SELECTION FOR INTRAMUSCULAR FAT IN RABBITS

Ph.D. Thesis by Marina Martínez Álvaro

Supervisors: Prof. Pilar Hernández Pérez

Prof. Agustín Blasco Mateu

Valencia, 6th April 2017

SELECTION FOR INTRAMUSCULAR FAT IN RABBITS

This Thesis has been submitted in fulfilment of the requirements for the degree of Doctor with International Mention at the Universitat Politècnica de València.

Esta tesis ha sido escrita y presentada como uno de los requisitos para optar al grado de Doctor con Mención Internacional por la Universitat Politècnica de València.

By: Marina Martínez Álvaro

Valencia, 6th April 2017

ACKNOWLEDGEMENTS

Esta tesis se ha podido realizar gracias al esfuerzo de mucha gente. En primer lugar, quiero agradecer a Agustín y a Pilar el tiempo que han dedicado a enseñarme, a corregirme y a mostrarme como se trabaja con rigor. Ha sido un proceso duro, pero reconfortante. Voy a intentar no olvidar nunca todo lo que he aprendido con vosotros.

Vorrei ringraziare il Professore Edi Piasentier per l'opportunità che mi è stata data, per lavorare con lui e il suo gruppo di ricerca nel Dipartimento di Scienze Agrarie e Ambientali nell`Università di Udine. Questa è stata un'ottima opportunità per introdurmi nel campo della valutazione della carne.

También quiero agradecer a Fede, Vero y Marina Morini su trabajo en este experimento, sin los cuales no habría podido desarrollar mi tesis. También a Vicent y a Ysaí por su ayuda en la granja y en el laboratorio, y por sus ganas de aprender.

Gracias a todos mis compañeros del departamento, en especial a Alberto, Marina, Samuel, Ahmed, Cristina, Mari Carmen, Saif, Luis y a todos los que me estoy olvidando, por haberme acompañado en estos cuatro años.

Quiero agradecer a todos mis amigos y a las bailarinas por haberme despejado la mente cuando lo he necesitado. También a toda mi familia, en particular a mi padre, madre y hermano, por estar conmigo siempre.

Esta tesis se la dedico a mi madre y a mi padre. Me siento muy afortunada de teneros. También quiero dedicar esta tesis a Emi. Resulta más fácil trabajar intensamente en algo si tienes a tu lado un refuerzo tan grande. Muchas gracias a los tres por entenderme siempre tan bien.

CONTENTS

INDEX OF TABLES

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5, I

CHAPTER 5, II

INDEX OF FIGURES

CHAPTER 1

ABSTRACT

A divergent selection experiment on intramuscular fat (IMF) content was performed in rabbits during eight generations. Selection criterion was the averaged phenotypic value of IMF measured in *Longissimus dorsi* (LD) muscle at 9 wk of age in two full-sibs of the candidate. The aim of this Thesis is to evaluate the direct response to selection for IMF and the correlated responses in other meat and carcass quality traits.

Divergent selection for IMF was successful. Direct response to selection in the eight generation was 2.4 standard deviations of the trait, which represents a genetic progress of 5% of the mean per generation. Genetic trends were symmetrical for both lines.

Correlated responses to selection were measured from fifth to eighth generations. Fatty acid composition of LD was affected by selection for IMF. High-IMF line showed greater monounsaturated (MUFA) but lower polyunsaturated (PUFA) fatty acids percentages, and their individual fatty acid percentages showed similar correlated responses, except for C18:3n-3 that was greater in the high-IMF line. We did not find differences between lines for saturated fatty acid (SFA) percentage, but C14:0 and C16:0 percentages were greater in the high-IMF line, and C18:0 was greater in the low-IMF line. A positive correlated response to selection for IMF was observed in the IMF content and fatty acid composition of other muscles with diverse oxidative pattern (*Biceps femoris*, *Supraspinatus* and *Semimembranosus proprius*). Protein content of LD was greater in the high-IMF line, whereas we did not observed differences between lines in color and pH meat quality traits. Instrumental firmness was 9.9% greater in the low-IMF line than in the high-IMF line, whereas we did not find differences between lines in other instrumental texture parameters and cooking loss. No effect of selection for IMF was observed in any sensory attribute. Regarding carcass quality traits, a positive correlated response to selection for IMF was observed in carcass fat depots, whereas we did not find differences between lines in other traits. Genetic parameters for IMF and some of the meat and carcass quality traits cited above were estimated and corrobated with the phenotypic responses to selection observed.

In this Thesis, we also studied the lipid metabolism of the divergent lines. High-IMF line showed greater glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme (EM) and fatty acid synthase (FAS) lipogenic activities in glycolytic LD muscle than low-IMF line, and greater G6PDH and FAS activities in the oxidative *Semimembranosus proprius* muscle and perirenal fat depot. However, differences between lines were observed only at 13 wk of age and not at 9 wk. Catabolic activities in muscle involved in the lipid metabolism did not explain the differences between lines for IMF. Liver size, which is the main lipogenic tissue in growing rabbits, was greater in the high-IMF line and showed greater G6PDH and EM activities than low-IMF line at 9 wk, although differences for EM were not relevant.

RESUMEN

Se ha realizado un experimento de selección divergente por grasa intramuscular (GIM) en conejo durante ocho generaciones. El criterio de selección fue el promedio del valor fenotípico de GIM medido en el músculo *Longissimus dorsi* (LD) a las 9 semanas de edad en dos hermanos completos del candidato a la selección. El objetivo de esta tesis es evaluar la respuesta directa a la selección por GIM y las respuestas correlacionadas en otros caracteres de calidad de la canal y de la carne.

La selección divergente por GIM ha tenido éxito. La respuesta directa en la octava generación de selección fue de 2.4 desviaciones típicas del carácter, lo que representa un progreso genético del 5% de la media en cada generación. Las líneas mostraron tendencias genéticas simétricas.

Las respuestas correlacionadas a la selección en caracteres de calidad de la canal y de la carne se estudiaron durante el periodo desde la quinta hasta la octava generación. La composición de ácidos grasos de LD se vió afectada por la selección. La línea de alta GIM mostró un mayor porcentaje de ácidos grasos monoinsaturados (MUFA) y un menor porcentaje de poliinsaturados (PUFA) que la línea de baja GIM, y los MUFA y PUFA individuales mostraron respuestas correlacionadas similares a los grupos, excepto el porcentaje de C18:3n-3 que fue mayor en la línea de alta GIM. No encontramos diferencias entre líneas en el porcentaje de ácidos grasos saturados (SFA), aunque los porcentajes de C14:0 y C16:0 fueron mayores en la línea de alta GIM, y el porcentaje de C18:0 fue mayor en la línea de baja GIM. Se observó una respuesta correlacionada positiva en el contenido de GIM y en la composición de ácidos grasos de otros músculos con un patrón oxidativo diferente al LD (*Biceps femoris*, *Supraspinatus* y *Semimembranosus proprius*). El contenido de proteína de LD fue mayor en la línea de alta GIM, mientras que no encontramos diferencias entre líneas en otros caracteres de calidad de carne como pH o color. El parámetro de textura instrumental firmeza fue 9.9% mayor en la línea de baja GIM, mientras que no se observaron diferencias entre líneas en otros parámetros de textura instrumental, pérdidas por cocinado, y atributos sensoriales. En cuanto a los caracteres de calidad de carne, solamente se observó una respuesta correlacionada positiva en los depósitos grasos de la canal, mientras que no se observaron diferencias en otros caracteres. Se

estimaron los parámetros genéticos de GIM y de otros caracteres de calidad de la carne y de la canal citados anteriormente, y se corroboraron con las respuestas correlacionadas a la selección observadas.

En esta tesis también se ha estudiado el metabolismo lipídico de las líneas divergentes. La línea de alta GIM mostró mayor actividad de las enzimas lipogénicas 6-glucosa-fosfato deshidrogenasa (G6PDH), enzima málico (EM) y ácido graso sintasa (FAS) en el músculo glicolítico LD respecto de la línea de baja GIM, y mayor actividad de las enzimas G6PDH y FAS en el músculo oxidativo *Semimembranosus proprius* y en el depósito de grasa perirrenal. Sin embargo, estas diferencias solo fueron observadas a las 13 semanas de edad, y no a las 9 semanas. En los músculos, las actividades de las enzimas catabólicas implicadas en el metabolismo de la grasa no mostraron diferencias entre líneas. El hígado, que es el principal tejido lipogénico en conejos en crecimiento, fué más grande en la línea de alta GIM, y mostró mayor actividad de las enzimas G6PDH y EM que la línea baja, aunque las diferencias en EM no fueron relevantes.

RESUM

S'ha realitzat un experiment de selecció divergent per greix intramuscular (GIM) en conill durant huit generacions. El criteri de selecció va ser la mitjana del valor fenotípic de GIM mesurat en el múscul *Longissimus dorsi* (LD) a les 9 setmanes d'edat en dos germans complets del candidat a la selecció. L'objectiu d'esta tesi és avaluar la resposta directa a la selecció per GIM i les respostes correlacionades en altres caràcters de qualitat de la canal i de la carn.

La selecció divergent per GIM ha tingut èxit. La resposta directa en la sèptima generació de selecció va ser de 2.4 desviacions típiques del caràcter, la qual cosa representa un progrés genètic del 5% de la mitjana en cada generació. Les línies van mostrar tendències genètiques simètriques.

Les respostes correlacionades a la selecció en caràcters de qualitat de la canal i de la carn es van estudiar durant el període de la quinta fins a l'octava generació. La composició d'àcids grassos de LD també es va veure afectada per la selecció. La línia d'alta GIM va mostrar un major percentatge d'àcids grassos monoinsaturats (MUFA) i un menor percentatge de poliinsaturats (PUFA) que la línia de baixa GIM, i els MUFA i PUFA individuals van mostrar respostes correlacionades semblants als grups, excepte el percentatge de C18:3n-3 que va ser major en la línia d'alta GIM. No trobem diferències entre línies en el percentatge d'àcids grassos saturats (SFA), encara que els percentatges de C14:0 i C16:0 van ser majors en la línia d'alta GIM, i el percentatge de C18:0 va ser major en la línia de baixa GIM. Es va observar una resposta correlacionada positiva en el contingut de GIM i en la composició d'àcids grassos d'altres músculs amb un patró oxidatiu diferent del LD (*Bíceps femoris, Supraspinatus* i *Semimembranosus proprius*). El contingut de proteïna de LD va ser major en la línia d'alta GIM, mentres que no trobem diferències entre línies en altres caràcters de qualitat de carn com pH o color. El paràmetre de textura instrumental fermesa va ser 9.9% major en la línia de baixa GIM, mentres que no es van observar diferències entre línies en altres paràmetres de textura instrumental, pèrdues per cuinat, i atributs sensorials. Quant als caràcters de qualitat de canal, només es va observar una resposta correlacionada positiva en els depòsits grassos de la canal, mentres que no es van observar diferències en altres caràcters. Es van estimar els paràmetres genètics de GIM i d'altres caràcters

de qualitat de la carn i de la canal esmentats anteriorment, i es van corroborar amb les respostes correlacionades a la selecció observades.

En esta tesi també s'estudia en metabolisme lipídic de les línies divergents. La línia d'alta GIM va mostrar major activitat dels enzims lipogénics 6-glucosa-fosfato deshidrogenasa (G6PDH), enzim màlic (EM) i àcid gras sintasa (FAS) en el múscul glicolític LD respecte de la línia de baixa GIM, i major activitat dels enzims G6PDH i FAS en el múscul oxidatiu *Semimembranosus proprius* i en el depòsit de greix perirrenal. No obstant això, estes diferències només van ser observades a les 13 setmanes d'edat, i no a les 9 setmanes. En els músculs, les activitats dels enzims catabòlics implicades en el metabolisme del greix no van mostrar diferències entre línies. El fetge, que és el principal teixit lipogénic en conills en creixement, va ser més gran en la línia d'alta GIM, i va mostrar major activitat dels enzims G6PDH i EM que la línia de baixa GIM, encara que les diferències en EM no van ser rellevants.

LITERATURE REVIEW

Consumers of developing countries are now demanding higher quality in food products. As a result, there is an increasing focus on improving the quality of meat from livestock species in meat industry. A main trait in meat quality is intramuscular fat content that largely affects the sensory quality of meat, increasing its juiciness, tenderness and flavor. The goal of the meat industry is increasing IMF enough to cover the eating quality demand, but avoiding an excess of fat that would affect human health when consuming meat. The Unit States Department of Agriculture (USDA) has proposed different beef carcass quality grades mainly based on the degree of marbling (Mermelstein, 2013).

Intramuscular fat can be increased by genetic selection due to its high heritability and moderate variability. However, it is required IMF measurements in a large amount of animals to perform a breeding programme, which is particularly expensive. In the rabbit market, IMF is not considered; however, rabbit can be a good genetic model for other livestock species due to its short generation interval, the low cost of the carcass and its low size, which facilitates carcass management. Moreover, sensory analysis can be performed with rabbit meat, which would not be possible with other laboratory animals as rodents. Measuring IMF by chemical methods is time-consuming and expensive. However, near infrared reflectance (NIR) technology allows analysing a high amount of samples accurately, in a short period of time, and at reasonable costs. A selection experiment for intramuscular fat content measured by NIRS was initiated by Zomeño et al., 2013 in rabbits. This thesis ends this experiment and studies the consecuences of the selection process.

1. Intramuscular fat role in the quality of meat

1.1. Effect of intramuscular fat in sensory properties of meat

Intramuscular fat content is one of the main parameters in meat quality because it affects the sensory attributes of meat as juiciness, tenderness and flavor (Warris, 2003 and Wood et al., 2008). Relationships between IMF and sensory quality of meat varies among studies, but in general, high amounts of IMF are associated to a juicy and tasty meat (Raes et al., 2001 in beef and Wood et al., 2004 in pig).

Juiciness increases with IMF due to the moisture trapping by lipids and to the increase of salivation during chewing stimulated by the content of meat lipids. The amount of IMF has been positively correlated with juiciness in several studies, reporting phenotypic correlations of 0.24 in rabbit (Hernández et al., 2000), 0.21 in pig (De Vol et al., 1988), 0.27 in beef (Mateescu et al., 2015), and 0.36 in lamb (Angood et al., 2008).

The content of IMF has been positively correlated with sensory tenderness and negative correlated with Warner-Bratzler shear force when measuring toughness instrumentally. De Vol et al. (1988) in pig and Mateescu et al. (2015) in beef, reported correlations between IMF and sensory tenderness of 0.32 and 0.21, respectively, and correlations between IMF and Warner-Bratzler shear force of -0.27 and -0.23, respectively. The association between IMF and tenderness could be related to the infiltration of IMF within the perimysium connective tissue that weakens the cross-linkage between collagen fibers, reducing the force required to break the connective tissue (Essén-Gustavsson et al., 1994; Nishimura et al., 1999).

Flavor is also affected by IMF by the generation of volatile compounds from fat during cooking (Warris, 2003). De Vol et al. (1988) reported a positive correlation of 0.21 between IMF and flavor in pig meat.

1.2. Effect of intramuscular fat in nutritional properties of meat

Intramuscular fat content and its fatty acid composition affect the nutritional value of meat. A higher IMF content implies a higher amount of almost all fatty acids. However, variation in IMF strongly affects the fatty acid profile of meat expressed as a percentage of total fatty acids, due to changes in the proportion of triglycerides, in comparison to phospholipids. Triglycerides fraction is rich in saturated (SFA) and monounsaturated (MUFA) fatty acids, whereas phospholipid fraction is rich in polyunsaturated fatty acids (PUFA). Thus, the proportion of PUFA respect SFA or MUFA is reduced when increasing fat deposition (Leseigneur-Meynier and Gandemer, 1991; De Smet et al., 2004).

In general, meat is an important source of fat in the diet of developed countries, particularly of SFA. High levels of SFA in diet increases low density

lipoprotein (LDL) cholesterol in humans, which has been correlated with coronary heart disease (Brown et al., 2009). Nutritional institutions recommend a total dietary fat intake between 15-35% of the daily energy intake. From those, SFA must account for less than 10% (World Health Organization, 2008), although recent recommendations suggest that SFA intake should be as low as possible (Brown et al., 2009). Among the SFA present in meat, stearic acid (C18:0) has a small effect on LDL cholesterol concentration in plasma, whereas lauric (C12:0), myristic (C14:0), and palmitic (C16:0) acids increases LDL cholesterol concentration, particularly myristic (Brown et al., 2009).

The main MUFA in meat is oleic acid (C18:1n-9), and it shows good properties for human health, lowering LDL cholesterol (Nestel et al., 1994 and Ruiz-Gutiérrez et al., 1996). The intake recommendations for MUFA in diet are usually determined by difference, i.e. total fat intake minus SFA minus PUFA minus trans fatty acids (World Health Organization, 2008).

Linoleic (C18:2n-6), aranchidonic (C20:4n-6) and linolenic (C18:3n-3) acids are the main PUFA present in meat. In general, meat from ruminants show less amounts of PUFA in comparison to monogastrics due to the extensive hydrogenation of dietary unsaturated fatty acids by rumen microorganisms (Wood et al., 2008). Nutritional recommendations from PUFA intake are between 6 and 11% of the daily energy intake (World Health Organization, 2008). Polyunsaturated fatty acids decrease LDL cholesterol and the total: high density lipoprotein (HDL) cholesterol ratio. Eicosanoids from n-3 EPA (C20:5n-3) and DHA (C22:6n-3), derived from C18:3n-3, play a major role decreasing the risk of cardiovascular diseases and they are widely recommended in human diets (World Health Organization, 2008). However, eicosanoids from n-6 fatty acids, derived from C18:2n-6 acid, are more related to inflammation states, promote platelet aggregation and cause vasoconstriction in comparison with eicosanoids produced from n-3 fatty acids. Nutrition advices recommend reducing the dietary n-6:n-3 ratio to values of 5-10 (ISSFAL, 2010 and World Health Organization, 2008), although a value < 4 is highly recommended to reduce the risk of many chronic diseases (Simopoulos, 2002).

In general, replacing SFA (C12:0-C16:0) by PUFA is largely recommended because prevents cardiovascular heart disease. The minimum PUFA:SFA ratio

stablished by nutritional institutions is 0.6 (World Health Organization, 2008). A similar but lesser effect is achieved by replacing these SFA by MUFA, but to our knowledge, nutritional institutions have not established recommended values for MUFA:SFA ratio.

1.3. Intramuscular fat in rabbit meat

The amount of fat in rabbit meat is largely dependent on the portion of the carcass considered (reviewed by Hernández and Gondret, 2006). In commercial Spanish carcasses (slaughtered at about 9 wk of age), the thoracic cage is the fattest part, with 12.8 g of fat/100g of edible meat. Abdominal walls and fore legs show a moderate fat content around 7.5 g of fat/100g whereas the fat content of the hind leg is lower, 3.0 g/100g. The leanest part of the carcass is the loin, with 1.2 g /100g. Besides, intramuscular fat content depends not only on the muscle type but also on the muscle site (Alasnier et al., 1996). In general, the range of fat variation in rabbit meat is similar to those of chickens, but lower than those of pigs (Dalle Zotte, 2002).

The main fatty acids in rabbit meat are PUFA and SFA, whereas MUFA are lower represented. Depending on the carcass portion, SFA ranges from 37-40%, PUFA from 24-37% and MUFA from 23-33% (reviewed by Dalle Zotte, 2002; Hernández and Gondret, 2006 and Dalle Zotte and Szendrö, 2011). The percentage of PUFA in rabbit meat is greater than in pigs, veal, beef and chickens and it shows a greater PUFA:SFA ratio (Dalle Zotte et al., 2002).

In rabbit meat, PUFA are mainly composed by n-6 whereas the amount of n-3 is lower. Linolenic acid (C18:2n-6), precursor of n-6 family of PUFA, is particularly high in rabbit meat. Besides, the amount of linolenic acid (C18:3n-3), precursor of n-3 family of PUFA, is also higher in rabbit meat than in other species (reviewed by Hernández and Gondret, 2006). Altogether comprise a n-6:n-3 ratio around 7 in the loin and around 11 in the hind leg (Ramírez et al., 2005 and Dalle Zotte and Szendrö, 2011). This value is far from the nutritional recommendations cited above, but it is more favourable than the n-6:n-3 ratio in pig, veal or chicken meats (Dalle Zotte, 2002). Together with the linoleic acid, palmitic (C16:0), and oleic (C18:1n-9) acids are the most abundant fatty acids in rabbit meat, showing percentages higher than the 20% of the total fatty acids (Hernández and Gondret, 2006 and Dalle Zotte, 2002).

Most sensory studies in rabbit meat are focused in the loin muscle, probably due to practical concerns (Gondret et al., 1998; Rødbotten et al., 2004; Gašperlin et al., 2006). Since the loin is a very lean portion of the rabbit carcass, its sensory properties should be poorly determined by its IMF content. Rabbit loin is considered particularly tender by other reasons than its IMF, such as its lower content of elastine (Ouhayoun and Lebas, 1987) and the high solubility of its collagen, in comparison with meat from other species (Combes et al., 2003). Besides, it is considered to have a mild flavor (Dalle Zotte, 2002 and Rødbotten et al., 2004) although its characteristic taste of wild game meat can be a cause of refusal by consumers (De Carlo, 1998).

2. Improving intramuscular fat by selection

Intramuscular fat content is easily modifiable by selection due to its moderate variability and high heritability. Estimates of IMF heritability range from 0.26 to 0.86 in pigs (Ciobanu et al., 2011) and from 0.34 to 0.77 in cattle (Mateescu, 2015). To our knowledge, there are no estimates of heritability in rabbits, apart from that estimated by Zomeño et al., (2013) of 0.38 [0.19, 0.59], within data from the first three generations of the selection experiment contained on this Thesis. Increasing IMF by selection can improve the quality of meat; however it can also lead to changes in other relevant carcass and meat quality traits due to their genetic relationships with IMF.

2.1. Selection experiments for intramuscular fat

To our knowledge, there are only three selection experiments for IMF, in pigs (Schwab et al., 2009), cattle (Sapp et al., 2002) and chickens (Zhao et al., 2007). After seven generations of selection in pigs (Schwab et al., 2009), five generations in chickens (Zhao et al., 2007), and only one generation in cattle (Sapp et al., 2002), all these experiments showed great direct responses to selection. Besides, the selection experiment in chickens, found a positive correlated response in IMF of the thigh muscle when selecting for IMF in breast (Zhao et al., 2007).

Selection experiments for IMF showed correlated responses to selection in other meat and carcass quality traits that should be considered. To our knowledge, the

effect of selection on the fatty acid composition of meat was only evaluated in the selection experiment in pigs (Burkett, 2009). In general, high IMF line showed greater MUFA percentages and lower PUFA percentages respect to a control line (Burkett, 2009). Moreover, these authors did not find significant differences between lines for SFA percentage.

