty acid profiles are increasingly requested, because they play an important role in the health properties of meat, but also

because the health properties of meat are improvable by means of animal breeding or environmental factors (De Smet et al., 2004). Therefore, it is essential to have information on fatty acid composition from a large number of samples in a fast and accurate fashion.

From an analytical point of view, the most common technique to obtain fatty acid profiles is gas chromatography (GC). The most usual methods of analysis involve lipid extraction and the conversion of the fatty acids into methyl esters (AOAC, 1997). However, other possibilities have been proposed for analysis, including the direct transesterification of fatty acids in freeze-dried raw materials (Rule, 1997; Eras et al., 2001) or the silylation of fatty acids (Drozd, 1975). Measurement time then depends on the number and type of fatty acids present in the oil, but these procedures are time-consuming, requiring at least 10 minutes to raise a fatty acid profile by GC. A good resolution of isomers requires longer analysis times (Hauff and Vetter, 2009).

More recently, as a consequence of the application of ionization techniques (Cai and Syage, 2006) such as electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI), lipid analysis by high-performance liquid chromatography/mass spectrometry (HPLC/MS) has become fairly common. Using these techniques, the derivatization of fatty acids prior to analysis is not essential, so measurements can be carried out rapidly and simply. Derivatization does have some advantages, however, such as working in the positive ion mode using common liquid chromatography mobile phases or increasing detection sensitivity (Yang et al., 2007). Despite these advantages, several methods have been proposed for using ESI as the ion source and operating in the negative ion mode to analyze fibroblasts (Valianpour et al., 2003), chocolate (Perret et al., 2004), vegetable and animal oils (Kurata et al., 2005) and blood plasma (Valianpour et al., 2003; Gagne et al., 2007).

In pork production systems in the Mediterranean, a characteristic way to improve meat health properties is by increasing oleic fatty acid content (Reixach and Estany, 2010). In practice, this means having to make a rapid oleic analysis on a large number of pigs. The objective of this study is then to create a fast and matrix-effect-free analytical

method for oleic fatty acid quantification in fresh pork. The main purpose of this new method is to shorten the time required for analysis by avoiding chromatography and using a flow injection system based on ESI-MS. This method is expected to reduce analysis costs because the boron trifluoride, which is used in the transesterification step, is around 20-fold more expensive than the potassium hydroxide in methanol/water (50:50, v/v), which is used in the saponification step.

2. Materials and methods

2.1. Equipment

A Trace 2000 series GLC (ThermoQuest, Milan, Italy) equipped with a flame ionization detector (FID) was used for analysis of all methyl esters of fatty acids. A Micromass ZMD 2000 MS single quadrupole instrument equipped with an ESI ion source and a Waters Alliance 2690 separation module (Milford, MA, USA) was used for all fatty acid flow injection analyses (FIA). All standard lines were prepared by serial dilution of working solutions by means of a GX-271 liquid handler (Gilson, Middleton, WI, USA).

2.2. Chemicals

Fatty acids, fatty acid methyl esters and triglycerides were purchased from Sigma-Aldrich (Madrid, Spain). Potassium hydroxide, isooctane, 2,6-di-tert-butyl-4-methylphenol and 2,2-dimethoxypropane were obtained from Panreac (Barcelona, Spain). Acetonitrile, petroleum spirit and boron trifluoride (20% methanolic solution) were from Prolabo (Vaugereau, France). Hexane and acetic acid were from Merck (Darmstadt, Germany). Ultrapure water was provided by a Milli-Q system (Millipore, Milford, MA, USA).

2.3. Samples and preparation

Samples of *m. gluteus medius* from 15 Duroc pigs were taken at 24 h post-mortem, vacuum-packaged, frozen and stored until used. Prior to derivatization, samples were

thawed, ground and homogenized using an electric grinder. Dry matter was determined by drying a precise weight at 100-102ºC in an air oven for 24 h. In addition, muscle samples were freeze-dried before the analysis of fatty acid methyl esters was conducted. After the freeze-drying process, samples were pulverized in a domestic electric grinder and fully homogenized by mixing with sand using a glass rod. Transesterification and hydrolysis methods are described in the following two sections.

2.4. GC analysis

Using an aliquot of the homogenate (160 mg), fatty acid methyl esters were obtained using a solution of boron trifluoride (20% in methanol) (Rule, 1997). Quantification of fatty acid methyl esters was performed by GC with a 30m x 0.25mm capillary column coated with a 0.20-µm film of poly(80% biscyanopropyl-20%cyanopropylphenyl siloxane) (SP2330; Supelco, Tres Cantos, Madrid, Spain) and a FID with helium as the carrier gas flowing at 1 mL/min. The oven temperature program increased from 150 to 225ºC at a rate of 7 ºC/min, and the injector and detector temperatures were 250ºC (Tor et al., 2005). Quantification of fatty acids was carried out in duplicate through area normalization after adding 1,2,3-tripentadecanoylglycerol into each sample as an internal standard.

2.5. ESI-MS analysis

For each fatty acid (tridecanoic, tetradecanoic, pentadecanoic, hexadecanoic, cis-9-hexadecenoic, octadecanoic, cis-9-octadecenoic, cis-9,12-octadecadienoic, cis-9,12,15-octadecatrienoic, eicosanoic and cis-5,8,11,14-eicosatetraenoic acids) a stock solution (approximately 10 mg/mL) was prepared in isooctane. Working solutions (200 µg/mL) were obtained from these stock solutions and were freshly prepared using the initial mobile phase (98/2, v/v, acetonitrile/water) as solvent. For initial instrument tuning, 100 µg/mL of tetradecanoic acid in acetonitrile/water (98/2, v/v) was infused by a syringe pump at a flow rate of 5 µL/min. Optimized tuning conditions are described below in the Results section. For standard scan analysis, 100 µg/mL of freshly prepared working solution of each fatty acid was infused at a flow rate of 5 µL/min. Full scan acquisition

data were recorded over the range 100–400 *m/z*. For optimization of signal-to-noise ratio (SN) and quantitative analysis, single ion recording (SIR) was performed using a function of 11 channels (one channel for each fatty acid *m/z* characteristic value) with a recording time of 1 minute, which gives 10 scans per function. Because the HPLC system worked without a column, the obtained peaks showed a flat shape and therefore, it was difficult to integrate them correctly using typical procedures. Accordingly, the sum of all the 10 single mass spectrometer values obtained for each fatty acid was used for quantification purposes instead of the peak area. For specificity evaluation, hexadecanoic, cis-9-octadecenoic and cis-9,12-octadecadienoic fatty acids were prepared by diluting a 200 µg/mL working solution with acetonitrile/water (98/2, v/v) to give standard lines with concentrations of 200, 100, 50, 25 and 12.5 µg/mL. The limits of detection (LODs) and quantitation (LOQs) were then calculated as the concentration of the analyte giving a signal equal to the average background plus either three or ten times the standard deviation of the blank.

For matrix effect evaluation, 15 muscle samples from different pigs were used. The total fatty acid profiles were obtained by saponification of 250 mg of sample meat using (5M) KOH in methanol/water (50:50, v/v). Then, glacial acetic acid was added to neutralize and the free fatty acids were extracted using petroleum spirit (Aldai et al., 2006). In addition, 52.3 µg of 1,2,3-titridecanoylglycerol was added before hydrolysis as an internal standard. The matrix effect on oleic acid quantification was then assessed by comparison of slopes between two sets of standard lines constructed using net solutions (n=5) or muscle extracts (n=15). Net solution standard lines were prepared by a 12-fold serial dilution of 200 µg/mL oleic acid with acetonitrile/water (98/2, v/v) containing 50 µg/mL tridecanoic acid, 50 µg/mL pentadecanoic acid and 2% petroleum spirit. In muscle extracts, standard lines were prepared similarly, but they were prepared without tridecanoic acid and were spiked with sample extract instead of petroleum spirit. The linearity of each standard line was confirmed by linear regression of the analyte/internal standard area ratio versus analyte concentration. Sensitivity was determined as the LOD and LOQ, calculated as 3.3 and 10 times the relative standard deviation (RSD)/Slope ratio (Christian, 2004) for each standard line, respectively. Oleic acid content of each sample was calculated both by the standard additions method and by the external

standard line. The precision was determined by replicate analyses (n=5) of each sample. Finally, the accuracy of the method was evaluated by comparison with the values of the samples obtained by GC.

2.6. Statistical analysis

Regression analysis was used to obtain standard lines. Data for the matrix effect evaluation were then fitted to a linear mixed model that included the standard line set, the oleic acid concentration and the interaction of fixed effects and random effects. The statistical analyses were performed using the PROC REG and PROC MIXED procedures (SAS System, Version 9; SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

3.1. Instrument tuning and optimization of the SN

Initial instrument tuning and the identification of major formed species were performed by direct infusion of tetradecanoic acid solution. The most abundant signal, which corresponded to the $[M-H]^-$ ion (*m/z* = 227), was used for visual establishment of the initial parameters set in the Masslynx tune page. These parameters were as follows: desolvation gas flow of 400 L/h, a capillary voltage of 2.7 kV, cone voltage of 30 V, extractor voltage of 5 V, source block temperature of 100ºC and a desolvation temperature of 350ºC. Afterwards, all the selected target fatty acids were assayed by direct infusion using the conditions described above and registered in the mass range of 200 to 400 *m/z*. As shown in **Figure 1**, all of the fatty acids gave major ions corresponding to the ionized $[M-H]^-$ form. In addition, no overlap was found for the most abundant signals between the studied fatty acids. From the analyses, it was discovered that 227, 241, 255, 253, 283, 281, 279, 277, 311 and 303 *m/z* ions should be the candidate ions for direct quantification of myristic, pentadecanoic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, arachidic and arachidonic fatty acids (or their isomers), respectively.

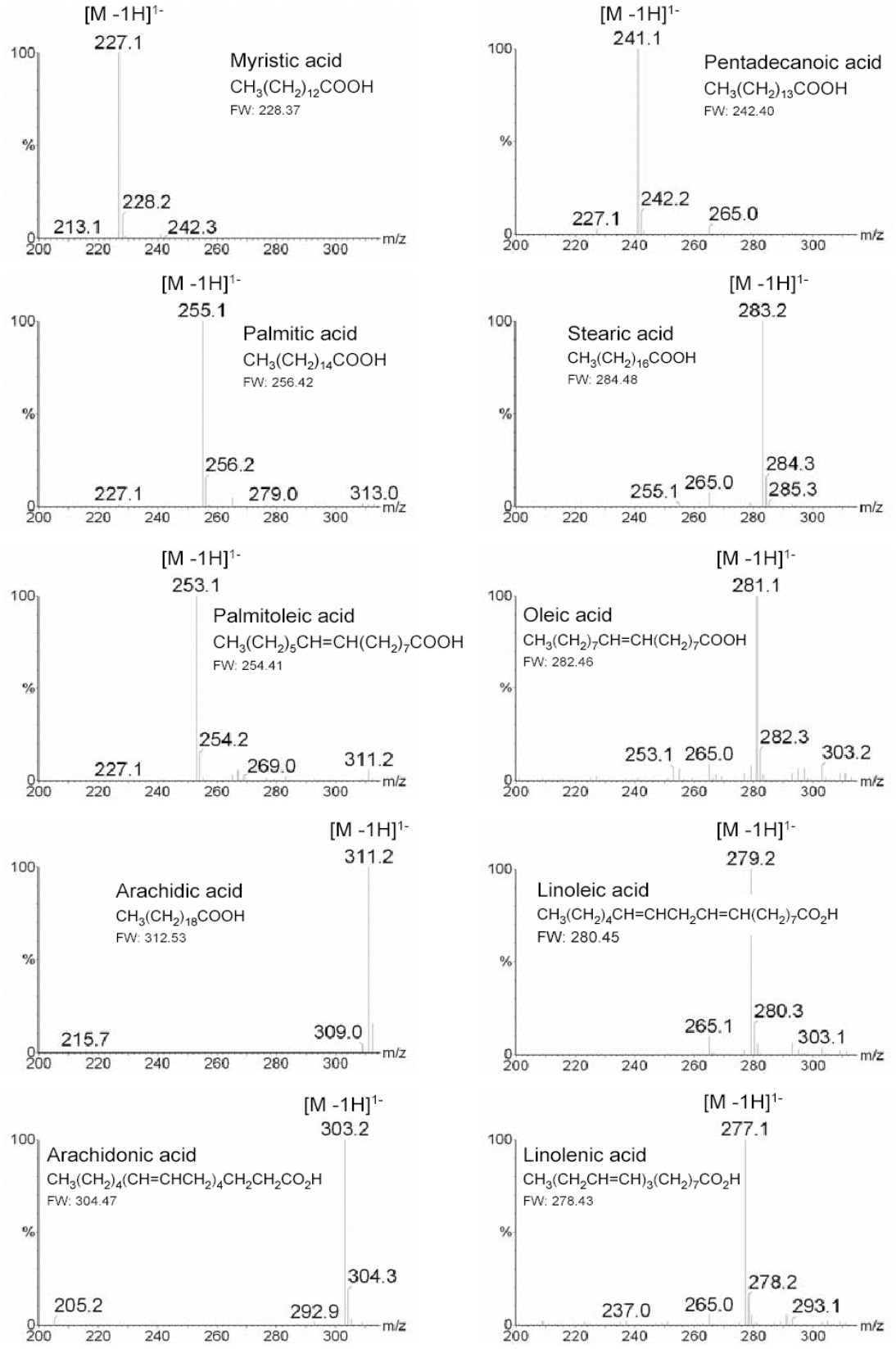

Figure 1. ESI mass spectrum in negative ion mode of the more common fatty acids in pork plus pentadecanoic acid.

In order to obtain a better SN, a mixture of 50 μg/mL of each fatty acid was used to optimize the parameters of the system. Using the initial conditions, phase composition, desolvation temperature, capillary voltage, cone voltage and injected volume were sequentially evaluated. In each concentration range, the standard mixture was injected in quintuplicate and, in order to determinate the SN, a blank was injected after every standard.

3.2. Phases

Phase composition

Between 1 and 20% we found a positive relationship between mobile phase water content and SN. The acetonitrile/water phase that gave the highest SN together with good repeatability (coefficient of variance (CV) <5%) was 92:8 (v/v).

Phase modifiers

As in previous reports, the use of mobile phase modifiers may improve the efficiency and sensitivity of lipid analysis by HPLC/ESI techniques (Cai and Syage, 2006). For free fatty acids, the best HPLC separation was obtained with acidic conditions, whereas the best ionization was achieved in the negative mode using basic mobile phase. In this study, a range of 0–20 mM ammonium acetate and a range of 0–0.25% ammonia were both assayed for lipid analysis. For ammonium acetate, although the signal intensity of the standards (recorded as 227, 241, 255, 253, 283, 281, 279, 277, 311 and 303 *m/z* ions) increased up to 5 mM, the SN was not improved. With regards to ammonia, the SN increased up to 0.005–0.02%, depending on the fatty acid considered, but then subsequently decreased at higher percentages. Taking into account all the studied fatty acids, the maximum of SN, or a value very close to it, was obtained at 0.01% of ammonia content in the mobile phase.

3.3. MS parameters

Desolvation temperature and capillary and cone voltages were evaluated between 250-450ºC, 2-3.5 kV and 10-60 V, respectively. The highest SN was attainted at 250 ºC, 2 kV and 10 V. In addition, the SN increased between 1 and 20 µL injection volume.

3.4. Cross-talk between the MS channels used for fatty acid quantification

Selectivity was assessed by monitoring the relative area of each MS channel for the individual fatty acid standard lines. In **Figure 2**, cross-talk between the three most abundant fatty acids in pork (palmitic, oleic and linoleic acids) is shown. The main quantitative response was in the target MS channel, but a slight increase in the noise level for the rest of the fatty acids was also detected. Using standards, an increase in the palmitic acid concentration from 12.5 to 200 µg/mL led to increases in the LOQs for oleic and linoleic acids from 3.01 and 2.85 to 11.81 and 12.50 µg/mL, respectively. Similarly, increasing the oleic (linoleic) fatty acid concentration from 12.5 to 200 µg/mL increased the LOQs of palmitic and linoleic (palmitic and oleic) fatty acids from 5.07 and 3.67 to 15.36 and 12.90 µg/mL, respectively (from 3.67 and 4.47 to 6.83 and 12.22, respectively). These results show that the LOD and LOQ depend on the fatty acid profiles of each sample and, therefore, that both must be recalculated in muscle tissue. This can lead to a decrease in the accuracy, particularly in samples with high fat and low oleic content, in which that value can be overestimated as a result of a cross-talk effect of the other fatty acids.

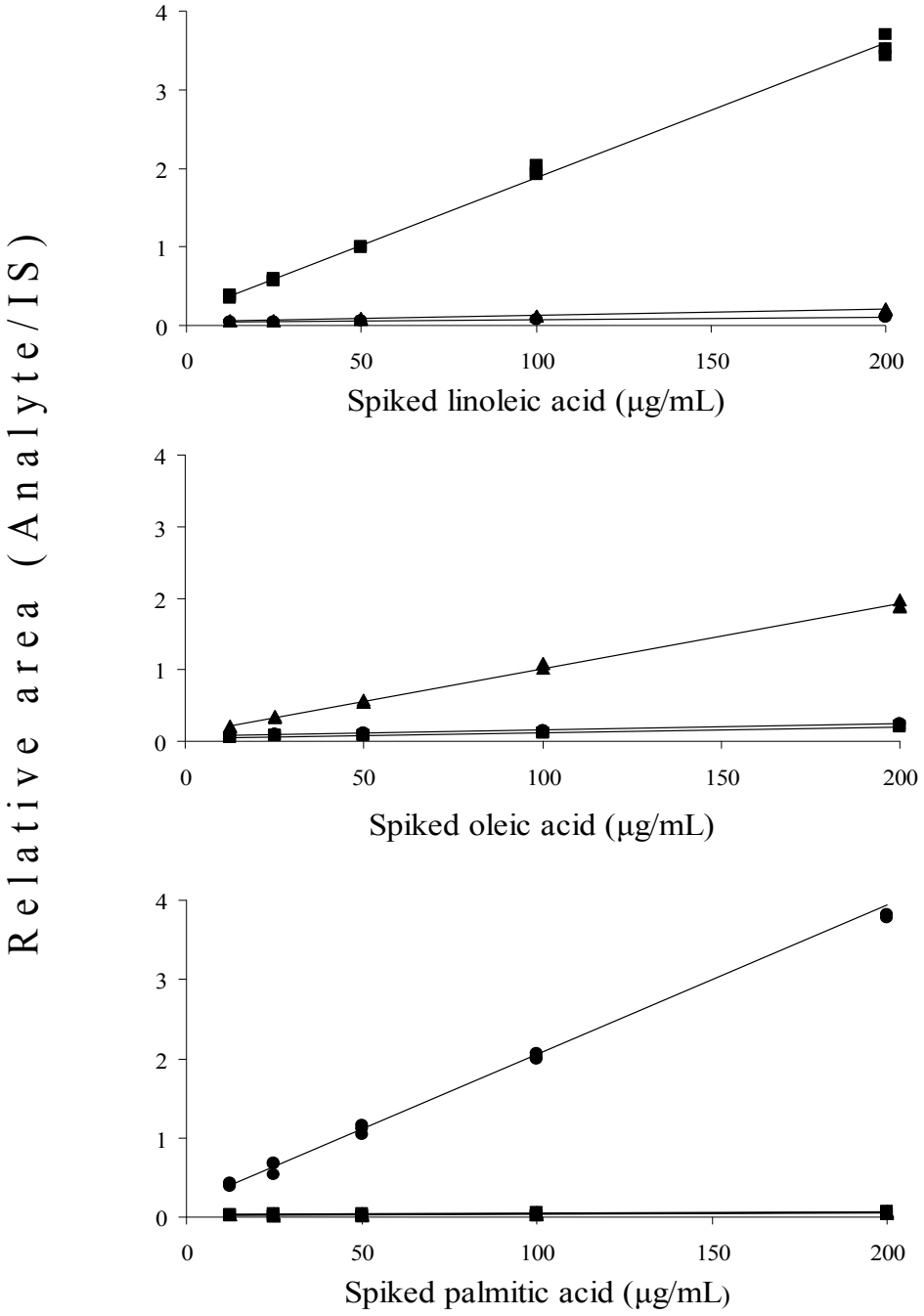

Figure 2. Cross-talk of relative responses for palmitic, oleic and linoleic fatty acids. Individual standard lines of the three most abundant fatty acids in pork. Greater response is observed in its own channel but a slight increase in the background noise of the other two fatty acids are detected. Analyte/IS: -■- linoleic fatty acid/pentadecanoic fatty acid (*m/z*/*m/z*: 279/241); -▲- oleic fatty acid/pentadecanoic fatty acid (*m/z*/*m/z*: 281/241); -●- palmitic fatty acid/pentadecanoic fatty acid (*m/z*/*m/z*: 255/241).

3.5. Matrix effect evaluation

The quantitative aspects of the method were studied by using two sets of oleic acid standard lines. The reference set of standards was prepared using solutions of standards (S), and the matrix set (M) was prepared as standard additions into hydrolyzed muscle extract. In both cases, pentadecanoic acid was used as an internal standard that was spiked into the samples just before the MS analyses. In order to evaluate the muscle matrix effect (Matuszewski et al., 2003), each line of the matrix set was constructed using a muscle sample from a different pig. The matrix effect was then studied by comparing standard line slopes between the two different sets. Although the MS absolute responses were different between the standards and the muscle samples, the use of an internal standard effectively overcame the matrix effect. Thus, no differences were found in the slope values between the standards and the matrix set lines (P>F 0.689, **Table 1**). It should be noted, however, that all the samples used in the study came from a single Duroc pig genetic line.

Even though no matrix effect was found, when an internal standard was used, the slope of the RSD of the standard lines (**Table 1**) was around 15%. It was difficult to attribute this phenomenon to a specific effect, particularly because it occurred both with and without matrix standard lines (16.1% vs. 15.8%) and, therefore, it was considered an intrinsic feature of the method. Thus, in order to be thoroughly accurate, the slope of the standard lines must be calculated from several measurement repetitions. Finally, standard values were fitted to regression lines, and the LOD and LOQ were calculated. The linearity of each standard line was confirmed by calculating the r-squared values (**Table 1**).

Table 1. Matrix effect for the standard lines of oleic fatty acid.

Sample	Regression equation	R^2	Slope		LOD ug/mL	LOQ ug/mL	Slope average	*P*>F
			SE	RSD				
S1	$y = 0.00803x + 0.04415$	0.9798	0.00014		3.83	11.61		
S2	$y = 0.01026x + 0.007$	0.9942	0.00008		2.04	6.17	0.009665	
S3	$y = 0.00986x + 0.01761$	0.9989	0.00004	16.1	0.87	2.65	±	
S4	$y = 0.00817x + 0.04496$	0.9994	0.00007		0.63	1.9	0.000959	
S5	$y = 0.01201x + 0.04423$	0.9990	0.00008		0.83	2.52		
M1	$y = 0.00858x + 0.27897$	0.9992	0.00003		0.76	2.31		
M2	$y = 0.00910x + 0.19987$	0.9870	0.00013		3.06	9.27		
M3	$y = 0.00936x + 0.31309$	0.9863	0.00013		3.14	9.51		
M4	$y = 0.00798x + 0.34566$	0.9941	0.00007		2.04	6.19		
M5	$y = 0.00877x + 0.24221$	0.9945	0.00008		1.98	6		0.689
M6	$y = 0.00756x + 0.38988$	0.9865	0.00011		3.12	9.44		
M7	$y = 0.00730x + 0.41404$	0.9878	0.00010		2.96	8.97	0.009215	
M8	$y = 0.00630x + 0.20482$	0.9943	0.00006	15.8	2.02	6.11	±	
M9	$y = 0.00985x + 0.40762$	0.9980	0.00005		1.19	3.6	0.001107	
M10	$y = 0.00973x + 0.1975$	0.9921	0.00010		2.38	7.21		
M11	$y = 0.01011x + 0.54991$	0.9773	0.00019		4.06	12.29		
M12	$y = 0.01114x + 0.50251$	0.9946	0.00010		1.96	5.94		
M13	$y = 0.01060x + 0.39648$	0.9981	0.00006		1.17	3.56		
M14	$y = 0.01063x + 0.75294$	0.9858	0.00016		3.24	9.82		
M15	$y = 0.01115x + 0.44835$	0.9957	0.00009		1.73	5.24		

S, net solutions of standards; M, standard additions of hydrolyzed muscle.

3.6. Internal standard method validation

In order to evaluate the accuracy and precision of the method, the oleic fatty acid concentration of each sample was calculated using either standard additions (used as a reference method due to the absence of a blank matrix) or by estimating the slope of standard lines using pentadecanoic fatty acid as an internal standard. The results of this validation are shown in **Table 2**. The correlation between the two methods proved to be high enough (r=0.9). This avoids the use of standard additions and greatly simplifies the process. The average RSD value (2.87) calculated from the 15 analyzed samples (in quintuplicate) ensured that the precision of the internal standard method was sufficient.

Table 2. Precision of oleic acid determination by the FIA/ESI-Q method with an internal standard spiked before and after sample hydrolysis.

	Oleic acid content (ug/mL)			
	Standard addition	Internal standard		
	Mean	Mean	SD	RSD
M1	32.51	29.713	0.111	0.373
M2	21.96	22.346	0.176	0.788
M3	33.45	36.971	1.883	5.093
M4	43.32	39.966	1.872	4.684
M5	27.62	27.483	1.273	4.631
M6	51.57	44.186	1.38	3.124
M7	56.72	42.893	0.619	1.444
M8	32.51	24.07	0.486	2.02
M9	41.38	40.839	2.629	6.439
M10	20.3	22.637	0.691	3.053
M11	54.39	63.454	0.617	0.972
M12	45.11	55.013	1.912	3.476
M13	37.4	43.061	0.921	2.138
M14	70.83	78.144	2.743	3.51
M15	40.21	46.765	0.63	1.348

3.7. Gas chromatography cross-validation

Cross-validation of the method was performed by correlating values of the oleic fatty acid content determined by FIA/MS using an internal standard with values obtained on the same samples by GC. Efficiency was taken into account by spiking samples with tridecanoic acid before hydrolysis (as 1,2,3-tritridecanoylglycerol) as a second internal standard in FIA/MS quantification. All values were expressed on a dry matter basis, and **Figure 3** shows the comparison between these two methods.

