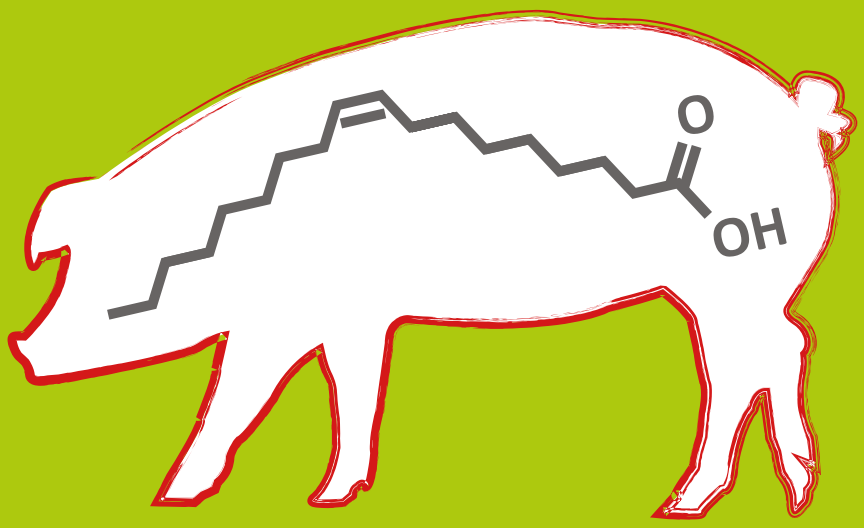
Genetic analysis and selection for intramuscular fat and oleic acid content in pigs

Roger Ros Freixedes

Dipòsit Legal: L224.-2015
http://hdl.handle.net/10803/285933

Universitat de Lleida

GENETIC ANALYSIS AND SELECTION FOR INTRAMUSCULAR FAT AND OLEIC ACID CONTENT IN PIGS



Roger Ros Freixedes

TESI DOCTORAL
Lleida, octubre 2014

Universitat de Lleida

GENETIC ANALYSIS AND SELECTION FOR INTRAMUSCULAR FAT AND OLEIC ACID CONTENT IN PIGS

Presentat per / *Presented by*
Roger Ros Freixedes

Aquesta tesi ha sigut presentada per optar al grau de Doctor en Ciència i Tecnologia Agrària i Alimentària per la Universitat de Lleida.
This thesis has been submitted in fulfillment of the requirements for the degree of Doctor of Philosophy in Agricultural and Food Science and Technology at the University of Lleida.

La recerca s'ha realitzat sota la supervisió del director de tesi:
Research was performed with the guidance of doctoral supervisor:

Dr. Joan Estany Illa
Departament de Producció Animal
Universitat de Lleida

Lleida, octubre 2014

Celebrem els funerals
amb diner i candela
de la mort del nostre porc,
que és gran meravella,
entre els amics i parents
que se'l troben entre dents
sens tenir-ne pensaments
si és mascle o femella.

Els funerals del porc (fragment),
cançó tradicional catalana

The funeral of the pig (fragment),
traditional Catalan song

Agraïments / Acknowledgments

Vull agrair al Dr. Joan Estany el suport que m'ha donat i les oportunitats ofertes des que vaig començar a treballar en el seu grup l'estiu de l'any 2009, llavors entubant mostres al laboratori amb la Teresa Giró i l'Anna Ñaco. En aquells moments no m'imaginava que uns quants anys més tard encara estaria aquí, a punt de presentar la meva tesi doctoral sota la seva direcció, i em sento molt feliç i afortunat d'haver pogut formar part d'aquest equip també juntament amb el Dr. Marc Tor, la Dra. Romi Pena, el Dr. Lluís Bosch, el Dr. Javi Álvarez, la Cristina Labella durant el seu pas pel laboratori i les meves companyes doctorandes i amigues: la Rebeca Muñoz i la Sofia Gol. De tots ells he après molt i amb ells m'he sentit com a casa. Vull agrair-los a tots la seva ajuda, sense la qual aquesta tesi no hauria esdevingut una realitat, i els bons moments viscuts.

A en Josep Reixach i la resta de personal de Selección Batallé per fer possible la realització dels experiments i la recerca en què es basa aquesta tesi, pel suport en la preparació de les bases de dades i per estar sempre disponible per donar el seu punt de vista i resoldre els dubtes o problemes que anessin apareixent.

In Summer 2010, just before formally starting my PhD research, I had the opportunity to visit Dr. Max Rothschild's group at Iowa State University as an intern. I would like to thank him for accepting me in the group back then. I learnt a lot and I have very good memories of those months and all the people I met there, particularly Dr. Suneel Onteru for teaching me everything I needed to know and for his kindness. Happily, I had the chance to collaborate again with Dr. Rothschild in 2013, together with Dr. Eui-Soo Kim, working on the analysis of selection signatures.

I visited Dr. Eli Gjerlaug-Enger and Dr. Eli Grindflek at Norsvin and the Norwegian University of Life Sciences in 2012. I thank them and the rest of Norsvin staff for their friendliness and for the very enjoyable experience of learning how this breeding company works and how they use near-infrared spectroscopy technology to analyze fatty acid composition in their pig lines.

When I went back to Iowa State University in 2013 I was glad to collaborate with Dr. Jack Dekkers for the analysis of our high-density genotyping data, as presented in Chapter 7 of this thesis dissertation. It was

very pleasing to meet again the people at the Animal Breeding and Genetics Group, to make new acquaintances, and to benefit from their knowledge on genomic selection and the last advances in animal breeding.

I am in debt with all of them.

No vull deixar de fer extensiu al meu agraïment a totes les persones que m'han acompanyat durant aquesta etapa de la meva vida, especialment als meus amics doctorands, companys de fatigues amb qui compartir més que l'hora de dinar, i molts d'ells ja feliçment doctorats. Als amics de tota la vida, per seguir sent-hi els caps de setmana. A la família, i especialment a la cosineta Anna per l'ajuda amb programes de disseny gràfic. A les meves àvies, els meus pares Sadurní i Núria i la meva germana Laura per la seva paciència i suport en tot moment. A la Nora pel seu amor.

Aquesta tesi s'ha realitzat en el Departament de Producció Animal de la Universitat de Lleida. La tesi ha sigut finançada amb una beca predoctoral de Formación de Personal Investigador (FPI) del Ministerio de Ciencia e Innovación (BES-2010-034607) i s'ha desenvolupat en el marc dels projectes d'investigació AGL2009-09779 i AGL2012-33529 finançats pel Ministerio de Economía y Competitividad.

Summary

Intramuscular fat (IMF) content and fatty acid composition affect the quality of pork. In particular, increasing oleic acid (C18:1) content would improve pork quality in terms of organoleptic and technological attributes and also of nutritional properties. This thesis dissertation is part of a line of research conducted by the Pig Breeding and Genetics Group of the University of Lleida, with the aim of finding strategies to genetically improve pork quality by increasing IMF and C18:1. It is divided into three parts. Part 1 discusses the implications of applying a specific statistical approach for compositional data to analyze these traits. It is shown that, because of the low variability of fatty acid composition in pork, the standard statistical techniques on raw percentages are robust enough for most genetic analyses, including those performed next. In Part 2, the genetic parameters associated to IMF and C18:1 were estimated in a purebred Duroc line. Both traits have a similar high heritability (0.51−0.56, for IMF, and 0.44−0.50, for C18:1) and a favorable genetic correlation between them (0.47). Furthermore, there exist selection scenarios where these traits and lean growth can be improved simultaneously. It was proved experimentally that (1) IMF and C18:1 respond effectively to selection on estimated breeding values based on phenotypic data of relatives, and (2) backfat thickness can be modified independently of IMF and C18:1. However, selection for IMF and C18:1 based on records from one muscle has unequal correlated responses on other muscles and fat tissues. In Part 3, the sequence variation of the stearoyl-CoA desaturase (*SCD*) gene, the gene producing the rate-limiting enzyme in the biosynthesis of C18:1, was analyzed. It was shown that there is a functional variant in the promoter of the *SCD* gene with an average additive effect of +0.75% and +1.00% on C18:1 and total monounsaturated fatty acids, respectively, but no effect on IMF or carcass fatness. This was confirmed in a genome-wide association study which also revealed nucleotide variations in the leptin receptor (*LEPR*) gene locus affecting overall fatness and, as a result, fat composition. The use markers at both loci substantially enhanced the accuracy of prediction of IMF and C18:1. It is concluded that it is possible to successfully select for increased IMF and C18:1 in pork. In light of the results obtained several scenarios are discussed on how to implement such selection in practice.

Resum

El contingut i la composició en àcids grassos del greix intramuscular (GIM) afecten la qualitat de la carn de porc. En particular, augmentar el contingut d'àcid oleic (C18:1) milloraria la seva qualitat pel que fa a atributs organolèptics i tecnològics i propietats nutricionals. Aquesta tesi doctoral forma part d'una línia de recerca del Grup de Millora Genètica del Porcí de la Universitat de Lleida, amb l'objectiu final de trobar estratègies per millorar genèticament la qualitat de la carn de porc a través de GIM i C18:1. Es divideix en tres parts. La Part 1 discuteix les implicacions d'aplicar un enfocament estadístic específic per a dades composicionals per analitzar aquests caràcters. Es va mostrar que, com que la variabilitat de la composició del greix de la carn de porc és baixa, les tècniques estadístiques estàndards sobre percentatges bruts són suficientment robustes per la majoria d'anàlisis, incloent les que es realitzen a continuació. En la Part 2, en una línia Duroc, es va estimar que GIM i C18:1 tenen una heretabilitat alta similar (0.51−0.56, per a GIM, i 0.44−0.50, per a C18:1) i una correlació favorable entre ells (0.47). A més, existeixen escenaris de selecció en què aquests caràcters i el creixement magre es poden millorar simultàniament. Es va demostrar experimentalment que (1) GIM i C18:1 responen a la selecció basada en valors de millora a partir de dades fenotípiques de parents, i (2) l'espessor de greix dorsal es pot modificar independentment de GIM i C18:1. No obstant, la selecció per GIM i C18:1 basada en dades preses en un múscul té respostes correlacionades desiguals en altres músculs i teixits adiposos. En la Part 3 es van analitzar les variacions de la seqüència del gen estearoil-CoA desaturasa (*SCD*), que codifica l'enzim limitant en la biosíntesi de C18:1. Es va mostrar que hi ha una variant funcional en el gen *SCD* amb un efecte additiu de +0.75% en C18:1 i +1.00% en contingut total d'àcids grassos monoinsaturats, però sense efecte en GIM o engreixament de la canal. Aquesta associació es va confirmar en un estudi d'associació genòmica que també va revelar variacions de nucleòtids en el locus del gen del receptor de la leptina (*LEPR*) que afecten el nivell d'engreixament i, en conseqüència, la composició del greix. L'ús de marcadors en aquests dos loci va millorar substancialment la precisió en les prediccions de GIM i C18:1. Es conclou que és possible seleccionar amb èxit per GIM i C18:1 en carn de porc i es discuteixen diversos escenaris sobre com implementar aquesta selecció a la pràctica.

Resumen

El contenido y la composición en ácidos grasos de la grasa intramuscular (GIM) afectan la calidad de la carne de cerdo. En particular, aumentar el contenido de ácido oleico (C18:1) mejoraría su calidad en cuanto a atributos organolépticos y tecnológicos y propiedades nutricionales. Esta tesis doctoral forma parte de una línea de investigación del Grupo de Mejora Genética del Porcino de la Universitat de Lleida, con el objetivo final de encontrar estrategias para mejorar genéticamente la calidad de la carne de cerdo a través de GIM y C18:1. Se divide en tres partes. La Parte 1 discute las implicaciones de aplicar un enfoque estadístico específico para datos composicionales para analizar estos caracteres. Se mostró que, como la variabilidad de la composición de la grasa de la carne de cerdo es baja, las técnicas estadísticas estándares sobre porcentajes brutos son suficientemente robustas para la mayoría de análisis, incluyendo los que se realizan a continuación. En la Parte 2, en una línea Duroc, se estimó que GIM y C18:1 tienen una heredabilidad alta similar (0.51−0.56, para GIM, y 0.44−0.50, para C18:1) y una correlación favorable entre ellos (0.47). Además, existen escenarios de selección en que estos caracteres y el crecimiento magro se pueden mejorar simultáneamente. Se demostró experimentalmente que (1) GIM y C18:1 responden a la selección basada en valores de mejora a partir de datos fenotípicos de parientes, y (2) el espesor de grasa dorsal se puede modificar independientemente de GIM y C18:1. No obstante, la selección por GIM y C18:1 basada en datos tomados en un músculo tiene respuestas correlacionadas desiguales en otros músculos y tejidos adiposos. En la Parte 3 se analizaron las variaciones de la secuencia del gen estearoil-CoA desaturasa (*SCD*), que codifica el enzima limitante en la biosíntesis de C18:1. Se mostró que hay una variante funcional en el gen *SCD* con un efecto aditivo de +0.75% en C18:1 y +1.00% en contenido total de ácidos grasos monoinsaturados, pero sin efecto en GIM o engrasamiento de la canal. Esta asociación se confirmó en un estudio de asociación genómica que también reveló variaciones de nucleótidos en el locus del gen del receptor de la leptina (*LEPR*) que afectan el nivel de engrasamiento y, en consecuencia, la composición de la grasa. El uso marcadores en estos dos loci mejoró substancialmente la precisión en las predicciones de GIM y C18:1. Se concluye que es posible seleccionar con éxito por GIM y C18:1 en carne de cerdo y se discuten varios escenarios sobre como implementar esta selección en la práctica.

Contents

Introduction

Pig meat represents 36% of the total meat produced in the world, and the 51% in the European Union (FAO, 2014). Spain is the 4th pig producer in the world and produces 3M tonnes/year, which represents 3% and 15% of the world and European Union productions, respectively. In Spain, where the production of pig meat reaches the 64% of total meat, processed and dry-cured products are of great importance. Particularly, the traditional dry-cured ham is very appreciated by consumers and can have a large added value according to its quality. Intramuscular fat (**IMF**) content and fatty acid composition are relevant factors for meat quality from the organoleptic, technological, and nutritional points of view (for a review see Wood et al., 2003), and thus, they are becoming two important issues for both the pig industry and the consumers.

Intramuscular fat comprises the lipids both in the proper intramuscular adipose tissue (fat cells located along the fibres and in the interfascicular areas) and in the muscle fibres (Gandemer, 2002). Whereas the intramuscular adipose tissue has a storage function and mostly contains neutral lipids (mainly triglycerides), the principal contribution of muscle fibres to IMF is phospholipids (lipids in the cell membrane with a structural function). Overall, the major fatty acids in pork are oleic acid (**C18:1**; >30% of total fatty acids, reaching 45% in our study population), palmitic acid (**C16:0**; ~20%), stearic acid (**C18:0**; ~10%), and linoleic acid (**C18:2**; ~10%). Both IMF content and composition are known to be interrelated and affected by several factors, including genetics, which makes them susceptible to being genetically improved through selection.

I.1. INTRAMUSCULAR FAT AND MEAT QUALITY

I.1.1. Organoleptic quality

There is a general agreement that IMF content has a favorable effect on the sensory attributes of pork, although there has been some debate because systematic effects have not been found in all studies (Cameron, 1990; Fernandez et al., 1999a,b; Ruiz-Carrascal et al., 2000; Fortin et al., 2005; Lonergan et al., 2007; Gjerlaug-Enger et al., 2010). The mechanisms by which IMF could improve the organoleptic quality are not totally clear either. It is speculated that the fat cells between muscle fibre fascicles might separate them physically and promote tenderness (Wood et al., 2003). Juiciness could be improved by lipids trapping moisture (Wood et al., 2003) and stimulating the secretion of saliva (Ruiz-Carrascal et al., 2000). Lipid oxidation and Maillard reactions during cooking transform the fatty acids into volatile compounds

that contribute to the characteristic flavor of pork (Mottram, 1998; Cameron et al., 2000; Gandemer, 2002). Some reports suggested that there is a threshold above which further increases of IMF levels do not improve sensory attributes. It has been estimated in 1.5% (Fortin et al., 2005), 2% (Bejerholm & Barton-Gade, 1986), or 2.5−3% (DeVol et al., 1988).

Nonetheless, the most important is the consumer acceptability of meat. Kempster et al. (1986) reported that although the meat from fat carcasses was juicier, less tough, and more flavorful than that from lean carcasses, no differences were found in consumer acceptability. Similarly, in the experiments by Fernandez et al. (1999a), a trained sensory panel gave higher juiciness and flavor scores to loin chops with IMF levels above approximately 2.5%. When the same chops were evaluated by non-trained consumers (Fernandez et al., 1999b), those with IMF levels of 2.5−3.5% were more favorably evaluated for both tenderness and taste, but it was observed that higher IMF levels could lead to rejection of the raw chops due to more visible fat and fat-associated health concerns (Fernandez et al., 1999b; Brewer et al., 2001). Marbling is the term used to refer to the visible fat in meat, in the form of white streaks of fat between the muscle fibres. It is only a part of the total IMF, but strongly related to total content, and it affects the consumer perception of meat (Brewer et al., 2001). Several studies determined the optimum IMF levels for consumer acceptability. It varies depending on cultural preferences but also on the kind of product. For example, for fresh pork, the optimum has been located at 1.5−3.5%, but for high-quality Iberian dry-cured products levels up to 12% are appreciated (Ventanas et al., 2007).

The composition of IMF also affects the organoleptic quality of meat. In general, SFA and MUFA are positively related to better attributes, in opposition to PUFA. This holds true for flavor, tenderness, and juiciness (Cameron & Enser, 1991; Cameron et al., 2000; Ruiz-Carrascal et al., 2000). In particular, Cameron et al. (2000) reported positive correlations of C18:1 with pork flavor and overall acceptability (0.36−0.40). However, because IMF content and composition are interrelated (Section I.2.1), it remains uncertain whether this reflects the effect of IMF content instead of the fatty acids themselves.

I.1.2. Technological quality

In general, greater concentrations of PUFA are undesirable regarding technological quality (Wood et al., 2003; Webb & O'Neill, 2008). The melting point of fatty acids declines with unsaturation and, as a consequence, PUFA content affects negatively the fat firmness and also the color of fat, which is more yellowish when fatty acids with low melting points are more abundant. At the same time, more unsaturated fatty acids are more prone to oxidation, either by direct chemical action or

by lipolytic enzymatic activity. Although lipid oxidation is an important reaction in the development of flavor of cooked pork, it affects negatively the product shelf life, causing problems of fat rancidity.

I.1.3. Nutritional value

Meat is a major source of fat in the diet and its fatty acid composition determines its nutritional value. Whereas total fat intake is clearly associated with diseases like obesity, no evidence for significant effects on cardiovascular disease or cancer incidence have been found (FAO, 2010). Rather than the total amount of fat, it is its fatty acid composition that has an impact on the risk of cardiovascular disease. The intake of SFA has been widely associated with increased low density lipoprotein (**LDL**) and total cholesterol levels in blood, considered indicators for the risk of cardiovascular disease (Williams, 2000; FAO, 2010). Reducing the total fat intake has been proved to be an inefficient strategy to overcome this problem (Hooper et al., 2012). Meta-analyses indicated that isocaloric substitutions of dietary SFA with carbohydrates have not succeeded in modifying the ratio between total and high-density lipoprotein (**HDL**) cholesterol in blood (Micha & Mozaffarian, 2010). On the other hand, isocaloric replacements of SFA with unsaturated fatty acids can reduce the cholesterol levels and the risk of cardiovascular diseases (Hooper et al., 2012). Particularly, replacing SFA with PUFA is effective in reducing LDL and total cholesterol and increasing HDL cholesterol and in reducing cardiovascular disease (Hu et al., 2001; Stewart et al., 2001; Micha & Mozaffarian, 2010). Results regarding the effects of replacement with MUFA are often mixed and contradictory. Some experiments proved that products with MUFA-enhanced compositions can be successful in reducing blood cholesterol levels in humans (Williams, 2000). A meta-analysis by Clarke et al. (1997) indicated that isocaloric substitutions of dietary SFA with MUFA succeeded only in increasing HDL cholesterol (about as much as PUFA) but failed to decrease LDL and total cholesterol. Substitutions of carbohydrates with MUFA resulted in increased HDL cholesterol levels without affecting LDL cholesterol (Hu et al., 2001) or in decreased LDL cholesterol without affecting HDL cholesterol (Micha & Mozaffarian, 2010). Overall, replacement of SFA with PUFA has had better results on blood cholesterol levels than replacement with MUFA. The FAO (2010) considered that there is convincing evidence that replacing SFA with PUFA reduces the risk of cardiovascular disease, and reckons that a similar effect may be expected for MUFA despite insufficient evidence.

Regarding PUFA, the omega-3 and omega-6 PUFA have to be distinguished. Both are essential in humans, i.e., they cannot be synthesized *de novo* and must be provided by the diet, mainly by meat and fish products. However, while omega-3

PUFA are widely regarded as beneficial for human health and have chemoprotective properties, some unfavorable effects have been reported for the omega-6 PUFA if their intake is excessive. These PUFA, and mainly arachidonic acid (**C20:4**), can easily oxidise and their peroxidation products could have adverse health effects such as increasing the risk of breast cancer development (Jiménez-Colmenero et al., 2001; de Lorgeril & Salen, 2012). Because the susceptibility to peroxidation increases with the number of double bonds of the molecule, Christophersen & Haug (2011) suggested replacing SFA with C18:1 instead of PUFA to overcome the adverse properties of the peroxidised products derived from PUFA.

Social concerns about the effects of fatty acid composition on health find their expression in the recommendations of national and international health authorities. The Food and Agriculture Organization and the World Health Organization of the United Nations (FAO, 2010) recommended a fat intake of 15−35% of total energy intake, a maximum SFA intake of 10% of total energy, and a PUFA intake of 6−11% of total energy (2.5−9% and 0.5−2% for omega-6 and omega-3 PUFA, respectively). No limits for MUFA intake were included in these recommendations. The MUFA intake can cover a wide range of values depending on total fat, SFA, and PUFA intakes. More recently, in 2011, Denmark was the first country to apply a "fat tax" of 2 €/kg of saturated fat on products that exceeded 2.3% of SFA (BBC News, 2011). Although it was abolished one year later due to the extra costs for the producers and the inflated prices for the consumers (BBC News, 2012), this is the first known attempt to regulate the fatty acid composition of food rather than only providing guidelines.

I.1.4. Intramuscular oleic acid and meat quality

While high contents of SFA and MUFA are desirable in terms of organoleptic and technological meat quality, dietary recommendations indicate unsaturated fatty acid profiles as healthier. A more monounsaturated fat can be regarded as a good alternative to improve simultaneously the organoleptic, technological, and nutritional properties of meat. The MUFA C18:1 is the main fatty acid in pork and, because of all this, intramuscular C18:1 content can be an interesting trait to be included in the selection objectives of pig lines aimed at high-quality products. As stated before, C18:1 has been associated with better pork flavor and overall pork acceptability (Cameron et al., 2000) and benefits on human health (Christophersen & Haug, 2011). Additionally, high intramuscular C18:1 content is one of the characteristics of the well-reputed dry-cured ham from acorn-fed Iberian pigs, sometimes also called "the olive with legs". Like olive oil, this kind of ham is already favorably perceived by consumers both for its taste and for its nutritional value. A higher C18:1 content in pork could help in changing the unjustified (from the scientific point of view) negative perception that some consumers have of pork (Verbeke et al., 1999). While nowadays pig carcasses

and meat are not directly priced based on its fat composition, meat products with better sensory attributes and a healthier fatty acid profile might find good acceptance in niche high-quality markets (Knap, 2014).

I.2. MAIN FACTORS AFFECTING INTRAMUSCULAR FAT COMPOSITION

Intramuscular fat content and composition are affected by several factors including diet, breed, sex, age, or tissue, among others. Because there exists a relationship between IMF content and its fatty acid composition, the effect of the aforementioned factors on IMF composition is difficult to separate from the effect of IMF content (De Smet et al., 2004; Wood et al., 2008). The diet is the factor with the greatest effect on fat composition. Because of this, multiple studies investigated diet-based strategies to modify the pork fatty acid profile, but their effectiveness regarding IMF composition is limited.

I.2.1. Relationship between fat content and composition

Fat depots can be divided into two fractions: neutral lipids (mainly triglycerides) and phospholipids. Because phospholipids are a constituent of cell membranes in muscle fibres, their amount in muscle remains almost constant throughout fattening (Wood et al., 2008). In the case of subcutaneous fat (**SF**), the proportion of phospholipids is low and unimportant. As the adipose tissue develops, the endogenous synthesis of SFA and MUFA increases and results in a greater accumulation of neutral lipids in the adipocytes. This leads to a differential composition of these two fractions. The phospholipids have a higher PUFA content than the neutral lipids (Leseigneur-Meynier & Gandemer (1991) reported values of 33−40% and 7−15%, respectively) and, particularly, C20:4 content is also proportionally higher in the phospholipid fraction with respect to the other PUFA. When IMF content increases, so does the neutral lipids fraction (and so, SFA and MUFA), while the phospholipids fraction (and so, PUFA) relatively decreases. Thus, the SFA and MUFA are positively correlated with the IMF content, and the PUFA negatively (e.g., Cameron & Enser, 1991; Yang et al., 2010). Also, because SFA and MUFA are mostly synthesized endogenously, the correlations among them are positive (mean value reported by Cameron & Enser (1991): 0.59), but they are negatively correlated with PUFA (−0.62), which, on the other hand, have a dietary origin and are also positively correlated among them (0.70).

I.2.2. Diet

In opposition to ruminant animals, in monogastric animals such as the pig dietary fatty acids are not modified during their pass through the digestive system and

are deposited in SF and in muscle as they are intaken. As a consequence, pig adipose tissues reflect the fatty acid composition of the diet. Feeds with supplementary fatty acids have been proved a successful strategy in modifying the composition of fat in pig. There has been particular interest for omega-3 PUFA. Because in pigs C18:2 and C18:3 are essential fatty acids (i.e., they cannot be synthesized endogenously), their contents can be easily modified by the diet. Reported experiments indicate that it is possible to increase the C18:2 content from 10-15% to over 30% only by supplementing it in the diet (Wood et al., 2003), but then adverse consequences on sensory attributes can appear. Similarly, the C18:1 content can also be modified by the diet, without negatively altering the organoleptic quality (Rhee et al., 1990; Myer et al., 1992; Klingenberg et al., 1995). The paragon of this strategy is the acorn-fed Iberian pigs, whose fresh ham can reach C18:1 contents up to ~55% (Tejeda et al., 2002). However, because the C16 and C18 SFA and MUFA can be synthesized endogenously, their final content is less affected by the diet than the PUFA content (Wood et al., 2008). In pigs, the *de novo* synthesis could produce 86% of total deposited non-essential fatty acids (Kloareg et al., 2007). This is likely to happen especially in genetically fatter pigs where *de novo* synthesis has a greater relative impact, although *de novo* synthesis could be reduced when high amounts of fat are fed (Flachowsky et al., 2008).

Another downside of this approach is that it has been observed that the fatty acid supplementation in the diet is less effective in modifying the IMF composition than that of the SF (Duran-Montgé et al., 2008; Flachowsky et al., 2008), probably because of the higher neutral lipids content of SF (the composition of structural lipids in IMF is more stable). For example, in a study by Klingenberg et al. (1995) pigs were fed during 8 weeks either with a control diet containing beef tallow or with an experimental diet containing high-oleic acid sunflower oil. The C18:1 content of the experimental diet was 1.92 folds that of the control, but C18:1 of animals in the experimental group resulted to be only 1.07-fold in IMF and 1.18-fold in SF. In this experiment, diet also affected the activity of the stearoyl-CoA desaturase (**SCD**), responsible of desaturing C18:0 into C18:1 and, consequently, the desaturation index C18:1/C18:0, in SF but not in IMF. Other experiments provided mixed results. In the trials by Mas et al. (2010, 2011), a high-oleic diet with 1.53 folds the C18:1 of the control treatment was fed to crosses of Landrace × Large White with either Pietrain or Yorkshire. While in one trial C18:1 in IMF increased more than in SF (1.13 vs. 1.08 folds those of the control treatment, respectively), in the other a significant increase was found only in SF.

An alternative strategy for modifying fat content and composition through the diet is feeding low-protein diets. In this case, the energy that cannot be used in muscle synthesis due to the unavailability of aminoacids is expended in synthesizing fat instead, increasing IMF (Wood et al., 2004). Doran et al. (2006) showed that in low-

protein diets, the intramuscular SCD protein expression and activity were also increased, resulting in increased desaturation index C18:1/C18:0 in muscle. Wang et al. (2012) provided further evidence of the up-regulation of the *SCD* gene expression in low-protein diets. Interestingly, the expression of lipogenic genes was up-regulated and that of lipolytic genes down-regulated. As a consequence of the increased IMF *de novo* synthesis, C18:1 and MUFA contents increase (Wang et al., 2012; Wood et al., 2013).

Cost of the high-oleic raw ingredients and penalty in the feed conversion ratio are other factors that may limit the application of these diet-based strategies.

I.3. GENETIC STRATEGIES TO IMPROVE INTRAMUSCULAR FAT CONTENT AND COMPOSITION

During the last decades, most breeding schemes have focused on performance traits, such as average daily gain, feed conversion ratio, and lean content. Due to the negative genetic correlation of IMF with carcass leanness (from −0.55 to −0.07, as reviewed by Sellier, 1998) and positive with carcass fatness (from 0.04 to 0.60, according to the same review), these breeding programs have reduced IMF content in pig meat below the recommended values (Bejerholm & Barton-Gade, 1986; DeVol et al., 1988; Wood et al., 2008). This led to a diminution in the sensory attributes of pork. During the 1970s and 1980s, some authors already alerted that the improvement of lean content in pig meat was leading to poorer meat sensorial quality (Judge, 1972, as cited by Martin & Fredeen, 1974; Kempster et al., 1986). However, despite of the positive genetic correlation between IMF and pork overall acceptability (from 0.54 to 0.68; Sellier, 1998), acceptability by consumers was not negatively affected (Kempster et al., 1986). Schwab et al. (2006) used frozen semen of Duroc sires from the 1980s to compare the meat quality of their offspring with that of current sires. Results showed that improvement of the leanness during the last decades had been accompanied by a reduction of IMF and instrumental tenderness, and by worse flavor scores. In a similar way, in another Duroc line selected for lean growth efficiency, a reduction of IMF content was observed together with reduced meat quality despite that a causal link between them was not established (Lonergan et al., 2001). This was also observed in Large White by Cameron et al. (2000), who showed that selection for the components of efficient lean growth reduced IMF and increased its PUFA content, but, in this case, unfavorable changes in flavor and acceptability were very low, maybe because IMF level was already very low (around 1%).

Nowadays, the negative effects of selection for efficient lean production on sensorial quality and the consumers concerns about health have stimulated the inclusion of criterion traits such as IMF content and fatty acid composition, particularly C18:1, in the selection schemes of the breeding companies for improving meat quality.

The new challenge for pig industry is to improve simultaneously the performance and meat quality traits or, at least, to improve some of them without penalizing the others. While diet modification is a feasible strategy, genetics can be an equally effective complementary approach.

I.3.1. Duroc crosses

The Duroc breed is characterized by its high IMF in relation to the other common breeds (Large White, Landrace, and Pietrain) and, because of this, it has been widely used in commercial crosses to improve meat quality (Oliver et al., 1994). In Spain, the Duroc breed is also used both as purebred and as Iberian-crossed (BOE, 2014) for dry-cured ham production. Duroc (sire) × Iberian (dam) crosses are aimed to improve production performance of Iberian pigs without penalyzing their IMF content as much as crossing with leaner breeds could (Tejeda et al., 2002; Ventanas et al., 2007).

I.3.2. Direct selection

Because there exists genetic variance within breed (De Smet et al., 2004; Cilla et al., 2006; Solanes et al., 2009), it is possible to select for IMF content and composition traits. The heritability of IMF has been found to be high, as well as that of fatty acids content. A wide range of heritability estimates have been reported for IMF, from 0.26 to 0.86, with an average of 0.50 (Sellier, 1998). Selection experiments have proved that IMF responds to selection but at the expense of increasing carcass fatness. In the experiment by Schwab et al. (2009), where IMF was selected without restrictions, IMF increased by 2.12% after six generations, but there were unfavorable correlated responses for BT (+6.17 mm) and loin muscle area (−3.62 cm^2). On the other hand, selection for IMF did not affect growth performance and the meat of selected animals showed greater instrumental tenderness and best flavor scores. It has been argued that, because the unfavorable genetic correlation between IMF and leanness traits is moderate, there is room for independent manipulation of IMF and carcass lean growth (Clutter, 2011). Indeed, quite low genetic correlations have been reported for IMF with BT (0.24 to 0.64; Suzuki et al., 2005b; Solanes et al., 2009; Schwab et al., 2010; Yang et al., 2010), loin muscle area (−0.15 to −0.27; Schwab et al., 2010), and carcass lean meat content (0.02 to −0.38; Knapp et al., 1997). However, such a selection objective has been proved difficult in practice. After seven generations of selection, Suzuki et al. (2005b) achieved more than the desired genetic gain for IMF (+1.2%) but renounced to BT reduction.

Estimates of genetic parameters of fatty acids content are scarcer, particularly of their genetic correlation with other economic traits. Early estimates were provided by

Cameron (1990) and Cameron & Enser (1991), who, using Duroc and Large White data, estimated a high heritability for C18:1 in SF (0.69) but a much lower one for C18:1 in loin IMF (0.28). Like IMF, C18:1 had an unfavorable correlation with lean weight (−0.41; Cameron & Enser, 1991). However, it was not until the last decade that genetic parameters of fatty acids were further studied for different breeds, genetic lines, adipose tissues, and analytical methods. The unweighted average heritability for C18:1 across previous studies was 0.38 (range: 0.26−0.58) in IMF, mostly in loin (Suzuki et al., 2006; Casellas et al., 2010; Ntawubizi et al., 2010; Sellier et al., 2010), 0.47 (range: 0.26−0.67) in SF (Fernández et al., 2003; Suzuki et al., 2006; Sellier et al., 2010; Gjerlaug-Enger et al., 2011), 0.44 in intermuscular fat (Suzuki et al., 2006), and 0.69 in perirenal fat (Sellier et al., 2010). Some of these studies, however, used few and heterogeneous data and were designed for other purposes than estimation of genetic parameters, which makes some of the estimates not conclusive enough. The few available genetic correlation estimates for intramuscular C18:1 indicate a correlation structure similar to that for IMF, being 0.20−0.25 with average daily gain, −0.75 with carcass lean meat content, −0.06 with BT, 0.22 with loin muscle area, and only 0.10 with IMF (Suzuki et al., 2006; Ntawubizi et al., 2010). Overall, the genetic parameter estimates indicate that selection for C18:1 should be effective, but to our knowledge, there are no reports of the response of C18:1 to direct selection, and only some results showing a slight favorable correlated response after selection for IMF (Burkett et al., 2008).

The main problem for including IMF and fatty acid composition traits in the selection objectives of the breeding companies is that they are difficult to measure. Determination by chemical methods such as gas chromatography is the most accurate but laborious, time-consuming, and expensive. Therefore, it may be unsuitable if a large number of records are needed, for example, for accurate genetic evaluations. A faster and more cost-effective method using flow injection analysis/mass spectrometry has been developed specifically for intramuscular C18:1 (Muñoz et al., 2011). However, it is still difficult to measure these traits *in vivo* in the selection candidates. Biopsies (Bosch et al., 2009) allow the determination of both IMF and fatty acid composition in live pigs but they are likely to be restricted to experimental purposes due to ethical concerns. During the last years, several technologies have been developed for the indirect determination of these traits. Real-time ultrasound predicts IMF *in vivo* (Newcom et al., 2002, 2005), while computed tomography predicts IMF in carcasses but it does not seem reliable *in vivo* (Kongsro & Gjerlaug-Enger, 2013). The on-line near infrared spectrometry (NIRS) systems that are currently being developed open up new opportunities to set routine determinations of fatty acid composition traits in the abattoir (González-Martín et al., 2002, 2005). This technology can be implemented to stablish regular genetic evaluations of the selection candidates for these traits based on data from their slaughtered relatives, provided that there exists, as

in all *post-mortem* measurements, full traceability of carcasses or primal cuts, which in practical terms can be rather difficult to achieve in most current pig breeding schemes.

Due to the difficulty and cost of sampling muscles of interest, measures of IMF and C18:1 are usually taken on a single muscle, mostly the loin, or on SF. However, it is known that the pattern of fatty acid deposition may differ between IMF and SF (Duran-Montgé et al., 2008; Sellier et al., 2010; Bosch et al., 2012), across muscles (Sharma et al., 1987; Leseigneur-Meynier & Gandemer, 1991; Kim et al., 2008), and even among locations within a specific tissue (Sharma et al., 1987, Faucitano et al., 2004; Franco et al., 2006). Thus, to develop adequate recording and genetic evaluation schemes for IMF and fatty acid composition traits, there is a need to know the correlation structure of these traits across valuable muscles and with SF. The few reports available giving phenotypic correlations of fatty acids contents in different tissues show positive but low-to-moderate values (0.19−0.57 between fatty acids in IMF and SF; Suzuki et al., 2006; Yang et al., 2010). Regarding C18:1, the phenotypic correlation between its content in IMF and in SF has been estimated to be 0.33−0.45 and the genetic correlation 0.66−0.72 (Suzuki et al., 2006; Yang et al., 2010). A phenotypic correlation of 0.35 was found between C18:1 in muscles *longissimus dorsi* and *gluteus medius* (Rauw et al., 2012), but genetic correlations between muscles are not available and, therefore, it remains unclear to what extent selection for C18:1 based on records from a particular muscle or SF would be successful in modifying C18:1 in other muscles.

I.3.3. Marker-assisted and genomic selection

Genetic markers have been proposed as a useful tool to select for traits that are difficult to measure in the selection candidates, such as meat quality traits. Under a marker-assisted selection scheme, genetic lag and prediction error are expected to be reduced in comparison to selection schemes based on phenotypes of slaughtered littermates (Grindflek et al., 2001). During the last decades, a lot of efforts have been put into the detection of quantitative trait loci (**QTL**) affecting IMF content and fatty acid composition. Using low-density microsatellite linkage maps, several QTL and candidate genes have been reported for IMF content (as summarized by Gao & Zhao, 2009) and several more for fatty acid composition of either SF or IMF (Pérez-Enciso et al., 2000; Grindflek et al., 2001; Clop et al., 2003; Kim et al., 2006; Nii et al., 2006; Sanchez et al., 2007; Guo et al., 2009; Quintanilla et al., 2011; Uemoto et al., 2012c). These first attempts provided large QTL confidence intervals, which limited the identification and validation of the positional candidate genes within them. Moreover, most of these studies were performed using experimental crosses designed to generate variability for the studied traits. Because, to our knowledge, their results have not been

validated in purebred lines, markers have not been introduced in commercial breeding programs (Dekkers, 2004, 2012). The onset of high-density genotyping arrays has enabled a more precise scanning of the genome to detect QTL and quantitative trait nucleotides (QTN). The first genome-wide association studies (**GWAS**) for fatty acid composition in swine have already been performed in Iberian × Landrace (Ramayo-Caldas et al., 2012; Muñoz et al., 2013b) and in White Duroc × Erhualian and Chinese Sutai pigs (Yang et al., 2013). This technology can also be used to make genomic predictions of breeding values (Meuwissen et al., 2001), but the accuracy of genomic prediction for IMF content and fatty acid composition in swine has not been assessed yet.

To date, association with fatty acid traits has been reported for some candidate genes. One of the most promising is the *SCD* gene. Stearoyl-CoA desaturase has a direct role in the MUFA biosynthesis pathway as it is the responsible for catalyzing the desaturation at the Δ^9 position of stearoyl-CoA and palmitoyl-CoA into oleoyl-CoA and palmitoleoyl-CoA, respectively. The *SCD* gene maps to *Sus scrofa* chromosome (SSC) 14 at 120.96−120.98 Mb, which co-localizes with some previously detected QTL for the C16 and C18 SFA and MUFA in purebred and crossed Duroc (Sanchez et al., 2007; Quintanilla et al., 2011; Uemoto et al., 2012c). Findings so far support that there is genetic variation in the *SCD* gene affecting fatty acid composition of muscle and adipose tissue. Several single nucleotide polymorphisms (SNPs) in the *SCD* promoter region have been associated to C16 and C18 SFA and MUFA content both in IMF and SF, but results are not conclusive yet, as either the location of haplotypes is not coincident (Uemoto et al., 2012b; Maharani et al., 2013), favorable alleles are swapped (Renaville et al., 2013), or even no association was found (Bartz et al., 2013). Suzuki et al. (2006) suggested that a selection strategy for fatty acid composition targeting *SCD* might be effective, but genetic markers in the *SCD* gene will not be suitable for implementation in breeding schemes until the association between these SNPs and pork fatty acid composition is further validated and better understood.

Other functional candidate genes that at some point have been found to affect fatty acid composition include the microsomal triglyceride transfer protein (*MTTP*) (Estellé et al., 2009), the acetyl-CoA carboxylase α (*ACACA*) (Gallardo et al., 2009), the heart fatty acid binding protein (*FABP3*) (Lee et al., 2010), the leptin receptor (*LEPR*) (Galve et al., 2012), the elongase of very long chain fatty acids 6 (*ELOVL6*) (Corominas et al., 2013), the insulin-like growth factor 2 (*IGF2*) (López-Buesa et al., 2014), and the melanocortin 4 receptor (*MC4R*) (López-Buesa et al., 2014). Confirmation of these associations (without neglecting possible pleiotropic effects on carcass fat content and other economic traits) and discovery of other informative markers related to IMF content and fatty acid composition could lead to the development of low-density marker panels aimed at the genetic improvement of these

traits (Weigel et al., 2009; Vazquez et al., 2010). The great cost of genotyping large amount of individuals in commercial conditions and the shorter generation interval than in cattle are some factors often referred as limiting the implementation of genomic selection schemes in the pig industry (Blasco & Toro, 2014). Low-density marker panels could reduce the genotyping costs, thereby offering an attractive opportunity.

I.4. IN THIS THESIS DISSERTATION

A priority of the Pig Breeding and Genetics Group of the University of Lleida is to carry on research on the genetic selection for IMF content and fatty acid composition in pig for the ultimate benefit of consumers and the industry. The present thesis dissertation has been developed in the frame of this on-going research and focuses mainly on exploring the genetic strategies for improving IMF and C18:1 of pork. It comprises seven chapters that are divided into three parts.

Part 1 comprises Chapter 1 and deals with the proper methodology for the statistical analysis of fatty acid composition. A fact about fatty acid compositions that has been widely ignored in meat science research is that from the mathematical point of view they are, as the name itself indicates, compositional data. Compositional data have specific mathematical properties because they represent relative, rather than absolute, information. Therefore, they should not be analyzed using standard statistical techniques that are defined in the real space, which has an absolute scale. Although specific methods for compositional data have been developed since the 1980s (Aitchison, 1986), there is no reference in the literature where they have been used for fatty acid data in meat research. In Chapter 1, we discussed the implications of adopting or not the compositional data statistical approach to analyze fatty acid compositions.

Part 2 examines the opportunities for improving IMF and C18:1 using only phenotypic records on slaughtered relatives. It is hypothesized that with such recording scheme it is possible to capture enough genetic variation to directly improve IMF and C18:1. Thus, Part 2 first estimates the genetic parameters associated to C18:1 and IMF together with those of other economic relevant traits and then discusses their expected responses under different selection scenarios accordingly (Chapter 2). It is not clear how response based on one muscle or fat tissue may affect others. Therefore, the genetic correlations of IMF and fatty acid composition traits across muscles and in SF are studied in Chapter 3. Moreover, the effectiveness of selection using different objetive and criteria muscles or fat tisues is discussed. It is known that selection for carcass lean content decreased both BT and IMF, but its effect on C18:1 is less understood. Chapter 4 presents the results from a selection experiment designed for reducing BT at restrained IMF. Chapter 5 provides the results from a selection

experiment specifically conceived to increase C18:1, as well as the correlated response of C18:1 to the selection described in Chapter 4.

Part 3 explores marker-assisted selection as a strategy to enhance the genetic responses of IMF and C18:1. Here, it is hipothesized that there are nucleotide polymorphisms in candidate genes with potential for being used as genetic markers for IMF and C18:1. Chapter 6 includes the search and validation of genetic variants in the *SCD* gene, the most relevant one in the biosynthesis of C18:1. Then, a GWAS is performed to detect other polymorphisms associated with IMF and fatty acid composition traits across the whole genome (Chapter 7). Chapter 7 concludes with a discussion on the use of genomic data for improving breeding value estimation and selection for IMF and C18:1.

Finally, in the Discussion section, opportunities for genetic breeding value evaluation combining phenotypic records on slaughtered relatives and information from genetic markers are considered. In light of the results obtained in Parts 2 and 3, several scenarios are discussed on how to implement selection for IMF and C18:1 in practice.

Objectives

The main objective of this research was to analyze the genetic determinism of intramuscular fat (**IMF**) content and fatty acid composition, particularly of oleic acid (**C18:1**) content, in pork. A second objective was to find strategies to genetically improve IMF and C18:1 in the context of a breeding programme of a Duroc line aimed at producing high quality fresh and cured pork products.

For this purpose the following specific objectives were formulated:

1. To examine the implications of using a compositional data approach in the statistical and genetic analyses of fatty acid compositions.
2. To estimate the genetic parameters of IMF and C18:1 and their expected selection responses if other economically important traits are included in the selection objective.
3. To prove experimentally whether backfat thickness and carcass lean growth can be modified regardless of IMF and C18:1.
4. To prove experimentally whether C18:1 responds to selection.
5. To assess how selection for IMF and C18:1 in a particular muscle or subcutaneous fat affects these traits in other muscles and fat tissues.
6. To search sequence variations in the stearoyl-CoA desaturase (*SCD*) gene associated with saturated and monounsaturated fatty acids content.
7. To identify other candidate regions associated to IMF content and fatty acid composition by means of a genome-wide association study.
8. To explore the use of genetic markers and genomic selection as a way to enhance the response to selection for IMF and C18:1.

Animals and Samples

Data from a purebred Duroc line were used for the analyses. This line is primarily used for producing high quality dry-cured hams. The line was completely closed in 1991 and since then it has been selected for an index including body weight (**BW**), backfat thickness (**BT**), and intramuscular fat (**IMF**) content (Tibau et al., 1999; Solanes et al., 2009). The data set currently consists of 119,390 pedigree-connected pigs, from which 110,855 have at least one recorded trait. Pigs with records were born from 1996 to 2013. At approximately 75 d of age, piglets were moved to the fattening units, where they were penned by sex (8 to 12 pigs/pen) until slaughter. All pigs were performance-tested at an average age of 180 d for BW (n=110,165) and BT (n=106,276). Backfat thickness was ultrasonically measured at 5 cm off the midline at the position of the last rib (Piglog 105, SFK-Technology, Herlev, Denmark). During the test period, pigs had *ad libitum* access to commercial diets.

Since 2002, 1,391 of the purebred barrows used for producing dry-cured ham were taken for recording IMF and C18:1. Barrows were castrated within the first week of age. Two barrows per litter were taken and raised in 17 batches. From 160 d onwards, barrows were fed a commercial pelleted finishing diet (Esporc, Riudarenes, Girona, Spain). Feed composition was similar along the years, with an average composition of 17.2% crude protein, 5.8% fiber, and 6.5% fat (C16:0, 18.0%; C18:0, 6.9%; C18:1, 34.4%; and C18:2, 29.7%). Feed in each batch was analyzed in triplicate as described in Cánovas et al. (2009). At the end of the finishing period, the barrows were slaughtered in the same commercial slaughterhouse at around 210 d (at ~125 kg of BW). After slaughter, the carcass weight (**CW**) and the carcass length were measured. The carcass length was measured from the anterior edge of the symphysis pubic to the recess of the first rib. Carcass BT and loin thickness at 6 cm off the midline between the third and fourth last ribs were measured by an on-line ultrasound automatic scanner (AutoFOM, SFK-Technology, Herlev, Denmark). The carcass lean percentage was estimated on the basis of 35 measurements of AutoFOM points by using the official approved equation (OJ, 2001) and the carcass lean weight was calculated using CW and lean percentage. Immediately after slaughter, samples of subcutaneous fat (**SF**; n=343), muscle *semimembranosus* (**SM**; n=200), and liver (n=96) were collected. After chilling for about 24 h at 2ºC, each carcass was divided into primal cuts and the left side ham was weighed. Each ham was trimmed according to customary procedure used for manufacturing traditional dry-cured Spanish ham. Immediately after quartering, samples of at least 50 g of muscle *gluteus medius* (**GM**; n=1,383) were collected from the left side ham. A section of around 1 kg from the left loin (muscle *longissimus dorsi*; **LD**) of each carcass at the level of the third and fourth last ribs was also taken following the same procedure (n=406). Samples of SF were

collected at the same location than either the LD (n=210) or the GM (n=133) samples. Additional samples of muscle *latissimus dorsi* (**LT**; n=85) were collected from the left side shoulder. Samples were immediately vacuum-packaged, and stored in deep-freeze conditions (at −20ºC) until required for IMF and C18:1 determination. Storage time does not affect fatty acid composition (De Pedro et al., 2000).

After muscle samples were completely defrosted and vacuum drip losses were eliminated, the dissected muscle, trimmed of subcutaneous and intermuscular fat, was minced. A representative aliquot from the pulverized freeze-dried muscle was used for fat analysis. Intramuscular fat content and composition was determined in duplicate by quantitative determination of the individual fatty acids by gas chromatography (Bosch et al., 2009). Fatty acid methyl esters 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 × 0.25 mm; Supelco, Bellefonte, PA) and a flame ionization detector with helium as carrier gas at 1 ml/min. Runs were made with a constant column head pressure of 172 kPa. The oven temperature program increased from 150 to 225°C at 7°C/min, and injector and detector temperatures were both 250°C. Quantification was carried out through area normalization after adding into each sample 1,2,3-tripentadecanoylglycerol as internal standard. Intramuscular fat content was calculated as the sum of each individual fatty acid expressed as triglyceride equivalents (AOAC, 1997) and expressed as percentage of fresh matter. Fatty acids were identified by comparing their relative retention times with those of the external standard and confirmed by comparing their mass spectra to the computer library of the gas-liquid chromatography/mass spectrometry databases Wiley 275 K and NBS 75 K (Agilent Technologies, Wilmington, DE). Fatty acids were analyzed on a simple quadrupole instrument (GC/MSD 6890N-5973N, Agilent Technologies, Wilmington, DE) equipped with an electron ionization source using the same temperature program as described above. The scanned mass range of fatty acids was m/z 35 to 450 and the scanning rate 3.46 scans/s. The complete profile for each sample included saturated (**SFA**; C14:0, C16:0, C18:0, and C20:0), monounsaturated (**MUFA**; C16:1n-7, C18:1n-9, and C20:1n-9), and polyunsaturated (**PUFA**; C18:2n-6, C18:3n-3, C20:2n-6, and C20:4n-6) fatty acids, expressed as their percentage relative to total fatty acids in IMF. Because oleic (C18:1n-9) and vaccenic (C18:1n-7) acids were not completely resolved with this chromatography program, their contents were summed up. Vaccenic acid represented ~10% of total C18:1. Preliminary results using either total C18:1 or individual C18:1n-9 did not differ substantially. Fatty acid profiles of SF were analyzed following the same procedure.

PART 1

Chapter 1.
On the compositional analysis of fatty acids in pork

R. Ros-Freixedes & J. Estany

Departament de Producció Animal, Universitat de Lleida − Agrotecnio Center, 191 Av. Alcalde Rovira Roure, 25198 Lleida, Catalonia, Spain.

The content of this chapter was published in Journal of Agricultural, Biological, and Environmental Statistics (2014), 19: 136−155. DOI: 10.1007/s13253-013-0162-x

The authors wish to thank Dr. Juan José Egozcue, Univeristat Politècnica de Catalunya, for his helpful advice.

ABSTRACT: The fatty acid composition of pork is an important issue for the pig industry and consumers. Fatty acid compositions are commonly described as the percentages of a set of fatty acids relative to total and therefore should be statistically treated as compositional data. To our knowledge there is no reference in the literature where specific methods for compositional data analysis have been applied to analyze fatty acid composition in meat quality research. The purposes of this study were (1) to present an overview of compositional data analysis techniques, (2) to apply them to the analysis of the fatty acid composition of muscles and subcutaneous fat from 971 pigs as a case study, and (3) to discuss and interpret the results with respect to those obtained using standard techniques. Results from both approaches indicate that fatty acid composition differed across tissues and muscles but also, for a given muscle, with the intramuscular fat content. It is concluded that fatty acid composition in pork did not display enough variability to become critical for standard statistics, particularly if the individual fatty acid parts remain the same across experiments. However, even in such case, compositional analysis may be useful to correctly interpret the correlation structure among fatty acids.

1.1. INTRODUCTION

The quality of fat is a feature becoming increasingly important for both the industry and consumers. Currently, there is enough evidence indicating that fat quantity and quality affect the nutritional, sensory, and technological properties of animal products, particularly pork (Wood et al., 2003; Schmid, 2010). Fat quality is chemically defined in terms of fatty acid composition, which is commonly presented as a set of percentages corresponding to the relative content of each individual fatty acid (or the sum of some of them) with respect to the total content of the fatty acids that had been determined, i.e., as a vector of positive values whose sum is a constant. Technically, this sort of data is what in statistics is known as compositional data, i.e., multivariate data where the variables represent parts of a whole (Pawlowsky-Glahn & Egozcue, 2006). Compositional data are intrinsically multivariate because each component cannot be interpreted without relating it to any of the other components. They only represent relative information and therefore standard statistical techniques, which were conceived to deal with variables measured on an absolute scale, are inappropriate. Consequently, specific methods for compositional data analysis have been developed since the 1980s (Aitchison, 1982; Aitchison, 1986; Aitchison & Egozcue, 2005; Bacon-Shone, 2011). To our knowledge there is no reference in the literature where compositional data analysis had been applied to meat quality research.

Much research has been undertaken in recent years to assess the effect of influential factors (such as diet, genotype, gender, body weight, age, or fat content, among others) on the fatty acid profile of pork fat and meat, mostly sampled from backfat and loin chops. However, it is also known that the pattern of fatty acid deposition differs not only between the adipose and muscle tissues (Franco et al., 2006; Duran-Montgé et al., 2008; Yang et al., 2010) but also among muscles (Sharma et al., 1987; Leseigneur-Meynier & Gandemer, 1991). The University of Lleida has assembled a biorepository of pig fat and muscle specimens for conducting research studies on meat quality, including samples from a Duroc genetic line used for producing premium quality pork cuts. Currently, the associated dataset to this line, with around 1,700 fatty acid profiles from different muscles and backfat locations (Section 1.2), provides a valuable resource for revisiting the pattern of fatty acid deposition in pork under a compositional data analysis setting. The purpose of this study was (1) to review the fundamentals of the compositional data analysis techniques (Sections 1.3−1.4), and then (2) to use this approach to examine the variations in the fatty acid profile of pork meat and fat as a case study (Section 1.5). The utility of adopting the compositional data approach in the statistical analysis of fatty acid compositions in meat products is discussed in light of the results of the case study.