Overall, IMF is well appreciated in meat industry due to its favorable effects on the sensory properties of meat, particularly, juiciness, tenderness and flavor. Sensory attributes were only evaluated in the selection experiment in pigs. Schwab et al. (2009) observed higher flavor in pigs selected for high IMF, whereas hardness and juiciness were not affected. Meat tenderness can be also evaluated through instrumental texture procedures, such as Warner-Bratzler or Instron tests. Instrumental texture parameters were evaluated in the selection experiments of chickens and pigs. In both, the fat line showed favorable texture parameters, lower instrumental shear force and lower Instron tenderness, respect to the control line (Zhao et al., 2007 and Schwab et al., 2009).

Other meat quality traits such us pH, meat color and cooking loss were also evaluated in these experiments. In pigs, Schwab et al., (2009) found no effect of selection for IMF on meat pH. They found a large positive correlated response in meat lightness (L*) when selecting for high IMF, although this change was not detected by a panel evaluating the color of fresh meat. In broilers, Zhao et al. (2007) did not find a selection effect on meat color parameters. Drip loss was not affected by selection for IMF in chickens (Zhao et al., 2007), and neither cooking loss in pigs (Schwab et al., 2009).

Selection for IMF in pigs (Schwab et al., 2009) and chickens (Zhao et al., 2007) showed a positive correlated response in carcass fat depots (abdominal fat weight and back fat content, respectively). However, in chickens, differences between lines were not relevant when abdominal fat weight was expressed as a percentage of body weight (Zhao et al., 2007). The selection experiment in cattle did not show a correlated response in the carcass fat depots; however, these results should be taken with caution because selection was performed only during one generation (Sapp et al., 2002). Selection for IMF showed correlated responses in other carcass quality traits. For instance, in chickens, body weight and carcass weight increased when selecting for

high IMF (Zhao et al., 2007), although carcass weight was not affected by selection for IMF in pigs (Schwab et al., 2009) or cattle (Sapp et al., 2002). Carcass meat content was differently affected depending on the specie. Schwab et al. (2009) showed a negative correlated response in loin muscle area when selecting for IMF in pigs. In cattle, Sapp et al. (2002) did not find a response in ribeye area after selection for IMF, and in chickens, the fat line showed greater breast muscle weight and muscle percentage than the control line, but this was not observed for thigh muscle (Zhao et al., 2007).

2.2. Genetic correlations of intramuscular fat with meat and carcass quality traits

Genetic correlations involving IMF and meat and carcass quality traits in general, are difficult to obtain because they need to be estimated with a great amount of data for acceptable accuracies. However, when data come from a selection experiment, the observed correlated responses to selection can be used for corroborating the estimates of genetic parameters. The selection experiments for IMF in pigs (Burkett et al., 2009 and Schwab et al., 2010) and chickens (Zhao et al., 2006) estimated genetic parameters.

The genetic correlation between IMF content in breast and in hind leg in the selection experiment in chickens was large and positive (0.89), corroborated by the positive response to selection observed (Zhao et al., 2006).

Burkett et al. (2009) calculated genetic correlations between IMF and fatty acid percentages in the selection experiment in pigs, obtaining parameters with sign and magnitudes according to their correlated responses observed. The genetic correlations of IMF with PUFA percentage was -0.80. The other genetic correlations between IMF and other fatty acid percentages were estimated with very low accuracy due to their low amount of data (n = 663).

Genetic correlations between IMF and texture traits (sensory juiciness and tenderness and instrumental Instron tenderness) reported in the selection experiment of pigs (Schwab et al., 2010) were low, and showed low accuracy. The genetic correlation between IMF and flavor was greater, 0.65 ± 0.25, in line with the positive correlated response to selection observed. The genetic correlations of IMF with meat

lightness reported in pigs was 0.52 ± 0.15, whereas the genetic correlations between IMF and other meat quality traits such as pH of meat and subjective color score were low, and were also estimated with low accuracy (Schwab et al., 2007). However, they should be not substantial, since these traits were unaffected after several generations of selection for IMF.

Intramuscular fat showed a positive genetic correlation with backfat content in pigs (0.42 ± 0.17, Schwab et al., 2010) and with carcass fat traits in chickens (from 0.26 to 0.66, Zhao et al., 2006). Their results suggest some genetic independence between fat depots. In this line, a selection experiment for reducing back fat thickness restraining IMF was performed during one generation in pigs (Ros-Freixedes et al., 2013). Back fat thickness was reduced, although with a slight reduction also in IMF that the authors attribute to inaccuracies estimating the breeding value of IMF.

3. Intramuscular fat metabolism

Intramuscular fat is the total of lipids present in meat and comprises mainly triglycerides (energy reserves) and phospholipids (constructing cell membranes). The contribution of phospholipids to IMF content is relatively constant in muscles of similar oxidative pattern, whereas the amount of triglycerides is highly variable depending of their fat content (reviewed by Gerbens, 2004, in several species). In rabbits, triglycerides range from 0.5 to 3.9 g/100g of muscle, whereas phospholipids are lower and less variable, between 0.7 and 0.9 g/100g of muscle (Alasnier et al., 1996).

In mammals and birds, triglycerides are mainly stored within intramuscular adipocytes (around 80%) and to a lesser extent, within cytoplasm of the myofibers, in droplets close to mitochondria (Hocquette et al., 2010). Gondret et al. (1998) studied the relative contribution of the lipid depots in myofibers and in adipocytes to the total IMF content in rabbits during growth. It was concluded that lipids were mainly accumulated within the myofibers during the first two weeks of age, while they were mainly accumulated within adipocytes thereafter.

In general, development of adipose tissue occurs by a combination of increase in adipocytes number (hyperplasia) and size (hypertrophy). Hyperplasia is due to the proliferation and differentiation of precursor cells (adipoblasts) or to the accumulation

of lipid differentiated cells devoid of lipids. Hypertrophy occurs primarily through triglycerides accumulation. Adipocyte size can increase or decrease depending on the energy balance of the animal (Flint and Vernon, 1993; Gerbens, 2004 and Hausman et al., 2009), i. e. to anabolic and catabolic pathways of lipids (Lee and Kauffman, 1974a and b in pigs and Baik et al., 2014 in cattle). Differences between animals at same ages in IMF content have been ascribed to differences in the number (Damon et al., 2006) and size (Hauser et al., 1997) of the intramuscular adipocytes.

3.1. Lipid biosynthesis *de novo*

Lipogenesis *de novo* or fatty acid synthesis *de novo* is the metabolic pathway that synthetizes fatty acids from excess of carbohydrates. Triglycerides can be also synthetized with fatty acids coming from diet, but this is a minor source in meat animals, since their diets are poor in fats.

Lipogenesis *de novo* in animals occurs in the cytosol cells. The first step is the carboxylation of acetyl-CoA to malonyl-CoA, catalysed by the enzyme acetyl-CoA carboxylase. Enzyme fatty acid synthase catalyses the following steps: the conversion of malonyl-Co A to a long chain saturated fatty acid, in presence of a carbon source and NADPH. In adipose tissue and liver, NADPH is generated by malic enzyme, involved in citrate-malate-pyruvate shuttle, and by glucose-6-phosphate dehydrogenase, involved in the pentose phosphate pathway (Hsu et al., 1965 and Melo and Cuamatzi, 2010). The main product of the fatty acid synthesis *de novo* is palmitic acid (C16:0). Palmitic acid may be converted to longer chain fatty acids by fatty acid elongases. Single double bound can be also introduced by stearoyl-CoA desaturase to convert the saturated fatty acids to their respective monounsaturated forms. Long chain polyunsaturated fatty acids can also be produced via elongation and desaturation of the essential precursors, linoleic (C18:2n-6) and linolenic (C18:3n-3) fatty acids that cannot be synthetized and have to be incorporated by the diet.

Lipogenesis *de novo* occurs in most tissues of the body, although liver and adipose tissue are the major sites. However, the relative contribution of liver or adipose tissue varies between species and age of animals. In pigs and ruminants the adipose tissue is the principal site of fatty acid synthesis; in humans, chickens and fish

the liver is the most important site, and in rat and rabbits, both tissues are contributing (reviewed by Shargo et al., 1971; Pullen et al., 1990 and Dodson et al., 2010). In rabbits, liver is the major site for lipogenesis *de novo* during their growing period whereas adipose tissue became more relevant in adults (Leung and Bauman, 1975 and Gondret et al., 1997).

In general, when lipogenesis *de novo* is produced in adipose tissue or muscles, lipids are stored as triglycerides for local consumption. However, the synthesis of triglycerides in liver is mainly destined to produce lipoproteins for their distribution through the bloodstream (Melo and Cuamatzi, 2010). In species where adipose tissue is a minor site of lipogenesis *de novo*, IMF deposition depends not only on the metabolism of intramuscular adipocytes, but also on metabolic activity of other organs, as liver (reviewed by Hocquette et al., 2010). Greater lipogenic activities or lipogenic gene expressions, both in muscle and liver, have been associated to a greater intramuscular fat deposition (Zomeño et al., 2010 in rabbits, Ramírez et al., 2007, Mourot and Kouba, 1998 and 1999 in pigs; Bonnet et al., 2007 and Ward et al., 2010 in cattle and Cui et al., 2012 in chickens).

3.2. Lipid catabolism

Lipolysis is the first step for the use of fatty acids as an energy source, and comprises the hydrolysis of triglycerides into free fatty acids. This step is required to fats for entering into cells. Gastrointestinal lipases mediates the catabolism of dietary fat; vascular lipases hydrolyse triglycerides associated to lipoproteins in plasma and intracellular lipases catalyses the hydrolysis of triglycerides stored in intracellular lipid droplets (Zechner et al., 2012). Vascular lipoprotein lipase is the limiting enzyme for release of plasma lipids to muscle and fat tissues and it has been suggested as a good indicator of lipid deposition in pigs (Allen et al., 1976). Regarding intracellular lipases activities in muscle, literature is not clear. Some studies have related greater IMF deposition to greater lipolytic activities (Hernández et al., 2008 in rabbits) or greater expression of genes involved in intracellular lipolysis (Cánovas et al., 2010 in pigs), while other studies have related greater IMF to lower lipolytic activities (Cava et al.,

2004 in pigs, Zomeño et al., 2010 in rabbits) or lower lipolysis gene expressions (Jeong et al., 2012 in cattle).

Once free fatty acids are in the cell, the fatty acid oxidation takes place in the mitochondria. Oxidation rate depends on the fatty acid structure. In general terms, unsaturated fatty acids are oxidised at faster rate than saturated. Between saturated fatty acids, the short chain fatty acids show faster oxidation rate than those with longer chain. Between unsaturated, C18:3n-3 and oleic acid (C18:1n-9) are oxidised at faster rate than C18:2n-6 or arachidonic acid (C20:4n-6) (Leyton et al., 1987). In several species, greater fatty acid oxidation has been related to lower IMF deposition (Kim et al., 2000 in humans, Young et al., 2002 in rats, Gondret and Lebret, 2007 in pigs, Zomeño et al., 2010 in rabbits and Hocquette et al., 2012 in cattle).

4. Literature cited

Alasnier, C., H. Rémington and G. Gandemer. 1996. Lipid characteristics associated with oxidative and glycolytic fibres in rabbit muscles. Meat Sci. 43:213-224.

Angood, K., J. Wood and G. Nute. 2008. A comparison of organic and conventionally-produced lamb purchased from three major UK supermarkets: Price, eating quality and fatty acid composition. Meat Sci. 78:176–184.

Baik, M., J.Y. Jeong, T.T.T. Vu, M.Y. Piao and H.J.Kang. 2014. Effects of castration on the adiposity and expression of lipid metabolism genes in various fat depots of Korean cattle. Livest. Sci. 168:168-176.

Bonnet, M., Y. Faulconnier, C. Leroux, C. Jurie, I. Cassar-Malek, D. Bauchart, P. Boulesteix, D. Pethick, J.F. Hocquette and Y. Chilliard. 2007. Glucose-6-phosphate dehydrogenase and leptin are related to marbling differences among Limousin and Angus or Japanese Black x Angus steers. J. Anim. Sci. 85:2882–2894.

Brown, W.V., W. Karmally, P. Kris-Etherton and L.R. Rudel. 2009. Discussion on dietary fat. J. Clin. Lipidol. 3:303-314.

Burkett, J.L. 2009. The effect of selection for intramuscular fat on fatty acid composition in Duroc pigs. Graduate Theses and Dissertations. Paper 10539.

Cánovas, A., R. Quintanilla, M. Amills, and R.N. Pena. 2010. Muscle transcriptomic profiles in pigs with divergent phenotypes for fatness traits. BMC Gen. 11:372, 1-15.

Cava, R., J.M. Ferrer, M. Estévez, D. Morcuende and F. Toldrá. 2004. Composition and proteolytic and lipolytic enzyme activities in muscle *Longissimus dorsi* from Iberian pigs and industrial genotype pigs. Food Chem. 88:25-33.

Ciobanu, D.C. S. M. Lonergan and E.J. Huff- Lonergan. 2011. Genetics of meat quality and carcass traits. In: M.F. Rothschild and A. Ruvinsky. The genetics of the pig. CAB Int. Oxfordshire (UK), pp. 355-389.

Combes, S., J. Lepetit, B. Darche, and F. Lebas. 2003. Effect of cooking temperature and cooking time on Warner–Bratzler tenderness measurement and collagen content in rabbit meat. Meat Sci. 66:91–96.

Cui, H.X., M.Q. Zheng, R.R. Liu, G.P. Zhao, J.L. Chen and J. Wen. 2012. Liver dominant expression of fatty acid synthase (FAS) gene in two chicken breeds during intramuscular-fat development. Mol. Biol. Rep. 39:3479-3484.

Damon, M., I. Loveau, L. Lefaucheur, B. Lebret, A. Vincent, P. Leroy, M.P. Sanchez, P. Herpin and F. Gondret. 2006. Number of intramuscular adipocytes and fatty acid binding protein-4 content are significant indicators of intramuscular fat level in crossbred Large White x Duroc pigs. J. Anim. Sci. 84:1083-1092.

Dalle Zotte, A. 2002. Perception of rabbit meat quality and major factors influencing the rabbit carcass and meat quality. Livest. Prod. Sci. 75:11–32.

Dalle Zotte, A. and Z. Szendrö. 2011. The role of rabbit meat as functional food. Meat Sci. 88:319-331.

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

De Vol, D.L., F.K. McKeith, P.J. Bechtel, J. Novakofski, R.D. Shanks and T.R. Carr. 1988. Variation in composition and palatability traits and relationships between muscle characteristics and palatability in a random sample of pork carcasses. J. Anim. Sci. 66:385-395.

De Carlo, N. 1998. Indagine sulla valorizzazione della qualitanel settore cunicolo Veneto. In Proc.: Workshop Il coniglio nell'alimentazione: aspettative dei consumatori e opportunitaper gli operatori. Padova, Italy, pp. 1-13.

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

Essén-Gustavsson, B., A. Karlsson, K. Lundström, and A.C. Enfält. 1994. Intramuscular fat and muscle fibre lipid contents in halothane-gene-free pigs fed high or low protein diets and its relation to meat quality. Meat Sci. 38:269–77.

Flint, D.J. and R.G. Vernon 1993. Hormones and adipose Growth. The endocrinology of growth, development, and metabolism in vertebrates. Academic Press, Inc. California (USA) Harcourt Brace Jovanovich Publishers, pp. 469-493.

Gašperlin, L., T. Polak, A. Rajar, M. Skvarèa, and B. Lender. 2006. Effect of genotype, age at slaughter and sex on chemical composition and sensory profile of rabbit meat. World Rabbit Sci. 14:157–166.

Gerbens, F. 2004. Genetic control of intramuscular fat accretion. Muscle development of livestock animals : physiology, genetics, and meat quality. CAB International, Wallingford, UK. pp. 343–361.

Gondret, F., J. Mourot and M. Bonneau. 1997. Developmental changes in lipogenic enzymes in muscle compared to liver and extramuscular adipose tissues in the rabbit (Oryctolagus cuniculus). Comp. Biochem. Physiol. 117B:259-265.

Gondret, F., H. Juin, J. Mourot, and M. Bonneau. 1998. Effect of age at slaughter on chemical traits and sensory quality of *Longissimus lumborum* muscle in the rabbit. Meat Sci. 48:181–187.

Gondret, F. and B. Lebret. 2007. Does feed restriction and re-alimentation differently affect lipid content and metabolism according to muscle type in pigs (Sus scrofa)? Comp. Bioch. Physiol. A: Mol. Int. Phys. 147:375-382.

Hauser, N., J. Mourot, L. De Clercq, C. Genart, and C. Remacle. 1997. The cellularity of developing adipose tissues in Pietrain and Meishan pigs. Reprod. Nutr. Dev. 37:617–625.

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

Hernández, P., M. Pla, M.A. Oliver and A. Blasco. 2000. Relationships between meat quality measurements in rabbits fed with three diets of different fat type and content. Meat Sci. 55:379–384.

Hernández, P. and F. Gondret. 2006. Rabbit meat quality and safety. Recent Advances in Rabbit Sciences. ILVO, Melle, Belgium, pp. 267-290.

Hernández, P., V. Cesari, and A. Blasco. 2008. Effect of genetic rabbit lines on lipid content, lipolytic activities and fatty acid composition of hind leg meat and perirenal fat. Meat Sci. 78:485–491.

Hocquette, J.F., F. Gondret, E. Baéza, F. Médale, C. Jurie, and D. W. Pethick. 2010. Intramuscular fat content in meat-producing animals: development, genetic and nutritional control, and identification of putative markers. Animal. 4:303-319.

Hocquette, J.F., I. Cassar-Malek, C. Jurie, D. Bauchart, B. Picard and G. Renand. 2012. Relationships between muscle growth potential, intramuscular fat content and different indicators of muscle fibre types in young Charolais bulls. Anim. Sci. J. 83:750-758.

Hsu, R. Y., G. Wasson and J.W. Porter. 1965. The purification and properties of the fatty acid synthetase of pigeon liver. J. Biol. Chem. 240:3736-3746.

ISSFAL, 2010. International Society for the Study of Fatty Acids and Lipids. Report of the Sub-Committee on “Recommendations for intake of polyunsaturated fatty acids in healthy adults”, http://www.issfal.org.uk.

Jeong, J., E.G. Kwon, S.K. Im, K.S. Seo, and M. Baik. 2012. Expression of fat deposition and fat removal genes is associated with intramuscular fat content in *Longissimus dorsi* muscle of Korean cattle steers. J. Anim. Sci. 90:2044-2053.

Kim, J.Y., R.C. Hickner, R.L. Cortright, G.L. Dohm and J. Houmard. 2000. Lipid oxidation is reduced in obese human skeletal muscle. Am. J. Physiol. Endocrinol. Metab. 279:E1039-E1044.

Lee, Y.B. and R.G. Kauffman. 1974a. Cellularity and lipogenic enzyme activities of porcine intramuscular adipose tissue. J. Anim. Sci. 38:538-544.

Lee, Y. B., and R.G. Kauffman. 1974b. Cellular and enzymatic changes with animal growth in porcine intramuscular adipose tissue. J. Anim. Sci. 38:532-537.

Leseigneur-Meyner, A. and G. Gandemer. 1991. Lipid composition of pork muscle in relation to the metabolic type of the fibres. Meat Sci. 29:229-241.

Leung, T.T. and D.E. Bauman. 1975 In vivo studies of the site of fatty acid synthesis in the rabbit. Int. J. Biochem. 6:801-805.

Leyton, J., P.J. Drury and M.A. Crawford. 1987. Differential oxidation of saturated and unsaturated fatty acids in vivo in the rat. Br. J. Nutr. 57:383-393.

Mateescu, R.G., D.J. Garrick, A.J. Garmyn, D.L. Vanoverbeke, G.G. Mafi and J.M. Reecy. 2015. Genetic parameters for sensory traits in *Longissimus* muscle and their associations with tenderness, marbling score, and intramuscular fat in Angus cattle. J. Anim. Sci. 93:21–27.

Mateescu, R.G. 2015. Genetics of meat quality. The genetics of cattle. CAB International, Oxfordshire, UK. pp. 544-570.

Melo, V. and O. Cuamatzi 2010. Bioquímica de los procesos metabólicos. Reverté Ediciones, México.

Mermelstein, N.H. 2013. Instrumental grading of meat. Food Technology 67:67-69.

Mourot, J. and M. Kouba. 1998. Lipogenic enzyme activities in muscle of growing Large White and Meishang pigs. Livest. Prod. Sci. 55:127-133.

Mourot, J. and M. Kouba. 1999. Development of intra- and intermuscular adipose tissue in growing Large White and Meishang pigs. Reprod. Nutr. Dev. 39:125-132.

Nestel, P., P. Clifton and M. Noakes. 1994. Effects of increasing dietary palmitoleic acid compared with palmitic and oleic acids on plasma lipids of hypercholesterolemic men. J Lipid Res. 35:656-662.

Nishimura, T., A. Hattori and K. Takahashi. 1999. Structural changes in intramuscular connective tissue during the fattening of Japanese Black cattle: effect of marbling on beef tenderization. J. Anim. Sci. 77:93–104.

Ouhayoun, J., and F. Lebas. 1987. Composition chimique de la viande de lapin. Cuniculture. 73:33-35.

Pullen, D. L., J.S. Liesman and R.S. Emery. 1990. A species comparison of liver slice synthesis and secretion of triacylglycerol from nonesterified fatty acids in media. J. Anim. Sci. 68:1395-1399.

Raes, K., S. De Smet and D. Demeyer. 2001. Effect of double-muscling in Belgian Blue young bulls on the intramuscular fatty acid composition with emphasis on conjugated linoleic acid and. Anim. Sci. 73:253-260.

Ramírez, J.A., I. Díaz, M. Plá, M. Gil, A. Blasco and M.A. Oliver. 2005. Fatty acid composition of leg meat and perirenal fat of rabbits selected by growth rate. Food Chem. 90:251-256.

Ramírez, M.R., D. Morcuende and R. Cava. 2007. Fatty acid composition and adipogenic enzyme activity of muscle and adipose tissue, as affected by Iberian × Duroc pig genotype. Food Chem. 104:500–509.

Rødbotten, M., E. Kubberød, P. Lea and O. Ueland. 2004. A sensory map of the meat universe. Sensory profile of meat from 15 species. Meat Sci. 68:137–144.

Ros-Freixedes, R., J. Reixach, L. Bosch, M. Tor and J. Estany. 2013. Response to selection for decreased backfat thickness at restrained intramuscular fat content in Duroc pigs. J. Anim. Sci. 91:3514–3521.

Ruiz-Gutiérrez, V., F.J. Muriana, A. Guerrero, A.M. Cert and J. Villar. 1996. Plasma lipids, erythrocyte membrane lipids and blood pressure of hypertensive women

after ingestion of dietary oleic acid from two different sources. J Hypertens. 14:1483-1490.