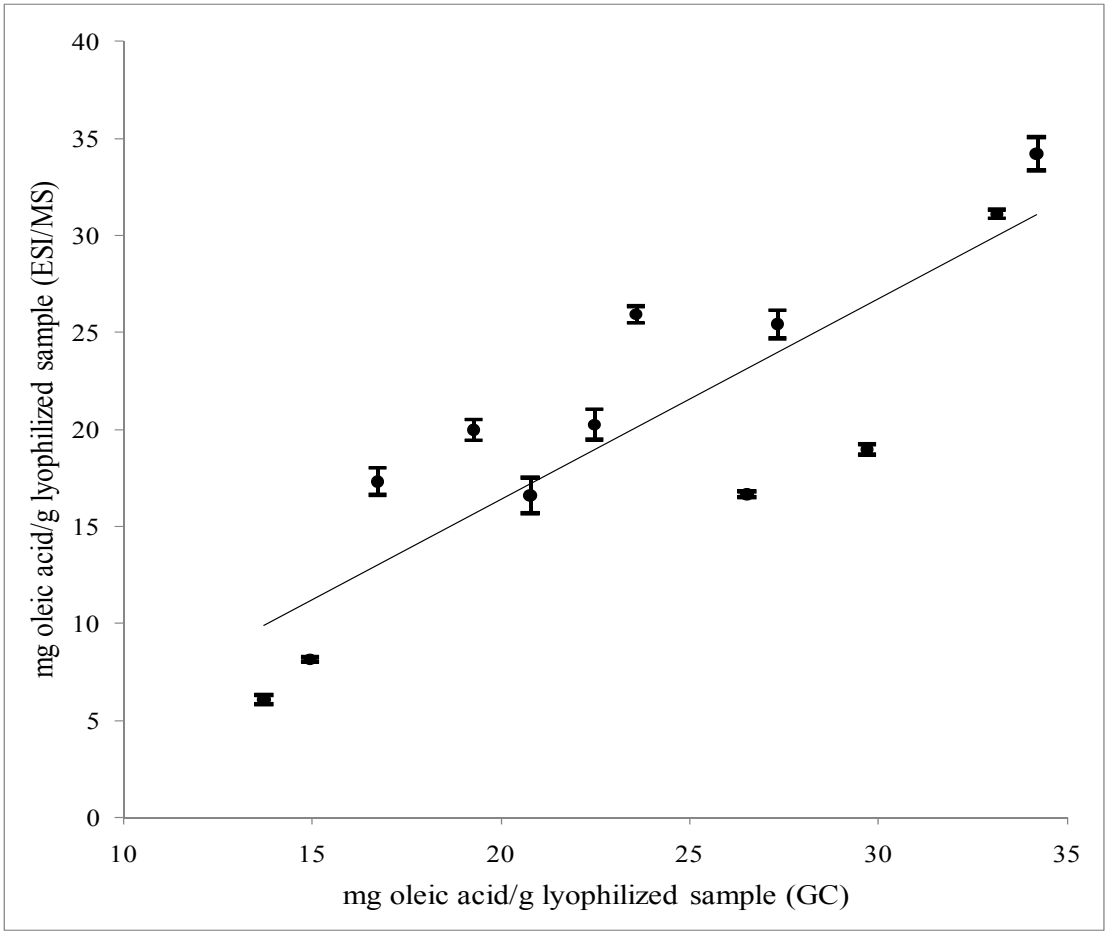

Figure 3. Relationship between oleic acid content in muscle analyzed by ESI-MS (mean ± SE; n=5) and gas chromatography. Line represents linear regression, (ESI-MS= 0.9692 GC – 2.7838; R^2 =0.7228, P<0.001).

When the regression line was raised using samples with less than 40 mg of oleic acid/g lyophilized meat, a linear approach sufficed (R^2=0.72). In this case, the ESI-MS method slightly underestimated the oleic fatty acid concentration (slope=0.96). However, when the whole sample set was fitted, a quadratic regression was required, as the curve flattened at higher values of oleic acid. The sensitivity of the method was observed to decrease in this final stretch because the ESI may become saturated in samples with high fat content, leading to response decreases. Alternatively, a higher dilution level was not possible because then the lower fat content would be below the LOQ. However, in view of a possible practical application of the method, it must be noted that the samples used in this work were from a Duroc line with high levels of

intramuscular fat content. Therefore, when sampling conventional pork carcasses, it is expected that few samples will fall in the upper stretch of the standard line. In any case, this method should always be linked to a previous screening of each meat batch by gas chromatography and mass spectrometry in order to provide a detailed list of the fatty acids present in the samples. In each batch, it should be proved that there is no significant presence of 18:1 fatty acids other than oleic acid.

A generalization of the method could be designed for a tandem mass spectrometer analysis. Such an extension could be particularly useful for those situations where a high specificity, accuracy and precision are needed and also in overcoming some drawbacks that decrease the feasibility and scope of the method as presented. In particular, the sensitivity of the method would be improved, as well as a reduction in the background noise. The selectivity of the single quadrupole, although appropriate for analyzing pork fatty acid profiles, did not eliminate the background noise completely, a circumstance that makes necessary to recalculate LOD and LOQ in muscle tissue. Reduced background noises would allow working with more diluted samples, a circumstance that in turn would reduce the matrix effect and would avoid the use of an internal standard before MS analyses. Note that to overcome the matrix effect, likely caused by a saturation of the ionization font by the other fatty acids in the sample, an internal standard has been added before the MS analysis. The high RSD for the slope of standard lines, around 15%, may be not enough for applications requiring high accuracy. For these situations, the use of a triple quadrupole mass spectrometer could be an interesting option to evaluate. On the contrary, the method described, should be useful in situations where the goal is more to discriminate superior samples (from animals, carcasses or retail cuts) by oleic content rather than to estimate its value very precisely. In particular, this could be the case when samples are processed for use as a criterion for selective breeding or for discriminating retail cuts for labeling purposes. In such cases, the main target is to identify superior animals or products and therefore ranking interchanges occurring in the mid-quartile are not very important, either because they would not lead to a status change in the corresponding item, or because, if misclassifications occur, they will not result in relevant changes in value. Note that the animals (or carcasses or retail cuts) more liable to be misclassified are those displaying very similar oleic contents.

This analytical method has been tuned specifically for oleic fatty acid analysis in pork. However, from our data, there is no impediment to adapting this method to fatty acids other than oleic acid or to monogastric species other than pig.

Conclusions

The FIA method presented here proved suitable for discriminating samples for selection and labeling purposes by oleic acid content, particularly when having large amounts of pork samples that must be processed in a short period of time. The possibility of avoiding extraction and chromatography steps, together with the use of a low-priced reagent in the hydrolysis step, should allow for a large number of analyses in a short period of time. The method can be cost-effective, provided that the type of mass spectrometer used here becomes a routine detector in many laboratories.

References

Aldai, N., K. Osoro, L. J. R. Barron, and A. I. Najera. 2006. Gas-liquid chromatographic method for analysing complex mixtures of fatty acids including conjugated linoleic acids (cis9trans11 and trans10cis12 isomers) and long-chain (n-3 or n-6) polyunsaturated fatty acids - Application to the intramuscular fat of beef meat. J. Chromatogr. A. 1110:133-139.

AOAC. 1997. Official Method 996.06. Official Methods of Analysis. 16th ed. Assoc. AOAC international. Arlington.

Cai, S. S., and J. A. Syage. 2006. Comparison of atmospheric pressure photoionization, atmospheric pressure chemical ionization, and electrospray ionization mass spectrometry for analysis of lipids. Anal. Chem. 78 (4):1191-1199.

Christian, G. D. 2004. In Analytical chemistry. In page 113 in Data handling and spreadsheets in analytical chemistry. John Wiley and Sons, ed. New York.

De Smet, S., K. Raes, and D. Demeyer. 2004. Meat fatty acid composition as affected by fatness and genetic factors: A review. Anim. Res. 53:91-98.

Dietary Guidelines for Americans. 2005. U.S. Department of Health and Human Services. U.S. Department of Agriculture.

Drozd J. 1975. Chemical derivatization in Gas-Chromatography. J. Chromatogr. A 113:303-356.

Eras, J., F. Montanes, J. Ferran, and R. Canela. 2001. Chlorotrimethylsilane as a reagent for gas chromatographic analysis of fats and oils. J. Chromatogr. A. 918:227-232.

Gagne, S., S. Crane, Z. Huang, C. S. Li, K. P. Bateman, and J. F. Levesque. 2007. Rapid measurement of deuterium-labeled long-chain fatty acids in plasma by HPLC-ESI-MS. J. Lipid Res. 48:252-259.

Hauff, S., and W. Vetter. 2009. Quantitation of cis- and trans-monounsaturated fatty acids in dairy products and cod liver oil by mass spectrometry in the select ion monitoring mode. J. Agric. Food Chem. 57:3423-3430.

Hooper, L., C. D. Summerbell, J. P. T. Higgins, R. L. Thompson, N. E. Capps, G. D. Smith, R. A. Riemersma, and S. Ebrahim. 2001. Dietary fat intake and prevention of cardiovascular disease: Systematic review. Brit. Med. J. 322:757-763.

Kurata, S., K. Yamaguchi, and M. Nagai. 2005. Rapid discrimination of fatty acid composition in fats and oils by electrospray ionization mass spectrometry. Anal. Sci. 21:1457-1465.

Matuszewski, B. K., M. L. Constanzer, and C. M. Chavez-Eng. 2003. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. Anal. Chem. 75:3019-3030.

Perret, D., A. Gentili, S. Marchese, M. Sergi, and L. Caporossi. 2004. Determination of free fatty acids in chocolate by liquid chromatography with tandem mass spectrometry. Rapid Commun. Mass Sp. 18:1989-1994.

Reixach, J., and J. Estany. 2010. Genetic parameters for intramuscular oleic fatty acid content in a Duroc line (Abstract). Proc. 9th World Congr. Genet. Appl. Livest. Produc., Leipzig, Germany: 294.

Rule, D. C. 1997. Direct transesterification of total fatty acids of adipose tissuee, and of freeze-dried muscle and liver with boro-trifluoride in methanol. Meat Sci. 46:23-32.

Tor, M., J. Estany, A. Francesch, and M. D. Cubilo. 2005. Comparison of fatty acid profiles of edible meat, adipose tissues and muscles between cocks and capons. Anim. Res. 54:413-424.

Valianpour, F., J. J. M. Selhorst, L. E. M. van Lint, A. H. van Gennip, R. J. A. Wanders, and S. Kemp. 2003. Analysis of very long-chain fatty acids using electrospray ionization mass spectrometry. Mol. Genet. Metab. 79:189-196.

Yang, W. C., J. Adamec, and F. E. Regnier. 2007. Enhancement of the LC/MS analysis of fatty acids through derivatization and stable isotope coding. Anal. Chem. 79:5150-5157.

CHAPTER II

Blood lipid indicators and fatness

ABSTRACT

The relationship between blood lipid indicators and subcutaneous and intramuscular fat (IMF) content and composition was investigated with the purpose to search for biomarkers specifically associated to one of them. The experiment was conducted on 111 purebred Duroc barrows, in which serum concentration of iron, glucose, total protein (TP), total cholesterol, HDL-Cholesterol (HDL-C), LDL-Cholesterol and total triglycerides, the percentage of lipoproteins (α-lipoprotein, β-lipoprotein, pre-β-lipoprotein and chylomicrons), and plasma insulin-like growth factor-I (IGF-I) were determined at 120, 160, and 185 days of age. Carcass backfat and loin thickness were measured at 215 days, as well as IMF content in *gluteus medius* and *longissimus dorsi* muscles. Fatty acid composition in *gluteus medius*, *longissimus dorsi* and in subcutaneous fat was analysed by gas chromatography. Blood lipid indicators displayed an age-related pattern and a low correlation structure with commercial traits. Circulating TP was the best biomarker for early estimation of fatness (with a correlation of 0.49 and 0.32 with backfat thickness and IMF in *gluteus medius*, respectively, $P<0.05$), although HDL-C was the most consistent throughout age. Circulating IGF-I at later stages of growth may help for specifically biomarking backfat thickness against IMF. Thus, whereas HDL-C at 185 days correlated similarly with backfat thickness (0.28, $P<0.05$) and IMF (0.22, in *gluteus medius*, and 0.20, in *longissimus dorsi*, $P<0.05$), IGF-I at this age was more specifically correlated to backfat thickness (0.33, $P<0.05$) than to IMF (0.01, $P>0.05$). However, none of the studied blood lipid indicators has been revealed as an effective potential biomarker for differential fat deposition in pigs.

1. Introduction

Fat content and composition play an important role in meat eating quality and human health. It is known that intramuscular fat (IMF) has a favorable effect on pork sensorial quality (Fernandez et al., 1999) and that some specific fatty acids, as the oleic fatty acid, may have beneficial cardiovascular properties (Williams, 2000). However, the continuous selection for lean meat content that happened in the last decades, due to the

positive genetic correlation among backfat thickness, IMF, and oleic fatty acid content (Solanes et al., 2009; Reixach and Estany, 2010), has not only led to reduce backfat thickness but also IMF and oleic content. Therefore, for increasing meat quality, particularly for dry-cured products, there is a need to find selection criteria allowing for an independent manipulation of lean and IMF content.

It has been demonstrated that selection against backfat thickness at restrained IMF content can be a useful strategy for reducing subcutaneous fat but not IMF (Reixach et al., 2009). However, the underlying mechanisms of fat distribution remain unexplained. Previous studies showed that there are both genetic (Gondret et al., 2001) and nutritional (Doran et al., 2006) factors that determine the site of fatty acid synthesis, providing evidence of differential metabolic properties of intramuscular and non-muscular adipocytes (Gardan et al., 2006; Cánovas et al., 2009a). Recent investigations have also identified genes involving physiological indicators that might be used as markers for assisted selection on meat quality and, specifically, for lipid deposition (Gao et al., 2007; Cánovas et al., 2009b).

Circulating serum concentrations of glucose, lipoproteins, cholesterol, and triglycerides are the result of the uptake and production by lipogenic tissues and therefore any diet or genetic-related changes in their levels may help to shed light on specific lipid metabolic pathways. Although subjected to complex control, involving hormonal, nutritional and genetic factors, such as insulin-like growth factors (Estany et al., 2007), they have the advantage that are easy to measure in live animals. Therefore, the present study aims at investigating the relationship of circulating lipid indicators at different ages with carcass and fat quality traits in commercial pigs, with regard to identification of potential biomarkers that may be associated specifically to subcutaneous fat or IMF content or composition.

2. Materials and methods

All the experimental procedures were approved by the Ethics Committee for Animal Experimentation of the University of Lleida.

2.1. Experiment, animals and sample collection

Pigs used in this study were from an experiment designed to test whether circulating lipid indicators were relevantly correlated (r>0.3) with carcass and fat quality traits. The experiment was conducted on 111 castrated Duroc pigs that were raised in two batches (57 and 54). Pigs had *ad libitum* access to commercial diets and were controlled at 185 days. The composition of the diet was different for each batch (**Table 1**) but not the energy content (DE, 3500 kcal/kg). In the first batch, pigs were also controlled at 120 days while in the second at 160 days. In each control three 10-mL samples of blood per pig were obtained with a syringe and kept in separate tubes. Blood collection took place between 8 and 10 a.m. after an overnight fast. Plasma was separated by centrifugation (3000g for 10 min at 4ºC) and stored at –40ºC until required for IGF-I determinations. The blood collected for serum analytical determinations was centrifuged and the serum was separated and stored at 4ºC until analysis. Once pigs were slaughtered at 215 days, carcass backfat thickness and loin thickness were measured at 6 cm off the midline between the third and fourth last ribs using an on-line ultrasound automatic scanner (Autofom®, SFK-Technology, Herlev, Denmark). At 24 h post-mortem, a sample of the *gluteus medius* and the *longissimus dorsi* muscles was removed. In 15 pigs per batch a sample of subcutaneous fat at the level where backfat thickness was measured was also taken. All samples were individually vacuum packaged and stored at –40ºC. The mean and the standard deviation of carcass and fat composition traits in each batch are given in **Table 2.**

Table 1. Composition of the diet fed during the finishing period.

Item	Batch 1	Batch 2
Dry matter (DM), g/kg	883.9	893.0
Crude protein, g/kg DM	168.0	163.4
Crude lipid, g/kg DM	69.3	73.3
Crude fibre, g/kg DM	51.7	62.9
Ash, g/kg DM	79.4	75.9
Fatty acid (FA)[1] composition, mg/g FA		
C14:0, myristic	9.2	11.1
C16:0, palmitic	173.5	200.4
C16:1, n-7 palmitoleic	13.1	17.6
C18:0, stearic	54.7	72.6
C18:1, n-9 oleic	311.7	338.9
C18:2, n-6 linoleic	383.6	308.7
C18:3, n-3 linolenic	38.3	31.0
C20:0, arachidic	2.9	2.5
C20:1, n-9 eicosenoic	7.4	9.4
C20:2, n-6 eicosadienoic	4.2	5.7
C20:4, n-6 arachidonic	1.6	2.2
SFA	240.1	286.6
MUFA	332.2	365.9
PUFA	427.7	347.6

[1]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).

Table 2. Mean (standard deviation) for carcass and fat composition traits in the pigs used in the experiment.

Trait	Batch 1	Batch 2
Number of pigs	57	54
Age at slaughter	214.3	216.4
Carcass weight, kg	93.2 (11.2)	91.4 (10.9)
Backfat thickness [1], mm	22.8 (0.5)	23.0 (0.5)
Loin thickness[1], mm	44.6[a] (0.9)	41.6 [b] (1.0)
Muscle *Gluteus medius* [2]		
IMF, % dry matter	15.3 (0.7)	15.3 (0.7)
SFA, % FA	34.2[a] (0.3)	29.8[b] (0.3)
MUFA, % FA	50.8[a] (0.4)	53.2[b] (0.4)
PUFA, % FA	15.0[a] (0.4)	17.0[b] (0.4)
Muscle *Longissimus dorsi*[2]		
IMF, % dry matter	12.6 (0.5)	12.7 (0.6)
SFA, % FA	35.2 (0.3)	34.6 (0.3)
MUFA, % FA	52.4 (0.3)	52.1 (0.3)
PUFA , % FA	12.4 (0.4)	13.3 (0.4)
Subcutaneous fat [2]		
SFA, % FA	36.7[a] (0.4)	27.1[b] (0.4)
MUFA, % FA	47.1[a] (0.3)	53.1[b] (0.3)
PUFA , % FA	16.3[a] (0.5)	19.7[b] (0.5)

[1] Ultrasonic backfat and loin thickness at 6 cm off the midline between the third and fourth last ribs.
[2] Intramuscular fat content (IMF) and saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids (see Table 1 for a complete definition).
[a,b] Means in the same row with unlike superscripts differ ($P<0.05$).

2.2. Laboratory analyses

Serum concentrations of iron (FE), glucose (GLU), total protein (TP), total cholesterol (TC), HDL-Cholesterol (HDL-C), LDL-Cholesterol (LDL-C), total triglycerides (TG), and percentage of lipoproteins (α-lipoprotein, β-lipoprotein, pre-β-lipoprotein and chylomicrons) were determined by using available kits in a commercial laboratory. Glucose and TP were determined by the hexokinase enzymatic (Bondar and Mead, 1974) and the Biuret method (Robinson and Hogden, 1940), respectively. Total

cholesterol, LDL-C and HDL-C were measured by enzymatic (CHOD-PAP method, Olympus diagnostics, Clare, Ireland) analytical chemistry (Allain et al., 1974), as well as plasma triglycerides (GPO-PAP colorimetric enzyme test, Olympus diagnostics, Clare, Ireland) (Fossati and Prencipe, 1982). The percentage of lipoproteins in serum was determined by agarose gel electrophoresis. Plasma insulin-like growth factor-I (IGF-I) concentration was measured in duplicate with a commercial available self-extraction ELISA kit by using an antibody raised against human IGF-I (Immunodiagnostic Systems, Boldon, UK) (Estany et al., 2007). Samples were analysed in four different assays. The intra-assay and inter-assay coefficients of variation were lower than 3% for all determinations except for IGF-I, which was 5.3% and 18.8%, respectively.

Once minced, a small quantity of subcutaneous fat and IMF samples was used to determine dry matter by drying 24 h at 100-102 ºC in an air oven, whereas the rest of the sample was freeze-dried. Fatty acid methyl esters were directly obtained by transesterification using a solution of boron trifluoride 20% in methanol (Rule, 1997) and determined by gas chromatography (Bosch et al., 2009). Intramuscular fat content was calculated as the sum of individual fatty acids expressed as triglyceride equivalents on a dry tissue basis. Then, IMF composition was calculated as the percentage of each individual fatty acid relative to total fatty acid and expressed as mg/g fatty acid. 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) FA contents were calculated.

2.3. Statistical analyses

The effect of age on blood lipid indicator concentrations was analyzed within batch by using a mixed model with age at control as fixed effect and animal as random effect. The age class was tested following the Kenward–Roger approach. Results are presented as the least-square means by age within batch. Correlation estimates between traits were obtained by the robust estimation method, which is useful in data that might have outliers. The method essentially ignores the outlying values by down-weighting them. Partial correlations of blood lipid indicators at 185 days with carcass and fat content and

composition traits were used for adjusting for the batch effect. All analyses were performed using JMP 9.0.0 statistical software (SAS Institute, 2010).

3. Results

3.1. Relationship between blood lipid indicators at different ages

The correlation between blood lipid indicator concentrations at different ages is shown in **Table 3**. Repeatability of TP, FE, TG, TC, and HDL-C were moderate but consistently positive, irrespective of the age interval used (from 0.26 to 0.62, for the correlation between 120 and 185 days, and from 0.32 to 0.54, for the correlation between 160 and 185 days). A less clear pattern was observed for LDL-C, β-lipoprotein, and IGF-I. Thus, for LDL-C, the only significant correlation ($P<0.01$) was between measurements at 120 and 185 days (r=0.59) while, for β-lipoprotein and IGF-I, the only one was between measurements at 160 and 185 days (r=0.35 and r=0.47, respectively, $P<0.01$). No significant correlation between measurements at different ages was found for GLU, chylomicron, α-lipoprotein, and pre-β-lipoprotein.

The concentration of blood lipid indicators differed by age (**Table 3**). Thus, GLU and TG decreased 12.5% and 16.7%, respectively, from 120 to 185 days while FE, IGF-I, and LDL-C levels increased by 61.6%, 36.1%, and 13.7% respectively. Chylomicrons and α-lipoprotein mostly increased from 160 to 185 days and TP, TC, HDL-C, and β-lipoproteins appeared to reach a maximum within the age period span studied. Circulating FE and IGF-I were higher in batch 1 than in batch 2 while chylomicrons showed higher values in batch 2 than in batch 1 ($P<0.01$).

Table 3. Least square means (± SE) for blood lipid indicator concentration by age (*n*: number of pigs per batch) and correlation between blood lipid indicators concentration at the two ages controlled within a batch.

	Batch 1			Batch 2		
	n=57			n=54		
Trait[1]	120 days	185 days	r	160 days	185 days	r
Glucose, mg/dl	77.7±1.6[a]	68.0±1.6[b]	-0.14	71.3±1.1[a]	65.8±1.1[b]	-0.02
Protein, g/l	64.9±0.6[a]	69.3±0.6[b]	0.44**	74.4±0.7[a]	69.6±0.7[b]	0.41**
Iron, mg/dl	83.5±4.4[a]	134.9±4.4[b]	0.26*	96.9±4.7	100.6±4.7	0.32*
Triglyceride, mg/dl	43.1±1.2[a]	35.9±1.2[b]	0.62**	42.8±1.8[a]	35.5±1.8[b]	0.37**
Cholesterol, mg/dl	105.2±2.1[a]	117.6±2.2[b]	0.52**	135.8±2.8[a]	118.8±2.8[b]	0.49**
HDL-C, mg/dl	38.4±0.8[a]	43.9±0.9[b]	0.39**	48.9±1.2[a]	44.1±1.2[b]	0.54**
LDL-C, mg/dl	68.1±1.6[a]	77.4±1.7[b]	0.59**	64.8±2.6[a]	79.3±2.6[b]	0.13
Chylomicron, %	1.6±0.1	1.7±0.1	-0.27	1.4±0.2[a]	2.7±0.2[b]	0.17
α-lipoprotein , %	43.0±0.7	43.8±0.7	0.01	41.2±0.7[a]	43.9±0.7[b]	0.27
β-lipoprotein, %	44.7±0.9	42.7±0.9	0.18	48.3±0.8[a]	42.4±0.8[b]	0.35**
Preβ-lipoprotein, %	10.5±0.7	11.8±0.7	-0.06	10.1±0.7	11.0±0.7	0.03
IGF-I, ng/ ml	77.1±7.1 [a]	104.9±7.2 [b]	0.19	49.8±3.3[a]	65.3±3.3[b]	0.47**

[1]HDL-C: HDL-Cholesterol; LDL-C: LDL-Cholesterol; IGF-I: Insulin-like growth factor-I.
[a,b] Means in the same row with unlike superscripts were significantly different within age class ($P<0.05$).
*$P<0.05$; **$P<0.01$.