1.2. DESCRIBING THE CASE STUDY

The case study comprises data from 971 purebred Duroc barrows (see Animals and Samples Section). The pigs were raised at a carcass market weight of around 95−100 kg in twelve commercial batches from 2001 to 2008 (Table 1.1). All pigs had *ad libitum* access to a commercial feed and were slaughtered at the same abattoir. There, a sample of the muscle *gluteus medius* (**GM**) was collected from the left ham of all pigs. Moreover, in randomly chosen subgroups of them, additional samples of the muscles *longissimus dorsi* (at the level of the third and fourth last ribs; **LD**), *semimembranosus* (**SM**), and *latissimus dorsi* (**LT**) were also taken, as representative muscles of the loin, ham, and shoulder, respectively. Finally, two samples of the subcutaneous backfat (**SF**) were obtained at the positions where GM (**SFGM**) and LD (**SFLD**) muscle samples were taken. The number of samples per muscle and backfat location by batch is detailed in Table 1.1. Samples were collected and analyzed for intramuscular fat (**IMF**) content and fatty acid composition as detailed in the Animals and Samples Section. The complete profile for each sample included saturated (**SFA**; C14:0, C16:0, C18:0, and C20:0), monounsaturated (**MUFA**; C16:1n-7, C18:1n-9, and C20:1n-9), and polyunsaturated (**PUFA**; C18:2n-6, C18:3n-3, C20:2n-6, and C20:4n-6) fatty acids (Figure 1.1). Because C20:0 is present at very low levels, it was not detectable in a few samples. The zero values represent a mathematical challenge for compositional data, which only represent relative magnitudes. To solve this problem several replacement strategies have been proposed (Martín-Fernández &

Table 1.1. Number of animals (n), age at slaughter (Age), carcass weight (CW), and number of samples per muscle and backfat location by batch.

Batch	n	Age, d (SD)	CW, kg (SD)	Sample[1]					
				GM	LD	SM	LT	SFGM	SFLD
1	109	215.8 (5.3)	90.9 (11.3)	109	52	51	42	15	-
2	105	214.0 (3.4)	95.9 (10.6)	104	54	54	43	15	-
3	68	203.1 (6.4)	94.7 (7.8)	66	-	27	-	-	27
4	72	200.9 (8.2)	91.0 (10.2)	72	-	20	-	-	48
5	112	223.2 (3.8)	104.3 (12.0)	112	24	-	-	-	24
6	74	220.6 (4.0)	100.1 (7.7)	73	33	-	-	-	32
7	32	220.7 (0.8)	100.0 (8.8)	31	31	-	-	-	31
8	58	195.8 (1.9)	92.0 (9.2)	58	-	-	-	28	-
9	51	206.5 (1.7)	97.5 (10.0)	51	-	-	-	30	-
10	94	230.9 (2.1)	104.0 (10.7)	93	-	-	-	15	-
11	110	217.5 (1.7)	107.4 (8.9)	110	-	-	-	15	-
12	86	204.1 (2.9)	94.2 (10.1)	85	-	-	-	15	-
Total	971	213.8 (10.7)	98.4 (11.7)	964	194	152	85	133	162

[1] GM: *gluteus medius* muscle; LD: *longissimus dorsi* muscle; SM: *semimembranosus* muscle; LT: *latissimus dorsi* muscle; SFGM (SFLD): subcutaneous backfat at the level where GM (LD) was taken.

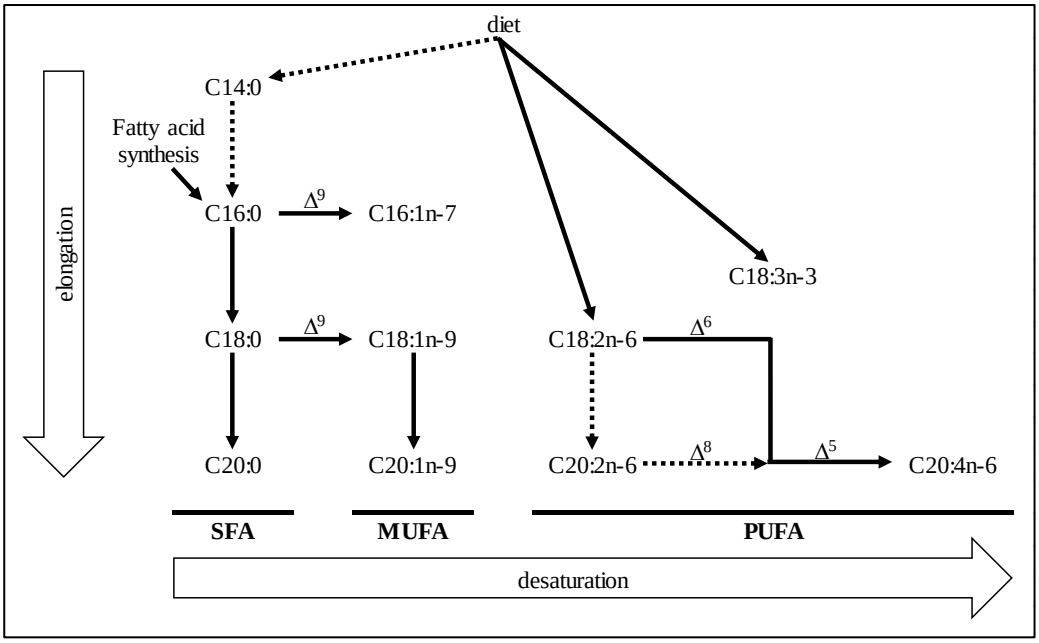


Figure 1.1. Main metabolic pathways for the fatty acids considered in the case study (adapted from Cook and McMaster, 2002). Discontinuous arrows indicate less important pathways.

Thió-Henestrosa, 2006; Palarea-Albaladejo et al., 2007). For its simplicity, we followed here the strategy in Sanford et al. (1993) and replaced the zeros by 0.55 times the lowest measured value in each tissue before calculating the fatty acid percentages.

1.3. SETTING THE PROBLEM

One of the drawbacks of analyzing compositional data with conventional methods is that the results can be subcompositionally incoherent (Aitchison, 1986, Chapter 3; Pawlowsky-Glahn & Egozcue, 2006). This becomes particularly evident in correlation analyses, where the correlation coefficient between two given components can differ depending on whether they are expressed relative to a set of components or another. In order to highlight this problem we calculated the correlation between pairs of fatty acids under two different compositional settings. In the first one, the correlation matrix among the complete 11-part fatty acid profile of GM was calculated (Table 1.2, rows a), while, in the second, the correlation was calculated between each SFA, MUFA, and PUFA expressed relative to the total SFA, MUFA, or PUFA, respectively, in such a way that, for instance, C14:0, C16:0, C18:0, and C20:0 summed up to 100% (i.e., the SFA subcomposition was closed). Then, the correlations among the fatty acids in each subcomposition (SFA, MUFA, and PUFA) were recalculated

Table 1.2. Correlations among raw fatty acid percentages in *gluteus medius* when expressed relative to either the full fatty acid composition (rows a) or the corresponding saturated (rows b), monounsaturated (rows c), and polyunsaturated (rows d) fatty acid subcompositions.

Fatty acid		C16:0	C18:0	C20:0	C16:1	C18:1	C20:1	C18:2	C18:3	C20:2	C20:4
C14:0	a	−0.10	−0.34	−0.02	0.14	0.10	0.14	0.00	0.31	0.05	0.12
	b	0.40	−0.75	−0.07	-	-	-	-	-	-	-
C16:0	a		0.80	0.39	−0.08	−0.73	−0.38	−0.44	−0.44	−0.35	−0.50
	b		−0.91	−0.26	-	-	-	-	-	-	-
C18:0	a			0.46	−0.20	−0.71	−0.21	−0.39	−0.44	−0.24	−0.48
	b			0.18	-	-	-	-	-	-	-
C20:0	a				−0.18	−0.20	0.01	−0.39	−0.13	−0.09	−0.34
C16:1	a					0.11	0.02	−0.24	−0.15	0.29	−0.15
	c					−0.98	−0.12	-	-	-	-
C18:1	a						0.43	−0.19	0.01	0.03	0.07
	c						−0.07	-	-	-	-
C20:1	a							−0.16	0.07	0.16	−0.04
C18:2	a								0.70	0.27	0.59
	d								0.21	−0.15	−0.89
C18:3	a									0.32	0.26
	d									0.22	−0.50
C20:2	a										0.13
	d										−0.24

(Table 1.2, rows b, c, and d, respectively). As can be seen in Table 1.2, the two correlations were not consistent, with the discrepancy being particularly relevant for those between C16:0 and C18:0, C16:1 and C18:1, and C18:2 and C20:4, which changed, respectively, from 0.80 to −0.91, 0.11 to −0.98, and 0.59 to −0.89. These changes, both in magnitude and sign, are due to the fact that components in compositional data do not vary independently. It can be proven that for a *D*-part composition $\mathbf{x} = [x_1, x_2, ..., x_D]$, if $x_1 + x_2 + ... + x_D = \kappa$ (where κ is a constant, often 1 or 100%), then $\text{cov}(x_1, x_2) + \text{cov}(x_1, x_3) + ... + \text{cov}(x_1, x_D) = -\text{var}(x_1)$. Therefore, at least one of the covariances of x_1 with the other components must be negative (Pearson, 1897; Aitchison, 1986, Chapter 3; Filzmoser & Hron, 2009). This negative bias causes that an increase in one of the components results in the decrease in, at least, another one. Hence, the correlations are not free to range over the interval $[-1, 1]$. The distribution of the bias over the covariance terms, along with the subsequent changes in the correlation matrix among components, depends upon which parts are included in the composition. As a consequence, the above correlations do not have any neat interpretation. This simple example highlights that the analysis of compositional data using standard techniques may lead to spurious and inconsistent results across subcompositions.

1.4. OVERVIEW OF COMPOSITIONAL ANALYSIS

Compositional data need to be statistically treated considering that they only carry relative information. Two general approaches have been developed to deal with them. The first is known as staying-in-the-simplex approach. It operates in the so-called simplex space (S^D, for D-part compositions) and uses the Aitchison geometry (Aitchison, 1986, Chapter 2). The second approach resorts to log-ratio transformations (Aitchison, 1986, Chapter 7; Egozcue et al., 2003) to map the simplex to the real space, where the more familiar Euclidean geometry is used and standard statistics methods can be applied. Both approaches can be used complementarily depending on which geometrical framework is preferred. A brief description of both approaches is given below. Some software has been developed to easily process and analyze compositional data, such as the freeware CoDaPack (Thió-Henestrosa & Martín-Fernández, 2005; Comas-Cufí & Thió-Henestrosa, 2011a,b) and the R packages *compositions* (van den Boogaart et al., 2011) and *robCompositions* (Templ et al., 2011).

1.4.1. Staying-in-the-simplex

The simplex vector space is defined by the internal simplicial operation of perturbation, the external operation of powering, and the simplicial metric. The operations of perturbation,

$$\mathbf{x} \oplus \mathbf{y} = [x_1, x_2, \dots, x_D] \oplus [y_1, y_2, \dots, y_D] = C[x_1 y_1, x_2 y_2, \dots, x_D y_D] \quad (1.1),$$

and powering,

$$a \odot \mathbf{x} = a \odot [x_1, x_2, \dots, x_D] = C[{x_1}^a, {x_2}^a, \dots, {x_D}^a] = C(\mathbf{x}^a) \quad (1.2),$$

where $\mathbf{x}$ ($\mathbf{y}$) is a D-part composition, x_i (y_i) are the percentages for each part (i = 1, 2, ..., D), a is a scalar, and C is the closure operator to constant κ (rescaling through division of each part by their total sum), are the equivalent to translation and scalar multiplication in the real space, respectively. The staying-in-the-simplex approach requires an algebra that differs from the one used in standard statistics.

An example of this algebra is found in the calculation of descriptive statistics. The mean and the variance are not suitable statistics for compositional exploratory analyses (Daunis-i-Estadella et al., 2006) and therefore they are replaced in the Aitchison geometry by the centre ($\mathbf{g}$) and the variation matrix ($\mathbf{T}$), respectively. The centre or geometric mean is defined as:

$$\mathbf{g} = C\left[\left(\prod_{j=1}^{n} x_{1j}\right)^{1/n}, \left(\prod_{j=1}^{n} x_{2j}\right)^{1/n}, \dots, \left(\prod_{j=1}^{n} x_{Dj}\right)^{1/n}\right] \quad (1.3),$$

where x_{ij} are the percentages for each part (i = 1, 2, ..., D) in sample j, and n is the number of samples. Moreover, the compositions can be centered, i.e., moved to the barycenter of the simplex, using $\mathbf{x} \oplus (-1 \odot \mathbf{g}) = \mathbf{x} \oplus \mathbf{g}^{-1}$ (Pawlowsky-Glahn & Egozcue, 2006). Centering is equivalent to subtracting the arithmetical mean in the Euclidean space. The variation matrix is defined as $\mathbf{T} = [\tau_{ij}]$, with $\tau_{ij} = \text{var}[\ln(X_i/X_j)]$, where X_i and X_j are the data vectors for the parts i and j across samples. Low variance of a log-ratio indicates proportionality between the parts involved. The total variability of the dataset is the sum of the variances of all log-ratios divided by 2D:

$$\text{total-variance} = \frac{1}{2D}\sum_{i=1}^{D}\sum_{j=1}^{D}\text{var}\left[\ln\frac{X_i}{X_j}\right] \tag{1.4}.$$

1.4.2. Log-ratio transformations

The two first log-ratio transformations were introduced by Aitchison (1986, Chapters 4 and 6) and the third by Egozcue et al. (2003). These log-ratio transformations make it possible to work on compositional data in the real space using Euclidean geometry.

1.4.2.1. Additive log-ratio

The additive log-ratio (**alr**) transformation is written in terms of log-ratios of $D-1$ components relative to an arbitrary D component:

$$\text{alr}(\mathbf{x}) = \left[\ln\frac{x_1}{x_D}, \ln\frac{x_2}{x_D}, \ldots, \ln\frac{x_{D-1}}{x_D}\right] \tag{1.5}.$$

This transformation has the obvious disadvantage that the results are dependent on the chosen divisor component, which in turn does not have an equivalent for further analyses. But, most importantly, the alr-transformation is not isometric, i.e., distances are not preserved in the new metric space (Filzmoser & Hron, 2009).

1.4.2.2. Centered log-ratio

The centered log-ratio (**clr**) transformation is written in terms of the log-ratio of each component relative to the geometric mean of all the components of an individual:

$$\mathbf{z} = \text{clr}(\mathbf{x}) = \left[\ln\frac{x_1}{\left(\prod_{i=1}^{D} x_i\right)^{1/D}}, \ln\frac{x_2}{\left(\prod_{i=1}^{D} x_i\right)^{1/D}}, \ldots, \ln\frac{x_D}{\left(\prod_{i=1}^{D} x_i\right)^{1/D}}\right] \tag{1.6}.$$

In the $\mathbf{z} = \mathrm{clr}(\mathbf{x})$ transformation all parts of the composition have a direct equivalent, so that transformed variables can be easily traced back to the originals. Although the clr transformation is isometric, it is subcompositionally incoherent. Moreover, the covariance matrix of the clr-transformed variables is singular, which difficults the use of the clr transformation in multivariate statistical analyses requiring the inversion of this matrix. The clr transformation is mostly used in exploratory analysis. The so-called clr-biplots allow for a graphical representation of the distribution of the samples based on their composition. Moreover, the depiction of links (i.e., the vectors connecting the apexes of two variable rays) provides an easy-to-interpret representation of the log-ratios between the two involved components, where their length represents the standard deviation of the log-ratios and the cosine of the angle between two links the correlation between the two involved log-ratios. A complete description of clr-biplots and their interpretation is given in Aitchison & Greenacre (2002) and Daunis-i-Estadella et al. (2006). Conclusions only should be drawn from biplots that explain a large percentage of the total variance. An example is presented in Section 1.5.1.

1.4.2.3. Isometric log-ratio

The isometric log-ratio (**ilr**) transforms the raw composition to its coordinates in an orthogonal system based upon an orthonormal basis ($\mathbf{\Psi}$) (Egozcue et al., 2003). If $\mathbf{\Psi}$ is chosen following a sequential binary partition (Egozcue & Pawlowsky-Glahn, 2005), the ilr-transformed components are called balances (b_k, where $k = 1, 2, ..., D-1$). In a sequential binary partition, $\mathbf{\Psi}$ is constructed by successive divisions of the set of parts into two mutually exclusive groups (parts in one group are marked with the symbol + while parts in the complementary group with the symbol −) until only one part per group is left (see Table 1.3 for an example). To be interpretable, partitions should be based on previous knowledge and experience. Then, $\mathbf{\Psi}$ is derived replacing the symbols + and – by $\frac{1}{r}\sqrt{\frac{rs}{r+s}}$ and $-\frac{1}{s}\sqrt{\frac{rs}{r+s}}$, respectively, where r (s) is the number of parts marked with + (−) in each balance, with blanks being zero. Then, the balances $\mathbf{w} = \mathrm{ilr}(\mathbf{x})$ are calculated as $\mathbf{w} = \mathbf{z}\mathbf{\Psi}^{T}$, or directly, in terms of normalized log-ratios between the geometric means of the two groups, as:

$$b_k = \sqrt{\frac{r_k s_k}{r_k + s_k}} \ln \frac{\left(\prod_{i=1}^{r_k} x_{ki}^{+}\right)^{\frac{1}{r_k}}}{\left(\prod_{j=1}^{s_k} x_{kj}^{-}\right)^{\frac{1}{s_k}}} \qquad (1.7),$$

where x_k^+ and x_k^- represent the subsets of r_k and s_k parts in group + and − of the kth balance, respectively.

Table 1.3. Sequential binary partition of the 11-fatty acid composition for ilr-transformation.

Balance	C14:0	C16:0	C18:0	C20:0	C16:1	C18:1	C20:1	C18:2	C18:3	C20:2	C20:4
1	−	−	−	−	−	−	−	+	+	+	+
2	−	+	+	+	+	+	+				
3		−	+	+	−	+	+				
4			−	+		−	−				
5		−			+						
6			−			+	+				
7						−	+				
8								+	−	+	+
9								−		−	+
10								−		+	

Note that, as happens with the alr transformation, there are only $D-1$ balances for a D-part composition, and that the balances may be different for each $\mathbf{\Psi}$. The balances are isometric and subcompositionally coherent and, as a result, they can be analyzed using standard statistical techniques. However, because they do not have a one-to-one relation to the original components, their interpretation is not straightforward. This can be overcome by choosing, if it exists, a sequential binary partition leading to interpretable balances or, alternatively, back-transforming them into interpretable D-part compositions lying in the simplex. Because compositions are intrinsically multivariate, estimates on the full set of $D-1$ balances (for instance, either least squares means or regression coefficients) must be jointly back-transformed as $\mathbf{x} = C(e^{\mathbf{w}\mathbf{\Psi}})$ (Tolosana-Delgado & van den Boogaart, 2011). In Sections 1.5.2 and 1.5.4 examples on the application of ilr-transforming and back-transforming are presented. However, it is not possible to back-transform the standard errors associated with least square estimates, but they can be substituted by the corresponding back-transformed confidence intervals. The use of balances is the best choice for correlations (Filzmoser & Hron, 2009), but they cannot be back-transformed either. If the sequential bipartition used does not lead to the desired balances, additional log-ratios can be calculated as linear combinations of the initial $D-1$ set derived from $\mathbf{\Psi}$. For example, apart from the balances derived from the sequential bipartition in Table 1.3 (b_1 to b_{10}) we could be interested in the log-ratios of C18:1 and C18:0:

$$\frac{1}{\sqrt{2}}\ln\frac{\mathrm{C18{:}1}}{\mathrm{C18{:}0}} = \frac{\sqrt{3}}{2}b_6 - \frac{1}{2}b_7 = \frac{\sqrt{3}}{2}\left(\sqrt{\frac{2}{3}}\ln\frac{\sqrt{\mathrm{C18{:}1}\cdot\mathrm{C20{:}1}}}{\mathrm{C18{:}0}}\right) - \frac{1}{2}\left(\frac{1}{\sqrt{2}}\ln\frac{\mathrm{C20{:}1}}{\mathrm{C18{:}1}}\right) \quad (1.8),$$

or, similarly, MUFA and SFA:

$$\sqrt{\frac{12}{7}}\ln\frac{\sqrt[3]{\mathrm{C16{:}1}\cdot\mathrm{C18{:}1}\cdot\mathrm{C20{:}1}}}{\sqrt[4]{\mathrm{C14{:}0}\cdot\mathrm{C16{:}0}\cdot\mathrm{C18{:}0}\cdot\mathrm{C20{:}0}}} = \frac{1}{2\sqrt{2}}b_2 - \frac{\sqrt{7}}{6}b_4 + \frac{\sqrt{7}}{2\sqrt{6}}b_5 + \frac{\sqrt{7}}{3\sqrt{2}}b_6 \quad (1.9).$$

The inclusion of more log-ratios can enrich the interpretation of the results but then it should be noted that the covariance matrix including the new log-ratios will be singular. An example of correlation analysis using balances is given in Section 1.5.5.

1.5. ANALYZING THE CASE STUDY

The basics of compositional analysis are illustrated in five examples using the pork fatty acid composition as a case study. The first is an exploratory analysis conducted to examine the differences between IMF and backfat for fatty acid composition (Section 1.5.1). The second and third introduce the procedures to compare the distinct tissues and muscles in terms of centers (Section 1.5.2) and variation matrixes (Section 1.5.3). In Section 1.5.4 a linear regression is used to assess the effect of IMF content on fatty acid composition. Finally, Section 1.5.5 illustrates how to interpret correlations among biologically meaningful balances. In Sections 1.5.2 and 1.5.4 the compositional and the standard approaches are compared.

1.5.1. Exploratory analysis

The distribution of fatty acid composition across muscles and backfat locations was first explored depicting the whole set of observations on a joint biplot (Figure 1.2). To this purpose the dataset **X** was clr-transformed to **Z**, and then singular value decomposed using standard procedures (Daunis-i-Estadella et al., 2011). The two first components accounted for 76% of the total variation. The projection of the samples (Figure 1.2a) in the biplot showed that IMF can be clearly discriminated from SF based on fatty acid composition. More specifically, the first component, which explained 56% of the total variation, was enough to separate IMF from SF samples. The most important fatty acid affecting this component was C20:4, whose ray was opposite to those of the other PUFA and formed with them a long link along the first component (as an example, the link of ln(C20:4/C18:2) is represented with a discontinuous line in Figure 1.2b). The length of these links, which relates to the standard deviation of the log-ratios of the two fatty acids involved, indicates that the log-ratio between C20:4 and other PUFA (C18:2 and C18:3) displayed a great variation along the gradient separating IMF and SF. The SF samples were allocated in a cluster at the left side of the biplot and the IMF samples were clustered at the right side, indicating that the ratios C20:4/C18:2 and C20:4/C18:3 were greater in IMF than in SF. Despite some overlapping, the samples from each muscle can also be singled out (Figure 1.2a), especially within batch (Figure 1.2b). In doing so, SM samples were mostly found in the upper region of the IMF cluster whereas those from GM (left), LT (middle), and LD (right) were in the lower. This could not be done for SF, where only one backfat location was analyzed per batch. The distribution pattern of the batch centers suggested

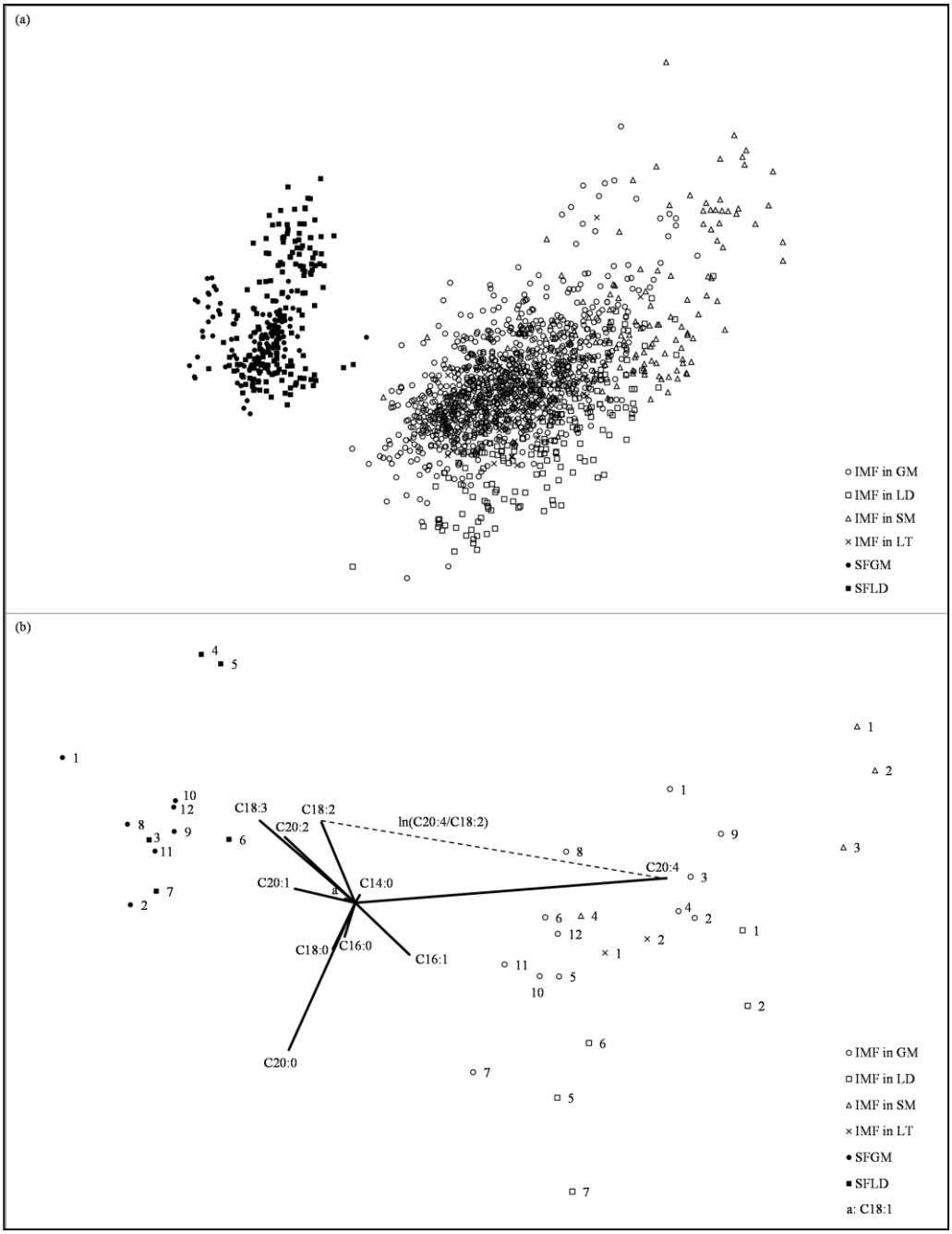


Figure 1.2. Score plot (a) and loading plot (b) of components 1 and 2 for fatty acid composition of intramuscular fat (IMF) across muscles (GM: *gluteus medius*; LD: *longissimus dorsi*; *SM: semimembranosus;* and LT: *latissimus dorsi*) and backfat locations (SFGM: at the level of GM; and SFLD: at the level of LD). The loading plot (b) includes one link (discontinuous) and the projection of the center of each of the twelve batches. Horizontal and vertical axes represent components 1 (56% of total variance) and 2 (20% of total variance), respectively.

that the effect of the batch on the fatty acid composition of IMF could be, at least partially, explained by differences in the age at slaughter (Table 1.1). Because IMF increases with age and saturation with IMF, pigs slaughtered at later ages are expected to have more saturated fat (Bosch et al., 2012). Accordingly, within muscle, the samples from pigs slaughtered at later ages (Table 1.1; batches 5−7 and 10−11) should tend to show greater SFA/PUFA ratios and therefore appear preferentially lower-left in the biplot relative to those from pigs slaughtered at earlier ages (Table 1.1; batches 1−4, 8−9, and 12).

A biplot for each muscle was also set up. The effect of batch was removed centering the data by batch (which is the equivalent in the simplex to subtract the mean of the batch) before they were clr-transformed and singular value decomposed. The IMF content was included in the biplots as a supplementary variable (Daunis-i-Estadella et al., 2011) to assess the relationship between IMF content and composition. The loading plots of the two first components by muscle are given in Figure 1.3. The two first components explained from 67% (GM) to 74% (SM) of the total variance. The loading plots showed a similar pattern among muscles, with SM being the most different. In all muscles, SFA and MUFA were in the opposite side to PUFA for the first component. The cosine of the angle between two links refers to the correlation between their log-ratios. In general, the angles between links involving two SFA (C16:0, C18:0), two MUFA (C16:1, C18:1), or a SFA with a MUFA, were small, indicating high correlations among them. Because C18:0 can be synthesized from precursor C16:0 by an elongase, and both C16:1 and C18:1 are synthesized by the same Δ^9 desaturase (stearoyl-CoA desaturase) from C16:0 and C18:0, respectively (Figure 1.1; Cook & McMaster, 2002), the product/substrate ratio C18:0/C16:0 is frequently used as an indicator of the elongase activity, and ratios C16:1/C16:0 and C18:1/C18:0 of the Δ^9 desaturase activity. Thus, the high correlations among ratios of these four fatty acids are biologically consistent and in line with the correlations found by other authors (Ntawubizi et al., 2010). The links involving C14:0, in all the muscles, and C20:0, in SM, had much greater angles, and thus lower correlations, with the other links. This might be because C14:0, unlike other SFA, is mainly of dietary origin (Figure 1.1; Wood et al., 2008) and because C20:0 is subjected to relatively larger instrumental error and greater number of zeros. Small angles, and thus high correlations, were also found between links corresponding to log-ratios of PUFA. However, in all the muscles, the links involving two SFA, two MUFA, or a SFA with a MUFA, on one side, and the links involving PUFA, on the other side, were almost perpendicular to each other. This indicates low correlations between these two groups of log-ratios, in accordance with the low association of PUFA with SFA and MUFA reported in literature (Cameron & Enser, 1991; Zhang et al., 2007; Ntawubizi et al., 2010; Yang et al., 2010). Overall, the results indicate that SFA and MUFA behave similarly to each other but differently from PUFA, in line with their different

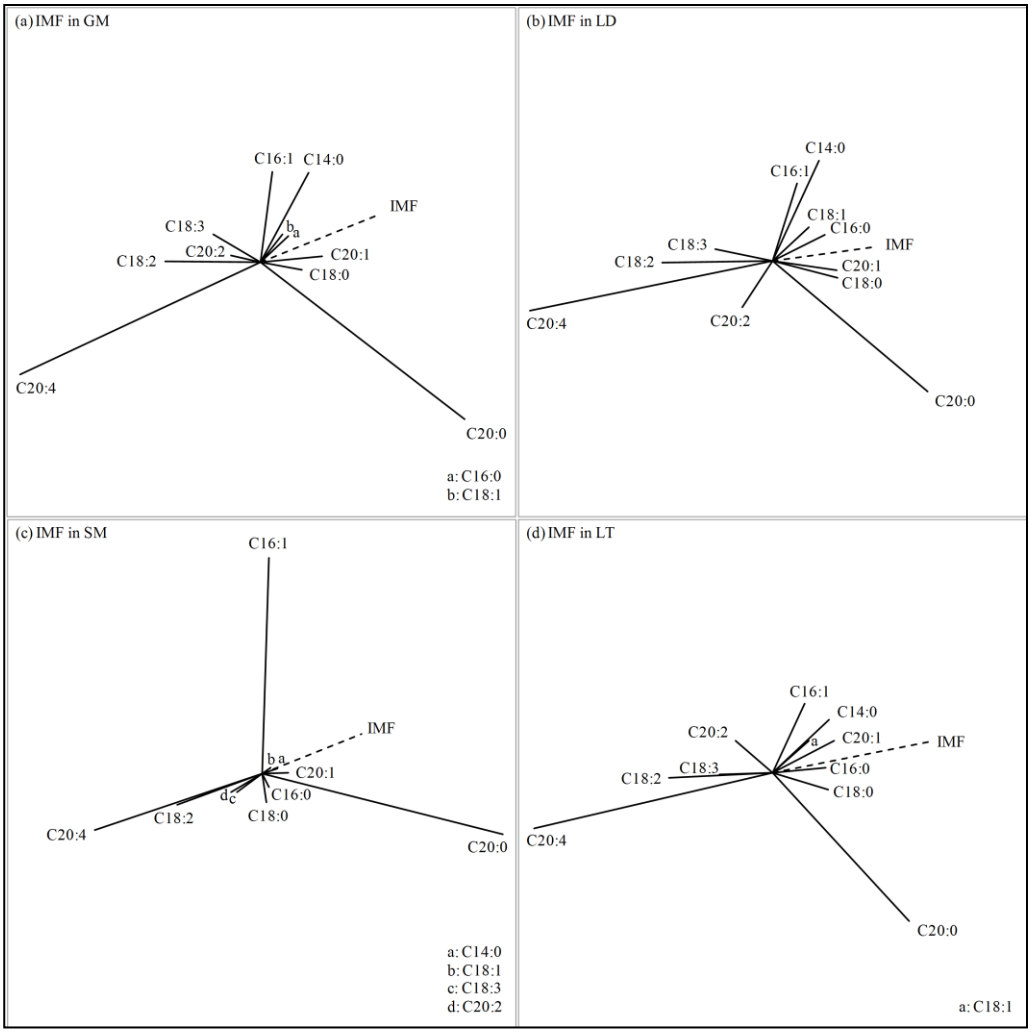


Figure 1.3. Loading plot of components 1 and 2 for the intramuscular fat (IMF) content and fatty acid composition in *gluteus medius* (GM, a), *longissimus dorsi* (LD, b), *semimembranosus* (SM, c), and *latissimus dorsi* (LT, d) muscles. Horizontal axis represents component 1 (46%, 51%, 47%, and 52% of the total variance in GM, LD, SM, and LT, respectively) and vertical axis component 2 (21%, 18%, 28%, and 18% of total variance in GM, LD, SM, and LT, respectively).

deposition patterns. Fat depots, IMF and SF, can be divided into two fractions: phospholipids and neutral lipids. Phospholipids have structural functions and have abundant PUFA, particularly C20:4, which is the major PUFA in cell membranes (Larsson et al., 2004), whereas neutral lipids, mainly composed of SFA and MUFA, have storage functions. It means that IMF increases with neutral lipids while phospholipids remain relatively constant (Cameron & Enser, 1991; De Smet et al.,

2004), which is the reason for the positive relationship of IMF with SFA and MUFA, but negative with PUFA (Cameron & Enser, 1991; Zhang et al., 2007; Yang et al., 2010). The IMF content displayed a negative collinearity with C20:4 in all the muscles, supporting that increased IMF is associated with decreased C20:4, namely phospholipids, and PUFA, as well as to increased SFA and MUFA (Cameron & Enser, 1991; De Smet et al., 2004; Bosch et al., 2012).

1.5.2. Differences among tissues and muscles

The centers of the fatty acid composition of IMF and SF (Eq 1.3) established that the most abundant fatty acids were C18:1 (44.0−46.1%), C16:0 (21.2−24.3%), C18:2 (9.2−16.2%), and C18:0 (10.6−12.1%) in all the studied muscles and backfat locations, in agreement with the general knowledge on meat fatty acid composition (Valsta et al., 2005). The centers revealed differences of fatty acid composition among the muscles and backfat locations. These differences were estimated and tested using the balances described in Table 1.3. The balances were analyzed using a linear mixed model, in which fixed effects included the batch (1 to 12), tissue (the four muscles and the two backfat locations), and carcass weight as a covariate. The pig and the residual were the random effects. Variances were estimated by restricted maximum likelihood and fixed effects were tested following a Kenward-Roger approach. The differences between tissues were contrasted with the Tukey HSD test at a significance level of 0.05. The analyses were performed using JMP 8 software (SAS Institute Inc., Cary, NC). The least squares means and confidence intervals for the balances were back-transformed as indicated in Section 1.4.2.3. Results were compared with those obtained using the same model for raw fatty acid percentages instead of balances.

The centers adjusted for batch and carcass weight are given in Table 1.4. The ordinary least squares means differed on average only by 0.1% (SD 0.1), with a maximum of 0.8% (C18:1). Significant differences among muscles and backfat locations were found, with compositional and standard approaches leading to similar conclusions. The two backfat locations showed greater contents of the PUFA C18:2, C18:3, and C20:2 than IMF in all muscles, but lower of C20:4. By contrast, IMF was more saturated and monounsaturated, although for some fatty acids the differences between IMF and SF were not significant. These findings were in line with the well-known result that essential PUFA, C18:2 and C18:3, which are from dietary origin (Figure 1.1), are preferentially deposited in SF (Kloareg et al., 2007; Duran-Montgé et al., 2008). That the C20:4 displays an opposite trend to other PUFA (see Figure 1.2b) could be explained by the much greater fraction of phospholipids in IMF as compared to SF. Among muscles, SM had higher concentrations of C18:2 and C20:4 than GM, LD, and LT, and lower of the main SFA and MUFA. The observed differences in muscle composition can be partly attributed to IMF content (Table 1.4).

Table 1.4. Centers[1] for fatty acid composition by muscle and backfat location adjusted for batch and carcass weight, and least squares means for intramuscular fat (IMF) content by muscle.

Fatty acid, %	Muscles[2]				Backfat locations[2]	
	GM	LD	SM	LT	SFGM	SFLD
C14:0	1.65^{A}_{a}	1.60^{B}_{ab}	1.45^{C}_{c}	1.55^{B}_{ab}	1.39^{D}_{c}	1.60^{B}_{b}
C16:0	23.23^{D}_{c}	24.37^{B}_{b}	23.56^{C}_{c}	26.04^{A}_{a}	21.72^{D}_{d}	20.89^{D}_{e}
C18:0	11.27^{D}_{d}	11.89^{B}_{bc}	12.08^{B}_{b}	14.10^{A}_{a}	11.42^{C}_{cd}	10.29^{D}_{e}
C20:0	0.14^{B}_{c}	0.16^{A}_{b}	0.09^{D}_{e}	0.16^{A}_{bc}	0.19^{A}_{a}	0.11^{C}_{d}
C16:1	3.81^{B}_{b}	4.04^{A}_{a}	2.94^{D}_{c}	3.03^{C}_{c}	2.11^{E}_{d}	2.11^{E}_{d}
C18:1	44.63^{B}_{bc}	45.48^{A}_{a}	41.78^{D}_{e}	42.83^{C}_{d}	43.90^{C}_{cd}	45.25^{A}_{ab}
C20:1	0.82^{B}_{b}	0.77^{B}_{c}	0.72^{C}_{d}	0.74^{B}_{cd}	1.12^{A}_{a}	1.14^{A}_{a}
C18:2	11.94^{C}_{c}	9.58^{D}_{d}	13.82^{B}_{b}	9.62^{D}_{d}	15.78^{A}_{a}	16.17^{A}_{a}
C18:3	0.73^{C}_{b}	0.48^{E}_{d}	0.58^{D}_{c}	0.55^{D}_{c}	1.19^{B}_{a}	1.31^{A}_{a}
C20:2	0.59^{C}_{c}	0.42^{E}_{e}	0.53^{D}_{d}	0.50^{D}_{d}	0.95^{A}_{a}	0.87^{B}_{b}
C20:4	1.19^{C}_{b}	1.21^{B}_{b}	2.44^{A}_{a}	0.88^{D}_{c}	0.23^{F}_{d}	0.28^{E}_{d}
IMF, % dry matter	$16.2\pm0.2_{b}$	$12.7\pm0.3_{c}$	$9.1\pm0.4_{d}$	$21.1\pm0.5_{a}$	-	-

[1] Adjusted centers were calculated following the compositional approach (i.e., using balances followed by back-transformation). The least squares means for each fatty acid based on the raw percentages are not shown because on average they only differed by 0.1% (SD 0.1).
[2] See abbreviations in Table 1.1.
[A-F] Differences tested on ilr-transformed variables. Within a row centers without a common superscript letter differ ($p<0.05$).
[a-e] Differences tested on raw percentages. Within a row means without common subscripts differ ($p<0.05$). Subscripts are given only for comparison purposes with superscripts.

1.5.3. Variation within tissue and muscle

The variation arrays and the total-variances (Eq 1.4) were calculated for each muscle and backfat location. The total-variance of the composition of IMF in GM was 0.57. After adjusting for batch (i.e., centering by batch), the total-variance decreased to 0.32. This indicates that around one half of the variability of the muscle fatty acid composition is due to common environmental effects in a batch. The adjusted total-variance was higher for IMF in SM (0.97) than in GM, LD, and LT, which were very similar to each other (0.27−0.32) and to SFLD (0.37). The total-variance for SFGM was much lower (0.10). In general, the log-ratios involving C18:1 were the ones displaying the lowest variances (0.01−0.33) in all cases. Interestingly, the log-ratios involving C20:4 showed the highest relative variability in all cases (0.02−0.73), except for IMF in SM and SFLD, where C20:0 was the most variable fatty acid. Nonetheless, the high variability of C20:0 could be due, because of its low content, to the relatively

large analytical errors and replaced zeros. The variability of C20:4 is partly due to the variance of the phospholipids fraction in the IMF content, which, as it will be shown in Section 1.5.4, is not neutral with respect to IMF content. Overall, the variation of fatty acid composition in pork is low. The largest element of the variation matrix of IMF in GM was 0.48 and the maximum across tissues was 1.13 for SM. These values are, for example, 10-fold and 4-fold lower than those reported by Daunis-i-Estadella et al. (2006) for geological compositional data, the area of expertise where compositional data techniques have been mostly applied.

1.5.4. Regression on intramuscular fat content

Results in Section 1.5.2 support that fat content influences fat composition (Wood et al., 2008; Bosch et al., 2012). This relationship can be assessed by performing a compositional regression analysis of fatty acid composition on IMF content (Aitchison, 1986, Chapter 7; Egozcue & Pawlowsky-Glahn, 2011; Egozcue et al., 2012). The 109 samples of GM in batch 1 were used for this purpose. The ten balances described in Table 1.3 were compositionally regressed on IMF content (JMP 8 software, SAS Institute Inc., Cary, NC) and then the results were compared with the simple regression of the raw fatty acid percentages on IMF content. The vectors of estimated intercepts (**i**) and slopes (**s**) in the ilr-setting were back-transformed to the simplex as $\mathbf{i'} = C(e^{\mathbf{i}\Psi})$ and $\mathbf{s'} = C(e^{\mathbf{s}\Psi})$. Then, the fatty acid composition at a given IMF content (**x**) can be predicted operating either in the simplex, with $\mathbf{x} = \mathbf{i'} \oplus (\text{IMF} \odot \mathbf{s'})$, or in the real space, with $\mathbf{w} = \text{ilr}(\mathbf{i'}) + \text{IMF} \times \text{ilr}(\mathbf{s'}) = \mathbf{i} + \text{IMF} \times \mathbf{s}$ and then back-transforming **w** to $\mathbf{x} = C(e^{\mathbf{w}\Psi})$.

The balances more influenced by IMF content were balances 1 and 8 (R^2=0.23 and 0.20, respectively). The R^2 associated to the other balances was lower than 0.08. The balance 1 was built to represent the ratio PUFA vs. SFA+MUFA, while balance 8 was associated to the ratio n-6 vs. n-3 PUFA (i.e., C18:2+C20:2+C20:4 vs. C18:3). This is consistent with results discussed in Section 1.5.1, where PUFA and, particularly, C20:4, more abundant in phospholipids, decrease as IMF content increases. Similar results were found for raw percentages, with C18:2 and C20:4 showing the highest R^2 (0.34 and 0.14). The relationship between fatty acids and IMF content is displayed in Figure 1.4. For simplicity, only three fatty acids are displayed, although the analyses were done using the whole 11-fatty acid composition. A relevant difference between compositional and standard regression is that in this latter case, at extreme values of the covariate, the predicted values can be non-sense. Thus, at high IMF contents negative percentages are predicted for C18:2 (IMF>65%) and C20:4 (IMF>35%). This does not happen in the compositional analysis. The back-transformed regressions of the 10 balances on IMF content were non-linear and

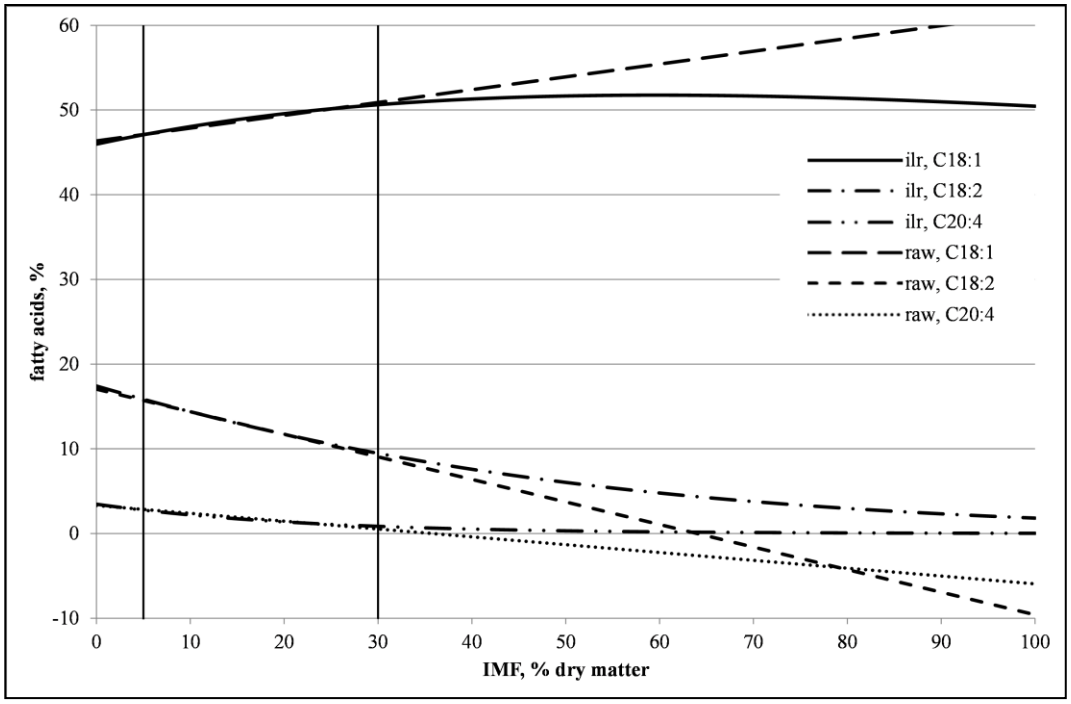


Figure 1.4. Regression of the percentage of C18:1, C18:2, and C20:4 on intramuscular fat (IMF) content in the muscle *gluteus medius* using the compositional (ilr) or ordinary (raw) regression analysis. Vertical lines delimit the range of observed values.

asymptotically bounded, with predicted values always lying within the [0, 100] range. However, within the expected range of values for IMF, from 5% to 30% on dry matter basis (equivalent to approximately 1% to 10% of fresh meat), the compositional regression is almost linear, overlapping with the standard regression. Predicted values, even using validation samples from other batches, were almost identical under the two approaches. In the expected range of values for IMF the standard regression led to similar results to the compositional analysis. A similar conclusion is reached in models other than the regression used here, which is deliberately simple for illustrative purposes.

1.5.5. Correlations among enzymatic indices

The correlations between balances for GM are given in Table 1.5. The balances described in Table 1.3 were established in accordance with known metabolic pathways for fatty acid synthesis in pigs (Figure 1.1). Because they are regulated by specific enzymes the balances can be thought in terms of enzymatic activity. The first balance can be interpreted as a polyunsaturation index (PUFA vs. SFA+MUFA), which separates the PUFA and the SFA and MUFA pathways. Balances 2 to 7 are associated

Table 1.5. Correlations among balances in muscle *gluteus medius*.

Balance	2	3	4	5	6	7	8	9	10
1	−0.35	−0.16	−0.42	0.12	0.40	−0.16	0.42	0.67	−0.22
2		0.24	0.24	−0.18	−0.40	0.12	0.08	−0.30	0.06^{ns}
3			0.64	−0.56	−0.02^{ns}	0.39	−0.12	−0.10	0.26
4				−0.29	−0.28	0.00^{ns}	−0.26	−0.19	0.17
5					0.61	0.02^{ns}	0.08	0.07	0.25
6						0.27	0.11	0.28	0.29
7							−0.08	−0.21	0.46
8								0.75	−0.11
9									−0.34

^{ns}: not significant (p>0.05).

to SFA and MUFA metabolism, where balances 2, 3, 4, and 7 can be interpreted as indexes of elongase activity, and balances 5 and 6 of Δ^9 desaturase activity. Note that although they are aimed at representing different elongation or desaturation steps, in general they are not ratios between single products and substrates. For instance, balance 3 accounts not only for the elongation of C16:0 to C18:0, but also for the amount of C16:0 that has alternatively been desaturated to C16:1 and the amount of C18:0 further transformed into C20:0, C18:1, and C20:1. The balances can be an interesting alternative to elementary indexes between only two fatty acids because they also include further or alternative products derived from the same substrate (Figure 1.1). However, because they are designed based upon a sequential bipartition, some balances cannot include all the desired fatty acids (e.g., balance 6 does not include C20:0, which can be elongated from C18:0). As expected, all the elongase balances were positively correlated among them, as well as the two desaturation indexes. However, interestingly, the correlation among the desaturation and the elongase indexes was negative. The polyunsaturation index was negatively correlated to the elongase activity but positively to the Δ^9 desaturase activity. Balances 8, 9, and 10 are associated with PUFA metabolism. Balance 8 is the ratio between n-6 and n-3 fatty acids, which is known to play a crucial role in the nutritional quality of fat (Schmid, 2010). The positive correlation between balance 1 and balance 8 indicates that the n-6/n-3 ratio increased with polyunsaturation. Balance 9 reflects the total efficiency of biosynthesizing C20:4 from any of the two pathways using C18:2 as a precursor, while balance 10 only accounts for the intermediate elongation step from C18:2 to C20:2 carried out in one of the two pathways (Figure 1.1). The positive correlation of balance 9 with balances 1 and 8 confirmed that the percentage of C20:4 increases with PUFA and with the n-6/n-3 ratio. A correct interpretation of the balances may help to gain new insight into fatty acid metabolism. Note that in this example we used only the $D-1$ balances described in Table 1.3, which derived from a unique sequential bipartition. More log-ratios could be calculated and added as discussed in

Section 1.4.2.3. For example, the correlation between the log-ratios of C18:1/C18:0 and MUFA/SFA (Eq 1.8 and 1.9) was 0.70.

1.6. CONCLUSIONS

Fatty acid compositions, which by nature are compositional data, should be statistically treated as such. There are two complementary approaches to analyze compositional data: either operate in the simplex space or make use of log-ratios to operate in the real space. The ilr transformation allows for a straightforward handling of geometric elements in the simplex using standard statistical procedures. Nonetheless, for the case study considered here we found that the inferences drawn from compositional analysis did not substantively differ from those obtained using standard statistics techniques on raw data. The low variability of fatty acid composition across fat pork depots may explain why the standard approach, although methodologically inconsistent, is robust enough for practical purposes. This is likely to happen to other unprocessed raw food products, where natural variability is subjected to homeostatic biological constraints. Results evidenced that IMF and SF behave differently in terms of fatty acid composition, with IMF showing more SFA, MUFA, and C20:4, and that fatty acid composition differs among muscles, with SFA and MUFA increasing with IMF. Compositional analysis proved to be useful in correctly interpreting the correlation structure among fatty acid components. Choosing an appropriate set of balances may help not only to avoid spurious results but also to better address the biological mechanisms involved in fatty acid deposition. Careful attention is recommended in cases of higher expected variability, such as when comparing differentiated processed products, where a compositional analysis may lead to more dramatic changes.

PART 2

Chapter 2.
Expected genetic response for oleic acid content in pork

R. Ros-Freixedes[1], J. Reixach[2], M. Tor[1], & J. Estany[1]

[1]Departament de Producció Animal, Universitat de Lleida − Agrotecnio Center, 191 Av. Alcalde Rovira Roure, 25198 Lleida, Catalonia, Spain.
[2]Selección Batallé S.A., Av. Segadors s/n, 17421 Riudarenes, Catalonia, Spain.

The content of this chapter was published in Journal of Animal Science (2012), 90: 4230−4238. DOI: 10.2527/jas.2011-5063

ABSTRACT: Intramuscular fat (IMF) and oleic acid (C18:1) content in pork are important issues for the pig industry and consumers. Data from a purebred Duroc line were used to (1) estimate the genetic parameters of IMF and C18:1 and their genetic correlations with lean growth components, and (2) evaluate the opportunities for genetically improving C18:1 in IMF. The data set used for estimating genetic parameters consisted of 93,920 pigs, from which 85,194 had at least one record for body weight (BW) or backfat thickness (BT) at 180 d and 943 for IMF and C18:1 at 210 d. Intramuscular fat content, expressed as percentage of fresh matter, and C18:1, expressed as percentage of total fatty acids, were determined in the *gluteus medius* muscle by gas chromatography. Genetic parameters for C18:1 were estimated under a Bayesian 4-trait multivariate animal mixed model. Heritability of C18:1 was 0.50, with a probability of 95% of being greater than 0.37. Genetic correlations of C18:1 with BW, BT, and IMF were 0.11, 0.22, and 0.47, respectively (with a probability of 95% of being greater than –0.07, 0.04, and 0.27, respectively). Genetic responses were evaluated by deterministic simulation using a half-sib recording scheme for C18:1 and the previously estimated parameters. The C18:1 content is expected to exhibit only minor changes in selection programs directed at growth rate but to decrease in those focusing on lean content. Maximum expected response in C18:1 at no lean growth loss (i.e., at no change in BW and BT) was 0.44%, with a resulting correlated response in IMF of 0.15%. However, because lean growth is emphasized in the breeding goal, the resulting response scenarios are more constrained. We concluded that there is evidence to support the idea that C18:1 in IMF is genetically determined and defined selection strategies can lead to response scenarios in which C18:1, IMF, BT, and BW can be simultaneously improved. However, if adopted, the potential for lean growth would be reduced. The extent to which it is affordable relies on how much consumers are prepared to pay for high-oleic pork products.

2.1. INTRODUCTION

Fat content and composition are important issues for the pig industry and consumers. Intramuscular fat (**IMF**) and oleic acid (**C18:1**) content are two of the traits that have attracted greatest interest in the last few years. The IMF content has been favorably related to tenderness and juiciness of cooked meat (Wood et al., 2008), as well as to technological and sensorial properties of dry-cured products (Ruiz-Carrascal et al., 2000). The C18:1 content has been traditionally considered a key quality criterion in dry-cured products because of its positive role in the manufacturing process and in flavor (Toldrá, 2002). More recently, because of its associated benefits for human health (Christophersen & Haug, 2011; Jiménez-Colmenero et al., 2010), C18:1 has become an appreciated trait in some niche markets of fresh meat.