Saap, R.L., J.K. Bertrand, T.D. Pringle and D.E. Wilson 2002. Effect of selection for ultrasound intramuscular fat percentage in Angus bulls on carcass traits of progeny. J. Anim. Sci. 80:2017-2022.

Schwab, C.R., T.J. Baas, K.J. Stalder and D. Nettleton. 2009. Results from six generations of selection for intramuscular fat in Duroc swine using real-time ultrasound. I. Direct and correlated phenotypic responses to selection. J. Anim. Sci. 87:2774-2780.

Schwab, C.R., T.J. Baas and K.J. Stalder. 2010. Results from six generations of selection for intramuscular fat in Duroc swine using real-time ultrasound. II. Genetic parameters and trends. J. Anim. Sci. 88:69-79.

Simopoulos, A.P. 2002. The importance of the ratio of omega-6:omega-3 essential fatty acids. Biomed. Pharmacother. 56:365−379.

Shrago, E., J.A. Glenon and E.S. Gordon E.S. 1971. Comparative aspects of lipogenesis in mammalian tissues. Metabolism, 20:54-62.

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

Warris, P.D. 2003. Calidad de la carne. Ciencia de la carne. Acribia, Zaragoza (España).

Wood, J.D., M. Enser, A.V. Fisher, G.R. Nute, P.R. Sheard, R.I. Richardson, S.I. Hughes, and F.M. Whittington. 2008. Fat deposition, fatty acid composition and meat quality: a review. Meat Sci. 78:343–58.

Wood, J.D., R.I. Richardson, G.R. Nute, A.V. Fisher, M.M. Campo, E. Kasapidou, P.R. Sheard, and M. Enser. 2004. Effects of fatty acids on meat quality: a review. Meat Sci. 66:21–32.

World Health Organization. 2008. Interim summary of conclusions and dietary recommendations on total fat and fatty acids. Joint FAO/WHO expert consultation on fats and fatty acids in human nutrition. pp. 10-14.

Young, M.E., P.H. Guthrie, P. Razeghi, B. Leighton, S. Abbasi, S. Patil, K.A. Youker and H. Teagtmeyer. 2002 Impaired long-chain fatty acid oxidation and contractile dysfunction in the obese Zucker rat heart. Diabetes 51:2587-2595.

Zechner, R., Zimmermannm R., Eichmann, T.O., Kohlwein, S.D., Haemmerle, G., Lass, A. and Madeo, F. 2012 Fat Signals- Lipases and lipolysis in lipid metabolism and signalling. Cell Metab. 15:279-291.

Zhao, G.P, J. Wen, J.L. Chen, M.Q. Zheng and X. Li. 2006. Selection response and estimation of the genetic parameters for intramuscular fat in a quality chicken line. Acta Vet. Zootech Sinica 37:870-873.

Zhao, G.P., J.L. Chen, M.Q. Zheng, J. Wen, and Y. Zhang. 2007. Correlated responses to selection for increased intramuscular fat in a Chinese quality chicken line. Poult. Sci. 86:2309–2314.

Zomeño, C., A. Blasco and P. Hernández. 2010. Influence of genetic line on lipid metabolism traits of rabbit muscle. J. Anim. Sci. 88:3419–3427.

Zomeño, C., P. Hernández and A. Blasco. 2013. Divergent selection for intramuscular fat content in rabbits. I. Direct response to selection. J. Anim. Sci. 91: 4526-4531.

Zomeño, C. 2013. Genetic analysis of intramuscular fat in rabbits. PhD Thesis. Universitat Politècnica de València.

OBJECTIVES

Intramuscular fat (IMF) is an important factor in meat quality. This trait can be improved by selection, although this can modify other important traits related with carcass and meat quality and lipid metabolism. This Thesis studies meat, carcass and lipid metabolism traits in two rabbit lines well differentiated for IMF by genetic selection.

The specific objectives of this Thesis are:

1. To evaluate the direct response to selection for IMF in *Longissimus dorsi* (LD) muscle and the correlated responses in carcass and meat quality traits. In particular we assessed the correlated response in the following traits:
    a. Carcass fatness, pH and carcass and meat color
    b. Fatty acid composition of LD and IMF and fatty acid composition of muscles with different metabolism
    c. Texture parameters and sensory attributes.

2. To estimate the genetic parameters of IMF and carcass and meat quality traits and corroborate them with the observed phenotypic responses to selection.

3. To study how selection for IMF of LD affects lipid metabolism of muscles having different metabolic profile, perirenal fat tissue and liver.

CHAPTER 1

Divergent selection on intramuscular fat in rabbits: responses to selection and genetic parameters

The content of this chapter has been published in *Journal of Animal Science*, *94*(12), 4993-5003.

ABSTRACT: A divergent selection experiment on intramuscular fat (IMF) was performed in rabbits. The aim of this study is to estimate the response to selection, the correlated responses in carcass and meat quality traits and their genetic parameters in the seventh generation of selection. Selection criterion was the averaged phenotypic value of IMF measured at 9 wk of age in 2 full sibs of the candidate. Traits considered were IMF, body weight, chilled carcass weight, reference carcass weight, scapular and perirenal fat weights, carcass and meat color, pH, protein and polyunsaturated (PUFA), monounsaturated (MUFA) and saturated (SFA) fatty acid percentages of meat. Total direct response to selection for IMF was 2.6 phenotypic standard deviations of the trait, around 5% of the mean (1.09 g/100g) per generation, both lines following a symmetrical trend. Heritability of IMF was high (0.54), and in general, all traits related to carcass fat depots and IMF fatty acid composition showed high heritabilities (dissectible fat of the carcass, 0.70; MUFA percentage 0.61; PUFA percentage 0.45; PUFA:SFA ratio 0.42), except SFA percentage (0.09). The other carcass and meat quality traits showed moderate to low heritabilities. Intramuscular fat and dissectible fat percentage showed a low genetic correlation (0.34). Intramuscular fat was genetically positively correlated with MUFA percentage (0.95) and negatively correlated with PUFA percentage (-0.89) and PUFA:SFA ratio (-0.98), corroborated with high correlated responses to selection. The rest of the traits did not show any substantial correlated response except protein content, which was greater in the high-IMF line than in the low-IMF line.

Keywords*:* genetic parameters, intramuscular fat, rabbits.

1. INTRODUCTION

Breeding goals in meat industry are now changing their focus towards meat quality traits to meet consumer expectations (Hermesch et al., 2000). Intramuscular fat (IMF) is a main goal because it has a large effect in the sensory properties of meat (Hocquette et al., 2010). Increasing IMF by selection improves meat quality, but can lead to unfavourable consequences in some carcass and meat quality traits, due to undesirable genetic correlations with IMF. For instance, in pigs, IMF and carcass fatness show a positive genetic correlation (Ciobanu et al., 2011), leading to an impairment of carcass quality when selecting for high IMF. Another example is the

negative genetic correlation between IMF and major polyunsaturated fatty acid percentages (reviewed by De Smet et al., 2004), leading to lower polyunsaturated to saturated fatty acids ratio when selecting for high IMF, which is against nutritional recommendations (World Health Organization, 2008).

Genetic parameters of IMF, particularly genetic correlations, are difficult to obtain because they need to be estimated with a large amount of data. Many genetic parameters of meat quality reported in literature are estimated with a low precision, without giving much information about their actual values (see, for example, Gjerlaug-Enger et al., 2010 in pigs or Buchanan et al., 2015 in cattle). However, when data come from a selection experiment, heritabilities and genetic correlations, although estimated with a limited precision, can be corroborated by the direct and correlated responses obtained. Selection experiments for IMF are scarce, and none of them have been performed in rabbits. Only Sapp et al. (2002) in cattle, Zhao et al. (2007) in chickens and Schwab et al. (2009) in pigs carried out selection for IMF.

Rabbit is a lean meat having a favourable fatty acid composition compared to beef, veal and pig meats (Dalle Zotte and Szendro, 2011). Besides, rabbit is a good model for genetic studies in other livestock species due to their short generation interval and low cost of the carcasses. A divergent selection experiment for IMF is being performed in rabbits (Zomeño et al., 2013). There are no published estimates of IMF heritability in rabbits, apart from that estimated by Zomeño et al. (2013) with the 3 first generations of this experiment, and there are no published genetic correlations among IMF and other meat and carcass quality traits. The aim of this study is to estimate the response to divergent selection for intramuscular fat in rabbits, the correlated responses in carcass and meat quality traits and their genetic parameters.

2. MATERIALS AND METHODS

2.1. Animals

A divergent selection experiment was performed during 7 generations. Animals came from a synthetic rabbit line formerly selected for ovulation rate for 10 generations (Laborda et al., 2011), and selection relaxed the following 2 generations. The base population consisted of 13 males and 83 females. Lines selected for high IMF

and low IMF had approximately 8 males and 40 females per generation. Two full sibs (male and female) of the first parity of each doe were slaughtered and their IMF was measured. All dams were then ranked according to the IMF values obtained by their offspring. The 20% best dams provided all females for the next generation. Each sire was mated with five dams, and one male of the best dam was selected for the next generation. This selection within male family was performed in order to reduce inbreeding. Normally, the first parity was used to collect the IMF data and the second parity to provide the rabbits for next generation, although exceptionally some IMF measurements were made on the second or third parity. A total of 2,365 rabbits were considered in the pedigree file, from which 1,337 were evaluated: 1,101 measurements were made in the first parity, 180 in the second and 56 in the third. A total of 154 rabbits from the seventh generation were used to study the correlated responses to selection in carcass and meat quality traits; 72 from high-IMF line and 82 from low-IMF line.

Rabbits were reared collectively from weaning to slaughter and were fed *ad libitum* with a commercial diet. Animals were slaughtered at 9 wk using electrical stunning and exsanguination. Before slaughter, the body weight (BW) was recorded. After slaughter, carcasses were chilled for 24h at 4ºC and the weight of the chilled carcass weight (CCW) was registered. Commercial rabbit carcass varies among countries; thus the World Rabbit Science Association (Toulouse, France) proposed to measure a reference carcass weight (the weight of the carcass without head, liver, lungs, thymes, esophagus, heart and kidneys) to allow comparisons between studies (Blasco et al., 1993; Blasco and Ouhayoun, 1996), thus reference carcass weight (RCW) was recorded. Scapular (SF) and perirenal (PF) fat depots were excised and weighed. The dissectible fat percentage (DF) was estimated as the sum of scapular and perirenal fat weights divided by RCW. The left leg was dissected to obtain the meat to bone ratio (M:B). Color parameters lightness (L*), redness (a*) and yellowness (b*) of the carcass were measured on the surface of the fourth lumbar vertebra, and color of the meat was measured at the seventh lumbar vertebra transversal section of *Longissimus dorsi*. Color euclidean distance Delta E (ΔE) was calculated (Sharma, 2002). Muscle pH was measured 24 hours *post mortem* in the *Longissimus dorsi* muscle at the level of the

fifth lumbar vertebra with a Crison pH-meter Basic +20 (Crison Instruments, Barcelona, Spain). Then, *Longissimus dorsi* muscle was excised, minced, freeze-dried and scanned with near infrared spectroscopy (a technique first proposed for rabbits by Masoero et al., 1992), to measure IMF, protein content and fatty acid composition applying the calibration equations previously developed by Zomeño et al. (2012). The potential use of this equation for animal breeding was evaluated by Zomeño et al. (2011). Intramuscular fat and protein content of *Longissimus dorsi* was expressed as g of IMF/100g of muscle on a fresh basis. Saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acid content was expressed as percentage of total fatty acids. The ratio between PUFA and SFA (PUFA:SFA) was calculated.

All experimental procedures involving animals were approved by the Universitat Politècnica de València Research Ethics Committee, according to Council Directives 98/58/EC (European Economic Community, 1998) and 2010/63/EU (European Commission Directive, 2010).

2.2. Statistical analysis

Descriptive statistics and phenotypic correlations between IMF and carcass and meat quality traits were estimated with data from all generations, after pre-correcting data by line-generation-season, parity order and sex fixed effects.

Direct and correlated responses to selection were calculated by two different ways. First, they were estimated as the phenotypic differences between high and low-IMF lines at the same generation of selection. Secondly, they were estimated as the genetic means of each line per generation.

Phenotypic differences between lines were estimated with the model

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Wc} + \mathbf{e}$$

Data were assumed to be conditionally distributed as

$$\mathbf{y} \mid \mathbf{b}, \mathbf{c}, \sigma_e^2 \sim \mathrm{N}\left(\mathbf{Xb} + \mathbf{Wc}, \mathbf{I}\sigma_e^2\right)$$

in which **b** is the vector with the fixed effects of line (high-IMF and low-IMF), month, sex and parity order; **c** is the vector of common litter random effect, σ_e^2 is the

residual variance, **X** and **W** are the known incidence matrices and **I** is an identity matrix. Common litter random effect was assumed to be distributed as

$$\mathbf{c} \sim \mathrm{N}\left(\mathbf{0}, \mathbf{I}\sigma_c^2\right)$$

In which σ_c^2 is the common litter variance.

Heritabilities, genetic correlations with IMF and genetic means per generation were estimated by fitting the following bivariate animal model, with the same effects for all traits:

$$\begin{bmatrix}\mathbf{y}_1\\ \mathbf{y}_2\end{bmatrix} = \begin{bmatrix}\mathbf{X}_1 & \mathbf{0}\\ \mathbf{0} & \mathbf{X}_2\end{bmatrix}\begin{bmatrix}\mathbf{b}_1\\ \mathbf{b}_2\end{bmatrix} + \begin{bmatrix}\mathbf{Z}_1 & \mathbf{0}\\ \mathbf{0} & \mathbf{Z}_2\end{bmatrix}\begin{bmatrix}\mathbf{u}_1\\ \mathbf{u}_2\end{bmatrix} + \begin{bmatrix}\mathbf{W}_1 & \mathbf{0}\\ \mathbf{0} & \mathbf{W}_2\end{bmatrix}\begin{bmatrix}\mathbf{c}_1\\ \mathbf{c}_2\end{bmatrix} + \begin{bmatrix}\mathbf{e}_1\\ \mathbf{e}_2\end{bmatrix}$$

Data were assumed to be conditionally distributed as

$$\begin{bmatrix}\boldsymbol{y_1}\\ \boldsymbol{y_2}\end{bmatrix} \Big| \ \mathbf{b_1}, \mathbf{b_2}, \mathbf{u_1}, \mathbf{u_2}, \mathbf{c_1}, \mathbf{c_2}, \mathbf{R} \sim \mathbf{N}\left(\begin{bmatrix}\mathbf{X} & 0\\ 0 & \mathbf{X}\end{bmatrix}\begin{bmatrix}\mathbf{b_1}\\ \mathbf{b_2}\end{bmatrix} + \begin{bmatrix}\mathbf{Z} & 0\\ 0 & \mathbf{Z}\end{bmatrix}\begin{bmatrix}\mathbf{u_1}\\ \mathbf{u_2}\end{bmatrix} + \begin{bmatrix}\mathbf{W} & 0\\ 0 & \mathbf{W}\end{bmatrix}\begin{bmatrix}\mathbf{c_1}\\ \mathbf{c_2}\end{bmatrix}, \mathbf{R}\right)$$

In which $\mathbf{b_1}$ and $\mathbf{b_2}$ are the vectors of fixed effects (month, sex and parity order); $\mathbf{u_1}$ and $\mathbf{u_2}$ are the vectors of additive genetic effects; $\mathbf{c_1}$ and $\mathbf{c_2}$ are the vectors of common litter effects; **X**, **Z** and **W** are known incidence matrices; and **R** is the residual co (variance) matrix between the two traits.

Sorting data by individuals, additive effects were distributed as

$$\mathbf{u} \sim \mathrm{N}\left(\mathbf{0}, \mathbf{A} \otimes \mathbf{G}_0\right)$$

common litter effects were distributed as

$$\mathbf{c} \sim \mathrm{N}\left(\mathbf{0}, \mathbf{I}_m \otimes \mathbf{C}_0\right)$$

and residuals were distributed as

$$\mathbf{e} \sim \mathrm{N}\left(\mathbf{0}, \mathbf{I}_n \otimes \mathbf{R}_0\right)$$

In which $\mathbf{G_0}$, $\mathbf{C_0}$ and $\mathbf{R_0}$ are the 2 x 2 genetic additive, common litter, and residual (co)variance matrices between the two traits; **A** is the relationship matrix, $\mathbf{I_m}$ is an identity matrix of the same order as the number of levels of common litter effects,

and $\mathbf{I}_n$ is an identity matrix of the same order as the number of individuals. All effects were assumed to be independent between them.

Bayesian inference was used, with bounded flat priors for all unknowns. Marginal posterior distributions were estimated using Gibbs sampling. Descriptive statistics and phenotypic differences between lines were computed with the programme Rabbit developed by the Institute for Animal Science and Technology (Valencia, Spain). After some exploratory analyses, results were based on Monte Carlo Markov chains (MCMC) consisting of 60,000 iterations, with a burn-in period of 10,000, and only 1 of every 10 samples were saved for inferences. Phenotypic correlations and genetic analyses were computed with the software TM (Legarra et al., 2008). In this case, after some exploratory analyses results were based on MCMC consisting of 1,000,000 iterations, with a burn-in period of 200,000; and only one of every 100 samples were saved for inferences. In all analyses, convergence was tested using the Z criterion of Geweke and Monte Carlo sampling errors were computed using time-series procedures (Sorensen and Gianola, 2002) included in Rabbit and TM programs. In all cases, Monte Carlo standard errors were small and lack of convergence was not detected by the Geweke test.

As Bayesian inference is based in probabilities, this gives a great flexibility to construct all kinds of confidence intervals. This allows asking questions that we could not ask within the classical inference approach. It may be important to know how big the phenotypic difference between lines for IMF is, with a chosen probability, for instance, 80%. Thus, we can calculate a guaranteed value; i.e. the minimum value k that the difference can take with 80% probability. This is the limit of the interval [k, $+\infty$) with 80% probability. To help in the discussion, we can also calculate the probability of the phenotypic difference between lines being greater than a relevant value. This relevant value is the minimum amount having economical or biological significance; it is normally used in experimental design as the difference to be detected when calculating sample sizes. For some traits, it may be difficult to assess the economical or biological importance for a relevant value; in these cases it can be taken as relevant around 10% of the phenotypic variance of the trait, or one third of the phenotypic standard deviation.

The parameters obtained from the marginal posterior distributions of the phenotypic differences between lines were: the median of the difference (D), the standard deviation, the highest posterior density region at 95% ($HPD_{95\%}$), the probability of the difference being greater than zero when D > 0 or lower than zero when D < 0 (P_0), and the guaranteed value of the difference with a probability of 80%, i.e. the limit of the interval [k, +∞) when D > 0 or the limit of the interval (-∞, k] when D < 0. We considered 1/3 of the phenotypic standard deviation of a trait as a relevant value (r), and we also calculated the probability of relevance (probability of the difference being greater than r when D > 0 or lower than r when D < 0) (P_r).

Regarding the genetic analyses, we calculated the median and the standard deviation of the marginal posterior distributions of genetic means in each generation. For heritabilities, we estimated the median of each marginal posterior distribution, the $HPD_{95\%}$, and the guaranteed value with probability of 80% or 95%; i. e. limit of the interval [k, 1] with 80% or 95% probability. For genetic and phenotypic correlations, we estimated the median of each marginal posterior distribution, the $HPD_{95\%}$ and the probability of being greater than 0 when the median is positive or lower than 0 when the median is negative (P_0). A more detailed description of these features can be found in Blasco (2005).

3. RESULTS AND DISCUSSION

3.1. Descriptive statistics of the traits

Table 1 shows descriptive statistics of carcass and meat quality traits. Descriptive statistics for carcass and meat quality traits are in line with those reported by Hernández et al. (2004). Coefficient of variation of yellowness (b*) is not reported because it is not defined, since b* takes positive and negative values.

Scapular and perirenal fat depots are the two main carcass fat depots in rabbits and, on average, account for 65% of carcass dissectible fat (Hernández et al., 2006). Carcass fat percentage of rabbits was very low in comparison with the fat percentage of pig and beef carcasses (Lawrie and Ledward, 2006). Rabbit meat showed a greater percentage of PUFA and a greater PUFA:SFA ratio in comparison with pig, cattle and sheep (Dalle Zotte, 2002; Dalle Zotte and Szendro, 2011). The PUFA:SFA ratio of rabbit

meat was greater than 0.60, in line with the nutritional recommendations for adults reported by the World Health Organization (2008).

Table 1. Descriptive statistics of carcass and meat quality traits.

Trait[1]	n	Mean	SD	CV x 100
Carcass quality traits				
BW, g	1,337	1717	127	7.42
CCW, g	1,335	978	83	8.50
RCW, g	1,336	774	71	9.15
SF, g	1,325	3.92	1.12	28.6
PF, g	1,333	8.44	2.98	35.3
DF, %	1,320	1.57	0.39	24.8
M:B ratio	954	4.44	0.49	10.9
L*	1,336	54.5	2.02	3.71
a*	1,327	3.27	0.86	26.3
b*	1,303	1.26	1.31	NA
Meat quality traits				
IMF, g/100g	1,337	1.09	0.15	13.9
Protein, g/100g	1,304	22.0	0.43	1.96
pH	1,329	5.57	0.09	1.58
L*	1,336	53.7	2.27	4.22
a*	1,323	3.60	0.97	27.1
b*	1,335	1.78	0.72	NA
SFA, %	1,298	36.3	1.85	5.10
MUFA, %	1,298	23.3	2.49	10.7
PUFA, %	1,298	39.9	3.71	9.29
PUFA:SFA ratio	1,297	1.10	0.11	9.66

[1]CCW = chilled carcass weight; RCW = reference carcass weight; SF = scapular fat weight; PF = perirrenal fat weight; DF = dissectible fat percentage; M:B ratio = meat to bone ratio of the hind leg; L* = lightness, a* = redness, b* = yellowness measured on the carcass surface (carcass quality traits) or in *Longissimus dorsi* muscle (meat quality traits); IMF = intramuscular fat; Saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids are expressed as % of total fatty acids; NA, not applicable since b* has positive and negative values.

3.2. Direct response to selection for IMF

In our experiment, direct and correlated responses to selection were estimated using two independent criteria: the observed phenotypic differences between lines

and the estimated genetic means of each line per generation. In divergent selection experiments, assuming that environment affects both lines alike, the genetic progress is the phenotypic difference between lines at the same generation, which is independent on any genetic model. However, to assess whether genetic response is symmetrical or not we need the estimates of the genetic trends, that are model dependent (Sorensen and Johansson, 1992); i.e., their results depend on the genetic parameters used to estimate them. When the phenotypic observed difference is coincident with the difference observed using genetic trends, this corroborates the model used in the estimation of the genetic values. It is, therefore, interesting to have independent criteria to evaluate the direct and correlated responses. For all the traits, differences between genetic means of the lines were coherent with the phenotypic differences. This corroborates the model used for the genetic analyses.