3.2. Relationship between blood lipid indicators and commercial traits

Blood concentration of lipid indicators measured either at 120 days (**Table 4**), 160 days (**Table 5**), or at 185 days (**Table 6**) displayed a low-correlation pattern with carcass and fat quality traits, with the maximum correlation being between circulating IGF-I at 120 days and MUFA content in subcutaneous fat (r=0.65). Circulating TP showed the maximum correlation with IMF, particularly in *gluteus medius* (r=0.32, both at 120 and 160 days), but also with backfat thickness (r=0.49, at 120 days). However, serum HDL-C concentration proved to have the most consistent correlation structure with fat traits throughout age, with positive correlations with backfat thickness (r=0.38, at 120 days,

and r=0.28, at 185 days) and IMF content, both in *gluteus medius* (r=0.22, at 185 days) and in *longissimus dorsi* (r=0.20, at 185 days) muscles, and negative correlations with loin thickness (r=-0.30, at 185 days) and PUFA content, both in *gluteus medius* (r=-0.23, at 185 days) and in *longissimus dorsi* (r=-0.31, at 120 days, r=-0.39, at 160 days, and r=-0.21, at 185 days) muscles. A similar pattern was observed for GLU at 185 days, with positive correlations with backfat thickness (r = 0.33) and IMF in *longissimus dorsi* (r=0.20) but negative with PUFA content, particularly in *gluteus medius* (r = -0.25). Blood chylomicron concentration resulted to be more related to backfat thickness than to IMF, although inconsistently throughout age (from r=0.30, at 120 days, to -0.33, at 160 days). Circulating FE and IGF-I concentration at 185 days, but not at earlier ages, resulted also to be more correlated to backfat thickness (r=0.30 and r=0.33, respectively) than to IMF (from 0.00 to 0.20). These three last traits, chylomicrons, FE and IGF-I, were the only ones affected by batch (**Table 3**), with pigs in batch 1 showing lower levels of chylomicrons and higher of FE and IGF-I than pigs in batch 2. Because loin thickness and SFA were also higher in batch 1 than in batch 2 (**Table 2**) a negative correlation across batches of chylomicrons with loin thickness (r=-0.24) and SFA (from -0.53 to -0.23), as well as a positive correlation of FE and IGF-I with SFA (from 0.36 to 0.60, results not shown) was found. Taken globally, circulating lipid indicators at 120 days resulted to be similarly correlated to fat traits than those measured at 185 days. However, blood lipid indicators showed a low potential for predicting IMF content and fatty acid composition (SFA, MUFA, and PUFA). Stepwise regression analysis demonstrated that blood lipid indicators at 185 days explained at most 6.1%, 22%, 4.8%, and 13.6% of the variation of IMF, SFA, MUFA, and PUFA, respectively.

Table 4. Correlation of blood biomarkers at 120 days of age with carcass and fat composition traits.

	Carcass traits[2] (n=55)			*Gluteus medius* muscle[2] (n=54)				*Longissimus dorsi* muscle[2] (n=54)				Subcutaneous fat[2] (n=15)		
Trait at 120 days[1]	CW	BT	LT	IMF	SFA	MUFA	PUFA	IMF	SFA	MUFA	PUFA	SFA	MUFA	PUFA
Glucose, mg/dl	0.18	0.07	-0.06	-0.02	-0.04	-0.08	-0.07	0.06	-0.17	0.08	0.05	-0.29	0.14	0.25
Protein, g/l	0.01	**0.49***	**-0.44****	**0.32***	-0.07	0.04	-0.17	0.15	-0.03	0.20	-0.13	0.22	0.40	-0.48
Iron, mg/dl	0.13	0.22	0.03	-0.08	0.06	0.03	0.14	0.01	-0.16	0.07	-0.04	-0.14	0.46	-0.07
Triglyceride, mg/dl	0.16	-0.21	-0.14	-0.07	0.04	-0.18	0.06	0.03	0.13	**-0.29***	0.02	0.05	-0.02	-0.10
Cholesterol, mg/dl	0.09	0.22	0.06	0.11	0.13	-0.11	-0.07	0.10	0.07	-0.11	-0.11	-0.30	0.38	-0.17
HDL-C, mg/dl	**0.28***	**0.38***	-0.04	0.20	0.13	0.10	-0.24	0.12	0.08	0.17	**-0.31***	0.13	0.38	-0.37
LDL-C, mg/dl	0.13	0.10	0.18	0.09	0.02	**-0.27***	0.05	-0.06	0.05	**-0.36****	0.13	-0.15	-0.48	0.28
Chylomicron, %	**0.32***	**0.30***	-0.12	-0.06	0.13	0.00	-0.09	0.01	-0.19	**0.27***	-0.11	0.50	0.49	-0.30
α-lipoprotein ,%	0.04	**0.39***	0.02	0.01	0.02	0.10	-0.06	-0.07	**-0.28***	0.14	0.02	0.22	0.47	-0.44
β-lipoprotein, %	-0.01	**-0.28***	0.14	0.00	-0.17	0.17	0.05	0.12	0.11	-0.03	-0.02	0.05	0.06	0.00
Preβ-lipoprotein,%	-0.25	0.08	-0.14	0.25	**0.29***	-0.06	-0.18	-0.07	0.04	-0.14	0.04	-0.02	-0.08	0.04
IGF-I, ng/ ml	-0.06	0.10	-0.01	0.13	0.13	-0.08	0.04	-0.11	**-0.27***	**-0.34***	0.10	0.32	**0.65****	**-0.51***

[1] See footnote in Table 3 for abbreviations.
[2] Carcass weight (CW) and ultrasonic backfat (BT) and loin (LT) thickness at 6 cm off the midline between the third and fourth last ribs; IMF: intramuscular fat content; see Table 1 for saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acid definition.
*$P<0.05$; **$P<0.001$.

Table 5. Correlation of blood biomarkers at 160 days of age with carcass and fat composition traits.

	Carcass traits[2] (n=53)			*Gluteus medius* muscle[2] (n=53)				*Longissimus dorsi* muscle[2] (n=52)				Subcutaneous fat[2] (n=15)		
Trait at 160 days[1]	CW	BT	LT	IMF	SFA	MUFA	PUFA	IMF	SFA	MUFA	PUFA	SFA	MUFA	PUFA
Glucose, mg/dl	-0.01	-0.20	0.02	0.13	0.10	0.00	0.00	-0.03	-0.15	0.01	0.13	0.01	0.04	0.23
Protein, g/l	0.09	0.17	-0.01	**0.32***	0.26	0.03	-0.11	0.24	0.22	-0.03	-0.24	0.23	0.02	-0.39
Iron, mg/dl	0.26	-0.07	0.02	0.02	-0.03	-0.22	0.08	0.05	-0.07	-0.03	-0.07	-0.38	0.45	0.01
Triglyceride, mg/dl	0.21	0.26	0.02	-0.10	-0.10	-0.21	0.06	0.11	**0.33***	-0.11	-0.14	0.16	0.17	-0.33
Cholesterol, mg/dl	0.17	0.14	-0.01	0.06	-0.14	0.14	-0.08	0.23	0.16	0.08	**-0.36****	0.05	0.28	-0.30
HDL-C, mg/dl	0.25	0.23	-0.15	0.13	-0.22	0.15	-0.15	0.25	0.18	0.23	**-0.39****	0.04	0.45	-0.18
LDL-C, mg/dl	0.14	0.23	-0.04	-0.04	-0.17	0.05	0.07	0.23	0.05	0.09	-0.24	0.22	0.09	-0.24
Chylomicron, %	-0.04	**-0.33***	**0.34***	-0.19	-0.18	0.17	0.09	0.04	-0.18	-0.20	0.03	0.00	-0.17	0.18
α-lipoprotein ,%	0.00	-0.07	0.06	0.05	-0.23	0.20	-0.04	-0.13	-0.28	0.26	0.08	-0.07	-0.18	0.17
β-lipoprotein, %	0.03	0.14	-0.05	-0.22	-0.17	-0.15	0.08	-0.16	0.09	-0.26	-0.26	-0.19	0.43	-0.12
Preβ-lipoprotein, %	0.01	-0.07	0.17	0.01	-0.07	-0.12	0.03	0.18	0.22	-0.16	-0.12	**0.62***	-0.10	-0.34
IGF-I, ng/ ml	0.13	-0.10	0.13	-0.02	**-0.37****	0.12	0.10	-0.26	**-0.28***	0.19	**0.31***	0.14	-0.02	-0.02

[1] See footnote in Table 3 for abbreviations.
[2] Carcass weight (CW) and ultrasonic backfat (BT) and loin (LT) thickness at 6 cm off the midline between the third and fourth last ribs; IMF: intramuscular fat content; see Table 1 for saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acid definition.
*$P<0.05$; **$P<0.001$.

Table 6. Correlation of blood biomarkers at 185 days of age with carcass and fat composition traits adjusted for batch.

	Carcass traits[2] (n=108)			*Gluteus medius* muscle[2] (n=107)				*Longissimus dorsi* muscle[2] (n=105)				Subcutaneous fat[2] (n=30)		
Trait at 185 days[1]	CW	BT	LT	IMF	SFA	MUFA	PUFA	IMF	SFA	MUFA	PUFA	SFA	MUFA	PUFA
Glucose, mg/dl	0.07	**0.33****	-0.18	0.13	0.14	0.16	**-0.25****	**0.20***	0.05	0.17	-0.19	0.20	0.11	-0.29
Protein, g/l	-0.10	0.15	-0.16	0.13	0.02	0.01	-0.06	0.13	-0.05	0.16	-0.06	0.11	0.15	-0.18
Iron, mg/dl	0.15	**0.30****	0.05	0.06	0.05	0.05	-0.14	**0.20***	0.10	0.16	-0.16	0.18	-0.05	-0.14
Triglyceride, mg/dl	-0.01	-0.12	-0.06	-0.09	0.03	**-0.25***	0.06	0.10	0.17	-0.15	-0.04	0.19	-0.31	-0.05
Cholesterol, mg/dl	0.06	0.16	-0.20	0.11	0.03	0.07	-0.16	0.10	0.07	0.06	-0.09	-0.16	0.17	-0.11
HDL-C, mg/dl	0.07	**0.28****	**-0.30****	**0.22***	0.07	0.15	**-0.23***	**0.20***	0.08	**0.21***	**-0.21***	0.00	0.12	-0.19
LDL-C, mg/dl	0.15	0.18	-0.14	0.11	0.10	0.07	**-0.20***	0.17	0.10	0.11	-0.17	-0.13	0.24	-0.18
Chylomicron, %	-0.05	-0.07	-0.11	0.02	-0.16	0.07	0.08	0.00	0.00	0.00	0.02	-0.33	-0.06	**0.36***
α-lipoprotein ,%	-0.03	-0.07	0.08	-0.02	-0.23	0.09	0.07	-0.03	-0.06	0.07	0.07	0.12	0.03	-0.02
β-lipoprotein, %	0.16	-0.06	0.19	-0.02	-0.13	0.03	0.06	-0.14	-0.11	-0.01	0.12	-0.32	0.33	0.10
Preβ-lipoprotein, %	-0.03	0.12	-0.14	-0.04	**0.34****	-0.15	-0.10	0.18	**0.29****	-0.14	-0.16	**0.43****	-0.30	-0.13
IGF-I, ng/ ml	0.10	**0.33****	0.09	0.00	0.05	0.09	-0.11	0.01	-0.02	0.11	-0.04	0.14	-0.03	-0.08

[1] See footnote in Table 3 for abbreviations.
[2] Carcass weight (CW) and ultrasonic backfat (BT) and loin (LT) thickness at 6 cm off the midline between the third and fourth last ribs; IMF: intramuscular fat content; see Table 1 for saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acid definition.
*$P<0.05$; **$P<0.001$.

4. Discussion

Circulating lipid indicators were in the range of the expected values (Coles, 1980; Kaneko, 1989; Cánovas et al., 2010), except for LDL-C, whose values were a little higher as compared to other studies (Fernandez-Robredo et al., 2005; Kojima et al., 2008). This might be due to an intrinsic metabolic feature of the Duroc pigs, since Gallardo et al. (2008), also in Duroc, found similar LDL-C concentrations as in the present study.

Despite the complex biological mechanisms involved in the regulation of fat deposition, several studies have investigated the use of blood parameters to predict IMF and backfat thickness. However, results were not very promising, excepting for TC, in pigs (Tremere et al., 1966), and for TG and GLU, in cattle (Adachi et al., 1999; Hocquette et al., 1999), where these lipid indicators displayed a positive correlation with fat deposition traits. Steele et al. (1972) found that the Duroc pigs selected against backfat thickness showed lower levels of serum GLU and TC than those selected for increased levels, and Etherton and Kris-Etherton (1980) encountered a marked alteration in the plasma lipoprotein composition between lean and obese swine. Contrarily, Mersmann et al. (1982) did not find differences between lean and obese pigs for serum lipids. Casellas et al. (2010) reported moderate estimates for heritability of serum lipid traits, thereby confirming that these traits may be altered by selection. In fact, genetically high and low plasma cholesterol pigs were developed by Pond et al. (1997) after seven generations of selection. This latter author found that pigs selected for low cholesterol had lower TC, HDL-C, TG and body weight than those selected for high cholesterol. However, as indicate the value of the correlations found here between blood indicators and fat traits, relevant correlated responses on blood lipid indicators should only be expected after intense selection on production traits. This is the case reported in Steele et al. (1972), whose lines for high and low backfat thickness were developed after seventeen generations of selection.

Circulating lipid indicators were weakly correlated with growth and fatness traits. Rauw et al. (2007), also in Duroc, found similar correlations between serum lipids and

production traits than in our research. Etherton and Kris-Etherton (1980) showed that plasma-TG, very low-density lipoproteins (VLDL), and HDL-C were highly and positively correlated in 1-year old pigs. Although HDL-C has been related to pig adiposity in several studies (Etherton and Kris-Etherton, 1980; Taylor et al., 1992), the physiological reason for the relationship of HDL-C with backfat thickness and IMF is unclear. HDL-Cholesterol plays a major role in recirculating cholesterol back to the liver from peripheral tissues but not in transporting triglycerides to adipose tissue, like VLDL, which circulate through the blood giving up their triglycerides to fat and muscle until their remnants are modified and converted into LDL (Pond and Mersmann, 1996). A possible explanation is that HDL-C and VLDL are metabolically related, for instance, increasing VLDL half-life. Thus, Birchbauer et al. (1992) showed that the half-life of VLDL increases by transferring apoE to HDL-C. Several studies observed a positive association between HDL-C and body weight at different ages (Pond et al., 1993; Rauw et al., 2007; Gallardo et al., 2008). In the present study, HDL-C was positively correlated to carcass weight at 120 days but not at 185 days, indicating that this relationship, at least in Duroc, is primarily found at earlier stages of growth.

Circulating IGF-I and FE at later ages displayed a similar correlation pattern with backfat thickness and IMF. The IGF-I system is implicated in the regulation of cellular response to energy-restriction (Simmen et al., 1998) and it has been demonstrated that IGF-I, together with growth hormone, plays an important role in hormonal regulation of adipogenesis, and in growth and differentiation of preadipocytes (Smith et al., 1988; Louveau and Gondret, 2004). In accordance with previous works in pigs (Bunter et al., 2005; Estany et al., 2007), the concentration of plasma IGF-I was found to be not neutral with respect to fatness. However, contrarily to results here, IGF-I, as well as FE (Pietruszka et al., 2009), has been found to be primarily related to increased growth and lean content. Because circulating IGF-I displayed a marked nonlinear trend from shortly after weaning to peripuberty, and it is also easily influenced by recent feeding events (Therkildsen et al., 2004), its effect on fat content and composition may change across genetic types and measurement time-points.

A relevant problem for using serum lipid as biomarkers is that they are sensitive to environmental conditions, as can be seen from changes of circulating FE or IGF-I by batch, and to age or pig's physiological state. Thus, GLU and TG gradually declined with age, whereas LDL-C and IGF-I increased. Total cholesterol, HDL-C, and TP followed a pattern suggesting a maximum around 160 days of age, which is rather consistent with findings in previous studies (Mersmann et al., 1982; Gondret et al., 2004). It has been suggested by these latter authors that the liver may use greater amounts of glucose to ensure increased lipogenic needs with advancing age. In fact, Scott et al. (1981) found that, in pigs, glucose incorporation into total lipids of adipose tissue was affected by age, with a maximum at 4 months and declined thereafter. Lipogenic tissues display a differential maturation capacity with age. Subcutaneous fat might have a major capacity of fatty acid synthesis until 4-5 months of age and from then onwards other fat depots would be more active in fat deposition (Kris-Etherton and Etherton, 1982). Moreover, substrate for TG formation shifts from fatty acids synthesized within the adipocytes to fatty acids taken from plasma (Etherton and Allen, 1980; Etherton et al., 1981). It has been suggested that around 160 days there can be an age-associated change in LDL-C metabolism, in connection with several studies that found that the LDL_1/LDL_2 ratio varied throughout the growing period in pigs (Jackson et al., 1976; Jürgens et al., 1981; Birchbauer et al., 1992). There is also evidence that chemical composition of lipoproteins is modified with pig development, as a result of changes occurring in the cellular metabolism and nutritional needs of the organism (Johansson and Karlsson, 1982).

Conclusions

Blood lipid indicator concentrations, likely because they are age-mediated, were only moderately repeatable across time, showing a very weak correlation structure with commercial traits. Circulating TP was the best biomarker for early estimation of fatness but HDL-C was the most consistent throughout age. Insulin-like growth factor-I at later stages of growth may help for specifically biomarking subcutaneous fat against IMF content. However, neither of them resulted to be good predictors. This fact, together

with their relatively high determination costs, prevents from using them as routine biomarkers for fat deposition in pigs.

References

Adachi, K., H. Kawano, K. Tsuno, Y. Nomura, N. Yamamoto, A. Arikawa, A. Tsuji, M. Adachi, T. Onimaru, and K. Ohwada. 1999. Relationship between serum biochemical values and marbling scores in Japanese black steers. J. Vet. Med. Sci. 61:961-964.

Allain, C. C., L. S. Poon, C. S. G. Chan, W. Richmond, and P. C. Fu. 1974. Enzymatic determination of total serum cholesterol. Clin. Chem. 20:470-475.

Birchbauer, A., W. Gerhard, and G. Knipping. 1992. Metabolism of very low density lipoproteins in the pig. An *in vivo* study. Int. J. Biochem. 24:1591-1597.

Bondar, R J. L. and D. C. Mead. 1974. Evaluation of Glucose-6-Phosphate Dehydrogenase from *Leuconostoc mesenteroides* in the hexokinase method for determining glucose in serum. Clin. Chem. 20:586-590.

Bosch, L., M. Tor, J. Reixach, and J., Estany. 2009. Estimating intramuscular fat content and fatty acid composition in live and post-mortem samples in pigs. Meat Sci. 82:432-437.

Bunter, K. L., S. Hermesch, B. G. Luxford, H-U. Graser, and R. E. Crump. 2005. Insulin-like growth factor-I measured in juvenile pigs is genetically correlated with economically important performance traits. Aust. J. Exp. Agric. 45:783-792.

Cánovas, A., J. Estany, M. Tor, R. N. Pena, and O. Doran. 2009a. Acetyl-CoA carboxylase and stearoyl-CoA desaturase protein expression in subcutaneous adipose tissue is reduced in pigs selected for decreased backfat thickness at constant intramuscular fat content. J. Anim. Sci. 87:3905-3914.

Cánovas, A., R. Quintanilla, D. Gallardo, I. Díaz, J. L. Noguera, O. Ramírez, and R. N. Pena. 2009b. Functional and association studies on the pig HMGCR gene, a cholesterol-synthesis limiting enzyme. Animal 4: 224-233.

Cánovas, A., R. Quintanilla, M. Amills, and R. N. Pena. 2010. Muscle transcriptomic profiles in pigs with divergent phenotypes for fatness traits. BMC Genomics 11: 372.

Casellas, J., J. L. Noguera, J. Reixach, I. Díaz, M. Amills, and R. Quintanilla. 2010. Bayes factor analyses of heritability for serum and muscle lipid traits in Duroc pigs. J. Anim. Sci. 88:2246-2254.

Coles, E. H. 1980. Veterinary clinical pathology, third ed. Saunders, Philadelphia.

Doran, O., S. K. Moule, G. A. Teye, F. M. Whittington, K. G. Hallett, and J. D. Wood. 2006. A reduced protein diet induces stearoyl-CoA desaturase protein expression in pig muscle but not in subcutaneous adipose tissue: Relationship with intramuscular lipid formation. Br. J. Nutr. 95:609-617.

Estany, J., M. Tor, D. Villalba, L. Bosch, D. Gallardo, N. Jiménez, L. Altet, J. L. Noguera, J. Reixach, M. Amills, and A. Sánchez. 2007. Association of CA repeat polymorphism at intron 1 of insulin-like growth factor (IGF-I) gene with circulating IGF-I concentration, growth, and fatness in swine. Physiol. Genomics 31:236-243.

Etherton, T. D., and C. E. Allen. 1980. Effects of age and adipocyte size on glucose and palmitate metabolism and oxidation in pigs. J. Anim. Sci. 50:1073-1084.

Etherton, T. D., and P. M. Kris-Etherton. 1980. Characterization of plasma lipoproteins in swine with different propensities for obesity. Lipids 15:823-829.

Etherton, T. D., E. D. Aberle, E. H. Thompson, and C. E Allen. 1981. Effects of cell size and animal age on glucose metabolism in pig adipose tissue. J. Lipid Res. 22: 72-80.

Fernandez, X., G. Monin, A. Talmant, J. Mourot, and B. Lebret. 1999. Influence of intramuscular fat content on the quality of pig meat-2. Consumer acceptability of *m. longissimus lumborum.* Meat Sci. 53:67-72.

Fernandez-Robredo, P., D. Moya, J. A. Rodriguez, and A. Garcia-Layana. 2005. Vitamins C and E reduce retinal oxidative stress and nitric oxide metabolites and prevent ultrastructural alterations in porcine hypercholesterolemia. Invest. Ophthalmol. Vis. Sci. 46:1140-1146.

Fossati, P., and L. Prencipe. 1982. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin. Chem. 28:2077-2080.

Gallardo, D., R. N. Pena, M. Amills, L. Varona, O. Ramírez, J. Reixach, I. Díaz, J. Tibau, J. Soler, J. M. Prat-Cuffi, J. L. Noguera, and R. Quintanilla. 2008. Mapping of quantitative trait loci for cholesterol, LDL, HDL, and triglyceride serum concentrations in pigs. Physiol. Genomics 35:199-209.

Gao Y., R. Zhang, X. Hu, and N. Li. 2007. Application of genomic technologies to the improvement of meat quality of farm animals. Meat Sci. 77:36-45.

Gardan, D., F. G. Gondret, and I. Louveau. 2006. Lipid metabolism and secretory function of porcine intramuscular adipocytes compared with subcutaneous and perirenal adipocytes. Am. J. Physiol. Endocrinol. Metab. 291: E372-E380.

Gondret, F., P. Ferré, and I. Dugail. 2001. ADD-1/SREBP-1 is a major determinant of tissue differential lipogenic capacity in mammalian and avian species. J. Lipid Res. 42:106-113.

Gondret, F., J-F. Hocquette, and P. Herpin. 2004. Age-related relationships between muscle fat content and metabolic traits in growing rabbits. Reprod. Nutr. Dev. 44:1-16.

Hocquette, J-F., P. Bas, D. Bauchart, M. Vermorel, and Y. Geay. 1999. Fat partitioning and biochemical characteristics of fatty tissues in relation to plasma metabolites and hormones in normal and double-muscled young growing bulls. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 122:127–138.

Jackson, R. L., O. D. Taunton, R. Segura, J. G. Gallagher, H. F. Hoff, and A. M. Gotto. 1976. Comparative studies on plasma low density lipoproteins from pig and man. Comp. Biochem. Physiol. 53 B:245-253.

Johansson, M. B. N., and B. W. Karlsson. 1982. Lipoprotein and lipids profiles in the blood serum of the fetal, neonatal and adult pig. Biol. Neonate 42:127-137.

Jürgens, G., G. M. J. Knipping, P. Zipper, R. Kayushina, G. Degovics, and P. Laggner. 1981. Structure of two subfractions of normal porcine (*Sus domesticus*) serum Low-Density Lipoproteins. X-ray small-angle scattering studies. Biochemistry 20:3231-3237.

Kaneko, J. J. 1989. Clinical biochemistry of domestic animals, fourth ed. Academic Press, San Diego.

Kojima, M., M. Sekimoto, and M. Degawa. 2008. Gender-related differences in the level of serum lipids in Meishan pigs. JHS. 54:97-100.

Kris-Etherton, P. M., and T. D. Etherton. 1982. The role of lipoproteins in lipid metabolism of meat animals. J. Anim. Sci. 55:804-817.

Louveau, I., and F. Gondret. 2004. Regulation of development and metabolism of adipose tissue by growth hormone and the insulin-like growth factor system. Domest. Anim. Endocrinol. 27:241-255.

Mersmann, H. J., W. G. Pond, and J. T. Yen. 1982. Plasma glucose, insulin and lipids during growth of genetically lean and obese swine. Growth 46:189-198.

Pietruszka, A., E. Jacyno, A. Kolodziej, M. Kawęcka, C. Elzanowski, and B. Matysiak. 2009. Effects of L-carnitine and iron diet supplementations on growth performance, carcass characteristics and blood metabolites in fattening pigs. Agric. Food Sci.18:27-34.

Pond, W. G., H. J. Mersmann, P. D. Klein, L. L. Ferlic, W. W. Wong, D. L. Hachey, P. A. Schoknecht, and S. Zhang. 1993. Body weight gain is correlated with serum

cholesterol at 8 weeks of age in pigs selected for four generations for low and high serum cholesterol. J. Anim. Sci. 71: 2406-2411.

Pond, W. G., and H. J. Mersmann. 1996. Genetically diverse pig models for neonatal cholesterol nutrition: a review. Nutr. Res. 16:707-721.