Both IMF and C18:1 are affected by dietary and genetic factors (De Smet et al., 2004). It is known that IMF, despite being unfavorably correlated with carcass lean content, can be efficiently selected (Suzuki et al., 2005a). However, there is little evidence on the opportunities for genetic change in fatty acid composition. Recent studies in this regard, although promising, were either based on small and heterogeneous data sets (Ntawubizi et al., 2010; Sellier et al., 2010) or, regarding C18:1, not conclusive (Casellas et al., 2010). Moreover, because the challenge for the industry is to develop selection criteria not only aimed at increasing C18:1 but at the whole profit of a line, the genetic correlation structure of C18:1 with other economic traits, particularly with lean growth, is needed. Therefore, the aims of this study were to (1) estimate the heritability of C18:1 and its genetic correlations with IMF and lean growth in pigs from a Duroc line primarily used for producing high quality dry-cured hams, and (2) discuss the opportunities for genetically improving C18:1 under different selection scenarios.

2.2. MATERIALS AND METHODS

2.2.1. Animals and sample collection

Data from a purebred Duroc line were used for the analyses (see Animals and Samples Section). The data set used for the estimation of genetic parameters consisted of 93,920 pedigree-connected pigs, from which 85,253 had at least one recorded trait. Pigs with records were born from 1996 to 2009. All pigs were performance-tested at an average age of 180 d for BW and BT. Since 2002, a sample of the purebred barrows used for producing dry-cured ham was taken for recording IMF and C18:1. These barrows were raised in twelve batches until slaughter at around 210 d. After chilling for approximately 24 h at 2°C, a sample of the *gluteus medius* muscle was taken from the left side ham and used for IMF and C18:1 determination, expressed as percentage of fresh matter and of total fatty acids, respectively. Complete details on the procedures

are given in the Animals and Samples Section. A summary of the population characteristics and number of records, sires, dams, and litters used for each analyzed trait in this analysis is given in Table 2.1.

Table 2.1. Description of the data set used in the analyses.

Item	No. of pigs	No. of sires	No. of dams	No. of litters	Mean	SD
Pedigree	93,920	731	18,516	32,315	-	-
Traits[1]						
BW at test, kg	85,002	641	16,548	32,211	104.8	12.5
BT at test, mm	80,687	642	16,335	31,197	15.6	3.5
IMF, %	943	141	543	546	4.9	1.9
C18:1, %	947	142	544	547	44.8	3.1
Covariates						
Age at test, d	85,194	642	16,601	32,310	180.2	10.7
Age at slaughter, d	2,098	298	1,313	1,370	206.5	14.6
Carcass weight, kg	937	142	542	545	98.4	11.6

[1] BW: body weight; BT: backfat thickness; IMF: intramuscular fat; C18:1: oleic acid.

2.2.2. Estimation of genetic parameters

Genetic parameters for BW, BT, IMF, and C18:1 were estimated fitting a 4-trait multivariate animal model. In matrix notation, the model was:

$$\mathbf{y}_i = \mathbf{X}_i\mathbf{b}_i + \mathbf{Z}_i\mathbf{a}_i + \mathbf{W}_i\mathbf{c}_i + \mathbf{e}_i ,$$

where $\mathbf{y}_i$ is the vector of observations for trait i (BW, BT, IMF, and C18:1); $\mathbf{b}_i$, $\mathbf{a}_i$, $\mathbf{c}_i$, and $\mathbf{e}_i$ are the vectors of systematic, additive genetic, litter, and residual effects, respectively; and $\mathbf{X}_i$, $\mathbf{Z}_i$, and $\mathbf{W}_i$ the known incidence matrices that relate $\mathbf{b}_i$, $\mathbf{a}_i$, and $\mathbf{c}_i$ with $\mathbf{y}_i$, respectively. Systematic effects for BW and BT were the batch (1,039 levels), gender (3 levels; males, females, and castrates), and age at measurement as a covariate. Pigs tested at the same time and in the same unit were considered as one batch. The same model was used for IMF and C18:1 but with systematic effects only including batch (12 levels) and age at measurement (or carcass weight). Because there were only 1.7 piglets/litter with records on IMF and C18:1, litter was dropped from the model for these two traits. Intramuscular fat content and C18:1 were analyzed using either the raw data or the following u_1 and u_2 isometric log-ratio (**ilr**) transformed variables (Egozcue et al., 2003):

$$u_1 = \frac{1}{\sqrt{6}} \ln \left[\frac{\mathrm{C18{:}1} \times (100 - \mathrm{C18{:}1}) \times \left(\frac{\mathrm{IMF}}{100}\right)^2}{(100 - \mathrm{IMF})^2} \right]$$

and

$$u_2 = \frac{1}{\sqrt{2}} \ln\left[\frac{C18:1}{(100 - C18:1)}\right],$$

where (100 – IMF) + [C18:1 + (100 – C18:1)] × IMF/100 = 100.

Genetic parameters were estimated in a Bayesian framework using Gibbs sampling with the TM software (Legarra et al., 2011). Observed phenotypes and missing records imputed by data augmentation were assumed to be conditionally normally distributed as follows:

$$\begin{bmatrix}\mathbf{y}_1\\ \mathbf{y}_2\\ \mathbf{y}_3\\ \mathbf{y}_4\end{bmatrix} | \mathbf{b}_1, \mathbf{b}_2, \mathbf{b}_3, \mathbf{b}_4, \mathbf{a}_1, \mathbf{a}_2, \mathbf{a}_3, \mathbf{a}_4, \mathbf{c}_1, \mathbf{c}_2, \mathbf{R} \sim N\left(\mathbf{X}\begin{bmatrix}\mathbf{b}_1\\ \mathbf{b}_2\\ \mathbf{b}_3\\ \mathbf{b}_4\end{bmatrix} + \mathbf{Z}\begin{bmatrix}\mathbf{a}_1\\ \mathbf{a}_2\\ \mathbf{a}_3\\ \mathbf{a}_4\end{bmatrix} + \mathbf{W}\begin{bmatrix}\mathbf{c}_1\\ \mathbf{c}_2\end{bmatrix}, \mathbf{R}\right),$$

where **R** was the (co)variance matrix. Sorting records by trait, and pig within trait, **R** could be written as $\mathbf{R}_0 \otimes \mathbf{I}$, with $\mathbf{R}_0$ being the 4 × 4 residual (co)variance matrix between the 4 traits analyzed and **I** an identity matrix of appropriate order. Flat priors were used for $\mathbf{b}_i$ and residual (co)variance components. Additive genetic and litter values, conditionally on the associated (co)variance components, were both assumed multivariate normally distributed with mean zero and with (co)variance $\mathbf{G} \otimes \mathbf{A}$ and $\mathbf{C} \otimes \mathbf{I}$, respectively, where **A** was the numerator relationship matrix, **G** was the 4 × 4 genetic relationship matrix between the 4 traits, and **C** was the 2 × 2 (co)variance matrix between litter effects of BW and BT. The matrix **A** was calculated using all the pedigree information summarized in Table 2.1. Flat priors were used for additive and litter (co)variance components. Statistical inferences were derived from the samples of the marginal posterior distribution using a unique chain of 2,000,000 iterations, where the first 250,000 were discarded and 1 sample out of 100 iterations was retained. Statistics of marginal posterior distributions and the convergence diagnostics were obtained using the *boa* package (Smith, 2005). Convergence was tested using the *Z*-criterion of Geweke (Geweke, 1992) and visual inspection of convergence plots.

2.2.3. Prediction of expected responses

The expected genetic response for C18:1 from a simulated breeding program was compared in two recording scenarios. In the first, it was assumed that records on C18:1 were not available and selection was only directed at either increasing BW (or IMF) or decreasing BT, while in the second, records on C18:1 were available and C18:1 was proactively selected. The selection objective in each case was derived as the linear combination of the appropriate breeding values weighted by their economic values. Economic weights were determined iteratively using a desired-gains approach

until the desired combination of genetic gains was achieved. For simplicity, only some illustrative cases in each scenario are presented. A population with discrete generations was simulated in which 40 boars were randomly mated to 400 sows with a mating ratio of 1 boar to 10 sows. The breeding scheme consisted of two selection stages resulting in the top 25% males and 50% females, with the same selection pressure in each stage. Two males and 2 females from the offspring of each sow were performance-tested at 180 d for BW and BT. In the second stage, 3 of the culled individuals per sire family were slaughtered to determine IMF, in the first scenario, and also C18:1, in the second. Pigs in the first stage were selected on the individual, full-sib, and half-sib phenotypic performance of BW and BT, and the pedigree information (best linear unbiased prediction, **BLUP**) of all recorded traits. Selection on the second stage was additionally based on the new half-sib records on IMF and, if available, C18:1. Only the first stage, but with the whole selection pressure, was applied in cases where neither IMF nor C18:1 were recorded. Selection response was predicted by deterministic simulation of a 2-stage selection scheme with discrete generations using the program SelAction (Rutten et al., 2002). The program accounts for reduction in variance due to selection (Bulmer, 1971) and corrects selection intensities for finite population size and for the correlation between index values of family members (Meuwissen, 1991).

2.3. RESULTS

2.3.1. Phenotypic values and environmental effects

The average phenotypic value of C18:1 in IMF was 44.8%, with an IMF content of 4.9% (Table 2.1). The effects of batch and age at slaughter on C18:1 are given in Table 2.2. On average, the variation among batches accounted for 2.4% of C18:1, with a maximum difference between batches of 7.5%. The effect of age at slaughter on

Table 2.2. Features of the posterior distribution of the effect of batch, age at slaughter, and carcass weight on oleic acid content (C18:1).

Parameter	Mean	SD	Mode	HPD95[1]	k^2
Batch					
Maximum difference	7.49	0.50	7.54	6.53, 8.49	6.65
Minimum difference	0.21	0.53	0.02	−1.24, 0.83	−1.12
SD among batch effects	2.35	0.10	2.34	2.14, 2.55	2.17
Covariates					
Age at slaughter, d	−0.02	0.02	−0.02	−0.05, 0.00	−0.05
Intramuscular fat, %	0.00	0.14	−0.02	−0.31, 0.26	−0.25
Carcass weight, kg	0.02	0.01	0.02	0.00, 0.05	0.00

[1] HPD95: highest posterior density interval at 95% of probability.
[2] k: limit for the interval $[k, +\infty)$ having a probability of 95%.

C18:1 was small but negative (–0.02%/d). There was not much evidence for the environmental effect of the IMF content on C18:1, with a mean value of 0 but showing a large highest density interval at 95% of probability (**HPD95**), ranging from –0.31 to 0.26%/percentage unit of IMF. The environmental effect of carcass weight was positive, with a mean value of 0.02%/kg, with a probability of 95% of being greater than 0.

2.3.2. Genetic parameters

Estimates of the variance components and heritabilities for BW, BT, IMF, and C18:1, together with the respective genetic and residual correlations among each other, can be seen in Table 2.3. Specific features concerning the posterior distribution of the heritability of C18:1 and the genetic and phenotypic correlations of C18:1 with BW, BT, and IMF are given in Table 2.4. The correlation between litter effects in BW and BT was 0.58 (SD 0.02). The heritability for C18:1 was 0.50 (SD 0.08) and similar to that for IMF (0.56, SD 0.09), with a probability of 95% of being greater than 0.37. The genetic and phenotypic correlations of C18:1 with IMF were moderate and positive, with a 95% probability of being greater than 0.27 and 0.29, respectively. The genetic and phenotypic correlations with BW and BT were also all positive, although lower, with values in the range of 0.11 to 0.22. Results did not provide conclusive evidence concerning the sign of the genetic correlation between C18:1 and BW, where the associated HPD95 ranged from –0.10 to 0.31. No substantial deviations in the estimates were observed after adjusting C18:1 for carcass weight or IMF content, or when the ilr-transformed variables u_1 and u_2 were used in the analyses instead of IMF and C18:1 (Table 2.5). Compared with the reference case, where C18:1 was only

Table 2.3. Posterior means (SD) of heritabilities (diagonal), genetic correlations (above diagonal), residual correlations (under diagonal), additive genetic variance (σ_a^2), litter variance (σ_c^2), and residual variance (σ_e^2) for body weight (BW), backfat thickness (BT), intramuscular fat content (IMF), and oleic acid content (C18:1).

Parameter	Trait			
	BW	BT	IMF	C18:1
Trait				
BW	**0.31** (0.01)	0.63 (0.02)	0.27 (0.10)	0.11 (0.11)
BT	0.60 (0.01)	**0.45** (0.01)	0.37 (0.10)	0.22 (0.10)
IMF	0.08 (0.07)	0.15 (0.08)	**0.56** (0.09)	0.47 (0.12)
C18:1	0.20 (0.07)	0.22 (0.08)	0.20 (0.12)	**0.50** (0.08)
Variance				
σ_a^2	29.75 (1.34)	4.11 (0.14)	1.85 (0.36)	2.22 (0.42)
σ_c^2	9.26 (0.37)	0.61 (0.03)	-	-
σ_e^2	57.25 (0.78)	4.45 (0.08)	1.41 (0.27)	2.22 (0.32)

Table 2.4. Features of the posterior distribution of the heritability of oleic acid content (C18:1) and the genetic and phenotypic correlations of C18:1 with body weight (BW), backfat thickness (BT), and intramuscular fat content (IMF).

Parameter	Mean	SD	Mode	HPD95[1]	k^2
Heritability	0.50	0.08	0.49	0.35, 0.65	0.37
Genetic correlations					
C18:1, BW	0.11	0.11	0.13	−0.10, 0.31	−0.07
C18:1, BT	0.22	0.10	0.22	0.01, 0.42	0.04
C18:1, IMF	0.47	0.12	0.51	0.24, 0.71	0.27
Phenotypic correlations					
C18:1, BW	0.15	0.03	0.15	0.09, 0.21	0.10
C18:1, BT	0.21	0.03	0.21	0.15, 0.27	0.16
C18:1, IMF	0.35	0.03	0.35	0.28, 0.41	0.29

[1] HPD95: highest posterior density interval at 95% of probability.
[2] k: limit for the interval $[k, +\infty)$ having a probability of 95%.

Table 2.5. Posterior means (SD) of heritability of oleic acid content (C18:1) and the genetic correlations of C18:1 with body weight (BW), backfat thickness (BT), and intramuscular fat content (IMF) under alternative models for C18:1.

Parameter	Covariate[1]				ilr[2]
	Age	Age+IMF	CW	CW+IMF	
Heritability	0.50 (0.08)	0.51 (0.08)	0.53 (0.09)	0.55 (0.07)	0.49 (0.08)
Genetic correlation					
C18:1, BW	0.11 (0.11)	0.15 (0.11)	0.02 (0.11)	0.02 (0.12)	0.12 (0.12)
C18:1, BT	0.22 (0.10)	0.21 (0.11)	0.16 (0.11)	0.16 (0.12)	0.18 (0.11)
C18:1, IMF	0.47 (0.12)	0.45 (0.14)	0.42 (0.13)	0.46 (0.13)	0.48 (0.13)

[1] C18:1 was adjusted for age at slaughter (Age), age at slaughter plus IMF (Age+IMF), carcass weight (CW), or carcass weight plus IMF (CW+IMF).
[2] The isometric log-ratio (ilr) transformed variables u_1 and u_2 were used instead of IMF and C18:1, respectively.

adjusted for age at slaughter, the estimates of the heritability of C18:1 after alternatively adjusting C18:1 for carcass weight, age plus IMF content, or carcass weight plus IMF content were only slightly greater with a maximum value of 0.55. Similar values were obtained for the differently adjusted genetic correlations of C18:1 with BW, BT, and IMF, except for the correlation between C18:1 adjusted for carcass weight and BW, where, as expected, values decreased to almost zero. When the ilr-transformed variables were used, the maximum change occurred for the genetic correlation between C18:1 and BT, which decreased from 0.22 to 0.18. Because only minor changes were seen across models and data transformation, responses below were calculated using the estimates in Table 2.3.

2.3.3. Expected responses

Indirect expected responses in C18:1 to selection for BW, BT, or IMF are given in Table 2.6. In the first scenario, where records on IMF are not available, at best no change in C18:1 is expected. In most sire lines, the breeding goal is directed at increasing lean growth. According to the emphasis put on each of the two components of the trait, the selection objective in these lines can be placed in-between maximizing BW at restrained BT, in one extreme, and minimizing BT at restrained BW, in the other. Thus, within this scenario, the best situation occurs when selection is for BW at restrained BT, in which case only little changes in C18:1 are expected. However, as selection against BT is emphasized, C18:1 decreases by up to 0.2% per generation when BW is constrained to remain unchanged. This decrease in C18:1 can be minimized if records on IMF are available. Thus, in this new scenario, if IMF is also restrained, the decrease in C18:1 is reduced 3-fold. Moreover, if IMF is proactively selected, there is room for favorable responses in C18:1. Increasing IMF at restrained BW and BT led to similar but opposite response in C18:1 than decreasing BT at restrained BW. Response in C18:1 can be further improved if it is directly selected (Figure 2.1). There are selection scenarios leading to favorable responses in all traits; for instance, 1 kg in BW, –0.25 mm in BT, 0.06% in IMF, and 0.25% in C18:1. Maximum expected response in C18:1 at no lean growth loss (i.e., at no change in BW and BT) is 0.40%, with a resulting correlated response in IMF of 0.15%. Increasing the emphasis on BW and against BT constricts the response curves.

Table 2.6. Indirect response per generation in oleic acid content (C18:1) to restricted selection for body weight (BW), backfat thickness (BT), or intramuscular fat content (IMF) by availability of IMF records.

Recorded traits	Objective[1]	Restriction	Expected response			
			BW, kg	BT, mm	IMF, %	C18:1, %
BW, BT						
	Max BW	$\Delta BT = 0$	+2.28	0.00	+0.03	−0.03
	Min BT	$\Delta BW = 0$	0.00	−0.93	−0.20	−0.17
BW, BT, IMF						
	Max BW	$\Delta BT = \Delta IMF = 0$	+2.26	0.00	0.00	−0.05
	Min BT	$\Delta BW = \Delta IMF = 0$	0.00	−0.80	0.00	−0.06
	Max IMF	$\Delta BW = \Delta BT = 0$	0.00	0.00	+0.35	+0.17

[1] Trait to maximize (Max) or minimize (Min).

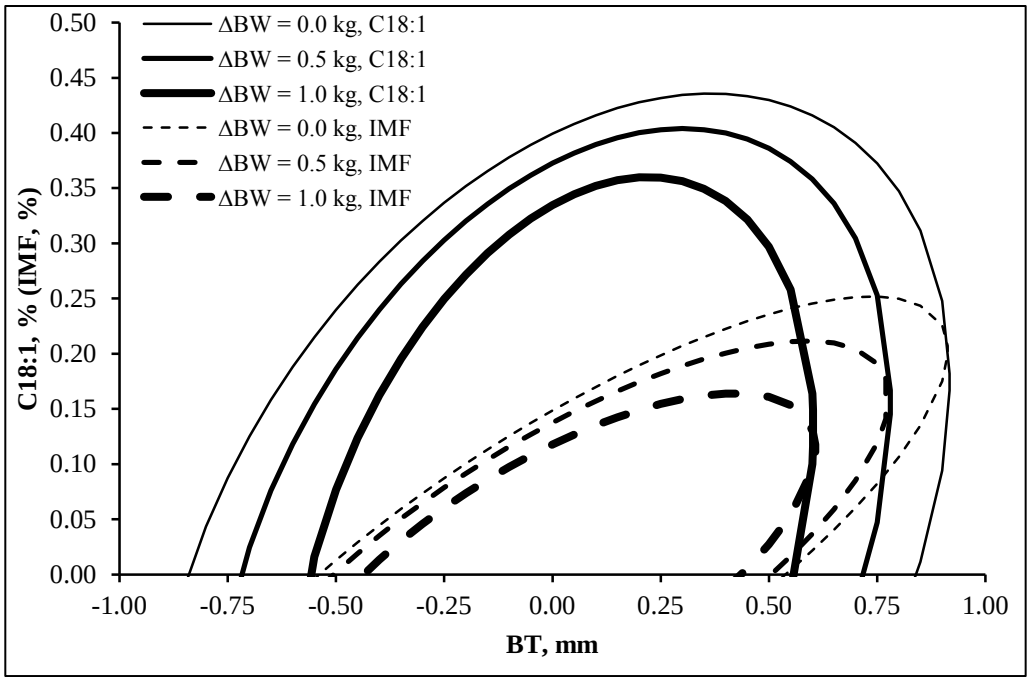


Figure 2.1. Maximum expected response for oleic acid (C18:1) (and correlated response for intramuscular fat content, IMF) at differing backfat thickness (BT) and fixed body weight (BW) responses (0, 0.5, and 1 kg). Responses are given per generation.

2.4. DISCUSSION

Results obtained provide strong evidence that C18:1 content in IMF is genetically determined. The estimate of the heritability of C18:1, with a value around 0.50, is in line with that obtained by Ntawubizi et al. (2010) with crossbred pigs but greater than other estimates, which were in the range of 0.26 (Sellier et al., 2010), in Landrace and Large White, to 0.36 (Suzuki et al., 2006), in Duroc. In the present study, as in Suzuki et al. (2006), inferences were based on a Duroc line with known selection trajectory, but using a bigger data set and a more representative family structure across generations. Similar values have been reported for the heritability of C18:1 in backfat of Duroc pigs, with values ranging from 0.26 (Suzuki et al., 2006) to 0.57 (Gjerlaug-Enger et al., 2011). Estimates obtained in other breeds for the heritability of C18:1 in subcutaneous fat showed a similar trend, with values from 0.30 in Iberian pigs (Fernández et al., 2003) to as great as 0.67 in Landrace (Gjerlaug-Enger et al., 2011).

The high value for the heritability of C18:1 is maintained even when adjusted for IMF, showing a negligible probability of being less than 0.28. This finding removes the concerns raised by Casellas et al. (2010) about the genetic determinism of C18:1 at fixed IMF. However, this result contrasts with the dramatic reduction, from 0.58 to

0.18, observed by Ntawubizi et al. (2010) for the heritability of C18:1 after adjusting for IMF. These later authors suggested that this might be due to the low IMF content showed by their experimental crossbred pigs (1.2%), a situation where small variations in IMF, mostly dominated by changes in polyunsaturated fatty acids, may have a great impact. Our results, which were obtained in a population displaying 4-fold greater IMF than theirs, would support this hypothesis. However, note that here, because estimates are based on a 4-trait analysis, with IMF being one of the traits, and not on a series of univariate analyses, the genetic effect is subtracted from IMF when acting as a covariate, then giving as a result a lesser effect of IMF on C18:1. In fact, the effect of IMF on C18:1 was much greater in a 3-trait analysis excluding IMF (0.33, SD 0.04) than in the full 4-trait analysis (0.01, SD 0.14). The heritability of C18:1 in the 3-trait analysis was lower (0.45, SD 0.08) but still conclusive with respect to the genetic determination of C18:1. Taken as a whole, the results indicate that C18:1 displays a moderate-to-high heritability and suggest that there is potential for improving C18:1 in IMF by selection.

Selection responses in C18:1 should be put into context with the correlated genetic change in other economic traits. In this study, C18:1 showed a favorable and moderately high genetic correlation with IMF, in accordance with the observed trend of fatty acid composition with IMF in this line (Bosch et al., 2012), but much greater than that reported by Suzuki et al. (2006), the only other study that examined the genetic relationship between C18:1 and IMF, which was 0.10. Although positive and low, there is less evidence on the magnitude of the genetic correlations of C18:1 with BW and BT, particularly for BW, where negative values cannot be discarded completely. Reported estimates for the correlation between C18:1 and BW are more consistent with the values encountered here than those for the correlation between C18:1 and BT (Suzuki et al., 2006; Ntawubizi et al., 2010). Suzuki et al. (2006) observed that C18:1 and BT are almost uncorrelated, but Ntawubizi et al. (2010) found that they are positively correlated (−0.75 with carcass lean meat content). Because genetic correlation among C18:1 at different fat depots is approximately 0.7 (Suzuki et al., 2006), complementary information can be retrieved from results on C18:1 in fat tissues other than IMF. Results for backfat C18:1 give a similar contradictory picture; some authors (Cameron, 1990; Fernández et al., 2003) found that C18:1 and BT are hardly correlated (around 0.10), and others reported that they are unfavorably related (Gjerlaug-Enger et al., 2011). Intramuscular fat content showed a similar genetic correlation structure with BW and BT as C18:1, in agreement with previous results in the same Duroc population (Solanes et al., 2009).

Discrepancies in the above estimates may arise because of differences in the age or weight at test and in the muscle where IMF and C18:1 were measured. In the present study, pigs were tested for BW and BT at 180 d, and IMF and C18:1 were determined

analytically in the *gluteus medius* muscle at 210 d. Results in Solanes et al. (2009) showed that the genetic correlation of IMF with BW and BT, both traits measured at 180 d, were greater than those found here for IMF at 210 d. This could indicate that the genetic relationship between performance traits, particularly for BW and IMF-related traits, including C18:1, decreases as age increases. In fact, in heavy Iberian pigs, Fernández et al. (2007) found that the correlation between BW and IMF was negative. This might be interpreted in light of the fact that C18:1 evolved linearly with age throughout the period studied, whereas BW and BT did not (Bosch et al., 2012). The muscle and the determination method of IMF may also influence the relationship among fat depots. Here C18:1 was measured in the *gluteus medius* muscle instead of the *longissimus*, as in most reported estimates, because sampling from *gluteus medius* is easier and cheaper, compared with *longissimus*. Muscles behave differently in terms of both IMF content and composition and, because *gluteus medius* is fatter than *longissimus* at a given age (Casellas et al., 2010), IMF in *gluteus medius* may be more correlated to overall fatness (Solanes et al., 2009). Variations in age, slaughter weight, and IMF content are commonly adjusted including a covariate in the model describing the data. The magnitude of these covariates for C18:1 in the 4-trait analysis was very small, and therefore inferences concerning C18:1 did not relevantly change across models. Major differences occurred when adjusting for carcass weight, likely because, in this case, the covariate is capturing part of the deviations between BW at 180 d and carcass weight at 210 d. Similarly, no relevant changes in the estimates of the genetic parameters were observed after the ilr transformation of IMF and C18:1. Note that both IMF and C18:1 are compositional data in nature (Aitchison, 1986), so conceptually they cannot be used in real space unless they are previously transformed (Egozcue et al., 2003). However, Estany et al. (2011), using real and simulated data, have already shown that, in regard to IMF and C18:1, transformed values only performed a little better when predicting future records of IMF.

Data on fatty acid composition have often been obtained from experiments designed for other purposes or from culled pigs, and, therefore, they are not necessarily randomly chosen. In such cases, data may be subjected to selective recording and inferences on genetic parameters may be biased. However, if the history of the selection process is contained in the data used in the analysis, the posterior distribution has the same mathematical form with or without selection (Gianola & Fernando, 1986). In this study, pigs in which IMF and C18:1 were determined were chosen exclusively on the BLUP of the breeding values of BW and BT from the pedigree and records used in the present analysis. All estimates shown here were derived under this principle, and they were implicitly adjusted for selective recording. Inferences obtained using only data from pigs with records on C18:1, although they did not affect the estimate of the heritability of C18:1, underestimated the genetic correlations of C18:1 and IMF with BW and BT, even suggesting a negative genetic relationship of BW with IMF and

C18:1 (results not shown). Including all data in the analysis removed the effect of selection and revealed the risks of estimating genetic parameters, particularly correlations, using data recorded for other purposes.

Expected responses suggest that breeding programs directed at increasing C18:1 are feasible but also that this genetic progress is achieved at the expense of decreasing lean content. In many instances, the correlated change in C18:1 to selection for production traits is likely more important than the execution of direct selection. In this scenario, our results show that selection for lean growth will not lead to favorable changes in C18:1, which will only be indirectly improved in breeding regimens selecting proactively for IMF. Some experiments have already demonstrated that it is possible to increase IMF through selection (Suzuki et al., 2005a; Schwab et al., 2009). The low expected responses in C18:1 and IMF to selection for BW at restrained BT indicate that, if selection gives a great emphasis on growth rate, little changes in both IMF and C18:1 should be expected. This result is consistent with experimental evidence indicating that continuous selection for lean growth did not necessarily lead to decreased IMF (Oksbjerg et al., 2000; Tribout et al., 2004).

Direct selection for C18:1 allows for convenient scenarios in which C18:1, IMF, BW, and BT can be simultaneously improved. A desired-gain approach was used to determine the weights for traits in the breeding objective. This is a useful approach for traits not yet included in the payment system or subjected to restrictions, as established in some labeled products. In fact, restricted values on fatty acids are a common feature in regulations for foods bearing nutritional or health claims concerning fat properties and, for example, when minimum C18:1 and maximum palmitic, stearic, and linoleic acid contents are required in grading Iberian cured products. However, proper economic weights are needed to achieve the optimum response profile in each situation. It has been proposed to use interviews with experts or market surveys as input for developing a pricing system based on a quantitative differentiation of willingness-to-pay values for carcasses of different qualities (von Rohr et al., 1999). The method has been used in the Swiss breeding program for calculating the economic value of fat quality, indirectly measured as the amount of double bonds in fatty acids in the outer layer of backfat (Hofer et al., 2006). To our knowledge, this is so far the only published attempt to select for fat composition in pigs, although no realized responses have been reported yet. A similar approach can be used to elucidate the economic value of traits, such as C18:1, reflecting possible future trends in the pork market.

Selection for C18:1 leads to an undesired correlated response in BT (i.e., lean content) and to genetic lag in BW (i.e., average daily gain). Then, for a given scenario, the opportunity cost of selecting for increased C18:1 can be derived by subtracting the total economic response weight in the adopted scenario from the maximum total economic response. Alternatively, in case of being negative, this difference can also be

interpreted as an estimation of the societal benefits of selecting for healthiness (Kanis et al., 2005). Other economic traits not included in the present analysis may also show undesired responses. There have not been reported estimates of the genetic correlation of C18:1 in IMF with feed conversion ratio, proportion of premium cuts, or prolificacy. However, results relating to C18:1 (Fernández et al., 2003) and linoleic acid (Hofer et al., 2006) in backfat lead to expected unfavorable correlated responses in both feed conversion ratio and proportion of premium cuts, although not to premium pieces weight. By contrast, in accordance with Solanes et al. (2009), who found that IMF was uncorrelated to prolificacy, no relevant genetic change in prolificacy is expected after selection for C18:1.

Genetic differences between individuals for C18:1 in IMF may come from differential ability of pigs either to incorporate dietary C18:1 to IMF or to synthesize C18:1 from palmitic and stearic acids via increased enzymatic activity of elongases and Δ^9 desaturases, respectively. Cánovas et al. (2009) found that selection for decreased BT at restrained IMF led to decreased expression of both enzymes in backfat but not in IMF, giving support to the hypothesis that the metabolic pathways underlying the synthesis of C18:1 are altered by selection. From a practical view, however, the question whether selection for increased C18:1 content is affordable must be contrasted with the cost/benefit ratio of alternative strategies. Diet and age at slaughter, which partly explain the variation among batches for C18:1, are the two most used practices to improve both IMF content and composition. However, experimental results indicate that the impact of dietary fatty acid additions mainly affects subcutaneous fat and polyunsaturated fatty acids rather than IMF and monounsaturated fatty acids (Wood et al., 2008). Even though feeding pigs high-oleic acid diets may increase C18:1 in IMF by up to 3% (Mas et al., 2010), this approach has not always been successful (Mas et al., 2011). In general, major changes in C18:1 are achieved indirectly by raising IMF content. Teye et al. (2006), using a low protein diet, and Bosch et al (2012), delaying the age at slaughter, two management practices aimed at improving IMF, increased C18:1 by values in the range of 4 to 7%. However, our data indicate that, on average, batch differences only accounted for around 2% of C18:1, approximately the expected genetic change that would be achieved after 5 generations of selection.

A limitation for implementing direct selection for C18:1 is that phenotypes cannot be observed on the selection candidates themselves and are costly to determine. It is difficult to measure C18:1 in live animals unless biopsies (Bosch et al., 2009) or genetic markers (Estellé et al., 2009) are used. However, the first approach is mostly restricted to experimental designs, and the second has not yet been able to translate advances into effective commercial improvements (Dekkers, 2004). The use of increasingly accurate on-line equipment, such as that based on near-infrared spectroscopy (Gjerlaug-Enger et al., 2011; Shackelford et al., 2011), represents an

opportunity for systematic recording of C18:1 on the slaughter chain. Due to greater measurement errors, lower heritability values may be expected using such records in relation to analytical methods (Fernández et al., 2003). However, the estimate of the heritability of IMF obtained here is consistent with a previous estimate obtained in the same Duroc population, but using data taken with a near-infrared transmittance spectrometry device (Solanes et al., 2009). Accordingly, no relevant changes should be expected by using on-line measurement technologies. Other direct alternative methods specifically for determining C18:1 content have also been proposed (Muñoz et al., 2011).

2.5. CONCLUSIONS

Two questions were addressed in this study. (1) Is there genetic variation in C18:1 content in IMF? (2) Which response scenarios are expected for indirect and direct selection? We concluded that selection for C18:1 content in IMF can be effective and that there are selection strategies leading to response scenarios in which C18:1, IMF, BT, and BW can be simultaneously improved. However, if adopted, a reduction in the potential for lean growth is also expected. The extent to which it is affordable relies on how much consumers are prepared to pay for high-oleic pork products.

Chapter 3.
Genetic correlations of intramuscular fat content and fatty acid composition among muscles and with subcutaneous fat in Duroc pigs

R. Ros-Freixedes[1], J. Reixach[2], L. Bosch[3], M. Tor[1], & J. Estany[1]

[1]Departament de Producció Animal, Universitat de Lleida − Agrotecnio Center, 191 Av. Alcalde Rovira Roure, 25198 Lleida, Catalonia, Spain.
[2]Selección Batallé S.A., Av. Segadors s/n, 17421 Riudarenes, Catalonia, Spain.
[3]Departament d'Enginyeria Química, Agrària i Tecnologia Agroalimentària, Universitat de Girona, Campus de Montilivi, 17071 Girona, Catalonia, Spain.

The content of this chapter has been accepted for publication in Journal of Animal Science (2014), 92: 5417−5425. DOI: 10.2527/jas.2014-8202

ABSTRACT: There is an increasing interest in including intramuscular fat (IMF) content and fatty acid composition, particularly oleic acid (C18:1) content, in the selection objectives of pig lines for quality pork markets. These traits are costly and can be measured in more than one location, so knowing their correlation structure across muscles and with subcutaneous fat (SF) is necessary for developing optimum sampling and recording schemes. We analyzed the genetic and phenotypic correlations of IMF content and composition among three of the most relevant muscles (GM: *gluteus medius*; LD: *longissimus dorsi*; and SM: *semimembranosus*) and with the fatty acid composition of SF. All genetic correlations were positive but variable. For IMF, the genetic correlation between GM and LD was 0.68 and, for fatty acids, ranged from 0.62, for C18:1, to 0.82, for total polyunsaturated fatty acids. Genetic correlations of GM and LD with SM were much lower: 0.13−0.19, for IMF, and 0.10−0.54, for fatty acids. Correlations for fatty acid composition in SF were moderate to high with GM and LD (0.29−0.53 and 0.43−0.75, respectively), but were null with SM. The expected responses for IMF in the three muscles and for C18:1 in each muscle and in SF to selection on records taken from only a single muscle or SF were estimated. Selection for IMF and C18:1 in GM is expected to lead to positive responses in IMF and C18:1 in LD and vice versa, although this can entail genetic lags of 20−45% in the muscle not directly selected for. Selection for C18:1 in SF is more effective for C18:1 in LD than in GM and of very limited value for IMF. In conclusion, the genetic correlations of IMF content and fatty acid composition among muscles and with SF, although positive, are variable enough to influence the genetic evaluation scheme for IMF and fat quality. They also indicate that GM and LD can be used alternatively for selection purposes.

3.1. INTRODUCTION

Intramuscular fat (**IMF**) content and fatty acid composition affect both the organoleptic and nutritional properties of pork and its derivatives (Wood et al., 2003). Particularly, oleic acid (**C18:1**) content has become an appreciated trait in dry-cured products and in some niche markets of fresh meat because of its association with flavor, technological properties, and health benefits (Toldrá, 2002; Christophersen & Haug, 2011; Jiménez-Colmenero et al., 2010). The strong economic importance of dry-cured ham in the Mediterranean area, where hams containing higher levels of C18:1 are premium-paid, together with the increased demand of healthy sources of meat, has triggered the interest of including IMF and fatty acid composition in the breeding goal of the pig lines producing for those markets. Because these traits are difficult and costly to measure, their genetic evaluation is usually based on indirect assessments (Jeremiah, 1998; Newcom et al., 2002, 2005) or on a limited number of records taken either on a single muscle (Chapter 2; Ntawubizi et al., 2010) or from the subcutaneous fat (**SF**) (Fernández et al., 2003; Hofer et al., 2006; Gjerlaug-Enger et al., 2011). However, it is known that the pattern of fatty acid deposition may differ between IMF and SF (Duran-Montgé et al., 2008; Sellier et al., 2010; Bosch et al., 2012), across muscles (Sharma et al., 1987; Leseigneur-Meynier & Gandemer, 1991; Kim et al., 2008), and even among locations within a specific tissue (Sharma et al., 1987; Faucitano et al., 2004; Franco et al., 2006). Therefore, to develop adequate recording and genetic evaluation schemes for IMF and fatty acid composition traits, there is a need to know the correlation structure of these traits across valuable muscles and with SF. The objective of this study is to estimate the genetic correlation of IMF and fatty acids content across three economically relevant muscles (the loin and two muscles from the ham) and with SF. The expected response for IMF and C18:1 in each muscle and SF to selection on records from only one of them is assessed.

3.2. MATERIALS AND METHODS

3.2.1. Animals and sample collection

Data from a purebred Duroc line were used for the analyses (see Animals and Samples Section). The data set used for the estimation of genetic parameters consisted of 111,305 pedigree-connected pigs, from which 102,915 had at least one recorded trait. Pigs with records were born from 1996 to 2011. All pigs were performance-tested at an average age of 180 d for body weight (**BW**) and backfat thickness (**BT**). Since 2002, 1,204 of the purebred barrows used for producing dry-cured ham were taken for recording IMF and C18:1. These barrows were raised in 15 batches until slaughter at around 210 d of age. At the end of the finishing period, all barrows were slaughtered in the same commercial slaughterhouse at ~125 kg of BW. Immediately after slaughter, a

sample of SF (n=333) and muscle *semimembranosus* (**SM**, n=198) was collected. After chilling for about 24 h at 2ºC, samples of muscles *gluteus medius* (**GM**, n=1,204) from the left side ham and *longissimus dorsi* at the level of the third and fourth ribs (**LD**, n=318) were also collected. Samples of SF were collected at the same location than either the LD (n=203) or the GM (n=130) samples. Samples were used for determination of IMF (expressed as percentage of fresh matter), individual C18:1, and total saturated (**SFA**), monounsaturated (**MUFA**), and polyunsaturated (**PUFA**) fatty acids content (expressed as percentage of total fatty acids). Complete details on the procedures are given in the Animals and Samples Section. A summary of the population characteristics and number of records, sires, dams, and litters used for each analyzed trait is given in Table 3.1.

Table 3.1. Description of the data set used in the analyses.

Item	No. of pigs	No. of sires	No. of dams	No. of litters	Mean	SD
Pedigree	111,305	830	22,634	40,658	-	-
Traits[1]						
BW at test, kg	102,325	747	20,722	39,594	104.8	12.3
BT at test, mm	98,397	748	20,582	38,724	15.6	3.5
Muscle *gluteus medius*						
IMF, %	1,200	169	678	681	4.8	1.9
C18:1, %	1,204	171	680	683	44.9	2.9
SFA, %	1,204	171	680	683	36.3	3.5
MUFA, %	1,204	171	680	683	49.4	3.1
PUFA, %	1,204	171	680	683	14.2	2.6
Muscle *longissimus dorsi*						
IMF, %	318	90	264	264	3.5	1.2
C18:1, %	318	90	264	264	45.8	2.7
SFA, %	318	90	264	264	38.0	3.4
MUFA, %	318	90	264	264	50.5	2.6
PUFA, %	318	90	264	264	11.6	2.5
Muscle *semimembranosus*						
IMF, %	146	59	138	138	2.7	1.7
C18:1, %	196	69	170	170	44.3	5.0
SFA, %	196	69	170	170	34.3	4.8
MUFA, %	196	69	170	170	48.3	5.3
PUFA, %	196	69	170	170	17.4	4.4
Subcutaneous fat						
C18:1, %	333	130	281	281	44.1	3.7
SFA, %	333	130	281	281	34.2	5.3
MUFA, %	333	130	281	281	47.3	4.0
PUFA, %	333	130	281	281	18.4	2.4
Covariates						
Age at test, d	102,915	748	20,848	39,837	179.3	10.6
Age at slaughter, d	4,317	392	2,480	2,633	207.2	16.1

[1] BW: body weight; BT: backfat thickness; IMF: intramuscular fat; C18:1: oleic acid; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

3.2.2. Estimation of genetic parameters

Genetic parameters for IMF and fatty acids content in GM, LD, SM, and SF were estimated fitting 4-trait multivariate models, where BW and BT were the two first traits and IMF or C18:1 in two different tissues the other two. In matrix notation, the model was:

$$\mathbf{y}_i = \mathbf{X}_i\mathbf{b}_i + \mathbf{Z}_i\mathbf{a}_i + \mathbf{W}_i\mathbf{c}_i + \mathbf{e}_i\,,$$

where $\mathbf{y}_i$ is the vector of observations for trait i; $\mathbf{b}_i$, $\mathbf{a}_i$, $\mathbf{c}_i$, and $\mathbf{e}_i$ are the vectors of systematic, additive genetic, litter, and residual effects, respectively; and $\mathbf{X}_i$, $\mathbf{Z}_i$, and $\mathbf{W}_i$ the known incidence matrices that relate $\mathbf{b}_i$, $\mathbf{a}_i$, and $\mathbf{c}_i$ with $\mathbf{y}_i$, respectively. Systematic effects for BW and BT were the batch (1,226 levels), gender (3 levels; males, females, and castrates), and age at measurement as a covariate. The model for IMF and fatty acids content only included the batch (15 levels) and age at measurement. Because there were only 1.2 piglets/litter with records on IMF and fatty acids content in LD, SM, and SF, litter was dropped from the model for these two traits. Genetic correlations between IMF and C18:1 in different tissues were estimated fitting 6-trait (or 5-trait) multivariate models including, besides BW and BT, IMF and C18:1 in two different tissues (only IMF in one muscle if the other tissue was SF). The genetic parameters were estimated in a Bayesian framework using Gibbs sampling with the TM software (Legarra et al., 2011). Observed phenotypes and missing records imputed by data augmentation were assumed to be conditionally normally distributed as follows:

$$\begin{bmatrix}\mathbf{y}_1\\ \mathbf{y}_2\\ \ldots\\ \mathbf{y}_n\end{bmatrix} \mid \mathbf{b}_1,\mathbf{b}_2,\ldots,\mathbf{b}_n,\mathbf{a}_1,\mathbf{a}_2,\ldots,\mathbf{a}_n,\mathbf{c}_1,\mathbf{c}_2,\mathbf{R} \sim \mathrm{N}\left(\mathbf{X}\begin{bmatrix}\mathbf{b}_1\\ \mathbf{b}_2\\ \ldots\\ \mathbf{b}_n\end{bmatrix} + \mathbf{Z}\begin{bmatrix}\mathbf{a}_1\\ \mathbf{a}_2\\ \ldots\\ \mathbf{a}_n\end{bmatrix} + \mathbf{W}\begin{bmatrix}\mathbf{c}_1\\ \mathbf{c}_2\end{bmatrix}, \mathbf{R}\right),$$

where $\mathbf{R}$ was the (co)variance matrix. Sorting records by trait, and pig within trait, $\mathbf{R}$ could be written as $\mathbf{R_0} \otimes \mathbf{I}$, with $\mathbf{R_0}$ being the $n \times n$ residual (co)variance matrix between the n traits analyzed and $\mathbf{I}$ an identity matrix of appropriate order. Flat priors were used for $\mathbf{b}_i$ and residual (co)variance components. Additive genetic and litter values, conditional on the associated (co)variance components, were both assumed multivariate normally distributed with mean zero and with (co)variance $\mathbf{G} \otimes \mathbf{A}$ and $\mathbf{C} \otimes \mathbf{I}$, respectively, where $\mathbf{A}$ was the numerator relationship matrix, $\mathbf{G}$ was the $n \times n$ genetic relationship matrix between the n traits, and $\mathbf{C}$ was the 2×2 (co)variance matrix between litter effects of BW and BT. The matrix $\mathbf{A}$ was calculated using all the pedigree information. Flat priors were used for additive and litter (co)variance components. Statistical inferences (means and highest posterior density intervals at 95% of probability (**HPD95**)) were derived from the samples of the marginal posterior distribution using a unique chain of 1,000,000 iterations, where the first 500,000 were

discarded and one sample out of 100 iterations retained. Statistics of marginal posterior distributions and the convergence diagnostics were obtained using the *boa* package (Smith, 2005). Convergence was tested using the *Z*-criterion of Geweke (Geweke, 1992) and visual inspection of convergence plots.

3.2.3. Prediction of expected responses

The expected genetic responses for IMF and C18:1 were evaluated in a simulated breeding program based on records on either IMF or C18:1, or both simultaneously, taken from a particular tissue. For a given scenario, we assumed that only records from one of the tissues were available. Intramuscular fat and C18:1 were assumed to have the same economic weight when both traits were included in the selection objective. The simulated breeding program was a simplified version of that described in Chapter 2. A population of 40 boars and 400 sows randomly mated was maintained on discrete generations. We assumed that 3 individuals per sire family were slaughtered to determine IMF or C18:1 or both. In each generation 25% of males and 50% of females were selected based on three half-sib plus pedigree records. Selection response was predicted deterministically by using the program SelAction (Rutten et al., 2002). The program accounts for reduction in variance due to selection (Bulmer, 1971) and corrects selection intensities for finite population size and for the correlation between index values of family members (Meuwissen, 1991).

3.3. RESULTS

The posterior mean of the genetic variance and the posterior mean and HPD95 of the heritability of IMF in GM, LD, and SM, as well as of the genetic correlations among them and with BT, are shown in Table 3.2. The corresponding posterior means and HPD95 for fatty acid composition in GM, LD, SM, and SF are given in Table 3.3. The heritability of IMF in the three muscles was high, particularly for LD. Although they had wide HPD95 (due to the low number of pigs with data on these traits), all of them showed 95% probability of being greater than 0.30. The heritabilities of C18:1, SFA, MUFA, and PUFA in the three muscles were of similar magnitude than those for IMF. In GM and LD, the heritability estimates for SFA were the lowest and those for PUFA the highest. The heritabilities estimated in SF tended to be lower than those estimated in the muscles for all fatty acids. The genetic variance of fatty acids was much higher in SM than in GM, LD, and SF.

The genetic correlation between IMF in GM and LD was high (0.68), but it decreased to ~0.15 for that between them and SM. Unlike for GM and LD, the HPD95 for the genetic correlation between IMF in SM and IMF in GM and LD included null

and negative values, thereby indicating very little evidence of correlation between them. Similarly, BT was positively correlated to IMF in GM and LD (~0.40), but uncorrelated to IMF in SM. The phenotypic correlations showed the same trends, but lower in magnitude than the genetic correlations. For all fatty acid traits, the highest genetic correlations were also found between GM and LD (0.62 to 0.82). The genetic correlations of GM and LD with SM were also positive but more moderate (0.29 to 0.44 and 0.10 to 0.54, respectively). However, the genetic correlations of LD with SF were consistently higher (0.43 to 0.75) than those of GM with SF (0.29 to 0.53). No evidence of genetic correlation between SM and SF was found.

The genetic parameters for C18:1 adjusted for IMF are shown in Table 3.4. Adjusted estimates did not relevantly differ from the unadjusted estimates reported in Table 3.2. Including IMF of the involved muscles as covariates only slightly decreased the correlations among muscles, although increased those between muscles and SF. Including IMF as additional traits in the multivariate model did not have any systematic effect on the genetic parameters.

The posterior mean and HPD95 of the genetic correlations of C18:1 in GM, LD, SM, and SF with IMF in the three muscles are given in Table 3.5. The IMF content of of GM and LD were moderately correlated with the C18:1 content in the same muscles (0.47−0.52), except for IMF in GM with C18:1 in LD (0.24). The genetic correlations between C18:1 and IMF were much lower when SM was involved (ranging from 0.14 to 0.37), although C18:1 and IMF in SM were highly correlated (0.69). The IMF content in any of the three muscles was uncorrelated with C18:1 in SF.

Table 3.2. Genetic variance, heritability (diagonal, in bold), genetic correlations (above diagonal), and phenotypic correlations (below diagonal) for intramuscular fat content (IMF) in three muscles and backfat thickness (BT).

Trait	Genetic variance	Genetic parameters[1]			
		IMF[2]			BT
		GM	LD	SM	
IMF, %					
GM	1.66	**0.51**	0.68	0.16	0.42
		(0.38, 0.65)	(0.48, 0.87)	(−0.25, 0.56)	(0.24, 0.59)
LD	0.76	0.47	**0.64**	0.13	0.40
		(0.38, 0.56)	**(0.44, 0.83)**	(−0.15, 0.42)	(0.14, 0.66)
SM	1.47	0.15	0.21	**0.53**	−0.09
		(−0.04, 0.33)	(0.02, 0.39)	**(0.30, 0.72)**	(−0.53, 0.30)
BT, mm	4.35	0.29	0.32	0.04	**0.48**
		(0.24, 0.34)	(0.23, 0.42)	(−0.12, 0.22)	**(0.46, 0.50)**

[1] Mean of the posterior density and, in parentheses, highest posterior density interval at 95% of probability.
[2] GM: *gluteus medius*; LD: *longissimus dorsi*; SM: *semimembranosus*.

Table 3.3. Genetic variance, heritability (diagonal, in bold), genetic correlations (above diagonal), and phenotypic correlations (below diagonal) for oleic (C18:1), saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acid content in three muscles and subcutaneous fat (SF).

Fatty acid	Genetic variance	Genetic parameters[1]			
		Muscle[2]			SF
		GM	LD	SM	
C18:1, %					
GM	1.92	**0.44**	0.62	0.40	0.29
		(0.32, 0.59)	(0.41, 0.80)	(0.14, 0.65)	(−0.06, 0.72)
LD	2.13	0.54	**0.59**	0.30	0.52
		(0.46, 0.62)	**(0.41, 0.80)**	(−0.08, 0.68)	(0.24, 0.78)
SM	12.69	0.24	0.30	**0.59**	0.02
		(0.12, 0.37)	(0.13, 0.45)	**(0.39, 0.78)**	(−0.47, 0.60)
SF	1.83	0.34	0.47	0.09	**0.41**
		(0.23, 0.45)	(0.31, 0.61)	(−0.16, 0.39)	**(0.22, 0.60)**
SFA, %					
GM	1.89	**0.42**	0.73	0.29	0.53
		(0.26, 0.57)	(0.53, 0.91)	(−0.03, 0.58)	(0.21, 0.81)
LD	2.39	0.67	**0.54**	0.10	0.75
		(0.60, 0.73)	**(0.36, 0.71)**	(−0.19, 0.46)	(0.57, 0.90)
SM	8.28	0.18	0.15	**0.57**	0.00
		(0.04, 0.30)	(0.00, 0.28)	**(0.37, 0.78)**	(−0.52, 0.49)
SF	3.33	0.37	0.63	0.13	**0.46**
		(0.27, 0.47)	(0.51, 0.74)	(−0.10, 0.38)	**(0.26, 0.65)**
MUFA, %					
GM	2.55	**0.50**	0.73	0.43	0.32
		(0.35, 0.66)	(0.56, 0.88)	(0.18, 0.70)	(−0.06, 0.66)
LD	2.56	0.62	**0.61**	0.30	0.58
		(0.55, 0.69)	**(0.45, 0.80)**	(0.02, 0.66)	(0.33, 0.79)
SM	14.63	0.28	0.30	**0.59**	0.02
		(0.16, 0.41)	(0.16, 0.43)	**(0.41, 0.78)**	(−0.42, 0.58)
SF	2.13	0.35	0.54	0.10	**0.41**
		(0.24, 0.45)	(0.40, 0.66)	(−0.15, 0.37)	**(0.22, 0.59)**
PUFA, %					
GM	2.79	**0.60**	0.82	0.44	0.41
		(0.47, 0.75)	(0.73, 0.92)	(0.20, 0.66)	(0.19, 0.63)
LD	3.23	0.76	**0.67**	0.54	0.43
		(0.72, 0.81)	**(0.48, 0.82)**	(0.32, 0.79)	(0.17, 0.68)
SM	10.30	0.38	0.47	**0.57**	0.11
		(0.26, 0.49)	(0.35, 0.58)	**(0.37, 0.76)**	(−0.28, 0.49)
SF	2.27	0.40	0.41	0.03	**0.57**
		(0.31, 0.49)	(0.25, 0.55)	(−0.18, 0.21)	**(0.37, 0.76)**

[1] Mean of the posterior density and, in parentheses, highest posterior density interval at 95% of probability.
[2] GM: *gluteus medius*; LD: *longissimus dorsi*; SM: *semimembranosus*.

Table 3.4. Heritability (diagonal, in bold), genetic correlations (above diagonal), and phenotypic correlations (below diagonal) for oleic acid content (C18:1) in muscles adjusted for intramuscular fat content (IMF) and C18:1 in subcutaneous fat (SF). Adjustment for IMF was performed either adding IMF of the corresponding muscles as covariates in the model for C18:1 or as additional traits in a multivariate analysis[1].

Model	Muscle[2]			SF
	GM	LD	SM	
IMF as covariate				
GM	**0.41**	0.56	0.35	0.31
	(0.28, 0.55)	(0.28, 0.82)	(0.10, 0.58)	(−0.07, 0.64)
LD	0.50	**0.55**	0.18	0.52
	(0.42, 0.59)	**(0.34, 0.75)**	(−0.22, 0.60)	(0.25, 0.81)
SM	0.19	0.19	**0.59**	0.14
	(0.06, 0.31)	(0.06, 0.33)	**(0.40, 0.79)**	(−0.22, 0.46)
SF	0.35	0.47	0.16	**0.44**
	(0.23, 0.46)	(0.29, 0.62)	(−0.05, 0.36)	**(0.24, 0.63)**
IMF as trait				
GM	**0.47**	0.64	0.33	0.32
	(0.34, 0.60)	(0.49, 0.80)	(0.11, 0.55)	(0.00, 0.62)
LD	0.55	**0.61**	0.36	0.49
	(0.47, 0.62)	**(0.43, 0.80)**	(0.13, 0.59)	(0.15, 0.76)
SM	0.21	0.30	**0.62**	0.18
	(0.10, 0.31)	(0.17, 0.43)	**(0.41, 0.82)**	(−0.24, 0.57)
SF	0.34	0.47	0.15	**0.45**
	(0.23, 0.45)	(0.30, 0.62)	(−0.09, 0.38)	**(0.24, 0.65)**

[1] Mean of the posterior density and, in parentheses, highest posterior density interval at 95% of probability.
[2] GM: *gluteus medius*; LD: *longissimus dorsi*; SM: *semimembranosus*.