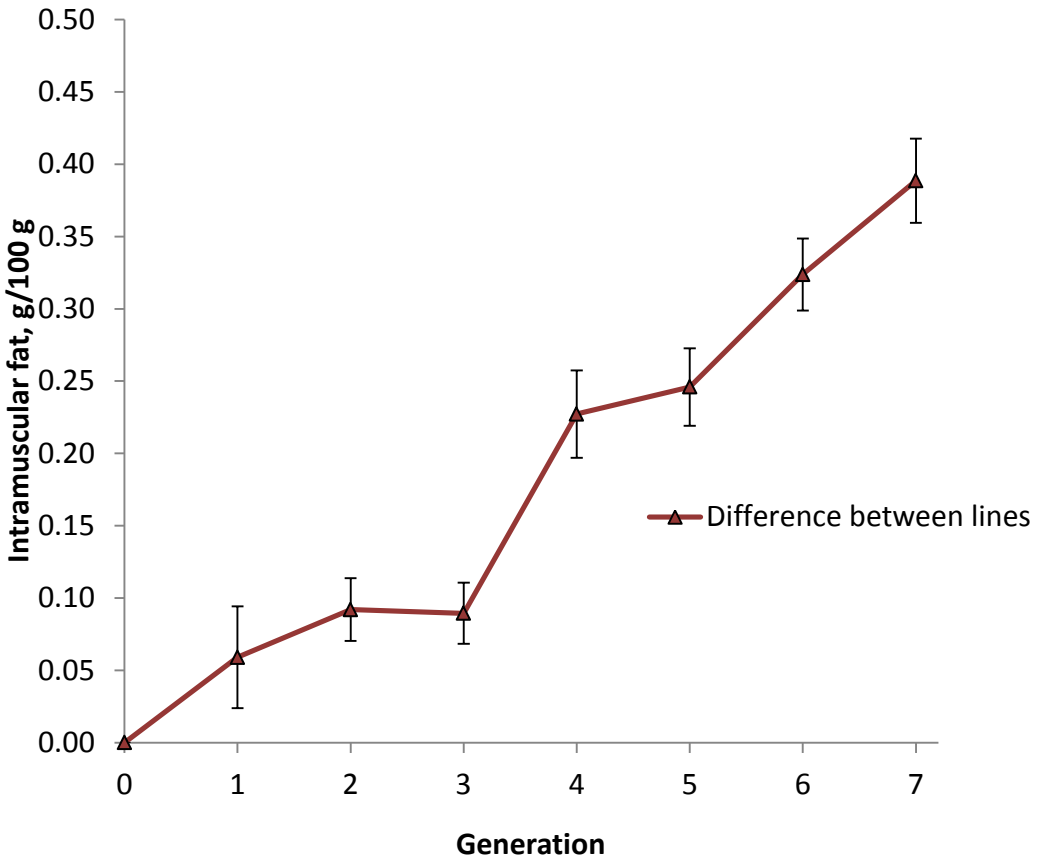

Figure 1. Medians and SD of the estimated marginal posterior distributions of the phenotypic differences for intramuscular fat (IMF) between the high-IMF line and the low-IMF line.

Figure 1 shows the phenotypic differences between lines for IMF in each generation. Comparisons between lines should be done at the same stage of maturity, and our lines were approximately at the same stage (Pascual et al., 2015). Phenotypic difference between lines in the seventh generation was 0.39 g/100g, around 2.6 standard deviations of the trait and a 5% of the mean per generation. The guaranteed value of the difference with 80% of probability was 0.36 g/100g.

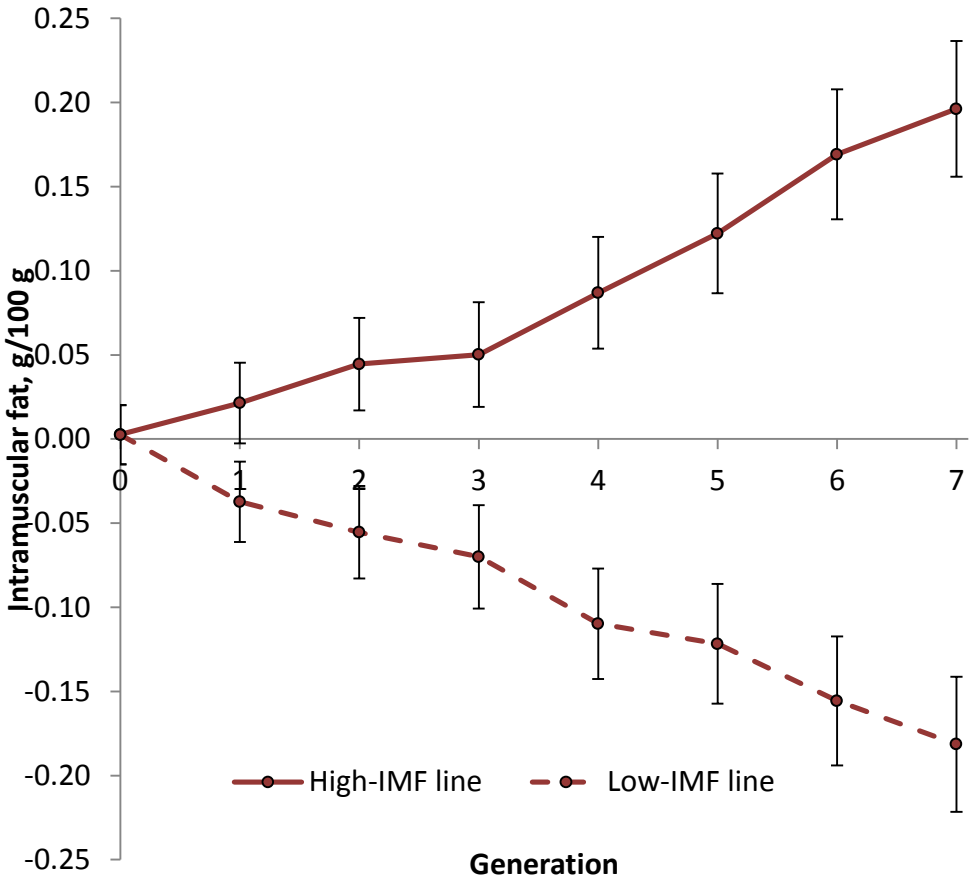

Figure 2. Medians and SD of the marginal posterior distributions of the estimated genetic means for intramuscular fat (IMF) per generation.

Figure 2 shows the genetic means of each line per generation. Direct response to selection was symmetrical for both lines. In the 7th generation, direct response was 0.20 g/100g in high-IMF and -0.18 g/100g in low-IMF line, which is 1.3 and -1.2 SD of the trait in high and low-IMF lines, respectively. Environmental trends were the same in both lines and did not show any particular pattern. Similar genetic trends were observed when lines were analysed separately. Currently, there is no other experiment

of selection for IMF in rabbits, and in other species they are scarce (Sapp et al., 2002 in cattle; Zhao et al., 2007 in chickens and Schwab et al., 2009 in pigs), all of them showing high responses to selection.

3.3. Correlated responses in carcass quality traits

Table 2 shows the correlated responses for carcass and meat quality traits estimated as the phenotypic differences between lines in the seventh generation.

Table 2. Features of the marginal posterior distributions of the differences between lines for carcass and meat quality traits in the seventh generation of selection.

Trait[1]	D[2]	$HPD_{95\%}$[3]	P_0[4]	r[5]	P_r[6]
Carcass quality traits					
RCW, g	27.9	2.30, 52.3	0.98	23.7	0.63
SF, g	0.28	-0.05, 0.60	0.96	0.37	0.30
PF, g	3.32	2.51, 4.14	1.00	0.99	1.00
DF, %	0.43	0.31, 0.55	1.00	0.13	1.00
M:B ratio	0.23	0.04, 0.42	0.99	0.16	0.76
L*	-0.54	-1.31, 0.22	0.92	0.67	0.37
a*	-0.39	-0.76, 0.01	0.98	0.29	0.71
b*	0.18	-0.36, 0.69	0.76	0.44	0.16
Meat quality traits					
IMF, g/100g	0.39	0.33, 0.44	1.00	0.05	1.00
Protein, g/100g	0.38	0.24, 0.53	1.00	0.14	1.00
pH	0.01	-0.02, 0.05	0.82	0.03	0.15
L*	-0.33	-1.48, 0.77	0.72	0.76	0.21
a*	-0.21	-0.60, 0.20	0.85	0.32	0.29
b*	-0.22	-0.57, 0.14	0.88	0.24	0.44
SFA, %	-0.12	-0.87, 0.60	0.61	0.62	0.09
MUFA, %	7.49	6.56, 8.49	1.00	0.83	1.00
PUFA, %	-10.1	-11.5, -8.61	1.00	1.24	1.00
PUFA:SFA ratio	-0.26	-0.30, -0.22	1.00	0.04	1.00

[1]RCW = reference carcass weight; SF = scapular fat weight; PF = perirenal fat weight; DF = dissectible fat percentage; M:B = meat to bone ratio of the hind leg; L* = lightness, a* = redness, b* = yellowness measured on the carcass surface (carcass quality traits) or in *Longissimus dorsi* muscle (meat quality traits); IMF = intramuscular fat; Saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids are expressed as % of total fatty acids; [2]D = the median of the difference (median of the marginal posterior distribution of the difference between the high-IMF line and

the low-IMF line); [3]$HPD_{95\%}$ = highest posterior density region at 95% of probability; [4]P_0 = probability of the difference being greater than zero when D > 0 or lower than zero when D < 0. [5]r = relevant value, proposed as 1/3 of the standard deviation of the trait; [6]P_r = probability of relevance (probability of the difference being greater than r when D > 0 or lower than r when D < 0.

Selection for IMF showed a positive correlated response on carcass fat depots. Perirenal fat weight was greater in high than in low-IMF line (P_0 = 1.00), and this difference was relevant (P_r = 1.00), although considering the low dissectible fat percentage in rabbit, this has no economic importance. Scapular fat was also greater in high-IMF than in low-IMF line, although the difference between lines was not relevant (P_r = 0.30).

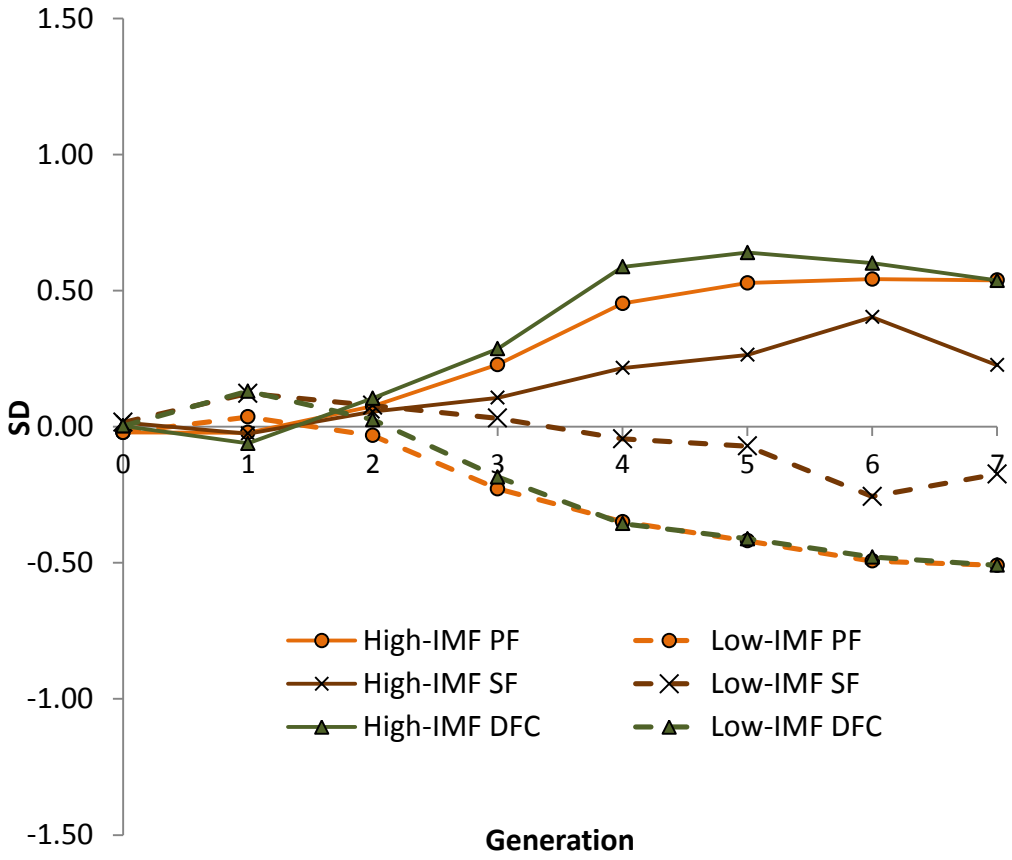

Figure 3. Genetic trends for perirenal fat weight (PF), scapular fat weight (SF) and dissectible fat percentage (DF). High-IMF line = the high-intramuscular fat line; Low-IMF line = the low-intramuscular fat line. Traits are expressed in units of SD of the trait.

Figure 3 shows the genetic trends for perirenal and scapular fat weights and dissectible fat percentage. To facilitate comparisons, genetic trends of correlated traits are expressed in SD units. Genetic response in both lines was symmetrical and, in the seventh generation, correlated response was ± 0.5 SD of perirenal fat weight and dissectible fat percentage. Other experiments of selection for IMF also showed positive correlated responses in the fat content of the carcass (Schwab et al., 2009 in pigs and Zhao et al., 2007 in chickens). A selection experiment for low backfat thickness restraining IMF was performed in pigs for one generation (Ros-Freixedes et al., 2013), reducing backfat thickness with a slight reduction in IMF.

Reference carcass weight was slightly greater in high-IMF than in low-IMF line, but the phenotypic difference between lines was not relevant (P_r = 0.63, Table 2). Other experiments of selection for IMF do not show any consistent pattern in the correlated response on carcass weight: Zhao et al. (2007) showed a significant increment in chickens, whereas Sapp et al. (2002) in cattle and Schwab et al. (2009) in pigs did not obtain any correlated response.

Meat to bone ratio of the hind leg is an estimator of the meat to bone ratio of the whole carcass in rabbits (Hernández et al., 1996). In the seventh generation, high-IMF line showed greater meat to bone ratio of the hind leg (P_0 = 0.99, Table 2) than low-IMF line but the phenotypic difference cannot be considered as relevant (P_r = 0.76).

Rabbit meat is usually commercialized as a whole carcass and to a less extent as retail cuts, thus carcass color is particularly important for consumers. Selection for IMF produced slight modifications in the color parameters of the carcass in the seventh generation (Table 2): low-IMF line showed greater lightness (L*) and redness (a*) than high-IMF line, although phenotypic differences were not relevant, whereas yellowness (b*) of the carcass was not affected by selection. Differences in L*, a* and b* color parameters have a difficult interpretation. To help in the discussion, color distance ΔE was calculated. Lines showed ΔE distance in carcass color of 0.80. Considering a ΔE of 2.3 noticeable for the human eye (Sharma, 2002), selection for IMF did not lead to noticeable changes in carcass color. Color of the carcass was not analyzed in other experiments of selection for IMF in other species. Genetic trends for reference carcass

weight, meat to bone ratio and carcass color parameters did not show any clear pattern along the experiment and are not reported.

3.4. Correlated responses in meat quality traits

A side effect of increasing IMF is the decline of PUFA percentage (De Smet et al., 2004; Sellier et al., 2010, Wood et al., 2008). This leads to unfavourable changes in the PUFA:SFA ratio that is an indicator of nutritional quality of meat. In the seventh generation of our experiment, low-IMF line showed relevant greater percentages of PUFA than high-IMF line (P_r = 1.00, Table 2) whereas SFA percentage was similar in both lines. Consequently, low-IMF line showed 0.26 greater PUFA:SFA ratio than high-IMF line (P_r = 1.00); however, PUFA:SFA ratio of both lines is still above the minimum nutritional recommendations (> 0.60, according to the World Health Organization, 2008). Regarding MUFA percentage, it was relevantly greater in high-IMF than in low-IMF line (P_r = 1.00, Table 2). Expressing the correlated responses in percentages leads to a decrease in PUFA in the high-IMF line, due to the faster increase of the other fatty acid groups (SFA and MUFA); however, it should be noticed that the amount in absolute terms of all fatty acid groups (PUFA, MUFA and SFA) was greater in high-IMF than in low-IMF line, since high-IMF line has more IMF. The differences between high and low-IMF lines were 58.8 g/100g of muscle for PUFA, 111 g/100g for MUFA and 106 g/100g for SFA in the seventh generation.

Figure 4 shows genetic trends for fatty acids profile of IMF. Percentages of MUFA and PUFA showed great responses to selection and their genetic trends were similar as those of IMF. In the seventh generation, correlated response in MUFA percentage was 1.5 SD in high-IMF and -1.3 SD in low-IMF line, and correlated response in PUFA percentage was -1.1 SD in high-IMF and 1.4 SD in low-IMF line. Percentage of SFA did not respond to selection. Correlated responses to selection for IMF in fatty acid profile of meat were estimated in the selection experiment for IMF in pigs with similar results (Burkett, 2009). Some studies in rabbit compared the fatty acid composition in several genetic lines differing in their IMF, but they did not show any common pattern (Gašperlin et al., 2006; Polak et al., 2006; Hernández et al., 2008).

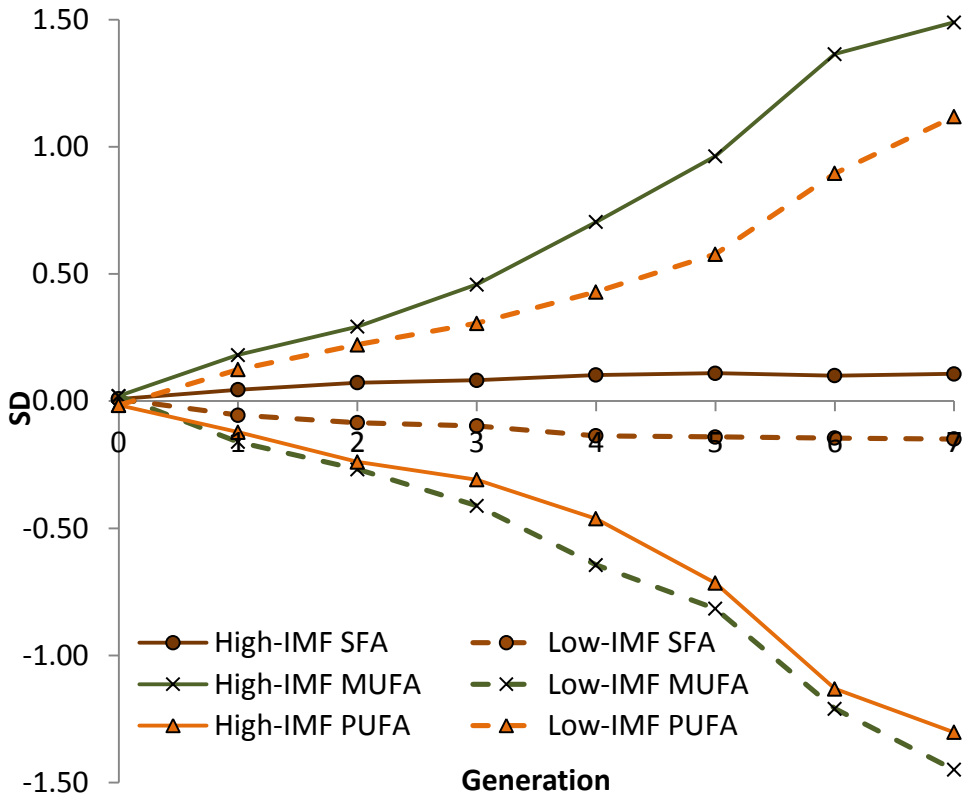

Figure 4. Genetic trends for saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acid percentages. High-IMF line = the high-intramuscular fat line; Low-IMF line = the low-intramuscular fat line. Traits are expressed in units of SD of the trait.

In the seventh generation, protein content was relevantly greater in the high-IMF than in low-IMF line (P_r = 1.00, Table 2). Genetic trends for protein content are reported in Figure 5. Protein content was modified by selection for IMF, increasing in the high-IMF and decreasing in the low-IMF line. Previous selection experiments for IMF did not measured protein content in any species.

The post mortem evolution of pH was not affected by selection, in agreement with the selection experiment for IMF in pigs (Schwab et al., 2009). Meat color was slightly affected by selection for IMF, as observed in the carcass. In the seventh generation of selection, meat redness (a*) and yellowness (b*) were greater in low-IMF than in high-IMF line but differences between lines were irrelevant, and lightness was similar in both lines. These differences between lines were not detectable for the human eye, according to Sharma (2002), since ΔE color distance between lines was

0.62. In other selection experiments for high IMF, lightness of the meat increased (Schwab et al., 2009 in pigs); however, when they measured the color of fresh meat by a sensory panel, they did not detect any effect of selection, in agreement to our results. In the experiment in chickens (Zhao et al., 2007), meat color parameters (L*, a* and b*) were not affected by selection for IMF. Genetic trends for pH and meat color parameters did not show a clear pattern and are not reported.

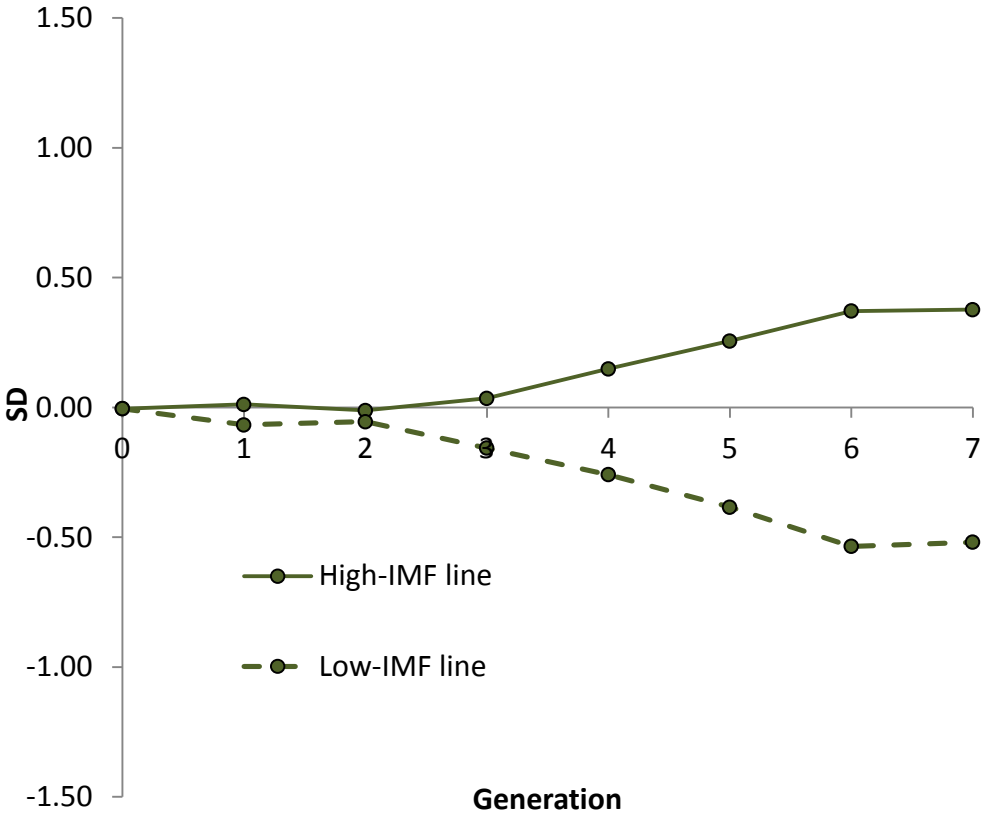

Figure 5. Genetic trends for meat protein content. High-IMF line = the high-intramuscular fat line; Low-IMF line = the low-intramuscular fat line. Traits are expressed in units of SD of the trait.