Pond, W. G., D. R. Su, and H. J. Mersmann. 1997. Divergent concentrations of plasma metabolites in swine selected for seven generations for high or low plasma total cholesterol. J. Anim. Sci. 75:311-316.

Rauw, W. M., O. Portolés, D. Corella, J. Soler, J. Reixach, J. Tibau, J. M. Prat, I. Diaz, and L. Gómez-Raya. 2007. Behaviour influences cholesterol plasma levels in a pig model. Animal 1: 865-871.

Reixach, J., M. Tor, and J. Estany. 2009. Respuesta a la selección contra grasa dorsal a grasa intramuscular constante en cerdos Duroc. Pages 120-122 in XIII Jornada sobre Producción Animal. M. Joy, J. H. Calvo, C. Calvete, M. A. Latorre, I. Casasus, A. Bernues, B. Panea, A. Sanz, and J. Balcells, ed. AIDA, Zaragoza, Spain.

Reixach, J., and J. Estany. 2010. Genetic parameters for intramuscular oleic fatty acid content in a Duroc line (Abstract). Proc. 9th World Congr. Genet. Appl. Livest. Produc., Leipzig, Germany: 294.

Robinson, H. W., and C. G. Hogden. 1940. The biuret reaction in the determination of serum proteins. J. Biol. Chem. 135:707-725.

Rule, D. C. 1997. Direct transesterification of total fatty acids of adipose tissue and of freeze-dried muscle and liver with boron-trifluoride in methanol. Meat Sci. 46:23-32.

SAS Institute (2010) Using JMP 9 (SAS Institute, Cary, NC).

Scott, R. A., S. G. Cornelius, and H. J. Mersmann. 1981. Effects of age on lipogenesis and lipolysis in lean and obese swine. J. Anim. Sci. 52:505-511.

Simmen, F. A., L. Badinga, M. L. Green, I. Kwak, S. Song, and R. C. M. Simmen. 1998. The porcine insulin-like growth factor system: at the interface of nutrition, growth and reproduction. J. Nutr. 128:315S-320S.

Smith, P. J., L. S. Wise, R. Berkowitz, C. Wan, and C. S. Rubin. 1988. Insulin-like growth factor-I is an essential regulator of the differentiation of 3T3-L1 adipocytes. J. Biol. Chem. 263:9402-9408.

Solanes, F. X., J. Reixach, M. Tor, J. Tibau, and J., Estany. 2009. Genetic correlations and expected response for intramuscular fat content in a Duroc pig line. Livest. Sci. 123:63-69.

Steele, N. C., L. T. Frobish, R. J. Davey, and M. Keeney. 1972. Effect of selection for backfat thickness in swine on lipogenic enzyme levels. J. Anim. Sci. 35:225.

Taylor, J. A., D. N. Salter, W. H. Close, and G. H. Laswai. 1992. Serum concentrations of insulin-like growth factor 1 and cholesterol in relation to protein and fat deposition in growing pigs. Anim. Prod. 55:257-264.

Therkildsen, M., M. Vestergaard, H. Busk, M. T. Jensen, B. Riis, A. H. Karlsson, L. Kristensen, P. Ertbjerg, and N. Oksbjerg. 2004. Compensatory growth in slaughter pigs-*in vitro* muscle protein turnover at slaughter, circulating IGF-I, performance and carcass quality. Livest. Prod. Sci. 88:63-75.

Tremere, A.W., B. D. Owen, and J. M. Bell. 1966. Relationship between blood serum lipid and protein constituents and carcass measurements in swine. J. Anim. Sci. 25: 138-141.

Williams, C. M. 2000. Dietary fatty acids and human health. Ann. Zootech. 49:165-180.

CHAPTER III

Effect of selection against backfat on hepatic lipogenic enzymes

ABSTRACT

This study investigated (i) whether genetic selection for decreased backfat thickness at constant intramuscular fat (IMF) affects the expression of lipogenic enzymes in pig liver and (ii) whether expression of the hepatic lipogenic enzymes is related to subcutaneous fat and IMF fatty acid composition. The enzymes investigated were fatty acid synthase (FAS), stearoyl-CoA desaturase and Δ6-desaturase (Δ6d). Experiments were conducted on 30 barrows (15 control and 15 selected). Selected pigs had lower backfat thickness, which was accompanied by a reduced expression of the hepatic FAS and Δ6d when compared to control pigs. There was a trend towards a positive relationship between FAS and Δ6d protein expression and saturated and polyunsaturated fatty acids content respectively, in subcutaneous fat but not in muscle. It was concluded that selection against backfat thickness is associated with changes in pig hepatic lipogenic proteins, which however poorly influence the fatty acid composition of tissues.

1. Introduction

Intramuscular fat (IMF) content is one of the important characteristics of meat quality (Fernandez et al., 1999). The amount and type of IMF have direct impact on human health (Valsta et al., 2005). During the last decades IMF content in pigs has been continuously decreasing as a result of selection towards leaner genotypes which compromised eating quality of pork (Merks, 2000; Hermesch, 2004). Although it is known that there is positive relationship between IMF and subcutaneous fat (Solanes et al., 2009), there is evidence that genetic correlation between these two fat depots is not always strong and that it is possible to manipulate IMF and subcutaneous fat independently by dietary or genetic means (Doran et al., 2006; Solanes et al., 2009). Manipulation of IMF and subcutaneous fat deposition is associated with regulation of expression of lipogenic enzymes in these tissues (Doran, et al., 2006; Cánovas et al., 2009a; Ntawubizi, et al., 2009). Furthermore it is unknown whether deposition of IMF and subcutaneous fat can be influenced by lipogenic processes taking place in other organs, and particularly in the liver. In spite of the fact subcutaneous adipose tissue is

the main site of *de novo* fatty acid synthesis in pigs (O´Hea and Leveille 1969), liver plays a crucial role in the remodelling of body lipid composition (Theil and Lauridsen, 2007; Reiter et al., 2007). Lipogenesis involves the biosynthesis of palmitic fatty acid, which in turn, may be then converted into a range of longer chain of fatty acids via elongation and/or desaturation reactions. Elongases (ELOVL) enzymes along the desaturases and fatty acid synthase (FAS) work coordinately to synthesize saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA), which are incorporated into complex lipids (Wang et al., 2006; Paton and Ntambi, 2008). Particularly, FAS, stearoyl-CoA desaturase (SCD), ELOVL3 and ELOVL6 are involved in the biochemical pathway of the long-chain MUFA biosynthesis. Furthermore, polyunsaturated fatty acids (PUFA) are obtained by a chain of desaturation and elongation reactions of the essential precursors linoleic and α-linolenic acids catalysed by Δ5-desaturase (Δ5d), Δ6-desaturase (Δ6d), ELOVL2 and ELOVL5 (Wang et al., 2006; Matsuzaka and Shimano, 2009; Guillou et al., 2010). Therefore, lipogenesis and biosynthesis of PUFA from precursors are important processes that influence tissue fatty acid composition (Ntawubizi et al., 2010).

In spite of increasing body of evidence that IMF and subcutaneous fat deposition can be regulated independently, the molecular mechanisms underlying fat partitioning in pigs remain unclear, and therefore, the main objectives of this study were (i) to investigate whether genetic selection for decreased subcutaneous fat thickness at constant IMF affects the expression of hepatic lipogenic enzymes and fatty acid profiles in pigs; and (ii) to determine whether expression of the hepatic lipogenic enzymes is related to fatty acid composition of subcutaneous fat and IMF. The study focused on the expression of the key lipogenic enzymes FAS, SCD, and Δ6d catalysing the biosynthesis of SFA, MUFA, and PUFA respectively.

2. Materials and methods

All the experimental procedures related to animal trials were approved by the Ethics Committee for Animal Experimentation of the University of Lleida, Spain. The pigs were reared and slaughtered at the facilities of the commercial company Grupo Batallé, Spain.

2.1. Animals, diet and sample collection

The experiment was conducted on 30 purebred Duroc barrows (the selected and the control groups, 15 animals per group) randomly chosen from 2 larger groups as described below. The groups differed in backfat thickness, but not in IMF content (Reixach, et al., 2009). The genetically selected and the control groups were constituted according to the mid-parent (litter) breeding values for backfat thickness at 180 days of age and for IMF content in the *gluteus medius* muscle adjusted for carcass weight, which were predicted using the model described in Solanes et al. (2009). Litters in the selected group were selected against backfat thickness while maintaining IMF content to the values most similar to those in the control group. At the age of 11 wk, 2 barrows per litter were randomly taken and moved to a finishing facility, where they were kept in pens with 12 animals per pen until the age of 30 wk. This selection process was repeated in 4 consecutive batches, in which pigs in both groups were raised under same conditions and managed in the same way. Pigs used in the present study were randomly taken from the last batch.

During the fattening period pigs were given *ad libitum* access to the feed. A commercial pelleted finishing diet (Esporc, Ruidarenes, Girona, Spain) was given from the day 160 onwards. Composition of this diet is given in **Table 1**. Feed analyses were performed in triplicate. Dry matter (DM) was determined by oven-drying at 100 to 102ºC for 24 h. Ash content was determined by muffle-heating at 550ºC until constant weight. Crude protein was analyzed by the Kjeldahl method (AOAC, 2000), crude lipid content was determined by Soxhlet fat analysis (AOAC, 2000), and crude fibre was analyzed by acid and alkaline digestion with a Dosi-Fiber extractor (Selecta, Barcelona, Spain; AOAC, 2000). Analyses of fatty acids composition of diet were performed as described in the section 2.2. after extracting the total lipids by the method of Hanson and Olley (1963).

Table 1. Composition of the diet during the finishing period.

Item	Amount
Dry matter (DM), g/kg	895.4
Crude protein, g/kg of DM	159.9
Crude lipid, g/kg of DM	68.4
Crude fibre, g/kg of DM	58.8
Ash, g/kg of DM	66.4
Fatty acid composition, mg/g of fatty acids	
C14:0, myristic	11.1
C16:0, palmitic	201.2
C18:0, stearic	69.9
C20:0, arachidic	2.2
Total SFA	284.4
C16:1, n-7 palmitoleic	23.8
C18:1, n-9 oleic	380.7
C20:1, n-9 eicosenoic	9.6
Total MUFA	414.1
C18:2, n-6 linoleic	258.8
C18:3, n-3 linolenic	23.9
C20:2, n-6 eicosadienoic	6.6
C20:4, n-6 arachidonic	2.3
Total PUFA	291.6

Weight of the pigs was recorded at 26 and 29 wk of age, and their backfat thickness was ultrasonically measured at 5 cm off the midline at the position of the last rib (Piglog 105, SFK-Technology, Herlev, Denmark). All the pigs were slaughtered at 30 wk of age, and carcass backfat was measured at 6 cm off the midline between the third and fourth last ribs using an on-line ultrasound automatic scanner (AutoFOM, SFK-Technology, Herlev, Denmark).

Samples of pig liver and subcutaneous fat were collected immediately after slaughter, snap-frozen and stored at –80ºC until analysed. The samples of subcutaneous fat were taken at the level of the third and fourth ribs. Samples of muscle were collected

after chilling the carcasses for about 24 h at 2ºC vacuum packaged, and stored in deep freeze until required. It has been previously demonstrated that these storage conditions do not affect protein abundance (Doran et al., 2006).

2.2. Analysis of fatty acid composition by gas chromatography

Once defrosted, the samples of liver, muscle, and subcutaneous fat were freeze-dried and homogenized by mixing with sand and using a glass stirring rod. Dry matter was calculated as the weight difference before and after freeze-drying. A representative aliquot from the homogenized samples was used for determining fatty acid composition. Fatty acid methyl esters (FAME) were directly obtained by transesterification using a solution of 20% boron trifluoride in methanol (Rule, 1997). Methyl esters were determined by gas chromatography using a capillary column SP2330 (30 m x 0.25 mm fused silica capillary coated with a 0.20 μm film of poly 80% bicyanopropyl-20% cyanopropylphenyl siloxane, Supelco, Bellefonte, PA) and a flame ionization detector with helium as carrier gas at 1mL/min. The oven temperature program increased from 150 to 225ºC at 7ºC per min and injector and detector temperatures were both 250ºC, (Bosch, et al., 2009). Response factors of methyl esters were calculated under the same chromatographic conditions using a commercially available standard mixture of FAME. Fatty acid quantification was carried out through area normalization by using as internal standards 1,2,3-tripentadecanoylglycerol, for muscle and subcutaneous fat, and the 1,2,3-triundecanoylglycerol, for the liver. Intramuscular fat was calculated as the sum of each individual fatty acid expressed as triglyceride equivalents (AOAC, 2000). In the case of liver, qualitative analysis was also performed using a GLC-MS system (Agilent 6890N GC coupled to a 5973 Mass Selective Detector; Agilent Technologies España, S.L. Las Rozas, Spain). The analytical column and chromatographic parameters were identical to those described above. Identification of each fatty acid was confirmed by comparing their mass spectra to the computer library of the GC/MS database Wiley 275. L and NBS75 K. L.

2.3. Isolation of microsomal and cytosolic fractions

Expression of SCD and Δ6d proteins was analyzed in microsomal fraction and FAS protein expression was analysed in cytosolic fraction of pig liver. Microsomes and cytosol were isolated by differential centrifugation with Ca^{2+} precipitation (Schenkman and Cinti, 1978) with minor modifications. Approximately 1.6 g of liver tissue was homogenized in 30 mL of cold sucrose buffer (250 m*M* sucrose, 10 m*M* Tris-HCl, pH 7.4) and centrifuged at 12,000 x *g,* for 10 min at 4ºC. Eight m*M* $CaCl_2$ was added to the supernatant to facilitate sedimentation of microsomes which was conducted as at 19,000 x *g,* for 50 min at 4ºC. The supernatant (cytosolic fraction) was collected and remaining microsomal pellet was re-suspended in a KCl-buffer (10 m*M* Tris-HCl, 250 m*M* KCl, pH 7.4) in the presence of inhibitors of proteolytic enzymes (1.5 μ*M* antipain, 1.5 μ*M* pepstatin, and 2 μ*M* leupeptin; Sigma, Dorset, UK). Total microsomal and cytosolic protein content was determined by the Bradford method (Bradford, 1976).

2.4. Analysis of protein expression

Analysis of lipogenic enzymes protein expression was analyzed by western blotting. This provided information about the amount of lipogenic enzymes of interest present in the tissues. Microsomal (25 μg and 30 μg) and cytosolic (10 μg) proteins were separated by SDS-PAGE and electroblotted onto a nitrocellulose membrane as described previously (Nicolau-Solano et al., 2006). The membranes with transferred proteins were incubated with one of the following primary antibodies: rabbit polyclonal anti-human antibody for FAS (Santa Cruz Biotechnology Inc., Santa Cruz, CA), and a rabbit polyclonal anti-human for SCD antibody (Abcam plc., Cambridge, UK). Delta6-desaturase antibody was custom-made in rabbits against the synthetic peptide containing amino acid sequence from conserved regions in the rat, pig and human, new C-terminus of the Δ6d protein (Sigma-Genosys Ltd, Cambridge, UK). Following incubation with primary antibody, the membranes were re-probed with commercially-available secondary antibody, which was horseradish peroxide-linked donkey anti-rabbit IgG (GE Healthcare, Amersham, Bucks, UK). The blots were developed using Enhanced Chemiluminescent Reagent (GE Healthcare, Amersham, Bucks, UK), and the intensity

of the corresponding bands was quantified using the ImageQuant Program (Molecular Dynamics, Sunnyvale, CA). In order to be able to compare intensity of signals between different blots, a reference sample was present on all the blots. The reference sample was a microsomal or cytosolic preparation from the liver of one randomly chosen pig. Intensity of signal of the reference sample was always taken as 100 arbitrary units, and the intensity of signals of other samples present on the blot was expressed as fractions of the reference sample. All the experiments were performed in duplicate. The duplicate samples were run on different blots and the average intensity of signals of the duplicates was calculated. The duplicates did not differ by more than 5%.

2.5. Statistical analyses

The effect of genetic selection for decreased backfat thickness at constant IMF content on hepatic lipogenic enzymes, production traits, and fatty acid composition in liver, muscle and fat was analysed by comparing mean values between the control and selected pigs using the *t*-test. Differences between tissues for fatty acid composition (expressed in %) were assessed following a 2 x 3 factorial design. Evaluation of significance of effects of the tissue was done using the F-test. The tissue means were separated using the Tukey test. A pair-wise correlations analysis was performed to test the relationship between expression of hepatic lipogenic enzymes and fatty acid composition in each tissue and the fatty acid composition of liver and production traits. All analyses were performed using JMP Version 8.0.2 statistical software (SAS Inst. Inc., Cary, NC).

3. Results

3.1. Effect of selection on production traits

Results of live measurements and carcass traits for pigs of control and selected groups are given in **Table 2**. As anticipated, subcutaneous fat thickness was lower ($P < 0.05$) in the selected group in all the cases, i.e. when the measurements were conducted on live animals at 26 and 29 wk (by 16% and 21%, respectively when

compared to the control group) and when the measurements were conducted on carcasses (by 15% when compared to the control group). Moreover, pigs from the selected group had leaner carcasses than those from the control group (by 7%, $P<0.05$). The control and selected groups did not differ for body and carcass weight, loin thickness and IMF.

Table 2. Effect of genetic selection on pig on-farm performance and carcass characteristics.

	Group		
Trait	C	S[1]	SEM
Live measurements at 26 wk			
Age, d	179.5	174.5	
Body weight, kg	105.8	102.3	2.7
Backfat thickness [2], mm	15.6 [a]	13.1 [b]	0.7
Loin thickness [2], mm	44.2	44.1	0.6
Live measurements at 29 wk			
Age, d	196.5	191.5	
Body weight, kg	117.1	112.9	2.8
Backfat thickness [2], mm	18.6 [a]	14.7 [b]	0.8
Loin thickness [2], mm	47.8	47.6	0.9
Carcass measurements at 30 wk			
Age, d	200.5	195.5	
Carcass weight, kg	88.3	85.1	2.2
Backfat thickness [2], mm	21.7 [a]	18.4 [b]	0.7
Loin thickness [2], mm	46.0	49.3	1.4
Lean content [2], %	45.5[a]	49.3[b]	0.9
Intramuscular fat, % of DM [3]	12.1	11.1	0.8

[a,b] Means within a row with different superscript differ ($P<0.05$). $n=15$ for control and selected groups.
[1] Pigs in the selected group (S) were selected for decreased backfat thickness at constant intramuscular fat content. (C) is the control (unselected) group.
[2] Ultrasonic backfat and loin thickness live measurements were determined at 5 cm off the midline at the position of the last rib using the Piglog technology (SFK-Technology, Herlev, Denmark) while in the carcass they were measured at 6 cm off the midline between the third and fourth last ribs using AutoFOM automatic scanner (SFK-Technology).
[3] Intramuscular fat was determined in the *gluteus medius* muscle and was expressed in % of dry matter (DM).

3.2. Effect of selection on fatty acid composition and fatty acid ratios

Effects of genetic selection on fatty acid composition are presented in **Table 3**. Fatty acid composition was only slightly affected by genetic selection. Thus, the control and selected groups did not differ in the level of individual and total SFA and PUFA. In the case of MUFA, the selected group had lower content of C20:1 (by 10.6%, $P=0.03$) in subcutaneous fat and a higher content of C24:1 in the liver (by 17.9%, $P=0.03$) when compared to the control group. No differences were observed in the muscle MUFA content between the control and selected groups. Selection for decreased backfat thickness did not affect n-6/n-3 and MUFA/PUFA ratios. The ratio of C18:0 to C16:0 was used to represent the enzymatic activity of ELOVL6, and the ratio of C20:1 to C18:1 to represent activity of ELOVL3. The very-long-chain elongase ELOVL6 is a lipogenic enzyme that elongates the C16:0 product of FAS to C18:0 (Jakobsson et al., 2006) and ELOVL3 controls the synthesis of MUFA with as many as 24-carbon atoms (Zadrevec et al., 2010). The ratio of C20:2 to C18:2 was used to represent the enzymatic index for ELOVL5, which is responsible for elongating of PUFA (Guillou, et al., 2010). The values of indices are estimations of enzymatic activities, which are often used in studies. The indices were estimated from ratios of product to precursor fatty acids (Zhang et al., 2007; Ntawubizi et al., 2009). In the case of ELOVL6 index, the selected group had a higher ratio of C18:0 to C16:0 (by 11%, $P<0.05$) when compared to the control group whereas ELOVL3 and ELOVL5 indices did no differ between groups.

Table 3. Effect of genetic selection in each tissue on fatty acid composition (mg/g of dry matter).

	Liver			Muscle			Subcutaneous fat		
	Group			Group			Group		
Fatty acid[1]	C	S[2]	SEM	C	S[2]	SEM	C	S[2]	SEM
C10:0, capric	0.07	0.06	0.00	nd	nd		nd	nd	
C12:0, lauric	0.03	0.03	0.00	nd	nd		nd	nd	
C13:0, tridecanoic	0.05	0.04	0.00	nd	nd		nd	nd	
C14:0, myristic	0.27	0.24	0.02	2.08	1.83	0.17	12.41	11.46	0.41
C15:0, pentadecanoic	0.34	0.25	0.04	nd	nd		nd	nd	
C16:0, palmitic	13.26	12.16	0.40	27.53	24.56	1.90	108.06	105.96	3.27
C17:0, margaric	0.97	1.10	0.09	nd	nd		nd	nd	
C18:0, stearic	20.79	21.9	0.94	13.23	12.11	0.93	52.05	50.60	1.79
C20:0, arachidic	0.10	0.11	0.01	0.18	0.17	0.02	0.55	0.53	0.02
C22:0, behenic	0.12	0.15	0.01	nd	nd		nd	nd	
ELOVL6 index, C18:0/C16:0	1.57[a]	1.82[b]	0.07	0.48	0.48	0.01	0.48	0.49	0.01
Total SFA	36.01	36.06	1.51	43.03	38.67	2.98	173.06	168.55	5.49
C16:1, n-7 palmitoleic	0.61	0.55	0.03	3.86	3.50	0.30	17.06	16.49	0.84
C17:1, heptadecenoic	0.17	0.13	0.02	nd	nd		nd	nd	
C18:1, n-9 oleic	13.86	12.88	0.50	48.84	44.69	3.32	374.08	349.59	11.79
C20:1, n-9 eicosenoic	0.30	0.27	0.01	0.88	0.79	0.07	8.18[a]	7.31[b]	0.27
C24:1, nervonic	0.22[a]	0.26[b]	0.01	nd	nd		nd	nd	
ELOVL3 index, C20:1/C18:1	0.02	0.02	0.00	0.02	0.02	0.00	0.02	0.02	0.00
Total MUFA	15.14	14.09	0.57	53.58	48.98	3.69	399.32	373.39	12.91
C18:3, n-3 linolenic	0.38	0.36	0.03	0.84	0.73	0.05	8.91	8.85	0.39
C22:6, docosahexanoic	1.61	1.42	0.10	nd	nd		nd	nd	
Total n-3PUFA	1.99	1.79	0.12	0.84	0.73	0.05	8.91	8.85	0.39
C18:2, n-6 linoleic	14.58	13.91	0.47	14.53	13.8	0.64	133.13	131.92	5.63
C20:2, n-6 eicosadienoic	0.56	0.51	0.02	0.69	0.60	0.04	7.16	6.76	0.24
C20:3, eicosatrienoic	1.09	0.99	0.08	nd	nd		nd	nd	
C20:4, n-6 arachidonic	15.83	14.00	1.15	1.97	1.92	0.04	1.83	1.93	0.09
ELOVL5 index, C20:2/C18:2	0.03	0.03	0.00	0.05	0.05	0.00	0.05	0.04	0.00
Total n-6PUFA	32.05	29.40	1.72	17.19	16.32	0.73	142.12	140.62	5.97
Total PUFA	33.97	30.96	1.68	18.02	17.06	0.74	151.04	149.47	6.31
Total fatty acids	85.18	81.34	3.91	114.64	104.71	1.86	723.42	691.41	6.19
n-6/n-3 ratio	16.55	16.97	0.12	21.30	22.74	0.08	15.95	15.90	0.01
MUFA/PUFA ratio	0.45	0.46	0.00	2.95	2.85	0.02	2.68	2.54	0.01

[a-b] Means with different superscripts within a row (within main factor) differ, (P<0.05).
[1] nd: non-detectable value. SEM: Standard error of the mean.
[2] Pigs in the selected group (S) were selected for decreased backfat thickness at constant intramuscular fat content. (C) is the control (unselected) group.

3.3. Effect of tissue on fatty acid composition (expressed as mg/ 100 g of total fatty acids)

Comparison between tissues showed that the total SFA percentage was the lowest in the fat and the highest in the liver (almost doubled when compared to the fat, $P<0.01$). In terms of individual SFA, the muscle had the highest percentage of C16:0 (1.5-fold higher than in the liver and fat). The highest percentage of total SFA in the liver was mostly due to an increased level of C18:0, which was 2 to 3-fold higher than in the other two tissues investigated. The lowest percentage of total MUFA was observed in the liver and was 3-fold lower ($P<0.01$) than in the muscle and fat. Differences in the percentage of individual MUFA between the muscle and fat were relatively small, with the muscle having higher values for C16:1 (by 1.4-fold, $P<0.01$) and lower values for C18:1 (by 1.2-fold, $P<0.01$) when compared to fat. In contrast to MUFA, the highest percentage of PUFA was found in the liver, and it was 1.8-fold ($P<0.01$) and 2.4-fold ($P<0.01$) higher than in fat and the muscle, respectively. In terms of PUFA, the greatest difference between liver and the other two tissues was observed for C20:4. This fatty acid accounted for almost one fifth of the hepatic fatty acids, with values 9.4-fold and 65.1-fold higher ($P<0.01$) than in the muscle and fat, respectively (**Table 4**).