Table 3.5. Genetic correlations of intramuscular fat (IMF) and oleic acid (C18:1) content in different muscles and subcutaneous fat (SF)[1].

IMF[2]	C18:1[2]			
	GM	LD	SM	SF
GM	0.47	0.24	0.29	−0.03
	(0.27, 0.66)	(−0.04, 0.50)	(−0.02, 0.59)	(−0.39, 0.32)
LD	0.51	0.52	0.37	0.10
	(0.30, 0.71)	(0.31, 0.72)	(0.19, 0.55)	(−0.29, 0.41)
SM	0.15	0.14	0.69	0.06
	(−0.15, 0.44)	(−0.14, 0.46)	(0.49, 0.86)	(−0.29, 0.42)

[1] Mean of the posterior density and, in parentheses, highest posterior density interval at 95% of probability.
[2] GM: *gluteus medius*; LD: *longissimus dorsi*; SM: *semimembranosus*.

The expected responses for IMF and C18:1 in the three sampled muscles and SF to selection on records from different tissues are shown in Table 3.6. The correlated response in IMF (or C18:1) in GM to selection for the same trait in LD, and vice versa, was 0.6−0.7 times the direct response obtained in the sampled muscle. For GM and LD, selection for C18:1 (or IMF) led to a correlated response for IMF (or C18:1, respectively) of around half of the response for the proactively selected trait. The correlated responses in SM to selection based on records on GD or LD were always very low. There was only a small opportunity cost for IMF and C18:1 (less than 20%) with respect to single-trait selection when both traits are measured and included in a selection objective with equal economic weights. Relevant genetic changes in C18:1 in SF were found only for direct selection or for selection for C18:1 in LD. Selection for C18:1 in SF led to the same correlated response of C18:1 in LD than selection for C18:1 in GM, but the first had the disadvantage that it was not accompanied by a correlated change in IMF.

Table 3.6. Direct (bold) and correlated (not bold) expected genetic response for intramuscular fat (IMF) and oleic acid (C18:1) content in a given tissue to selection on records taken on different muscles or subcutaneous fat (SF)[1].

Response[2]	Tissue and trait used as a selection criterion[3]						
	GM			LD			SF
	IMF	C18:1	IMF+C18:1[4]	IMF	C18:1	IMF+C18:1[4]	C18:1
IMF[3]							
GM	**28**	12	**24**	20	7	15	−1
LD	18	14	20	**30**	15	**25**	2
SM	4	4	5	4	4	5	2
C18:1[3]							
GM	13	**26**	**23**	15	18	19	7
LD	7	16	14	16	**29**	**27**	13
SM	8	11	11	11	9	11	1
SF	−1	7	4	3	15	12	**25**

[1] In each generation 25% of males and 50% of females were selected based on three half-sib plus pedigree records.
[2] Genetic standard deviation units (×100).
[3] GM: *gluteus medius*; LD: *longissimus dorsi*; SM: *semimembranosus*.
[4] Same economic weights for both traits in the selection objective.

3.4. DISCUSSION

Three economically relevant muscles were considered in this study, two of them located in the ham (GM and SM) and one in the loin (LD). Sampling of central LD for chemical analysis is laborious and depreciates the loin as a primal cut. Instead, a big sample of GM can be easily obtained on the cutting line from the superior edge of the

ham at no cost. Because of this, GM has been frequently used as the reference muscle in studies conducted under field conditions (Chapters 2 and 4; Casellas et al., 2010). It is also feasible to sample SM from its exposed surface at no cost, but this sampling scheme has the limitation that only allows obtaining small off-line samples. Since SF samples are much easier to obtain than muscle samples, SF has been often used as the reference tissue where to determine the fatty acid profile, both for research and genetic evaluation purposes (Fernández et al., 2003; Hofer et al., 2006; Gjerlaug-Enger et al., 2011). Although alternative non-destructive methods can be used in substitution of chemical determinations, such as near infrared technology (González-Martín et al., 2002, 2005), the nature of the problem still persists and it is still needed to know the correlation structure between target and measured muscles for IMF content and fatty acid composition. The present study investigates the genetic implications of using alternative muscles or SF for phenotyping IMF and fatty acid composition in pigs.

The estimates of the heritability were slightly higher than those previously reported (Suzuki et al., 2006; Casellas et al., 2010; Sellier et al., 2010) for IMF, C18:1, MUFA, and PUFA, but similar for SFA. Among muscles, GM and LD showed high correlations between them for IMF and fatty acids content, but not with SM, the correlations of which were much lower, particularly for IMF and SFA. An explanation for this result is that SM is subjected to greater sampling errors. To avoid depreciation of the ham, SM was sampled by cutting a small slice from the exposed surface of the carcass at the slaughterhouse. In contrast, a much bigger sample of GM and LD was obtained from the ham and the loin retail cuts, respectively. As a result, samples from GM and LD are likely more representative of the whole muscle than the small slices of SM. This result would confirm that sampling can be a critical factor for an adequate interpretation of the correlations across muscles (Bosch et al., 2009).

The genetic correlations of fatty acids content between muscles GM and LD were higher than those between them and SF, in line with the results of Cánovas et al. (2009), who found different expression patterns between IMF and SF. The only exception was the correlation between SFA in LD and SF. In general, the correlations of fatty acid composition between LD and SF were higher than those between GM or SM and SF. This can be attributed to the fact that SF samples were mostly collected at the same anatomical location as LD, thereby suggesting that SF composition correlates better to the IMF composition of an adjacent muscle. In line with this, the remaining SF samples were taken at the same location as GM and, consequently, SF showed a higher correlation with GM than with SM. Note, however, that, due to the low number of samples at each location, genetic parameters for SF are based on pooled estimates at both locations. An additional source of sampling error may be incurred by sampling SF across fat layers. Although it is known that fatty acid composition differs between SF layers, its effect on the estimates of genetic parameters is likely small. For the main

fatty acids, Suzuki et al. (2006) found that the correlation between the inner and outer SF layers was very high, from 0.84 to 0.96. The correlation structure of IMF fat content and composition with BT and SF composition has practical implications. On one hand, it indicates that there is room for improving IMF content independently from overall fatness (Chapter 4; Tribout et al., 2004; Solanes et al., 2009), but, on the other hand, that measuring fatty acids content in SF can be a good criterion for improving IMF traits only in certain retail cuts. Thus, regarding C18:1, SF (as measured in this study at the level of the third and fourth ribs) could be a good criterion for loin but not for ham.

The IMF content is known to affect fatty acid composition, being positively related to SFA and MUFA and negatively to PUFA (Chapter 1; Wood et al., 2008). Using C18:1 as an example, genetic parameters were adjusted for IMF of the involved muscles, including them either as covariates in the respective models or as additional traits in a multivariate approach. In general, the estimates based on (co)variances adjusted for IMF as a covariate were lower than those obtained when adding IMF as additional traits. Although the interpretation of this result is not straightforward, what is important here is that the differences of both approaches with the unadjusted estimates are minor, particularly in terms of HPD95.

Results in the literature regarding the correlation of IMF and fatty acid composition among tissues are scarce but in line with those obtained here. Rauw et al. (2012) reported a phenotypic correlation of IMF between GM and LD higher than ours (0.69), but in contrast, for the correlation among the main fatty acids, their estimates were below our lower HPD95 limit, with values below 0.38. A genetic correlation of 0.65 between IMF in GM and LD and much lower ones with BT (0.36−0.38) were found by Hernández-Sánchez et al. (2013) using genomic markers information. These estimates were similar to ours. The phenotypic correlations reported by Yang et al. (2010) between *longissimus* muscle and SF in a White Duroc × Erhualian cross were in the same range of values than ours (their values were included in our HPD95), with the exception of SFA, which were lower. Cameron & Enser (1991) reported much lower values for C18:1 (0.19) but more moderate for the main SFA and PUFA (0.31−0.54), using data from Duroc and Landrace. These latter results are in contrast with those obtained by Suzuki et al. (2006) in Duroc for the genetic correlation of MUFA and SFA between LD and SF (~0.70). For PUFA, this genetic correlation was as low as ~0.18. Although part of the discrepancies among estimates may be explained by the age of the pigs, much younger in Cameron & Enser (1991) as compared to other works, and part by the relatively high standard errors associated to them, they provide sufficient evidence indicating that the pattern of fat deposition can differ widely across muscles and fat tissues.

Low correlations between muscles have also been found for other meat quality traits. Huff-Lonergan et al. (2002), in Large White, reported phenotypic correlations of 0.47 and 0.30 between LD and SM for pH and color (relative lightness) at 24 h post-mortem, respectively. Similarly, Gjerlaug-Enger et al. (2010) reported high genetic correlations (~0.8) between ultimate pH in GM and LD, both in Landrace and Duroc, but the estimates between these muscles and *gluteus profundus* were only in the range of 0.10 to 0.55. The phenotypic correlations among these three muscles did not exceed 0.5. As in our study, correlations were positive but moderate in magnitude.

It has been shown that there is room for improving IMF and fatty acid composition of pork through genetic selection (Chapter 2). This involves setting up a feasible routine of recording these data on a commercial basis. The definition of an optimum design for such schemes requires knowing the correlation structure of IMF and fatty acid composition among target and sampled tissues. One of the main costs of sampling is the depreciation cost, which is likely to occur if measures are taken from the inner side of a high value retail cut such as loin. For its sampling simplicity, an alternative is to sample a portion of GM from the superior edge of the hams. Although it implies an opportunity cost with respect to LD, the target muscle, our results indicate that selection based on GM still leads to acceptable genetic gains in LD, both for IMF and C18:1. In some cases, however, selecting for C18:1 in SF can be a good criterion to increase C18:1 in LD without increasing IMF, at least if SF is taken at the same location as LD. However, in general, C18:1 in SF is of very limited value for improving IMF or its fatty acid composition. A full description of the consequences of alternative selection and sampling schemes must take into account both the economic value of each muscle and its relative proportion in the carcass, as well as the genetic variation of IMF and fatty acid composition traits within each of them (Faucitano et al., 2004).

3.5. CONCLUSIONS

The genetic correlations of IMF and fatty acid composition across muscles and fat tissues, although positive, are variable enough to influence the genetic evaluation schemes for IMF and fat quality. The results obtained indicate that, in terms of genetic response, GM and LD can be used alternatively as the reference muscle for selection purposes. Moreover, they also reveal that using fatty acid composition of SF as selection criterion should cause more changes in LD than in GM, but not in IMF.

Chapter 4.
Response to selection for decreased backfat thickness at restrained intramuscular fat content in Duroc pigs

R. Ros-Freixedes[1], J. Reixach[2], L. Bosch[3], M. Tor[1], & J. Estany[1]

[1]Departament de Producció Animal, Universitat de Lleida − Agrotecnio Center, 191 Av. Alcalde Rovira Roure, 25198 Lleida, Catalonia, Spain.
[2]Selección Batallé S.A., Av. Segadors s/n, 17421 Riudarenes, Catalonia, Spain.
[3]Departament d'Enginyeria Química, Agrària i Tecnologia Agroalimentària, Universitat de Girona, Campus de Montilivi, 17071 Girona, Catalonia, Spain.

The content of this chapter was published in Journal of Animal Science (2013), 91: 3514−3521. DOI: 10.2527/jas.2013-6282

ABSTRACT: Intramuscular fat (IMF) content is a relevant trait for the pig industry and consumers. However, selection for IMF has the undesired correlated effect of decreasing lean growth. A selection experiment was performed to investigate the effects of selection against backfat thickness (BT) at restrained IMF. Barrows from a purebred Duroc line were allocated into a selected (n=165) or a control (n=185) group based upon their litter predicted breeding values. Litters in the selected group were selected against BT at 180 d at restrained IMF in *gluteus medius* muscle (GM) whereas those in the control group were chosen randomly. Realized selection intensities and genetic responses for BT, IMF in GM, and body weight (BW) were estimated using a 3-trait multivariate animal mixed model under a Bayesian setting. Correlated responses for other traits were estimated similarly but using a 4-trait model, where other traits were added to the previous 3-trait model one at a time. Selected pigs had less BT than control pigs (−1.22 mm, with highest posterior density interval at 95% of probability (HPD95) [−2.47, −0.75]) with restrained decrease in IMF, both in GM (−0.16%, HPD95 [−0.36, +0.05]) and in *longissimus dorsi* muscle (−0.15%, HPD95 [−0.37, +0.09]). However, the realized selection intensity for IMF in GM denotes that the restriction on IMF was incomplete (−0.18, HPD95 [−0.36, +0.02]). Selection decreased BW (−1.64 kg, HPD95 [−2.47, −0.75]) but increased carcass lean weight (+0.66 kg, HPD95 [+0.14, +1.22]), indicating that the response in BT offsets the unfavorable correlated response in BW. Selected pigs were shorter (−0.50 cm, HPD95 [−0.81, −0.20]) but with similar ham weight and loin depth. These results provide evidence that lean weight can be improved restraining the genetic change in IMF. However, they also stress that a complete restriction on IMF is difficult to achieve unless selection is practiced on a big population where IMF is accurately predicted.

4.1. INTRODUCTION

Intramuscular fat (**IMF**) content is a key trait for marketing cured pork products, but it is also increasingly becoming relevant for fresh pork. Because IMF is unfavorably correlated with lean content, the selection for leanness undertaken in the last decades has led to develop genetic lines with a level of IMF that does not match the requirements of those specialized markets (Lonergan et al., 2001; Wood et al., 2008). However, the reported genetic correlations between lean-related traits and IMF are only moderate (Clutter, 2011), suggesting that there is room for improving lean growth independently from IMF.

Bosch et al. (2009, 2012) estimated the IMF content and backfat thickness (**BT**) at different age-points and muscles in a Duroc line. The values obtained by these authors proved that in some lines the problem is not IMF, which is already within the optimum range for dry-cured production, but overall fatness. Therefore, a suggestive breeding goal for such situations could be to increase leanness (reducing BT) subjected to minor change in IMF. It has been proved theoretically that this can be a feasible strategy (Chapter 2; Solanes et al., 2009), but there is only little experimental evidence to support this approach. Results in the two experiments reported so far involving IMF in the selection objective (Suzuki et al., 2005a,b; Schwab et al., 2009, 2010) confirmed that IMF responds to selection, but also that selection for increased IMF is accompanied by increased overall fatness.

In this paper the results of a selection experiment conducted to investigate the effects of selection against BT at restrained IMF are presented.

4.2. MATERIALS AND METHODS

4.2.1. Selection experiment

A selection experiment was conducted to study the effects of selection for decreased BT at restrained IMF. Selection was practiced in a purebred Duroc population that was completely closed in 1991 and since then it has been selected for an index including body weight (**BW**), BT, and IMF (see Animals and Samples Section). Selection was practiced among available litters at four established dates throughout 2006 and 2007 (selection batches 1 to 4). A litter born within two weeks before the set date was considered available for selection. In each batch, around 50 litters were allocated into a selected (**S**) or a control (**C**) group according to their litter (mid-parent) best linear unbiased prediction (BLUP) estimated breeding value (**EBV**) for BT and IMF. Litters in group C were chosen randomly whereas those in group S were selected against BT at 180 d at restrained IMF in *gluteus medius* muscle (**GM**). Linear programming was used to select the litters in group S. These litters were those

with the lowest EBV for BT while satisfying the restriction of having the same mean EBV for IMF than the litters in group C (±0.03%). The EBV for BT and IMF were obtained from, respectively, 37,698 and 3,066 records at 180 d from full pedigree-connected pigs born since 1996. The IMF content was determined in GM by near infrared transmittance spectrometry (Valero et al., 1999). The genetic evaluations were performed univariately using basically the same animal models described below (Solanes et al., 2009) but with heritabilities 0.19 and 0.40 for BT and IMF, respectively. Two males per litter were randomly chosen shortly after birth. Pigs from both groups were mixed and reared together. The number of litters and pigs used in the experiment by selection group and batch is given in Table 4.1.

Table 4.1. Number of pigs, litters, and sires, and mean (SD) of backfat thickness (BT) at 180 d, intramuscular fat (IMF) in *gluteus medius* (GM), and body weight (BW) at 180 d by selection group and batch.

Selection group	No. of pigs	No. of litters	No. of sires	Traits		
				BT, mm	IMF in GM, %	BW, kg
Batch 1						
Selected	55	31	12	17.89 (3.98)	4.32 (2.23)	99.52 (12.94)
Control	52	30	19	19.54 (5.10)	4.50 (2.05)	101.37 (17.93)
Batch 2						
Selected	47	30	14	16.77 (3.21)	4.72 (2.79)	104.50 (9.87)
Control	58	31	20	17.09 (3.54)	4.78 (2.33)	104.82 (10.41)
Batch 3						
Selected	30	22	12	16.45 (2.25)	4.74 (2.42)	105.92 (9.40)
Control	36	24	14	19.22 (3.28)	4.89 (2.94)	109.95 (9.44)
Batch 4						
Selected	33	20	9	14.38 (2.89)	3.36 (1.43)	105.18 (14.08)
Control	39	23	10	16.05 (2.96)	3.71 (1.99)	113.87 (11.23)

4.2.2. Management of pigs and sample collection

All pigs were performance-tested at an average age of 180 d for BW and BT. At the end of the finishing period, all barrows were slaughtered in the same commercial slaughterhouse at ~125 kg of BW. After slaughter, the carcass weight (**CW**), length, BT, loin thickness, and lean percentage were measured. After chilling for about 24 h at 2ºC, each carcass was divided into primal cuts and the left side ham was weighed. Immediately after quartering, a sample of GM was taken from the ham. A section of around 1 kg from the left loin (*longissimus dorsi*; **LD**) of each carcass at the level of the third and fourth last ribs was also taken. Intramuscular fat content was determined by gas chromatography in GM and LD. Complete details on the procedures are given in the Animals and Samples Section.

4.2.3. Analysis of response to selection

The response to selection was estimated as the difference between the average EBV of the pigs in group S and the pigs in group C. A description of the selection groups by batch is given in Table 4.1. The genetic parameters and EBV of the pigs for BT, IMF in GM, and BW were estimated fitting a 3-trait multivariate animal model under a Bayesian setting, in line with the methodology described in Chapter 2. The genetic parameters and EBV of other correlated traits were obtained using a 4-trait model, where each of them was added one at a time to the previous 3-trait model. A summary of the data used for the analyses is given in Table 4.2. Records for BT and BW were collected in pigs born from 1996 to 2009 while carcass traits only in pigs born since 2002 onwards.

The model used was:

$$\mathbf{y}_i = \mathbf{X}_i\mathbf{b}_i + \mathbf{Z}_i\mathbf{a}_i + \mathbf{W}_i\mathbf{c}_i + \mathbf{e}_i \,,$$

where $\mathbf{y}_i$ is the vector of observations for the *i*th trait; $\mathbf{b}_i$, $\mathbf{a}_i$, $\mathbf{c}_i$, and $\mathbf{e}_i$ are the vectors of systematic, additive genetic, litter, and residual effects, respectively; and $\mathbf{X}_i$, $\mathbf{Z}_i$, and $\mathbf{W}_i$, the known incidence matrices that relate $\mathbf{b}_i$, $\mathbf{a}_i$, and $\mathbf{c}_i$ with $\mathbf{y}_i$, respectively. Systematic effects for BW and BT were the batch (1,039 levels), gender (3 levels; males, females, and castrates), and age at test as a covariate. Pigs tested at the same time and in the same unit were considered as one batch. The model for the other traits

Table 4.2. Description of the data set used in the analysis of the response to selection.

Item	No. of pigs	No. of litters	No. of sires	No. of dams	Mean	SD
Pedigree	93,920	32,315	731	18,516	-	-
Traits[1]						
BW at test, kg	85,002	32,211	641	16,548	104.8	12.5
BT at test, mm	80,687	31,197	642	16,335	15.6	3.5
IMF in GM, %	943	546	141	543	4.9	1.9
Carcass weight, kg	937	545	142	542	98.4	11.6
Carcass length, cm	446	270	85	270	86.8	3.0
Carcass BT, mm	921	538	142	535	23.4	3.8
Carcass loin thickness, mm	921	538	142	535	43.7	7.9
Carcass lean percentage, %	921	538	142	535	42.9	5.2
Carcass lean weight, kg	920	538	142	535	42.0	5.7
Ham weight, kg	431	268	85	268	12.1	1.2
IMF in LD, %	189	149	65	149	3.9	1.2
Covariates						
Age at test , d	85,194	32,310	642	16,601	180.2	10.7
Age at slaughter, d	2,098	1,370	298	1,313	206.5	14.6

[1] BW: body weight; BT: backfat thickness; IMF in GM (LD): intramuscular fat in *gluteus medius* (*longissimus dorsi*).

only included the batch (12 levels) and the age at slaughter. The litter effect was not included in the model for carcass traits because there were only 1.7 piglets/litter with these data.

The genetic parameters and EBV for all traits were estimated in a Bayesian framework using Gibbs sampling with the TM software (Legarra et al., 2011). Observed phenotypes and missing records imputed by data augmentation were assumed to be conditionally normally distributed as follows:

$$\begin{bmatrix}\mathbf{y}_1\\ \mathbf{y}_2\\ \mathbf{y}_3\\ \mathbf{y}_4\end{bmatrix} | \, \mathbf{b}_1, \mathbf{b}_2, \mathbf{b}_3, \mathbf{b}_4, \mathbf{a}_1, \mathbf{a}_2, \mathbf{a}_3, \mathbf{a}_4, \mathbf{c}_1, \mathbf{c}_2, \mathbf{R} \sim \mathrm{N}\left(\mathbf{X}\begin{bmatrix}\mathbf{b}_1\\ \mathbf{b}_2\\ \mathbf{b}_3\\ \mathbf{b}_4\end{bmatrix} + \mathbf{Z}\begin{bmatrix}\mathbf{a}_1\\ \mathbf{a}_2\\ \mathbf{a}_3\\ \mathbf{a}_4\end{bmatrix} + \mathbf{W}\begin{bmatrix}\mathbf{c}_1\\ \mathbf{c}_2\end{bmatrix}, \mathbf{R} \right),$$

where **R** was the (co)variance matrix. Sorting records by trait, and pig within trait, **R** could be written as $\mathbf{R}_0 \otimes \mathbf{I}$, with $\mathbf{R}_0$ being, in the most general case, the 4 × 4 residual (co)variance matrix between the four traits analyzed and **I** an identity matrix of appropriate order. Flat priors were used for $\mathbf{b}_i$ and residual (co)variance components. Additive genetic and litter values, conditional on the associated (co)variance components, were both assumed multivariate normally distributed with mean zero and with (co)variance $\mathbf{G} \otimes \mathbf{A}$ and $\mathbf{C} \otimes \mathbf{I}$, respectively, where **A** was the numerator relationship matrix, **G** was the 4 × 4 genetic relationship matrix between the four traits, and **C** was the 2 × 2 (co)variance matrix between litter effects of BW and BT. The matrix **A** was calculated using all the pedigree information summarized in Table 4.2. Flat priors were used for additive and litter (co)variance components. Statistical inferences for all unknowns were derived from the samples of the marginal posterior distribution using a unique chain of 1,000,000 iterations, where the first 250,000 were discarded and one sample out of 100 iterations retained. Statistics of marginal posterior distributions and the convergence diagnostics were obtained using the *boa* package (Smith, 2005). Convergence was tested using the *Z*-criterion of Geweke (Geweke, 1992) and visual inspection of convergence plots.

The response to selection for the *i*th trait ($R_{(i)}$) was calculated as:

$$R_{(i)} = \bar{a}_{S(i)} - \bar{a}_{C(i)},$$

where $\bar{a}_{S(i)}$ and $\bar{a}_{C(i)}$ are the average of the EBV for the *i*th trait in pigs from group S and C, respectively. Overall responses to selection and by batch were calculated. In this latter case only the pigs from the corresponding batch were used in the above expression. The realized selection intensities for BT and IMF in GM ($i_{S(i)}$ and $i_{C(i)}$, for the *i*th trait and group S and C, respectively) were obtained by calculating the standardized selection differentials as follows:

$$i_{S(i)} = (\bar{a}_{S(i)} - \bar{a}_{all(i)})/\sigma_{a(i)}$$

and

$$i_{C(i)} = (\bar{a}_{C(i)} - \bar{a}_{all(i)})/\sigma_{a(i)},$$

where $\bar{a}_{all(i)}$ is the average EBV of pigs from all candidate litters (i.e., available litters at each selection time-point) for the *i*th trait, and $\sigma_{a(i)}$ the genetic standard deviation of the trait. Both $i_{S(i)}$ and $i_{C(i)}$ were calculated independently for each batch, with the EBV obtained using only the data collected up to the selection time-point of the batch. The average realized selection intensity of the experiment was calculated weighting the realized selection intensity across the four batches. Statistical inferences for genetic parameters, realized selection intensities, and responses to selection were derived from random samples of the corresponding marginal posterior distributions. In particular, the mean, the SD, the mode, and the highest posterior density interval at 95% of probability (**HPD95**) of the marginal posterior distributions were calculated. Response to selection was assessed using the HPD95 and the probability of $R_{(i)}$ being negative.

4.3. RESULTS

4.3.1. Genetic parameters

Estimates of the variance components and the heritability for each of the analyzed traits, as well as the genetic and residual correlations of BT, IMF in GM, and BW with carcass traits and IMF in LD are given in Table 4.3. The estimates of the heritability were within the expected range, from 0.31 (SD 0.01), for BW, to 0.69 (SD 0.09), for IMF in LD. The genetic correlations of BT with carcass traits were positive, except for the lean-related traits loin thickness (−0.40, SD 0.13), lean percentage (−0.88, SD 0.04), and lean weight (−0.49, SD 0.08). A similar genetic correlation structure was found for IMF in GM but, in general, lower in magnitude. The genetic correlations of IMF in GM with carcass loin thickness (−0.58, SD 0.07), lean percentage (−0.45, SD 0.11), and lean weight (−0.38, SD 0.12) were also negative. However, for IMF in GM, the genetic correlation with ham weight was much lower (0.09, SD 0.16) than for BT (0.36, SD 0.09). The genetic correlation of BT with IMF, both in GM (0.38, SD 0.10) and in LD (0.41, SD 0.12), was lower than observed between IMF in GM and LD (0.64, SD 0.10).

4.3.2. Realized selection intensities

The realized selection intensities are given in Table 4.4. As expected, in group S, the overall realized selection intensity for BT was negative (−0.49, HPD95 [−0.62, −0.35]) whereas that for IMF in GM was much closer to zero (−0.18, HPD95 [−0.36, +0.02]). By contrast, the values in group C confirmed that pigs in this group were

Table 4.3. Posterior means (SD) of variance components (σ_a^2: additive genetic, σ_e^2: residual) and heritability (h^2) of all analyzed traits, and genetic (r_g) and residual (r_e) correlations of backfat thickness (BT) at 180 d, intramuscular fat (IMF) in *gluteus medius* (GM), and body weight (BW) at 180 d with other carcass traits and IMF of *longissimus dorsi* (LD).

Trait	Variance components		h^2	r_g			r_e		
	σ_a^2	σ_e^2		BT	IMF in GM	BW	BT	IMF in GM	BW
BT	4.11	4.44	0.45	-	0.38	0.63	-	0.14	0.60
	(0.61)	(0.08)	(0.01)		(0.10)	(0.02)		(0.08)	(0.01)
IMF in GM	1.77	1.45	0.55	0.38	-	0.28	0.14	-	0.06
	(0.31)	(0.23)	(0.08)	(0.10)		(0.10)	(0.08)		(0.07)
BW	29.76	57.23	0.31	0.63	0.28	-	0.60	0.06	-
	(1.34)	(0.78)	(0.01)	(0.02)	(0.10)		(0.01)	(0.07)	
Carcass traits									
Weight	39.77	47.89	0.45	0.59	0.17	0.96	0.58	0.14	0.88
	(4.22)	(3.30)	(0.04)	(0.05)	(0.11)	(0.02)	(0.03)	(0.09)	(0.01)
Length	5.05	3.72	0.57	0.27	0.22	0.70	0.29	−0.07	0.76
	(0.91)	(0.63)	(0.08)	(0.11)	(0.15)	(0.08)	(0.08)	(0.15)	(0.05)
Carcass BT	6.73	5.66	0.54	0.91	0.36	0.61	0.48	0.20	0.32
	(0.97)	(0.70)	(0.06)	(0.03)	(0.10)	(0.06)	(0.04)	(0.10)	(0.05)
Loin thickness	19.77	41.05	0.32	−0.40	−0.58	0.04	0.05	0.14	0.14
	(4.37)	(3.77)	(0.06)	(0.13)	(0.13)	(0.13)	(0.07)	(0.11)	(0.06)
Lean percentage	13.00	10.21	0.56	−0.88	−0.45	−0.50	−0.36	−0.11	−0.18
	(1.92)	(1.37)	(0.07)	(0.04)	(0.11)	(0.07)	(0.05)	(0.12)	(0.06)
Lean weight	12.34	14.14	0.46	−0.49	−0.38	0.18	0.23	0.05	0.62
	(2.23)	(1.66)	(0.07)	(0.08)	(0.12)	(0.09)	(0.06)	(0.11)	(0.05)
Ham weight	0.49	0.71	0.41	0.36	0.09	0.83	0.46	0.03	0.87
	(0.09)	(0.07)	(0.06)	(0.09)	(0.16)	(0.06)	(0.05)	(0.11)	(0.03)
IMF in LD	1.11	0.49	0.69	0.41	0.64	0.14	0.24	0.19	0.25
	(0.20)	(0.15)	(0.09)	(0.12)	(0.10)	(0.15)	(0.15)	(0.18)	(0.15)

randomly chosen both for BT (+0.09, HPD95 [−0.03, +0.21]) and IMF in GM (0.00, HPD95 [−0.15, +0.15]). The corresponding realized selection differentials were −0.93 mm (HPD95 [−1.18, −0.67]), −0.25% (HPD95 [−0.53, +0.02]), +0.17 mm (HPD95 [−0.06, +0.40]), and 0.00% (HPD95 [−0.23, +0.21]), respectively. These results were consistent across the four selection batches. The associated HPD95 indicate that selection for BT was effective in all batches, but also that the constraint imposed on IMF was not fully accomplished.

Table 4.4. Realized selection intensity for backfat thickness (BT) at 180 d and intramuscular fat (IMF) in *gluteus medius* (GM) by selection group.

Selection group	BT		IMF in GM	
	Mean	HPD95[1]	Mean	HPD95[1]
Average				
Selected	−0.49	−0.62, −0.35	−0.18	−0.36, +0.02
Control	+0.09	−0.03, +0.21	0.00	−0.15, +0.15
Batch 1				
Selected	−0.40	−0.62, −0.20	−0.17	−0.51, +0.17
Control	+0.16	−0.05, +0.38	−0.05	−0.33, +0.23
Batch 2				
Selected	−0.45	−0.69, −0.23	−0.13	−0.45, +0.16
Control	+0.08	−0.12, +0.27	+0.03	−0.21, +0.29
Batch 3				
Selected	−0.40	−0.69, −0.10	−0.14	−0.52, +0.22
Control	+0.18	−0.09, +0.44	+0.10	−0.24, +0.45
Batch 4				
Selected	−0.76	−1.11, −0.39	−0.27	−0.77, +0.24
Control	−0.07	−0.33, +0.23	−0.06	−0.46, +0.32

[1] HPD95: highest posterior density interval at 95% of probability.

4.3.3. Direct response to selection

The phenotypic values of BT and IMF in GM by selection group and batch are given in Table 4.1. The features of the posterior distribution of the direct response to selection on these traits are given in Table 4.5. Selection against BT was effective (the probability of the response of BT being negative was greater than 0.99 in all batches), with an overall reduction of 1.22 mm (HPD95 [−1.51, −0.93]). The results also indicated that selection was not completely neutral with respect to IMF in GM. The IMF content in GM showed an overall decrease of 0.16% (HPD95 [−0.36, +0.05]), with a probability of 94% of getting a negative response. However, this probability was lower within each selection batch, where it ranged from 72 to 88%.

Table 4.5. Features of the posterior distribution of the response to selection to decreased backfat thickness (BT) at 180 d at restrained intramuscular fat content (IMF) in *gluteus medius* (GM).

Trait	Response				
	Mean	SD	Mode	HPD95[1]	$P(<0)$[2]
BT, mm					
Overall	−1.22	0.15	−1.26	−1.51, −0.93	>0.99
Batch 1	−1.35	0.26	−1.37	−1.89, −0.85	>0.99
Batch 2	−0.76	0.26	−0.67	−1.27, −0.23	>0.99
Batch 3	−1.55	0.33	−1.55	−2.20, −0.91	>0.99
Batch 4	−1.43	0.32	−1.39	−2.07, −0.82	>0.99
IMF in GM, %					
Overall	−0.16	0.10	−0.15	−0.36, +0.05	0.94
Batch 1	−0.11	0.18	−0.12	−0.45, +0.25	0.74
Batch 2	−0.10	0.17	−0.07	−0.44, +0.24	0.72
Batch 3	−0.21	0.23	−0.18	−0.67, +0.24	0.82
Batch 4	−0.27	0.23	−0.31	−0.70, +0.19	0.88

[1] HPD95: highest posterior density interval at 95% of probability.
[2] $P(<0)$: probability of having a negative response.

4.3.4. Correlated response to selection

The features of the posterior distribution of the correlated responses are given in Table 4.6. Selection reduced BW (−1.64 kg, HPD95 [−2.47, −0.75]), CW (−1.83 kg, HPD95 [−2.71, −0.85]), and carcass length (−0.50 cm, HPD95 [−0.81, −0.20]), whereas it increased lean percentage (+1.47%, HPD95 [+0.98, +1.97]). The favorable response in lean percentage more than offset the unfavorable correlated response in CW, thereby resulting in a favorable correlated response in carcass lean weight (+0.66 kg, HPD95 [+0.14, +1.22]). Despite the loss in CW, no correlated change in ham weight was detected. The correlated response in IMF in LD was similar to that in GM (−0.15%, HPD95 [−0.37, +0.09]), but with a lower probability of being negative (90%). In general, the overall correlated responses were consistent across selection batches (results not shown). Nonetheless, in this regard it is worth noting that in batch 2 there was found a relatively high probability (82%) of a positive response in IMF in LD, a result proving that there exist scenarios where BT and IMF can be improved simultaneously.

4.4. DISCUSSION

The selection experiments undertaken so far for increased IMF proved that IMF responds to selection but at the expense of increasing BT (Suzuki et al., 2005a,b; Schwab et al., 2009, 2010). Previous theoretical studies using the estimates of the

Table 4.6. Features of the posterior distribution of the overall correlated responses to selection to decreased backfat thickness (BT) at 180 d at restrained intramuscular fat content (IMF) in *gluteus medius*.

Trait	Response				
	Mean	SD	Mode	HPD95[1]	*P*(<0)[2]
Body weight, kg	−1.64	0.44	−1.76	−2.47, −0.75	>0.99
Carcass traits					
Weight, kg	−1.83	0.47	−1.73	−2.71, −0.85	>0.99
Length, cm	−0.50	0.16	−0.42	−0.81, −0.20	>0.99
Carcass BT, mm	−1.15	0.19	−1.18	−1.51, −0.78	>0.99
Loin thickness, mm	+0.48	0.45	0.31	−0.41, +1.34	0.14
Lean percentage, %	+1.47	0.25	+1.51	+0.98, +1.97	<0.01
Lean weight, kg	+0.66	0.27	+0.62	+0.14, +1.22	0.01
Ham weight, kg	−0.07	0.06	−0.06	−0.18, +0.05	0.87
IMF in *longissimus dorsi*, %	−0.15	0.12	−0.10	−0.37, +0.09	0.90

[1] HPD95: highest posterior density interval at 95% of probability.
[2] *P*(<0): probability of having a negative response.

genetic parameters obtained in this population showed that, despite the positive genetic correlation between BT and IMF, there are response scenarios where BT can be reduced with no change in IMF (Chapter 2; Solanes et al., 2009). The results presented here confirmed experimentally that such goal is feasible but difficult. Thus, even though the response in IMF was restrained, there is not compelling evidence that the constraint had been fully achieved.

The expected correlated response in IMF to one generation of unrestricted selection against BT can be approached as (Falconer & Mackay, 1996):

$$R_{(IMF)} = r_{g(IMF,BT)} \frac{\sigma_{a(IMF)}}{\sigma_{a(BT)}} R_{(BT)},$$

where $r_{g(IMF,BT)}$ is the genetic correlation between BT and IMF. In such situation, with the genetic parameters given in Table 4.4, decreasing BT by 1.22 mm is expected to result in a correlated reduction in IMF of 0.30%, in GM, and of 0.26%, in LD, values that are around 2 folds those realized. Therefore, in practical terms, the imposed restriction on IMF served to halve the correlated response in IMF. That the restriction had not been fully effective is in line with the negative value of the realized selection differential for IMF in the selected group. A reason for that could be the poor predictive capacity of the mid-parent EBV for IMF used for selection. It can be retrospectively assessed by correlating the litter EBV with the phenotypic values of the offspring. This correlation was 0.12, for IMF, and 0.27, for BT, and increased to 0.20 and 0.34, respectively, for the realized EBV, which were calculated using the multivariate model and data used for estimating the realized selection intensities. These

predictive capacities are consistent with the precision of the EBV in the experimental pigs, calculated as $1 - \sigma^2_{EBV}/\sigma^2_e$, where σ^2_{EBV} is the variance of the EBV of an individual between iterations and σ^2_e the residual variance of the corresponding trait. The average precisions were 0.45 (0.33 to 0.50), for IMF, and 0.62 (0.47 to 0.64), for BT. These results explain why selection response for lower BT was more successful than the restriction on IMF. Moreover, they evidenced that there is scope for improvement. In fact, although retrospectively, it can be proved that there is a subset of 90 barrows in group S showing, as compared to pigs in group C, much lower BT (−1.79 mm, HPD95 [−2.13, −1.44]) but identical IMF in GM (0.00%, HPD95 [−0.28, +0.28]). This result highlights the fact that selection against BT does not necessarily lead to decrease IMF if accurate EBV for IMF are available and the population is big enough to allow the pigs with low BT and high IMF to be sorted out.

The selected pigs were lighter and had lighter carcasses. Because BW is shown to be genetically more correlated to BT than to IMF (Table 4.4), selection for BT is expected to cause greater changes in BW than selection for IMF. This is in line with results from the experiments in Schwab et al. (2009, 2010), who found no correlated response in growth performance to selection for IMF, and in Solanes et al. (2009), who showed in this population that selection for BW at restrained BT did not affect IMF. Results from commercial lines suggest that changes in IMF depend on the selection emphasis that has been put on growth as compared to lean content, with pigs that had been more intensively selected for daily gain than for lean content showing higher IMF (Oksbjerg et al., 2000; Tribout et al., 2004). In this regard, carcass lean weight is a more appropriate trait for the industry (Fowler et al., 1976; Chen et al., 2002, 2003). Lighter carcasses at a fixed age mean that there has been a loss in either fat or lean mass or both during the fattening period. The results here support the hypothesis that decreased CW is mostly due to fat loss. The selected pigs not only increased carcass lean weight, but also they were able to decrease carcass BT without adversely affecting loin thickness. Thus, the detrimental effect of selection on CW (BW) becomes less relevant when expressed in terms of lean growth. This is in line with the findings in Gjerlaug-Enger et al. (2012), who in a recent study on body composition using computerized tomography found that the genetic variation in carcass lean percentage is more determined by fat than by muscle growth. No data on feed intake was available for this research, but feed efficiency is known to be negatively correlated to fatness. Some authors reported a similar genetic correlation of feed efficiency with both BT and IMF (Hermesch et al., 2000; Cai et al., 2008) while others found it more correlated to BT than to IMF (Suzuki et al., 2005b). In either case, the selected pigs should be at least as efficient as the control.

The two more important retail pork cuts are ham and loin, particularly for the dry-cured market. Even though the relationship between fatness and carcass quality can

be negligible in light white pigs (Hermesch et al., 2000), the correlation pattern observed here between BT and IMF with ham weight, loin thickness, and carcass length, together with previously reported estimates in Iberian (Fernández et al., 2003) and Duroc (Suzuki et al., 2005a; Solanes et al., 2009) heavy pigs, indicate that selection against fatness may lead to undesired effects on primal cuts. However, in terms of correlated responses, side effects were only found in carcass length, but not in ham weight and loin thickness, thereby suggesting that the loin may be more sensitive than the ham to simultaneous selection for BT and IMF. The results of our selection experiment indicate that selection for BT at restrained IMF may lead to shorter (lower carcass length), but not narrower (loin thickness did not decrease) loins, in agreement with the positive genetic correlation observed between BT and carcass length, both here and elsewhere (Johnson & Nugent, 2003; Chimonyo & Dzama, 2007). These results contradict the findings in Schwab et al. (2009), who found that selected pigs for increased IMF had lower loin muscle area but similar carcass length. However, it is worth noting that in this latter experiment BW did not significantly change by selection. Because the weight of primal cuts greatly depends on BW, their correlated responses must be interpreted in light of the correlated changes observed in BW.

The metabolism of IMF may differ among muscles (Sharma et al., 1987; Leseigneur-Meynier & Gandemer, 1991; Muriel et al., 2002) and even among locations within muscle (Sharma et al., 1987). The molecular mechanisms of the differential deposition patterns are not well known, and therefore it still remains uncertain whether changes in a muscle cause correlated changes into another. Most research so far concerning IMF in pigs used *longissimus* as the reference muscle. However, neither *longissimus* is the only valuable muscle nor likely, because of depreciation costs, it is the most convenient for sampling purposes. In this experiment GM has been used as the reference muscle for determining IMF. It has been shown that IMF in LD is not only highly genetically determined, but also that it displays a high genetic correlation with IMF in GM. Therefore, the correlated response for IMF in LD was very similar to that for IMF in GM. While this is a comforting outcome of the experiment, it needs to be assessed in other muscles differing in IMF content and fiber composition.

4.5. CONCLUSIONS

The results of the present selection experiment provide evidence that lean weight can be improved restraining the genetic change in IMF, both in GM and LD. The selection practiced may lead to lighter pigs, mainly due to decreased body fat rather than lean. Nonetheless, attention should be paid to primal cuts, which can be lighter too. Simultaneous genetic improvement of BT, IMF, and BW should be feasible if the accuracy of the EBV for IMF, along with the selection intensity, is high enough. While

accuracy for IMF can be easily increased with a well-designed recording scheme, selection intensity may be a problem in small populations. The experimental design used here was based on a series of one-generation selection batches aimed at proving that BT and IMF can be manipulated independently. Selecting for more traits would have reduced the response in BT and therefore the power of the experiment. However, in practice, pigs are continuously selected across generations for an objective including all relevant traits. Short-term responses are lower, but in the long-term the population can be better accommodated to specific needs.

Chapter 5.
Response to selection for intramuscular oleic acid content in Duroc pigs

The content of this chapter is currently under preparation for publication.

ABSTRACT: Intramuscular oleic acid (C18:1) is an interesting trait to improve pork quality from the organoleptic, technological, and nutritional points of view. It has been shown to be moderately to highly heritable, but there are no reports of realized selection responses for this trait. A selection experiment was performed to investigate the effectiveness of selection for C18:1. Barrows from a purebred Duroc line were allocated into a selected (n=137) or a control (n=136) group based upon their litter estimated breeding values (EBV). Litters in the selected group were selected for C18:1 in *gluteus medius* muscle whereas those in the control group were chosen randomly. The average selection differential was +0.57%. Realized genetic responses for C18:1, as well as correlated responses for intramuscular fat (IMF) content, backfat thickness (BT), and body weight (BW), were estimated using a 4-trait multivariate animal mixed model under a Bayesian setting. Selected pigs had 0.24% more C18:1 (with highest posterior density interval at 95% of probability (HPD95) [−0.03, +0.53]). While selection was successful in two out of three batches (+0.35% and +0.33%, respectively), no evidence of response in batch 3 was found (+0.04%) probably due to poor accuracy when predicting litter EBV. The correlated responses for IMF, BT, and BW were +0.13%, −0.32 mm, and −0.31 kg, respectively. The same 4-trait model was used to evaluate the correlated responses for C18:1 in two other selection experiments, one for reduced BT at restrained IMF and the other for BW at restrained BT. These experiments were performed following a similar design. Correlated responses for C18:1 showed the same trend than responses for IMF. To our knowledge, this is the first report of realized responses to selection for fat composition in pigs. Results proved that direct selection for C18:1 is possible but emphasized the need for a systematic recording to accurately predict C18:1 EBV.

5.1. INTRODUCTION

Intramuscular fat (**IMF**) composition affects the quality of pork. While high contents of saturated and monounsaturated fatty acids are desirable in terms of organoleptic and technological meat quality, dietary recommendations point towards unsaturated fatty acid profiles. The monounsaturated oleic acid (**C18:1**) is the main fatty acid in pork and, therefore, intramuscular C18:1 can be a good alternative to improve simultaneously the organoleptic (Cameron et al., 2000), technological, and nutritional (Christophersen & Haug, 2011) properties of pork. Although dietary modifications are a strategy for increasing intramuscular C18:1 (Rhee et al., 1990; Myer et al., 1992; Klingenberg et al., 1995), the increased costs associated to high-oleic raw ingredients and higher feed conversion ratio are factors that may limit its application. Other strategies based on genetic selection have been theoretically postulated (Chapter 2). However, to our knowledge, there are no reports of the direct response of C18:1 and those assessing its correlated response to selection for other economically important traits are very scarce (Burkett et al., 2008). The objective of this study is to analyze the realized responses of C18:1 in three selection experiments, one for increased intramuscular C18:1 and the other two for other carcass fatness and performance traits.

5.2. MATERIALS AND METHODS

5.2.1. Animals

Three selection experiments were performed in a purebred Duroc population that was completely closed in 1991 and since then it has been selected for an index including body weight (**BW**), backfat thickness (**BT**), and IMF (see Animals and Samples Section). The pigs in all the experiments were castrated within the first week of age. All pigs were performance-tested at an average age of 180 d for BW and BT. At the end of the finishing period the barrows were slaughtered in a commercial slaughterhouse at 210 d of age and a sample of subcutaneous fat (**SF**) was taken at the level of the third and fourth last ribs. After slaughter and chilling for about 24 h at 2ºC, a sample of *gluteus medius* muscle (**GM**) was taken from the ham, as well as a section from the left loin of each carcass at the level of the third and fourth last ribs (*longissimus dorsi*, **LD**). The IMF content (expressed as percentage of fresh matter) and fatty acid composition (expressed as percentage of total fatty acids) of the samples were determined by gas chromatography. Complete details on the procedures are given in the Animals and Samples Section.

5.2.2. Selection experiment 1: Increased intramuscular oleic acid

A selection experiment was conducted to study the effects of selection for increased C18:1 (Exp 1). Selection was practiced among available litters at three established dates throughout 2009 and 2010 (selection batches 1 to 3). A litter born within two weeks before the set date was considered available for selection. In each batch, around 50 litters were allocated into a selected (**S1**) or a control (**C1**) group according to their litter (mid-parent) best linear unbiased prediction (**BLUP**) estimated breeding value (**EBV**) for C18:1. Litters in group C1 were chosen randomly whereas those in group S1 were selected for increased C18:1 in GM. The EBV for C18:1 were obtained from 943 records on full pedigree-connected pigs born since 1996. The genetic evaluations were performed univariately using the same animal model described below but with heritability 0.41. The selection differential was calculated as the difference between the weighted average litter EBV of the pigs in group S1 and C1. The average realized selection differential was 0.57%. Two males per litter were randomly chosen shortly after birth to be performance-tested according to the procedures indicated in the Animals and Samples Section. Pigs from both groups were mixed and reared together. The number of litters and pigs used in the experiment by selection group and batch is given in Table 5.1.

Table 5.1. Number of pigs, litters, and sires, and mean (SD) of oleic acid (C18:1), intramuscular fat (IMF), backfat thickness (BT), and body weight (BW) by selection group and batch in Exp 1.

Selection group	No. of pigs	No. of litters	No. of sires	Traits			
				C18:1, %	IMF, %	BT, mm	BW, kg
Batch 1							
Selected	43	21	7	45.82 (2.98)	4.65 (1.84)	18.93 (3.49)	114.90 (9.14)
Control	43	26	11	45.56 (2.81)	4.23 (2.16)	19.44 (3.53)	114.70 (7.70)
Batch 2							
Selected	49	25	7	44.81 (1.58)	4.28 (2.06)	16.78 (3.83)	105.73 (8.97)
Control	49	24	10	44.53 (1.21)	4.34 (1.48)	17.77 (4.45)	108.22 (9.26)
Batch 3							
Selected	45	20	6	45.71 (1.18)	5.33 (1.72)	18.21 (3.45)	106.33 (9.72)
Control	44	21	9	45.88 (1.20)	5.08 (1.61)	18.25 (2.64)	108.23 (7.29)

5.2.3. Selection experiment 2: Reduced backfat thickness at restrained intramuscular fat

Another experiment was performed to study the effect of selection for decreased BT at restrained IMF on C18:1 (Exp 2). This experiment followed a similar procedure than experiment 1. Selection was practiced among available litters at four established dates throughout 2006 and 2007. In this experiment, 50 litters per batch were allocated

into a selected (**S2**) or a control (**C2**) group according to their litter BLUP EBV for BT and IMF. Litters in group C2 were chosen randomly whereas linear programming was used to select the litters with the lowest EBV for BT while satisfying the restriction of having the same mean EBV for IMF than the litters in group C2. Full details of this selection experiment are given in Chapter 4.

5.2.4. Selection experiment 3: Increased and reduced body weight at restrained backfat thickness

A third selection experiment was performed to assess the correlated response of C18:1 after selection for BW at restrained BT (Exp 3). This experiment took place in two established dates throughout 2002. Similarly to the experiments above, 50 litters per batch were allocated into a high (**H3**), average (**C3**), or low (**L3**) group according to their litter BLUP EBV for BW and BT. Linear programming was used to select litters displaying the maximum difference in the EBV for BW of the groups H3 and L3 respect to C3 while having the most similar average EBV for BT. Full details of this selection experiment are given in Solanes et al. (2009).

5.2.5. Analysis of response to selection

The response to selection was estimated as the difference between the average EBV of the pigs in the selected groups (S1, S2, and H3 or L3) and the pigs in the respective control groups (C1, C2, and C3). The genetic parameters and EBV of the pigs for C18:1, IMF, BT, and BW were estimated fitting a 4-trait multivariate animal model under a Bayesian setting, in line with the methodology described in Chapter 4. A unique analysis was used for all experiments. A summary of the data used for the analyses is given in Table 5.2. Records for BT and BW were collected in pigs born from 1996 to 2012 while carcass traits only in pigs born since 2002 onwards.

The model used was:

$$\mathbf{y}_i = \mathbf{X}_i\mathbf{b}_i + \mathbf{Z}_i\mathbf{a}_i + \mathbf{W}_i\mathbf{c}_i + \mathbf{e}_i\,,$$

where $\mathbf{y}_i$ is the vector of observations for the *i*th trait; $\mathbf{b}_i$, $\mathbf{a}_i$, $\mathbf{c}_i$, and $\mathbf{e}_i$ are the vectors of systematic, additive genetic, litter, and residual effects, respectively; and $\mathbf{X}_i$, $\mathbf{Z}_i$, and $\mathbf{W}_i$, the known incidence matrices that relate $\mathbf{b}_i$, $\mathbf{a}_i$, and $\mathbf{c}_i$ with $\mathbf{y}_i$, respectively. Systematic effects for BW and BT were the batch (1,311 levels), gender (3 levels; males, females, and castrates), and age at test as a covariate. Pigs tested at the same time and in the same unit were considered as one batch. The model for C18:1 and IMF only included the batch (17 levels) and the age at slaughter. The litter effect was not included in the model for carcass traits. Correlated responses for C18:1 in LD and SF were estimated using the same procedure but adding each trait in a 5-trait model.

Table 5.2. Description of the data set used in the analysis of the response to selection.

Item	No. of pigs	No. of litters	No. of sires	No. of dams	Mean	SD
Pedigree	119,390	46,723	863	24,612	-	-
Traits[1]						
BW at test, kg	110,165	42,870	780	22,683	104.9	12.2
BT at test, mm	106,276	42,017	781	22,549	15.6	3.5
IMF, %	1,275	718	175	715	4.9	1.9
C18:1, %	1,280	719	176	716	44.9	2.8
Covariates						
Age at test , d	110,795	43,130	781	22,815	178.9	10.5
Age at slaughter, d	4,545	2,707	401	2,553	207.3	15.7

[1] BW: body weight; BT: backfat thickness; IMF: intramuscular fat; C18:1: oleic acid.

The genetic parameters and EBV for all traits were estimated in a Bayesian framework using Gibbs sampling with the TM software (Legarra et al., 2011). For the 4-trait model, observed phenotypes and missing records imputed by data augmentation were assumed to be conditionally normally distributed as follows:

$$\begin{bmatrix}\mathbf{y}_1\\ \mathbf{y}_2\\ \mathbf{y}_3\\ \mathbf{y}_4\end{bmatrix} | \, \mathbf{b}_1, \mathbf{b}_2, \mathbf{b}_3, \mathbf{b}_4, \mathbf{a}_1, \mathbf{a}_2, \mathbf{a}_3, \mathbf{a}_4, \mathbf{c}_1, \mathbf{c}_2, \mathbf{R} \sim \mathrm{N}\left(\mathbf{X}\begin{bmatrix}\mathbf{b}_1\\ \mathbf{b}_2\\ \mathbf{b}_3\\ \mathbf{b}_4\end{bmatrix} + \mathbf{Z}\begin{bmatrix}\mathbf{a}_1\\ \mathbf{a}_2\\ \mathbf{a}_3\\ \mathbf{a}_4\end{bmatrix} + \mathbf{W}\begin{bmatrix}\mathbf{c}_1\\ \mathbf{c}_2\end{bmatrix}, \mathbf{R} \right),$$

where **R** was the (co)variance matrix. Sorting records by trait, and pig within trait, **R** could be written as $\mathbf{R}_0 \otimes \mathbf{I}$, with $\mathbf{R}_0$ being, in the 4-trait case, the 4 × 4 residual (co)variance matrix between the four traits analyzed and **I** an identity matrix of appropriate order. Flat priors were used for $\mathbf{b}_i$ and residual (co)variance components. Additive genetic and litter values, conditional on the associated (co)variance components, were both assumed multivariate normally distributed with mean zero and with (co)variance $\mathbf{G} \otimes \mathbf{A}$ and $\mathbf{C} \otimes \mathbf{I}$, respectively, where **A** was the numerator relationship matrix, **G** was the 4 × 4 genetic relationship matrix between the four traits, and **C** was the 2 × 2 (co)variance matrix between litter effects of BW and BT. The matrix **A** was calculated using all the pedigree information summarized in Table 5.2. Flat priors were used for additive and litter (co)variance components. Statistical inferences for all unknowns were derived from the samples of the marginal posterior distribution using a unique chain of 1,000,000 iterations, where the first 250,000 were discarded and one sample out of 100 iterations retained. Statistics of marginal posterior distributions and the convergence diagnostics were obtained using the *boa* package (Smith, 2005). Convergence was tested using the *Z*-criterion of Geweke (Geweke, 1992) and visual inspection of convergence plots.