3.5. Heritabilities of the traits

Table 3 shows the heritabilities (h^2) of carcass and meat quality traits. The h^2 of IMF was high (0.54), showing a guaranteed value of 0.47 with 80% probability, or 0.40 with 95% probability (Table 3). No other estimates of IMF heritability have been published in rabbits, apart from that estimated by Zomeño et al. (2013) with the 3 first generations of this experiment (0.37). In other species, there is a large range of IMF

heritabilities (from 0.26 to 0.86 reviewed by Ciobanu et al., 2011 in pigs and from 0.34 to 0.77 reviewed by Mateescu, 2015 in cattle).

Table 3. Heritabilities of carcass and meat quality traits.

Trait[1]	Median[2]	$HPD_{95\%}$[3]	$k_{80\%}$[4]	$k_{95\%}$[5]
Carcass quality traits				
RCW	0.18	0.04, 0.35	0.11	0.06
SF	0.32	0.14, 0.53	0.24	0.17
PF	0.54	0.33, 0.74	0.46	0.38
DF	0.70	0.51, 0.90	0.61	0.53
M:B ratio	0.10	0.01, 0.26	0.05	0.02
L*	0.11	0.01, 0.24	0.05	0.03
a*	0.37	0.19, 0.56	0.29	0.22
b*	0.06	0.00, 0.16	0.03	0.01
Meat quality traits				
IMF	0.54	0.37, 0.71	0.47	0.40
Protein	0.25	0.12, 0.42	0.19	0.14
pH	0.08	0.01, 0.20	0.05	0.03
L*	0.19	0.04, 0.35	0.12	0.07
a*	0.25	0.10, 0.43	0.18	0.13
b*	0.14	0.02, 0.29	0.09	0.05
SFA	0.09	0.01, 0.21	0.05	0.02
MUFA	0.61	0.45, 0.77	0.54	0.48
PUFA	0.45	0.31, 0.63	0.39	0.33
PUFA:SFA ratio	0.42	0.26, 0.58	0.35	0.29

[1]RCW = reference carcass weight; SF = scapular fat weight; PF = perirenal fat weight; DF = dissectible fat percentage; M:B = meat to bone ratio of the hind leg; L* = lightness, a* = redness, b* = yellowness measured on the carcass surface (carcass quality traits) or in *Longissimus dorsi* muscle (meat quality traits); IMF = intramuscular fat; Saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids are expressed as % of total fatty acids; [2]Median = median of the marginal posterior distribution of the heritability; [3]$HPD_{95\%}$ = highest posterior density region at 95% of probability; [4]$k_{80\%}$ = limit of the interval [k, 1] at 80% of probability; [5]$k_{95\%}$ = limit of the interval [k, 1] at 95% of probability.

In general, all traits related to carcass fat depots presented high h^2 (Table 3), with scapular fat weight showing the lowest estimate. These results are in agreement with previous reports in rabbits (Larzul et al., 2005; Larzul and Rochambeau, 2005 and

Garreau et al., 2008). Regarding IMF fatty acid composition, MUFA and PUFA percentages and PUFA:SFA ratio showed high h^2, whereas SFA percentage showed a low h^2, close to zero. We can corroborate these results with the positive correlated responses in PUFA and MUFA percentages and PUFA:SFA ratio, and a lack of correlated response in SFA percentage (Table 2). There are no previous estimates of fatty acids h^2 in rabbits. In other species, Ros-Freixedes et al. (2014) and Ibáñez-Escriche et al. (2016) in pigs and Nogi et al. (2011) in cattle, showed high h^2 for MUFA, PUFA and also for SFA percentages.

Protein content had a moderate heritability, in agreement with the study of Al-Saef et al., 2008 in rabbits. Color parameters showed low heritabilies, with the exception of carcass and meat a* that had a high to moderate h^2 (Table 3), in line with the previous values reported in rabbits by Larzul and Rochambeau, (2005). The other carcass and meat quality traits showed a low h^2, in agreement with the estimates reported by Hernández and Gondret, (2006) in a rabbit review.

3.6. Correlations between IMF and carcass and meat quality traits

Table 4 gives the phenotypic and genetic correlations between IMF and carcass and meat quality traits. No previous genetic correlations among IMF and carcass and meat quality traits are reported in rabbits. Our results show that, in general, genetic (r_g) and phenotypic (r_p) correlations were of the same order (Table 4).

The relationships between IMF and the fatty acid composition of meat has interest because both affect sensory and nutritional properties of meat (Wood et al., 2004). In our study, strong genetic and phenotypic correlations were found between IMF and MUFA and PUFA percentages and between IMF and PUFA:SFA ratio. Intramuscular fat was positively correlated with MUFA percentage (r_g = 0.95 and r_p = 0.88) and negatively correlated with PUFA percentage and PUFA:SFA ratio, both genetically (-0.89 and -0.98) and phenotypically (-0.74 and -0.76). These genetic correlations were estimated accurately, as shown by their narrow $HPD_{95\%}$ (Table 4). The great correlated responses to selection on these traits (Table 2) corroborate the strong genetic correlations between these traits and IMF. In contrast, genetic correlation between IMF and SFA percentage had a wide $HPD_{95\%}$, providing scarce information about the actual value of the parameter, but we can state that it was

positive with a high probability (P_0 = 0.95, Table 4). Phenotypic correlation was also positive but low (Table 4). The possibility of increasing IMF independently of SFA percentage is beneficial from a nutritional point of view, since it is recommended to reduce the consumption of SFA (World Health Organization, 2008). To our knowledge, no genetic correlations between IMF and fatty acids have been published in rabbits, although they have been studied in other species, for example in pigs (Ibáñez-Escriche et al., 2016) and beef (Buchanan et al., 2015). Our results showed stronger correlations between IMF and fatty acids than the correlations reported in these studies, except for SFA percentage.

Table 4. Phenotypic and genetic correlations between intramuscular fat and carcass and meat quality traits.

Trait[1]	Phenotypic correlation			Genetic correlation		
	Median[2]	$HPD_{95\%}$[3]	P_0[4]	Median[2]	$HPD_{95\%}$[3]	P_0[4]
Carcass quality traits						
RCW	0.26	0.21, 0.31	1.00	0.27	-0.11, 0.63	0.91
SF	0.29	0.24, 0.34	1.00	0.37	0.04, 0.64	0.98
PF	0.35	0.30, 0.39	1.00	0.33	0.05, 0.59	0.98
DF	0.34	0.29, 0.39	1.00	0.34	0.08, 0.60	0.99
M:B ratio	0.17	0.10, 0.23	1.00	0.38	-0.06, 0.93	0.93
Meat quality traits						
Protein	0.32	0.27, 0.37	1.00	0.43	0.10, 0.76	0.99
pH	-0.01	-0.06, 0.05	0.61	0.29	-0.17, 0.71	0.88
SFA	0.12	0.07, 0.18	1.00	0.37	-0.08, 0.88	0.95
MUFA	0.88	0.86, 0.89	1.00	0.95	0.90, 0.99	1.00
PUFA	-0.74	-0.76, -0.71	1.00	-0.89	-0.98, -0.78	1.00
PUFA:SFA ratio	-0.76	-0.79, -0.74	1.00	-0.98	-1.00, -0.91	1.00

[1]RCW = reference carcass weight; SF = scapular fat weight; PF = perirenal fat weight; DF = dissectible fat percentage; M:B = meat to bone ratio of the hind leg; Saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids are expressed as % of total fatty acids; [2]Median = median of the marginal posterior distribution of the correlation.[3]$HPD_{95\%}$ = highest posterior density region at 95% probability.[4]P_0 = probability of the correlation being greater than 0 when positive or lower than 0 when negative.

Improving the eating quality of meat without impairing carcass quality is a critical point in meat industry due to the positive genetic correlation between IMF and

carcass fat content (Ciobanu et al., 2011). In our experiment, we can state that genetic correlations among IMF and carcass fat content were also positive (P > 0.98), but the estimate was inaccurate, showing wide $HPD_{95\%}$ (Table 4). However, in Bayesian analysis we can calculate the maximum value that r_g can take with 80% probability, and in our case this value was 0.46 for the genetic correlation between IMF and dissectible fat percentage, showing that IMF and carcass fat should have a moderate to low genetic correlation. Phenotypic correlations between IMF and carcass fat content were also positive (P_0 = 1.00), with estimates ranging from 0.29 to 0.35 (Table 4).

Genetic and phenotypic correlations between IMF and protein content were positive (P_0 = 0.99 and P_0 = 1.00, Table 4), with medians of 0.43 and 0.32, respectively. Although this genetic correlation shows a wide $HPD_{95\%}$, the actual value of this parameter should be substantial, since high-IMF line showed a relevant higher content of protein than low-IMF line (Table 2). Our correlations did not agree with those estimated by Gjerlaug-Enger et al. (2010) in pigs, which showed negative genetic and phenotypic correlations.

Our estimates of genetic correlations between IMF and reference carcass weight, meat to bone ratio and pH were inaccurate; nevertheless, we can state that they were positive, with probabilities of 0.91, 0.93 and 0.88, respectively. The phenotypic correlations between IMF and reference carcass weight and meat to bone ratio were low but positive, whereas phenotypic correlation between IMF and pH was close to 0 (Table 4). Due to the inaccuracy of the estimates, we cannot make statements about the genetic correlations between IMF and color parameters, and they are not reported. The phenotypic correlations between IMF and color parameters were low or close to 0, ranging from -0.09 to 0.03.

4. CONCLUSIONS

Divergent selection for IMF led to a difference between high and low-IMF lines of 2.6 standard deviations of the trait, both lines following a symmetrical trend. Heritability of IMF was high, and in general, all traits related to carcass fat depots and IMF fatty acid composition showed high heritabilities, except for SFA percentage. High-IMF line showed greater carcass dissectible fat percentage than low-IMF line, although

the genetic correlation between both traits was low. Strong genetic correlations were estimated between IMF and fatty acid percentages, positive for MUFA and negative for PUFA, corroborated with high correlated responses to selection, high-IMF line showing lower PUFA and greater MUFA percentages than low-IMF line. Protein content was greater in the high than in low-IMF line, whereas the remaining traits did not show any substantial change due to selection.

5. LITERATURE CITED

Al-Saef, A.M., M.H. Khalil, S.N. Al-Dobaib, A.H. Al-Homidan, M.L. García and M. Baselga. 2008. Comparing Saudi synthetic lines of rabbits with the founder breeds for carcass, lean composition and meat quality traits. Livest. Res. Rural Dev. 20.

Blasco, A., J. Ouhayoun and G. Masoero. 1993. Harmonization of criteria and terminology in rabbit meat research. World Rabbit Sci. 1: 3-10.

Blasco, A. and J. Ouhayoun. 1996. Harmonization of Criteria and Terminology in Rabbit Meat Research. Revised Proposal. World Rabbit Sci. 4:93–99.

Blasco, A. 2005. The use of Bayesian statistics in meat quality analyses: A review. Meat Sci. 69:115-122.

Buchanan, J.W., J.M. Reecy, D.J. Garrick, Q. Duan, D.C. Beitz and R.G. Mateescu. 2015. Genetic parameters and genetic correlations among triacylglycerol and phospholipid fractions in Angus cattle. J. Anim. Sci. 93:522–528.

Burkett, J.L. 2009. The effect of selection for intramuscular fat on fatty acid composition in Duroc pigs. Graduate Theses and Dissertations. Paper 10539.

Ciobanu, D.C., S.M. Lonergan and E.J. Huff- Lonergan. 2011. Genetics of meat quality and carcass traits. In: M.F. Rothschild and A. Ruvinsky. The genetics of the pig. CAB Int. Oxfordshire (UK).pp. 355-389.

Dalle Zotte, A. 2002. Perception of rabbit meat quality and major factors influencing the rabbit carcass and meat quality. Livest. Prod. Sci. 75:11–32.

Dalle Zotte, A. and Z. Szëndro. 2011. The role of rabbit meat as functional food. Meat Sci. 88.319-331.

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

European Economic Community. 1998. Council Directive 98/58/EC of 20 July 1998 concerning the protection of animals kept for farming purposes. Official Journal L, 221(8), 8.

European Commission Directive. 2010. Council, E.P.A.E. 2010/63/EU on the protection of animals used for scientific purposes. Institute for Health and Consumer Protection, Ispra, Italy, b7.

Garreau, H., S.J. Eady, J. Hurtaud and A. Legarra. 2008. Genetic parameters of production traits and resistance to digestive disorders in a commercial rabbit population. In: Xiccato, G. Trocino, A. Lukefahr, S. (eds.) Proceedings of the 9th World Rabbit Congress. Fondazione Iniziative Zooprofilattiche e Zootechniche, Verona, Italy, pp. 103-108.

Gašperlin, L., T. Polak, A. Rajar, M. Skvarèa and B. Lender. 2006. Effect of genotype, age at slaughter and sex on chemical composition and sensory profile of rabbit meat. World Rabbit Sci. 14:157–166.

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

Hernández, P., M. Plá and A. Blasco. 1996. Prediction of carcass composition in the rabbit. Meat Sci. 44: 75-83.

Hernández, P., S. Aliaga, M. Pla, and A. Blasco. 2004. The effect of selection for growth rate and slaughter age on carcass composition and meat quality traits in rabbits. J. Anim. Sci. 82:3138–3143.

Hernández, P. and F. Gondret. 2006. Rabbit meat quality and safety. In: L.Maertens and P. Coudert, editors. Recent avances in rabbit sciences. ILVO, Melle, Belgium P267-290.

Hernández, P., B. Ariño, A. Grimal and A. Blasco. 2006. Comparison of carcass and meat characteristics of three rabbit lines selected for litter size or growth rate. Meat Sci. 73: 645-650.

Hernández, P., V. Cesari and A. Blasco. 2008. Effect of genetic rabbit lines on lipid content, lipolytic activities and fatty acid composition of hind leg meat and perirenal fat. Meat Sci. 78:485–491.

Hermesch, S., B.G. Luxford and H.U. 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. Livest. Prod. Sci. 65:249–259.

Hocquette, J.F., F. Gondret, E. Baéza, F. Médale, C. Jurie and D.W. Pethick. 2010. Intramuscular fat content in meat-producing animals: development, genetic and nutritional control, and identification of putative markers. Animal 4:303-319.

Ibáñez-Escriche, N., E. Magallón, E. González, J.F. Tejeda, and J.L. Noguera. 2016. Genetic parameters and crossbreeding effects of fat deposition and fatty acid profiles in Iberian pig lines. J. Anim. Sci. 94:28-37.

Laborda, P., M.L. Mocé, M.A. Santacreu and A. Blasco. 2011. Selection for ovulation rate in rabbits: Genetic parameters, direct response, and correlated response on litter size. J. Anim. Sci. 89:2981-2987.

Larzul, C., Gondret, F. Combes, S. de Rochambeau, H. 2005. Divergent selection on 63-day body weight in the rabbit: response on growth, carcass and muscle traits. Genet. Sel. Evol. 37:105-122.

Larzul, C., and Rochambeau, H. 2005. Selection for residual feed consumption in the rabbit. Livest. Prod. Sci. 95: 67-72.

Lawrie, R.A., and D.A. Ledward. 2006. The structure and growth of muscle. In: Meat Science. 7th ed. Woodhead Publising limited, Cambrige, England. pp. 41–73.

Legarra, A., L. Varona, and E. López de Maturana. 2008. TM Threshold Model. Available at http://snp.toulouse.inra.fr/~alegarra/manualtm.pdf. GenoToul Bioinformatics, Toulouse, France.

Masoero, G., G. Bergoglio, L. Riccioni, G. Destefanis, M.T. Barge. 1992. Near Infrared spectroscopy applied to living rabbits to estimate body composition and carcass and meat traits: a calibration study. Journal of Applied Rabbit Research, 15:810-818.

Mateescu, R.G. 2015. Genetics of meat quality. In: D.J. Garrick and A. Ruvinsky, editors. The genetics of cattle 2nd Edn. CAB Int, Oxfordshire, UK. pp. 544-570.

Nogi, T., T. Honda, F. Mukai, T. Okagaki and K. Oyama. 2011. Heritabilities and genetic correlations of fatty acid compositions in *Longissimus* muscle lipid with carcass traits in Japanese Black cattle. J. Anim. Sci. 89:615–21.

Pascual, M., de los D.E., W. Calle and A. Blasco. 2015. Comparison of degrees of maturity of rabbit lines selected for different traits. World Rabbit Sci. 23:155-161.

Polak, T., L. Gašperlin, A. Rajar and B. Žlender. 2006. Influence of genotype lines, age at slaughter and sexes on the composition of rabbit meat. Food Technol. Biotechnol. 44:65–73.

Ros-Freixedes, R., J. Reixach, L. Bosch, M. Tor and J. Estany. 2013. Response to selection for decreased backfat thickness at restrained intramuscular fat content in Duroc pigs. J. Anim. Sci. 91:3514–3521.

Ros-Freixedes, R., J. Reixach, L. Bosch, M. Tor and J. Estany. 2014. Genetic correlations of intramuscular fat content and fatty acid composition among muscles and with subcutaneous fat in Duroc pigs. J. Anim. Sci. 92:5417–5425.

Sapp, R.L., J.K. Bertrand, T.D. Pringle and D.E. Wilson. 2002. Effects of selection for ultrasound intramuscular fat percentage in Angus bulls on carcass traits of progeny. J. Anim. Sci. 80:2017–2022.

Schwab, C.R., T.J. Baas, K.J. Stalder and D. Nettleton. 2009. Results from six generations of selection for intramuscular fat in Duroc swine using real-time ultrasound. I. Direct and correlated phenotypic responses to selection. J. Anim. Sci. 87:2774–2780.

Sellier, P., L. Maignel, and J.P. 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.

Sharma, G. 2002. Color fundamentals for digital imaging. In: Digital color imaging handbook. pp. 1–114.

Sorensen, D. and D. Gianola. 2002. Likelihood, Bayesian, and MCMC methods in quantitative genetics. Springer, New York, NY.

Sorensen D.A. and K. Johansson. 1992. Estimation of direct and correlated responses to selection using univariate animal models. J. Anim. Sci. 70: 2038-2044.

World Health Organization. 2008. Interim summary of conclusions and dietary recommendations on total fat and fatty acids. Joint FAO/WHO expert consultation on fats and fatty acids in human nutrition, 10-14.

Wood, J.D., R.I. Richardson, G.R. Nute, A.V. Fisher, M.M. Campo, E. Kasapidou, P.R. Sheard and M. Enser. 2004. Effects of fatty acids on meat quality: A review. Meat Sci. 66:21–32.

Wood, J.D., M. Enser, A.V. Fisher, G.R. Nute, P.R. Sheard, R.I. Richardson, S.I. Hughes and F.M. Whittington. 2008. Fat deposition, fatty acid composition and meat quality: A review. Meat Sci. 78:343–58.

Zhao, G.P., J.L. Chen, M.Q. Zheng, J. Wen, and Y. Zhang. 2007. Correlated Responses to Selection for Increased Intramuscular Fat in a Chinese Quality Chicken Line. Poult. Sci. 86:2309–2314.

Zomeño, C., P. Hernández, and A. Blasco. 2011. Use of near infrared spectroscopy for intramuscular fat selection in rabbits. World Rabbit Sci. 19:203–208.

Zomeño, C., V. Juste, and P. Hernández. 2012. Application of NIRS for predicting fatty acids in intramuscular fat of rabbit. Meat Sci. 91:155-159.

Zomeño, C., P. Hernández, and A. Blasco. 2013. Divergent selection for intramuscular fat content in rabbits. I. Direct response to selection. J. Anim. Sci. 91:4526–4531.

CHAPTER 2

Effect of selection for intramuscular fat on the fatty acid composition of rabbit meat: correlated responses and genetic parameters

The content of this chapter has been submitted to *Animal*

ABSTRACT: Intramuscular fat (IMF) content and its fatty acid composition are interesting points in meat industry due to their effect in human health and sensory and technological properties of meat. A divergent selection experiment for IMF of *Longissimus dorsi* (LD) muscle was performed in rabbits during eight generations. The aim of this study is to estimate the correlated responses to selection for IMF on the fatty acid composition of LD and their genetic parameters. Response to selection for IMF was 0.34 g/100g of LD, representing 2.4 SD of the trait. Selection for IMF led to relevant modifications in the fatty acid composition of LD. High-IMF line showed increased monounsaturated fatty acids (MUFA) and decreased n-3, n-6 and polyunsaturated fatty acids (PUFA) percentages, in comparison to low-IMF line, and percentages of the main MUFA and PUFA individual fatty acids followed a similar pattern as groups, except for C18:3n-3 that was greater in the high-IMF line. We did not observe differences between lines for the percentage of saturated fatty acids (SFA) group, although we found greater C14:0 and C16:0 percentages and lower C18:0 percentage in the high than in the low-IMF line. Heritability estimates were high for all fatty acids percentages, with medians ranging from 0.43 to 0.59; except for n-3, C18:3n-3, C16:0 and SFA which were low (between 0.12 and 0.18). Genetic correlations between IMF and LD fatty acid percentages were strong and positive for C14:0, C16:1, C18:1n-9 and MUFA, with medians ranging from 0.88 to 0.97, and strong and negative for C18:0, C18:2n-6, C20:4n-6, n-6 and PUFA, with medians ranging from -0.83 to -0.91. Genetic correlations between IMF and C18:3n-3, C16:0 and SFA percentages were positive, and between IMF and n-3 it was negative, but all of them were estimated with low accuracy and we do not have much information about their actual value. In general, phenotypic and genetic correlations were of the same order.