Table 4. Effect of type of tissue on fatty acid composition (mg/100 g of total fatty acids).

	Tissue			
Fatty acid [1]	Liver	Muscle	Subcutaneous fat	SEM
No. of pigs	30	30	30	
C10:0	0.08	nd	nd	0.00
C12:0	0.03	nd	nd	0.00
C13:0	0.05	nd	nd	0.00
C14:0	0.31[a]	1.76[b]	1.68[b]	0.02
C15:0	0.35	nd	nd	0.03
C16:0	15.34[a]	23.63[b]	15.28[a]	0.32
C17:0	1.25	nd	nd	0.08
C18:0	25.90[a]	11.51[b]	7.33[c]	0.45
C20:0	0.13[a]	0.16[b]	0.08[c]	0.00
C22:0	0.15	nd	nd	0.00
Total SFA	43.61[a]	37.05[b]	24.40[c]	0.65
C16:1	0.69[a]	3.33[b]	2.36[c]	0.06
C17:1	0.18	nd	nd	0.01
C18:1	16.07[a]	42.46[b]	50.96[c]	0.39
C20:1	0.34[a]	0.75[b]	1.09[c]	0.01
C24:1	0.29	nd	nd	0.01
Total MUFA	17.57[a]	46.54[b]	54.42[c]	0.43
C18:3	0.44[a]	0.72[b]	1.25[c]	0.02
C22:6	1.84	nd	nd	0.08
Total n-3PUFA	2.28[a]	0.72[b]	1.25[c]	0.06
C18:2	17.17[a]	13.22[b]	18.72[c]	0.36
C20:2	0.61[a]	0.59[a]	0.98[b]	0.02
C20:3	1.18	nd	nd	0.07
C20:4	17.59[a]	1.88[b]	0.27[c]	0.44
Total n-6PUFA	36.54[a]	15.69[b]	20.00[c]	0.56
Total PUFA	38.82[a]	16.41[b]	21.22[c]	0.57

[a-c] Means with different superscripts within a row differ, ($P<0.05$).
[1] nd: non-detectable value. SEM: Standard error of the mean. See Table 3 for trait abbreviations.

3.4. Effect of selection on lipogenic enzyme expression

This study determined the presence of inmunoreactive bands for FAS, SCD, and Δ6d proteins of 250, 37 and 50 kDa respectively. This is consistent with the molecular weights of FAS, SCD and Δ6d reported for pigs (Smith, et al., 2003; Ren et al., 2004; Missotten et al., 2009).

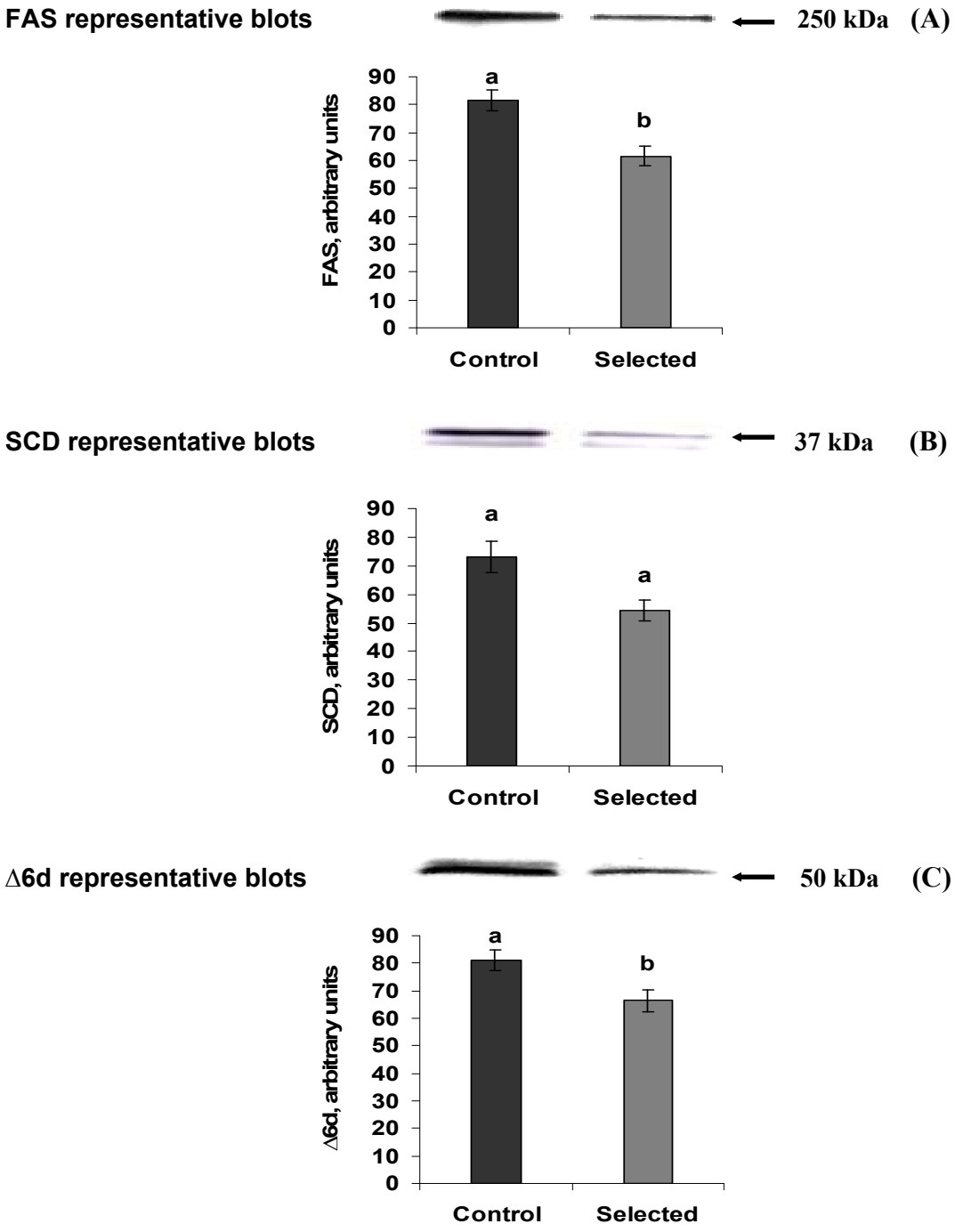

Figure 1. Representative blots and average values for expression of (A) fatty acid synthase (FAS), (B) stearoyl-CoA desaturase (SCD), and (C) Δ6-desaturase (Δ6d) proteins in liver of pigs of the control and selected groups. The amount of protein used for western blot analyses was: 10 µg of cytosolic protein for FAS, 25 µg and 30 µg of microsomal protein for SCD and Δ6d respectively. Immunoreactive bands of 250, 37 and 50 kDa were detected for FAS, SCD and Δ6d, respectively. Pigs in the selected group were selected for decreased backfat thickness at constant intramuscular fat content. Bars represent average of measurements for 15 animals. All measurements were done in duplicate. Error bars represent the standard error of the mean. [a,b]Means with different superscripts differ ($P<0.05$).

The selection for reduced subcutaneous fat thickness in pigs was accompanied by significant decreases in FAS and Δ6d protein expression in the liver. The FAS protein expression was 25% lower ($P = 0.02$; **Figure 1A**) and Δ6d was 18% lower ($P = 0.04$; **Figure 1C**) in pigs from the selected group when compared to control group. No difference between control and selected groups was found for SCD protein expression, although there was a trend towards reduction in SCD protein expression in the selected group ($P=0.06$; **Figure 1B**).

3.5. Relationship between enzyme expression and fatty acid composition

The relationship between expression of hepatic lipogenic enzymes and fatty acid composition of subcutaneous fat and muscle has been investigated in order to determine whether hepatic lipogenic processes might influence tissue-specific fat deposition (**Table 5**). In this case the relationship was analysed for the whole data set (i.e. the control and selected data were used as one data set). No relationship was found between the expression of hepatic FAS and total SFA, hepatic SCD and total MUFA and hepatic Δ6d and total n-3 PUFA or n-6 PUFA content in either liver, fat or the muscle. However, in case of fat, there was a trend towards positive correlation between the hepatic FAS protein expression and total SFA content ($r = 0.35$, $P = 0.06$), and between the hepatic Δ6d protein expression and total PUFA content ($r = 0.34$, $P = 0.06$). Statistically significant correlations were only found between the expression of SCD protein and C24:1 content in the liver ($r = -0.37$, $P = 0.04$), and between Δ6d protein expression and C20:2 content in fat ($r = 0.42$, $P = 0.02$).

Table 5. Relationship between expression of the hepatic lipogenic enzymes and fatty acid content (mg/g of dry matter) for all the pigs used in the study (n=30).

		Tissue		
Enzyme[1]	Fatty acid	Liver	Muscle	Subcutaneous fat
FAS vs.	Total SFA	0.03	0.14	0.35‡
	C14:0	-0.01	0.25	0.21
	C16:0	0.04	0.16	0.33‡
	C18:0	-0.02	0.06	0.28
	C20:0	-0.20	0.14	0.29
SCD vs.	Total MUFA	-0.10	-0.13	0.24
	C16:1	0.00	-0.15	-0.09
	C18:1	-0.11	-0.13	0.26
	C20:1	-0.07	-0.16	0.34‡
	C24:1	-0.37*	-	-
Δ6d vs.	Total PUFA	0.19	-0.15	0.34‡
	Total n-3PUFA	0.20	-0.11	0.32‡
	C18:3	0.33	-0.11	0.32‡
	Total n-6PUFA	0.18	-0.15	0.34‡
	C18:2	0.21	-0.15	0.34‡
	C20:2	0.10	-0.07	0.42*
	C20:4	0.17	-0.17	0.12

[1] FAS: fatty acid shynthase; SCD: stearoyl-CoA desaturase; Δ6d: delta 6-desaturase.
*$P<0.05$, ‡ $P<0.1$.

3.6. Effect of selection on association pattern between hepatic fatty acid composition and production traits

Selection did not affect to association pattern between hepatic fatty acids and the body and carcass weight and loin thickness. However, a regression x group interaction analysis showed that selection against backfat thickness triggered a change in the association pattern between the content of the SFA C18:0 in liver and IMF content. Thus, in pigs from selected group there was a positive relationship between hepatic C18:0 and IMF content (r=0.63, $P<0.01$), but not in the control group (r =-0.36, $P<0.1$), **Figure 2.**

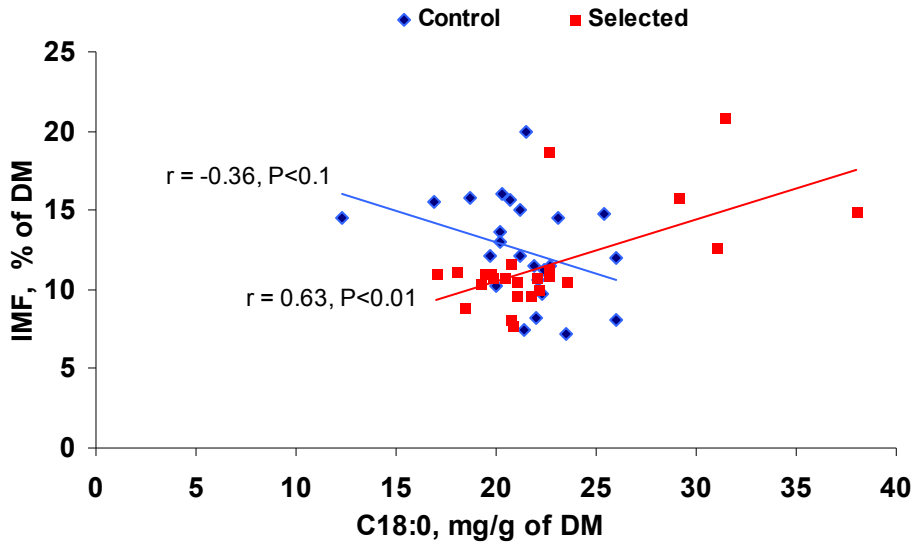

Figure 2. Relationship between IMF content (% of DM: dry matter) in *gluteus medius* muscle and C18:0 content (mg/g of DM) in liver of pigs in the control and selected groups (n=48).

4. Discussion

This study reported, for the first time, that genetic selection for reduced subcutaneous fat thickness at constant IMF is accompanied by reduction of expression of the key lipogenic enzymes FAS and Δ6d in pig liver. Relationship between enzyme expression and fatty acid composition has been studied previously (Cánovas et al. 2009a; Missotten et al., 2009), but these studies focused on enzymes and fatty acids from the same tissue (i.e. either fat or muscle). The question whether hepatic lipogenesis contribute to regulation of fatty acid composition of pig subcutaneous fat, muscle of other tissues, remains open. Cánovas et al. (2009a) reported that selection for decreased subcutaneous fat reduced the expression of the lipogenic enzymes Acetyl-CoA carboxylase (ACC) and SCD in pig subcutaneous fat. This is in a line with results of the present study, where we observed a reduced expression of another enzyme involved in the biosynthesis of SFA, namely FAS, under the genetic selection. However, in contrast to Cánovas et al. (2009a), the present study did not find significant effect of genetic selection on the expression of the hepatic SCD, the key enzyme in MUFA biosynthesis. This discrepancy can be related to the use of hepatic tissue in the present study, whilst

Cánovas et al. (2009a) investigated subcutaneous adipose tissue. Tissue-specific responses in porcine enzyme expression are not unusual, and have been previously observed under dietary manipulations. Thus, Doran et al. (2006) established an activation of SCD protein expression in pig muscle, but not in subcutaneous fat, in response to a reduced protein diet. Furthermore, impact of genetics on tissue-specific enzyme expression has been described in cattle (Dance et al., 2009). Effects of breed and diet on FAS, ACC, SCD, Δ5d and Δ6d protein expression have been previously reported in a range of genetic and dietary studies on pigs (Mourot et al., 1999; De Smet et al., 2004; Doran et al., 2006; Zhang et al., 2007; Ward et al., 2010). Furthermore, Gao and Zhao (2009) established different quantitative trait for IMF and subcutaneous fat content, which provides further evidence of tissue-specific regulation of fat deposition in subcutaneous fat and muscle depots. However, the mechanism and specific genes involved in the tissue-specific fat deposition remain unknown. The aim of the present study was not only to determine an effect of the genetic selection on the hepatic enzymes expression, but also to investigate potential link between hepatic enzyme expression and fat partitioning in pigs. It is known that each fat depot has a different function with regards to lipid biosynthesis or lipid storage (O´Hea and Leveille, 1969; Mourot et al., 1995). In pigs, lipogenesis occurs predominantly in subcutaneous fat, although the liver also has input in this process (Ponsuksili et al., 2007). Furthermore, a number of hepatic biomarkers have been shown to be associated with IMF and subcutaneous fat content (Jiang et al., 2006; Fan, et al., 2010). Finding of this study that genetic selection for reduced subcutaneous fat content at constant IMF level is accompanied by reduction of some hepatic lipogenic enzyme expression indicates that hepatic lipogenesis might affect fat partitioning in pigs. To investigate this further, this study looked into potential link between hepatic enzymes expression and fatty acid composition of pig liver, muscle and subcutaneous fat. This study did not find statistically significant correlations between the expression of the lipogenic enzymes in the liver and fatty acid composition of fat and muscle. However, there was a trend towards positive relationships between expression of the hepatic FAS and total SFA, and between expression of the hepatic Δ6d and total PUFA in fat, but not in the muscle. Lack of statistical significance might be related to effects on factors other than enzyme expression, contributing to net accretion of fatty

acid. These might include effect of lipolysis, and tissue-specific impact of dietary fatty acids on fat deposition (Mourot et al., 1995; Bernlohr et al., 2002).

It is interesting to note that the activity of ELOVL6 represented as ratio of C18:0 to C16:0 was affected by selection against backfat. In particular, the ELOVL6 activity in liver showed significant differences between control and selected groups. The activity of ELOVL6 was higher in liver than in muscle and subcutaneous fat, supporting studies that showed that elongases are regulated by tissue-specific mechanisms (Wang et al., 2005a). It is in line with Landriscina et al. (1972) and Caputi Jambrenghi et al. (2007), who detected a remarkably ELOVL6 activity in liver of rats and lambs. An aim of the present study was not only to determine the effect of the genetic selection on protein expression, enzymatic indices and fatty acid composition, but also to investigate the physiological mechanisms which are involved in achieving that pigs selected against backfat thickness at constant IMF, resulted to have less backfat thickness and similar IMF level when compared to control pigs. Liver plays a central role in the modulation of lipid metabolism by changing the activity of enzymes in relevant metabolic pathways (Ponsuksili et al., 2007; Matsuzaka and Shimano, 2009). Results in this study, suggest that selection against backfat thickness may make to change the association pattern between C18:0 pathway synthesis and IMF content in selected pigs, in which C18:0 content in liver was positively related to IMF whereas this did not occur in control pigs. The fact that activity of ELOVL6 was higher in the selected group and at the same time the selected animals with higher C18:0 content had also higher IMF levels, might suggest that the activity of hepatic ELOVL6 influenced on IMF content. Morcillo et al. (2011) reported that genetic variations in the ELOVL6 gene are related with insulin sensitivity in humans, so that with this finding we can speculate that selected animals could have a specific variation of this gene that increases the insulin sensitivity in muscle, and subsequently *de novo* fatty acid synthesis and therefore the IMF content. The mechanism by which the liver might regulate fat partitioning of other pig tissues remains unknown and information on this issue is limited. Several studies coincide in the importance of fatty acid profile in tissues. In fact, the alterations in the hepatic ratio of C18:0 to C16:0 and MUFA derived from endogenous synthesis, could reflect the underlying mechanisms that are involved in maintaining fatty acid homeostasis (Pan et

al., 1995; Okada et al., 2005; Matsuzaka et al., 2007; Paton and Ntambi, 2009; Morcillo et al., 2011). Furthermore, several studies realized in transgenic mouse models lacking specific lipogenic enzymes, demonstrated that the interruption of these enzymatic activities altered the whole body lipid (Guillou et al., 2010). Other studies established a model of whole body lipid metabolism, in which adipose-derived palmitoleic acid as a lipokine, contributed to regulation of the lipogenic activity in liver and muscle (Cao et al., 2008).

Present study reported that percentage of fatty acid composition significantly differed between the liver, the muscle and fat. The percentage of total SFA, n-3 and n-6 PUFA in this study was found to be the highest in liver, whereas the highest percentage of total MUFA was found in fat when compared to the liver and the muscle (Duran-Montgé et al., 2009). Moreover, the present study observed that the percentage of C20:4 and C22:6 were the highest in the liver when compared to the fat and the muscle. A lower percentage of long-chain PUFA in fat when compared to other tissue was also reported by other authors (Enser et al., 2000). This might be, at least partially, due to the fact that the liver is considered to be an important site for biosynthesis of long-chain PUFA, and C22:6 in particular (Scott and Bazan, 1989), and at the same time, adipose tissue is responsible for synthesizing and storing great amount of SFA and MUFA. The majority of hepatic C20:4 is known to be stored in the phospholipid fraction of cell membranes (Ray et al., 1969). Physiological significance of this fact is that the hepatic membrane C20:4 can be released by the action of the phospholipase A2 to provide a substrate for the biosynthesis of some bioactive lipids mediators, which can be transported to other tissues (Jump and Clarke, 1999).

Conclusions

The present study demonstrated for the first time that genetic selection for reduced subcutaneous fat thickness at constant IMF is accompanied by a reduced expression of Δ6d and FAS proteins in pig liver. It has been suggested that expression of lipogenic enzymes in liver might influence fat partition in pigs.

References

AOAC. 2000. Official Methods of Analysis. 17th ed. Assoc. Off. Anal. Chem., Gaithersburgh, MD.

Bernlohr, D. A., A. E, Jenkins, and A. A. Bennaars. 2002. Adipose tissue and lipid metabolism. In pages 263-269 in Biochemistry of lipids, lipoproteins and membranes. G. Bernardi, D. E. Vance, and J. E. Vance, 4th ed. Amsterdam: Elsevier.

Bosch, L., M. Tor, J. Reixach, and J. Estany. 2009. Estimating intramuscular fat content and fatty acid composition in live and post-mortem samples in pigs. Meat Sci. 82:432-437.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254.

Cánovas, A., J. Estany, M. Tor, R. N. Pena, and O. Doran. 2009a. Acetyl-CoA carboxylase and stearoyl-CoA desaturase protein expression in subcutaneous adipose tissue is reduced in pigs selected for decreased backfat thickness at constant intramuscular fat content. J. Anim. Sci. 87:3905-3914.

Cao, H., K. Gerhold, J. R. Mayers, M. M. Wiest, S. M. Watkins, and G. S. Hotamisligil. 2008. Identification of a lipokine, a lipid hormone linking adipose tissue to systemic metabolism. Cell 134:933-944.

Caputi Jambrenghi, A., G. Paglialonga, A. Gnoni, F. Zanotti, F. Giannico, G. Vonghia, and G. V. Gnoni. 2007. Changes in lipid composition and lipogenic enzyme activities in liver of lambs fed ω-6 polyunsaturated fatty acids. Comp. Biochem. Physiol. B:147: 498-503.

Dance, L. J. E., K. R. Matthews, and O. Doran. 2009. Effect of breed on fatty acid composition and stearoyl-CoA desaturase protein expression in the *semimembranosus* muscle and subcutaneous adipose tissue of cattle. Livest. Sci. 125:291-297.

De Smet, S., K. Raes, and D. Demeyer. 2004. Meat fatty acid composition as affected by fatness and genetic factors: A review. Anim. Res. 53:81-98.

Doran, O., S. K. Moule, G. A. Teye, F. M. Whittington, K. G. Hallett, and J. D. Wood. 2006. A reduced protein diet induces stearoyl-CoA desaturase protein expression in pig muscle but not in subcutaneous adipose tissue: Relationship with intramuscular lipid formation. Br. J. Nutr. 95:609-617.

Duran-Montgé, P., P. K. Theil, C. Lauridsen, and E. Esteve-García. 2009. Fat metabolism is regulated by altered gene expression of lipogenic enzymes and regulatory factors in liver and adipose tissue but not in *semimembranosus* muscle of pigs during fattening period. Animal 3:11:1580-1590.

Enser, M., R. I. Richardson, J. D. Wood, B. P. Gill, and P. R. Sheard. 2000. Feeding linseed to increase the n-3 PUFA of pork: Fatty acid composition of muscle, adipose tissue, liver and sausages. Meat Sci. 55:201-212.

Fan, B., Z. Q. Du, and M. F. Rothschild. 2010. The hepatocyte nuclear factor-1 alpha (HNF1A) gene is associated with fatness and loin muscle area in the pig. Animal 4:10:1619-1627.

Fernandez, X., G. Monin, A. Talmant, J. Mourot, and B. Lebret. 1999. Influence of intramuscular fat content on the quality of pig meat −1. Composition of the lipid fraction and sensory characteristics of m. *longissimus lumborum*. Meat Sci. 53:59-65.

Gao, S. Z., and S. M. Zhao. 2009. Physiology, affecting factors and strategies for control of pig meat intramuscular fat. Recent Pat. Food Nutr. Agric. 1(1):59-74.

Guillou, H., D. Zadravec, G. P. Martin, and A. Jacobsson. 2010. The key roles of elongases and desaturases in mammalian fatty acid metabolism: Insights from transgenic mice. Prog. Lipid Res. 49:186-199.

Hanson, S.W. F., and J. Olley. 1963. Application of the method of lipid extraction to tissue homogenate. Biochem. J. 89:101-102.

Hermesch, S. 2004. Genetic improvement of lean meat growth and feed efficiency in pigs. Aust. J. Exp. Agr. 44:383-391.

Jakobsson, A., R. Westerberg, and A. Jacobsson. 2006. Fatty acid elongases in mammals: Their regulation and roles in metabolism. Prog. Lipid Res. 45:237–249.

Jiang, Y. Z., X. W. Li, and G. X. Yang. 2006. Sequence characterization, tissue-specific expression and polymorphism of the Porcine (*Sus scrofa*) liver-type fatty acid binding protein gene. Acta Genetica Sinica 33(7):598-606.

Jump, D. B., and S. D. Clarke. 1999. Regulation of gene expression by dietary fat. Annu. Rev. Nutr.19:63-90.

Landriscina, C., G. V. Gnoni, and E. Quagliariello. 1972. Fatty-acid biosynthesis. The physiological role of the elongation system present in microsomes and mitochondria of rat liver. Eur. J. Biochem. 29:188-196.

Matsuzaka, T., H. Shimano, N. Yahagi, T. Kato, A. Atsumi, T. Yamamoto, N. Inoue, M. Ishikawa, S. Okada, N. Ishigaki, H. Iwasaki, Y. Iwasaki, T. Karasawa, S.

Kumadaki, T. Matsui, M. Sekiya, K. Ohashi, A. H. Hasty, Y. Nakagawa, A. Takahashi, H. Suzuki, S.Yatoh, H. Sone, H. Toyoshima, J-i. Osuga, and N. Yamada. 2007. Crucial role of a long-chain fatty acid elongase, Elovl6, in obesity-induced insulin resistance. Nat. Med. 13(10):1193-1202.

Matsuzaka, T., and H. Shimano. 2009. Elovl 6: A new player in fatty acid metabolism and insulin sensitivity. Mol. Med. 87:379-384.

Merks, J. W. M. 2000. One century of genetic changes in pigs and the future needs. Pages 8-19 in The Challenge of Genetic Change in Animal Production. W. G. Hill, S. C. Bishop, B. McGuirk, J. C. McKay, G. Simm, and A. J. Webb, ed. Occasional Publication No. 27. Br. Soc. Anim. Sci., Edinburgh, UK.

Missotten, J., S. De Smet, K. Raes, and O. Doran. 2009. Effect of supplementation of the maternal diet with fish oil or linseed oil on fatty-acid composition and expression of Δ5- and Δ6-desaturase in tissues of female piglets. Animal. 3:8:1196-1204.