The response to selection for the *i*th trait was calculated as $\bar{a}_{S(i)} - \bar{a}_{C(i)}$, where $\bar{a}_{S(i)}$ and $\bar{a}_{C(i)}$ are the average of the EBV for the *i*th trait in pigs from group S1 (or S2, H3, or L3) and C1 (or C2 or C3), respectively. Overall responses to selection and by batch were calculated. In this latter case only the pigs from the corresponding batch were used in the above expression. Statistical inferences for genetic parameters, realized selection intensities, and responses to selection were derived from random samples of the corresponding marginal posterior distributions. In particular, the mean, the mode, the SD, and the highest posterior density interval at 95% of probability (**HPD95**) of the marginal posterior distributions were calculated. Response to selection was assessed using the HPD95 and the probability of being positive.

5.3. RESULTS

5.3.1. Selection for increased intramuscular oleic acid

The phenotypic values of C18:1, IMF, BT, and BW by selection group and batch of Exp 1 are given in Table 5.1. The differentials of selection applied in Exp 1 and the features of the posterior distribution of the direct and correlated responses to selection for C18:1 in GM are given in Table 5.3. Overall, selection for C18:1 was effective, with a 95% probability of obtaining a positive response. However, responses by batch indicate that response was effective in batches 1 and 2, with C18:1 increases of 0.33−0.35%, but that response in batch 3 was null. In batches 1 and 2 response to selection was approximately half the expected. The increase of C18:1 in GM was accompanied by an increase of C18:1 also in LD in batch 1 but there was less evidence of a correlated response in SF. The overall correlated responses indicated that IMF increased by 0.13% with a probability of 85% and BT decreased by 0.32 mm with a probability of 96%. Selection for C18:1 appeared neutral to BW.

5.3.2. Selection for other carcass fatness and performance traits

The features of the posterior distribution of the responses to selection for reduced BT at restrained IMF are given in Table 5.4, and those to selection for BW at restrained BT in Table 5.5. Because the constraint on IMF was not fully achieved in Exp 2 (Chapter 4), selection against BT was accompanied by a small reduction in IMF. A correlated decrease was also observed for C18:1, with a similar magnitude than the increase obtained by direct selection in Exp 1 (−0.34%, HPD95 [−0.56, −0.08]). In Exp 3, selection succeeded in increasing or reducing BW (+2.27 and −3.17 kg, respectively) without relevantly altering BT. In both cases, moderate increases were observed for both IMF (+0.10−0.22%) and C18:1 (+0.19−27%).

Table 5.3. Differential of selection in Exp 1 and features of the posterior distribution of the response to selection to increased oleic acid content (C18:1) in *gluteus medius* (GM) and of the correlated responses of C18:1 in *longissimus dorsi* (LD) and subcutaneous fat (SF), intramuscular fat content (IMF) in GM, backfat thickness (BT), and body weight (BW).

Trait	Differential	Response				
		Mean	SD	Mode	HPD95[1]	$P(>0)$[2]
C18:1 in GM, %						
Overall	+0.57	+0.24	0.14	+0.26	−0.03, +0.53	0.95
Batch 1	+0.57	+0.35	0.25	+0.25	−0.16, +0.85	0.92
Batch 2	+0.61	+0.33	0.23	+0.38	−0.12, +0.76	0.93
Batch 3	+0.51	+0.04	0.24	+0.10	−0.44, +0.48	0.58
C18:1 in LD, %						
Overall	-	+0.18	0.17	+0.14	−0.15, +0.51	0.86
Batch 1	-	+0.67	0.28	+0.73	+0.09, +1.20	0.99
Batch 2	-	0.00	0.27	+0.03	−0.53, +0.52	0.51
Batch 3	-	−0.09	0.27	−0.15	−0.62, +0.42	0.36
C18:1 in SF, %						
Overall	-	+0.13	0.24	+0.17	−0.31, +0.63	0.71
Batch 1	-	+0.02	0.38	−0.03	−0.74, +0.76	0.53
Batch 2	-	+0.24	0.36	+0.17	−0.44, +0.95	0.74
Batch 3	-	+0.13	0.37	+0.26	−0.62, +0.83	0.64
IMF in GM, %	-	+0.13	0.12	+0.13	−0.10, +0.37	0.85
BT, mm	-	−0.32	0.18	−0.29	−0.67, +0.02	0.04
BW, kg	-	−0.31	0.56	−0.29	−1.34, +0.81	0.29

[1] HPD95: highest posterior density interval at 95% of probability.
[2] $P(>0)$: probability of having a positive response.

Table 5.4. Features of the posterior distribution of the correlated response of oleic acid content (C18:1) to selection for reduced backfat thickness (BT) at restrained intramuscular fat content (IMF) in Exp 2.

Trait	Response				
	Mean	SD	Mode	HPD95[1]	$P(>0)$[2]
BT, mm	−1.24	0.15	−1.26	−1.54, −0.96	<0.01
IMF, %	−0.18	0.10	−0.16	−0.39, +0.01	0.04
C18:1, %	−0.34	0.12	−0.36	−0.56, −0.08	<0.01

[1] HPD95: highest posterior density interval at 95% of probability.
[2] $P(>0)$: probability of having a positive response.

5.4. DISCUSSION

Results of Exp 1 proved that C18:1 responds to selection. However, although selection increased C18:1 in two experimental batches, response was less than expected. Moreover, selection failed to modify C18:1 in a third batch. A possible

Table 5.5. Features of the posterior distribution of the correlated response of oleic acid content (C18:1) and intramuscular fat content (IMF) to selection for increased (High) or reduced (Low) body weight (BW) at restrained backfat thickness (BT) in Exp 3.

Selection for BW	Trait	Response				
		Mean	SD	Mode	HPD95[1]	$P(>0)$[2]
High	BW, kg	+2.27	0.80	+2.28	+0.65, +3.79	>0.99
	BT, mm	+0.06	0.27	+0.11	−0.45, +0.59	0.59
	C18:1, %	+0.27	0.21	+0.32	−0.15, +0.66	0.90
	IMF, %	+0.10	0.18	+0.08	−0.27, +0.44	0.72
Low	BW, kg	−3.17	0.81	−3.00	−4.76, −1.59	<0.01
	BT, mm	−0.06	0.27	−0.08	−0.57, +0.47	0.41
	C18:1, %	+0.19	0.21	+0.31	−0.24, +0.58	0.82
	IMF, %	+0.22	0.18	+0.19	−0.13, +0.59	0.88

[1] HPD95: highest posterior density interval at 95% of probability.
[2] $P(>0)$: probability of having a positive response.

explanation for these results is the low precision of the litter EBV used to select the piglets. For the genetic evaluation of the candidate litters, we used 943 records from pigs born during the years 2002−2007. Candidate litters in batches 1 and 2 were born two years later, in October and November 2009, respectively, and those in batch 3 were born in November 2010. The Spearman's correlation coefficient between the C18:1 EBV used for litter selection (i.e., univariate model and no own phenotypic records) and those used for the response estimation (i.e., multivariate model and own phenotypic records) was 0.20 and 0.17 for batches 1 and 2, respectively, but only 0.04 for batch 3. This indicated that the ranking for C18:1 EBV was (poorly) conserved in batches 1 and 2 but not in batch 3. This emphasized the need for a sampling scheme allowing recording of C18:1 for estimating accurate EBV. A retrospective analysis was performed by including the phenotypic data of one littermate per litter during the estimation of the litter EBV. This increased the Spearman's correlations (calculated as above but excluding the littermates whose data was used) to 0.67 (n=27), 0.71 (n=32), and 0.65 (n=29) for experimental batches 1 to 3, respectively. If selection had been performed picking up the top half of these subsets, selection responses would have been +1.32%, +1.15%, and +0.67%, respectively. Overall, the results of this experiment, together with theoretical predictions (Chapter 2), indicated that C18:1 would effectively respond to selection if an adequate recording system is implemented.

This selection experiment was based on C18:1 and IMF records from GM. In batch 1, a positive correlated response on C18:1 was observed also in LD, but not in SF. This was consistent with the higher genetic correlation estimated between these two muscles than between them and SF (Chapter 3). In this study, the genetic correlation of C18:1 in GM was estimated to be 0.66 with LD and 0.33 with SF. Other studies have found higher genetic correlations (0.66−0.72) between fatty acids content

of muscles and SF (Suzuki et al., 2006; Yang et al., 2010), but these estimates could be highly dependent on the anatomical location of the samples (as suggested in Chapter 3). More surprising was the lack of correlated responses in LD in batch 2, where a positive response in GM was achieved. The reasons for the unexpected correlated responses of IMF and C18:1 in LD in batch 2 are not clear. Previous results also supported that genetic gains in one muscle can indirectly affect other non-selected muscles; e.g., those in Chapter 4 showing similar responses for IMF content in GM (directly selected) and in LD (correlated). Taking these results together, direct selection for C18:1 based on records from GM is expected to favorably modify C18:1 in LD, although this effect may be not always observed. More accurate EBV could increase the chance of favorable correlated responses in LD but maybe less in SF. We have proved that by using littermates data, EBV accuracy can be significantly increased and higher responses obtained. In this retrospective scenario, C18:1 correlated responses in LD would have been +0.91%, +0.88%, and +0.90% in batches 1 to 3, respectively, and in SF +0.37%, +0.28%, and +0.05%, respectively. Correlated responses may need to be assessed locally in other muscles of interest as they could be unaffected by selection (Chapter 3).

The correlated genetic changes of other traits of economic interest were analyzed. The greatest genetic correlation of C18:1 was found with IMF (0.44; Chapter 2). An overall positive correlated response was observed for IMF, but it tended to decrease in batch 2 (results not shown). Interestingly, on the other hand, BT decreased in both batches where selection was effective. This result was unexpected in light of the positive correlation between C18:1 and BT (0.30). Several studies have found C18:1 (in either IMF or SF) either hardly correlated with BT (Suzuki et al., 2006) or negatively correlated with carcass leanness traits (Ntawubizi et al., 2010; Gjerlaug-Enger et al., 2011), but, to our knowledge, no negative correlations have been reported with BT or carcass fatness that could explain this correlated response. Consistently with the very low correlation between C18:1 and BW, selection for C18:1 was almost neutral to BW. Overall the variability of the correlated responses per batch indicated that the selection for C18:1 led to little changes in other economic traits.

In Exp 2, BT decreased as intended but IMF was constrained only partially (Chapter 4). Despite that theoretically IMF and carcass fatness are susceptible to independent modification (Clutter, 2011), experience has shown that this goal is difficult to achieve in practice (Suzuki et al., 2005a,b; Schwab et al., 2009, 2010). Because IMF decreased to some extent, so did C18:1. The results from Exp 3, on the other hand, showed that selecting for high BW at restrained BT tended to cause a small positive correlated response on IMF, but surprisingly, a positive correlated response in IMF was also achieved when selecting for low BW at restrained BT. Despite that the magnitude of the correlated responses of IMF in this last experiment was smaller than

expected (Solanes et al., 2009), it is interesting to note that they were accompanied by a similar correlated response on C18:1. Taken together, results of Exp 2 and 3 indicated that C18:1 and IMF behave similarly in terms of correlated responses. Accordingly, genetic strategies aimed at improving IMF would indirectly improve also C18:1. This is consistent with the simulation of the expected responses based on the genetic parameters reported in Chapter 2, which already indicated that favorable C18:1 correlated changes would only be achieved following proactive selection for IMF but not for BT or BW. Unfortunately, there are scarce reports on correlated responses of C18:1 to selection on other traits. In a selection experiment that raised IMF by 1.95%, total monounsaturated fatty acids (mostly C18:1) in LD increased by 1% (although not significantly), while polyunsaturated fatty acids decreased (Burkett et al., 2008). Similar results were obtained in a divergent selection experiment performed in rabbits (Zomeño et al., 2009), where lines selected for high and low IMF differed by 1.16% and 1.58% for C18:1 and total monounsaturated fatty acids in LD, respectively. It is known that as IMF develops, the endogenous synthesis of saturated and monounsaturated fatty acids, including C18:1, increases and results in a greater accumulation of neutral lipids in the adipocytes respect to the phospholipids fraction (Wood et al., 2008).

5.5. CONCLUSIONS

To our knowledge, this is the first report on realized responses to selection for fat composition in pigs. Results indicated that direct selection for C18:1 is possible but they also emphasized the need for accurate EBV, which requires a systematic recording that in general is not available yet. Records taken on a particular muscle like GM could successfully improve C18:1 not only in the criterion muscle but also in LD but caution is recommended for other muscles and SF. Oleic acid is expected to respond to selection similarly to IMF. Results reported here prove that there are opportunities for genetically improving the fatty acid profile of pork for high-quality products.

PART 3

Chapter 6.
A functional variant in the stearoyl-CoA desaturase gene promoter enhances fatty acid desaturation in pork

J. Estany, R. Ros-Freixedes, M. Tor, & R. N. Pena

Departament de Producció Animal, Universitat de Lleida − Agrotecnio Center, 191 Av. Alcalde Rovira Roure, 25198 Lleida, Catalonia, Spain.

The content of this chapter was published in PLoS One (2014), 9: e86177. DOI: 10.1371/journal.pone.0086177

ABSTRACT: There is growing public concern about reducing saturated fat intake. Stearoyl-CoA desaturase (SCD) is the lipogenic enzyme responsible for the biosynthesis of oleic acid (C18:1) by desaturating stearic acid (C18:0). Here we describe a total of 18 mutations in the promoter and 3' non-coding region of the pig *SCD* gene and provide evidence that allele T at AY487830:*g.2228T>C* in the promoter region enhances fat desaturation (the ratio C18:1/C18:0 in muscle increases from 3.78 to 4.43 in opposite homozygotes) without affecting fat content (C18:0+C18:1, intramuscular fat content, and backfat thickness). No mutations that could affect the functionality of the protein were found in the coding region. First, we proved in a purebred Duroc line that the C-T-A haplotype of the 3 single nucleotide polymorphisms (SNPs) (*g.2108C>T*, *g.2228T>C*, *g.2281A>G*) of the promoter region was additively associated to enhanced C18:1/C18:0 both in muscle and subcutaneous fat, but not in liver. We show that this association was consistent over a 10-year period of overlapping generations and, in line with these results, that the C-T-A haplotype displayed greater *SCD* mRNA expression in muscle. The effect of this haplotype was validated both internally, by comparing opposite homozygote siblings, and externally, by using experimental Duroc-based crossbreds. Second, the *g.2281A>G* and the *g.2108C>T* SNPs were excluded as causative mutations using new and previously published data, restricting the causality to *g.2228T>C* SNP, the last source of genetic variation within the haplotype. This mutation is positioned in the core sequence of several putative transcription factor binding sites, so that there are several plausible mechanisms by which allele T enhances C18:1/C18:0 and, consequently, the proportion of monounsaturated to saturated fat.

6.1. INTRODUCTION

Good eating habits are conducive to good health. Total fat and fatty acid content in food affect both human health and food quality and, consequently, they are becoming increasingly important to consumers. There is convincing evidence that a high dietary intake of saturated fat (**SFA**) increases the risk of lipid metabolism disorders which are common to many human chronic diseases (FAO, 2010). Conversely, the intake of monounsaturated (**MUFA**) and polyunsaturated (**PUFA**) fat has beneficial effects over human health (de Lorgeril & Salen, 2012). In this regard, dietary guidelines advice that optimal intake of SFA should account for no more than 10% of the total diet energy, in line with recent findings suggesting that dietary composition may matter for longevity more than calorie count (Mattison et al., 2012). Worldwide, the demand for meat, but specifically pork, has increased from the 1980s onwards driven by growing human population and incomes (Smith et al., 2010). Although pork is rich in bioavailable macro- and micronutrients, it is also a source of dietary SFA (McAfee et al., 2010). In addition to nutritional aspects, fat content and fatty acid composition also influence relevant manufacturing and organoleptic properties of pork (Wood et al., 2003, 2008). Thus, high levels of intramuscular fat (**IMF**) and MUFA are favorably associated to texture, juiciness, flavor, and general acceptability of high-quality products (Wood et al., 2003, 2008) (Figure 6.1). Therefore, a reasonable strategy to deal with both healthy and quality constraints is to substitute dietary SFA with MUFA.

The pork fatty acid composition varies across fat tissues and muscles and it is greatly influenced by the genetic type of the pig, the diet, and, in general, by any factor

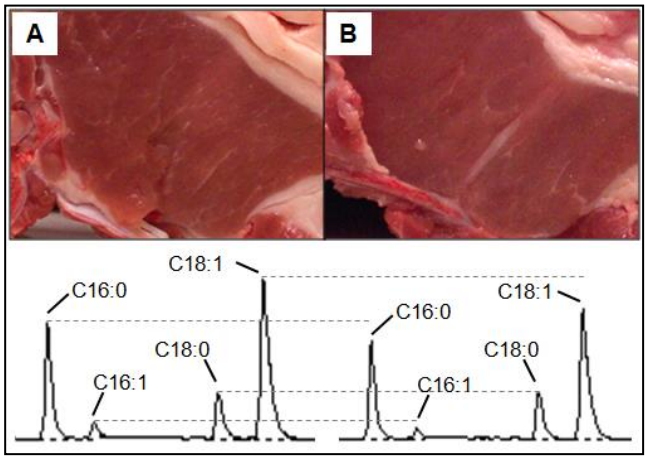


Figure 6.1. Pork loins with optimal intramuscular fat but different monounsaturated fatty acid content. The monounsaturated pamitoleic (C16:1) and oleic (C18:1) acids are more abundant in the loin in panel A (4.0% and 44.2%, respectively) than in the loin in panel B (3.0% and 41.4%), expressed as percentage with respect to total fatty acids. The peaks of these two fatty acids in the chromatograms below are labelled accordingly, along with those of their respective precursors, palmitic (C16:0) and stearic (C18:0) acids. The desaturation ratios C16:1/C16:0 and C18:1/C18:0 are higher in loin A (0.16 and 3.7, respectively) than in loin B (0.12 and 2.8, respectively). Genotyping for *g.2228T>C* in the promoter region of the *SCD* gene revealed that loin A was homozygous for allele T and loin B homozygous for allele C.

affecting fatness, such as gender or age (Nürnberg et al., 1998; Bosch et al., 2012). In this regard, the use of the Duroc breed is becoming very popular in quality conscious consumer segments because of their high level of IMF relative to subcutaneous fat. However, regardless of the genetic type, the deposition of dietary fatty acids is small compared to fatty acid synthesis, with endogenous oleic (**C18:1**), palmitic (**C16:0**), and stearic (**C18:0**) acids representing more than 80% of the total deposited fatty acids (Kloareg et al., 2007). The stearoyl-CoA desaturase (**SCD**) is the rate-limiting enzyme required for the biosynthesis of MUFA from SFA. In particular, SCD catalyzes the desaturation of palmitoyl-CoA and stearoyl-CoA substrates at the Δ^9 position to produce *de novo* palmitoleoyl-CoA and oleoyl-CoA, respectively. Maintaining a balance in the SCD activity is paramount to optimize health (Paton & Ntambi, 2009; Merino et al., 2010) and, therefore, *SCD* expression, both in normal and in disease states, is tightly controlled by dietary and hormonal factors (Mauvoisin & Mounier, 2011). *SCD* is largely expressed in liver and adipose tissue, responding positively to high carbohydrate diets and negatively to starvation and PUFA rich diets. The ratio of C18:1 to C18:0 (**C18:1/C18:0**) is commonly used as an indirect indicator of SCD activity. Alterations in this desaturation ratio have been linked to cardiovascular disease, obesity, diabetes, and cancer (Miyazaki & Ntambi, 2003; Ntambi & Miyazaki, 2004; Paton & Ntambi, 2009; Merino et al., 2010; Mauvoisin & Mounier, 2011), and correlated with longevity (Hulbert, 2010). Recent evidence indicates that SCD also plays an important role in defining plasma and tissue lipid profiles (Merino et al., 2010).

In pigs, the *SCD* gene is assigned to chromosome SSC14q27 (Ren et al., 2003). The position of this gene co-localizes with quantitative trait loci for muscle content of C18:0 and C18:1 described in Duroc-based populations (Sanchez et al., 2007; Quintanilla et al., 2011). *SCD* is, therefore, an attractive positional candidate gene (Uemoto et al., 2012c). In fact, findings so far support that there is genetic variation in the *SCD* gene affecting fatty acid composition of muscle and adipose tissue. Several single nucleotide polymorphisms (**SNPs**) in the *SCD* promoter region have been associated to C18:0 and C18:1 content. Yet, results are inconclusive, as either the location of haplotypes is not coincident (Uemoto et al., 2012b; Maharani et al., 2013), favorable alleles are swapped (Renaville et al., 2013), or even no association was found (Bartz et al., 2013). We have been collecting since 2002 samples of subcutaneous fat, muscle, and liver from a full-pedigreed Duroc line (Animals and Samples Section) and muscle samples from three *ad hoc* pig crossbreds divergent for fatness. Fat content and composition data is currently available for all these samples. Here we use this repository to provide evidence that allele T at SNP AY487830:*g.2228T>C* in the *SCD* gene is a causative mutation that promotes fat desaturation in muscle and subcutaneous fat.

6.2. MATERIALS AND METHODS

6.2.1. Animals and tissue sampling

An association analysis (Exp 1) was done using genomic DNA and phenotypic data of twelve batches (n=891) of purebred Duroc barrows from the line described in the Animals and Samples Section (Duroc-1). In two of these batches, crossbred Duroc (DU-3 × DU-1), Duroc × Iberian (IB-2 × DU-1), and Large White × Landrace (LW-1 × L-2) barrows (Exp 2) were contemporaneously raised to Duroc-1 barrows, for validation purposes (n=170). Pigs in the same batch were raised from 75 d of age until slaughter at 210 d in the same farm under identical conditions. All batches were managed following the same standard protocol for data recording and tissue sampling (Animals and Samples Section). Barrows had *ad libitum* access to commercial diets. In two of the Duroc batches at 180 d of age three 10-ml samples of blood per barrow were obtained between 8 and 10 a.m. after an overnight fast. All pigs were slaughtered in the same commercial abattoir, where lean content and other carcass traits were measured by using an on-line ultrasound automatic scanner. Immediately after slaughter, samples of the *semimembranosus* muscle, subcutaneous adipose tissue at the level of the third and fourth ribs, and liver were collected, snap-frozen, and stored at −20°C. After chilling for about 24 h at 2ºC, a sample of the *gluteus medius* muscle was excised from the left side ham, vacuum packaged, and stored at −20ºC. A sample of *longissimus dorsi* muscle was collected for a subset of animals. Finally, we used genomic DNA representing European wild boar and several domestic breeds of pigs and commercial crossbreds for monitoring haplotype segregation.

6.2.2. Fatty acid and blood lipid indicator analysis

Fat content and fatty composition was determined in duplicate by quantitative determination of the individual fatty acids by gas chromatography as detailed in the Animals and Samples Section. Blood triglycerides, cholesterol, leptin, and insulin-like growth factor-1 were determined using available kits (Muñoz et al., 2013a).

6.2.3. Nucleic acids isolation

Genomic DNA was isolated from freeze-dried muscle samples using standard protocols (Sambrook & Russell, 2001). Total RNA was isolated from fat, liver, and *semimembranosus* muscle. Samples (50 mg) were homogenized in 1 ml of TRI Reagent (Sigma-Aldrich, Madrid, Spain) using a mechanical rotor (IKA Werke, Staufen, Germany) following the manufacturer's instructions.

6.2.4. Sequencing of promoter and exonic regions of the pig *SCD* gene

Based on genomic and cDNA sequences (GenBank accession numbers AY487830 and NM_213781, respectively) primers were designed in order to amplify and sequence 780 bp of the *SCD* proximal promoter and the entire exonic regions of the gene. Seven primer sets were designed with the Primer3Plus online oligonucleotide design tool (http://primer3plus.com; Untergasser et al., 2007) (Table 6.1). The promoter and 3' non-coding region were amplified from approximately 60 ng of genomic DNA from twelve Duroc pigs selected to represent extreme levels of C18:1 in *gluteus medius*. PCR reaction of a final volume of 25 µl contained 200 nM of each primer, 160 mM dNTPs, 3 mM $MgCl_2$, and 0.4 U of *Taq* DNA polymerase (Biotools, Madrid, Spain). PCR conditions were as follows: 95ºC for 5 minutes, 35 cycles of 95ºC for 20 s, annealing temperature as in Table 6.1 for 40 s, and 72ºC for 90 s, and completed by an extension step at 72ºC for 5 min. The 5' non-coding and coding regions were amplified using the same reaction and cycling conditions from total RNA of *semimembranosus* muscle retrotranscribed to cDNA as indicated in Section 6.2.6. PCR amplicons were sequenced on an ABI-3100 capillary sequencer (Applied Biosystems, Foster City, CA) with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequences were aligned with the ClustalW

Table 6.1. Sequence of DNA primers used in the characterisation of the porcine *SCD* gene. A list of the primers used to amplify and sequence seven fragments of the porcine *SCD* gene encompassing 780 bp of the promoter and the entire coding and 5' and 3' non-coding regions (3'UTR). The annealing temperature used in the PCR cycling program is also indicated.

Primer name	Sequence 5' → 3'	Amplicon size	Annealing temperature
promoter_F	ACTTCCCTAGTGCCCATCCT	980 bp	58ºC
promoter_R	GATCACTTTCCCAGGGATGA		
cDNA_F	GTCTCATCCCTGGGAAAGTG	1160 bp	62ºC
cDNA_R	CAGCTGGCTTTCAGAAAAGG		
3'UTR_F1	AAGTATCCAAGGCTGCCATC	866 bp	58ºC
3'UTR_R1	CAATTCCGGAAAGAACCTCA		
3'UTR_F2	TGGGGAAGAAGTCTTTCTTGT	990 bp	58ºC
3'UTR_R2	GGTTCAGTGACCCTGAGCAT		
3'UTR_F3	TTTCCTGCCGGTTCTATCTC	945 bp	60ºC
3'UTR_R3	GAGTAGGTGCTTGGGTCTGG		
3'UTR_F4	ATGGAGGATAAAGGGGTTGG	648 bp	60ºC
3'UTR_R4	ACTTGCCCAGGGTCACATAG		
3'UTR_F5	GTCAAGGTTACACGGGTGGT	742 bp	58ºC
3'UTR_R5	CAGGACATAGGGTGGCAGAT		

Table 6.2. Primers used for genotyping the three single nucleotide polymorphisms (SNPs) in the porcine *SCD* gene promoter with an allelic discrimination assay.

Polymorphism in AY487830	Primer name	Sequence 5' → 3'	Final concentration
g.2108C>T	Primer Forward	AGTGTCTGCAGCATCCAGTTTT	900 nM
	Primer Reverse	GCATGGGCGGGAGAGG	900 nM
	Probe for G allele	VIC-CCAGCAAGCCCC-NFQ	200 nM
	Probe for A allele	FAM-CCCAGTAAGCCCC-NFQ	200 nM
g.2228T>C	Primer Forward	CCCTTCTTGGCAGCGAATAAAA	900 nM
	Primer Reverse	CAGGCTGGGTATTTAAAGGCTAGAG	900 nM
	Probe for C allele	VIC-CGACCGTGTCCTGTATT-NFQ	200 nM
	Probe for T allele	FAM-CGACCGTATCCTGTATT-NFQ	200 nM
g.2281A>G	Primer Forward	TGCCAGCTCTAGCCTTTAAATACC	900 nM
	Primer Reverse	CACGTTGGGTCGGTGTCT	900 nM
	Probe for G allele	VIC-ACCCGCGCACAGCA-NFQ	200 nM
	Probe for A allele	FAM-AGACCCACGCACAGCA-NFQ	200 nM

alignment tool (http://www.ebi.ac.uk/Tools/msa/clustalw2/) and compared to identify polymorphic sites. All sequences have been submitted to the GenBank data base (accession numbers KC736975 and KC736976).

6.2.5. Genotyping the pig *SCD* promoter

Three *SCD* promoter polymorphisms (AY487830:*g.2108C>T*, *g.2228T>C*, and *g.2281A>G*) were genotyped with allele discrimination assays (Custom TaqMan SNP Genotyping Assays, Applied Biosystems) using the primers and probes described in Table 6.2. For all of them, 15 ng of genomic DNA were used in 8 µl reactions containing 1x TaqMan Universal PCR Master Mix (Applied Biosystems) and 900 nM primers and 200 nM probes. Cycling conditions were as follows: initial denaturation at 95ºC for 10 min and 40 cycles at 93ºC for 5 sec and 60ºC for 1 min.

6.2.6. Gene expression analysis

SCD expression levels were measured by quantitative real-time PCR (qPCR) in *semimembranosus* muscle, subcutaneous fat, and liver and from a subset of 45 animals representing all diplotypes. Total RNA (1 µg) was treated with Turbo DNA-free DNase (Ambion, Austin, TX) according to the manufacturer's protocol and retrotranscribed with 0.5 pmol of random hexamers using 100 U of MuMLV reverse transcriptase (Fermentas, St. Leon-Rot, Germany) at 25ºC for 10 min, 42°C for 1 h,

and 70ºC for 10 min. cDNA was diluted 1:10 in DEPC-treated H_2O prior to qPCR analysis. Primers, PCR conditions, and data normalization was conducted as in Cánovas et al. (2010).

6.2.7. Estimating haplotype effects

The haplotype effect was estimated within tissue using a linear model including the diplotype and the batch (JMP 8, SAS Institute Inc., Cary, NC). The age at slaughter and fat content were tested as covariates in the model. The haplotype additive (a) and dominant (d) effects were tested replacing the diplotype effect by the covariates a (coded as +1 and −1 for homozygous and 0 for heterozygous diplotypes) and d (coded as 0 for homozygous and +1 for heterozygous diplotypes). The effects of the diplotype and covariates were tested using the F-statistic and the differences among diplotypes were contrasted with the Tukey-HSD test. The batch was removed from the model when results were expressed on a batch basis (Exp 1). The haplotype effect in the validation experiment (Exp 2) was estimated within genetic type using the same procedure. In IB-2 × DU-1 and LW-1 × L-2 crossbreds, the sire effect was included in the model because only two IB-2 and LW-1 sires were used. A paired t-test was used for comparing homozygote siblings. The additive fraction of the genetic variance accounted for by the diplotype was calculated as $2pqa^2$ (Falconer & Mackay, 1996) divided by the additive genetic variance. The genetic variance for fatty acids and their ratios were estimated using the approach in Chapter 2 and univariate animal models including the full pedigree since 1991.

6.2.8. *In silico* analysis of the *SCD* promoter

To characterize the *SCD* promoter, a computer-assisted identification of putative promoter/enhancer elements was performed using the GENOMATIX software suite (Genomatix Software GmbH) (Cartharius et al., 2005). Genomatix Matrix Library 8.3 was used with a core similarity threshold of 0.85 and an optimized matrix similarity threshold (program default). The Gene2Promoter application was used to retrieve the *SCD* promoter from pig, human, cow, and sheep. Common transcription factor binding motifs were explored using the CommonTF, DiAlignTF, and MatInspector applications for pattern search and analysis.

6.3. RESULTS

6.3.1. Sequence variation in the *SCD* gene in Duroc pigs

The 5’ and 3’ non-coding regions, coding region, and 680 bp upstream on the proximal promoter of the pig *SCD* gene were sequenced in twelve Duroc pigs

representing extreme phenotypes for muscle C18:1 content. A total of 18 polymorphisms were identified: three in the promoter and 15 in the 3' non-coding region (Table 6.3). No variation was found in the sequence corresponding to the *SCD* coding and 5' non-coding regions.

The *SCD* transcription unit spans 16,186 bp and includes a coding region of 1,079 bp plus an unusually long 3'UTR of 4,047 bp. Despite being over 12 kb apart, in the Duroc animals analyzed the polymorphisms of promoter and 3'UTR regions formed one haplotype block which displayed >95% overall linkage disequilibrium (r^2=0.965 between SNPs *g.2108C>T* in the promoter and *g.15109A>G* in the 3'UTR). The three SNPs in the promoter region were close together in a 173 bp fragment (Figure 6.2). Given the lack of sequence variation in the coding region of the gene, we focus on the study of the three SNPs in the promoter region as these might potentially influence the *SCD* mRNA expression levels affecting, therefore, the total SCD activity of the cells.

Table 6.3. Description of the polymorphisms identified at *SCD* gene. Eighteen polymorphisms in the *SCD* gene were found to be segregating in the investigated Duroc population by comparing the DNA sequence of six pigs with extreme high and low values for oleic acid content in *gluteus medius* muscle. Position numbering is relative to the translation start codon and the genomic sequence AY487830. Three of the polymorphisms are single-nucleotide substitutions in the promoter region.

Polymorphism in AY487830	Gene region	Sequence change	Position relative to ATG	Variant 1	Variant 2
g.2108C>T	Promoter	Y	−353	C	T
g.2228T>C	Promoter	Y	−233	T	C
g.2281A>G	Promoter	R	−180	A	G
g.14924G>A	3'UTR	R	+1382	G	A
g.14981C>T	3'UTR	Y	+1439	C	T
g.15013T>C	3'UTR	Y	+1471	T	C
g.15060A>G	3'UTR	R	+1518	A	G
g.15109A>G	3'UTR	R	+1566	A	G
g.15115_15119insATGG	3'UTR	ins(ATGG)	+1572	-	ATGG
g.15157C>T	3'UTR	Y	+1618	C	T
g.15294G>A	3'UTR	R	+1755	G	A
g.16195G>A	3'UTR	R	+2656	G	A
g.16617A>G	3'UTR	R	+3078	A	G
g.16623A>G	3'UTR	R	+3084	A	G
g.16663T>C	3'UTR	Y	+3124	T	C
g.17305G>C	3'UTR	S	+3766	G	C
g.17313G>T	3'UTR	K	+3774	G	T
g.17437A>C	3'UTR	M	+3898	A	C

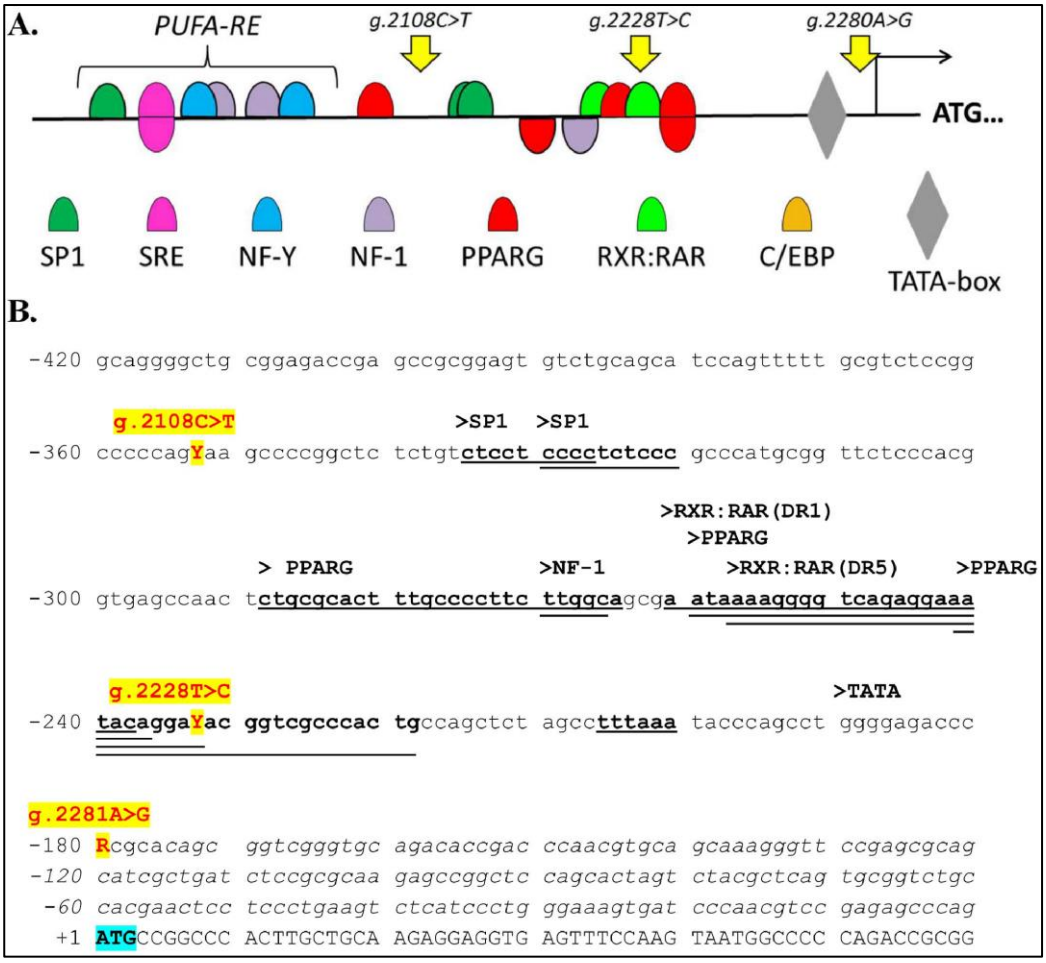


Figure 6.2. Characterization of the 5' flanking region to the transcription start site of the pig *SCD* gene. (A) Schematic representation of recognition motifs for several transcription factor binding sites in the proximal 5' flanking region of the pig *SCD* gene. The relative position of the three SNPs polymorphisms identified in this promoter (AY487830:*g.2108C>T*, *g.2228T>C*, and *g.2281A>G*) are indicated. (B) Sequence encompassing three SNPs polymorphisms in the promoter region of the pig *SCD* gene. Position numbering is relative to the translation START codon (in blue). The transcription start site is at position −175 (arrow in panel A). Coding sequence and the 5' non-coding region is shown in uppercase and italics, respectively. The motifs for transcription factors SP1, PPARγ, NF-1, RXR:RARα, and the TATA-box are underlined and notated above the sequence.

6.3.2. Association of *SCD* haplotypes with desaturation ratios

In a first experiment we genotyped all the available purebred Duroc pigs in the repository (n=891) which had at least one tissue analyzed for fatty acid composition (Exp 1; Table 6.4). The segregation analysis of the three SNPs in this population

Table 6.4. Haplotype frequencies of single nucleotide polymorphisms AY487830:*g.2108C>T*, *g.2228T>C*, and *g.2281A>G* at the promoter region of the *SCD* gene in different pig populations.

Haplotype	Polymorphism in AY487830			Genetic type[1]										
				Purebred						Crossbred				
				Exp 1	Other					Exp 2			Other	
	g.2108C>T	*g.2228T>C*	*g.2281A>G*	Duroc-1	Duroc-2	Landrace-1	Pietrain	Iberian-1	Wild boar	DU-3 × DU-1	IB-2 × DU-1	LW-1 × L-2	LW-2 × L-3	(LW-3 × L-4) × DU-4
1	C	T	A	783	26	40	39	81	14	67	76	81	37	18
2	T	C	G	988	11	0	1	0	0	48	39	0	0	11
3	T	C	A	0	3	0	0	0	0	0	0	26	3	9
4	T	T	G	2	0	0	0	0	0	0	0	0	0	0
5	T	T	A	4	0	0	0	1	0	0	1	0	0	2
6	C	C	G	2	0	0	0	0	0	1	0	0	0	0
7	C	C	A	0	0	0	0	0	0	0	0	1	0	0
8	C	T	G	3	0	0	0	0	0	0	0	0	0	0
No. of animals				891	20	20	20	41	7	58	58	54	20	20

[1] Purebred pigs include Duroc (DU), Landrace (L), Pietrain, Iberian (IB), and wild boar. Numbers after the breed refer to independent lines from the same breed. The Duroc-1 was the line used for the association analysis (Exp 1) and crossbreds in Exp 2 where those used for the validation analysis.

revealed that they are in strong linkage disequilibrium (r^2>0.97), with two clearly predominant haplotypes (**H1**: C-T-A, frequency 43.7%; and **H2**: T-C-G, frequency 55.5%). The results of the association analysis confirmed that pigs carrying the H1 haplotype had higher C18:1/C18:0 ratio in the three muscles analyzed (*gluteus medius*, *longissimus dorsi*, and *semimembranosus*) and subcutaneous fat but not in liver (Figure 6.3). We proved that this haplotype behaved additively, with an average additive effect for C18:1/C18:0 in the muscle *gluteus medius* of 0.33 (Table 6.5), but also that it did not affect the amount of C18:0+C18:1. Moreover, these effects were consistent across batches, thereby showing both genetic stability over generations and environmental stability against occasional dietary and management changes. A similar trend was found for the ratios of palmitoleic acid (**C16:1**) to C16:0 (**C16:1/C16:0**) and of MUFA to SFA (**MUFA/SFA**) (Table 6.6). As a result, the substitution effect of H1 for H2 for MUFA, C18:1, and C16:1 in the *gluteus medius* muscle was 1.02%, 0.70%, and 0.30%, respectively. Adjusting these values for the age at slaughter and fat content did not change the results. Because segregation was at intermediate frequencies, the above

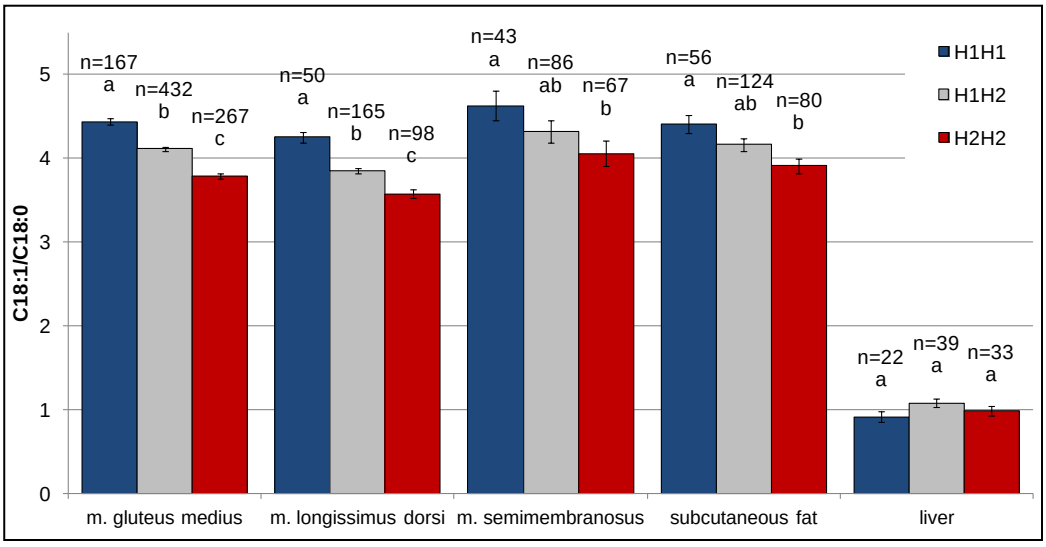


Figure 6.3. Desaturation ratio by *SCD* diplotype and tissue in purebred Duroc. The presence of haplotype H1 is associated to higher C18:1/C18:0 ratio both in intramuscular and subcutaneous fat. The H1H1 pigs have a greater C18:1/C18:0 ratio than the H2H2 animals in the muscles *gluteus medius* (H1H1−H2H2: 0.65), *longissimus dorsi* (H1H1−H2H2: 0.67), and *semimembranosus* (H1H1−H2H2: 0.57), and in the subcutaneous fat (H1H1−H2H2: 0.50), with the heterozygote H1H2 showing an intermediate effect. No difference is observed among diplotypes in liver. Error bars represent standard errors. Columns lacking a common letter within tissue differ (p<0.01, for *gluteus medius* and *longissimus dorsi*; p<0.05, for *semimembranosus* and subcutaneous fat).

Table 6.5. Desaturation ratio C18:1/C18:0 and content of C18:0+C18:1 by batch and *SCD* diplotype in purebred Duroc. The haplotype H1 affects the C18:1/C18:0 ratio in the muscle *gluteus medius* but not the C18:0+C18:1 content (in percentage of total fatty acids). H1 exerts a consistent favorable additive effect on the desaturation ratio across all time-batches. Analyses were performed both within batch (1 to 12) and across batches (All). Values are expressed as the least square mean for each trait by genotype. Means lacking a common superscript within trait differ (p<0.05). The number of pigs (n) genotyped per batch ranged from 22 to 109. The frequency of the haplotype H1 (f(H1)) by batch ranged from 0.33 to 0.57.

Batch	Year	n	f(H1)	C18:1/C18:0								C18:0+C18:1 (%)			
				Diplotype				Additive (a) and dominant (d) values				Diplotype			
				H1H1	H1H2	H2H2	*p*-value	a	*p*-value	d	*p*-value	H1H1	H1H2	H2H2	*p*-value
1	2002	109	0.33	3.37^{a}	3.34^{a}	3.04^{b}	<0.001	0.16	0.004	0.14	0.07	53.98	54.55	54.35	0.53
2	2003	71	0.51	3.45^{a}	3.19^{b}	3.05^{b}	0.002	0.20	<0.001	−0.06	0.36	55.03^{b}	56.13^{a}	55.72^{ab}	0.04
3	2003	28	0.46	3.18^{a}	2.96^{a}	2.65^{b}	0.001	0.27	<0.001	0.04	0.62	55.09	55.64	56.14	0.30
4	2006	28	0.57	4.33	3.98	3.70	0.10	0.32	0.038	−0.04	0.88	55.00	54.24	53.90	0.53
5	2006	22	0.55	4.86^{a}	4.43^{b}	3.75^{c}	<0.001	0.55	<0.001	0.12	0.42	55.49	55.84	54.37	0.38
6	2006	109	0.44	6.20^{a}	5.83^{a}	5.37^{b}	<0.001	0.42	<0.001	0.05	0.69	57.10	57.31	56.77	0.55
7	2007	101	0.46	4.92^{a}	4.54^{b}	4.29^{b}	<0.001	0.31	<0.001	−0.07	0.50	56.38	56.94	56.90	0.75
8	2008	66	0.44	5.96^{a}	5.12^{b}	4.54^{b}	<0.001	0.71	<0.001	−0.13	0.56	58.26	56.65	56.64	0.05
9	2008	72	0.38	4.35^{a}	3.77^{b}	3.50^{c}	<0.001	0.43	<0.001	−0.15	0.07	55.46^{a}	53.90^{b}	54.46^{ab}	0.02
10	2010	84	0.39	4.35^{a}	4.29^{a}	3.89^{b}	0.006	0.23	0.011	0.17	0.20	56.91	56.60	56.50	0.80
11	2010	95	0.50	4.34^{a}	4.04^{b}	3.72^{c}	<0.001	0.31	<0.001	0.01	0.87	55.79	55.75	56.30	0.36
12	2011	81	0.49	4.11^{a}	3.82^{b}	3.64^{c}	<0.001	0.23	<0.001	−0.05	0.43	57.78	57.71	58.15	0.22
All	-	866	0.44	4.43^{a}	4.11^{b}	3.78^{c}	<0.001	0.33	<0.001	0.00	>0.99	56.10	56.03	55.98	0.81

Table 6.6. Carcass weight, fat content, and fatty acid composition by *SCD* diplotype and fat tissue in purebred Duroc. The haplotype H1 showed a favorable effect on fatty acid composition traits resulting from increased *SCD* activity (C16:1/C16:0, C18:1/C18:0, MUFA/SFA, C18:1, C16:1, and MUFA) and no effect on fat content-related traits (carcass weight, lean content, intramuscular fat content, C16:0+C16:1, C18:0+C18:1, and SFA+MUFA). This pattern was more evident in muscle than in subcutaneous fat. Values are expressed as the least square mean (±standard error) for each trait by diplotype. Means lacking a common superscript within trait differ (p<0.05).

Trait[1]	Diplotype			
	H1H1	H1H2	H2H2	*p*-value
No. of pigs	166	435	268	-
Age at sampling (d)	212.1	212.0	211.9	-
Carcass weight (kg)	95.7±0.8	96.9±0.5	96.7±0.6	0.38
Backfat depth (mm)	22.6±0.3	23.1±0.2	22.9±0.2	0.33
Lean content (%)	43.9±0.4	43.3±0.2	43.5±0.3	0.30
Muscle *gluteus medius*				
No. of pigs	167	432	267	-
IMF (% dry matter)	16.57±0.37	16.42±0.23	16.55±0.30	0.91
C16:1 (%)	4.10±0.05^{a}	3.76±0.03^{b}	3.50±0.04^{c}	<0.001
C16:1/C16:0 (×100)	18.04±2.46^{a}	16.18±1.58^{b}	14.90±2.00^{c}	<0.001
C16:0+C16:1 (%)	27.32±0.11	27.24±0.07	27.16±0.09	0.52
C18:1 (%)	45.38±0.16^{a}	44.71±0.10^{b}	43.97±0.13^{c}	<0.001
C18:1/C18:0	4.43±0.04^{a}	4.11±0.03^{b}	3.78±0.03^{c}	<0.001
C18:0+C18:1 (%)	56.10±0.15	56.03±0.10	55.98±0.12	0.81
MUFA (%)	50.30±0.17^{a}	49.27±0.11^{b}	48.27±0.14^{c}	<0.001
MUFA/SFA	1.44±0.01^{a}	1.37±0.01^{b}	1.31±0.01^{c}	<0.001
SFA+MUFA (%)	86.02±0.17	85.88±0.11	85.75±0.14	0.44
Muscle *longissimus dorsi*				
No. of pigs	50	165	98	-
IMF (% dry matter)	12.88±0.47	12.96±0.26	12.47±0.34	0.51
MUFA (%)	51.43±0.26^{a}	50.23±0.14^{b}	49.22±0.19^{c}	<0.001
MUFA/SFA	1.40±0.01^{a}	1.32±0.01^{b}	1.27±0.01^{c}	<0.001
SFA+MUFA (%)	88.76±0.31	88.73±0.17	88.49±0.23	0.67
Muscle *semimembranosus*				
No. of pigs	43	86	67	-
IMF (% dry matter)	10.22±0.70	10.01±0.54	11.03±0.58	0.36
MUFA (%)	49.78±0.70	48.32±0.53	47.94±0.57	0.11
MUFA/SFA	1.48±0.05	1.44±0.04	1.39±0.04	0.37
SFA+MUFA (%)	83.73±0.62	82.88±0.47	83.34±0.51	0.51
Subcutaneous fat				
No. of pigs	56	124	80	-
C16:1 (%)	2.20±0.04	2.18±0.03	2.08±0.03	0.029
C16:1/C16:0 (×100)	10.54±2.79	10.32±1.96	9.78±2.38	0.07
C16:0+C16:1 (%)	23.86±0.22	24.10±0.15	24.17±0.19	0.53
C18:1 (%)	44.87±0.28^{a}	43.76±0.19^{b}	43.41±0.24^{b}	<0.001
C18:1/C18:0	4.38±0.11^{a}	4.16±0.07^{ab}	3.90±0.09^{b}	0.002
C18:0+C18:1 (%)	55.74±0.22^{a}	54.95±0.16^{b}	55.16±0.19^{ab}	0.018
MUFA (%)	48.23±0.29^{a}	47.04±0.21^{b}	46.56±0.25^{b}	<0.001
MUFA/SFA	1.47±0.03^{a}	1.41±0.02^{ab}	1.36±0.03^{b}	0.031
SFA+MUFA (%)	82.31±0.26	81.78±0.18	82.02±0.22	0.24

[1] IMF: intramuscular fat; C16:0: palmitic acid; C16:1: palmitoleic acid; C18:0: stearic acid; C18:1: oleic acid; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids.

Table 6.7. Blood lipid indicators by *SCD* diplotype in purebred Duroc. The diplotype did not affect (p<0.05) blood plasma lipid indicators at 180 d. Values are expressed as the least square mean (±standard error) for each trait by diplotype.

Trait[1]	Diplotype			
	H1H1	H1H2	H2H2	*p*-value
No. of pigs	20	52	36	-
Triglycerides, mg/dl	33.9±3.2	37.8±1.9	36.1±2.4	0.57
Cholesterol, mg/dl	119.1±4.2	118.8±2.6	114.6±3.2	0.54
HDL cholesterol, mg/dl	44.3±1.6	44.44±1.0	42.8±1.2	0.57
LDL cholesterol, mg/dl	78.6±3.0	78.8±1.9	75.7±2.2	0.56
VLDL, mg/dl	7.4±0.8	7.8±0.6	7.0±0.8	0.71
Leptin				
No. of pigs	11	39	26	-
Concentration, ng/ml	44.7±9.5	47.2±4.9	40.0±6.3	0.68
IGF-1				
No. of pigs	42	138	84	-
Concentration, ng/ml	75.6±5.0	75.8±2.8	82.9±3.6	0.27

[1] HDL: high-density lipoprotein; LDL: low-density lipoprotein; VLDL: very low density lipoprotein; IGF-1: insulin-like growth factor 1.