Key words: genetic parameters, correlated responses, fatty acids, intramuscular fat, selection.

1. INTRODUCTION

Intramuscular fat (IMF) content and its fatty acid composition are important traits due to their effect in human health and sensory and technological properties of meat. Intramuscular fat shows a high heritability around 0.50 (Martínez-Álvaro et al.,

2016a in rabbits and Estany et al., 2016 in pigs) and a moderate variability, which are favourable characteristics to be modified by genetic selection. In the literature there are three selection experiments for IMF, in pigs (Schwab et al., 2009), cattle (Sapp et al., 2002), and chickens (Zhao et al., 2007). We are performing a divergent selection experiment for IMF in rabbits (Zomeño et al., 2013 and Martínez-Álvaro et al., 2016a). Rabbit is a good model for genetic studies in other livestock species due to its short generation interval and the low cost of its carcass.

Selection for IMF could produce changes in the fatty acid composition that can influence nutritional, sensory and technological properties of meat. Great amounts of monounsaturated (MUFA) and saturated (SFA) fatty acids improve meat flavor (Carrapiso et al., 2003 and Burkett, 2009) but nutritional institutions recommend reducing the intake of SFA (World Health Organization, 2008). By other side, polyunsaturated fatty acids (PUFA) are beneficial from a nutritional point of view, but can led to undesirable flavors, to a decrease in the melting point of fat and to a shortened shelf life of the meat (Wood et al., 2004).

Genetic parameters of IMF, particularly genetic correlations with other meat quality traits, are difficult to estimate because they need a large amount of data for obtaining reasonable accuracies. However, when data come from a selection experiment, heritabilities and genetic correlations, although estimated with a limited precision, can be corroborated by the direct and correlated responses observed. Only the experiment of selection for IMF in pigs reported genetic parameters of fatty acid composition of meat (Burkett, 2009). To our knowledge, there are no genetic parameters of IMF and meat fatty acid composition published in rabbits, apart from that estimated by Martínez-Álvaro et al. (2016a) for SFA, MUFA and PUFA groups in the selection experiment for IMF previously quoted. The aim of this study is to estimate the correlated responses to selection for IMF on the fatty acid composition of rabbit meat, and their genetic parameters.

2. MATERIALS AND METHODS

2.1. Animals

A divergent selection experiment for IMF in rabbits was performed during 8 generations. Animals came from a synthetic rabbit line. The base population consisted of 13 males and 83 females, and then, lines selected for high and low IMF had approximately 8 males and 40 females per generation. Two full sibs (a male and a female) of the first parity of each doe were slaughtered at 9 wk of age and their IMF content was measured in *Longissimus dorsi* (LD) muscle. The average between these two phenotypic values was calculated. All dams were then ranked according to the IMF values obtained by their offspring. The 20% best dams provided all females for the next generation. Each sire was mated with five does, and to reduce inbreeding only one male progeny of the sire, from highest ranked mate, was selected for breeding the next generation. Normally, the first parity was used to collect the IMF data and the second parity to provide the rabbits for next generation, although exceptionally some IMF measurements were made on the second or third parity. More details of this experiment can be found in Martínez-Álvaro et al. (2016a). A total of 2,713 rabbits were considered in the pedigree file, from which 1,511 were evaluated. A total of 173 rabbits from the eighth generation were used to study the correlated responses to selection on fatty acid composition of LD; 82 from the high-IMF line and 91 from the low-IMF line.

Litters were homogenised at birth up to 9 kits per litter. Rabbits were reared collectively from weaning to slaughter, and were fed *ad libitum* with a commercial diet with an average composition of 15.1% CP, 14.5% crude fibre and 2.48% of fat. Fatty acid composition of the diet (% of total fatty acids) was 0.49% of C14:0, 19.4% of C16:0, 0.68% of C16:1, 2.77% of C18:0, 20.5% C18:1n-9, 48.1% of C18:2n-6, 6.80% of C18:3n-3 and 1.26% of C > 20. Animals were slaughtered using electrical stunning and exsanguination. After slaughter, carcasses were chilled for 24h at 4ºC.

All experimental procedures involving animals were approved by the Universitat Politècnica de València Research Ethics Committee, according to Council

Directives 98/58/EC (European Economic Community, 1998) and 2010/63/EU (European Commission Directive, 2010).

2.2. Intramuscular fat and fatty acids measurements

After refrigeration, LD was excised, minced, freeze-dried and scanned with near infrared spectroscopy to measure IMF and fatty acid composition, applying the calibration equations previously developed by Zomeño et al. (2012) with some modifications. Intramuscular fat and fatty acid contents were obtained in g/100g of muscle on a fresh basis. Then, fatty acids were expressed as % of total fatty acids. Fatty acids studied were: the major individual fatty acids C14:0, C16:0, C16:1, C18:0, C18:1n-9, C18:2n-6, C18:3n-3 and C20:4n-6 and the SFA, MUFA, n-3, n-6 and PUFA groups, which included the major fatty acids cited above and all identified minor fatty acids (i.e. C15:0 and C17:0 for SFA, C18:1n-7 for MUFA and C20:2n-6, C20:3n-6, C20:5n-3, C22:4n-6, C22:5n-3 and C22:6n-3 for PUFA).

2.3. Statistical analysis

Descriptive statistics and phenotypic correlations between IMF and fatty acid percentages of LD were estimated with data from all generations, after correcting data by line-generation-season, parity order and sex fixed effects.

Direct and correlated responses to selection were estimated as the phenotypic differences between high and low-IMF lines at the eight generation of selection. Phenotypic differences between lines were estimated with the model

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Wc} + \mathbf{e}$$

Data were assumed to be conditionally distributed as

$$\mathbf{y} \mid \mathbf{b}, \mathbf{c}, \sigma_e^2 \sim \mathrm{N}\left(\mathbf{Xb} + \mathbf{Wc}, \mathbf{I}\sigma_e^2\right)$$

in which **b** is the vector with the fixed effects of line (high-IMF and low-IMF), month, sex and parity order; **c** is the vector of common litter random effects, σ_e^2 is the residual variance, **X** and **W** are known incidence matrices and **I** is an identity matrix. Common litter random effects were assumed to be distributed as

$$\mathbf{c} \sim \mathrm{N}\left(\mathbf{0}, \mathbf{I}\sigma_c^2\right)$$

in which σ_c^2 is the common litter variance.

Heritabilities and genetic correlations with IMF were estimated by fitting the following bivariate animal model, with the same effects for all traits:

$$\begin{bmatrix} \boldsymbol{y_1} \\ \boldsymbol{y_2} \end{bmatrix} = \begin{bmatrix} \boldsymbol{X} & 0 \\ 0 & \boldsymbol{X} \end{bmatrix} \begin{bmatrix} \boldsymbol{b_1} \\ \boldsymbol{b_2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{Z} & 0 \\ 0 & \boldsymbol{Z} \end{bmatrix} \begin{bmatrix} \boldsymbol{u_1} \\ \boldsymbol{u_2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{W} & 0 \\ 0 & \boldsymbol{W} \end{bmatrix} \begin{bmatrix} \boldsymbol{c_1} \\ \boldsymbol{c_2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{e_1} \\ \boldsymbol{e_2} \end{bmatrix}$$

Data were assumed to be conditionally distributed as

$$\begin{bmatrix} \boldsymbol{y_1} \\ \boldsymbol{y_2} \end{bmatrix} \Big| \ \mathbf{b_1}, \mathbf{b_2}, \mathbf{u_1}, \mathbf{u_2}, \mathbf{c_1}, \mathbf{c_2}, \mathbf{R} \sim \mathrm{N}\left(\begin{bmatrix} \mathbf{X} & 0 \\ 0 & \mathbf{X} \end{bmatrix} \begin{bmatrix} \mathbf{b_1} \\ \mathbf{b_2} \end{bmatrix} + \begin{bmatrix} \mathbf{Z} & 0 \\ 0 & \mathbf{Z} \end{bmatrix} \begin{bmatrix} \mathbf{u_1} \\ \mathbf{u_2} \end{bmatrix} + \begin{bmatrix} \mathbf{W} & 0 \\ 0 & \mathbf{W} \end{bmatrix} \begin{bmatrix} \mathbf{c_1} \\ \mathbf{c_2} \end{bmatrix}, \mathbf{R} \right)$$

in which $\mathbf{b_1}, \mathbf{b_2}$ are the vectors of fixed effects (month, sex and parity order); $\mathbf{u_1}, \mathbf{u_2}$ are the vectors of additive genetic effects; $\mathbf{c_1}, \mathbf{c_2}$ are the vectors of common litter effects; **X**, **Z** and **W** are known incidence matrices, and **R** is the residual co (variance) matrix between the two traits.

Sorting data by individuals, additive effects were distributed as

$$\mathbf{u} \sim \mathrm{N}\left(\mathbf{0}, \mathbf{A} \otimes \mathbf{G}_0\right)$$

common litter effects were distributed as

$$\mathbf{c} \sim \mathrm{N}\left(\mathbf{0}, \mathbf{I}_m \otimes \mathbf{C}_0\right)$$

and residuals were distributed as

$$\mathbf{e} \sim \mathrm{N}\left(\mathbf{0}, \mathbf{I}_n \otimes \mathbf{R}_0\right)$$

in which $\mathbf{G}_0$, $\mathbf{C}_0$ and $\mathbf{R}_0$, the 2 x 2 genetic additive, common litter, and residual (co)variance matrices between the two traits, **A** is the relationship matrix, $\mathbf{I}_m$ is an identity matrix of the same order as the number of levels of common litter effects, and $\mathbf{I}_n$ is an identity matrix of the same order as the number of individuals. All effects were assumed to be independent between them.

Bayesian inference was used, with bounded flat priors for all unknowns. Marginal posterior distributions were estimated using Gibbs sampling. Descriptive statistics and phenotypic differences between lines were computed with the programme Rabbit developed by the Institute for Animal Science and Technology (Valencia, Spain). After some exploratory analyses, results were based on Monte Carlo Markov chains consisting of 60,000 iterations, with a burn-in period of 10,000, and only one of every 10 samples were saved for inferences. Phenotypic correlations and genetic analyses were computed with the software TM (Legarra et al., 2008). In this case, after some exploratory analyses results were based on Monte Carlo Markov chains consisting of 1,000,000 iterations, with a burn-in period of 200,000; only one of every 100 samples were saved for inferences. In all analyses, convergence was tested using the Z criterion of Geweke and Monte Carlo sampling errors were computed using time-series procedures (Sorensen and Gianola, 2002) included in the Rabbit and TM programs. In all cases, Monte Carlo standard errors were small and lack of convergence was not detected by the Geweke test.

The parameters obtained from the marginal posterior distributions of the phenotypic differences between high and low-IMF lines were: the median of the difference (D), the highest posterior density region at 95% ($HPD_{95\%}$), and the probability of the difference being greater than zero when $D > 0$ or lower than zero when $D < 0$ (P_0). We considered 1/3 of the phenotypic standard deviation of a trait as a relevant value (r), and we also calculated the probability of relevance (probability of the difference being greater than r when $D > 0$ or lower than r when $D < 0$) (P_r). For heritabilities we estimated the median of each marginal posterior distribution, the $HPD_{95\%,}$ and the limit of the interval [k, 1] with 80% probability, i.e. the guaranteed value with probability of 80% ($k_{80\%}$). For genetic and phenotypic correlations, we estimated the median of each marginal posterior distribution, the $HPD_{95\%}$ and the probability of being greater than 0 when the median is positive or lower than 0 when the median is negative (P_0), and the guaranteed value with probability of 80%, i. e. limit of the interval [k, 1] when the median is positive or [-1, k] when the median is negative with 80% probability. A more detailed description of these features can be found in Blasco (2001, 2005, 2017).

3. RESULTS AND DISCUSSION

Table 1 shows descriptive statistics of IMF content and fatty acid composition of LD. On average, IMF was 1.04 g/100g of LD. Percentages of SFA and PUFA were similar (38.2% and 41.8%, respectively), while MUFA percentage was lower (24.8%). Polyunsaturated fatty acids were mainly composed by n-6 (39.5%), whereas n-3 represented a lower percentage (2.92%). Linoleic (C18:2n-6), palmitic (C16:0), and oleic (C18:1n-9) acids were the most abundant fatty acids in rabbit meat (28.1%, 26.6% and 21.5% respectively). They were followed by stearic (C18:0) and arachidonic acids (C20:4n-6) with percentages of 9.83% and 7.10% respectively, whereas other fatty acids (C14:0, C16:1 and C18:3n-3) represented minor percentages. These results are in agreement with other studies in rabbits (reviewed by Dalle Zotte, 2002).

Table 1. Descriptive statistics of intramuscular fat (g/100g of muscle) and fatty acid composition of *Longissimus dorsi* muscle (% of total fatty acids).

Trait[1]	Mean	SD	CV x 100
IMF	1.04	0.14	13.4
C14:0	1.41	0.32	22.4
C16:0	26.6	1.05	3.95
C18:0	9.83	0.66	6.67
SFA	38.2	1.28	3.35
C16:1	1.60	0.53	33.1
C18:1n-9	21.5	1.85	8.60
MUFA	24.8	2.47	9.95
C18:2n-6	28.1	1.59	5.65
C18:3n-3	1.92	0.19	9.87
C20:4n-6	7.10	0.95	13.4
n-3	2.92	0.27	9.39
n-6	39.5	2.64	6.68
PUFA	41.8	2.74	6.54

[1]IMF, intramuscular fat; SFA = saturated fatty acids, C14:0 + C15:0 + C16:0 + C17:0 + C18:0; MUFA = monounsaturated fatty acids, C16:1 + C18:1n-7 + C18:1n-9; n-3 = C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3; n-6 = C18:2n-6 + C20:2n-6 + C20:3n-6 + C20:4n-6 + C22:4n-6; PUFA = polyunsaturated fatty acids, C18:2n-6 + C18:3n-3 + C20:2n-6 + C20:3n-6 + C20:4n-6 + C20:5n-3 + C22:4n-6 + C22:5n-3 + C22:6n-3.

Ratios PUFA:SFA and n-6:n-3 are healthy indicators of meat. In our experiment, PUFA:SFA ratio was 1.09, above the minimum of 0.60 recommended by the World Health Organization (2008). However, the n-6:n-3 ratio was not favourable (13.5), higher than the nutritional recommendations for human diets of 5-10 reviewed by ISSFAL (2010), due to the high amount of C18:2n-6 in rabbit meat. In comparison with other species, rabbit meat shows greater PUFA, C18:2n-6 and C18:3n-3 percentages and PUFA:SFA ratio than pig, cattle and sheep, and lower n-6:n-3 ratio than pig, cattle and chickens (reviewed by Dalle Zotte, 2002), which makes it a high quality meat from a nutritional point of view.

3.1. Response to selection and correlated responses in fatty acid composition of LD

Table 2 shows the direct response to selection for IMF and correlated responses in fatty acid composition of LD estimated as differences between high-IMF and low-IMF lines. Comparisons between lines should be done at the same stage of maturity, and our lines were approximately at the same stage (Pascual et al., 2015).

Response to selection for IMF was 0.34 g/100g of LD, representing 2.4 SD of the trait, with a probability of the difference between lines being relevant of P_r = 1.00. This great response to selection was expected, due to the high heritability of the trait (0.54 with $HPD_{95\%}$ between 0.37 and 0.71, Martínez-Álvaro et al., 2016a) and its moderate variability (Table 1). Other authors obtained great responses to selection for IMF in pig (Schwab et al., 2009), cattle (Sapp et al., 2002) and chickens (Zhao et al., 2007), in line with our results.

Selection for IMF led to great modifications in the fatty acid composition of LD, expressed as % of total fatty acids (Table 2). High-IMF line showed increased MUFA and decreased PUFA percentages in comparison to low-IMF line. The differences between high-IMF and low-IMF lines for these fatty acid groups were both relevant (P_r = 1.00) and of similar magnitude. Within PUFA, both n-6 and n-3 were relevantly lower in the high-IMF line (P_r = 1.00), being the differences between lines greater for n-6 (3.8 SD) than for n-3 (1.4 SD). However, we did not observe differences between lines for the SFA percentage.

High-IMF line showed relevantly greater amounts of SFA, MUFA, n-6, n-3 and PUFA groups and of all individual fatty acids in absolute terms (g/100 g of LD) respect to the low-IMF line (data not shown), due to its greater amount of IMF. Differences in the fatty acid percentages between lines are the consequence of a greater proportion of triglycerides (stored in adipocytes) respect to phospholipids (located in cells membranes) in the high-IMF than in the low-IMF line. In general, phospholipid fraction shows greater percentages of all individual PUFA whereas triglycerides fraction is richer in all MUFA and SFA. The faster increase of MUFA and SFA respect to PUFA when fatness increases is well documented (reviewed by De Smet et al., 2004 and Wood et al., 2008 in several farm species).

Table 2. Features of the marginal posterior distributions of the differences between high and low intramuscular fat (IMF) lines for IMF (g/100g of muscle) and fatty acid composition of *Longissimus dorsi* (% of total fatty acids) in the eighth generation of selection.

Trait[1]	D[2]	HPD$_{95\%}$[3]		P_0[4]	r[5]	P_r[6]
IMF	0.34	0.29,	0.39	1.00	0.05	1.00
C14:0	0.75	0.60,	0.90	1.00	0.11	1.00
C16:0	0.63	0.18,	1.08	1.00	0.35	0.89
C18:0	-1.87	-2.22,	-1.54	1.00	0.22	1.00
SFA	-0.31	-0.91,	0.33	0.83	0.43	0.36
C16:1	1.15	0.89,	1.41	1.00	0.18	1.00
C18:1n-9	6.66	5.69,	7.67	1.00	0.62	1.00
MUFA	9.20	7.88,	10.6	1.00	0.82	1.00
C18:2n-6	-4.70	-5.36,	-4.03	1.00	0.53	1.00
C18:3n-3	0.20	0.10,	0.30	1.00	0.06	1.00
C20:4n-6	-3.36	-3.84,	-2.86	1.00	0.32	1.00
n-3	-0.39	-0.50,	-0.29	1.00	0.09	1.00
n-6	-9.97	-11.2,	-8.68	1.00	0.88	1.00
PUFA	-10.3	-11.6,	-8.98	1.00	0.91	1.00

[1]SFA = saturated fatty acids, C14:0 + C15:0 + C16:0 + C17:0 + C18:0; MUFA = monounsaturated fatty acids, C16:1 + C18:1n-7 + C18:1n-9; n-3 = C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3; n-6 = C18:2n-6 + C20:2n-6 + C20:3n-6 + C20:4n-6 + C22:4n-6; PUFA = polyunsaturated fatty acids, C18:2n-6 + C18:3n-3 + C20:2n-6 + C20:3n-6 + C20:4n-6 + C20:5n-3 + C22:4n-6 + C22:5n-3 + C22:6n-3. [2]D = median of the marginal posterior distribution of the difference between lines[3]HPD$_{95\%}$ = highest posterior

density region at 95% of probability.[4]P_0 = probability of the difference being greater than zero when D > 0 or lower than zero when D < 0. [5]r = relevant value, proposed as 1/3 of the standard deviation of the trait. [6]P_r = probability of relevance (probability of the difference being greater than r when D > 0 or lower than r when D < 0.

We have studied the effect of selection for IMF on the fatty acid ratios PUFA:SFA, MUFA:SFA and n-6:n-3 dividing the medians of the fatty acid groups within each line in the eighth generation. The ratio between MUFA and SFA is interesting from a healthy point of view, since it is recommended to replace the intake of SFA by unsaturated fatty acids (World Health Organization, 2008). The line selected for high IMF showed greater MUFA:SFA ratio (0.57) respect to the low-IMF line (0.33), which implies an improvement of meat quality in the high-IMF line. In contrast, selection for high IMF led to a detriment of the PUFA:SFA ratio respect to selection for low IMF, which was 0.98 in the high-IMF line and 1.23 in the low-IMF line. However, in both cases, PUFA:SFA ratio was above the minimum of 0.60 recommended by the World Health Organization (2008). Within PUFA, the n-6:n-3 ratio was more favourable in the high-IMF (11.6) than in the low-IMF line (13.1), due to the greater differences between lines in n-6 than in n-3. Notice that the median for the n-6:n-3 ratio in the eight generation was 12.4, differing from the median of the whole selection experiment showed in Table 1.

In general, individual MUFA and PUFA showed similar patterns as their groups (Table 2). We did not observe differences between lines for the percentage of SFA group, but we found positive and negative differences between lines for individual SFA. High-IMF line showed greater percentages of C14:0 and C16:0 but lower percentage of C18:0 than low-IMF line. Differences between lines were relevant (P_r = 1.00 for C14:0 and C18:0, and P_r = 0.89 for C16:0). Rabbits and mammals in general, are able to synthetize SFA and MUFA from glucose through lipogenesis *de novo*, which produces primarily C16:0. In contrast, PUFA are entirely derived from the diet. Previous studies observed that high-IMF line showed greater lipogenic activities than low-IMF line in several tissues such as LD, perirenal fat and liver (Martínez-Álvaro et al., 2017a and b). These findings explain the greater proportion of C14:0 and C16:0

individual SFA and total MUFA observed in the high-IMF line in comparison to the low-IMF line, and consequently, its lower proportion of PUFA. However, high-IMF line showed lower percentage of C18:0. This is explained because in rabbits, C18:0 percentage is greater in phospholipids than in triglycerides fraction (Alasnier et al., 1996; Cambero et al., 1991a and b; Otake et al., 1971). This particularity is not observed in other species (Lesigneur-Meynier and Gandemer, 1991 and Burkett, 2009 in pigs and Wood et al., 2004 in a review including pigs, lambs and cattle).

Concerning individual MUFA, high-IMF line had relevantly greater C18:1n-9 and C16:1 percentages (P_r = 1.00, Table 2). The ratio between C18:1n-9 and C18:0 is a common indicator of the stearoyl-CoA desaturase (SCD) activity (Attie et al., 2002), enzyme responsible for the synthesis of main MUFA from their SFA forms. This ratio was 1.98 for high-IMF line and 1.09 for low-IMF line, indicating greater SCD activity in the high-IMF line.

Within PUFA, high-IMF line showed lower C18:2n-6 and C20:4n-6 percentages than low-IMF line (P_r = 1.00), but greater percentage of C18:3n-3 (P_r = 1.00). In rabbits, C18:3n-3 percentage is much greater in triglycerides than in phospholipids (Alasnier et al., 1996 and Otake et al 1971). In other species such as pig, C18:3n-3 is similar in both fractions (reviewed by De Smet et al., 2004, Wood et al., 2004 and 2008), or it is only slightly greater in triglycerides (Burkett, 2009).