Morcillo S., G. M. Martín-Nuñez, G. Rojo-Martinez , M. C. Almaraz, E. García-Escobar, M. L. Mansego, G. de Marco, F. J. Chaves, and F. Soriguer. 2011. ELOVL6 genetic variation is related to insulin sensitivity: A new candidate gene in energy metabolism. PLoS ONE 6(6):1-7.

Mourot, J., M. Kouba, and P. Peiniau. 1995. Comparative study of *in vitro* lipogenesis in various adipose tissues in the growing domestic pig (*Sus domesticus*). Comp. Biochem. Physiol. 111B(3):379-384.

Mourot, J., M. Kouba, and G. Salvatori. 1999. Facteurs de variation de la lipogenèse dans les adipocytes et les tissus adipeux chez le porc. INRA Prod. Anim. 12(4):311-318.

Nicolau-Solano, S. I., J. D. McGivan, F. M. Whittington, G. J. Nieuwhof, J. D. Wood, and O. Doran. 2006. Relationship between the expression of hepatic but not testicular 3β-hydroxysteroid dehydrogenase with androstenone deposition in pig adipose tissue. J. Anim. Sci. 84:2809-2817.

Ntawubizi, M., K. Raes, N. Buys, and S. De Smet. 2009. Effect of sire and sex on the intramuscular fatty acid profile and indices for enzyme activities in pigs. Livest. Sci. 122:264-270.

Ntawubizi, M., E. Colman, S. Janssens, K. Raes, N. Buys, and S. De Smet. 2010. Genetic parameters for intramuscular fatty acid composition and metabolism in pigs. J. Anim. Sci. 88:1286-1294.

O´Hea, E. K., and G. A. Leveille. 1969. Significance of adipose tissue and liver as sites of fatty acid synthesis in the pig and the efficiency of utilization of various substrates for lipogenesis. J. Nutr. 99:338-344.

Okada, T., N. Furuhashi, Y. Kuromori, M. Miyashita, F. Iwata, and K. Harada. 2005. Plasma palmitoleic acid content and obesity in children. J. Clin. Nutr. 82:747-50.

Pan, D. A., S. Lillioja, M. R. Milner, A. D. Kriketos, L. A. Baur, C. Bogardus, and L. H. Storlienil. 1995. Skeletal muscle membrane lipid composition is related to adiposity and insulin action. J. Clin. Invest. 96:2802-2808.

Paton, C. M., and J. M. Ntambi. 2009. Biochemical and physiological function of stearoyl-CoA desaturase. Am. J. Physiol. Endocrinol. Metab. 297:E28-E37.

Ponsuksili, S., E. Murani, C. Walz, M. Schwerin, and K. Wimmers. 2007. Pre- and postnatal hepatic gene expression profiles of two pig breeds differing in body composition: Insight into pathways of metabolic regulation. Physiol. Genomics 29:267-279.

Ray, T. K., V. P. Skipski, M. Barclay, E. Essner, and F. M. Archibald. 1969. Lipid composition of rat liver plasma membranes. J. Biol. Chem. 25:5528-5536.

Reiter, S. S., C. H. C. Halsey, B. M. Stronach, J. L. Bartosh, W. F. Owsley, and W. G. Bergen. 2007. Lipid metabolism related gene-expression profiling in liver, skeletal muscle and adipose tissue in crossbred Duroc and Pietrain Pigs. Comp. Biochem. Physiol. D2:200-206.

Reixach, J., M. Tor, and J. Estany. 2009. Respuesta a la selección contra grasa dorsal a grasa intramuscular constante en cerdos Duroc. Pages 120-122 in XIII Jornada sobre Producción Animal. M. Joy, J. H. Calvo, C. Calvete, M. A. Latorre, I. Casasus, A. Bernues, B. Panea, A. Sanz, and J. Balcells, ed. AIDA, Zaragoza, Spain.

Ren, J., C. Knorr, L. Huang, and B. Brenig. 2004. Isolation and molecular characterization of the porcine stearoyl-CoA desaturase gen. Gene. 340:19-30.

Rule, D. C. 1997. Direct transesterification of total fatty acid of adipose tissue and freeze dried muscle and liver with boron-trifluoride in methanol. Meat Sci. 46(1):23-32.

Schenkman, J. B., and D. L. Cinti. 1978. Preparation of microsomes with calcium. Methods Enzymol. 52:83-89.

Scott, B. L., and N. G. Bazan, 1989. Membrane docosahexaenoate is supplied to the developing brain and retina by the liver. Proc. Natl. Acad. Sci. USA. 86:2903-2907.

Smith, S., A. Witkowski, and A. K. Joshi. 2003. Structural and functional organization of the animal fatty acid synthase. Prog. Lipid Res. 42:289-317.

Solanes, F. X., J. Reixach, M. Tor, J. Tibau, and J. Estany. 2009. Genetic correlations and expected response for intramuscular fat content in a Duroc pig line. Livest. Sci. 123:63-69.

Theil, P. K., and C. Lauridsen. 2007. Interactions between dietary fatty acids and hepatic gene expression in livers of pigs during the weaning period. Livest. Sci. 108: 26-29.

Valsta, L. M., H. Tapanainen, and S. Männistö. 2005. Meat fats in nutrition. Meat Sci. 70:525-530.

Wang, Y., D. Botolin, B. Christian, J. Busik, J. Xu, and D. B. Jump. 2005a. Tissue-specific, nutritional, and developmental regulation of rat fatty acid elongases. J. Lipid Res. 46:706-715.

Wang, Y., D. Botolin, J. Xu, B. Christian, E. Mitchell, B. Jayaprakasam, M. Nair, J. M. Peters, J. Busik, and D. B. Jump. 2006. Regulation of hepatic fatty acid elongase and desaturase expression in diabetes and obesity. J. Lipid Res. 47:2028-2041.

Ward, R. E., B. Woodward, N. Otter, and O. Doran. 2010. Relationship between the expression of key lipogenic enzymes, fatty acid composition, and intramuscular fat content of Limousin and Aberdeen Angus cattle. Livest. Sci. 127:22-29.

Zadravec, D., A. Brolinson, R. M. Fisher, C. Carneheim, R. I. Csikasz, J. Bertrand-Michel, J. Borén, H. Guillou, M. Rudling, and A. Jacobsson. 2010. Ablation of the very-long-chain fatty acid elongase ELOVL3 in mice leads to constrained lipid storage and resistance to diet-induced obesity. FASEB J. 24(11):4366-4377.

Zhang, S., T. J. Knight, K. J. Stalder, R. N. Goodwin, S. M. Lonergan, and D. C. Beitz. 2007. Effects of breed, sex, and halothane genotype on fatty acid composition of pork *longissimus* muscle. J. Anim. Sci.85:583-591.

GENERAL DISCUSSION

GENERAL DISCUSSION

The industry's challenge is both producing low-cost meat but increasing meat quality. A strategy to obtain an optimum IMF content without affecting negatively to the efficiency of livestock production could be selective lipid deposition. Particularly, Reixach et al. (2009), selected pigs against backfat thickness at constant IMF content, obtaining animals with less backfat, but with similar levels of IMF. This strategy involves routinely IMF determination and therefore an added-cost to the product. For this reason determination of IMF content and composition needs to be optimised. Previous studies (Reixach et al., 2010) have shown that selection for oleic fatty acid content may be an interesting alternative for improving IMF and oleic. This approach, using the methodology proposed in Chapter I, may help to reduce the cost of the chemical analysis and to short the time required for analysing a great number of samples. For this reason, the aim of the first study was to develop a high-throughput analytical method for oleic fatty acid quantification in pork.

Several studies have determined fatty acid content of food samples using HPLC/MS technique. These methods have in common the use of ESI as the ion source, operating in the negative ion mode and moreover, fatty acids obtained after extraction are analysed as underivatised compounds. In our case, oleic acid content was determined with the same analytical conditions that used in these previous studies, but the lipid extraction procedure was a little different (Kerwin and Wiens, 1996; Perret et al., 2004; Kurata et al., 2005). The suitable combination of the analytical conditions, and the extraction procedure was very important to continue developing the new method, because only fatty acids obtained following the Aldai´s extraction procedure and ionised in negative ion mode were able to generate an abundant signal of ionised fatty acids.

Some important points of the method are the evaluation of matrix effect and quantitative analysis. In order to avoid matrix interference, a good strategy is to build a standard calibration curve based on the determination of the analyte extracted from a "blank matrix", previously spiked with the analytes itself at different concentrations levels. But in our case, this technique was impossible to work, since all types of meat contain the oleic fatty acid. Therefore, to quantify oleic fatty acid, the method of the

standard additions had to be utilized (Gosetti et al., 2007). The matrix effect was studied by using two sets of oleic acid standard lines. The reference set of standards was prepared using solutions of standards and the lines of the matrix set were prepared as standard additions into hydrolysed muscle extract and in both cases, pentadecanoic acid was used as an internal standard. The matrix effect was evaluated by comparing standard line slopes between the two different sets. It was observed that the use of internal standard overcame the matrix effect and no differences were found between the standards and the matrix set lines (**Table 1, study 1**). The high correlation obtained between values determined by the reference method of standards additions and with values obtained by estimating the slope of standard lines, means the oleic acid calculated with a standard line is as valid as that obtained with the standard additions. Thus, to make a calibration curve for each sample would be not need, and the analytical process could simplify greatly (**Table 2, study 1**).

Cross-validation of the method was performed by correlating values of the oleic fatty acid content determined by FIA/MS with values obtained on the same samples by GC. The value of coefficient of the regression line was 0.72 when the samples contained less 40 mg of oleic/g of dry matter (**Figure 3, study 1**). However, when samples had more than 40 mg of oleic fatty acid, a quadratic regression was required. This means that sensitivity of method decreases in samples with high oleic content. An explanation to this fact could be the ESI font becomes saturated. Other possible cause may be these samples lost more oleic fatty acid due to the selectivity of MS channels decreased. Although was assessed that selectivity in each MS channel for individual fatty standard lines was acceptable, a slight noise level for the rest of the fatty acids was also detected, suggesting there was a little cross-talk between the three most abundant fatty acids in pork (palmitic, oleic and linoleic acids) and therefore this can lead to a decrease in the accuracy (**Figure 2, study 1**).

Flow inyection analysis is a method that can be coupled to large variety of detectors (mass spectrometer, sensors, spectrophotometer, pHmeter) without need to use a column and it is suitable for a rapid quantification of analytes and processing large number of samples (Ruzicka and Hansen, 1975; Hlabangana et al., 2006). The FIA/MS method is

faster than the GC/FID because the phase of chromatographic separation is eliminated. This explains why the time consumed by the mass spectrometer is less than one min., whereas in the case of samples analysed by GC, the process takes 15 min. Moreover, in the FIA/MS method it is not necessary to freeze-dry the sample before the saponification and hydrolysis. A fast alternative to FIA method is the fast GC. This technique provides cost-effective determinations of compounds by reducing analysis time. This has been achieved by improving GC instrumentation and working conditions (Korytár et al., 2002). Particularly, two studies realized with fast GC methodology in pork (Mondello et al., 2004; Ficarra et al., 2010) achieved to separate the chromatographic peaks in 3.6 min. However, when compared to our method, the time consumed by the mass spectrometer per sample was less than one min. This supports the idea to try developing firstly a FIA/MS method that to optimise GC working conditions. Another fast and economic method used to estimate content and fatty acid composition is NIRS. However, the instrument that estimates the fatty acid content is easily affected by several factors such as, calibration, the environment and the sample preparation procedure, resulting in a decrease of precision and periodical evaluations of the prediction ability of NIRS equations (Fernández-Cabanás et al., 2007). The variability of the oleic acid content obtained in both methods with and without matrix standard lines was around 15%. This value means precision of FIA/MS method may not be sufficient for some applications. However, this phenomenon is considered an intrinsic feature of the method and is not dependent uncontrolled variations. Moreover, FIA/MS is a chemical method that quantifies realiable the amount of an analyte, whereas NIRS technology estimates this amount. Therefore, the FIA/MS technique is more suitable for our purposes than NIRS.

The higher selectivity of a triple quadrupole compared to a simple one, could reduce background noise completely, and this would allow working with more diluted samples, which would reduce the matrix effect and therefore, precision and accuracy of the method would improve. However, the use of a triple quadrupole had increased the cost of samples analysis (Mastovská and Lehotay, 2003), and in our case is more important to reduce the cost of analysis than to determine the oleic content very precisely. In fact, the main application of the method is to use the oleic fatty acid content as a criterion for

selective breeding or for discriminating retail cuts for labeling purposes, in which the main target is to identify superior animals or products (Kurata et al., 2005).

The study 2 investigated the relationship between blood lipid indicators and subcutaneous backfat and IMF content and composition with the purpose to search for biomarkers specifically associated to one of them. Circulating cholesterol and triglycerides containing lipoproteins exert an influence on the rates of fatty acid synthesis in tissues (Kris-Etherton and Etherton, 1982) and to learn about lipoprotein metabolism may contribute to a better understanding of the biological determination of IMF and subcutaneous backfat. These physiological indicators have the advantage that are easy to measure in live animals at any age and therefore they may be useful for being used as early predictors of IMF and subcutaneous fat content and composition. Moreover, several studies have identified genes encoding physiological indicators that might be explored as markers for assisted selection on lipid deposition (Gao et al., 2007; Cánovas et al., 2009b). The experiment was conducted on Duroc barrows, in which serum concentration of iron, glucose, TP, TC, HDL-C, LDL-C and TG, the percentage of lipoproteins (α-lipoprotein, β-lipoprotein, pre-β-lipoprotein and chylomicrons), and plasma IGF-I were determined at 120, 160, and 185 days of age and consequently correlated to carcass and fat composition traits.

In general, the relationship between blood lipid indicators displayed an age-related pattern and a low correlation structure with commercial traits. Particularly, circulating TP was the best biomarker for early estimation of fatness, since TP at 120 days of age was relevantly correlated ($r > 0.3$) with subcutaneous backfat thickness and IMF in *gluteus medius*. High-density lipoprotein was the most consistent indicator throughout age, and it was negatively correlated with PUFA content of *longissimus dorsi* in all ages of control, and positively related to subcutaneous backfat thickness at 120 and 185 days. In this late age of control, HDL-C was also associated positively to IMF content in *gluteus medius* and *longissimus dorsi*. Circulating IGF-I at later stages of growth may help for specifically biomarking subcutaneous backfat thickness against IMF, since IGF-I at 185 days was more specifically correlated to backfat than to IMF (**Tables 4, 5 and 6, study 2**). Whereas positive correlations between HDL-C and carcass traits at different

ages are in accordance to previous studies (Taylor et al., 1992; Pond et al., 1993; Rauw et al., 2007; Gallardo et al., 2008), the correlations found between IGF-I and carcass traits differed to some studies, in which the concentration of plasma IGF-I have been found to be primarily related to increased growth and lean content (Owens et al., 1999). Contrarily, Luxford (1998), Cameron et al. (2003) and Bunter et al. (2005) estimated positive genetic and phenotypic correlations between IGF-I and backfat. These contradictory results among different studies could be due to IGF-I levels and performance traits have a marked age-related pattern (Louveau et al., 1991; Carter et al., 2002).

A relevant problem for using serum lipids as biomarkers is that they are sensitive to environmental conditions, age and pig's physiological state. This affirmation is supported by previous studies, where investigated the use of blood parameters to predict IMF and subcutaneous backfat thickness and none of the serum lipids measured were enough relevant as biomarkers of carcass quality in pigs (Tremere et al., 1966; Adachi et al., 1999; Hocquette et al., 1999). In our study, blood lipid concentrations were moderately repeatable across time, showing a weak correlation structure with commercial traits and therefore this fact, together with their high determination costs, prevents from using them as routine biomarkers for fat deposition in pigs.

The animals used on the experiments of the study 3 were randomly chosen from a previous genetic trial at the University of Lleida (Reixach et al., 2009). Pigs in the experimental group were selected against backfat thickness while maintaining IMF content to the values most similar to those in the control group. The goal of the selection was achieved, and as expected groups differed in backfat thickness, but not in IMF content. However, the mechanisms underlying alteration in fat repartition have not been unravelled and therefore the genetic selection could cause side effects that are unknown at the moment (Rauw et al., 1998). On one hand, the studies of protein expression and enzymatic indices were carried out in liver because this plays a crucial role in the remodelling of body lipid composition (Reiter et al., 2007; Theil and Lauridsen, 2007). Furthermore, the mechanisms by which the liver might influence on lipogenic processes taking place in other organs remains unknown and the information on this subject is

limited and fragmental. On the other hand, lipogenic enzymes and serum lipoproteins influence on rates on fatty acid synthesis and therefore any genetic-related changes in their levels may help to shed light on specific lipid metabolic pathways (Kris-Etherton and Etherton, 1982; Bernlohr et al., 2002; Zhao et al., 2010). Thus, the aim of the study 3 was not only to determine the effect of the genetic selection on lipogenic protein expression and fatty acid composition, but also to investigate the physiological mechanisms which are involved in achieving that pigs selected against backfat thickness at constant IMF, resulted to have less backfat thickness and similar IMF level when compared to control pigs.

The studies reported, for the first time, that genetic selection for reduced backfat thickness at constant IMF is accompanied by reduction of expression of the key lipogenic enzymes FAS and Δ6d in pig liver (**Figure 1, study 3**). However, selection against backfat thickness hardly altered the fatty acid profile in liver, muscle or subcutaneous fat (**Table 3, study 3**). In fact, control and selected groups only differed in the content of the two MUFA: C20:1 in subcutaneous fat and C24:1 in liver. The finding of that genetic selection for reduced subcutaneous backfat content at constant IMF level is accompanied by reduction of FAS and Δ6d protein expression in liver, indicates that hepatic lipogenesis might affect fat partitioning in pigs. To investigate this further, this study looked into relationship between the hepatic lipogenic enzyme expression and fatty acid composition of subcutaneous fat and the *gluteus medius* muscle. The results showed, that there was a trend towards a positive relationship between FAS and Δ6d protein expression and SFA and PUFA content respectively, in subcutaneous fat but not in muscle.

One interesting result that could help to unravel how liver influenced on fat repartition is that selection against backfat thickness triggered a change in the association pattern between the content of C18:0 in liver and IMF level. Thus, in pigs from selected group there was a positive relationship between the hepatic C18:0 and IMF content, but not in the control group (**Figure 2, study 3**). In order to investigate further this finding, several indices for enzyme activities involved in elongation of SFA, MUFA and PUFA (Wang et al., 2006; Matsuzaka and Shimano, 2009) were studied,

specially ELOVL6, which elongates the C16:0 product of FAS to C18:0 (Jakobsson et al., 2006). The results showed activity of ELOVL6 was higher in liver than in muscle and subcutaneous fat, and in liver only, groups differed for ELOVL6 index. Both results suggest that ELOVL6 may play a key role in fat repartition (**Table 3, study 3**). It is in line with previous studies that found a number of hepatic biomarkers were associated with IMF and subcutaneous fat content (Jiang et al., 2006; Fan et al., 2010), pointing once more that liver has a significant input in lipid metabolism. This fact is in accordance to studies that showed that elongases are regulated by tissue-specific mechanisms (Wang et al., 2005a) and the ELOVL6 activity is remarkably high in liver of rats and lambs (Landriscina et al., 1972; Caputi Jambrenghi et al., 2007).

In spite of the fact that relationship between protein expression and fatty acid composition has been studied previously in pigs (Cánovas et al., 2009a; Missotten et al., 2009), these studies focused on enzymes and fatty acids from the same tissue (i.e. either fat or muscle). Cánovas et al. (2009a) reported that selection for decreased backfat reduced the expression of the lipogenic enzymes ACC and SCD in pig subcutaneous fat. This is consistent with results of the present study that expression of the hepatic FAS, which is an enzyme involved in the biosynthesis of SFA alongside with ACC, was reduced under the genetic selection. However, in contrast to Cánovas et al. (2009a), this investigation did not find significant effect of genetic selection on the hepatic SCD protein expression. This discrepancy can be explained by the use of hepatic tissue in this study, whilst Cánovas et al. (2009a) investigated subcutaneous fat enzyme expression. Furthermore, tissue–specific expression may be subjected to genetic constraints as in Dance et al. (2009), who demonstrated that the mechanisms regulating SCD protein expression in beef cattle are tissue and breed-specific.

It is difficult to explain why decreased protein expression of hepatic FAS and Δ6d in the selected group was not accompanied by a correlative change in the products they catalysed. Several reports have shown that activity of the enzymes involved in *de novo* fatty acid synthesis is an indicator of the lipogenic activity in a tissue (Clarke, 1993; Mourot et al., 1999). However, although lipogenic activity has been shown to be paralleled by protein and mRNA expression (Gondret et al., 2001; Zhao et al., 2010),

increased lipogenic protein expression does not always involve a proportional rise in the activation of proteins, and consequently in the amount of the fatty acids biosynthesised by them (Doran et al., 2006; Cánovas et al., 2009a).

CONCLUSIONS

CONCLUSIONS

1.- The FIA/MS method allows a rapid quantification of oleic acid from small pork samples.

2.- Therefore, the FIA/MS may be used for decreasing the cost of the analyses in cases where oleic acid content needs to be routinely determined over a large number of samples from high-valued pork cuts.

3.- The FIA/MS method is a suitable method for ranking pigs, carcasses, cuts or pork products according to IMF or oleic content.

4.- The serum concentration of lipid indicators, namely cholesterol, lipoproteins, tryglicerides, and insulin-like growth factor-I, are only moderately repeatable across age.

5.- The serum lipid indicators show a low correlation structure with commercial traits and in particular with fat content and composition.

6.- Therefore, none of the serum lipid indicators investigated is recommended to be used as an early biomarker of fatness.

7.- Genetic selection for decreased backfat at restrained intramuscular fat content is accompanied by reduction of expression of the lipogenic enzymes FAS and Δ6d in pig liver.

8.- There is only a little effect of genetic selection for decreased backfat on fatty acid composition of liver, muscle, and subcutaneous fat.

9.- There is evidence that the hepatic expression of FAS is positively related to SFA and hepatic expression of Δ6d to PUFA content in subcutaneous fat but not in muscle.

10.- Expression of lipogenic enzymes in pig liver might influence fat partition and fatty acid composition of pig subcutaneous fat.

CONCLUSIONES

1.- El método FIA/MS permite una rápida cuantificación del ácido oleico en muestras pequeñas de carne de cerdo.

2.- Por lo tanto el FIA/MS puede ser utilizado para disminuir el coste de los análisis en casos dónde el contenido de ácido oleico necesite ser determinado rutinariamente en un gran número de piezas de carne de porcino de elevado valor.

3.- El método FIA/MS es adecuado para clasificar cerdos, canales, piezas ó productos de carne de porcino según su contenido en GIM ó oleico.

4.- La concentración sérica de los indicadores lipídicos: colesterol, lipoproteínas, triglicéridos y el factor de crecimiento insulínico tipo-I, son moderadamente repetibles a lo largo de la edad.

5.- Los indicadores lipídicos muestran una estructura de correlación baja con los carácteres comerciales, y en particular con la composición y contenido de la grasa.

6.- Por lo tanto, ninguno de los indicadores lipídicos séricos investigados son recomendados para ser usados como biomarcadores tempranos de la grasa.

7.- La selección genética contra grasa dorsal a constante grasa intramuscular es asociada con la disminución de la expresión de las enzimas lipogénicas FAS y Δ6d en hígado de cerdo.

8.- Hay únicamente un pequeño efecto de la selección genética contra grasa dorsal en la composición de ácidos grasos en hígado, músculo y grasa subcutánea.

9.- Hay indicios de que la expresión hepática de FAS es relacionada positivamente con el contenido en SFA, y la expresión hepática de Δ6d con el contenido en PUFA en grasa subcutánea pero no en músculo.

10.- La expresión de las enzimas lipogénicas en hígado de cerdo podría influir en la repartición de la grasa y en la composición de ácidos grasos de la grasa subcutánea de cerdo.

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REFERENCES

REFERENCES

Adachi, K., H. Kawano, K. Tsuno, Y. Nomura, N. Yamamoto, A. Arikawa, A. Tsuji, M. Adachi, T. Onimaru, and K. Ohwada. 1999. Relationship between serum biochemical values and marbling scores in Japanese black steers. J. Vet. Med. Sci. 61:961-964.

Aldai, N., K. Osoro, L. J. R. Barron, and A. I. Najera. 2006. Gas-liquid chromatographic method for analysing complex mixtures of fatty acids including conjugated linoleic acids (cis9trans11 and trans10cis12 isomers) and long-chain (n-3 or n-6) polyunsaturated fatty acids - Application to the intramuscular fat of beef meat. J. Chromatogr. A. 1110:133-139.

Agren, J. J., A. Julkunen, and I. Penttila. 1992. Rapid separation of serum lipids for fatty acid analysis by a single aminopropyl column. J. Lipid Res. Note on Methodology. 33:1871-1876.

Anderson, D. B., and R. G. Kauffman. 1973. Cellular and enzymatic changes in porcine adipose tissue during growth. J. Lipid Res. 14:593-599.

AOAC. 2006. Official Methods of Analysis. 17th ed. Assoc. Off. Anal. Chem., Gaithersburgh, MD.

Arnau Arboix. 2001. Tecnología, microbiología y principales problemas tecnológicos del jamón curado. Pages 1177-1204. Enciclopedia de la carne y de los productos cárnicos. S. Martín Bejarano, ed. Martín & Macías, DL., Plasencia.

Azain, M. J. 2004. Role of fatty acids in adipocyte growth and development. J. Anim. Sci. 82:916-924.

Berg, H., M. Magard, G. Johansson, and L. Mathiasson. 1997. Development of a supercritical fluid extraction method for determination of lipid classes and total fat in meats and its comparison with conventional methods. J. Chromatogr. 785:345-352.