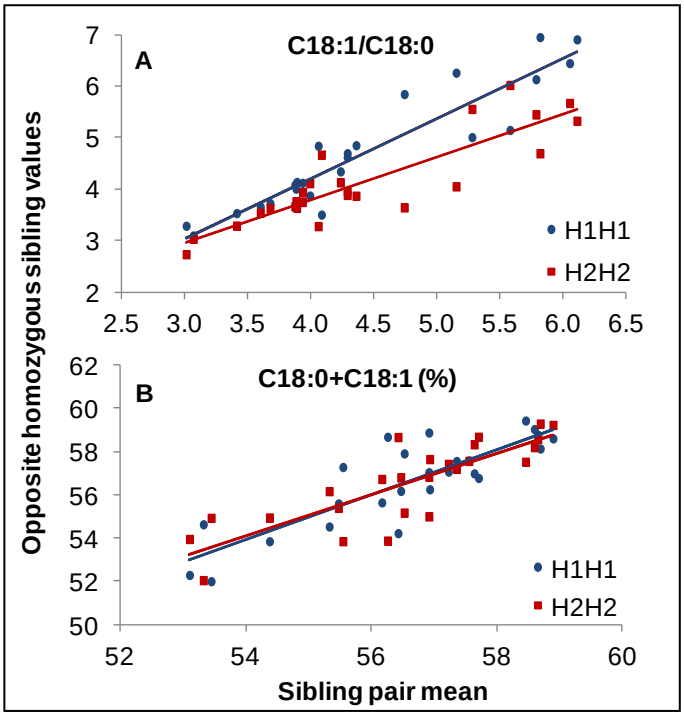


Figure 6.4. Desaturation ratio in opposite homozygous siblings for *SCD* haplotypes H1 and H2. (A) Ratio C18:1/C18:0, and (B) C18:0+C18:1 content (in percentage of total fatty acids) in the muscle *gluteus medius* of homozygous H1H1 and H2H2 sibling pairs (n=25) are plotted against the sibling pair mean value. The H1H1 pigs showed a greater desaturation ratio (p<0.01) than their H2H2 sibs but the same C18:0+C18:1 (p=0.94) content. The associated *p*-values were determined using a paired t-test. Regression lines were fitted for each diplotype (blue: H1H1; red: H2H2). The difference between homozygotes for C18:1/C18:0 increased with C18:1/C18:0 sibling pair mean (p<0.05), with H1H1 sibs showing a trend higher than the expected (1.17±0.10) and H2H2 sibs lower (0.83±0.10). The regression of C18:0+C18:1 on the litter mean value was not different from the average trend (unity) in both genotypes (p=0.89).

haplotype variants were able to explain a relevant fraction of the total additive genetic variance for MUFA/SFA (31%), C18:1/C18:0 (37%), C16:1/C16:0 (35%), MUFA (20%), C18:1 (13%), and C16:1 (25%). However, they did not affect fat content-related traits, including carcass weight, backfat thickness, lean content, and IMF content (Table 6.6), or standard blood lipid indicators (Table 6.7). The favorable effect of H1 on C18:1/C18:0 was internally validated by comparing opposite homozygote siblings (Figure 6.4). In line with the population-wide results, H1H1 pigs had a greater C18:1/C18:0 ratio in *gluteus medius* muscle than their corresponding H2H2 sib pairs, with no change in the total content of C18:0+C18:1.

To assess the functional impact of the haplotype association we analyzed the *SCD* mRNA expression in muscle, subcutaneous adipose tissue, and liver across diplotypes. In accordance with the association results, we found that H1H1 animals showed greater *SCD* mRNA expression than H2H2 pigs in muscle (Figure 6.5). Despite the trend was the expected, we were not able to detect significant differences in *SCD* mRNA expression between diplotypes in subcutaneous fat. The haplotype had no effect on the *SCD* mRNA expression in liver.

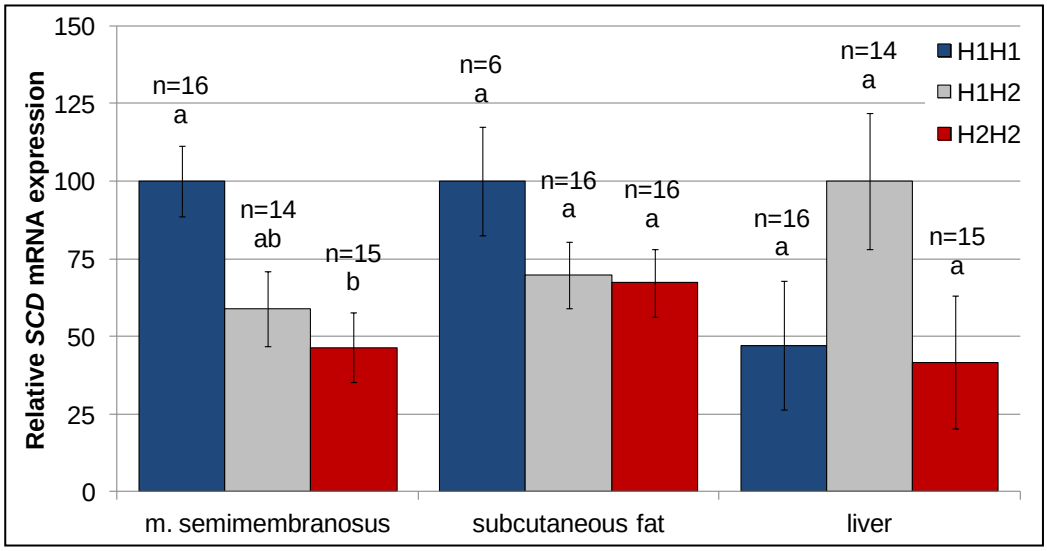


Figure 6.5. The haplotype H1 upregulates *SCD* mRNA expression in muscle. Pigs H1H1 had higher *SCD* mRNA expression than the H2H2 pigs in muscle *semimembranosus* but not in subcutaneous fat and liver. Values are expressed relative to the mean expression in the diplotype with the greater expression in each tissue. Error bars represent standard errors. Columns lacking a common letter within tissue differ ($p<0.05$). Haplotype H1 had a favorable additive effect on *SCD* mRNA expression in muscle (24.9±8.2, $p<0.01$) but not in subcutaneous fat (7.2±12.5, $p=0.57$) and liver (−1.5±15.0, $p=0.91$).

6.3.3. Validation and haplotype determination

We next validated the effect of the haplotypes on experimental Duroc crossbreds (Exp 2; Table 6.4). To that end, Duroc sows from the line used in Exp 1 were mated, in addition to Duroc boars from the same line (genetic type control), to either Duroc boars from a leaner commercial line or to Iberian boars where the H1 haplotype was fixed. Barrows from contemporary offspring of the three mating types were raised in two batches. The Duroc crossbred types reproduced not only the favorable effect of H1 on the C18:1/C18:0 ratio, but also, when compared to purebred Duroc, replicated the magnitude of the effect as well (Figure 6.6). Thus, the substitution effect of H1 for H2 for C18:1/C18:0 remained close to 0.40. Moreover, as expected, the H1 variant increased the C16:1/C16:0 ratio, but did not affect body growth and fatness (Table 6.8).

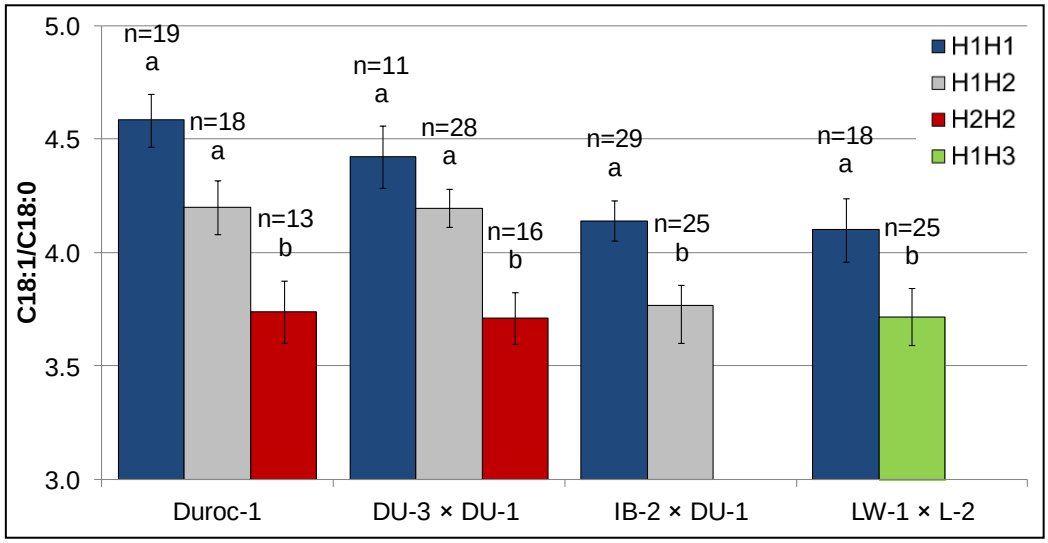


Figure 6.6. Desaturation ratio by *SCD* diplotype in experimental crossbreds. The effect of *SCD* haplotypes on the C18:1/C18:0 ratio was validated in three experimental genetic types. Sows from the investigated Duroc line (Duroc-1), which was used as control, were sired by boars from an independent Duroc line (DU-3 × DU-1) and by Iberian boars (IB-2 × DU-1), and their progeny contemporarily compared with Large White × Landrace barrows (LW-1 × L-2). The results confirmed that the H1 haplotype increased the C18:1/C18:0 ratio in the *gluteus medius* muscle in all genetic types. The H1H1 pigs showed a higher desaturation ratio than H2H2 (0.81 more in Duroc-1 and 0.61 more in DU-3 × DU-1), H1H2 (0.37 more in IB-2 × DU-1), and H1H3 (0.38 more in LW-1 × L-2) pigs. All LW-1 × L-2 pigs were AA for SNP *g.2281A>G*, thereby excluding this SNP as a causative mutation. Error bars represent standard errors. Columns lacking a common letter within genetic type differ ($p<0.05$).

To refine the haplotype block determination and disentangle which SNP was the responsible of the haplotype effect, we investigated the progeny of two heterozygote C-T-A/T-C-A (**H1H3**) Large White boars. Barrows from mating these boars with H1H1 Landrace sows were contemporaneously raised with pigs in Exp 2, with the expectation to obtain half of the offspring C-T-A/C-T-A (H1H1) and half C-T-A/T-C-A (H1H3) (i.e., segregating at *g.2108C>T* and *g.2228T>C*, while fixed at *g.2281A>G*). The haplotype segregation was as expected (Table 6.4). This experiment showed that, similarly to contemporaneously purebred and crossbreds Duroc in Exp 2, H1H1 Large White × Landrace barrows still displayed a greater C18:1/C18:0 ratio than heterozygotes carrying the H3 haplotype (Figure 6.6). As with other genetic types in Exp 2, the haplotype in Large White × Landrace had no effect on traits other than those directly affected by SCD (Table 6.8). Importantly, the results of this last validation experiment allow us to exclude SNP *g.2281A>G* as the causative mutation of the effect on the desaturation index.

Table 6.8. Carcass weight, fat content, and fatty acid composition by *SCD* diplotype in experimental crossbred pigs. The haplotype H1 showed a favorable effect on C16:1/C16:0 and C18.1/C18:0 ratios and no effect on fat content-related traits (carcass weight, lean content, intramuscular fat content, C16:0+C16:1, and C18:0+C18:1). Values are expressed as the least square mean (±standard error) for each trait by diplotype. Means lacking a common superscript within trait differ (p<0.05).

Genetic type	Trait[1]	Diplotype			
		H1H1	H1H2 / H1H3[2]	H2H2	*p*-value
DU-3 × DU-1	No. of pigs	11	28	16	-
	Age at sampling (d)	206.0	206.1	205.7	-
	Carcass weight (kg)	94.9±2.8	95.9±1.7	91.5±2.3	0.31
	Backfat depth (mm)	20.0±1.2	19.6±0.7	19.1±0.9	0.85
	Lean content (%)	47.9±1.5	48.4±0.9	48.8±1.2	0.90
	Muscle *gluteus medius*				
	IMF (% dry matter)	10.63±1.00	12.15±0.62	12.11±0.82	0.41
	C16:1 (%)	4.01±0.15^{a}	3.82±0.10^{a}	3.32±0.13^{b}	0.001
	C16:1/C16:0 (×100)	17.46±0.72^{a}	16.81±0.45^{a}	14.43±0.60^{b}	0.002
	C16:1+C16:0 (%)	27.00±0.46	26.66±0.29	26.37±0.38	0.58
	C18:1 (%)	43.28±0.77	43.08±0.48	42.30±0.63	0.53
	C18:1/C18:0	4.42±0.14^{a}	4.20±0.08^{a}	3.71±0.11^{b}	<0.001
	C18:1+C18:0 (%)	53.10±0.85	53.46±0.53	53.83±0.70	0.80
	MUFA (%)	48.03±0.77	47.69±0.48	46.39±0.63	0.18
	MUFA/SFA	1.39±0.04	1.36±0.02	1.28±0.03	0.07
	MUFA+SFA (%)	82.75±0.82	82.90±0.51	82.89±0.67	0.99

[1] IMF: intramuscular fat; C16:0: palmitic acid; C16:1: palmitoleic acid; C18:0: stearic acid; C18:1: oleic acid; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids.
[2] H1H2 for DU-3 × DU-1 and IB-2 × DU-1; H1H3 for LW-1 × L-2.

Table 6.8. Continued.

Genetic type	Trait[1]	Diplotype			
		H1H1	H1H2 / H1H3[2]	H2H2	*p*-value
IB-2 × DU-1	No. of pigs	29	25	-	-
	Age at sampling (d)	206.6	206.6	-	-
	Carcass weight (kg)	93.2±1.8	91.8±1.9	-	0.57
	Backfat depth (mm)	24.4±0.3	24.4±0.4	-	0.96
	Lean content (%)	45.5±0.9	44.9±0.9	-	0.61
	Muscle *gluteus medius*				
	IMF (% dry matter)	18.15±0.93	17.77±0.97	-	0.77
	C16:1 (%)	3.87±0.09[a]	3.46±0.09[b]	-	0.002
	C16:1/C16:0 (×100)	15.80±0.42[a]	14.18±0.43[b]	-	0.009
	C16:1+C16:0 (%)	28.48±0.34	27.97±0.35	-	0.30
	C18:1 (%)	45.12±0.42	44.68±0.44	-	0.47
	C18:1/C18:0	4.14±0.09[a]	3.77±0.09[b]	-	0.005
	C18:1+C18:0 (%)	56.11±0.42	56.67±0.43	-	0.35
	MUFA (%)	49.77±0.44	48.90±0.45	-	0.17
	MUFA/SFA	1.33±0.03	1.28±0.03	-	0.15
	MUFA+SFA (%)	87.38±0.37	87.31±0.38	-	0.89
LW-1 × L-2	No. of pigs	18	25	-	-
	Age at sampling (d)	205.5	205.5	-	-
	Carcass weight (kg)	106.8±1.9	102.6±1.7	-	0.10
	Backfat depth (mm)	18.4±0.8	17.7±0.7	-	0.46
	Lean content (%)	50.7±1.0	51.7±0.9	-	0.45
	Muscle *gluteus medius*				
	IMF (% dry matter)	7.62±0.41	7.38±0.37	-	0.66
	C16:1 (%)	3.57±0.13	3.29±0.12	-	0.12
	C16:1/C16:0 (×100)	15.89±0.66	14.38±0.59	-	0.09
	C16:1+C16:0 (%)	26.12±0.41	26.22±0.37	-	0.85
	C18:1 (%)	39.90±0.49	39.42±0.44	-	0.45
	C18:1/C18:0	4.10±0.14[a]	3.72±0.13[b]	-	0.04
	C18:1+C18:0 (%)	49.85±0.54	50.31±0.49	-	0.51
	MUFA (%)	44.16±0.55	43.40±0.50	-	0.30
	MUFA/SFA	1.29±0.03	1.23±0.03	-	0.11
	MUFA+SFA (%)	78.55±0.69	79.02±0.62	-	0.60

[1] IMF: intramuscular fat; C16:0: palmitic acid; C16:1: palmitoleic acid; C18:0: stearic acid; C18:1: oleic acid; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids.
[2] H1H2 for DU-3 × DU-1 and IB-2 × DU-1; H1H3 for LW-1 × L-2.

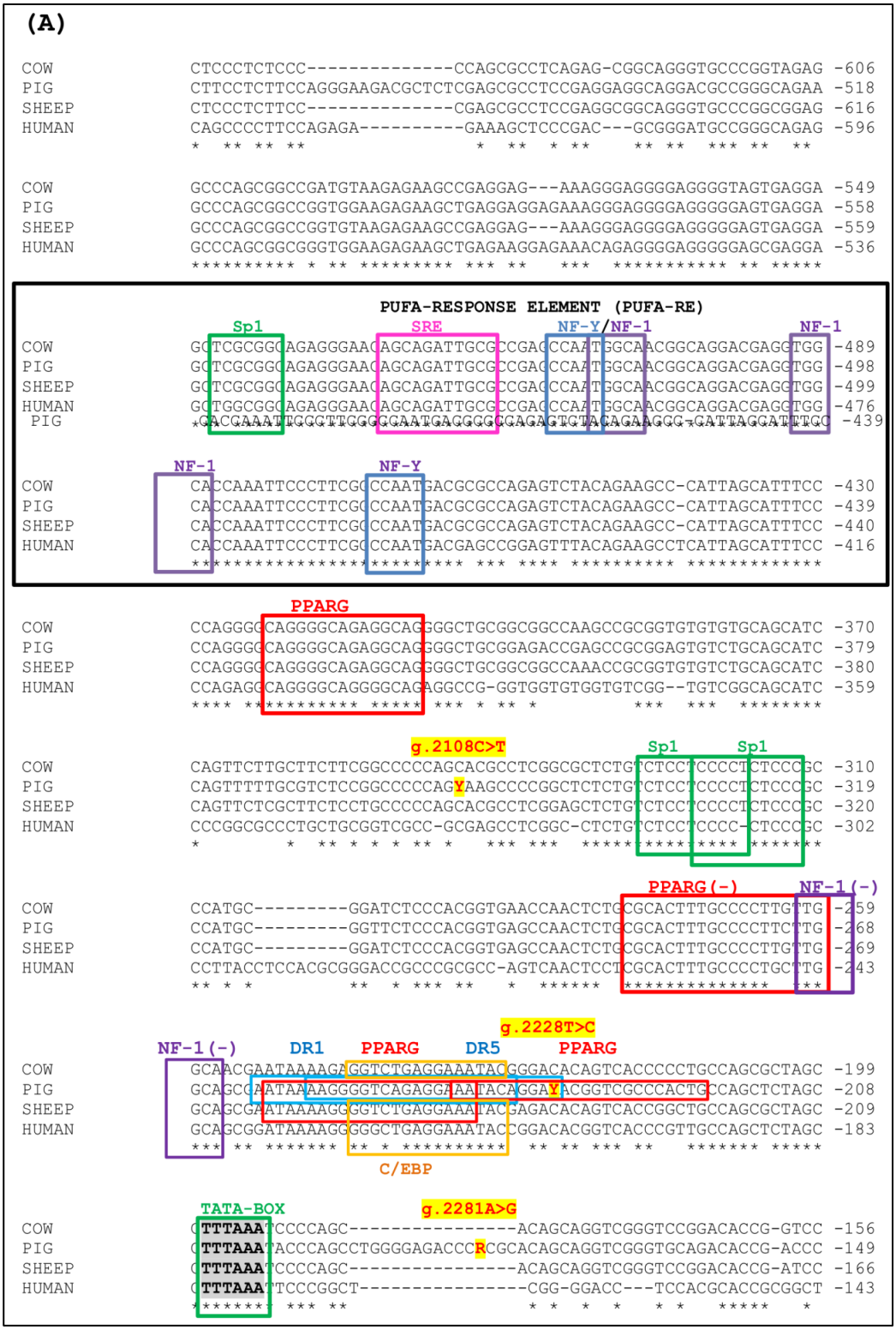
6.3.4. *In silico* analysis of *SCD* promoter polymorphisms

To assess if polymorphisms in the promoter region could affect *SCD* expression through the disruption of transcription factor binding sites, a computer-assisted identification of potential *cis*-acting DNA-sequence motifs was carried out. As a first step, we analyzed in parallel the promoter region (−500 to +100 from the transcriptional start site) of human, cow, pig, and sheep *SCD* gene with the view of identifying common regulatory modules. The promoter sequence displays stretches of

strong conservation between these four species interspersed with fragments of lower conservation (Figure 6.7). A conserved PUFA response element (**PUFA-RE**), which includes a sterol regulatory element (**SRE**), two CCAAT boxes (**NF-Y**), and two nuclear factor (**NF**)-1 binding sites, has been described approximately at positions −450/−550, which is highly conserved between species (Ntambi, 1999; Bené et al., 2001; Ren et al., 2004b; Ohsaki et al., 2007) (Figure 6.7) and is essential for correct *SCD* gene regulation by PUFA and cholesterol (Ntambi, 1999; Bené et al., 2001). The transcription factor SRE binding protein-1 down-regulates *SCD* expression through the interaction with the SRE element in this regulatory region (Bené et al., 2001). In addition to the PUFA-RE element, our *in silico* analyses revealed a conserved peroxisome proliferator-activated receptor gamma (**PPARγ**) motif at position −400/−420. Another region containing many potential binding motifs lays in the sequence around the *g.2228T>C* polymorphism, about 40 bp upstream of the TATA-box. Several transcription factor-binding motifs partially overlap in this region. There is a conserved PPARγ and NF-1 motif on the negative strand, which lay adjacent to a CCAAT/enhancer binding protein motif (**C/EBP**) in cow, sheep, and humans. However, our analysis failed to recognize this C/EBP motif in the pig sequence, although it has been postulated before (Ren et al., 2004b). In pig this motif is replaced by two PPARγ binding sites, a half-site in the positive strand and a full homodimer motif with 3-bp inter half-site spacing between inverted repeats (**IR3**) where PPARγ binds to both complementary strands (data available at: http://www.plosone.org/article/fetchSingleRepresentation.action?uri=info:doi/10.1371/journal.pone.0086177.s006). In addition, bridging these PPARγ sites together, there are two binding motifs for the retinoid X receptor and the retinoic acid receptor α (**RXR:RARα**) response elements (direct repeats with 1-bp (**DR1**) and 5-bp (**DR5**) spacer sequence, respectively). The *g.2228T>C* polymorphism lies in the core of the IR3 motif in the positive strand and at the end of the DR5 element (Figure 6.7).

6.4. DISCUSSION

We have shown that the C-T haplotype at SNPs *g.2108C>T* and *g.2228T>C* in the promoter region of the *SCD* gene increases fatty acid desaturation in muscle and subcutaneous fat, in line with some previous findings in Duroc (Uemoto et al., 2012b). The third polymorphism screened in the promoter (*g.2281A>G*) was excluded as a causal by the results in our external validation experiment (Exp 2), where Large White × Landrace pigs were all homozygous for this SNP but still presented the same effect on the desaturation index. Conversely, as in all the screened populations SNPs *g.2108C>T* and *g.2228T>C* were in almost complete linkage disequilibrium, we were not able to disentangle which one of the two is the causative mutation. However, considering the results from a Landrace × Korean native pig intercross in which these


(A)
COW CTCCCTCTCCC----------CCAGCGCCTCAGAG-CGGCAGGGTGCCCGGTAGAG -606
PIG CTTCCTCTTCCAGGGAAGACGCTCTCGAGCGCCTCCGAGGAGGCAGGACGCCGGGCAGAA -518
SHEEP CTCCCTCTTCC----------CGAGCGCCTCCGAGGCGGCAGGGTGCCCGGCGGAG -616
HUMAN CAGCCCCTTCCAGAGA----------GAAAGCTCCCGAC---GCGGGATGCCGGGCAGAG -596
COW GCCCAGCGGCCGATGTAAGAGAAGCCGAGGAG---AAAGGGAGGGGAGGGGTAGTGAGGA -549
PIG GCCCAGCGGCCGGTGGAAGAGAAGCTGAGGAGGAGAAAGGGAGGGGAGGGGGAGTGAGGA -558
SHEEP GCCCAGCGGCCGGTGTAAGAGAAGCCGAGGAG---AAAGGGAGGGGAGGGGGAGTGAGGA -559
HUMAN GCCCAGCGGCGGGTGGAAGAGAAGCTGAGAAGGAGAAACAGAGGGGAGGGGGAGCGAGGA -536
PUFA-RESPONSE ELEMENT (PUFA-RE)
Sp1
SRE
NF-Y/NF-1
NF-1
COW GCTCGCGGCAGAGGGAACAGCAGATTGCGCCGACCCAATGGCAACGGCAGGACGAGGTGG -489
PIG GCTCGCGGCAGAGGGAACAGCAGATTGCGCCGACCCAATGGCAACGGCAGGACGAGGTGG -498
SHEEP GCTCGCGGCAGAGGGAACAGCAGATTGCGCCGACCCAATGGCAACGGCAGGACGAGGTGG -499
HUMAN GCTGGCGGCAGAGGGAACAGCAGATTGCGCCGACCCAATGGCAACGGCAGGACGAGGTGG -476
PIG *GACGAAATTGGGTTGGGGGAATGAGGGGGAGAGTGTACAGAAGGG-GATTAGGATTTGC -439
NF-1
NF-Y
COW CACCAAATTCCCTTCGCCCAATGACGCGCCAGAGTCTACAGAAGCC-CATTAGCATTTCC -430
PIG CACCAAATTCCCTTCGCCCAATGACGCGCCAGAGTCTACAGAAGCC-CATTAGCATTTCC -439
SHEEP CACCAAATTCCCTTCGCCCAATGACGCGCCAGAGTCTACAGAAGCC-CATTAGCATTTCC -440
HUMAN CACCAAATTCCCTTCGCCCAATGACGAGCCGGAGTTTACAGAAGCCTCATTAGCATTTCC -416
PPARG
COW CCAGGGGCAGGGGCAGAGGCAGGGGCTGCGGCGGCCAAGCCGCGGTGTGTGTGCAGCATC -370
PIG CCAGGGGCAGGGGCAGAGGCAGGGGCTGCGGAGACCGAGCCGCGGAGTGTCTGCAGCATC -379
SHEEP CCAGGGGCAGGGGCAGAGGCAGGGGCTGCGGCGGCCAAACCGCGGTGTGTCTGCAGCATC -380
HUMAN CCAGAGGCAGGGGCAGGGGCAGAGGCCG-GGTGGTGTGGTGTCGG--TGTCGGCAGCATC -359
g.2108C>T
Sp1
Sp1
COW CAGTTCTTGCTTCTTCGGCCCCCAGCACGCCTCGGCGCTCTGTCTCCTCCCCTCTCCCGC -310
PIG CAGTTTTTGCGTCTCCGGCCCCCAGYAAGCCCCGGCTCTCTGTCTCCTCCCCTCTCCCGC -319
SHEEP CAGTTCTCGCTTCTCCTGCCCCCAGCACGCCTCGGAGCTCTGTCTCCTCCCCTCTCCCGC -320
HUMAN CCCGGCGCCCTGCTGCGGTCGCC-GCGAGCCTCGGC-CTCTGTCTCCTCCCC-CTCCCGC -302
PPARG(-)
NF-1(-)
COW CCATGC---------GGATCTCCCACGGTGAACCAACTCTGCGCACTTTGCCCCTTGTTG -259
PIG CCATGC---------GGTTCTCCCACGGTGAGCCAACTCTGCGCACTTTGCCCCTTCTTG -268
SHEEP CCATGC---------GGATCTCCCACGGTGAGCCAACTCTGCGCACTTTGCCCCTTGTTG -269
HUMAN CCTTACCTCCACGCGGGACCGCCCGCGCC-AGTCAACTCCTCGCACTTTGCCCCTGCTTG -243
g.2228T>C
NF-1(-)
DR1
PPARG
DR5
PPARG
C/EBP
COW GCAACGAATAAAAGAGGTCTGAGGAAATACGGGACACAGTCACCCCCTGCCAGCGCTAGC -199
PIG GCAGCGAATAAAAGGGGTCAGAGGAAATACAGGAYACGGTCGCCCACTGCCAGCTCTAGC -208
SHEEP GCAGCGAATAAAAGGGGTCTGAGGAAATACGAGACACAGTCACCGGCTGCCAGCGCTAGC -209
HUMAN GCAGCGGATAAAAGGGGGCTGAGGAAATACCGGACACGGTCACCCGTTGCCAGCTCTAGC -183
TATA-BOX
g.2281A>G
COW CTTTAAATCCCCAGC----------ACAGCAGGTCGGGTCCGGACACCG-GTCC -156
PIG CTTTAAATACCCAGCCTGGGGAGACCCRCGCACAGCAGGTCGGGTGCAGACACCG-ACCC -149
SHEEP CTTTAAATCCCCAGC----------ACAGCAGGTCGGGTCCGGACACCG-ATCC -166
HUMAN CTTTAAATTCCCGGCT----------CGG-GGACC---TCCACGCACCGCGGCT -143

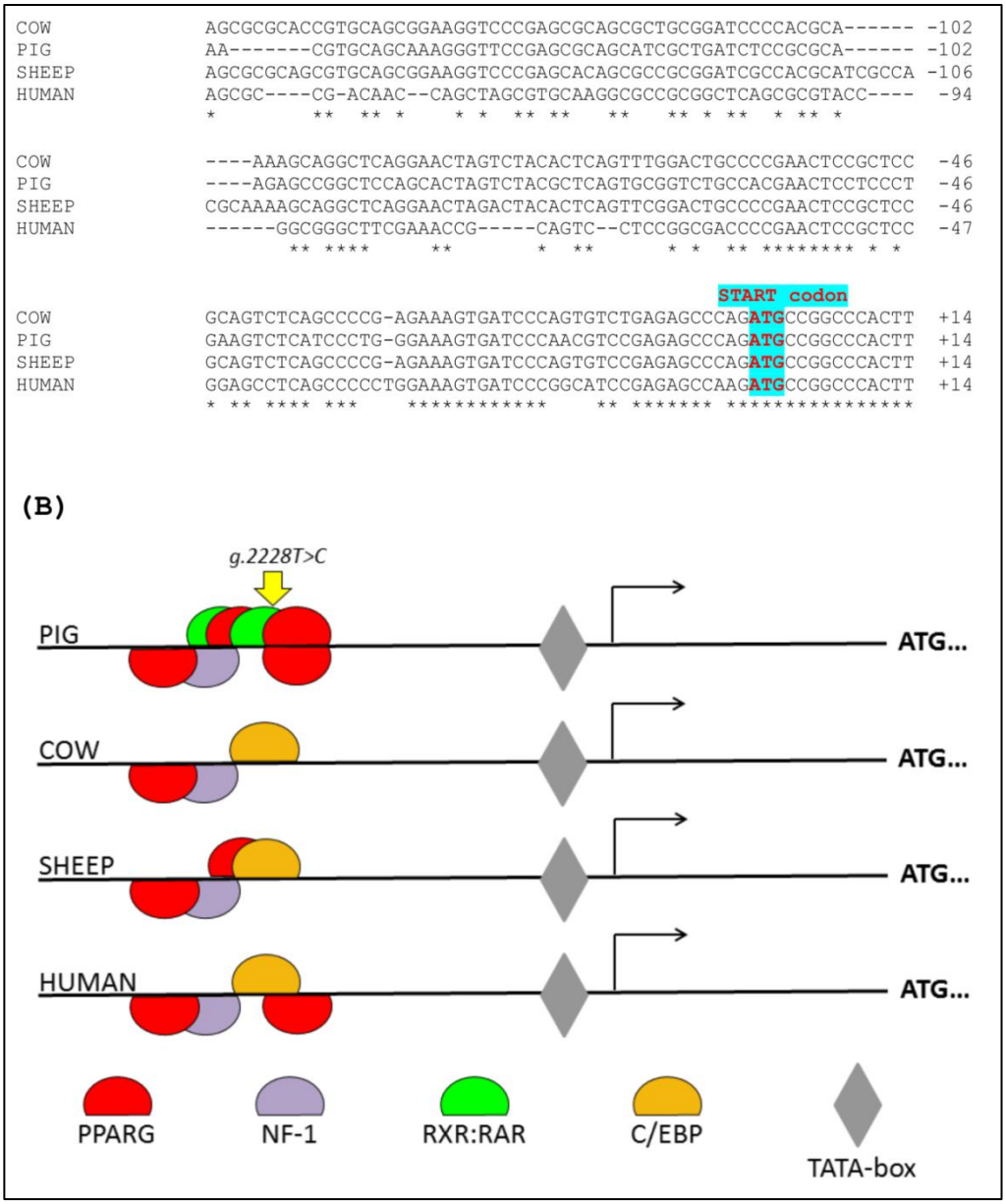


Figure 6.7. Comparative promoter sequence between cow, pig, sheep and human *SCD* gene. (A) Sequence alignment of a 700 bp homologous 5' flanking sequence of the gene using ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/). The conserved PUFA response element including a sterol response element (SRE), two CCAAT-box (NF-Y), two nuclear factor (NF)-1, and one stimulator protein 1 (SP1) binding site is boxed. Other common motifs (TATA-box, NF-1, and PPARγ) are also indicated along with the position of the three pig promoter SNPs genotyped. Several putative transcription factor binding sites close to the *g.2228T>C* polymorphism are depicted in the four species. These include a putative CCAAT enhancer binding protein (C/EBP) element, NF-1, two PPARγ binding sites, and two RXR:RARα motifs (DR1 and DR3). (B) Potential binding of these transcription factors in the sequence around the *g.2228T>C* polymorphism.

two SNPs segregated independently (Maharani et al., 2013), we can conclude that fatty acid composition was associated to an haplotype comprised, in its 5' extreme, not beyond the g.2109 position and, in its 3' end, not past the g.2280 nucleotide (Figure 6.2). However, no other mutations have been described in this short 171 bp region in other studies which have extensively sequenced the *SCD* promoter in independent Duroc lines (Uemoto et al., 2012b; Bartz et al., 2013; Maharani et al., 2013; Renaville et al., 2013), including the present study. In contrast, the *g.2228T>C* SNP is common to all the studies which have found a significant relationship between the *SCD* promoter genotype and fatty acid composition (Uemoto et al., 2012b; Maharani et al., 2013). Taken together, these findings strongly support that allele T at *g.2228T>C* is the causative mutation leading to increased fatty acid desaturation. Interestingly, this allele is virtually absent in the Asian breeds (Ren et al., 2004a) and, in contrast, almost fixed in other breeds, including Landrace, Pietrain, Iberian, and wild boar (Table 6.4; Bartz et al., 2013). This explains why whole-genome analyses based on these latter breeds failed to identify *SCD* as a positional candidate gene for fatty acid composition. It remains unclear why Duroc is the only breed where *g.2228T>C* is segregating at intermediate frequencies.

In pig, the *g.2228T>C* SNP is positioned at 58 nucleotides from the *SCD* transcription start site, in a stretch of moderate sequence conservation with cow, sheep, and human *SCD* sequences (Figure 6.7). *In silico* analysis of this region has identified several overlapping putative transcription factor binding sites, some of which are unique to the pig promoter and contain the T>C mutation at position g.2228 (Figure 6.2). Among them, there are the two putative DR1 and DR5 retinoic acid response elements overlapping to two PPARγ motifs. The DR1 is a high affinity response element for RXR:RARα and PPARγ:RARα heterodimers (Lefterova et al., 2008), which regulate gene expression in response to their ligands, all-trans or 9-cis retinoic acid. A recent genome-wide study revealed that the consensus PPARγ/RXRα DR1-binding motifs co-localized at nearly all locations tested (Lefterova et al., 2008). Bioinformatics analysis also revealed C/EBP-binding motifs in the vicinity of most PPARγ-binding sites in genes induced in adipogenesis. Thus, PPARγ and C/EBP factors cooperatively regulate adipocyte-specific gene expression by adjacent binding (Lefterova et al., 2008). Unlike other authors (Ntambi, 1999; Ren et al., 2004b), we failed to identify the C/EBP motif in the pig promoter, although it has been described for instance in the human, mouse, sheep, and cow promoters (Bené et al., 2001; Ohsaki et al., 2007; Zulkifli et al., 2010).

By which mechanism the *g.2228T>C* polymorphism enhances *SCD* expression is unknown, although we can postulate three possible scenarios. In the first one, the T>C mutation, which affects a core nucleotide of the PPARγ homodimer motif, might alter the PPARγ binding affinity to this site. In a second scenario, the mutation might

alter the affinity of the RXR:RARα to their target DNA motifs, enhancing or repressing transcription depending on the nature of the motif. And lastly, our third possible scenario relies on the cooperative binding between RXR:RARα and PPARγ sites, which is a wide-spread feature in the genome (Lefterova et al., 2008), and that the *g.2228T>C* mutation alters the relative affinity of one or both of these regulatory partners. This mechanism is additionally fine-tuned by the availability and concentration of different ligands, which not only modulates their affinity for the DNA binding sites, but also their ability to interact with other co-activators, thus defining their enhancing or inhibitory action over gene expression (Pérez et al., 2012).

In this regard, we were able to prove increased *SCD* transcription in TT pigs as compared to CC pigs in muscle, indicating that higher product-to-precursor ratios in pigs carrying the allele T are a consequence of increased *SCD* expression rather than a more active version of the protein, as the two main haplotypes did not differ in the coding region sequence. Moreover, our results indicate that the enhanced activity of the allele T of the *SCD* gene is tissue-specific, with preference for muscle, and substrate-specific, with preference for C18:0 rather than C16:0. In contrast to subcutaneous fat, IMF is less sensitive to dietary fat and, conversely, more prone to endogenous fatty acid synthesis and remodeling, particularly regarding C18:1 (Bosch et al., 2012). Therefore, differences across *SCD* genotypes are expected to be better accounted for in muscle than in the subcutaneous tissue. We have seen in a previous experiment that genetic selection of pigs against fatness led to differential responses in SCD protein expression in muscle and subcutaneous adipose tissue (Cánovas et al., 2009). The tissue-specific behavior of the pig *SCD* gene is also shown by distinct patterns of CpG methylation in the proximal promoter in muscle as compared to subcutaneous fat (Cho et al., 2011). In contrast, the *SCD* promoter genotypes had no impact on liver fatty acid composition, which is in line with the fact that, in pigs, the adipose tissue, and not the liver, is the principal site of *de novo* fatty acid synthesis (Dodson et al., 2010). Moreover, in liver, genes encoding for fatty acid remodeling enzymes, such as *SCD*, respond differently to steroid hormone stimulation than genes involved in the fatty acid biosynthesis. For instance, unlike fatty acid synthase or malic enzyme gene, the hepatic pig *SCD* gene undergoes a negative response to thyroid hormone occurring through a thyroid receptor response element located downstream the *g.2228T>C* (Waters et al., 1997). Although indirectly, the results here also indicate that the expected extra SCD produced by allele T prefers C18:0 rather than C16:0 as a substrate. Thus, we observed that allele T has a consistent negative side effect on the C18:0/C16:0 ratio. Because there is no reason for differential dietary deposition of fatty acids across genotypes (they were subjected to the same diet), a likely interpretation is that C18:0 is consumed more steadily than C16:0, which may occur if SCD desaturates C18:0 to C18:1 more efficiently than C16:0 to C16:1 (Kloareg et al., 2007). Comparison of the means of C16:0, C16:1, C18:0, and C18:1

for the two extreme genotypes (Table 6.6) shows that, in *gluteus medius*, TT homozygotes desaturate 10.9% more C18:0 than the CC but only 2.1% more C16:0. As for the subcutaneous fat, these values were 8.5% and 3.0%, respectively, thereby reproducing the same pattern. The substrate specificity may be due to different SCD isoforms (Miyazaki & Ntambi, 2003). A recent update of the pig *SCD* annotation in *Ensembl*, corresponding to assembly Sscrofa10.2 release 72 (performed on June 2013) reported three new isoforms for the *SCD* gene, bringing the total number to four. They are translated into four different peptides. The tissue-dependent expression of these isoforms is another level of complexity of the activity of the *SCD* expression that has not yet been explored in pigs.

In addition, the regulation of *SCD* expression is a complex phenomenon. The intracellular concentration of desaturases fluctuates in response to a large number of effectors including hormonal and dietary factors (Mauvoisin & Mounier, 2011). However, the influence of dietary treatment on muscle fatty acid composition is not evident (Duran-Montgé et al., 2009), likely because deposition of dietary fat can be offset by endogenous synthesis. It has been shown experimentally in pigs that a reduced protein diet enhances *SCD* expression in muscle but not in subcutaneous adipose tissue (Doran et al., 2006). The favorable effect of the allele T on C18:1/C18:0, although consistent, varied across batches. A key component of all the environmental factors accounted for in the batch effect is the diet. We have seen that there is a negative relationship of the additive effect of this allele in muscle with dietary protein (R^2=0.38, p<0.05). In contrast, the dietary C18:1/C18:0 ratio exerted a positive effect on the additive effect of allele T in muscle (R^2=0.39, p<0.05). These effects were not detected in the subcutaneous fat. Overall, these findings not only give additional evidence that the effect of the *SCD* genotypes is most noticeable in muscle, but also that it is tuned by the diet. In this regard, an interesting topic for future research will be to study the effect of these haplotype variants in pigs subjected at diets differing in vitamin A, or some other metabolic precursor of retinoic acid. In line with two of our hypothetical scenarios, it has been shown experimentally that retinoic acid inhibits porcine preadipocyte differentiation by upregulating RAR and downregulating RXR (Brandebourg & Hu, 2005) but the effects of dietary vitamin A on IMF content and fatty acid composition in pigs are scarce and inconclusive (Olivares et al., 2009a), with results depending on the genetic type (Olivares et al., 2009b). The study of the *g.2228T>C* mutation may contribute to unravel the biological causes of the interaction between dietary vitamin A and gene expression. Moreover, because the RAR and RXR mRNA levels decline with age (Enderlin et al., 1997), it may also help to explain the favorable evolution of the C18:1/C18:0 ratio with age (Bosch et al., 2012).

6.5. CONCLUSIONS

We provide evidence that there exists genetic variation in the *SCD* gene with the potential to increase MUFA content in pork. Strict values on fatty acid content are becoming a common feature in regulations for foods bearing nutritional or health claims concerning fat properties. The MUFA content can be also subjected to such regulations. Selective lipid deposition in meat animals is a relevant issue not only in terms of animal agriculture but also in biomedicine. Evidence is also emerging indicating the existence of allelic variations in the human *SCD* gene affecting enzyme activity and, consequently, disease risk factors (Merino et al., 2010). Therefore, research in meat animals may well not only lead to a new understanding of the regulation of lipid metabolism (Dodson et al., 2010) but also to integrate agriculture science, nutrition, and pharmacology for improved treatment of important chronic diseases (Christophersen & Haug, 2011).

Chapter 7.
Genome-wide association study singles out the *SCD* and *LEPR* as the two main loci influencing intramuscular fat content and fatty acid composition in Duroc pigs

R. Ros-Freixedes[1], S. Gol[1], R. N. Pena[1], M. Tor[1], J. C. M. Dekkers[2], & J. Estany[1]

[1]Departament de Producció Animal, Universitat de Lleida − Agrotecnio Center, 191 Av. Alcalde Rovira Roure, 25198 Lleida, Catalonia, Spain.
[2]Department of Animal Science, Iowa State University, Ames, Iowa 50011, USA.

The content of this chapter has been submitted for publication.

ABSTRACT: Intramuscular fat (IMF) content and fatty acid composition affect the organoleptic quality and nutritional value of pork. A genome-wide association study was performed on 138 purebred Duroc barrows genotyped with a 60k single nucleotide polymorphism (SNP) chip. We detected strong associations with IMF traits for two chromosomal regions co-localizing with the *SCD* (SSC14) and *LEPR* (SSC6) genes. The *SCD* gene is responsible for the biosynthesis of oleic acid (C18:1) from stearic acid. This locus affected the oleic to stearic desaturation index (C18:1/C18:0), C18:1, and saturated (SFA) and monounsaturated (MUFA) fatty acids and was consistently detected in *gluteus medius*, *longissimus dorsi*, and subcutaneous fat. The association of *LEPR* with fatty acid composition was detected only in muscle and was, at least in part, a consequence of its effect on IMF content, with increased IMF resulting in more SFA, less polyunsaturated fatty acids (PUFA), and greater SFA/PUFA ratio. Marker substitution effects estimated with a subset of 65 animals were used to predict the genomic estimated breeding values of 70 animals born 7 years later. Although predictions using all chip SNPs were relatively highly correlated with observed SFA, MUFA, and C18:1/C18:0 (0.48−0.60), IMF content and composition were in general better predicted by using only the SNPs at the *SCD* and *LEPR* loci, in which case the correlation between predicted and observed values was in the range of 0.32 to 0.54 for all traits. It is concluded that markers in these two genes can be useful to select for optimum fatty acid profiles of pork.

7.1. INTRODUCTION

Intramuscular fat (**IMF**) content and fatty acid composition affect both organoleptic quality and nutritional value of pork and, therefore, there is increasing interest in including these traits in the selection objectives of pigs bred for quality pork markets. Particularly, oleic acid (**C18:1**) is the most abundant fatty acid in pork and can be regarded as a good alternative for the simultaneous improvement of organoleptic, technological, and nutritional attributes of pork (Cameron et al., 2000; Christophersen & Haug, 2011). The onset of high-density single nucleotide polymorphism (**SNP**) genotyping arrays has enabled a more precise scanning of the genome to detect quantitative trait loci (**QTL**) and nucleotides (QTN) and to make genomic predictions of breeding values. While some genome-wide association studies (**GWAS**) have already been reported for fatty acid composition in pig, there are no reports for purebred pigs. Moreover, the accuracy of genomic prediction for IMF content and fatty acid composition in swine has not been assessed. Thus, the objectives of this study were to use GWAS techniques to detect genomic regions affecting IMF content and composition in a purebred Duroc population, and to discuss the potential use of genomic prediction for these traits.

7.2. MATERIALS AND METHODS

7.2.1. Animals and data

We genotyped 138 purebred Duroc barrows from the line described in the Animals and Samples Section using the PorcineSNP60 v2 Genotyping BeadChip (Illumina, CA). Animals were chosen to be as unrelated as possible and representative of the whole population. For this purpose, the offspring of 54 sires and 126 dams were chosen to be genotyped. Half of the animals (n=66, from 29 sires and 57 dams) were born in 2002−2003, and the other half (n=72, from 25 sires and 69 dams) in 2009−2010. All animals were raised in 6 batches (3 batches for each period, with 19 to 26 genotyped animals per batch) under commercial conditions and slaughtered in the same commercial abattoir at ~210 d of age. Carcass backfat thickness (**BT**, n=131) was measured by an on-line ultrasound automatic scanner (AutoFOM, SFK-Technology, Herlev, Denmark). Samples of muscle *gluteus medius* (**GM**, n=138), muscle *longissimus dorsi* at the level of the third and fourth ribs (**LD**, n=138), and subcutaneous fat at the same location (**SF**, n=112) were collected. The IMF content (expressed as percentage of fresh sample) and individual and total saturated (**SFA**), monounsaturated (**MUFA**), and polyunsaturated (**PUFA**) fatty acid contents (expressed as the percentage relative to total fatty acids) were determined in duplicate by gas chromatography. The desaturation ratios of C18:1 to stearic acid (**C18:1/C18:0**) and SFA/PUFA were calculated. Full details of the procedures are given in the

Animals and Samples Section. Means and range of values observed for each trait are detailed in Table 7.1. The range of values in the genotyped animals was found to be representative of those observed in the whole population. DNA was extracted as described in Chapter 6. The concentration of leptin in blood plasma at 180 d of age after overnight fasting was analyzed in a subset of animals (n=37) using a porcine leptin ELISA kit (Diagnostic Systems Laboratories Inc., Webster, TX) (Amills et al., 2008).

Table 7.1. Mean and range of values of trait phenotypes and posterior means of marker-based additive genetic (σ_a^2) and residual (σ_e^2) variances and heritability (h^2).

Trait[1]	Phenotypes		Posterior mean of variance components		
	Mean	Range	σ_a^2	σ_e^2	h^2
Backfat thickness, mm	22.98	12.7−30.1	4.19	7.14	0.37
Muscle *gluteus medius*					
IMF, %	5.07	2.2−9.5	1.00	0.90	0.53
SFA, %	38.62	34.9−45.5	1.48	0.73	0.67
MUFA, %	48.41	42.0−52.9	1.76	0.70	0.72
C18:1, %	44.06	38.1−48.7	1.42	0.62	0.70
PUFA, %	12.97	8.6−17.7	1.50	1.05	0.59
C18:1/C18:0	3.65	2.4−4.8	0.087	0.044	0.66
SFA/PUFA	3.05	2.0−4.7	0.095	0.118	0.45
Muscle *longissimus dorsi*					
IMF, %	3.49	1.5−6.8	0.60	0.50	0.54
SFA, %	39.58	33.5−48.2	1.81	0.93	0.66
MUFA, %	49.48	44.8−54.8	1.73	1.00	0.63
C18:1, %	44.86	39.1−50.5	1.46	1.11	0.57
PUFA, %	10.94	6.9−16.3	1.99	0.85	0.70
C18:1/C18:0	3.58	2.1−5.2	0.082	0.046	0.64
SFA/PUFA	3.76	2.2−7.0	0.132	0.297	0.31
Subcutaneous fat					
SFA, %	37.94	29.7−44.5	1.41	2.06	0.41
MUFA, %	44.94	39.1−50.9	1.63	1.49	0.52
C18:1, %	41.89	36.4−47.3	1.48	1.31	0.53
PUFA, %	17.12	12.1−22.1	1.22	1.48	0.45
C18:1/C18:0	3.34	2.3−4.9	0.072	0.069	0.51
SFA/PUFA	2.26	1.4−3.5	0.050	0.050	0.47

[1] IMF: intramuscular fat; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; C18:1: oleic acid; C18:0: stearic acid.

7.2.2. High-density SNP data quality control

The PLINK software (Purcell et al., 2007) was used to filter out SNPs with minor allele frequency below 0.05 and genotyping rate below 0.95, and individuals

with more than 10% missing genotypes. Unmapped SNPs based on the current pig genome assembly *Sus scrofa* Build 10.2 were also excluded. The remaining data comprised 135 individuals and 36,432 SNPs.

7.2.3. Genome-wide association study

Associations of SNP genotypes with the phenotypes were analyzed using the Bayes B approach (Meuwissen et al., 2001) implemented in the GenSel software (Fernando & Garrick, 2009). The basic model was:

$$\mathbf{y} = \mathbf{Xb} + \sum_{j=1}^{k} \mathbf{z}_j \alpha_j \delta_j + \mathbf{e},$$

where **y** is the phenotype vector, **X** is the incidence matrix relating fixed factors to phenotypes, **b** is the vector of fixed effects, $\mathbf{z}_j$ is the vector of (coded) genotypes for a SNP at locus j (j= 1 to k), α_j is the allele substitution effect of the SNP at locus j, δ_j is a random 0/1 variable that represents the absence or presence (with prior probabilities π and $1-\pi$, respectively) of SNP j in the model for a given iteration of the Markov chain Monte Carlo procedure, and **e** is the vector of random residuals (assumed to be normally distributed). Alternate homozygous genotypes were coded as −10 and 10, heterozygotes as 0, and missing genotypes as the average value in the population. Fixed effects included batch as a class variable and age at slaughter as a covariate. Intramuscular fatty acid composition traits were analyzed with and without IMF content as an additional covariate. Due to the limited number of animals in the study, the prior proportion of SNPs considered to have no effect on the trait (δ_j=0) was fixed to π=0.997, so that the model fitted ~110 SNPs per iteration. Variance components used as priors were estimated as in Chapter 2 with the full pedigree and all available phenotypic data. A total of 750,000 iterations with a burn-in of 250,000 were run for the analyses. Posterior means and posterior samples of the effects of all SNPs within 1-Mb non-overlapping windows (based on Build 10.2 of the swine genome) were collectively used to predict the genomic merit of the window for each individual and the proportion of total genetic variance that the window accounted for. Windows that accounted for at least 2.5% of the genetic variance of a trait were considered as candidate regions. To take account of potential linkage disequilibrium between SNPs, both single 1-Mb windows and combinations of contiguous or nearby windows that accounted for at least 0.25% of the genetic variance were considered. Linkage disequilibrium in candidate regions was analyzed using Haploview software (Barrett et al., 2005). Candidate genes in these regions were retrieved from Ensembl (EMBL-EBI) and functional gene annotation was based on Enrichr gene analysis tool (Chen et al., 2013). For strong candidate genes, a tag SNP was selected based on its position relative to the gene. The association of the tag SNP with the studied traits was further analyzed

using an animal model with batch and genotype of the tag SNP as class variables. This analysis was performed using the full pedigree under a Bayesian setting with TM software (Legarra et al., 2011).

7.2.4. Genomic prediction

We used the animals born in 2002−2003 as training data to estimate the SNP effects and then to predict the GEBV of the animals born in 2009−2010. The effect of each SNP was re-estimated using the same procedure as for GWAS but with π=0.9985 because only the training set (n=65) was used instead of the whole population. The GEBV of an individual *i* in the testing dataset was predicted as:

$$\mathrm{GEBV}_i = \sum_{j=1}^{k} z_{ij}\,\hat{\alpha}_j ,$$

where z_{ij} is the genotype of animal *i* for a SNP at locus *j* (*j*= 1 to *k*, where *k* is the number of SNPs) coded as above, and $\hat{\alpha}_j$ is the allele substitution effect estimate for the SNP at locus *j* based on the analysis of the training dataset. The correlation between GEBV and the adjusted phenotypic values of the testing dataset was used as a measure of the prediction accuracy. Phenotypes were adjusted for batch and age at slaughter using a fixed model. Different sets of SNPs were used for both training and prediction: (1) all SNPs in the chip, (2) only SNPs from selected regions with the strongest associations, or (3) all SNPs in the chip but excluding those in the selected regions. For case (2) only, some SNPs not available in the chip but genotyped independently in a previous study (AY487830:*g.2228T>C* and *g.2281A>G*; Chapter 6) were also used.

7.3. RESULTS AND DISCUSSION

7.3.1. Genome-wide association study

The posterior means of variance components and heritabilities based on the genotypic data are given in Table 7.1. Variance estimates of individual markers for BT and IMF content and composition of GM are shown in Figure 7.1, and those of LD and SF are in Figures 7.2 and 7.3, respectively. A summary of the 1-Mb windows explaining more than 2.5% of genetic variance of any trait is given in Table 7.2. The GWAS analysis only found weak associations for carcass BT, with the highest values of explained genetic variance hardly reaching 2%. In contrast, strong signals for IMF content and fatty acid composition of GM and LD were located on SSC6 and SSC14, which are zoomed in in Figures 7.4 and 7.5.