Modifications in the IMF content and in its fatty acid composition could affect sensory and technological meat quality traits such as flavor, fat consistence and shelf life (Wood et al., 2004). In a previous study our rabbit lines did not shown differences in sensory properties when their LD were evaluated by a trained sensory panel, despite their differences in IMF and fatty acid composition. However, low-IMF showed greater instrumental firmness than high-IMF line (Martínez-Álvaro et al., 2016b).

Our results are in close agreement with findings of the selection experiment for IMF in pigs (Burkett, 2009). The line of pigs selected for high IMF showed more MUFA percentages and lower PUFA percentages respect to a control line with the exception of C18:3n-3 that was greater in the former (Burkett, 2009). Moreover, these authors also did not find significant differences for SFA percentage, as a result of greater

percentages of C15:0 and C17:0 but lower of C18:0 and C20:0 in the line selected for high IMF respect to the control line. In a simulation study in pigs, Ros-Freixedes et al., (2012) expected a positive response to selection in C18:1n-9 percentage when selecting by IMF and other traits, including IMF. Some studies in rabbit compared the fatty acid composition in several genetic lines differing in their IMF, but they did not show any common pattern (Gašperlin et al., 2006; Polak et al., 2006; Hernández et al., 2008).

3.2. Heritabilities of the traits

For all the traits, the differences between the genetic means of the high-IMF and low-IMF lines estimated with the animal model agreed with the phenotypic differences between lines (Table 2). This corroborates the model used for the genetic analysis and the genetic parameters reported below.

Table 3. Heritabilities of fatty acid composition of *Longissimus dorsi* muscle (in % of total fatty acids).

Trait[1]	Median[2]	$HPD_{95\%}$[3]	$k_{80\%}$[4]
C14:0	0.43	0.29, 0.60	0.37
C16:0	0.16	0.04, 0.31	0.11
C18:0	0.42	0.29, 0.56	0.36
SFA	0.12	0.02, 0.25	0.07
C16:1	0.43	0.30, 0.59	0.37
C18:1n-9	0.53	0.39, 0.68	0.46
MUFA	0.56	0.41, 0.72	0.50
C18:2n-6	0.50	0.35, 0.67	0.43
C18:3n-3	0.18	0.05, 0.33	0.12
C20:4n-6	0.50	0.36, 0.65	0.44
n-3	0.15	0.06, 0.26	0.11
n-6	0.59	0.44, 0.74	0.52
PUFA	0.59	0.44, 0.75	0.52

[1]SFA = saturated fatty acids, C14:0 + C15:0 + C16:0 + C17:0 + C18:0; MUFA = monounsaturated fatty acids, C16:1 + C18:1n-7 + C18:1n-9; n-3 = C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3; n-6 = C18:2n-6 + C20:2n-6 + C20:3n-6 + C20:4n-6 + C22:4n-6; PUFA = polyunsaturated fatty acids, C18:2n-6 + C18:3n-3 + C20:2n-6 + C20:3n-6 + C20:4n-6 + C20:5n-3 + C22:4n-6 + C22:5n-3 + C22:6n-3; [2]Median = median of the

marginal posterior distribution of the heritability; [3]$HPD_{95\%}$ = highest posterior density region at 95% of probability; [4]$k_{80\%}$ = limit of the interval [k, 1] at 80% of probability.

Table 3 shows the heritabilities (h^2) of fatty acid composition of LD. In general, fatty acid composition of LD showed high h^2. Percentages of MUFA, n-6 and PUFA groups displayed the greatest h^2 estimates (from 0.56 to 0.59), showing guaranteed values from 0.50 to 0.52 with 80% of probability. Their major fatty acids, C18:1n-9 and C16:1 for MUFA and C18:2n-6 and C20:4n-6 for n-6 and PUFA, also showed great h^2 estimates (from 0.43 to 0.53), with guaranteed values at 80% probability of 0.37, 0.46, 0.43 and 0.44, respectively. However, n-3 group and its major fatty acid C18:3n-3, showed lower estimates (0.15-0.18) and their guaranteed values with 80% probability were between 0.11 and 0.12. Percentage of SFA group showed a low h^2 (0.12 with a $k_{80\%}$ = 0.07) because of the low h^2 of its main component, C16:0 (0.16 with a $k_{80\%}$ = 0.11). However, other important individual SFA percentages such as C18:0 and C14:0 displayed high h^2 (0.42-0.43) with guaranteed values at 80% probability of 0.36 and 0.37 respectively.

Heritability estimates were high for the major fatty acids of LD (except for C18:3n-3 and C16:0), indicating an important genetic background regulating their synthesis and management. Even though C18:2n-6 and C20:4n-6 must come from diet (C20:4n-6 can be also synthetized in mammals, but only from dietary C18:2n-6), our results show that there is an important genetic control for their accumulation in IMF, whereas this was not observed for C18:3n-3 (Table 3). Several studies report high to moderate h^2 estimates for all IMF fatty acid percentages in general (Burkett et al., 2009; Sellier et al., 2010 and Ibáñez-Escriche et al., 2016 in pigs and Saatchi et al., 2013 and Nogi et al., 2016 in cattle). However, the h^2 estimate of C18:3n-3 percentage reported by Ibáñez-Escriche et al. (2016) was moderate (0.22) and those reported by Sellier et al. (2010) and Nogi et al. (2011) were null, in line with our results in rabbits. Only Burkett et al. (2009) reported h^2 for C16:0 near 0 (0.06±0.08).

3.3. Correlations between IMF and fatty acid composition

Table 4 shows the phenotypic and genetic correlations (r_g) between IMF and fatty acid composition of LD. To our knowledge, there are no previous reports of genetic correlations among IMF and fatty acid composition of meat in rabbits.

Estimates of r_g between IMF and fatty acid percentages of LD were strong and positive for C14:0, C16:1, C18:1n-9 and MUFA, with medians ranging between from 0.88 to 0.97, and strong and negative for C18:0, C18:2n-6, C20:4n-6, n-6 and PUFA, with medians ranging from -0.83 to -0.91. Because of the high values of these correlations, 1,511 data were enough to obtain quite accurate estimates (see their narrow $HPD_{95\%}$) and their guaranteed values at 80% of probability were very close to medians (see $k_{80\%}$ in Table 4). Phenotypic correlations between IMF and these fatty acids were of the same order or slightly lower than r_g (Table 4). The great correlated responses to selection for IMF observed on these traits (Table 2) corroborates their strong r_g with IMF.

Table 4. Phenotypic and genetic correlations between intramuscular fat and fatty acid composition of *Longissimus dorsi* muscle (in % of total fatty acids).

Trait[1]	Phenotypic correlation					Genetic correlation				
	M[2]	$HPD_{95\%}$[3]		P_0[4]	$k_{80\%}$[5]	M[2]	$HPD_{95\%}$[3]		P_0[4]	$k_{80\%}$[5]
C14:0	0.75	0.73,	0.78	1.00	0.74	0.97	0.93,	1.00	1.00	0.96
C16:0	0.34	0.29,	0.38	1.00	0.32	0.48	0.13,	0.80	0.99	0.32
C18:0	-0.68	-0.71,	-0.66	1.00	-0.67	-0.91	-0.98,	-0.80	1.00	-0.86
SFA	0.21	0.15,	0.26	1.00	0.18	0.30	-0.06,	0.66	0.94	0.14
C16:1	0.83	0.81,	0.85	1.00	0.82	0.96	0.90,	1.00	1.00	0.94
C18:1n-9	0.76	0.74,	0.78	1.00	0.75	0.88	0.79,	0.95	1.00	0.84
MUFA	0.79	0.77,	0.81	1.00	0.78	0.89	0.81,	0.96	1.00	0.85
C18:2n-6	-0.62	-0.66,	-0.59	1.00	-0.61	-0.83	-0.94,	-0.69	1.00	-0.77
C18:3n-3	0.33	0.28,	0.38	1.00	0.30	0.59	0.23,	0.92	1.00	0.44
C20:4n-6	-0.78	-0.80,	-0.75	1.00	-0.77	-0.89	-0.96,	-0.81	1.00	-0.86
n-3	-0.37	-0.42,	-0.32	1.00	-0.35	-0.71	-0.99,	-0.43	1.00	-0.58
n-6	-0.88	-0.89,	-0.87	1.00	-0.87	-0.89	-0.96,	-0.81	1.00	-0.85
PUFA	-0.82	-0.83,	-0.80	1.00	-0.81	-0.88	-0.95,	-0.81	1.00	-0.85

[1]SFA = saturated fatty acids, C14:0 + C15:0 + C16:0 + C17:0 + C18:0; MUFA = monounsaturated fatty acids, C16:1 + C18:1n-7 + C18:1n-9; n-3 = C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3; n-6 = C18:2n-6 + C20:2n-6 + C20:3n-6 + C20:4n-6 + C22:4n-6; PUFA = polyunsaturated fatty acids, C18:2n-6 + C18:3n-3 + C20:2n-6 + C20:3n-6 + C20:4n-6 + C20:5n-3 + C22:4n-6 + C22:5n-3 + C22:6n-3. [2]M = median of the marginal

posterior distribution of the correlation. [3]$HPD_{95\%}$ = highest posterior density region at 95% probability.[4]P_0 = probability of the correlation being greater than 0 when the median is positive or lower than 0 when the median is negative.[5]$k_{80\%}$ = limit of the interval [k, 1] at 80% of probability.

The r_g of IMF with n-3 was negative (P_0 = 1.00), whereas with C18:3n-3 was positive (P_0 = 1.00), and their estimates showed medians of -0.71 and 0.59, respectively. These results suggest that other n-3 longer-chain fatty acids percentages show different relationships with IMF than C18:3n-3. The r_g of IMF with C16:0 and SFA percentages were positive with a high probability (P_0 = 0.99 and 0.94, respectively), but their medians were lower (0.30-0.48). All these r_g estimates showed a wide $HPD_{95\%}$ providing scarce information about their actual value. However, except for SFA percentage, their value should be substantial, since selection for IMF led to relevant correlated responses on these traits (Table 2). Phenotypic correlations between IMF and percentages of n-3, C18:3n-3, C16 and SFA showed the same sign as their corresponding r_g (P_0 = 1.00) but their medians were lower. Our study reports strong r_g between IMF and most of the major fatty acid percentages in rabbit meat (except for C16:0 and C18:3n-3), suggesting that, as IMF increases, there is a rapid dilution of PUFA in mainly MUFA but also in C14:0. This dilution is due to the difference in fatty acid composition between the muscle phospholipids and triglycerides (De Smet et al., 2004).

The genetic relationships between IMF and the fatty acid composition of meat has interest because both affect sensory, technological and nutritional properties of meat (Wood et al., 2004). In the selection experiment for IMF in pigs, Burkett et al., 2009 calculated r_g between IMF and fatty acid percentages. The genetic correlations of IMF with PUFA and C18:2n-6 percentages were negative and strong (-0.80 and -0.84, respectively) in line with our estimates, and between IMF and C14:0 was 0.50, lower than our results. The other r_g between IMF and other fatty acid percentages were estimated with very low accuracy due to their low amount of data (n = 663). In general, our results showed stronger correlations between IMF and fatty acid composition of meat than the correlations reported in other studies in pigs (Suzuki et al., 2006 and Ros-Freixedes et al., 2014) and cattle (Nogi et al., 2011 and Buchanan et al., 2015).

There have been detected a couple of genes (*ELOVL6* and *SCD*) affecting MUFA and SFA content without modifying IMF (reviewed by Estany et al., 2016). However, the strong genetic correlations between IMF and most fatty acids estimated in rabbits leave few options to change the fatty acid composition of LD without varying IMF.

4. CONCLUSIONS

Selection for IMF led to relevant modifications in the fatty acid composition of LD. High-IMF line showed increased MUFA and decreased n-6, n-3 and PUFA percentages in comparison to low-IMF line, and percentages of the main MUFA and PUFA individual fatty acids followed a similar pattern as groups, except for C18:3n-3 that was greater in the high-IMF line. We did not observe differences between lines for the percentage of SFA group, but we found greater C14:0 and C16:0 percentages in the high-IMF and lower percentage of C18:0. Heritability estimates were high for the major individual fatty acid percentages (except for C18:3n-3 and C16:0) and fatty acid groups (except for n-3 and SFA), indicating an important genetic component under these traits. Intramuscular fat showed strong genetic correlations with most of fatty acids except for n-3, C18:3n-3, C16:0 and SFA.

5. LITERATURE CITED

Alasnier, C., H. Rémington and G. Gandemer. 1996 Lipid characteristics associated with oxidative and glycolytic fibres in rabbit muscles. Meat Sci., 43:213-224.

Alasnier, C. and G. Gandemer. 1998. Fatty acid and aldehyde composition of individual phospholipids classes of rabbit skeletal muscles in related to the metabolic type of the fibre. Meat Sci. 48:225-235.

Attie A.D., R.M. Krauss, M.P. Gray-Keller, A. Brownlie, M. Miyazaki, J.J. Kastelein, A.J. Lusis, F.H. Stalenhoef, J.P. Stoehr, M.R. Hayden and J.M. Ntambi. 2002. Relationship between stearoyl-CoA desaturase activity and plasma triglycerides in human and mouse hypertriglyceridemia. J. Lipid Res. 43:1899-1907.

Blasco, A. 2001. The Bayesian controversy in animal breeding. J. Anim. Sci. 79:2023-2046.

Blasco, A. 2005. The use of Bayesian statistics in meat quality analyses: A review. Meat Sci. 69:115-122.

Blasco, A. 2017. Bayesian analysis for animal scientists. Springer, N.Y., USA

Buchanan, J.W., J.M. Reecy, D.J. Garrick, Q. Duan, D.C. Beitz, and R.G. Mateescu. 2015. Genetic parameters and genetic correlations among triacylglycerol and phospholipid fractions in Angus cattle. J. Anim. Sci. 93:522–528.

Burkett, J.L. 2009. The effect of selection for intramuscular fat on fatty acid composition in Duroc pigs. Graduate Theses and Dissertations. Paper 10539.

Cambero, M.I., L. de la Hoz, B. Sanz and A. Ordóñez. 1991a. Lipid and fatty acid composition of rabbit meat: Part 1.- Apolar fraction. Meat Sci., 29:153-166.

Cambero, M.I., L. de la Hoz, B. Sanz and A. Ordóñez. 1991b. Lipid and fatty acid composition of rabbit meat: Part 2.- Phospholipids. Meat Sci., 29:167-176.

Carrapiso, A.I., F. Bonilla and C. Garcia. 2003. Effect of crossbreeding and rearing system on sensory characteristics of Iberian ham. Meat Sci. 65:623-629.

Dalle Zotte, A. 2002. Perception of rabbit meat quality and major factors influencing the rabbit carcass and meat quality. Livest. Prod. Sci. 75:11–32.

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

Estany, J., R. Ros-Freixedes, M. Tor and R. N. Pena. 2016. Genetics and breeding for intramuscular fat and oleic acid content in pigs. J. Anim. Sci. 94:380-381.

European Economic Community. 1998. Council Directive 98/58/EC of 20 July 1998 concerning the protection of animals kept for farming purposes. Official Journal L, 221(8), 8.

European Commission Directive. 2010. Council, E. P. A. E. 2010/63/EU on the protection of animals used for scientific purposes. Institute for Health and Consumer Protection, Ispra, Italy, b7.

Gašperlin, L., T. Polak, A. Rajar, M. Skvarèa, and B. Lender. 2006. Effect of genotype, age at slaughter and sex on chemical composition and sensory profile of rabbit meat. World Rabbit Sci. 14:157–166.

Hernández, P., V. Cesari, and A. Blasco. 2008. Effect of genetic rabbit lines on lipid content, lipolytic activities and fatty acid composition of hind leg meat and perirenal fat. Meat Sci. 78:485–491.

Ibáñez-Escriche, N., E. Magallón, E. González, J.F. Tejeda, and J.L. Noguera. 2016. Genetic parameters and crossbreeding effects of fat deposition and fatty acid profiles in Iberian pig lines. J. Anim. Sci. 94:28-37.

ISSFAL, 2010. International Society for the Study of Fatty Acids and Lipids. http://www.issfal.org/statements/pufa-recommendations/recommendations-by-others.

Legarra, A., L. Varona and E. López de Maturana. 2008. TM Threshold Model. Available at http://snp.toulouse.inra.fr/~alegarra/manualtm.pdf . GenoToul Bioinformatics, Toulouse, France.

Leseigneur-Meyner, A. and G. Gandemer. 1991. Lipid composition of pork muscle in relation to the metabolic type of the fibres. Meat Sci., 29:229-241.

Martínez-Álvaro, M., P. Hernández, and A. Blasco. 2016a. Divergent selection on intramuscular fat in rabbits: Responses to selection and genetic parameters. J. Anim. Sci. 94:4993-5003.

Martínez-Álvaro, M., V. Penalba, A. Blasco, and P. Hernández. 2016b. Effect of divergent selection for intramuscular fat on sensory traits and instrumental texture in rabbit meat1. J. Anim. Sci. 94:5137-5143.

Martínez-Álvaro, M., S. Agha, A. Blasco, and P. Hernández. 2017a. Muscle lipid metabolism in two rabbit lines divergently selected for intramuscular fat. J. Anim. Sci. (Accepted).

Martínez-Álvaro, M., Y. Paucar, K. Satué, A. Blasco, and P. Hernández. 2017b. Liver metabolism traits in two rabbit lines divergently selected for intramuscular fat. Animal (Submitted).

Nogi, T., T. Honda, F. Mukai, T. Okagaki, and K. Oyama. 2011. Heritabilities and genetic correlations of fatty acid compositions in *Longissimus* muscle lipid with carcass traits in Japanese Black cattle. J. Anim. Sci. 89:615–21.

Otake, Y., M. Watanabe and T. Nakazato. 1971. Fatty acid composition of rabbit meat lipid. Jap. J. Zootech. Sci., 42:162-167.

Pascual, M., de los D.E., W. Calle and A. Blasco. 2015. Comparison of degrees of maturity of rabbit lines selected for different traits. World Rabbit Sci. 23:155-161.

Polak, T., L. Gašperlin, A. Rajar, and B. Žlender. 2006. Influence of genotype lines, age at slaughter and sexes on the composition of rabbit meat. Food Technol. Biotechnol. 44:65–73.

Ros-Freixedes, R., J. Reixach, M. Tor and J. Estany. 2012. Expected genetic response for oleic acid content in pork. J. Anim. Sci., 90:4230-4238.

Ros-Freixedes, R., J. Reixach, L. Bosch, M. Tor and J. Estany. 2014. Genetic correlations of intramuscular fat content and fatty acid composition among muscles and with subcutaneous fat in Duroc pigs. J. Anim. Sci. 92:5417–5425.

Saatchi, M., D.J. Garrick, R.G. Tait Jr, M.S. Mayes, M. Drewnoski, J. Schoonmaker, C. Diaz, D.C. Beitz and J.M. Reecy. 2013 Genome-wide association and prediction of direct genomic breeding values for composition of fatty acids in Angus beef cattl. BMC Genomics, 14:730.

Sapp, R.L., J.K. Bertrand, T.D. Pringle, and D.E. Wilson. 2002. Effects of selection for ultrasound intramuscular fat percentage in Angus bulls on carcass traits of progeny. J. Anim. Sci. 80:2017–2022.

Schwab, C.R., T.J. Baas, K.J. Stalder, and D. Nettleton. 2009. Results from six generations of selection for intramuscular fat in Duroc swine using real-time ultrasound. I. Direct and correlated phenotypic responses to selection. J. Anim. Sci. 87:2774–2780.

Sellier, P.L. Maignel, and J.P. 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.

Sorensen, D.A. and D. Gianola. 2002. Likelihood, Bayesian, and MCMC methods in quantitative genetics. Springer, New York, NY.

Wood, J.D., R.I. Richardson, G.R. Nute, A. V. Fisher, M.M. Campo, E. Kasapidou, P.R. Sheard and M. Enser. 2004. Effects of fatty acids on meat quality: A review. Meat Sci. 66:21–32.

Wood, J.D., M. Enser, A.V. Fisher, G.R. Nute, P.R. Sheard, R.I. Richardson, S.I. Hughes and F.M. Whittington. 2008. Fat deposition, fatty acid composition and meat quality: a review. Meat Sci. 78:343-358.

World Health Organization. 2008. Interim summary of conclusions and dietary recommendations on total fat and fatty acids. Joint FAO/WHO expert consultation on fats and fatty acids in human nutrition, 10-14.

Zhao, G.P., J.L. Chen, M.Q. Zheng, J. Wen and Y. Zhang. 2007. Correlated responses to selection for increased intramuscular fat in a chinese quality chicken line. Poult. Sci. 86:2309–2314.

Zomeño, C., V. Juste and P. Hernández. 2012. Application of NIRS for predicting fatty acids in intramuscular fat of rabbit. Meat Sci. 91:155-159.

Zomeño, C., P. Hernández and A. Blasco. 2013. Divergent selection for intramuscular fat content in rabbits. I. Direct response to selection. J. Anim. Sci. 91: 4526-4531.

CHAPTER 3

Correlated responses to selection for intramuscular fat in several muscles in rabbits

The content of this chapter has been submitted to *Meat Science*

ABSTRACT: A divergent selection experiment for intramuscular fat (IMF) in muscle *Longissimus dorsi* (LD) at 9 wk of age was performed in rabbits. The aim of this study was to evaluate the correlated reponses to selection on IMF content and fatty acid composition of muscles with diverse metabolic profile in the sixth generation of selection. The muscles studied were *Biceps femoris* (BF), *Supraspinatus* (SS) and *Semimembranosus proprius* (SP). Additionally, reference carcass and perirenal and scapular fat weights were recorded. Traits were measured at two commercial ages for rabbit carcasses, 9 and 13 wk. Our results suggest that there is a common genetic background for the IMF deposition and its composition in muscles with different metabolism profile in rabbits. Direct response to selection was 0.33 g of IMF/100 g of LD muscle, or 2.38 SD of the trait. A positive correlated response was observed in IMF of other muscles. The differences between high and low-IMF lines expressed in units of SD of the traits were 1.18 in BF, 1.27 in SS and 1.19 in SP at 9 wk, and all the differences between lines increased at 13 wk. Selection for IMF content in LD affected similarly the fatty acid composition of LD, BF and SS muscles, although the greatest differences between lines were observed in LD. High-IMF line showing greater monounsaturated (MUFA) but lower polyunsaturated (PUFA) fatty acid percentages than low-IMF line, whereas no differences between lines were observed for saturated fatty acids (SFA). Results were similar at 13 wk. Phenotypic correlations between IMF of muscles were positive, but low. Selection for IMF showed a positive and relevant correlated response in carcass fat depot weights.

Key words: correlated responses, intramuscular fat, muscles, selection.