Bergen, W. G., and H. J. Mersmann. 2005. Comparative aspects of lipid metabolism: Impact on contemporary research and use of animal models. J. Nutr. 135:2499-2502.

Bernlohr, D. A., A. E. Jenkins, and A. A. Bennaars. 2002. Adipose tissue and lipid metabolism. Pages 263-269 in Biochemistry of lipids, lipoproteins and membranes. D. E., Vance, J. E. Vance, 4th ed. Elsevier, Amsterdam.

Birchbauer, A., W. Gerhard, and G. Knipping. 1992. Metabolism of very low density lipoproteins in the pig. An *in vivo* study. Int. J. Biochem. 24(10):1591-1597.

Blake, W. L., and S. D. Clarke. 1990. Induction of adipose fatty acid binding protein (a-FABP) by insulin-like growth factor-1 (IGF-1) in 3T3-L1 preadipocytes. Biochem. Biophys. Res. Comm.173(1):87-91.

Blanchard, P. J., M. B. Willis, C. C. Warkup, and M. Ellis. 2000. The influence of carcass backfat and intramuscular fat level on pork eating quality. J. Sci. Food Agric. 80:145-151.

Bligh, E. G., and E. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911-917.

Breier, B. H. 1999. Regulation of protein and energy metabolism by the somatotropic axis. Domest. Anim. Endocrin.17:209–218.

Brewer, M. S., and C. J. Prestat. 2002. Consumer attitudes toward food safety issues. J. Food Saf. 22:67-83.

Brown, M. S., and J. L. Goldstein. 1984. Progress in understanding the LDL receptor and HMG-CoA reductase, two membrane proteins that regulate the plasma cholesterol. J. Lipid Res. 25:1450-1461.

Bunter, K. L., S. Hermesch, B. G. Luxford, H-U. Graser, and R. E. Crump. Insulin-like growth factor-I measured in juvenile pigs is genetically correlated with economically important performance traits. 2005. Aust. J. Exp. Agr. 45:783-792.

Cai, S. S., and J. A. Syage. 2006. Comparison of atmospheric pressure photoionization, atmospheric pressure chemical ionization, and electrospray ionization mass spectrometry for analysis of lipids. Anal. Chem. 78(4):1191-1199.

Cameron, N. D., and M. B. Enser. 1991. Fatty acid composition of lipid in *longissimus dorsi* muscle of Duroc and British Landrace pigs and its relationship with eating quality. Meat Sci. 29:295-307.

Cameron, N. D., M. Enser, G. R. Nute, F. M. Whittington, J. C. Penman, A. C. Fisken, A. M. Perry, and J. D. Wood. 2000. Genotype with nutrition interaction on fatty acid composition of intramuscular fat and the relationship with flavour of pig meat. Meat Sci. 55:187-195.

Cameron, N. D., E. McCullough, K. Troup, and J. C. Penman. 2003. Serum insulin-like growth factor-1 concentration in pigs divergently selected for daily food intake or lean growth rate. J. Anim. Breed. Genet. 120:228-236.

Cánovas, A., J. Estany, M. Tor, R. N. Pena, and O. Doran. 2009a. Acetyl-CoA carboxylase and stearoyl-CoA desaturase protein expression in subcutaneous adipose tissue is reduced in pigs selected for decreased backfat thickness at constant intramuscular fat content. J. Anim. Sci. 87:3905-3914.

Cánovas, A., R. Quintanilla, D. Gallardo, I. Díaz, J. L. Noguera, O. Ramírez, and R. N. Pena. 2009b. Functional and association studies on the pig HMGCR gene, a cholesterol-synthesis limiting enzyme. Animal 4: 224-233.

Cánovas, A., R. Quintanilla, M. Amills, and R. N. Pena. 2010. Muscle transcriptomic profiles in pigs with divergent phenotypes for fatness traits. BMC Genomics 11: 372.

Cao, H., K. Gerhold, J. R. Mayers, M. M. Wiest, S. M. Watkings, and G. S. Hotamisligil. 2008. Identification of a lipokine, a lipid hormone linking adipose tissue to systemic metabolism. Cell. 134:933-944.

Caputi Jambrenghi, A., G. Paglialonga, A. Gnoni, F. Zanotti, F. Giannico, G. Vonghia, and G. V. Gnoni. 2007. Changes in lipid composition and lipogenic enzyme activities in liver of lambs fed ω-6 polyunsaturated fatty acids. Comp. Biochem. Physiol. B: 147: 498-503.

Carpenter, D. E., J. Ngeh-Ngwainbi, and S. Lee. 1993. Lipid Analysis. Page 85-104 in Methods for Analysis for Nutrition Labeling. D. M. Sullivan, and D. E, Carpenter, ed. AOAC Internacional, Arlington.

Carrapiso, A. I., and C. García. 2000. Development in lipid analysis: Some new extraction techniques and *in situ* transesterification. Lipids. 35(11):1167-1177.

Carrapiso, A. I., M. L. Timón, M. J. Petrón, J. F. Tejeda, and C. García. 2000. *In situ* transesterification of fatty acids from Iberian pig subcutaneous adipose tissue. Meat Sci. 56:159-164.

Carter, C. S., M. M. Ramsey, and W. E. Sonntag. 2002. A critical analysis of the role of growth hormone and IGF-1 in aging and lifespan. TRENDS in Genetics. 18(6):295-301.

Casellas, J., J. L. Noguera, J. Reixach, I. Díaz, M. Amills, and R. Quintanilla. 2010. Bayes factor analyses of heritability for serum and muscle lipid traits in Duroc pigs. J. Anim. Sci. 88:2246-2254.

Chen, H. C., and R. V. Farese Jr. 2002. Fatty acids, triglycerides, and glucose metabolism: recent insights from knockout mice. Curr. Opin. Clin. Nutr. Metab. Care. 5:359-363.

Chen, P., T. J. Baas, J. C. M. Dekkers, K. J. Koehler and J. W. Mabry. 2003. Evaluation of strategies for selection for lean growth rate in pigs. J. Anim. Sci. 81:1150-1157.

Christie, W. W. 1992. Pages 1-17 in Advances in Lipid Methodology – One. W. W. Christie, ed. Oily Press, Ayr.

Cilla, I., J. Altarriba, L. Guerrero, M. Gispert, L. Martínez, C. Moreno, J. A. Beltrán, M. D. Guàrdia, A. Diestre, J. Arnau, and P. Roncalés. 2006. Effect of different Duroc line sires on carcass composition, meat quality and dry-cured ham acceptability. Meat Sci. 72: 252-260.

Clarke, S. D. 1993. Regulation of fatty acid synthase gene expression: An approach for reducing fat accumulation. J. Anim. Sci. 71:1957-1965.

Coppo, N. B., J. A. Coppo, and M. A. Lazarte. 2003. "Intervalos de confianza para colesterol ligado a lipoproteínas de alta y baja densidad en suero de bovinos, equinos, porcinos y caninos ". Rev.Vet. 14:1:3-10.

Da Costa, N., C. McGillivray, Q. Bai, J. D. Wood, G. Evans, and K-C. Chang. 2004. Restriction of dietary energy and protein induces molecular changes in young porcine skeletal muscles. J. Nutr. 134:2191-2199.

Dambergs, N. 1956. Acetone-water mixtures for the extraction and rapid estimation of fats of biological materials, particularly fish products. J. Fish. Res. Bd. Canada. 13:791-796.

Dance, L. J. E., K. R. Matthews, and O. Doran. 2009. Effect of breed on fatty acid composition and stearoyl-CoA desaturase protein expression in the *semimembranosus* muscle and subcutaneous adipose tissue of cattle. Livest. Sci. 125: 291-297.

Daniels, T. F., K. M. Killinger, J. J. Michal, R. W. Wright Jr., and Z. Jiang. 2009. Lipoproteins, cholesterol homeostasis and cardiac health. Int. J. Biol. Sci. 5:474-488.

David, F., D. R. Gere, F. Scanlan and P. Sandra. 1999. Instrumentation and applications of fast high-resolution capillary gas chromatography. J. Chromatogr. A. 842: 309-319.

De Pedro, E., M. Casillas, and C. M. Miranda. 1997. Microwave oven application in the extraction of fat from the subcutaneous tissue of iberian pig ham. Meat Sci. 45:45-51.

De Smet, S., K. Raes, and D. Demeyer. 2004. Meat fatty acid composition as affected by fatness and genetic factors: A review. Anim. Res. 53:81-98.

Dekkers, J. C. M. 2004. Commercial application of marker- and gene-assisted selection in livestock: Strategies and lessons. J. Anim. Sci. 82:E313-328.

Dodson, M. V., G. J. Hausman, L. Guan, M. Du, T. P. Rasmussen, S. P. Poulos, P. Mir, W. G. Bergen, M. E. Fernyhough, D. C. McFarland, R. P. Rhoads, B. Soret, J. M. Reecy, S. G. Velleman, and Z. Jiang. 2010. Lipid metabolism, adipocyte depot physiology and utilization of meat animals as experimental models for metabolic research. Int. J. Biol. Sci. 6(7):691-699.

Dömötörová, M., and E. Matisová. 2008. Fast gas chromatography for pesticide residues analysis. J. Chromatogr. A. 1207(1-2): 281-294.

Donato, P., P. Q. Tranchida, P. Dugo, G. Dugo, and L. Mondello. 2007. Rapid analysis of food products by means of high speed gas chromatography. J. Sep. Sci. 30(4):508-526.

Doran, O., S. K. Moule, G. A. Teye, F. M. Whittington, K. G. Hallett, and J. D. Wood. 2006. A reduced protein diet induces stearoyl-CoA desaturase protein expression in pig muscle but not in subcutaneous adipose tissue: Relationship with intramuscular lipid formation. Br. J. Nutr. 95:609-617.

Dransfield, E., T. M. Ngapo, N. A. Nielsen, L. Bredahl, P. O. Sjödén, M. Magnusson, M. M. Campo, and G. R. Nute. 2005. Consumer choice and suggested price for pork as influenced by its appearance, taste and information concerning country of origin and organic pig production. Meat Sci. 69:61-70.

Driscoll, D. M., and G. S. Getz. 1984. Extrahepatic synthesis of apolipoprotein E. Lipid Res. 25:1368-1379.

Duran-Montgé, P., C. E. Realini, A. C. Barroeta, R. Lizardo and E. Esteve-Garcia. 2008. Tissue fatty acid composition of pigs fed different fat sources. Animal. 2:12:1753-1762.

Eller, F. J., and J. W. King. 1996. Determination of fat content in foods by analytical SFE I. Seminars in Food Analisis. 1:145-162.

Eras, J., J. Ferran, B. Perpiña, and R. Canela. 2004. Chlorotrimethylsilane, a reagent for the direct quantitative analisis of fats and oils present in vegetable and meat samples. J. Chromatogr. A. 1047:157-161.

Estany, J., M. Tor, D. Villalba, L. Bosch, D. Gallardo, N. Jiménez, L. Altet, J. L. Noguera, J. Reixach, M. Amills, and A. Sánchez. 2007. Association of CA repeat polymorphism at intron 1 of insulin-like growth factor (IGF-I) gene with circulating IGF-I concentration, growth, and fatness in swine. Physiol Genomics. 31:236-243.

Etherton, T. D., and P. M. Kris-Etherton. 1980. Characterization of plasma lipoproteins in swine with different propensities for obesity. Lipids. 15(10): 823-829.

Etherton, T. D. 2000. The biology of somatotropin in adipose tissue growth and nutrient partitioning. J. Nutr. 130:2623-2625.

Fan, B., Z. Q. Du, and M. F. Rothschild. 2010. The hepatocyte nuclear factor-1 alpha (HNF1A) gene is associated with fatness and loin muscle area in the pig. Animal 4:10:1619-1627.

Farese R. V. Jr., S. Cases, and S. J. Smith. 2000. Triglyceride synthesis: Insights from the cloning of diacylglycerol acyltransferase. Curr. Opin. Lipidol.11:229-234.

Fernandez, X., G. Monin, A. Talmant, J. Mourot, and B. Lebret. 1999. Influence of intramuscular fat content on the quality of pig meat −1. Composition of the lipid fraction and sensory characteristics of m. *longissimus lumborum*. Meat Sci. 53:59-65.

Fernández-Cabanás, V. M., A. Garrido-Varo, J. G. Olmo, E. De Pedro, and P. Dardenne. 2007. Optimisation of the spectral pre-treatments used for Iberian pig fat NIR calibrations. Chemometr. Intell. Lab. 87:104-112.

Fernández-Cabanás, V. M., O. Polvillo, R. Rodríguez-Acuña, B. Botella, and A. Horcada. 2011. Rapid determination of the fatty acid profile in pork dry-cured sausages by NIR spectroscopy. Food Chem. 124:373-378.

Ficarra, A., D. P. Lo Fiego, M. Giovanna, and A. Antonelli. 2010. Ultra fast analysis of subcutaneous pork fat. Food Chem. 121:809-814.

Fielding, C. J., and F. E. Fielding. 1995. Molecular transport physiology of reverse cholesterol. J. Lipid Res. 36:211-228.

Firestone, D., and M. M. Mossoba. 1997. Newer methods for fat analysis in foods. Pages 1-33 in new techniques and applications in lipid analysis. R. E. McDonald, and M. M. Mossoba, ed. JAOCS Press, Champaign, Illinois.

Folch, J., M. Less, and G. H. Sloane. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-509.

Fortin, A., W. M. Robertson, and A. K. W. Tong. 2005. The eating quality of Canadian pork and its relationship with intramuscular fat. Meat Sci. 69:297-305.

Fujii, J., K. Otsu, F. Zorzato, S. DeLEon, V. K. Khanna, J. E. Weiler, P. J. O'Brien, and D. H. MacLennon. 1991. Identification of a mutation in the porcine ryanodine receptor associated with malignant hypothermia. Science (Wash. DC) 253:448-451.

Gallardo, D., R. N. Pena, M. Amills, L. Varona, O. Ramírez, J. Reixach, I. Díaz, J. Tibau, J. Soler, J. M. Prat-Cuffi, J. L. Noguera, and R. Quintanilla. 2008. Mapping of quantitative trait loci for cholesterol, LDL, HDL, and triglyceride serum concentrations in pigs. Physiol. Genomics. 35:199-209.

Ganzler, K., A. Salgò, and K. Valkó. 1986. Microwave extraction. A novel sample preparation for chromatography. J. Chromatogr. 371: 299-306.

Gao, Y., R. Zhang, X. Hu, and N. Li. 2007. Application of genomic technologies to the improvement of meat quality of farm animals. Meat Sci. 77:36-45.

Gao, S-Z., and S-M. Zhao. 2009. Physiology, affecting factors and strategies for control of pig meat intramuscular fat. Recent. Pat. Food Nutr. Agric. 1. 59-74.

García-Macías, J. A., M. Gispert, M. A. Oliver, A. Diestre, P. Alonso, A. Muñoz-Luna, K. Siggens, and D. Cuthbert-Heavens. 1996. The effects of cross, slaughter weight and halothane genotype on leanness and meat and fat quality in pig carcasses. Animal. 63:487-496.

García-Olmo, J., E. De Pedro, A. Garrido, A. Paredes, C. Sanabria, M. Santolalla, J. Salas, J. R. García-Hierro, I. Gonzalez, M. D. García-Cachan, and J. Guirao. 2002. Determination of the precision of fatty acid analysis of Iberian pig fat by gas chromatography. Results of a mini collaborative study. Meat Sci. 60:103-109.

Gardan, D., F. G. Gondret, and I. Louveau. 2006. Lipid metabolism and secretory function of porcine intramuscular adipocytes compared with subcutaneous and perirenal adipocytes. Am. J. Physiol. Endocrinol. Metab. 291:E372-E380.

Gondret, F., P. Ferré, and I. Dugail. 2001. ADD-1/SREBP-1 is a major determinant of tissue differential lipogenic capacity in mammalian and avian species. J. Lipid Res.42:106-113.

Gondret, F., N. Guitton, C. Guillerm-Regost, and I. Louveau. 2008. Regional differences in porcine adipocytes isolated from skeletal muscle and adipose tissues as identified by a proteomic approach. J. Anim.Sci. 86:2115-2125.

Gonzalez-Baró, M. R., T. M. Lewin, and R. A. Coleman. 2007. Regulation of triglyceride metabolism II. Function of mitochondrial GPAT1 in the regulation of triacylglycerol biosynthesis and insulin action. Am. J. Physiol. Gastrointest. Liver Physiol. 292:G1195–G1199.

González-Martín, I., C. González-Pérez, N. Alvarez-García, and J. M. González-Cabrera. 2005. On-line determination of fatty acid composition in intramuscular fat of Iberian pork loin by NIRs with a remote reflectance fibre optic probe. Meat Sci. 69:243-248.

Gosetti, F., E. Mazzucco, V. Gianotti, S. Polati, and M. C Gennaro. 2007. High performance liquid chromatography/tandem mass spectrometry determination of biogenic amines in typical Piedmont cheeses. J. Chrom. A. 1149:151-157.

Gregoire, F. M., C. M. Smas, and H. S. Sul. 1998. Understanding adipocyte differentiation. Physiol. Rev. 78(3):783-809.

Guillou, H., D. Zadravec, G. P. Martin, and A. Jacobsson. 2010. The key roles of elongases and desaturases in mammalian fatty acid metabolism: Insights from transgenic mice. Prog. Lipid Res. 49:186-199.

Gunnlaugsdottir, H., and R. G. Ackman. 1993. Extraction methods for determination of lipids in fish-meal. Evaluation of a hexane isopropanol method as an alternative to chloroform-based method. J. Sci. Food Agr. 61(2):235-240.

Hausman, D. B., M. DiGirolamo, T. J. Bartness, G. J. Hausman, and R. J. Martin. 2001. The biology of white adipocyte proliferation. Obes. Rev. 2:239-254.

Hausman, G. J., M. V. Dodson, K. Ajuwon, M. Azain, K. M. Barnes, L. L. Guan, Z. Jiang, S. P. Poulos, R. D. Sainz, S. Smith, M. Spurlock, J. Novakofski, M. E. Fernyhough and W. G. Bergen. 2009. Board-Invited Review: The biology and regulation of preadipocytes and adipocytes in meat animals. J. Anim. Sci. 87:1218-1246.

Heinemann, F. S. and J. Ozols. 2003. Stearoyl-CoA desaturase, a short-lived protein of endoplasmic reticulum with multiple control mechanisms. Prostaglandins Leukot. Essent. Fatty Acids. 68:123-133.

Hermesch, S., K. L. Bunter, and B. G. Luxford. 2001. Estimates of genetic correlations between IGF-1 recorded at 4 weeks of age and individual piglet weights at birth and 14 days along with lifetime growth rate and backfat. Proc. Assoc. Advan. Anim. Breed. Genet. 14:227-230.

Hermesch, S. 2004. Genetic improvement of lean meat growth and feed efficiency in pigs. Aust. J. Exp. Agr. 44:383-391.

Hervás, C., A. Garrido, B. Lucena, N. García, and E. De Pedro. 1994. Near infrared spectroscopy for classification of Iberian pig carcasses using an artificial neural network. J. Near Infrared Spectrosc. 2:177-184.

Hlabangana, L., S. Hernandez-Cassou, and J. Saurina. 2006. Multicomponent determination of drugs using flow-injection analysis. Curr. Phar. Anal. 2:127-140.

Hocquette, J-F., P. Bas, D. Bauchart, M. Vermorel, and Y. Geay. 1999. Fat partitioning and biochemical characteristics of fatty tissues in relation to plasma metabolites and hormones in normal and double-muscled young growing bulls. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 122:127-138.

Hodgson, R. R., W. Davis, G. C. Smith, J. W. Savell, and H. R. Cross. 1991. Relationships between pork loin palatability traits and physical characteristics of cooked chops. J. Anim. Sci. 69: 4858-4865.

Hood, R. L., and C. E. Allen. 1973. Lipogenic enzyme activity in adipose tissue during the growth of swine with different propensities to fatten. J. Nutr. 103:353-362.

Hood, R. L., and C. E. Allen. 1977. Cellularity of porcine adipose tissue: Effects of growth and adiposity. J. Lipid. Res.18:275-284.

Hossner, K. L., R. H. McCusker, and M. V. Dodson. 1997. Insulin-like growth factors and their binding proteins in domestic animals. Anim. Sci. 64:1-15.

Huff-Lonergan, E., and S. M. Lonergan. 2005. Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. Meat sci. 71:194-204.

Issanchou, S. 1996. Consumer expectations and perceptions of meat and meat product quality. Meat Sci. 43: S5-S19.

Iverson, J. L., and A. J. Sheppard. 1986. Determination of fatty acids in butter fat using temperature-programmed gas chromatography of the butyl esters. Food Chem. 21:223-234.

Iverson, J. L., and A. J. Sheppard. 1989. Detection of adulteration in cow, goat, and sheep cheeses utilizing gas-liquid chromatographic fatty acid data. J. Dairy Sci. 72(7):1707-1712.

Iverson, S. J., S. L. C. Lang, and M. H. Cooper. 2001. Comparison of the Bligh and Dyer and Folch methods for total lipid determination in a broad range of marine tissue. Lipids. 36:1283-1287.

Jakobsson, A., R. Westerberg, and A. Jacobsson. 2006. Fatty acid elongases in mammals: Their regulation and roles in metabolism. Prog. Lipid Res. 45:237-249.

Jiang, Y. Z., X. W. Li, and G. X. Yang. 2006. Sequence characterization, tissue-specific expression and polymorphism of the Porcine (*Sus scrofa*) liver-type fatty acid binding protein gene. Acta Genetica Sinica 33(7): 598-606.

Jump, D. B., and S. D. Clarke. 1999. Regulation of gene expression by dietary fat. Annu. Rev. Nutr.19:63-90.

Jump, D. B. 2004. Fatty acid regulation of gene transcription. Critical Reviews in clinical laboratory sciences. 41(1):41-78.

Jump, D. B., Y. Wang, J. Xu, B. Christian, and O. Demeure. 2005. Recent advances in nutritional sciences fatty acid regulation of hepatic gene transcription. J. Nutr. 135:2503-2506.

Kaluzny, M. A., L. A. Duncan, M. V. Merritt, and D. E. Epps. 1985. Rapid separation of lipid classes in high yield and purity using bonded phase columns. J. Lipid. Res. 26:135-140.

Karlsson, A., A. C. Enfält, B. Essén-Gustavsson, K. Lundström, L. Rydhmer and S. Stern. 1993. Muscle histochemical and biochemical properties in relation to meat quality during selection for increased lean tissue growth rate in pigs. J. Anim. Sci. 71:930-938.

Keele, J. W., R. K. Johnson, L. D. Young, and T. E. Socha. 1988. Comparison of methods of predicting breeding values of swine. J. Anim. Sci. 66:3040-3048.

Kerwin, J. L., and A. M. Wiens. 1996. Identification of fatty acids by electrospray mass spectrometry and tandem mass spectrometry. J. Mass Spectrum. 31:184-192.

Kim, J. B., P. Sarraf, M. Wright, K. M. Yao, E. Mueller, G. Solanes, B. B. Lowell, and B. M. Spiegelman. 1998a. Nutritional and insulin regulation of fatty acid synthetase and leptin gene expression through ADD1/SREBP1. J. Clin. Invest. 101:1-9.

Kim, J. B., H. M. Wright, M. Wright, and B. M. Spiegelman. 1998b. ADD1 y SREBP1 activates PPARγ through the production of endogenous ligand. Proc. Natl. Acad. Sci. USA. 95:4333-4337.

King, J. W., F. J. Eller, J. M. Snyder, J. H. Johnson, F. K. McKeith, and C. R. Suites. 1996. Extraction of fat from ground beef for nutrient analysis using analytical supercritical fluid extraction. J. Agric. Food Chem. 44:2700-2704.

Knap, P. W. 1999. Simulation of growth in pigs: evaluation of a model to relate thermoregulation to body protein and lipid content and deposition. Anim. Sci. 68:655-679.

Korytár, P., J. Hans-Gerd, E. Matisová, and U.A.Th. Brinkman. 2002. Practical fast gas chromatography: Methods, instrumentation and applications. Trends Analyt. Chem. 21(9-10):558-572.

Kostecká, Z., and J. Blahovec. 2002. Animal insulin-like growth factor binding proteins and their biological functions. Vet. Med. – Czech. 47(2-3):75-84.

Kouba, M., M. Enser, F. M. Whittington, G. R. Nute, and J. D. Wood. 2003. Effect of a high-linolenic acid diet on lipogenic enzyme activities, fatty acid composition, and meat quality in the growing pig. J. Anim. Sci. 81:1967-1979.

Kovács, L., A. Zalka, R. Dobó, and J. Pucsok. 1986. One-dimensional thin-layer chromatographic separation of lipids into fourteen fractions by two successive developments on the same plate. J. Chromatogr. 382:308-313.

Kris-Etherton, P. M., and T. D. Etherton. 1982. The role of lipoproteins in lipid metabolism of meat animals. J. Anim. Sci. 55:804-817.

Kurata, S., K. Yamaguchi, and M. Nagai. 2005. Rapid discrimination of fatty acid composition in fats and oils by electrospray ionization mass spectrometry. Anal. Sci. 21:1457-1465.

Laaksonen, D. E., K. Nyyssönen, L. Niskanen, T. H. Rissanen, and J. T. Salonen. 2005. Prediction of cardiovascular mortality in middle-aged men by dietary and serum linoleic and polyunsaturated fatty acids. Arch. Intern. Med. 165:193-199.

Lahti, K.G., K. Bunter, J. Mercer, and S. Clearkin. 2001. Genetic relationships between insulin-like growth factor-I and performance traits in purebred swine. J. Anim. Sci. 79 (Suppl. 1):192.

Lamberson, W. R., T. J. Safranski, R. O. Bates, D. H. Keisler, and R. L. Matteri. 1995. Relationships of serum insulin-like growth factor I concentrations to growth, composition, and reproductive traits of swine. J. Anim. Sci. 73:3241-3245.