The region on SSC6, at 132−137 Mb, accounted for 5.8% of the genetic variance of IMF in GM. This region includes two overlapping genes, the leptin receptor (*LEPR*)

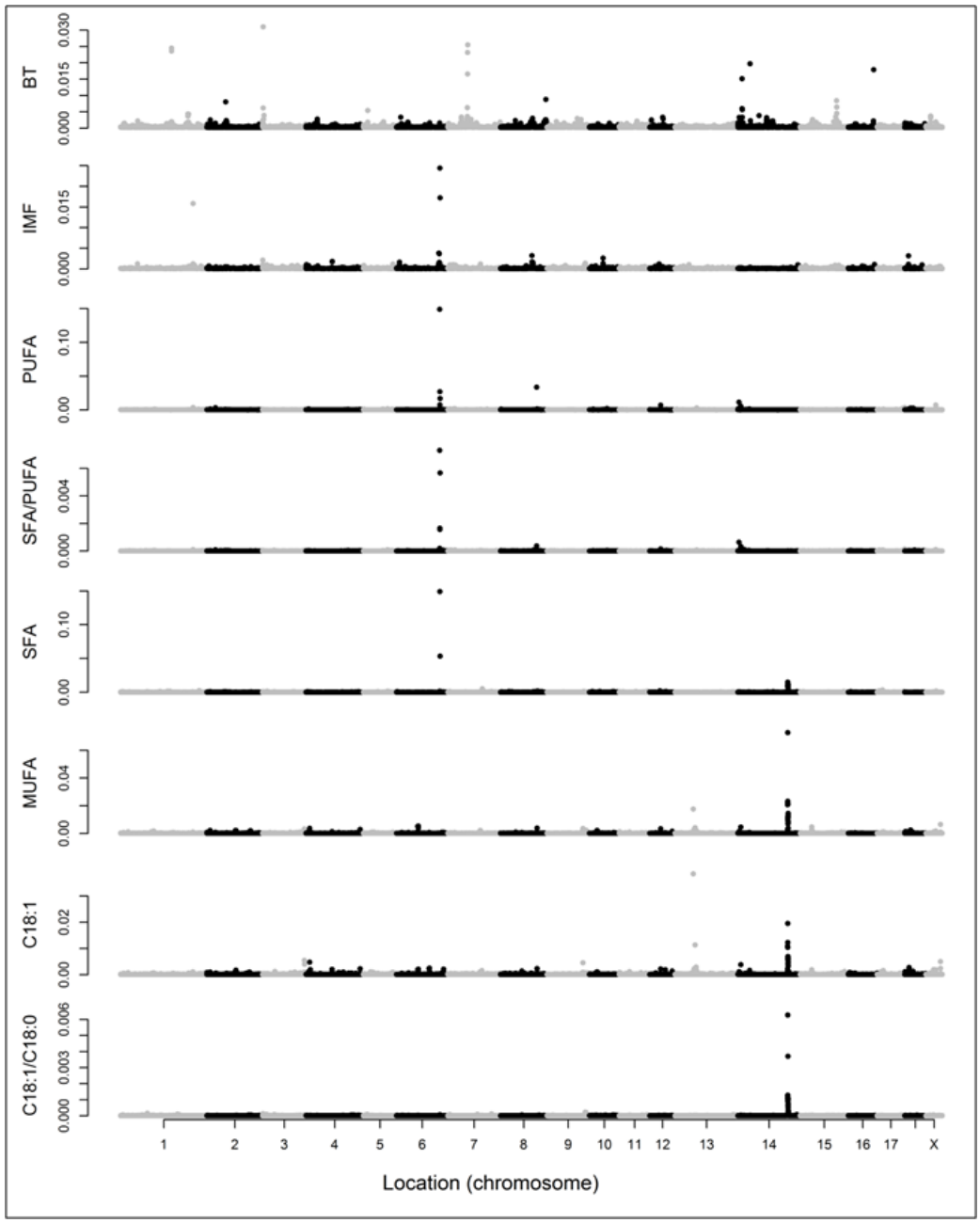


Figure 7.1. Variance estimates of individual markers for backfat thickness (BT, mm) and intramuscular fat (IMF, %) content and composition of *gluteus medius*, including saturated (SFA, %), monounsaturated (MUFA, %), and polyunsaturated (PUFA, %) fatty acids, individual oleic acid (C18:1, %), and the ratios of oleic to stearic acid (C18:1/C18:0) and SFA/PUFA.

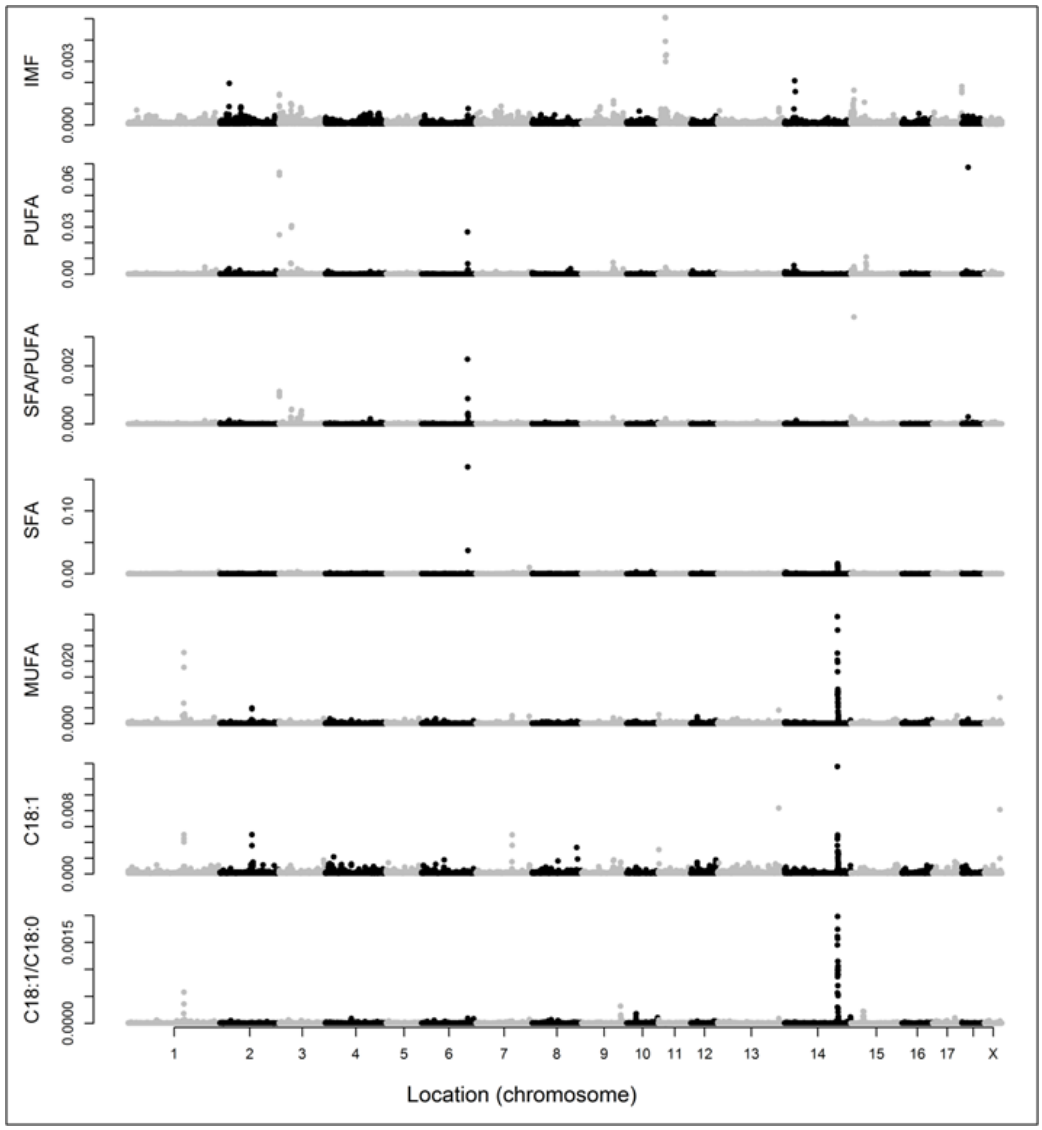


Figure 7.2. Variance estimates of individual markers for intramuscular fat (IMF, %) content and composition of *longissimus dorsi*, including saturated (SFA, %), monounsaturated (MUFA, %), and polyunsaturated (PUFA, %) fatty acids, individual oleic acid (C18:1, %), and the ratios of oleic to stearic acid (C18:1/C18:0) and SFA/PUFA.

and the leptin receptor overlapping transcript (*LEPROT*), which share the two first exons (Figure 7.4). Leptin is an adipocytokine that acts as an adiposity signal that regulates energy intake and expenditure through interaction with its receptor. The *LEPROT* gene encodes a protein that negatively regulates the presence of leptin receptors in the cell surface, decreasing the response to leptin. A non-synonymous

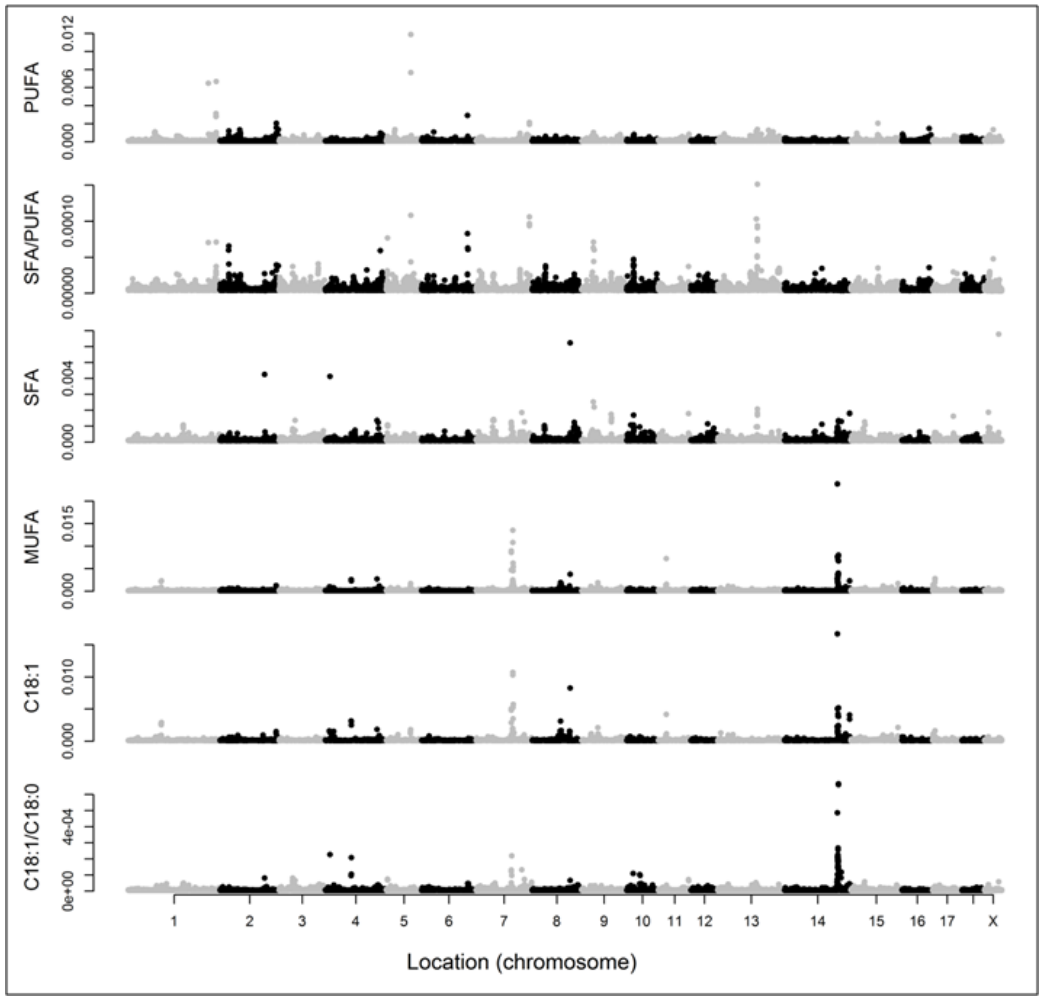


Figure 7.3. Variance estimates of individual markers for subcutaneous fat composition, including saturated (SFA, %), monounsaturated (MUFA, %), and polyunsaturated (PUFA, %) fatty acids, individual oleic acid (C18:1, %), and the ratios of oleic to stearic acid (C18:1/C18:0) and SFA/PUFA.

polymorphism in the exon 14 of *LEPR* has been reported as the possible causative mutation associated with increased feed intake and fatness, affecting both BT and IMF (Óvilo et al., 2005; Galve et al., 2012; Uemoto et al., 2012a). Moreover, several QTL for IMF have been reported in this region (animalgenome.org/QTLdb/pig.html). In our study, this region (135−137 Mb) was also strongly associated with fatty acid composition, both in GM and LD. In particular, it explained a great percentage of the genetic variance for SFA (17.6%), PUFA (16.7%), and SFA/PUFA (21.7%) in GM and, to a lesser extent, in LD (14.7, 2.5, and 3.6%, respectively). However, when adjusting for IMF, the variance explained by this region decreased to 0−7%, indicating

Table 7.2. Candidate regions for intramuscular fat (IMF) content and fatty acid composition of muscles *gluteus medius* (GM) and *longissimus dorsi* (LD) and fatty acid composition of subcutaneous fat (SF).

SSC	Position[1] (Mb)	Trait[2]	Tissue	Genetic variance[3] (%)	Top 1-Mb window		
					Position (Mb)	Genetic variance[3] (%)	P(>0)[4]
1	181−186	MUFA	LD	4.4	182−183	3.0	0.31
3	1−3	PUFA	LD	10.7	1−2	9.3	0.68
		SFA/PUFA	LD	3.2	1−2	2.5	0.17
3	28−30	PUFA	LD	5.1	29−30	3.8	0.41
5	83−85	PUFA AdjIMF	GM	3.0	84−85	2.5	0.35
6	132−137	IMF	GM	5.8	135−136	3.1	0.28
6	135−137	SFA	GM	17.6	135−136	13.2	0.72
			LD	14.7	135−136	12.2	0.72
		PUFA	GM	16.9	135−136	15.4	0.76
			LD	2.5	135−136	2.2	0.31
		SFA/PUFA	GM	21.7	135−136	21.7	0.58
			LD	3.6	135−136	3.0	0.22
		SFA AdjIMF	GM	4.1	135−136	3.0	0.34
			LD	7.0	135−136	6.1	0.47
		PUFA AdjIMF	GM	1.2*	135−136	1.0	0.16
			LD	0.4*	135−136	0.4	0.10
		SFA/PUFA AdjIMF	GM	1.2*	135−136	0.9	0.14
			LD	0.6*	135−136	0.5	0.11
7	87−96	SFA	GM	2.8	90−91	0.8	0.17
		MUFA	SF	5.3	90−91	1.7	0.18
		C18:1	SF	4.8	90−91	1.7	0.17
8	107−113	IMF	GM	2.6	109−110	0.6	0.09
9	145−148	PUFA AdjIMF	LD	2.9	146−147	1.6	0.22
11	19−21	IMF	LD	3.7	19−20	2.0	0.20
12	24−25	SFA AdjIMF	GM	2.5	24−25	2.5	0.39
13	40−50	MUFA	GM	3.3	40−41	1.3	0.20
		C18:1	GM	6.7	40−41	3.3	0.32
		C18:1 AdjIMF	GM	3.9	40−41	1.8	0.22
14	120−124	SFA	GM	18.2	121−122	10.1	0.57
			LD	17.3	121−122	9.1	0.54
			SF	2.2*	122−123	0.9	0.14
		MUFA	GM	27.8	121−122	17.5	0.64
			LD	24.1	121−122	12.2	0.53
			SF	11.5	122−123	3.7	0.29
		C18:1	GM	14.7	121−122	8.4	0.55
			LD	8.0	121−122	2.9	0.31
			SF	7.5	122−123	2.3	0.24
		C18:1/C18:0	GM	45.0	121−122	31.1	0.71
			LD	38.7	121−122	22.4	0.61
			SF	15.2	122−123	5.2	0.33

[1−4,*] See footnotes in next page.

Table 7.2. Continued.

SSC	Position[1] (Mb)	Trait[2]	Tissue	Genetic variance[3] (%)	Top 1-Mb window		
					Position (Mb)	Genetic variance[3] (%)	P(>0)[4]
14	120−124	SFA AdjIMF	GM	22.9	121−122	12.3	0.57
			LD	23.7	121−122	13.6	0.59
		MUFA AdjIMF	GM	28.6	121−122	18.0	0.65
			LD	25.2	121−122	12.8	0.53
		C18:1 AdjIMF	GM	16.0	121−122	9.0	0.55
			LD	9.5	121−122	3.8	0.35
		C18:1/C18:0 AdjIMF	GM	44.8	121−122	30.6	0.70
			LD	38.5	121−122	22.4	0.61
15	7−8	SFA/PUFA	LD	3.1	7−8	3.1	0.20
18	13−15	PUFA	LD	3.4	14−15	3.2	0.37

[1] Regions that explained at least 2.5% of genetic variance. To take account of potential linkage disequilibrium between SNPs, combinations of contiguous or nearby 1-Mb windows that accounted for at least 0.25% of the genetic variance were considered. Details of the most associated 1-Mb window are also given.
[2] SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; C18:1: oleic acid; C18:0: stearic acid; AdjIMF: trait with intramuscular fat content fitted in the model as a covariate.
[3] Posterior mean of the percentage of total genetic variance explained by this region or 1-Mb window.
[4] Probability that the percentage of total genetic variance explained by the top 1-Mb window is greater than 0.
* Below 2.5% but shown due to the importance of the locus.

Table 7.3. Mean of the estimated marginal posterior distribution of differences between ASGA0089937 genotypes and probability of the difference being positive (*P*(>0)) for leptin concentration in plasma and fat-related traits.

Trait[1]	CC−AA		CC−AC		AC−AA	
	Mean	*P*(>0)	Mean	*P*(>0)	Mean	*P*(>0)
Leptin in plasma, ng/ml	+32.21	0.91	+25.50	0.97	+6.71	0.69
Backfat thickness, mm	+2.50	0.99	+1.15	0.93	+1.35	0.94
Muscle *gluteus medius*						
Intramuscular fat, %	+0.91	0.99	+0.59	0.98	+0.33	0.85
SFA, %	+1.30	>0.99	+1.25	>0.99	+0.04	0.56
MUFA, %	+0.56	0.89	+0.17	0.69	+0.40	0.85
PUFA, %	−1.91	<0.01	−1.41	<0.01	−0.51	0.08
Muscle *longissimus dorsi*						
Intramuscular fat, %	+0.51	0.97	+0.37	0.98	+0.14	0.75

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

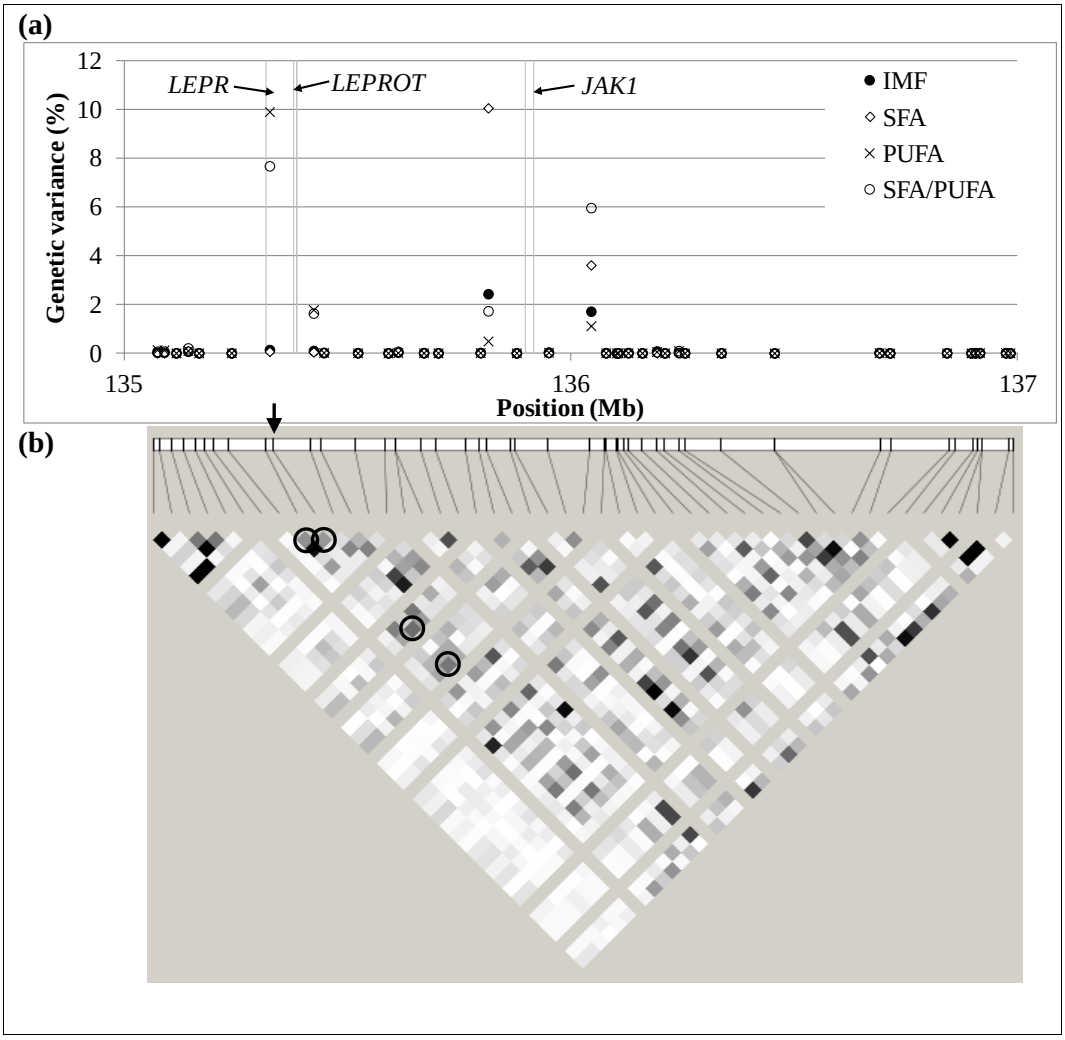


Figure 7.4. Detail of the individual markers in the SSC6 at 135−137 Mb region. Panel (a) shows the variance estimates for intramuscular fat content (IMF, %), saturated fatty acids (SFA, %), polyunsaturated fatty acids (PUFA, %), and SFA/PUFA of muscle *gluteus medius*, with vertical grey lines indicating the location of candidate genes *LEPR* (tentative), *LEPROT*, and *JAK1*. Panel (b) shows the linkage disequilibrium in the region (white: r^2=0; black: r^2=1). An arrow indicates the SNP described by Óvilo et al. (2005) in exon 14 of *LEPR*, not provided in the chip and not included in the genome-wide association study but in high linkage disequilibrium with the four SNPs picking up the strongest signals (circled; from left to right: ASGA0089937, ASGA0093565, ALGA0037129, and H3GA0053839).

that, at least in part, the observed associations of the SNPs in this region with SFA and PUFA are an indirect effect of differences in IMF. It is well known that the endogenous synthesis of SFA and MUFA increases with IMF content, which leads PUFA to proportionally decrease (Wood et al., 2008). The association of the *LEPR*

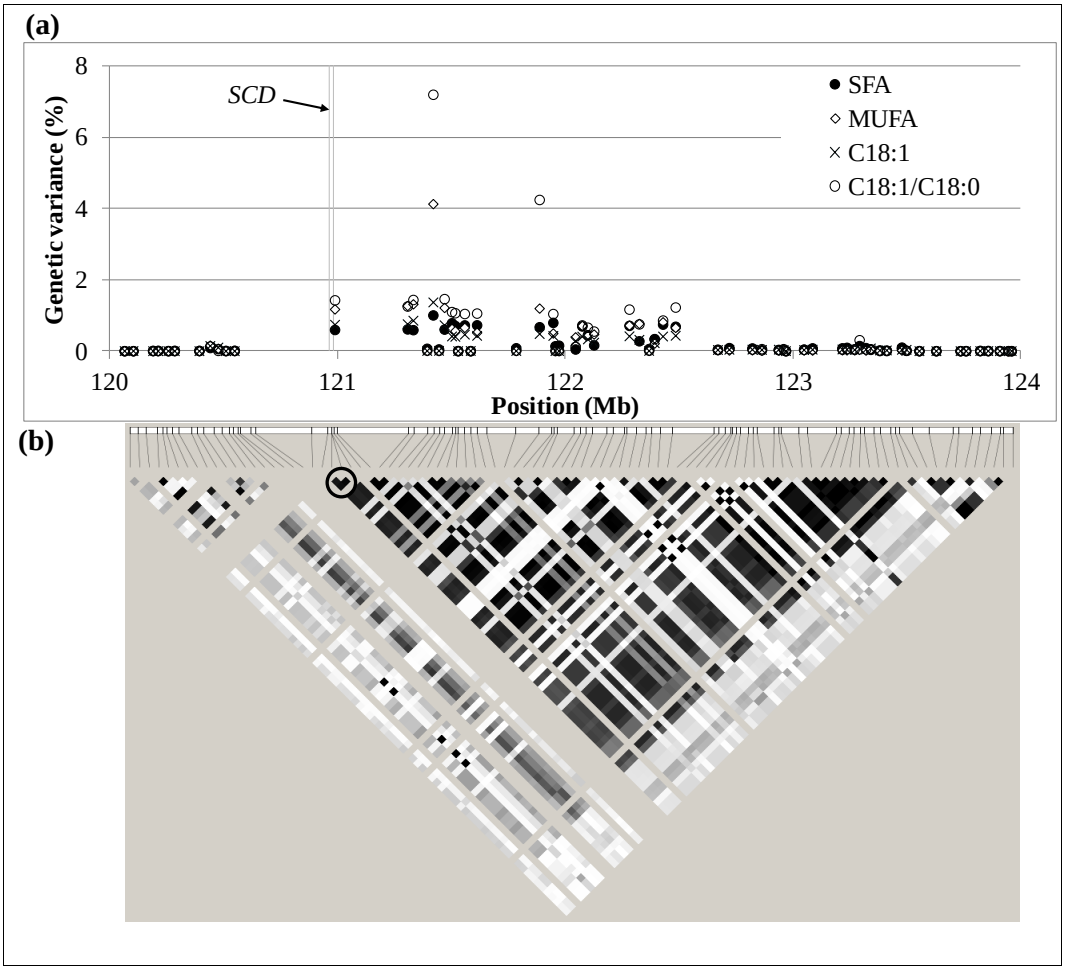


Figure 7.5. Detail of the individual markers in the SSC14 at 120−124 Mb region. Panel (a) shows the variance estimates for saturated fatty acids (SFA, %), monounsaturated fatty acids (MUFA, %), oleic acid (C18:1, %), and the desaturation ratio C18:1/C18:0 of muscle *gluteus medius*, with vertical grey lines indicating the location of candidate gene *SCD*. Panel (b) shows the linkage disequilibrium in the region (white: r^2=0; black: r^2=1). Circled, the haplotype described in Chapter 6, not provided in the chip and not included in the genome-wide association study but in high linkage disequilibrium with several SNPs downstream.

locus with fat-related traits was further evaluated using ASGA0089937 (in intron 3 of the *LEPR* gene; Genbank accession number FN677933.1) as a tag SNP. Pigs with CC genotype (allele C frequency: 0.55) for this SNP had higher leptin concentration in plasma, were fatter (both BT and IMF), and had more saturated fat than AA pigs (Table 7.3). Taken together, these results suggest that a mutation in or near the *LEPR* gene may affect the leptin regulatory system (Clément et al., 1998) and, as a consequence, feed intake and overall carcass fatness. The significant signal in this region reaches out about 0.6 Mb, starting in the *LEPR*/*LEPROT* locus and finishing

downstream the *JAK1* gene. Interestingly, the signal transductor coded by *JAK1*, which maps to 135.9 Mb (Figure 7.4), is also involved in the adipocytokine signaling pathway, promoting the leptin-induced transactivation of the satiety neuropeptide *NPY* gene (Muraoka et al., 2003). Mutations in *JAK1* have not been related to fattening traits in pigs. The *JAK1*-located SNPs are in strong linkage disequilibrium with the exon 14 mutation in *LEPR* (Figure 7.4), which, in our view, remains the strongest candidate mutation for this QTL effect.

On the other hand, the region on SSC14 at 120−124 Mb was found to be strongly associated with SFA, MUFA, C18:1, and the desaturation index C18:1/C18:0. This region, which was estimated to capture up to 45% of the genetic variance of C18:1/C18:0, corresponds to the location of the *SCD* gene (Figure 7.5), thereby confirming the association already found in the same population in Chapter 6 of an haplotype in the promoter of the *SCD* gene with C18:1/C18:0 and related fatty acid traits. The SCD enzyme is rate-limiting for the biosynthesis of MUFA C18:1 from SFA C18:0. Due to the high linkage disequilibrium downstream the *SCD* position (Figure 7.5), the signal detected spanned 4 Mb and included other genes involved in lipid metabolism, such as *ELOVL3* (responsible for the elongation of long-chain SFA and MUFA). The percentages of genetic variance explained by this region for SFA (18.2% in GM and 17.3% in LD), MUFA (27.8% and 24.1%, respectively), C18:1 (14.7% and 8.0%, respectively), and C18:1/C18:0 (45.0% and 38.7%, respectively) were close to those obtained in Chapter 6 when only accounting for the effect of the *SCD* haplotypes. Moreover, in accordance with the findings in Chapter 6, the explained genetic variance did not depend on IMF, which confirms that sequence variation at this locus affects fatty acid composition but not total IMF content (Chapter 6). The same association was found, although to a lesser extent, in SF.

A previous GWAS performed by Yang et al. (2013), using a Duroc × Erhualian F_2 cross and a larger population size, did not reach much different results from ours, with the *SCD* locus being the only reported QTL for major fatty acids in IMF. The aforementioned *SCD* haplotypes have not been found to segregate in the Iberian and Landrace breeds (Chapter 6), but neither the *SCD* nor the *LEPR* locus were identified in the GWAS experiments using Iberian × Landrace crossbreds by Ramayo-Caldas et al. (2012) and Muñoz et al. (2013).

7.3.2. Genomic prediction

The different sets of SNPs used were: (1) all SNPs in the chip, (2) only SNPs from the *LEPR* and/or *SCD* loci, and (3) all SNPs in the chip but excluding those in the *LEPR* and *SCD* windows defined in Table 7.2. For case (2), we used SNPs ASGA0089937 and ASGA0093565, as representatives of the *LEPR* locus, and

AY487830:*g.2228T>C* and *g.2281A>G*, from the promoter of gene *SCD* (not available in the chip but genotyped independently in Chapter 6). The correlation between GEBV and adjusted phenotypic values of the testing dataset are given in Table 7.4. Note that these correlations should be divided by the square root of heritability of the trait to convert them to accuracies of GEBV as predictors of true breeding values. Accuracies of GEBV based on 36,432 SNPs were low (0.04−0.10) for IMF, PUFA, and SFA/PUFA, moderate (0.28) for C18:1, and high (0.48−0.60) for SFA, MUFA, and C18:1/C18:0. The accuracy of SFA, MUFA, and C18:1/C18:0 only showed a slight decline when predictions were based only on the two SNPs at the promoter of the *SCD* gene, and improved for C18:1. Similarly, using only two SNPs at the *LEPR* locus raised the accuracy of predictions for IMF, PUFA, and SFA/PUFA to 0.33−0.40, although that for SFA was halved as compared to whole genome predictions. The combination of the four SNPs in *SCD* and *LEPR* provided similar or better accuracies than the whole chip, with values ranging from 0.32, for IMF, to 0.54, for C18:1/C18:0. Consistently, the rest of SNPs predicted the phenotypes very poorly.

On one hand, these results confirm the relevance of the effects of the SNPs at *SCD* and *LEPR* loci and, because pigs in the predicted set were separated by a span of seven years from those in the training set, that their effects are consistent across generations. On the other hand, these results suggest that using many SNPs does not necessarily lead to improved predictive ability. To our knowledge, the only attempt to assess the value of genomic prediction for IMF fatty acid composition has been in Angus cattle using the BovineSNP50 BeadChip (Saatchi et al., 2013). Interestingly,

Table 7.4. Correlation between genomic estimated breeding value and adjusted phenotype of the 2009-born pigs using the 2002-born as training set and using different sets of SNPs for both training and prediction.

Trait[1]	SNPs used for training and prediction[2]				
	36k	*SCD*	*LEPR*	*SCD+LEPR*	36k−*SCD*−*LEPR*
IMF	0.04	-[3]	0.33	0.32	0.03
SFA	0.48	0.38	0.23	0.43	0.17
MUFA	0.50	0.43	-	0.46	0.14
C18:1	0.28	0.36	-	0.37	0.14
PUFA	0.07	-	0.40	0.42	0.04
C18:1/C18:0	0.60	0.54	-	0.54	0.04
SFA/PUFA	0.10	-	0.38	0.36	0.03

[1] See abbreviations in Table 7.1.
[2] 36k: using all 36,432 SNPs; *SCD*: using only AY487830:*g.2228T>C* and *g.2281A>G* from the *SCD* promoter; *LEPR*: using only ASGA0089937 and ASGA0093565; *SCD+LEPR*: using all four SNPs at the *SCD* and *LEPR* loci; 36k−*SCD*−*LEPR*: all SNPs except the SSC14 at 120−124 Mb (*SCD*) and SSC6 at 135−137 Mb (*LEPR*) windows.
[3] A hyphen indicates lack of convergence of the model.

their reported correlations between GEBV and phenotypes using the whole genome SNPs were in line with ours, i.e., very low for PUFA and SFA/PUFA (0.07 and 0.10) and moderate for C18:1, MUFA, and SFA (0.26−0.34).

7.4. CONCLUSIONS

We have been able to confirm the association of known SNPs at the *SCD* gene with fatty acid composition and to identify an association between SNPs in the *LEPR*/*LEPROT* region and IMF traits, which had not yet been detected in the Duroc population used in this study. The described SNPs in these two loci can be used conjointly for marker-assisted selection for IMF and fatty acid composition. The other minor candidate regions detected require further research, but they provide a good basis for exploring the development of low-density SNP panels aimed at improving meat quality.

Discussion

The first issue that was assessed was the correct methodology for statistically analyzing the fatty acid compositions. As the name itself indicates, fatty acid compositions belong to the mathematical category of compositional data (Aitchison, 1986), which require specific treatment according to their properties and own geometry. The contents of each individual fatty acid are expressed in relative terms, commonly respect to the amount of total fatty acids, and, because of this, the fatty acid percentages of a composition are not independent: when one fatty acid percentage increases at least another one has to decrease, or, in other terms, there must be at least one negative covariance between each fatty acid and any other. This has been commonly ignored in meat quality research, and to our knowledge there was no study assessing the practical implications of neglecting the compositional data approach. Application of the compositional data techniques in Chapter 1 brought a new perspective on how to perform exploratory data analyses and interpret correlations among ratios of fatty acids. However, in the particular case of pork, the isometric log-ratio (**ilr**) transformation (Egozcue et al., 2003) did not lead to relevant changes in the results of variance and regression analyses when compared to standard analysis of raw percentages.

Additionally, Estany et al. (2011) assessed the impact of introducing the compositional data approach to genetic evaluations of intramuscular fat (**IMF**) and oleic acid (**C18:1**) contents. Raw and ilr-transformed percentages were used to estimate the genetic parameters and estimated breeding values (**EBV**) of pigs. Although the ilr-transformed data showed a capacity for predicting future records slightly greater than the raw percentages, no relevant differences were observed either in the estimation of genetic parameters, as reported in Chapter 2, or in the ranking of the selection candidates. In this latter case, Spearman's correlation coefficients of >0.95 were found between the EBV estimated based on the raw or ilr-transformed data (>0.99 when only animals with own registers of IMF and C18:1 were considered). Based on these results, we decided to neglect the compositional data approach in further analyses. Although analyzing raw percentages under the Euclidean metrics is methodologically inconsistent, results were robust enough for genetic evaluations, selection, and other practical applications. The reason is that fatty acid composition of pork is subjected to biological homeostasis and, as a consequence, it has low variability. Only a narrow range of possible fatty acid percentages is observed and, in this range, fatty acid data approximately follow the Euclidean geometry in the real space.

Two genetic breeding value evaluation strategies for C18:1 were explored. The first was based on best linear unbiased prediction (**BLUP**) of EBV using only phenotypic data on relatives, while the second relied on the use of genetic markers. Our results indicated that direct selection for C18:1 based only on phenotypes from relatives is theoretically feasible (Chapter 2). Oleic acid content in IMF is a highly heritable trait. Its heritability (h^2) was estimated to be 0.44−0.50 in muscle *gluteus medius* (**GM**) and 0.59 in both muscles *longissimus dorsi* (**LD**) and *semimembranosus* (**SM**) (Chapters 2 and 3). The heritability estimates in our population were higher than the unweighted average (0.38) and close to the upper bound of the range (0.26−0.58) of previous reports (Suzuki et al., 2006; Casellas et al., 2010; Ntawubizi et al., 2010; Sellier et al., 2010). In subcutaneous fat (**SF**), our estimate for heritability of C18:1 was 0.41 (Chapter 3), which is similar to the unweighted average of previous estimates (Fernández et al., 2003; Suzuki et al., 2006; Sellier et al., 2010; Gjerlaug-Enger et al., 2011). However, we did not consider the litter effect for C18:1 and IMF in our analyses. Preliminary results indicated that the litter effect (c^2) for C18:1 and IMF might be around 0.05−0.10 and their heritabilities after accounting for the litter effect ~0.40. As a consequence, their genetic responses to selection may be slightly overestimated. In contrast, the genetic correlations among traits did not seem to be substantially affected by the litter effect.

Results from a selection experiment proved that C18:1 responds to direct selection (Chapter 5). Unfortunately, the responses displayed in this experiment fell short as compared to expected values. The reason for this is the poor predictive capacity of the EBV of the selection candidates, particularly in the third batch. Intramuscular C18:1 has a high heritability and considerable genetic variation, but accurate EBV predictions are needed. One of the main difficulties in C18:1 (and also IMF) breeding schemes appears during the phenotyping of animals. Whereas IMF can be indirectly measured via real-time ultrasound *in vivo* in selection candidates (Newcom et al., 2002, 2005), this technology is unsuitable for fatty acid composition traits. Genetic evaluation for fatty acids content is more likely to be based on phenotypes of slaughtered littermates and other relatives of the selection candidates. In our experiment, slaughtered barrows with C18:1 records were distantly related to selection candidates. Further analyses showed that if records from littermates had been available to ensure a high accuracy for the genetic evaluation of candidates, genetic responses had been significantly improved.

The muscle sampled for measuring fatty acid content is a relevant factor that should not be neglected when designing a selection programme for C18:1 or any other fatty acid. It is known that muscles differ among them and from SF regarding fatty acid metabolism and deposition (Chapter 1; Sharma et al., 1987; Faucitano et al., 2004; Franco et al., 2006; Duran-Montgé et al., 2008; Bosch et al., 2012). The fatty acid

content and composition of muscles GM and LD are highly correlated (0.62−0.82), but their correlation with other muscles, such as SM, can be much lower (Chapter 3). It is advisable, therefore, to analyze the correlation between the fatty acid composition of the criterion and target muscles, given that all muscles cannot be sampled. Our results indicated that selection performed on records from GM would indirectly improve also C18:1 in LD, although with an opportunity cost. This was validated in our selection experiment, where we found evidence of a positive correlated response of C18:1 in LD after selection based on GM (Chapter 5). This correlated response in LD was observed in experimental batch 1 but not in batch 2, but results also indicated that if higher accuracies had been obtained for C18:1 in GM, a positive correlated response in LD had been expected in all batches. To our knowledge, there are no studies assessing the correlations between fatty acid traits in two different muscles other than Rauw et al. (2012), who reported phenotypic correlations for fatty acids in GM and LD much lower than ours. On the other hand, measuring fatty acid composition traits in SF could be informative only for anatomically close muscles (Chapter 3). In particular, SF composition may be of interest for improving the fatty acid composition of the loin (Suzuki et al., 2006; Yang et al., 2010).

Selection responses of C18:1 should be put into context with the correlated genetic changes in other economic traits. A positive genetic correlation was estimated between C18:1 and IMF (0.47; Chapters 2 and 3). Genetic correlations with performance traits backfat thickness (**BT**) and body weight (**BW**) were positive (0.22 and 0.11 for C18:1, respectively) but low enough to allow the simultaneous genetic improvement of all analyzed traits (Chapter 2). Our selection experiment for C18:1 applied no restriction on the correlated traits (Chapter 5). Interestingly, BT decreased in both experimental batches where C18:1 successfully responded to selection. Correlated responses for IMF and BW differed across batches and suggested that, if restrictions were applied, scenarios of simultaneous improvement for the four traits could have been met. On the other hand, C18:1 is expected to respond to selection similarly to IMF.

Like C18:1, IMF is positively correlated to BT (0.37−0.42; Chapters 2, 3, and 4) but there is room for BT to be genetically improved independently to IMF (Chapter 2; Solanes et al., 2009; Clutter, 2011). We proved that selection for reduced BT at restrained IMF was possible after a one-generation selection experiment (Chapter 4). These results have been validated by the genetic trends of the studied population for the last decade, which are plotted in Figure D.1. The EBV for all pedigreed animals were estimated using the same 4-trait multivariate animal model as in Chapters 2 and 5 with the complete data described in the Animals and Samples Section. The average EBV per trait and year of birth was calculated and expressed in genetic standard deviation units. The displayed genetic trends show that it is possible to reduce BT

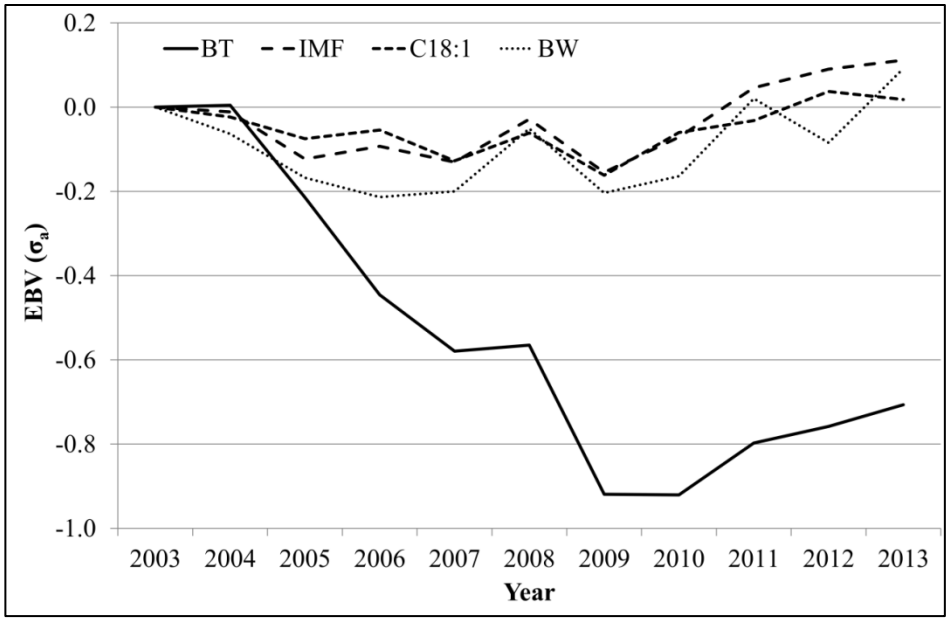


Figure D.1. Evolution of the average estimated breeding value (EBV, expressed in additive genetic standard deviation units) by year of birth for backfat thickness (BT), intramuscular fat (IMF), oleic acid (C18:1), and body weight (BW) during the last decade.

while maintaining IMF, C18:1, and BW. After one decade of selection, BT has been reduced by approximately 0.7 genetic standard deviations while the other traits did not change substantially.

We identified a haplotype block in the promoter region of the stearoyl-CoA desaturase (***SCD***) gene that affects fatty acid composition of IMF and SF but neither IMF content nor carcass fatness (Chapter 6). The haplotype is formed by three single nucleotide polymorphisms (**SNPs**): AY487830:*g.2108C>T*, *g.2228T>C*, and *g.2281A>G*. Although in complete linkage disequilibrium with the two other SNPs, our results provide evidence that the SNP *g.2228T>C* is the causative mutation of the effect of the haplotype on C18:1 and the monounsaturated fatty acids (**MUFA**) content. This haplotype presents several advantages in terms of their use in selection: (1) its effect is additive, (2) it explains a high percentage of the additive genetic variance, (3) the frequency of the favorable haplotype (C-T-A) is intermediate, (4) it does not influence IMF and production traits, and (5) its effect is consistent across muscles and fat tissues. The effect has been validated across generations, in different Duroc lines, and in Duroc crossbreds, which is important because the Duroc, in accordance with the current regulations (BOE, 2014), is the only breed allowed for use in the production of Iberian products.

The response of C18:1 to the selection experiment in Chapter 5 was reexamined in light of the effect of the *SCD* marker (Table D.1). Decomposing the response into polygenic and gene effects showed unexpected results. Responses in each batch gave a different picture when the underlying effects of selection are unraveled. The components of the response in batch 1 were the expected: the realized response was partly explained by the polygenic effect and partly by the marker (~30% of total response). Total response in batch 2 was similar to that obtained in batch 1, but here the realized change was only due to the polygenic effect, since the frequency of allele T did not differ between the selected and the control group. Finally, in batch 3, although there was no response, the frequency of the allele T increased significantly. Thus, the impact of selection on the polygenic and marker effects varied across batches. Polygenic response was negative and marker response was positive in batch 3. The negative polygenic response in batch 3 could be attributed to the poor accuracy of breeding values in this batch as compared to the other two. In such situation, the marker is the dominant driving-selection force and therefore, in line with the results, when it works better. These results highlight the fact that a successful selection for C18:1 needs to account for both effects.

The genome-wide association study confirmed the *SCD* locus as the most determining for the fatty acid composition together with polymorphisms in the leptin receptor (***LEPR***) locus. The *LEPR* polymorphisms affected IMF content and, as a

Table D.1. Number of genotypes (AY487830:*g.2228T>C*), allelic frequency of T (f(T)), and response to selection for oleic acid content (due to the *SCD* marker and polygenic effect) by batch and selection group.

Selection group	Genotype (AY487830:*g.2228T>C*)				Components of response (% of total fatty acids)		
	TT	CT	CC	f(T)	Total[1]	Polygenic[2]	*SCD*[3]
Batch 1							
Selected	7	23	12	0.44	+0.35	+0.27	+0.11
Control	8	14	21	0.35	-	-	-
Batch 2							
Selected	9	29	10	0.49	+0.33	+0.27	−0.03
Control	13	22	13	0.50	-	-	-
Batch 3							
Selected	12	26	6	0.57*	+0.04	−0.13	+0.21
Control	5	26	12	0.42	-	-	-

[1] Polygenic response obtained using the model described in Chapter 5.
[2] Polygenic response as in Chapter 5 but including the *SCD* genotype as an additional effect.
[3] Response due to changes in the allelic frequency of the *SCD* marker, with genotype effects estimated as in footnote 2.
* Significant difference between the allele frequency of T between selected and control groups with a χ^2-test ($p<0.05$).

result, saturated (**SFA**) and polyunsaturated (**PUFA**) fatty acids content (Chapter 7). This association was tested using a tag SNP and confirmed to consistently affect BT and IMF content and composition in muscle. The other associations found in the genome-wide association study were much weaker in comparison with those of loci *SCD* and *LEPR* and need further validation.

Both *SCD* and *LEPR* genetic markers proved to be useful for predicting IMF content and composition traits (Chapter 7). However, because of the genotyping costs, the benefits of marker-assisted and genomic selection should be carefully evaluated against selection based exclusively on phenotypes and pedigree. Moreover, there exist genetic evaluation strategies that combine both sources of information. In Table D.2 the accuracy (calculated as the correlation with adjusted phenotypes) of different methodologies for estimating the EBV are compared. Case A summarizes the genomic EBV (**GEBV**) predicted using Bayes B in Chapter 7. In case B, EBV were predicted by BLUP without genetic markers information. In case C, a single-step genomic BLUP (**ssGBLUP**; Legarra et al., 2014) was performed with high-density genotyping data. Finally, in case D a BLUP was performed incorporating information of the genetic markers at the *SCD* and *LEPR* loci.

Genomic prediction by Bayes B using all SNPs available had disparate accuracies: high (0.48−0.60) for SFA, MUFA, and C18:1/C18:0, moderate (0.28) for C18:1, and low (0.04−0.10) for IMF, PUFA, and SFA/PUFA. Interestingly, when only four markers at the *SCD* and *LEPR* loci were used instead of all available SNPs, the accuracies for the more poorly predicted traits raised to 0.32−0.42, with only a small penalty for the traits with the highest accuracies (0.43−0.54). When records from littermates were available, estimates using BLUP from about 1,000 records provided accuracies of 0.31−0.39 for all traits except SFA and C18:1/C18:0, consistently with the results reported in Chapter 5. However, because BW and BT are recorded routinely in commercial breeding units, they can be included in a multivariate model for a joint evaluation with IMF and fatty acid traits. In doing so, the accuracies of BLUP exceeded those of Bayes B, excepting for the C18:1/C18:0 ratio, which is uncorrelated with BW and BT (results not shown). For the other traits, genetic correlations with BW and BT are high enough to improve substantially the accuracy of BLUP. Genetic markers resulted more beneficial when phenotypic data contribute with little information.

Accuracies did not improve with ssGBLUP, probably because of the low number of genotyped animals and the poor degree of relationship between the reference and the testing sets. In contrast, BLUP accounting for the *SCD* and *LEPR* genetic markers provided the best accuracies. With univariate models and no records from littermates, accuracies from BLUP with genetic markers where similar to those obtained for Bayes B with only the *SCD* and *LEPR* markers. By adding records from

Table D.2. Correlation of estimated breeding value (EBV) with adjusted phenotype[1] in a testing set of pigs born in 2009−2010 (n=70) by prediction methodology.

Methodology[2]	Trait[3]						
	IMF	SFA	MUFA	C18:1	PUFA	C18:1/C18:0	SFA/PUFA
(A) Bayes B							
36k	0.04	0.48	0.50	0.28	0.07	0.60	0.10
SCD+LEPR	0.32	0.43	0.46	0.37	0.42	0.54	0.36
(B) BLUP							
U, NL	0.11	0.11	0.08	0.12	0.16	0.07	0.13
U, L	0.31	0.15	0.32	0.32	0.39	0.14	0.39
M, NL	0.41	0.39	0.30	0.29	0.61	0.08	0.60
M, L	0.42	0.41	0.40	0.38	0.67	0.15	0.67
(C) ssGBLUP							
U, NL	0.13	0.14	0.07	0.11	0.26	0.02	0.23
U, L	0.31	0.15	0.34	0.34	0.48	0.15	0.45
M, NL	0.39	0.40	0.27	0.27	0.61	0.05	0.61
M, L	0.39	0.38	0.39	0.38	0.67	0.17	0.65
(D) BLUP accounting for genetic markers							
U, NL	0.34	0.50	0.39	0.31	0.41	0.51	0.41
U, L	0.42	0.52	0.51	0.44	0.51	0.53	0.51
M, NL	0.47	0.59	0.48	0.41	0.65	0.50	0.63
M, L	0.47	0.62	0.55	0.48	0.70	0.53	0.70

[1] Phenotypes were adjusted for batch and age at slaughter using a fixed model.

[2] Methodology:

(A) Genomic prediction on the effects of either 36,432 SNPs (36k) or only SNPs at *SCD* and *LEPR* loci (*SCD+LEPR*) estimated by Bayes B in a training set of 65 pigs born in 2002−2003. See Chapter 7 for further details.

(B) Univariate (U) and multivariate (M) best linear unbiased prediction (BLUP) using phenotypic data and pedigree. Multivariate models included body weight and backfat thickness at 180 d. Further model details as in Chapter 5. Data on IMF and fatty acids were from pigs born in 2002−2007 (NL; n=936) or included also littermates of the testing set (L; n=1,132). AIREMLF90 software (Misztal et al., 2002) was used.

(C) Single-step genomic BLUP using the 36k SNPs. Other details as in case B.

(D) Same as in case B but adding genotypes of *SCD* (n=915) and *LEPR* (n=803) genetic markers. The breeding value of animal *j* for trait *i* was calculated as $\hat{v}_{(SCD)ij} + \hat{v}_{(LEPR)ij} + \hat{u}_{ij}$, where $\hat{v}_{(SCD)ij}$ and $\hat{v}_{(LEPR)ij}$ are the genotypic values of markers at *SCD* and *LEPR* loci, respectively, and $\hat{u}_{ij}$ the polygenic effect adjusted for the markers.

[3] IMF: intramuscular fat; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; C18:1: oleic acid; C18:0: stearic acid.

littermates and on BW and BT, accuracies were 0.47−0.48 for IMF and C18:1, 0.53−0.55 for MUFA and C18:1/C18:0, and as high as 0.62−0.70 for SFA, PUFA, and SFA/PUFA. As compared with BLUP, the *SCD* and *LEPR* markers substantially improved the accuracy for SFA, MUFA, C18:1, and C18:1/C18:0. Contrarily, the contribution of these markers to the accuracy of IMF, PUFA, and SFA/PUFA is

irrelevant as compared to predictions based on data from routine recording of pedigree-connected relatives.

Overall, our results indicate that a breeding program involving IMF and C18:1 should first focus on designing a system for recording IMF and C18:1 at slaughter and then on developing a BLUP genetic evaluation procedure based on phenotypes and relationships among relatives. This requires to set a system for individual traceability of pigs until slaughter and the implementation there of a feasible recording routine, preferably on muscle rather than on SF. Fortunately, new non-destructive on-line equipments, mostly based on near infrared spectrometry (NIRS), are becoming available to cope with this need with very promising results (González-Martín et al., 2002, 2005). Some genetic markers, as those found here in the *SCD* and *LEPR* loci, may enhance the accuracy of BLUP-based breeding values for some meat quality traits. If additional markers are incorporated, this may lead to set a panel of several markers or even to develop low-density SNP panels (Weigel et al., 2009; Vazquez et al., 2010) with which to improve selection decisions at any stage of the breeding scheme. In light of our results, the use of genomic selection, involving the genotyping of a big number of pigs with high-density SNP chips, is not crucial. With current technical and economic standards, the use of genomic selection for improving IMF and C18:1 needs further assessment. Although it may open up new selection opportunities (Ibáñez-Escriche et al., 2014), it still entails many unsolved questions to be the method of choice at commercial level (Blasco & Toro, 2014). Instead, given the cost of feed, the input of nutrigenomics, with a better knowledge of the potential interactions between genetics and dietary factors, may give new insights into integrated strategies directed at producing pork with optimum IMF and fatty acid profiles.

Conclusions

1. Statistical analyses based on raw fatty acid percentages are robust enough for most genetic analyses and practical applications due to the low variability of the fatty acid composition of pork. However, because fatty acids are compositional data in nature, using the compositional data statistical approach may help to avoid inconsistencies and to correctly interpret results from exploratory and correlation analyses.

2. The intramuscular fat and oleic acid contents present a high heritability and are positively correlated to each other. Their genetic correlation with backfat thickness is unfavorable, particularly for intramuscular fat, but favorable with body weight, albeit lower. Yet, there are selection scenarios where intramuscular fat and oleic acid can be simultaneously improved with lean growth.

3. It has been shown experimentally that, with proper selection objectives, it is possible to decrease backfat thickness and increase carcass lean growth at no change in intramuscular fat and oleic acid contents.

4. It has been shown experimentally that oleic acid content responds to selection on predicted breeding values based only on phenotypic data from relatives.

5. The response to selection for intramuscular fat and oleic acid contents is unequal across muscles and fat tissues. Because fat content and composition traits in muscles *gluteus medius* and *longissimus dorsi* are highly genetically correlated, records taken on one of them can be used as a selection criterion for the other. However, genetic correlations with other muscles and fat tissues may be lower. In particular, the oleic acid content in subcutaneous fat could be of limited value for the oleic acid content in muscles other than *longissimus dorsi*.

6. The C-T-A haplotype (AY487830:*g.2108C>T*, *g.2228T>C*, and *g.2281A>G*) in the promoter of the stearoyl-CoA desaturase gene enhances fat desaturation in pigs without affecting total fat content. The haplotype additively increases oleic and total monounsaturated fatty acid contents, reaching to explain around 40% of the genetic variance of the stearic to oleic acid desaturation ratio. Evidence is provided that *g.2228T>C* is the causative mutation of this effect.