Lan, Y. H., F. K. McKeith, J. Novakofski, and T. R. Carr. 1993. Carcass and muscle characteristics of Yorkshire, Meishan, Yorkshire x Meishan, Meishan x Yorkshire, Fengjing x Yorkshire, and Minzhu x Yorkshire pigs. J. Anim. Sci. 71:3344-3349.

Landriscina, C., G. V. Gnoni, and E. Quagliariello. 1972. Fatty-acid biosynthesis. The physiological role of the elongation system present in microsomes and mitochondria of rat liver. Eur. J. Biochem. 29:188-196.

Lauritzen, L., H. S. Hansen, M. H. Jørgensen, and K. F. Michaelsen. 2001. The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. Progr. Lipid Res. 40:1-94.

Lee, Y. B., and Y. I. Choi. 1999. PSE (pale, soft, exudative) pork: The causes and solutions-Review. Asian-Australas J. Anim. Sci. 12:244-252.

Lengi, A. J., and B. A., Corl. 2007. Identification and characterization of a novel bovine stearoyl-CoA desaturase isoform with homology to human SCD5. Lipids. 42:499-508.

Lengi, A. J., and B. A. Corl. 2008. Comparison of pig, sheep and chicken SCD5 homologs: Evidence for an early gene duplication event. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 150:440-446.

Le Roith, D., L. Scavo, and A. Butler. 2001. What is the role of circulating IGF-I? TRENDS in Endocrinology & Metabolism.12(2):48-52.

Lewis, K. J., P. C. Molan, and J. J. Bass. 1988. The lipolytic activity of low concentrations of insulin-like growth factors in ovine adipose tissue. Endocrinology. 122:2554-2557.

Liu, K. S. 1994. Preparation of fatty acid methyl esters for gas-chromatographic analysis of lipids in biological materials. J. Am. Oil Chem. Soc. 71:1179-1187.

Louveau, I., M. Bonneau, and D. N. Salter. 1991. Age-related changes in plasma porcine growth hormone (GH) profiles and insulin-like growth factor-I (IGF-I)

concentrations in Large White and Meishan pigs. Reprod. Nutr. Dev. 31:205-216.

Louveau, I., and M. Bonneau. 1996. Effect of a growth hormone infusion on plasma insulin-like growth factor-I in Meishan and Large White pigs. Reprod. Nutr. Dev. 36:301-310.

Louveau, I., and F. Gondret. 2004. Regulation of development and metabolism of adipose tissue by growth hormone and the insulin-like growth factor system. Domestic Animal Endocrinology. 27:241-255.

Luxford, B. G., K. L. Bunter, P. C. Owens, and R. G. Campbell. 1998. Use of IGF-1 as a selection criteria in pig breeding. Pages 17-21 in AGBU Pig Genetics Workshop.

Macala, L. J., R. K. Yu, and S. Ando. 1983. Analysis of brain lipids by high-performance thin-layer chromatography and densitometry. J. Lipid. Res. 24(9):1243-1250.

Mahley, R. W., T. L. Innerarity, S. C. Rall, Jr., and K. H. Weisgraber. 1984. Plasma lipoproteins: Apolipoprotein structure and function. J. Lipid Res. 25:1277-1294.

Majewska, M., K. Krosowiak, A. Raj, and K. Smigielski. 2008. Solid phase extraction in food analysis. Food Chem. Biotechnol.72:1-12.

Mastovská, K., and S. J. Lehotay. 2003. Practical approaches to fast gas chromatography–mass spectrometry. J. Chromatogr. A. 1000:153-180.

Matisová, E., M. Simeková, S. Hrouzková, P. Korytár, and M. Dömötörová. 2002. Factors influencing chromatographic data in fast gas chromatography with on-column injection. J. Sep. Sci. 25:1325-1331.

Matisová, E., and M. Dömötörová. 2003. Fast gas chromatography and its use in trace analysis. J. Chromatogr. A. 1000:199-221.

Matsuzaka T., H. Shimano, N. Yahagi, T. Kato, A. Atsumi, and T. Yamamoto. 2007. Crucial role of a long-chain fatty acid elongase, Elovl6, in obesity-induced insulin resistance. Nat. Med. 13:1193-202.

Matsuzaka, T., and H. Shimano. 2009. Elovl 6: A new player in fatty acid metabolism and insulin sensitivity. Mol. Med. 87:379-384.

Mattson, F. H., and S. M. Grundy. 1985. Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. J. Lipid Res. 26:194-202.

Mersmann, H. J., W. G. Pond, and J. T. Yen. 1982. Plasma glucose, insulin and lipids during growth of genetically lean and obese swine. Growth. 46:189-198.

Missotten, J., S. De Smet, K. Raes, and O. Doran. 2009. Effect of supplementation of the maternal diet with fish oil or linseed oil on fatty-acid composition and expression of Δ5- and Δ6-desaturase in tissues of female piglets. Animal. 3:8:1196-1204.

Miyazaki, M., Y-C. Kim, M. P. Gray-Keller, A. D. Attie, and J. M. Ntambi. 2000. The biosynthesis of hepatic cholesterol esters and triglycerides is impaired in mice with a disruption of the gene for stearoyl-CoA desaturase 1. J. Biol. Chem. 275:30132-30138.

Miyazaki, M., M. J. Jacobson, W. C. Man, P. Cohen, E. Asilmaz, J. M. Friedman, and J. M. Ntambi. 2003. Identification and characterization of murine SCD4, a novel heart-specific stearoyl-CoA desaturase isoform regulated by leptin and dietary factors. J. Biol. Chem. 278:33904-33911.

Miyazaki, M., and J. M. Ntambi. 2003. Role of stearoyl-coenzyme A desaturase in lipid metabolism. Prostaglandins Leukot. Essent. Fatty Acids. 68:113-121.

Mondello, L., R. Costa, B. Chiofalo, and V. Chiofalo. 2004. Pages 439-441 in Qualité Sicurezza degli Alimenti, Morgan Scientifiche, ed. Milano.

Morrison, W. R., and L. M. Smith. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J. Lipid Res. 5:600-608.

Mourot, J., M. Kouba, and G. Salvatori. 1999. Facteurs de variation de la lipogenèse dans les adipocytes et les tissus adipeux chez le porc. INRA Prod. Anim. 12(4):311-318.

Mourot, J., and D. Hermier. 2001. Lipids in monogastric animal meat. Reprod. Nutr. Dev. 41:109-118.

Nafikov, R. A., and D. B. Beitz. 2007. Carbohydrate and lipid metabolism in farm animals. J. Nutr. 137:702-705.

Nakamura, M. T., and T. Y. Nara. 2004. Structure, function, and dietary regulation of Δ6, Δ5, and Δ9 desaturases. Annu. Rev. Nutr. 24:345-376.

Niemelä, S., S. Miettinen, J. R. Sarkanen, and N. Ashammakhi. 2008. Adipose tissue and adipocyte differentiation: Molecular and cellular aspects and tissue engineering applications. Vol 4. in Topics in issue engineering. N. Ashammakhi, R. Reis, and F. Chiellini, ed. © 2008.

Ntambi, J. M. 1999**.** Regulation of stearoyl-CoA desaturase by polyunsaturated fatty acids and cholesterol: A review. J. Lipid Res. 40:1549-1558.

Ntambi, J. M., and Y-C. Kim. 2000. Adipocyte differentiation and gene expression. Symposium: Adipocyte function, differentiation and metabolism. J. Nutr. 130:3122S-3126S.

Ntambi, J. M., and M. Miyazaki. 2004. Regulation of stearoyl-CoA desaturases and role in metabolism. Prog. Lipid Res. 43:91-104.

.

Ntawubizi, M., K. Raes, N. Buys, and S. De Smet. 2009. Effect of sire and sex on the intramuscular fatty acid profile and indices for enzyme activities in pigs. Livest. Sci. 122:264-270.

Nürnberg, K., J. Wegner, and K. Ender. 1998. Factors influencing fat composition in muscle and adipose tissue of farm animals. Livest. Prod. Sci. 56:145-156.

O´Hea, E. K., and G. A. Leveille. 1969. Significance of adipose tissue and liver as sites of fatty acid synthesis in the pig and the efficiency of utilization of various substrates for lipogenesis. J. Nutr. 99:338-344.

Ohlsson, C., S. Mohan, K. Sjogren, A. Tivesten, J. Isgaard, O. Isaksson, J. O. Jansson, and J. Svensson. 2009. The role of liver-derived insulin-like growth factor-I. Endocr. Rev. 30(5):494-535.

Olivares, A., A. I. Rey, A. Daza, and C. J. Lopez-Bote. 2011. Low levels of dietary vitamin A increase intramuscular fat content and polyunsaturated fatty acid proportion in liver from lean pigs. Livest. Sci. 137:31-36.

Oliver, M. A., P. Gou, M. Gispert, A. Diestre, J. Arnau, J. L. Noguera and A. Blasco. 1994. Comparison of five types of pig crosses. II. Fresh meat quality and sensory characteristics of dry cured ham. Livest. Prod. Sci. 40:179-185.

Olson, R. E. 1998. Symposium: Evolution of ideas about the nutritional value of dietary fat. Discovery of the lipoproteins, their role in fat transport and their significance as risk factors. J. Nutr. 128:439S-443S.

Owens, P. C., K. L. Gatford, P. E. Walton, W. Morley, and R. G. Campbell. 1999. The relationship between endogenous insulin-like growth factors and growth in pigs. J. Anim. Sci. 77:2098-2103.

Owens, P. C., R. G. Campbell, B. G. Luxford, and P. E. Walton. 2000. Selection of livestock using IGF levels. United States Patent. Patent number: 6,090,569.

Panagiotopoulou, P. M., and M. Tsimidou. 2002. Solid phase extraction: Applications to the chromatographic analysis of vegetables oils and fats. Grasas y aceites. 53(1):84-95.

Paré, J. R. J., G. Matni, J. M. R. Bélanger, K. Li, C. Rule, and B. Thibert. 1997. Use of the microwave-assisted process in extraction of fat from meat, dairy, and egg products under atmospheric pressure conditions. J. AOAC Int. 80:928-933.

Pégorier, J. P., P. H. Duée, J. Girard, and J. Peret. 1983. Metabolic fate of non-esterified fatty acids in isolated hepatocytes from newborn and young pigs. Evidence for a

limited capacity for oxidation and increased capacity for esterification. Biochem. J. 212:93-97.

Perret, D., A. Gentili, S. Marchese, M. Sergi, and L. Caporossi. 2004. Determination of free fatty acids in chocolate by liquid chromatography with tandem mass spectrometry. Rapid Commun. Mass Sp. 18:1989-1994.

Pettigrew, J. E., and M. A. Esnaola. 2001. Swine nutrition and pork quality: A review. J. Anim. Sci. 79:E316-E342.

Pond, W.G., H. J. Mersmann, and L. D. Young. 1986. Heritability of plasma cholesterol and triglyceride concentrations in swine. Proc. Soc. Exp. Biol. Med. 182:221-224.

Pond, W. G., H. J. Mersmann, P. D. Klein, L. L. Ferlic, W. W. Wong, D. L. Hachey, P. A. Schoknecht, and S. Zhang. 1993. Body weight gain is correlated with serum cholesterol at 8 weeks of age in pigs selected for four generations for low and high serum cholesterol. J. Anim. Sci. 71:2406-2411.

Pond, W. G., D. R. Su, and H. J. Mersmann. 1997. Divergent concentrations of plasma metabolites in swine selected for seven generations for high or low plasma total cholesterol. J. Anim. Sci. 75:311-316.

Postic, C., and J. Girard. 2008. The role of the lipogenic pathway in the development of hepatic steatosis. Diabetes Metab. 34:643-648.

Rajaram, S., D. J. Baylink and S. Mohan. 1997. Insulin-like growth factor-binding proteins in serum and other biological fluids: Regulation and functions. Endoc. Rev. 18(6): 801-831.

Ramsay, T. G., M. E. White, and C. K. Wolverton. 1989. Insulin-like growth factor 1 induction of differentiation of porcine preadipocytes. J. Anim.Sci. 67:2452-2459.

Randolph, T. W. 1990. Supercritical fluid extractions in biotechnology. Trends Biotechnol. 8:78-82.

Rauw, W. M., E. Kanis, E. N. Noordhuizen-Stassen, and F. J. Grommers. 1998. Undesirable side effects of selection for high production efficiency in farm animals: A review. Livest. Prod. Sci. 56:15-33.

Rauw, W. M., O. Portolés, D. Corella, J. Soler, J. Reixach, J. Tibau, J. M. Prat, I. Diaz, and L. Gómez-Raya. 2007. Behaviour influences cholesterol plasma levels in a pig model. Animal. 1:6:865-871.

Rauw, W. M. 2009. Resource allocation theory applied to farm animal production W. M. Rauw, ed. Cambridge, MA:CABI, cop., Wallingford, UK.

Rechler, M. M., and S. P. Nissley. 1985. The nature and regulation of the receptors for insulin-like growth factors. Annu. Rev. Physiol. 47:425-442.

Reiter, S. S., C. H. C. Halsey, B. M. Stronach, J. L. Bartosh, W. F. Owsley, and W. G. Bergen. 2007. Lipid metabolism related gene-expression profiling in liver, skeletal muscle and adipose tissue in crossbred Duroc and Pietrain Pigs. Comp. Biochem. Physiol. D2:200-206.

Reixach, J., M. Tor, C. Díaz, and J. Estany. 2008. Between and within breed variation for lean growth and intramuscular fat content and fatty acid composition in pigs. Page 9.13. in Proceedings of the 54th International Congress of Meat Science and Technology, Cape Town, South Africa, 10-15 August.

Reixach, J., M. Tor, and J. Estany. 2009. Respuesta a la selección contra grasa dorsal a grasa intramuscular constante en cerdos Duroc. Pages 120-122 in XIII Jornada sobre Producción Animal. M. Joy, J. H. Calvo, C. Calvete, M. A. Latorre, I. Casasus, A. Bernues, B. Panea, A. Sanz, and J. Balcells, ed. AIDA, Zaragoza, Spain.

Reixach, J., and J. Estany. 2010. Genetic parameters for intramuscular oleic fatty acid content in a Duroc line (Abstract). Proc. 9th World Congr. Genet. Appl. Livest. Produc., Leipzig, Germany: 294.

Ren, J., C. Knorr, L. Huang and B. Brenig. 2004. Isolation and molecular characterization of the porcine stearoyl-CoA desaturase gene. Gene. 340:19-30.

Renaville, R., M. Hammadi, and D. Portetelle. 2002. Role of the somatotropic axis in the mammalian metabolism. Domestic Animal Endocrinology. 23:351-360.

Roongta, U. V., J. G. Pabalan, X. Wang, R-P. Ryseck, J. Fargnoli, B. J. Henley, W-P. Yang, J. Zhu, M. T. Madireddi, R. M. Lawrence, T. W. Wong and B. A. Rupnow. 2011. Cancer cell dependence on unsaturated fatty acids implicates Stearoyl-CoA desaturase as a target for cancer therapy. Mol. Cancer Res. 9 (11):1551-1561.

Rosen, C. J., and M. Pollak. 1999. Circulating IGF-I: New perspectives for a new century. 1999. TEM. 10(4):136-141.

Ruiz-Capillas, C., P. Aller-Guiote, and F. Jimenez-Colmenero. 2007. Application of flow injection analysis to determine protein-bound nitrite in meat products. Food Chem. 101:812-816.

Rule, D. C. 1997. Direct transesterification of total fatty acid of adipose tissue and freeze dried muscle and liver with boron-trifluoride in methanol. Meat Sci. 46(1):23-32.

Ruzicka, J., and E. H. Hansen. 1975. Flow injection analyses 1. New concept of fast continuous-flow analysis Anal. Chim. Acta. 78(1):145-157.

Saleh, J., A. D. Sniderman, and K. Cianflone. 1999. Regulation of plasma fatty acid metabolism. Clin. Chim. Acta. 286 (1-2):163-180.

Scheider, J. F., L. L. Christian, and D. L. Kuhlers. 1982. Crossbreding in swine: Genetic effects on litter performance. J. Anim. Sci. 54:739-746.

Schuartz, J., and H. M. Goodman. 1976. Comparison of the delayed actions of growth hormone and somatomedin on adipose tissue metabolism. Endocrinology. 98: 730-737.

Scott, B. L., and N. G. Bazan. 1989. Membrane docosahexaenoate is supplied to the developing brain and retina by the liver. Proc. Natl. Acad. Sci. USA. 86:2903-2907.

Sellier, P. 1976. The basis of crossbreeding in pigs: a review. Livest. Prod. Sci. 3:203-226.

Sellier, P. 1998. Genetics of meat and carcass traits. Pages 463-510 in The genetics of the pig. M. F. Rothschild and A. Ruvinsky, ed. CAB International, Wallingford, UK.

Shimizu, M., F. Torti, and R. A. Roth. 1986. Characterization of the insulin and insulin-like growth factor receptors and responsitivity of a fibroblast/adipocyte cell line before and after differentiation. Biochem. Biophys. Res. Commun. 137(1):552-558.

Sherma, J. 2000. Thin-layer chromatography in food and agricultural analysis. J. Chromatogr. A. 880:129-147.

Simmen, F. A., L. Badinga, M. L. Green, I. Kwak, S. Song, and R. C. M. Simmen. 1998. The porcine insulin-like growth factor system: At the interface of nutrition, growth and reproduction. J. Nutr. 128:315S-320S.

Smith, P. J., L. S. Wise, R. Berkowitz, C. Wan, and C. S. Rubin. 1988. Insulin-like growth factor-I is an essential regulator of the differentiation of 3T3-L1 adipocytes. J. Biol. Chem. 263 (19):9402-9408.

Smith, S. B., H. J. Mersmann, E. O. Smith, and K. G. Britain. 1999. Stearoyl-coenzyme A desaturase gene expression during growth in adipose tissue from obese and crossbred pigs. J. Anim. Sci. 77:1710-1716.

Smith, S., A. Witkowski, and A. K. Joshi. 2003. Structural and functional organization of the animal fatty acid synthase: A Review. Prog. Lipid Res. 42:289-317.

Smith, J., M. Al-Amri, P. Dorairaj, and A. Sniderman. 2006. The adipocyte life cycle hypothesis. Clin. Sci. 110:1-9.

Solanes, F. X., J. Reixach, M. Tor, J. Tibau, and J. Estany. 2009. Genetic correlations and expected response for intramuscular fat content in a Duroc pig line. Livest. Sci. 123:63-69.

Soxhlet, F. 1879. Die gewichtsanalytische Bestimmung des Milchfettes, Polytechnisches J. (Dingler's). 232:461.

Suryawan, A., L. V. Swanson, and C. Y. Hu. 1997. Insulin and hydrocortisone, but not triiodothyronine, are required for the differentiation of pig preadipocytes in primary culture. J. Anim. Sci. 75:105-111.

Suzuki, K., M. Nakagawa, K. Katoh, H. Kadowaki, T. Shibata, H. Uchida, Y. Obara and A. Nishida. 2004. Genetic correlation between serum insulin-like growth factor-1 concentration and performance and meat quality traits in Duroc pigs. J. Anim. Sci. 82:994-999.

Suzuki, K., M. Irie, H. Kadowaki, T. Shibata, M. Kumagai and A. Nishida. 2005. Genetic parameter estimates of meat quality traits in Duroc pigs selected for average daily gain, *longissimus* muscle area, backfat thickness, and intramuscular fat content. J. Anim. Sci. 83:2058-2065.

Suzuki, K., M. Ishida, H. Kadowaki, T. Shibata, H. Uchida and A. Nishida. 2006. Genetic correlations among fatty acid compositions in different sites of fat tissues, meat production, and meat quality traits in Duroc pigs**.** J. Anim. Sci. 84:2026-2034.

Taylor, J. A., D. N. Salter, W. H. Close, and G. H. Laswai. 1992. Serum concentrations of insulin-like growth factor 1 and cholesterol in relation to protein and fat deposition in growing pigs. Anim. Prod. 55:257-264.

Terpstra, A. H. M., A. F. Stucchi, T. L. Foxall, G. T. Shwaery, D. B. Vespa and R. J. Nicolosi. 1993. Unidirectional transfer *in vivo* of high-density lipoprotein cholesteryl esters to lower-density lipoproteins in the pig, an animal species without plasma cholesteryl ester transfer activity. Metabolism. 42(12):1524-1530.

Theil, P. K., and C. Lauridsen. 2007. Interactions between dietary fatty acids and hepatic gene expression in livers of pigs during the weaning period. Livest. Sci. 108:26-29.

Tong, L. 2005. Acetyl-coenzyme A carboxylase: Crucial metabolic enzyme and attractive target for drug discovery. CMLS. 62:1784-1803.

Tremere, A.W., B. D. Owen, and J. M. Bell. 1966. Relationship between blood serum lipid and protein constituents and carcass measurements in swine. J. Anim. Sci. 25:138-141.

Urkijo, E., P. Eguinoa, and J. Labairu. 2008. Valorización commercial de canales de porcino en Navarra. Navarra Agraria. 166:45-55.

Valsta, L. M., H. Tapanainen, and S. Männistö. 2005. Meat fats in nutrition. Meat Sci. 70:525-530.

Van der Wal, P. G., A. H. Bolink, and G. S. M. Merkus. 1988. Research note. Differences in quality characteristics of normal, PSE and DFD pork. Meat Sci. 24: 79-84.

Verbeke, W., M. J. Van Oeckel, N. Warnants, J. Viaene, and Ch.V. Boucqué. 1999. Consumer perception, facts and possibilities to improve acceptability of health and sensory characteristics of pork. Meat Sci. 53:77-99.

Wabitsch, M., H. Hauner, E. Heinze, and W. M. Teller. 1995. The role of growth hormone/ insulin-like growth factors in adipocyte differentiation. Metabolism. 44(10):45-49.

Wakil, S. J. 1989. Fatty acid synthase, a proficient multifunctional enzyme? Perspect. Biochem. 28(11): 4524-4530.

Wakil, S. J., and L. A. Abu-Elheiga. 2009. Fatty acid metabolism: Target for metabolic síndrome. J. Lipid Res. 50: S138-S143.

Wang, Y., D. Botolin, B. Christian, J. Busik, J. Xu, and D. B. Jump. 2005a. Tissue-specific, nutritional, and developmental regulation of rat fatty acid elongases. J. Lipid Res. 46:706-715.

Wang, J., L.Yu, R. E. Schmidt, C. Su, X. Huang, K. Gould and G. Cao. 2005b. Characterization of HSCD5, a novel human stearoyl-CoA desaturase unique to primates. Biochem. Biophys. Res. Común. 332:735-742.

Wang, Y., D. Botolin, J. Xu, B. Christian, E. Mitchell, B. Jayaprakasam, M. Nair, J. M. Peters, J. Busik, and D. B. Jump. 2006. Regulation of hepatic fatty acid elongase and desaturase expression in diabetes and obesity. J. Lipid Res. 47:2028-2041.

Warriss, P. D., S. N. Brown, J. G. Franklin, and S. C. Kestin. 1990. The thickness and quality of backfat in various pig breeds and their relationship to intramuscular fat and the setting of joints from the carcasses. Meat Sci. 28(1):21-29.

Webb, E. C., and H. A. O 'Neill. 2008. The animal fat paradox and meat quality. Meat Sci. 80:28-36.

Williams, C. M. 2000. Dietary fatty acids and human health. Ann. Zootech. 49:165-180.

Wood, J. D., S. N. Brown, G. R. Nute, F. M. Whittington, A. M. Perry, S. P. Johnson and M. Enser. 1996. Effects of breed, feed level and conditioning time on the tenderness of pork. Meat Sci. 44(1-2):105-112.

Wood, J. D., G. R. Nute, R. I. Richardson, F. M. Whittington, O. Southwood, G. Plastow, R. Mansbridge, N. Da Costa, and K. C. Chang. 2004. Effects of breed, diet and muscle on fat deposition and eating quality in pigs. Meat Sci. 67:651-667.

Wu, A-L., and H. G. Windmueller. 1979. Relative contributions by liver and intestine to individual plasma apolipoproteins in the rat. J. Biol. Chem. 254(15):7316-7322.

Xueqing, L., M. S. Strable, and J. M. Ntambi. 2011. Stearoyl CoA Desaturase 1: Role in cellular inflammation and stress. Adv. Nutr. 2(1):15-22.

Yang, W. C., J. Adamec, and F. E. Regnier. 2007. Enhancement of the LC/MS analysis of fatty acids through derivatization and stable isotope coding. Anal. Chem. 79: 5150-5157.

Young, L. D., W. G. Pond, and H. J. Mersmann. 1993. Direct and correlated responses to divergent selection for serum cholesterol concentration on day 56 in swine. J. Anim. Sci. 71:1742-1753.

Zadravec, D., A. Brolinson, R. M. Fisher, C. Carneheim, R. I. Csikasz, J. Bertrand-Michel, J. Borén, H. Guillou, M. Rudling, and A. Jacobsson. 2010. Ablation of the very-long-chain fatty acid elongase ELOVL3 in mice leads to constrained lipid storage and resistance to diet-induced obesity. FASEB J. 24(11):4366-4377.

Zhang, L., L. Ge, S. Parimoo, K. Stenn, and S. M. Prouty. 1999. Human stearoyl-CoA desaturase: Alternative transcripts generated from a single gene by usage of tandem polyadenylation sites. Biochem. J. 340:255-264.

Zhao, S., J. Wang, X. Song, X. Zhang, C. Ge, and S. Gao. 2010. Impact of dietary protein on lipid metabolism-related gene expression in porcine adipose tissue. Nutr. Metab. 7:6:1-13.

Zheng, Y., S. M. Prouty, A. Harmon, J. P. Sundberg, K. S. Stenn, and S. Parimoo. 2001. Scd3-A novel gene of the stearoyl-CoA desaturase family with restricted expression in skin. Genomics 71:182-191.