7. A genome-wide association study detected single nucleotide polymorphisms around the leptin receptor gene that are associated to backfat thickness and intramuscular fat content and fatty acid composition. However, unlike for the stearoyl-CoA desaturase gene polymorphism, the effects on intramuscular fat content and fatty acid composition are not specific but a result of increased carcass fatness.

8. High accuracies for the predicted breeding values of intramuscular fat and oleic acid content can be achieved based on phenotypic data of close relatives and correlated performance traits. The accuracies can be substantially enhanced with the genetic markers described at the stearoyl-CoA desaturase and the leptin receptor genes. With the benefits of genomic selection needing further assessment, selection combining pedigree-connected phenotypic data and some singled-out genetic markers is presented as a suitable alternative.

References

Aitchison J. 1982. The statistical analysis of compositional data (with discussion). *Journal of the Royal Statistical Society. Series B (Methodological)* 44: 139-177.

Aitchison J. 1986. *The Statistical Analysis of Compositional Data*. Chapman and Hall Ltd (reprinted in 2003 with additional material by The Blackburn Press), London, UK.

Aitchison J, & JJ Egozcue. 2005. Compositional data analysis: Where are we and where should be heading? *Mathematical Geology* 37: 829-850.

Aitchison J, & M Greenacre. 2002. Biplots of compositional data. *Journal of the Royal Statistical Society. Series C (Applied Statistics)* 51: 375-392.

Amills M, D Villalba, M Tor, A Mercadé, D Gallardo, B Cabrera, N Jiménez, JL Noguera, A Sànchez, & J Estany. 2008. Plasma leptin levels in pigs with different leptin and leptin receptor genotypes. *Journal of Animal Breeding and Genetics* 125: 228-233.

AOAC. 1997. Supplement to "AOAC Official Method 996.06: Fat (Total, Saturated, and Monounsaturated) in Foods Hydrolytic Extraction Gas Chromatographic Method". In: *Official Methods of Analysis*, 16th ed. (Supplement, March 1997), Association of Official Analytical Chemists (ed.), Arlington, VA.

Bacon-Shone J. 2011. A short history of compositional data analysis. In: *Compositional Data Analysis: Theory and Applications*, V Pawlowsky-Glahn, & A Buccianti (eds.), John Wiley & Sons Ltd., West Sussex, UK, pp. 3-11.

Barrett JC, B Fry, J Maller, & MJ Daly. 2005. Haploview: Analysis and visualization of LD and haplotype maps. *Bioinformatics* 21: 263-265.

Bartz M, M Szydlowski, B Kociucka, S Salamon, HH Jelen, & M Switonski. 2013. Transcript abundance of the pig *stearoyl-CoA desaturase* gene has no effect on fatty acid composition in muscle and fat tissues, bat its polymorphism within the putative microRNA target site is associated with daily body weight gain and feed conversion ratio. *Journal of Animal Science* 91: 10-19.

BBC News. 2011. Denmark introduces world's first food fat tax. 1 October 2011. Available at (26 June 2014): http://www.bbc.co.uk/news/world-europe-15137948

BBC News. 2012. Denmark to abolish tax on high-fat foods. 10 November 2012. Available at (26 June 2014): http://www.bbc.com/news/world-europe-20280863

Bejerholm C, & P Barton-Gade. 1986. Effect of intramuscular fat level on eating quality of pig meat. In: *Proceedings of the 32nd European Meeting of Meat Research Workers*, Meat Research Workers (ed.), Ghent, Belgium, pp. 389-391.

Bené H, D Lasky, & JM Ntambi. 2001. Cloning and characterization of the human stearoyl-CoA desaturase gene promoter: Transcriptional activation by sterol regulatory element binding protein and repression by polyunsaturated fatty acids and cholesterol. *Biochemical and Biophysical Research Communications* 284: 1194-1198.

Blasco A, & MA Toro. 2014. A short critical history of the application of genomics to animal breeding. *Livestock Science* 166: 4-9.

BOE. 2014. Real Decreto 4/2014, de 10 de enero, por el que se aprueba la norma de calidad para la carne, el jamón, la paleta y la caña de lomo ibérico. *Boletín Oficial del Estado* BOE-A-2014-318.

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

Bosch L, M Tor, J Reixach, & J Estany. 2012. Age-related changes in intramuscular and subcutaneous fat content and fatty acid composition in growing pigs using longitudinal data. *Meat Science* 91: 358-363.

Brandebourg TD, & CY Hu. 2005. Regulation of differentiating pig preadipocytes by retinoic acid. *Journal of Animal Science* 83: 98-107.

Brewer MS, LG Zhu, & FK McKeith. 2001. Marbling effects on quality characteristics of pork loin chops: Consumer purchase intent, visual and sensory characteristics. *Meat Science* 59: 153-163.

Bulmer MG. 1971. The effect of selection on genetic variability. *The American Naturalist* 105: 201-211.

Burkett J, TJ Baas, DC Beitz, CR Schwab, N Berry, & S Zhang. 2008. Correlated response in fatty acid composition after five generations of selection for intramuscular fat in Duroc pigs. *Animal Industry Report* AS 645, ASL R2350. Available at (26 June 2014): http://lib.dr.iastate.edu/ans_air/vol654/iss1/99

Cai W, DS Casey, & JCM Dekkers. 2008. Selection response and genetic parameters for residual feed intake in Yorkshire swine. *Journal of Animal Science* 86: 287-298.

Cameron ND. 1990. Genetic and phenotypic parameters for carcass traits, meat and eating quality traits in pigs. *Livestock Production Science* 26: 119-135.

Cameron ND, & MB Enser. 1991. Fatty acid composition of lipid in *longissimus dorsi* muscle of Duroc and British Landrace pigs and its relationship with eating quality. *Meat Science* 29: 295-307.

Cameron ND, M Enser, GR Nute, FM Whittington, JC Penman, AC Fisken, AM Perry, & JD Wood. 2000. Genotype with nutrition interaction on fatty acid composition of intramuscular fat and the relationship with flavor of pig meat. *Meat Science* 55: 187-195.

Cánovas A, J Estany, M Tor, RN Pena, & O Doran. 2009. 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. *Journal of Animal Science* 87: 3905-3914.

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

Cartharius K, K Frech, K Grote, B Klocke, M Haltmeier, A Klingenhoff, M Frisch, M Bayerlein, & T Werner. 2005. MatInspector and beyond: Promoter analysis based on transcription factor binding sites. *Bioinformatics* 21: 2933-2942.

Casellas J, JL Noguera, J Reixach, I Díaz, M Amills, & R Quintanilla. 2010. Bayes factor analyses of heritability for serum and muscle lipid traits in Duroc pigs. *Journal of Animal Science* 88: 2246-2254.

Chen P, TJ Baas, JCM Dekkers, KJ Koehler, & JW Mabry. 2003. Evaluation of strategies for selection for lean growth rate in pigs. *Journal of Animal Science* 81: 1150-1157.

Chen P, TJ Baas, JW Mabry, JCM Dekkers, & KJ Koehler. 2002. Genetic parameters and trends for lean growth rate and its components in US Yorkshire, Duroc, Hampshire, and Landrace pigs. *Journal of Animal Science* 80: 2062-2070.

Chen EY, CM Tan, Y Kou, Q Duan, Z Wang, GV Meirelles, NR Clark, & A Ma'ayan. 2013. Enrichr: Interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC Bioinformatics* 14: 128.

Chimonyo M, & K Dzama. 2007. Estimation of genetic parameters for growth performance and carcass traits in Mukota pigs. *Animal* 1: 317-323.

Cho ES, SG Kwon, JH Kim, DH Park, TW Kim, J Nam, IS Jang, JS Choi, WY Bang, & CW Kim. 2011. Study for the expression of adiponectin, fatty acid binding protein (FABP)4, stearoyl-CoA desaturase (SCD) genes and the methylation of SCD promoter in porcine muscle and fat tissues. *African Journal of Agricultural Research* 6: 6425-6431.

Christophersen OA, & A Haug. 2011. Animal products, diseases and drugs: A plea for better integration between agricultural sciences, human nutrition and human pharmacology. *Lipids in Health and Disease* 10: 16.

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

Clarke R, C Frost, R Collins, P Appleby, & R Peto. 1997. Dietary lipids and blood cholesterol: Quantitative meta-analysis of metabolic ward studies. *BMJ* 314: 112.

Clément K, C Vaisse, N Lahlou, S Cabrol, V Pelloux, D Cassuto, M Gourmelen, C Dina, J Chambaz, JM Lacorte, A Basdevant, P Bougnères, Y Lebouc, P Froguel, & B Guy-Grand. 1998. A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature* 392: 398-401.

Clop A, C Ovilo, M Perez-Enciso, A Cercos, A Tomas, A Fernandez, A Coll, JM Folch, C Barragan, I Diaz, MA Oliver, L Varona, L Silio, A Sanchez, & JL Noguera. 2003. Detection of QTL affecting fatty acid composition in the pig. *Mammalian Genome* 14: 650-656.

Clutter AC. 2011. Genetics of performance traits. In: *The Genetics of the Pig, 2nd Edition*, MF Rothschild, & A Ruvinsky (eds.), CAB International, New York, NY, pp. 325-354.

Comas-Cufí M, & S Thió-Henestrosa. 2011a. CoDaPack 2.0: A stand-alone, multi-platform compositional software. In: *CoDaWork'11: 4th International Workshop on Compositional Data Analysis*, JJ Egozcue, R Tolosana-Delgado, & MI Ortego (eds.), Sant Feliu de Guíxols, Spain.

Comas-Cufí M, & S Thió-Henestrosa. 2011b. CoDaPack 2.0. Manual and software, Version 2.01. Universitat de Girona. Available at (26 June 2014): http://ima.udg.edu/codapack/

Cook HW, & CR McMaster. 2002. Fatty acid desaturation and chain elongation in eukaryotes. In: *Biochemistry of Lipids, Lipoproteins and Membranes* (4th ed.), DE Vance, & JE Vance (eds.), Elsevier, New York, NY, pp. 181-204.

Corominas J, Y Ramayo-Caldas, A Puig-Oliveras, D Pérez-Montarelo, JL Noguera, JM Folch, & M Ballester. 2013. Polymorphism in the *ELOVL6* gene is associated with a major QTL effect on fatty acid composition in pigs. *PLoS One* 8: e53687.

Daunis-i-Estadella J, C Barceló-Vidal, & A Buccianti. 2006. Exploratory compositional data analysis. In: *Compositional Data Analysis in the Geosciences: From Theory to Practice*, A Buccianti, G Mateu-Figueras, & V Pawlowsky-Glahn (eds.), London, UK. *Geological Society Special Publications* 264, pp. 161-174.

Daunis-i-Estadella J, S Thió-Henestrosa, & G Mateu-Figueras. 2011. Including supplementary elements in a compositional biplot. *Computers & Geosciences* 37: 696-701.

de Lorgeril M, & P Salen. 2012. New insights into the health effects of dietary saturated and omega-6 and omega-3 polyunsaturated fatty acids. *BMC Medicine* 10: 50.

De Pedro E, M Murillo, J Salas, & F Peña. 2000. Effect of storage time on fatty acid composition of subcutaneous fat. *Options Méditerranéenes, Series A* 41: 251-255.

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

Dekkers JCM. 2004. Commercial application of marker- and gene-assisted selection in livestock: Strategies and lessons. *Journal of Animal Science* 82: E313-E328.

Dekkers JCM. 2012. Application of genomics tools to animal breeding. *Current Genomics* 13: 207-212.

DeVol DL, FK McKeith, PJ Bechtel, J Novakofski, RD Shanks, & TR Carr. 1988. Variation in composition and palatability traits and relationships between muscle characteristics and palatability in a random sample of pork carcasses. *Journal of Animal Science* 66: 385-395.

Dodson MV, GJ Hausman, L Guan, M Du, TP Rasmussen, SP Poulos, P Mir, WG Bergen, ME Fernyhough, DC McFarland, RP Rhoads, B Soret, JM Reecy, SG Velleman, & Z Jiang. 2010. Lipid metabolism, adipocyte depot physiology and utilization of meat animals as experimental models for metabolic research. *International Journal of Biological Sciences* 6: 691-699.

Doran O, SK Moule, GA Teye, FM Whittington, KG Hallett, & JD 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. *British Journal of Nutrition* 95: 609-617.

Duran-Montgé P, CE Realini, AC Barroeta, R Lizardo, & E Esteve-Garcia. 2008. Tissue fatty acid composition of pigs fed different fat sources. *Animal* 2: 1753-1762.

Egozcue JJ, J Daunis-i-Estadella, V Pawlowsky-Glahn, K Hron, & P Filzmoser. 2012. Simplicial regression: The normal model. *Journal of Applied Probability and Statistics* 6: 87-108.

Egozcue JJ, & V Pawlowsky-Glahn. 2005. Groups of parts and their balances in compositional data analysis. *Mathematical Geology* 37: 795-828.

Egozcue JJ, & V Pawlowsky-Glahn. 2011. Basic concepts and procedures. In: *Compositional Data Analysis: Theory and Applications*, V Pawlowsky-Glahn, & A Buccianti (eds.), John Wiley & Sons Ltd., West Sussex, UK, pp. 12-28.

Egozcue JJ, V Pawlowsky-Glahn, G Mateu-Figueras, & C Barceló-Vidal. 2003. Isometric logratio transformations for compositional data analysis. *Mathematical Geology* 35: 279-300.

Enderlin V, V Pallet, S Alfos, E Dargelos, R Jaffard, H Garcin, & P Higueret. 1997. Age-related decreases in mRNA for brain nuclear receptors and target genes are reversed by retinoic acid treatment. *Neuroscience Letters* 229: 125-129.

Estany J, R Ros, M Tor, & J Reixach. 2011. A compositional genetic analysis of oleic acid content in pig meat. In: *CoDaWork'11: 4th International Workshop on Compositional Data Analysis*, JJ Egozcue, R Tolosana-Delgado, & MI Ortego (eds.), Sant Feliu de Guíxols, Spain.

Estellé J, AI Fernández, M Pérez-Enciso, A Fernández, C Rodríguez, A Sánchez, JL Noguera, & JM Folch. 2009. A non-synonymous mutation in a conserved site of the *MTTP* gene is strongly associated with protein activity and fatty acid profile in pigs. *Animal Genetics* 40: 813-820.

Falconer DS, & TFC Mackay. 1996. *Introduction to Quantitative Genetics*, 4th ed. Longman Group Ltd, Harlow, UK.

FAO. 2010. Fats and fatty acids in human nutrition: Report of an expert consultation. Geneva, Switzerland. *FAO Food and Nutrition Paper* 91. Available at (26 June 2014): http://www.who.int/nutrition/publications/nutrient/en/

FAO. 2014. FAOSTAT. Available at (26 June 2014): http://faostat3.fao.org/faostat-gateway/go/to/home/E

Faucitano L, J Rivest, JP Daigle, J Lévesque, & C Gariepy. 2004. Distribution of intramuscular fat content and marbling with the longissimus muscle of pigs. *Canadian Journal of Animal Science* 84: 57-61.

Fernández A, E de Pedro, N Núñez, L Silió, J García-Casco, & C Rodríguez. 2003. Genetic parameters for meat and fat quality and carcass composition traits in Iberian pigs. *Meat Science* 64: 405-410.

Fernández A, J García-Casco, E de Pedro, L Silió, & MC Rodríguez. 2007. Genetic antagonism between intramuscular fat content and primal cuts in Iberian pigs? *Options Méditerranéenes, Series A* 76: 43-46.

Fernandez X, G Monin, A Talmant, J Mourot, & B Lebret. 1999a. 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 Science* 53: 59-65.

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

Fernando R, & DJ Garrick. 2009. Gensel: User manual for a portfolio of genomic selection related analyses. 3rd edition. Iowa State University. Available at (26 June 2014): http://bigs.ansci.iastate.edu/bigsgui/help.html.

Filzmoser P, & K Hron. 2009. Correlation analysis of compositional data. *Mathematical Geosciences* 41: 905-919.

Flachowsky G, E Schulz, R Kratz, & P Glodek. 2008. Effects of different dietary fat sources on the fatty acid profile of backfat and intramuscular fat of pigs of various sire breeds. *Journal of Animal and Feed Sciences* 17: 363-371.

Fortin A, WM Robertson, & AKW Tong. 2005. The eating quality of Canadian pork and its relationship with intramuscular fat. *Meat Science* 69: 297-305.

Fowler VR, M Bichard, & A Pease. 1976. Objectives in pig breeding. *Animal Production* 23: 365-387.

Franco I, MC Escamilla, J García, MC García Fontán, J Carballo. 2006. Fatty acid profile of the fat from Celta pig breed fattened using a traditional feed: Effect of the location in the carcass. *Journal of Food Composition and Analysis* 19: 792-799.

Gallardo D, R Quintanilla, L Varona, I Díaz, O Ramírez, RN Pena, & M Amills. 2009. Polymorphism of the pig *acetyl-coenzyme A carboxylase α* gene is associated with fatty acid composition in a Duroc commercial line. *Animal Genetics* 40: 410-417.

Galve A, C Burgos, L Silió, L Varona, C Rodríguez, C Ovilo, & P López-Buesa. 2012. The effects of leptin receptor (LEPR) and melanocortin-4 receptor (MC4R) polymorphisms on fat content, fat distribution and fat composition in a Duroc×Landrace/Large White cross. *Livestock Science* 145: 145-152.

Gandemer G. 2002. Lipids in muscles and adipose tissues, changes during processing and sensory properties of meat products. *Meat Science* 62: 309-321.

Gao SZ, & SM Zhao. 2009. Physiology, affecting factors and strategies for control of pig meat intramuscular fat. *Recent Patents on Food, Nutrition & Agriculture* 1: 59-74.

Geweke J. 1992. Evaluating the accuracy of sampling-based approached to the calculation of posterior moments (with discussion). In: *Bayesian Statistics*, JM Bernardo, JO Berger, AP Dawid, & AFM Smith (eds.), Oxford University Press, Oxford, UK, pp. 169-193.

Gianola D, & RL Fernando. 1986. Bayesian methods in animal breeding theory. *Journal of Animal Science* 63: 217-244.

Gjerlaug-Enger E, L Aass, J Ødegård, J Kongsro, & O Vangen. 2011. Genetic parameters of fat quality in pigs measured by near-infrared spectroscopy. *Animal* 5: 1495-1505.

Gjerlaug-Enger E, L Aass, J Ødegård, & O Vangen. 2010. Genetic parameters of meat quality traits in two pig breeds measured by rapid methods. *Animal* 4: 1832-1843.

Gjerlaug-Enger E, J Kongsro, J Ødegård, L Aass, & O Vangen. 2012. Genetic parameters between slaughter pig efficiency and growth rate of different body tissues estimated by computed tomography in live boars of Landrace and Duroc. *Animal* 6: 9-18.

González-Martín I, C González-Pérez, N Álvarez-García, & JM 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 Science* 69: 243-248.

González-Martín I, C González-Pérez, J Hernández-Méndez, N Álvarez-García, & J.L. Hernández Andaluz. 2002. On-line non-destructive determination of proteins and infiltrated fat in Iberian pork loin by near infrared spectrometry with a remote reflectance fibre optic probe. *Analytica Chimica Acta* 453: 281-288.

Grindflek E, J Szyda, Z Liu, & S Lien. 2001. Detection of quantitative trait loci for meat quality in a commercial slaughter pig cross. *Mammalian Genome* 12: 299-304.

Guo T, J Ren, K Yang, J Ma, Z Zhang, & L Huang. 2009. Quantitative trait loci for fatty acid composition in longissimus dorsi and abdominal fat: Results from a White Duroc × Erhualian intercross F_2 population. *Animal Genetics* 40: 185-191.

Hermesch S, BG Luxford, & HU Graser. 2000. Genetic parameters for lean meat yield, meat quality, reproduction and feed efficiency traits for Australian pigs: 2. Genetic relationships between production, carcase and meat quality traits. *Livestock Production Science* 65: 249-259.

Hernández-Sánchez J, M Amills, RN Pena, A Mercadé, A Manunza, & R Quintanilla. 2013. Genomic architecture of heritability and genetic correlations for intramuscular and back fat contents in Duroc pigs. *Journal of Animal Science* 91: 623-632.

Hofer A, H Luther, D Kaufmann, & D Schwörer. 2006. Genetic evaluation and selection for backfat quality in pigs. In: *Proceedings of the 8th World Congress on Genetics Applied to Livestock Production*, Belo Horizonte, Brazil.

Hooper L, CD Summerbell, R Thompson, D Sills, FG Roberts, HJ Moore, & G Davey Smith. 2012. Reduced or modified dietary fat for preventing cardiovascular disease. *Cochrane Database of Systematic Reviews* 5: CD002137.

Hu FB, JE Manson, & WC Willett. 2001. Types of dietary fat and risk of coronary heart disease: A critical review. *Journal of the American College of Nutrition* 20: 5-19.

Huff-Lonergan E, TJ Baas, M Malek, JC Dekkers, K Prusa, & MF Rothschild. 2002. Correlations among selected pork quality traits. *Journal of Animal Science* 80: 617-627.

Hulbert AJ. 2010. Metabolism and longevity: Is there a role for membrane fatty acids? *Integrative and Comparative Biology* 50: 808-817.

Ibáñez-Escriche N, S Forni, JL Noguera, & L Varona. 2014. Genomic information in pig breeding: Science meets industry needs. *Livestock Science* 166: 94-100.

Jeremiah LE. 1998. Marling and pork tenderness. National Pork Producers Council and American Meat Science Association, Des Moines, IA. *Pork Fact Sheet* 04310. Available at (26 June 2014): http://www.pork.org/filelibrary/factsheets/porkscience/q-colorandmarb04310.pdf

Jiménez-Colmenero F, J Carballo, & S Cofrades. 2001. Healthier meat and meat products: Their role as functional foods. *Meat Science* 59: 5-13.

Jiménez-Colmenero F, J Ventanas, & F Toldrá. 2010. Nutritional composition of dry-cured ham and its role in a healthy diet. *Meat Science* 84: 585-593.

Johnson ZB, & RA Nugent III. 2003. Heritability of body length and measures of body density and their relationship to backfat thickness and loin muscle area in swine. *Journal of Animal Science* 81: 1943-1949.

Judge MD. 1972. A review of possible methods to detect animal stress susceptibility and potential low quality pork. In: *The Proceedings of the Pork Quality Symposium*, R Cassens, F Giesler, & Q Kolb (eds.), Madison, WI, pp. 68-90.

Kanis E, KH De Greef, A Hiemstra, & JAM van Arendonk. 2005. Breeding for societally important traits in pigs. *Journal of Animal Science* 83: 948-957.

Kempster AJ, AW Dilworth, DG Evans, & KD Fisher. 1987. The effects of fat thickness and sex on pig meat quality with special reference to the problems associated with overleanness: 1. Butcher and consumer panel results. *Animal Production* 43: 517-533.

Kim Y, M Kong, YJ Nam, & C Lee. 2006. A quantitative trait locus for oleic fatty acid content on *Sus scrofa* chromosome 7. *Journal of Heredity* 97: 535-537.

Kim JH, PN Seong, SH Cho, BY Park, KH Hah, LH Yu, DG Lim, IH Hwang, DH Kim, JM Lee, & CN Ahn. 2008. Characterization of nutritional value for twenty-one pork muscles. *Asian-Australian Journal of Animal Science* 21: 138-143.

Klingenberg IL, DA Knabe, & SB Smith. 1995. Lipid metabolism in pigs fed beef tallow or high-oleic acid sunflower oil. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 110: 183-192.

Kloareg M, J Noblet, & J van Milgen. 2007. Deposition of dietary fatty acids, *de novo* synthesis and anatomical partitioning of fatty acids in finishing pigs. *British Journal of Nutrition* 97: 35-44.

Knap P. 2014. Pig breeding goals in competitive markets. In: *Proceedings of the 10th World Congress on Genetics Applied to Livestock Production*, Vancouver, Canada.

Knapp P, A Willam, & J Sölkner. 1997. Genetic parameters for lean meat content and meat quality traits in different pig breeds. *Livestock Production Science* 52: 69-73.

Kongsro J, & E Gjerlaug-Enger. 2013. *In vivo* prediction of intramuscular fat in pigs using computed tomography. *Open Journal of Animal Sciences* 3: 321-325.

Larsson SC, M Kumlin, M Ingelman-Sundberg, & A Wolk. 2004. Dietary long-chain n-3 fatty acids for the prevention of cancer: A review of potential mechanisms. *American Journal of Clinical Nutrition* 79: 935-945.

Lee SH, YM Choi, JH Choe, JM Kim, KC Hong, HC Park, & BC Kim. 2010. Association between polymorphisms of the heart fatty acid binding protein gene and intramuscular fat content, fatty acid composition, and meat quality in Berkshire breed. *Meat Science* 86: 794-800.

Lefterova MI, Y Zhang, DJ Steger, M Schupp, J Schug, A Cristancho, D Feng, D Zhuo, CJ Stoeckett Jr, XS Liu, & MA Lazar. 2008. PPARγ and C/EBP factors orchestrate adipocyte biology via adjacent binding on a genome-wide scale. *Genes and Development* 22: 2941-2952.

Legarra A, OF Christensen, I Aguilar, & I Misztal. 2014. Single step, a general approach for genomic selection. *Livestock Science* 166: 54-65.

Legarra A, L Varona, & E López de Maturana. 2011. TM Threshold Model. Available at (26 June 2014): http://snp.toulouse.inra.fr/~alegarra/

Leseigneur-Meynier A, & G Gandemer. 1991. Lipid composition of pork muscle in relation to the metabolic type of the fibres. *Meat Science* 29: 229-241.

Lonergan SM, E Huff-Lonergan, LJ Rowe, DL Kuhlers, & SB Jungst. 2001. Selection for lean growth efficiency in Duroc pigs influences pork quality. *Journal of Animal Science* 79: 2075-2085.

Lonergan SM, KJ Stalder, E Huff-Lonergan, TJ Knight, RN Goodwin, KJ Prusa, & DC Beitz. 2007. Influence of lipid content on pork sensory quality within pH classification. *Journal of Animal Science* 85: 1074-1079.

López-Buesa P, C Burgos, A Galve, & L Varona. 2014. Joint analysis of additive, dominant and first-order epistatic effects of four genes (*IGF2*, *MC4R*, *PRKAG3* and *LEPR*) with known effects on fat content and fat distribution in pigs. *Animal Genetics* 45: 133-137.

Maharani D, HB Park, JB Lee, CK Yoo, HT Lim, SH Han, SS Lee, MS Ko, IC Cho, & JH Lee. 2013. Association of the gene encoding stearoyl-CoA desaturase (SCD) with fatty acid composition in an intercross population between Landrace and Korean native pigs. *Molecular Biology Reports* 40: 73-80.

Martin AH, & HT Fredeen. 1974. Pork quality in relation to carcass fatness and muscling. *Canadian Journal of Animal Science* 54: 137-143.

Martín-Fernández JA, & S Thió-Henestrosa. 2006. Rounded zeros: Some practical aspects for compositional data. In: *Compositional Data Analysis in the Geosciences: From Theory to Practice*, A Buccianti, G Mateu-Figueras, & V Pawlowsky-Glahn (eds.), London, UK. *Geological Society Special Publications* 264, pp. 191-201.

Mas G, M Llavall, D Coll, R Roca, I Diaz, M Gispert, MA Oliver, & CE Realini. 2010. Carcass and meat quality characteristics and fatty acid composition of tissues from Pietrain-crossed barrows and gilts fed an elevated monounsaturated fat diet. *Meat Science* 85: 707-714.

Mas G, M Llavall, D Coll, R Roca, I Diaz, MA Oliver, M Gispert, & CE Realini. 2011. Effect of an elevated monounsaturated fat diet on pork carcass and meat quality traits and tissue fatty acid composition from York-crossed barrows and gilts. *Meat Science* 89: 419-425.

Mattison JA, GS Roth, TM Beasley, EM Tilmont, AM Handy, RL Herbert, DL Longo, DB Allison, JE Young, M Bryant, D Barnard, WF Ward, W Qi, DK Ingram, & R de Cabo. 2012. Impact of caloric restriction on health and survival in rhesus monkeys from the NIA study. *Nature* 489: 318-321.

Mauvoisin D, & C Mounier. 2011. Hormonal and nutritional regulation of SCD1 gene expression. *Biochimie* 93: 78-86.

McAfee AJ, EM McSorley, GJ Cuskelly, BW Moss, JMW Wallace, MP Bonham, & AM Fearon. 2010. Red meat consumption: An overview of the risks and benefits. *Meat Science* 84: 1-13.

Merino DM, DWL Ma, & DM Mutch. 2010. Genetic variation in lipid desaturases and its impact on the development of human disease. *Lipids in Health and Disease* 9: 63.

Meuwissen THE. 1991. Reduction of selection differentials in finite populations with a nested full-half sib family structure. *Biometrics* 47: 195-203.

Meuwissen THE, BJ Hayes, & ME Goddard. 2001. Prediction of total genetic value using genome-wide dense marker maps. *Genetics* 157: 1819-1829.

Micha R, & D Mozaffarian. 2010. Saturated fat and cardiometabolic risk factors, coronary heart disease, stroke, and diabetes: A fresh look at the evidence. *Lipids* 45: 893-905.

Misztal I, S Tsuruta, T Strabel, B Auvray, T Druet, & DH Lee. 2002. BLUPF90 and related programs (BGF90). In: *Proceedings of the 7th World Congress on Genetics Applied to Livestock Production*, Montpellier, France.

Miyazaki M, & JM Ntambi. 2003. Role of stearoyl-coenzyme A desaturase in lipid metabolism. *Prostaglandins, Leukotrienes and Essential Fatty Acids* 68: 113-121.

Mottram DS. 1998. Flavour formation in meat and meat products: A review. *Food Chemistry* 62: 415-424.

Muñoz R, J Estany, M Tor, & O Doran. 2013a. Hepatic lipogenic enzyme expression in pigs is affected by selection for decreased backfat thickness at constant intramuscular fat content. *Meat Science* 93: 746-751.

Muñoz M, MC Rodríguez, E Alves, JM Folch, N Ibañez-Escriche, L Silió, & AI Fernández. 2013b. Genome-wide analysis of porcine backfat and intramuscular fat fatty acid composition using high-density genotyping and expression data. *BMC Genomics* 14: 845.

Muñoz R, F Vilaró, J Eras, J Estany, & M Tor. 2011. Fast determination of oleic acid in pork by flow injection analysis/mass spectrometry. *Rapid Communications in Mass Spectrometry* 25: 1082-1088.

Muraoka O, B Xu, T Tsurumaki, S Akira, T Yamaguchi, & H Higuchi. 2003. Leptin-induced transactivation of *NPY* gene promoter mediated by JAK1, JAK2 and STAT3 in the neural cell lines. *Neurochemistry International* 42: 591-601.

Muriel E, J Ruiz, J Ventanas, & T Antequera. 2002. Free-range rearing increases (n-3) polyunsaturated fatty acids of neutral and polar lipids in swine muscles. *Food Chemistry* 78: 219-225.

Myer RO, DD Johnson, DA Knauft, DW Gorbet, JH Brendemuhl, & WR Walker. 1992. Effect of feeding high-oleic-acid peanuts to growing-finishing swine on resulting carcass fatty acid profile and on carcass and meat quality characteristics. *Journal of Animal Science* 70: 3734-3741.

Newcom DW, TJ Bass, & JF Lampe. 2002. Prediction of intramuscular fat percentage in live swine using real-time ultrasound. *Journal of Animal Science* 80: 3046-3052.

Newcom DW, TJ Baas, KJ Stalder, & CR Schwab. 2005. Comparison of three models to estimate breeding values for percentage of loin intramuscular fat in Duroc swine. *Journal of Animal Science* 83: 750-756.

Nii M, T Hayashi, F Tani, A Niki, N Mori, N Fujishima-Kanaya, M Komatsu, K Aikawa, T Awata, & S Mikawa. 2006. Quantitative trait loci mapping for fatty acid composition traits in perirenal and back fat using a Japanese wild boar × Large White intercross. *Animal Genetics* 37: 342-347.

Ntambi JM. 1999. Regulation of stearoyl-CoA desaturase by polyunsaturated fatty acids and cholesterol. *Journal of Lipid Research* 40: 1549-1558.

Ntambi JM, & M Miyazaki. 2004. Regulation of stearoyl-CoA desaturases and role in metabolism. *Progress in Lipid Research* 43: 91-104.

Ntawubizi M, E Colman, S Janssens, K Raes, N Buys, & S De Smet. 2010. Genetic parameters for intramuscular fatty acid composition and metabolism in pigs. *Journal of Animal Science* 88: 1286-1294.

Nürnberg K, J Wegner, & K Ender. 1998. Factors influencing fat composition in muscle and adipose tissue of farm animals. *Livestock Production Science* 56: 145-156.

Ohsaki H, T Sawa, S Sasazaki, K Kano, M Taniguchi, F Mukai, & H Mannen. 2007. Stearoyl-CoA desaturase mRNA expression during bovine adipocyte differentiation in primary culture derived from Japanese Black and Holstein cattle. *Comparative Biochemistry and Physiology, Part A* 148: 629-634.

OJ. 2001. Comission Decision 2001/775/EC of 7 November 2001 amending Decision 88/479/EEC authorising methods for grading pig carcasses in Spain. *Official Journal of European Communities* L291: 50-51.

Oksbjerg N, JS Petersen, IL Sørensen, P Henckel, M Vestergaard, P Ertbjerg, AJ Møller, C Bejerholm, & S Støier. 2000. Long-term changes in performance and meat quality of Danish Landrace pigs: A study on a current compared with an unimproved genotype. *Animal Science* 71: 81-92.

Olivares A, A Daza, AI Rey, & CJ López-Bote. 2009a. Dietary vitamin A concentration alters fatty acid composition in pigs. *Meat Science* 81: 295-299.

Olivares A, A Daza, AI Rey, & CJ Lopez-Bote. 2009b. Interactions between genotype, dietary fat saturation and vitamin A concentration on intramuscular fat content and fatty acid composition in pigs. *Meat Science* 82: 6-12.

Oliver MA, P Gou, M Gispert, A Diestre, J Arnau, JL Noguera, & A Blasco. 1994. Comparison of five types of pig crosses: 2. Fresh meat quality and sensory characteristics of dry cured ham. *Livestock Production Science* 40: 179-185.

Óvilo C, A Fernández, JL Noguera, C Barragán, R Letón, C Rodríguez, A Mercadé, E Alves, JM Folch, L Varona, & M Toro. 2005. Fine mapping of porcine chromosome 6 QTL and *LEPR* effects on body composition in multiple generations of an Iberian by Landrace intercross. *Genetical Research* 85: 57-67.

Palarea-Albaladejo J, JA Martín-Fernández, & J Gómez-García. 2007. A parametric approach for dealing with compositional rounded zeros. *Mathematical Geology* 39: 625-645.

Paton CM, & JM Ntambi. 2009. Biochemical and physiological function of stearoyl-CoA desaturase. *American Journal of Physiology, Endocrinology and Metabolism* 297: E28-E37.

Pawlowsky-Glahn V, & JJ Egozcue. 2006. Compositional data and their analysis: An introduction. In: *Compositional Data Analysis in the Geosciences: From Theory to Practice*, A Buccianti, G Mateu-Figueras, & V Pawlowsky-Glahn (eds.), London, UK. *Geological Society Special Publications* 264, pp. 1-10.

Pearson K. 1987. Mathematical contributions to the theory of evolution: On a form of spurious correlation which may arise when indices are used in the measurement of organs. *Proceedings of the Royal Society of London* 60: 489-498.

Pérez E, W Bourguet, H Gronemeyer, & AR de Lera. 2012. Modulation of RXR function through ligand design. *Biochimica et Biophysica Acta* 1821: 57-69.

Pérez-Enciso M, A Clop, JL Noguera, C Ovilo, A Coll, JM Folch, D Babot, J Estany, MA Oliver, I Díaz, & A Sánchez. 2000. A QTL on pig chromosome 4 affects fatty acid metabolismo: Evidence from an Iberian by Landrace intercross. *Journal of Animal Science* 78: 2525-2531.

Purcell S, B Neale, K Todd-Brown, L Thomas, MAR Ferreira, D Bender, J Maller, P Sklar, PIW de Bakker, MJ Daly, & PC Sham. 2007. PLINK: A toolset for whole-genome association and population-based linkage analysis. *American Journal of Human Genetics* 81: 559-575.

Quintanilla R, RN Pena, D Gallardo, A Cánovas, O Ramírez, I Díaz, JL Noguera, & M Amills. 2011. Porcine intramuscular fat content and composition are regulated by quantitative trait loci with muscle-specific effects. *Journal of Animal Science* 89: 2963-2971.

R Development Core Team. 2010. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at (26 June 2014): http://www.R-project.org.

Ramayo-Caldas Y, A Mercadé, A Castelló, B Yang, C Rodríguez, E Alves, I Díaz, N Ibáñez-Escriche, JL Noguera, M Pérez-Enciso, AI Fernández, & JM Folch. 2012. Genome-wide association study for intramuscular fatty acid composition in an Iberian × Landrace cross. *Journal of Animal Science* 90: 2883-2893.

Rauw WM, I Diaz, F Francés, D Corella, J Soler, J Tibau, & L Gomez-Raya. 2012. The relationship between feed intake behaviour with intramuscular fat, cholesterol and fatty acid composition in pork. *Journal of Animal Breeding and Genetics* 129: 289-297.

Ren J, C Knorr, YM Guo, NS Ding, HS Ai, B Brenig, & LS Huang. 2004a. Characterization of five single nucleotide polymorphisms in the porcine stearoyl-CoA desaturase (*SCD*) gene. *Animal Genetics* 35: 255-257.

Ren J, C Knorr, F Habermann, R Fries, LS Huang, & B Brenig. 2003. Assignment of the porcine stearoyl-CoA desaturase (*SCD*) gene to SSC14q27 by fluorescence *in situ* hybridization and by hybrid panel mapping. *Animal Genetics* 34: 471-473.

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

Renaville B, A Prandi, B Fan, A Sepulcri, MF Rothschild, & E Piasentier. 2013. Candidate gene marker associations with fatty acid profiles in heavy pigs. *Meat Science* 93: 495-500.

Rhee KS, TL Davidson, HR Cross, & YA Ziprin. 1990. Characteristics of pork products from swine fed a high monounsaturated fat diet: 1. Whole muscle products. *Meat Science* 27: 329-341.

Ruiz-Carrascal J, J Ventanas, R Cava, AI Andrés, & C García. 2000. Texture and appearance of dry cured ham as affected by fat content and fatty acid composition. *Food Research International* 33: 91-95.

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

Rutten MJM, P Bijma, JA Woolliams, & JAM van Arendonk. 2002. SelAction: Software to predict selection response and rate of inbreeding in livestock breeding programs. *The Journal of Heredity* 93: 456-458.

Saatchi M, DJ Garrick, RG Tait, MS Mayes, M Drewnoski, J Schoonmaker, C Diaz, DC Beitz, & JM Reecy. 2013. Genome-wide association and prediction of direct genomic breeding values for composition of fatty acids in Angus beef cattle. *BMC Genomics* 14: 730.

Sambrook J, & D Russell. 2001. *Molecular Cloning: A Laboratory Manual*, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sanchez MP, N Iannuccelli, B Basso, JP Bidanel, Y Billon, G Gandemer, H Gilbert, C Larzul, C Legault, J Riquet, D Milan, & P Le Roy. 2007. Identification of QTL with effects on intramuscular fat content and fatty acid composition in a Duroc × Large White cross. *BMC Genetics* 8: 55.

Sanford RF, CT Pierson, & RA Crovelli. 1993. An objective replacement method for censored geochemical data. *Mathematical Geology* 25: 59-80.

Schmid A. 2010. The role of meat fat in the human diet. *Critical Reviews in Food Science and Nutrition* 51: 50-66.

Schwab CR, TJ Baas, & KJ Stalder. 2010. Results from six generations of selection for intramuscular fat in Duroc swine using real-time ultrasound: 2. Genetic parameters and trends. *Journal of Animal Science* 88: 69-79.

Schwab CR, TJ Baas, KJ Stalder, & JW Mabry. 2006. Effect of long-term selection for increased leanness on meat and eating quality traits in Duroc swine. *Journal of Animal Science* 84: 1577-1583.

Schwab CR, TJ Baas, KJ Stalder, & D Nettleton. 2009. Results from six generations of selection for intramuscular fat in Duroc swine using real-time ultrasound: 1. Direct and correlated phenotypic responses to selection. *Journal of Animal Science* 87: 2774-2780.

Sellier P. 1998. Genetics of meat and carcass traits. In: *The Genetics of the Pig*, MF Rothschild, & A Ruvinsky (eds.), CAB International, New York, NY, pp. 463-510.

Sellier P, L Maignel, & JP Bidanel. 2010. Genetic parameters for tissue and fatty acid composition of backfat, perirenal fat and *longissimus* muscle in Large White and Landrace pigs. *Animal* 4: 497-504.

Shackelford SD, DA King, & TL Wheeler. 2011. Development of a system for classification of pork loins for tenderness using visible and near-infrared reflectance spectroscopy. *Journal of Animal Science* 89: 3803-3808.

Sharma N, G Gandemer, & R Goutefongea. 1987. Comparative lipid composition of porcine muscles at different anatomical locations. *Meat Science* 19: 121-128.

Smith BJ. 2005. Bayesian Output Analysis Program (BOA), Version 1.1.5. University of Iowa. Available at (26 June 2014): http://www.public-health.uiowa.edu/boa

Smith JW, C Opio, H Steinfeld, & A Lufafa. 2010. Global demand and trade in livestock and livestock products in a globalizing world. In: *Adapting Animal Production to Changes for a Growing Human Population*, J Estany, C Nogareda, & MF Rothschild (eds.), Lleida, Spain, pp. 25-34.

Solanes FX, J Reixach, M Tor, J Tibau, & J Estany. 2009. Genetic correlations and expected response for intramuscular fat content in a Duroc pig line. *Livestock Science* 123: 63-69.

Stewart JW, ML Kaplan, & DC Beitz. 2001. Pork with a high content of polyunsaturated fatty acids lowers LDL cholesterol in women. *American Journal of Clinical Nutrition* 74: 179-187.

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

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

Suzuki K, H Kadowaki, T Shibata, H Uchida, & A Nishida. 2005b. Selection for daily gain, loin-eye area, backfat thickness and intramuscular fat based on desired gains over seven generations of Duroc pigs. *Livestock Production Science* 97: 193-202.

Tejeda JF, G Gandemer, T Antequera, M Viau, & C García. 2002. Lipid traits of muscles as related to genotype and fattening diet in Iberian pigs: Total intramuscular lipids and triacylglycerols. *Meat Science* 60: 357-363.

Templ M, K Hron, & P Filzmoser. 2011. robCompositions: Robust estimation for compositional data. Manual and Package, Version 1.5.0. Available at (26 June 2014): http://cran.r-project.org/web/packages/robCompositions

Teye GA, PR Sheard, FM Whittington, GR Nute, A Stewart, & JD Wood. 2006. Influence of dietary oils and protein level on pork quality: 1. Effects on muscle fatty acid composition, carcass, meat and eating quality. *Meat Science* 73: 157-165.

Thió-Henestrosa S, & JA Martín-Fernández. 2005. Dealing with compositional data: The freeware CoDaPack. *Mathematical Geology* 37: 773-793.

Tibau J, J Reixach, & MT Batallé. 1999. Tendencias genéticas en líneas maternales de raza Duroc. In: *VIII Jornadas sobre Producción Animal AIDA*, Asociación Interprofesional para el Desarrollo Agrario (ed.), Zaragoza, Spain, pp. 333-335.

Toldrá F. 2002. Manufacturing of dry-cured ham. In: *Dry-cured meat products*. Wiley-Blackwell, Ames, IA, pp. 27-62

Tolosana-Delgado R, & KG van der Boogaart. 2011. Linear models with compositions in R. In: *Compositional Data Analysis: Theory and Applications*, V Pawlowsky-Glahn, & A Buccianti (eds.), John Wiley & Sons Ltd., West Sussex, UK, pp. 356-371.

Tribout T, JC Caritez, J Goguè, J Gruand, M Bouffaud, Y Billon, C Péry, H Griffon, S Brenot, MH Le Tiran, F Bussières, P Le Roy, & JP Bidanel. 2004. Estimation, par utilisation de semence congelée de progrès génétique réalisé en France entre 1977 et 1998 dans le race porcine Large White: résultats pour quelques caractères de production et de qualité des tissus gras et maigres. *Journées Recherche Porcine* 36: 275-282.

Uemoto Y, Kikuchi T, H Nakano, S Sato, T Shibata, H Kadowaki, K Katoh, E Kobayashi, & K Suzuki. 2012a. Effects of porcine leptin receptor gene polymorphisms on backfat thickness, fat area ratios by image analysis, and serum leptin concentrations in a Duroc purebred population. *Animal Science Journal* 83: 375-385.

Uemoto Y, H Nakano, T Kikuchi, S Sato, M Ishida, T Shibata, H Kadowaki, E Kobayashi, & K Suzuki. 2012b. Fine mapping of porcine SSC14 QTL and *SCD* gene effects on fatty acid composition and melting point of fat in a Duroc purebred population. *Animal Genetics* 43: 225-228.

Uemoto Y, Y Soma, S Sato, M Ishida, T Shibata, H Kadowaki, E Kobayashi, & K Suzuki. 2012c. Genome-wide mapping for fatty acid composition and melting point of fat in a purebred Duroc pig population. *Animal Genetics* 43: 27-34.

Untergasser A, H Nijveen, X Rao, T Bisseling, R Geurts, & JAM Leunissen. 2007. Primer3Plus, an enhanced web interface to Primer3. *Nucleic Acids Research* 35: W71-W74.

Valero A, M Gispert, & JA García-Regueiro. 1999. Determinación de la grasa intramuscular de diferentes músculos de cerdo por transmitancia en el infrarrojo cercano (NIT). *Eurocarne* 79: 87-90.

Valsta LM, H Tapanainen, & S Männistö. 2005. Meat fats in nutrition. *Meat Science* 70: 525-530.

van den Boogaart KG, R Tolosana, & M Bren. 2011. compositions: Compositional Data Analysis. Manual and Package, Version 1.10-2. Available at (26 June 2014): http://www.stat.boogaart.de/compositions

Vazquez AI, GJM Rosa, KA Weigel, G de los Campos, D Gianola, & DB Allison. 2010. Predictive ability of subsets of single nucleotide polymorphisms with and without parent average in US Holsteins. *Journal of Diary Science* 93: 5942-5949.

Ventanas S, J Ruiz, C García, & J Ventanas. 2007. Preference and juiciness of Iberian dry-cured loin as affected by intramuscular fat content, crossbreeding and rearing system. *Meat Science* 77: 324-330.

Verbeke W, MJ Van Oeckel, N Warnants, J Viaene, & CV Boucqué. 1999. Consumer perception, facts and possibilities to improve acceptability of health and sensory characteristics of pork. *Meat Science* 53: 77-99.

von Rohr P, A Hofer, & N Kunzi. 1999. Economic values for meat quality traits in pigs. *Journal of Animal Science* 77: 2633-2640.

Wang J, SM Zhao, XL Song, HB Pan, WZ Li, YY Zhang, SZ Gao, & DW Chen. 2012. Low protein diet up-regulate intramuscular lipogenic gene expression and down-regulate lipolytic gene expression in growth-finishing pigs. *Livestock Science* 148: 119-128.

Waters KM, CW Miller, & JM Ntambi. 1997. Localization of a negative thyroid hormone-response region in hepatic stearoyl-CoA desaturase gene 1. *Biochemical and Biophysical Research Communications* 233: 838-843.

Webb EC, & HA O'Neill. 2008. The animal fat paradox and meat quality. *Meat Science* 80: 28-36.

Weigel KA, G de los Campos, O González-Recio, H Naya, XL Wu, N Long, GJM Rosa, & D Gianola. 2009. Predictive ability of direct genomic values for lifetime net merit of Holstein sires using selected subsets of single nucleotide polymorphism markers. *Journal of Dairy Science* 92: 5248-5257.

Williams CM. 2000. Dietary fatty acids and human health. *Annales de Zootechnie* 49: 165-180.

Wood JD, M Enser, AV Fisher, GR Nute, PR Sheard, RI Richardson, SI Hughes, & FM Whittington. 2008. Fat deposition, fatty acid composition and meat quality: A review. *Meat Science* 78: 343-358.

Wood JD, NR Lambe, GA Walling, H Whitney, S Jagger, PJ Fullarton, J Bayntun, K Hallett, & L Bünger. 2013. Effects of low protein diets on pigs with a lean genotype: 1. Carcass composition measured by dissection and muscle fatty acid composition. *Meat Science* 95: 123-128.

Wood JD, GR Nute, RI Richardson, FM Whittington, O Southwood, G Plastow, R Mansbridge, N da Costa, & KC Chang. 2004. Effects of breed, diet and muscle on fat deposition and eating quality in pigs. *Meat Science* 67: 651-667.

Wood JD, RI Richardson, GR Nute, AV Fisher, MM Campo, E Kasapidou, PR Sheard, & M Enser. 2003. Effects of fatty acids on meat quality: A review. *Meat Science* 66: 21-32.

Yang KX, JW Ma, YM Guo, TF Guo, YG Zhao, NS Ding, M Betti, GS Plastow, & LS Huang. 2010. Correlations between fat depot traits and fatty acid composition in abdominal subcutaneous adipose tissue and longissimus muscle: Results from a White Duroc × Erhualian intercross F_2 population. *Journal of Animal Science* 88: 3538-3545.

Yang KX, JW Ma, YM Guo, TF Guo, YG Zhao, NS Ding, M Betti, GS Plastow, & LS Huang. 2010. Correlations between fat depot traits and fatty acid composition in abdominal subcutaneous adipose tissue and longissimus muscle: Results from a White Duroc × Erhualian intercross F_2 population. *Journal of Animal Science* 88: 3538-3545.

Yang B, W Zhang, Z Zhang, Y Fan, X Xie, H Ai, J Ma, S Xiao, L Huang, & J Ren. 2013. Genome-wide association analyses for fatty acid composition in porcine muscle and abdominal fat tissues. *PLoS One* 8: e65554.

Zhang S, TJ Knight, KJ Stalder, RN Goodwin, SM Lonergan, & DC Beitz. 2007. Effects of breed, sex, and halothane genotype on fatty acid composition of pork longissimus muscle. *Journal of Animal Science* 85: 583-591.

Zomeño C, A Blasco, & P Hernández. 2013. Divergent selection for intramuscular fat content in rabbits: 2. Correlated responses on carcass and meat quality traits. *Journal of Animal Science* 91: 4532-4539.

Zulkifli R, T Parr, A Salter, & J Brameld. 2010. Regulation of ovine and porcine stearoyl coenzyme A desaturase gene promoters by fatty acids and sterols. *Journal of Animal Science* 88: 2565-2575.

Other research outputs

Scientific publications

Kim ES, R Ros-Freixedes, RN Pena, T Baas, J Estany, & MF Rothschild. Identification of signatures of selection for intramuscular fat in two Duroc populations. *Submitted.*

Ros-Freixedes R, LJ Sadler, SK Onteru, RM Smith, JM Young, AK Johnson, SM Lonergan, E Huff-Lonergan, JCM Dekkers, & MF Rothschild. 2014. Relationship between gilt behavior and meat quality using principal component analysis. *Meat Science* 96: 264-269.

Divulgation publication

Bosch L, R Ros-Freixedes, RN Pena, M Tor, & J Estany. 2013. Mejora del contenido de grasa intramuscular y ácido oleico en porcino. *Albéitar* 166: 6-7. [2013. Melhoria do conteúdo de gordura intramuscular e do ácido oleico em suínos. *Albéitar* (Portugal) X(5): 8-9.]

Conference proceedings

Ros-Freixedes R, S Gol, RN Pena, M Tor, JCM Dekkers, & J Estany. 2014. Genome-wide association study for intramuscular fat content and composition in Duroc pigs. *10th World Congress of Genetics Applied to Livestock Production WCGALP*, Vancouver, Canada, August 17-22.

Ros-Freixedes R, E Henríquez, J Reixach, M Tor, & J Estany. 2014. Genetic correlations of intramuscular fat and oleic acid content among muscles and with subcutaneous fat in Duroc pigs. *10th World Congress of Genetics Applied to Livestock Production WCGALP*, Vancouver, Canada, August 17-22.

Ros-Freixedes R, S Gol, RN Pena, M Tor, JCM Dekkers, & J Estany. 2014. Asociación genómica de los loci de SCD y LEPR con la composición de la grasa intramuscular en cerdos Duroc. *XVII Reunión de Mejora Genética Animal*, Bellaterra, Spain, June 5-6.

Ros-Freixedes R, RN Pena, M Tor, & J Estany. 2013. Breeding for high oleic acid content in pork using direct selection and molecular markers. *XV Jornadas sobre Producción Animal AIDA*, Zaragoza, Spain, May 14-15.

Ros-Freixedes R, E Gjerlaug-Enger, & E Grindflek. 2012. Fatty acid composition determined by near-infrared spectroscopy and its relationship with feed conversion ratios. *Excelmeat workshop: The Omics of Pork Quality and Biosensing Technology*, Lleida, Spain, October 25-26.

Ros-Freixedes R, RN Pena, M Tor, & J Estany. 2012. Expresión del gen estearoil-coA desaturasa y desaturación de la grasa intramuscular en porcino. *XVI Reunión de Mejora Genética Animal*, Ciutadella de Menorca, Spain, May 31-June 2.

Reixach J, R Ros, M Tor, & J Estany. 2011. Selección por contenido de oleico en carne de cerdos Duroc. *XIV Jornadas sobre Producción Animal AIDA*, Zaragoza, Spain, May 17-18.

Estany J, R Ros, M Tor, & J Reixach. 2011. A compositional genetic analysis of oleic acid content in pig meat. *4th International Workshop on Compositional Data Analysis CoDaWork 2011*, Sant Feliu de Guíxols, Spain, May 9-13.

Ros R, J Reixach, M Tor, & J Estany. 2011. Exploratory data analysis for fatty acid composition in pig meat. *4th International Workshop on Compositional Data Analysis CoDaWork 2011*, Sant Feliu de Guíxols, Spain, May 9-13.

Ros R, SK Onteru, DJ Garrick, & MF Rothschild. 2011. Whole genome association analyses for principal components of lifetime reproduction and structural soundness traits in the pig. *Plant and Animal Genome Conference PAG XIX*, San Diego, CA, January 15-19.

Ros R, J Reixach, & J Estany. 2010. Using fatty acid composition for discrimination between subcutaneous and intramuscular fat. *Adapting Animal Production to Changes for a Growing Human Population International Conference AAP 2010*, Lleida, Spain, May 19-21.