1. INTRODUCTION

Intramuscular fat (IMF) is a main factor in meat quality because affects sensory, nutritional and technologic properties of meat (Wood et al., 2004). Intramuscular fat shows favorable characteristics for being improved by selection, such as its moderate variability and high heritability (Ciobanu et al., 2011 in pigs, Mateescu et al., 2015 in cattle and Martínez-Álvaro et al., 2016 in rabbits). Selection experiments for IMF are scarce (Sapp et al., 2002 in cattle; Zhao et al., 2007 in chickens and Schwab et al., 2009 in pigs). At the Universitat Politècnica de València we are performing a divergent

selection experiment for IMF in rabbits (Zomeño et al., 2013 and Martínez-Álvaro et al., 2016). Rabbit is a good model for genetic studies in other livestock species due to its short generation interval and the low cost of its carcasses.

Muscles with diverse metabolism profile show differences in meat quality (Wilson et al., 1976 in several species). In general, there is scarce information about the genetic mechanisms involved in IMF deposition in muscles with different metabolic profile. Only the selection experiment for IMF in chicken breast cited above (Zhao et al., 2007) studied the correlated response to selection for IMF in the hind leg, showing a positive correlated response. Other authors have suggested that IMF content and composition is regulated by QTL´s with different muscle-specific effects (Quintanilla et al., 2011 in pigs). To our knowledge, there is no information about the correlated responses to selection for IMF in several muscles with different metabolic profile.

The aim of this work is to study the correlated responses to selection for IMF of *Longissimus dorsi* (LD) in rabbits, in the IMF content and fatty acid composition of other muscles with different metabolic profile.

2. MATERIALS AND METHODS

2.1. Animals

This study was performed with rabbits from the sixth generation of a divergent selection experiment for IMF. The base population consisted in 13 males and 83 females coming from a synthetic rabbit line, and the following generations had 8 males and 40 females per line (high-IMF and low-IMF lines). Intramuscular fat was measured in LD muscle in rabbits slaughtered at 9 wk of age. Two rabbits (a male and a female) from the first parity of each doe were evaluated for IMF, and the average between these two phenotypic values was calculated. Then, all dams were ranked according to this average, and selection for high or low IMF was performed on rabbits from the second parity. All females of the approximately 20% best dams were selected for next generation. Each sire was mated with five does, and to reduce inbreeding only one male progeny of the sire, from highest ranked mate, was selected for the next generation. Lines selected for high-IMF and low-IMF were reared contemporary at the

farm of the Universitat Politècnica de València. The housing had a constant photoperiod of 16:8 h and controlled ventilation. Litters were homogenized by performing adoptions at birth up to 9 kits per litter. From weaning to slaughter, rabbits were reared collectively and fed *ad libitum*. More details of this experiment can be found in Zomeño et al. (2013) and Martínez-Álvaro et al. (2016).

All the rabbits from this study came from the first parity. A total of 134 rabbits were used to estimate the direct response to selection for IMF in LD muscle and the correlated responses in carcass and fat depot weights, pH and fatty acid composition of LD at 9 wk of age (68 from the high-IMF line and 66 from the low-IMF line). From those, a subsample of 60 animals (30 per line) was taken to study the correlated responses in IMF, fatty acid composition and pH of muscles with diverse metabolic profile: *Biceps femoris* (BF), *Supraspinatus* (SS) and *Semimembranosus proprius* (SP). Additionally, 51 rabbits (26 from the high-IMF line and 25 from the low-IMF line) were slaughtered at 13 wk of age to study the correlated responses to selection in previous carcass traits and meat quality traits in the four muscles (LD, BF, SS and SP). Animals were slaughtered at 9 or 13 wk of age by electrical stunning and exsanguination.

All experimental procedures involving animals were approved by the Universitat Politècnica de València Research Ethics Committee, according to council directives 98/58/EC (European Economic Community, 1998) and 2010/63/EU (European Commission Directive, 2010).

2.2. Intramuscular fat, pH and fatty acid measurements

Carcasses were chilled for 24 h at 4 ºC. The reference carcass weight (i.e., the weight of the carcass without the head, liver, lungs, thymes, esophagus, heart and kidneys) carcass was recorded according to the World Rabbit Science Association recommendations (Blasco and Ouhayoun, 1996). Perirenal and scapular fat depots were excised from the carcass and weighed. Muscles LD, BF, SS and SP were excised from the carcass and muscle pH was measured 24 h *post mortem* with a Crison pH-meter Basic +20 (Crison Instruments, Barcelona, Spain). In LD, pH was measured at the level of the fifth lumbar vertebra and in BF, SS and SP pH was measured in the central

area, always in the left muscles. Muscles LD, BF and SS were minced, freeze-dried and scanned with Near Infrarred Spectrophotometry (model 5000, FOSS NIRSystems INC., Hilleroed, Denmark). Intramuscular fat and saturated, (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acid contents were determined in g/100g of muscle on a fresh basis applying the calibration equations previously developed by Zomeño et al. (2011, 2012) with some modifications. Then, fatty acid groups SFA (C14:0 + C15:0 + C16:0 + C17:0 + C18:0), MUFA (C16:1n-9 + C18:1n-7 + C18:1n-9) and PUFA (C18:2n-6 + C18:3n-3 + C20:2n-6 + C20:3n-6 + C20:4n-6 + C20:5n-3 + C22:4n-6 + C22:5n-3 + C22:6n-3) were expressed as a percentage of total fatty acids. Because of the lack of enough sample, IMF content of SP muscle was quantified by ether extraction (Soxtec 1043 extraction unit, Tecator, Höganäs, Sweden), and was expressed in g of IMF/100g of muscle on a fresh basis.

2.3. Statistical analysis

Descriptive statistics of carcass and meat quality traits at 9 and 13 wk were estimated after correcting data by line, sex, and month-season fixed effects. Direct and correlated responses to selection were estimated as the phenotypic differences between high and low-IMF lines at 9 and 13 wk. All the differences were estimated with a model including line, sex and month-season fixed effects and common litter random effect. Phenotypic correlations between IMF of muscles were estimated with all data after correcting data by age, line, sex, and month-season fixed effects.

Bayesian inference was used (Blasco, 2005 and 2017). Common litter effect and residuals of the models were assumed to be independently normally distributed. Bounded flat priors were assumed for all fixed effects and variances. Marginal posterior distributions were estimated using Gibbs sampling. Descriptive statistics and differences between lines were performed with programme “Rabbit”, developed by the Institute for Animal Science and Technology (Valencia, Spain). After some exploratory analyses, results were based on Monte Carlo Markov chains runs consisting of 60,000 iterations, with a burn-in period of 10,000, and only one of every 10 samples were saved for inferences. Phenotypic correlations were computed with the software TM (Legarra et al., 2008). In this case, after some exploratory analyses

results were based on Monte Carlo Markov chains runs consisting of 1, 000,000 iterations, with a burn-in period of 200,000, and only one of every 100 samples were saved for inferences. Convergence was tested using the Z criterion of Geweke and Monte-Carlo sampling errors were computed using time-series procedures (Blasco, 2017).

The parameters obtained from the marginal posterior distributions of the differences between lines were: the median, the highest posterior density region at 95% ($HPD_{95\%}$) and the probability of the difference being greater than zero when the median is positive or lower than zero when the median is negative (P_0). Additionally, we considered 1/3 of the SD of a trait as a relevant value (r) and we calculated the probability of relevance (probability of the difference between lines being greater than r when the median is positive or lower than r when the median is negative) (P_r). From the marginal posterior distributions of the phenotypic correlations we estimated the median, $HPD_{95\%}$, P_0 and the guaranteed value with probability of 80% ($k_{80\%}$); i. e. limit of the interval [k, 1] with 80% probability. A more detailed description of these features can be found in Blasco (2001, 2005 and 2017).

3. RESULTS AND DISCUSSION

3.1. Descriptive statistics

Table 1 presents descriptive statistics of carcass traits at 9 and 13 wk of age. Since commercial carcass of rabbits varies between countries from 9 wk of age (Spain) to 13 wk of age (North of Italy), correlated responses to selection have been evaluated at both ages. Descriptive statistics are similar than those previously reported by Hernández et al. (2004) in rabbits at the same ages. Perirenal and scapular fat depots are the greater fat depots in rabbits (Hernández et al., 2006). The sum of both fat depots weights represented a 1.45% of the reference carcass weight at 9 wk, and 2.25% at 13 wk, showing the leanness of rabbit carcass, in comparison to carcasses from other meat species such as pig and beef (Lawrie and Leward, 2006).

Table 1. Descriptive statistics of carcass traits (g) at 9 and 13 wk of age.

	9 wk		13 wk	
Trait[1]	Mean	SD	Mean	SD
RCW	766	59.8	1262	91.9
SF	3.78	1.22	7.79	2.85
PF	7.48	2.32	20.6	7.13

[1]RCW, reference carcass weight; SF, scapular fat weight; PF, perirenal fat weight.

Table 2 presents descriptive statistics of meat quality traits in LD, BF, SS and SP muscles at 9 and 13 wk of age. These muscles were chosen due to their different physicochemical properties (see their pH in Table 2) and metabolic type, according to Delmas and Ouhayoun (1990). These authors reported a high glycolytic activity in LD and a high oxidative activity in SS and SP, whereas BF was intermediate between them. In our study, IMF was lower in glycolytic LD muscle than in SS, SP and BF. A greater lipid content in oxidative than in glycolytic muscles has been previously observed in rabbits (Alasnier et al., 1996 and Gondret et al., 1998) and pigs (Hernández et al., 1998). However, BF showed similar IMF than SS, despite its different metabolism. There are several studies supporting that the total lipid content is not strictly related to the metabolic type of fiber (Alasnier et al., 1996 in rabbits, and Leisegneur-Meyner and Gandemer, 1991 and Larzul et al., 1997 in pigs).

In LD, BF and SS muscles, SFA percentage ranged from 31.2 to 37.4, MUFA percentage from 20.8 to 32.1 and PUFA percentage from 31.9 to 42.4. The ratio between PUFA and SFA is a healthy indicator in diet and it is recommended to replace the intake of SFA by PUFA (World Health Organization, 2008). This ratio was 1.28 for SS, 1.15 for LD and 0.95 for BF at 9 wk, and 1.13 for SS, 0.98 for LD and 0.88 for BF at 13 wk. The three muscles showed PUFA:SFA ratios above nutritional recommendations of 0.60 (World Health Organization, 2008). In general, rabbit meat shows greater PUFA:SFA ratio than other species as pig, beef or lamb (Enser et al., 1996 and Dalle Zotte, 2002).

Table 2. Descriptive statistics of meat quality traits in several muscles at 9 and 13 wk of age.

		9 wk		13 wk	
Muscle	**Trait[1]**	**Mean**	**SD**	**Mean**	**SD**
Longissimus dorsi	**pH**	5.66	0.11	5.75	0.12
	IMF	1.05	0.14	1.42	0.17
	SFA	36.8	1.28	37.4	1.11
	MUFA	20.8	3.04	26.1	2.25
	PUFA	42.4	3.39	36.5	2.38
Biceps femoris	**pH**	5.81	0.10	5.86	0.12
	IMF	2.08	0.33	2.55	0.57
	SFA	35.8	1.07	36.3	0.77
	MUFA	29.9	2.17	31.8	1.62
	PUFA	34.2	2.43	31.9	1.73
Supraspinatus	**pH**	6.22	0.14	6.31	0.18
	IMF	2.13	0.35	2.91	0.58
	SFA	31.2	1.59	31.9	1.31
	MUFA	28.7	2.19	32.1	1.73
	PUFA	40.1	3.39	36.0	2.28
Semimembranosus proprius	**pH**	6.40	0.11	6.38	0.12
	IMF	2.64	0.51	4.18	0.55

[1]IMF; intramuscular fat expressed as g of IMF/100g muscle; Saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA) expressed as a percentage of total fatty acids.

3.2. Correlated responses to selection on carcass traits

Table 3 shows the differences between high and low-IMF lines for carcass traits at 9 and 13 wk of age. Comparisons should be made at the same state of maturity. High and low-IMF lines were at the same state of maturity along all their growing period (Pascual et al., 2015). High-IMF line showed relevantly greater perirenal fat weight at both ages (P_r = 1.00), whereas scapular fat weight was relevantly higher only at 9 wk (P_r = 0.99). Considering the low fat percentage of rabbit carcass, these results should not be a problem from a healthy or economical point of view. Other experiments of selection for IMF also showed positive correlated responses in the carcass fat content (Schwab et al., 2009 in pigs and Zhao et al., 2007 in broilers).

Correlated responses to selection for IMF in rabbits on fat depots and reference carcass weight have been previously discussed in Zomeño et al. (2013) and Martínez-Álvaro et al. (2016).

Table 3. Differences between high and low intramuscular fat lines for carcass traits (g) at 9 and 13 wk of age.

	9 wk						13 wk					
Trait[1]	**D**[2]	**HPD$_{95\%}$**[3]		**P$_0$**[4]	**r**[5]	**P$_r$**[6]	**D**[2]	**HPD$_{95\%}$**[3]		**P$_0$**[4]	**r**[5]	**P$_r$**[6]
RCW	53.8	29.7,	80.4	1.00	19.9	0.99	23.0	-39.9,	80.8	0.78	30.6	0.40
SF	1.00	0.45,	1.50	1.00	1.26	0.99	-0.05	-1.69,	1.65	0.48	2.60	0.14
PF	2.89	2.01,	3.90	1.00	2.49	1.00	9.31	5.30,	13.5	1.00	6.86	1.00

[1]RCW, reference carcass weight; SF, scapular fat weight; PF, perirenal fat weight; [2]D = median of the marginal posterior distribution of the difference between lines; [3]HPD$_{95\%}$ = highest posterior density region at 95% of probability; [4]P$_0$ = probability of the difference being greater than zero when D > 0 or lower than zero when D < 0; [5]r = relevant value, proposed as 1/3 of the standard deviation of the trait; [6]P$_r$ = probability of relevance (probability of the difference being greater than R when D > 0 or lower than R when D < 0.

3.3. Correlated responses to selection in several muscles

Table 4 shows the differences between high and low-IMF lines for IMF and fatty acid composition of LD, BF, SS and SP muscles at 9 wk of age. Direct response to selection on IMF of LD was large, 0.33 g of IMF/100g muscle (P$_r$ = 1.00), representing a 31.4% of the mean and 2.38 SD of the trait. Other selection experiments for IMF also reported great responses to selection (Sapp et al., 2002 in cattle, Zhao et al., 2007 in chickens and Schwab et al., 2009 in pigs). Correlated responses in IMF content of BF, SS and SP muscles were positive and relevant (P$_r$ = 1.00). The differences between high and low-IMF lines at 9 wk of age expressed in SD of the traits were 1.18 in BF, 1.27 in SS and 1.19 in SP. All the differences between lines increased at 13 wk (data not shown). Differences between lines at 13 wk were 0.71 g of IMF/100g in LD (which represents 4.12 SD of the trait), 0.92 g of IMF/100g in BF (1.60 SD), 0.83 g of IMF/100g in SSP (1.43 SD) and 1.76 g of IMF/100g in SP (3.20 SD). This is due to the later

development of IMF with respect to other tissues (Gondret et al., 1998 and Pascual et al., 2008, both in rabbits). Gondret et al. (1998) reported acceleration in the rate of intramuscular lipid deposition in rabbits around 14 wk of age, which was particularly large for SP in comparison to *Longissimus lumborun* and BF muscles. The pH value was not relevantly modified by selection in any muscle at any age. Schwab et al. (2009) found no effect of selection for IMF on pH in porcine.

Table 4. Differences between high and low intramuscular fat (IMF) lines for meat traits in several muscles at 9 wk of age.

Muscle	Trait[1]	D[2]	$HPD_{95\%}$[3]	P_0[4]	r[5]	P_r[6]
Longissimus dorsi	**pH**	0.06	0.01, 0.11	0.98	0.04	0.72
	IMF	0.33	0.27, 0.38	1.00	0.05	1.00
	SFA	0.67	0.30, 1.29	1.00	0.43	0.93
	MUFA	7.11	5.76, 8.49	1.00	1.01	1.00
	PUFA	-7.90	-9.41, -6.42	1.00	1.13	1.00
Biceps femoris	**pH**	0.02	-0.04, 0.08	0.77	0.03	0.62
	IMF	0.39	0.20, 0.60	1.00	0.11	1.00
	SFA	0.38	-0.33, 1.15	0.86	0.36	0.52
	MUFA	2.14	0.96, 3.39	1.00	0.72	0.99
	PUFA	-2.54	-3.95, -0.98	1.00	0.81	0.99
Supraspinatus	**pH**	0.08	-0.02, 0.18	0.94	0.05	0.72
	IMF	0.45	0.22, 0.68	1.00	0.12	1.00
	SFA	0.52	-0.42, 1.54	0.87	0.53	0.51
	MUFA	3.25	1.95, 4.74	1.00	0.73	1.00
	PUFA	-3.80	-6.02, -1.72	1.00	1.13	0.99
Semimembranosus proprius	**pH**	-0.05	-0.11, 0.02	0.94	0.04	0.62
	IMF	0.61	0.32, 0.89	1.00	0.17	1.00

[1]IMF; intramuscular fat expressed as g of IMF/100g muscle; Saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA) expressed as a percentage of total fatty acids. [2]D = median of the marginal posterior distribution of the difference between lines; [3]$HPD_{95\%}$ = highest posterior density region at 95% of probability; [4]P_0 = probability of the difference being greater than zero when D > 0 or lower than zero when D < 0; [5]r = relevant value, proposed as 1/3 of the standard deviation of the trait; [6]P_r = probability of relevance (probability of the difference being greater than R when D > 0 or lower than R when D < 0.

In general, selection for IMF affected similarly the fatty acid composition of LD, BF and SS muscles (Table 4). Selection for high IMF in LD relevantly increased MUFA

and decreased PUFA percentages in all muscles at 9 and 13 wk ($P_r \geq 0.99$, Table 4). Differences between lines were greater in LD than in BF and SS. These results were similar at 13 wk (data not shown). Correlated responses to selection in the SFA percentage did not shown a clear pattern; we did not find differences between lines for SFA percentage except in LD at 9 wk and in BF and SS at 13 wk, that was greater in high-IMF line with $P_r = 0.93$, 0.94 and 0.94, respectively.

This study provides strong evidence that selection for IMF in LD muscle affects positively the IMF content and fatty acid composition of other muscles with diverse metabolic profile. From the point of view of animal breeders, this is an interesting result, since selection for increasing IMF content in one muscle, leads to an improvement of the IMF content of several muscles at the same time. Likewise, changes in the fatty acid composition of IMF due to selection are similarly observed in several muscles, regardless of their metabolic profile. To our knowledge, only the selection experiment for IMF in chickens evaluated the correlated response in IMF of other muscle. In line with our results, they found a positive correlated response in the IMF of the hind leg when selecting for high IMF in breast (Zhao et al., 2007). In a commercial population of Duroc pigs, Quintanilla et al. (2011) observed that some lipid QTL maps were different between *Longissimus* and *Gluteus medius* muscles, suggesting that IMF content and composition of these two muscles were partially regulated by different genes.

Our results suggest a common genetic background for the IMF deposition and fatty acid composition in several muscles with different metabolism profile in rabbits. Few studies have reported genetic correlations between muscles, providing results in the same direction as our findings. In chickens, Zhao et al. (2006) reported a genetic correlation between IMF of breast muscle and IMF of hind leg of 0.89 In pigs, Ros-Freixedes et al., 2014 estimated the genetic correlation between IMF in LD and IMF in *Gluteus medius* muscles and it was 0.68 with $HPD_{95\%}$ [0.48, 0.87]. The genetic correlations between these two muscles for SFA, MUFA and PUFA percentages were also positive and high, ranging from 0.62 to 0.82 (Ros-Freixedes et al., 2014).

3.4. Correlations between IMF content of the muscles

Table 5 shows the phenotypic correlations between muscles for IMF. All the phenotypic correlations between IMF of LD, BF, SS and SP muscles were positive with P_0 = 1.00, but in general, they were low. The medians of the phenotypic correlations ranged between 0.24 and 0.52. The guaranteed values at 80% of probability of the correlations ranged from 0.16 to 0.45, being the greatest between LD and BF and the lowest between BF and SP. These results indicate that IMF phenotypes are not strictly controlled by the same environmental factors. To our knowledge, there are no previous estimates of correlations between IMF from different muscles of the carcass in rabbits. Phenotypic correlations between IMF of five different muscles in lambs were positive and variable, ranging from 0.30 to 0.75, the strongest correlations found between muscles located within the same region of the carcass (Anderson et al., 2015). In beef, IMF of LD was linearly related to IMF of fourteen muscles of the carcass, with R^2 ranging from 0.67 to 0.84 (Brackerbrush et al., 1991). In pigs, a significant and positive correlation for IMF between muscles *Longissimus* and *Gluteus medius* of 0.71 was reported by Quintanilla et al. (2011).

Table 5. Medians and highest posterior density regions at 95% of probability (in brackets) of the marginal posterior distributions of the phenotypic correlations[1] between intramuscular fat of muscles.

Muscles	***Biceps femoris***	***Supraspinatus***	***Semimembranosus proprius***
Longissimus dorsi	0.41 [0.25, 0.56]	0.46 [0.31, 0.60]	0.32 [0.13, 0.49]
Biceps femoris		0.52 [0.37, 0.65]	0.24 [0.06, 0.42]
Supraspinatus			0.49 [0.32, 0.63]

[1]All the phenotypic correlations between IMF content of muscles were positive with a probability of 1.00.

4. CONCLUSIONS

Our results show that there is a common genetic background for the IMF deposition and fatty acid composition in muscles with different metabolism type in rabbits. Selection for IMF in muscle LD at 9 wk of age showed a positive correlated response in IMF of other muscles with different metabolic profile (BF, SS and SP). Besides, all the differences between lines increased at 13 wk. Selection for IMF in LD affected similarly the fatty acid composition of LD, BF and SS muscles, high-IMF line showing greater MUFA but lower PUFA percentages than low-IMF lines, whereas no differences between lines were observed for SFA. Results at 13 wk were similar.

5. LITERATURE CITED

Alasnier, C., H. Rémington and G. Gandemer. 1996 Lipid characteristics associated with oxidative and glycolytic fibres in rabbit muscles. Meat Sci., 43:213-224.

Anderson, F., D.W. Pethick and G.E. Gardner. 2015. The correlation of intramuscular fat between muscles of the lamb carcass and the use of computed tomography to predict intramuscular fat percentage in lambs. Animal 9:1239-1249.

Blasco, A. and J. Ouhayoun. 1996 Harmonization of criteria and terminology in rabbit meat research. World Rabbit Sci. 4:93-99.

Blasco, A. 2001. The Bayesian controversy in animal breeding. J. Anim. Sci. 79:2023-2046.

Blasco, A. 2005. The use of Bayesian statistics in meat quality analyses: A review. Meat Sci. 69:115-122.

Blasco, A. 2017. Bayesian analysis for animal scientists. Springer, N.Y., USA

Brackebush, S.A., F.K. McKeith, T.R. Carr and D.G. McLaren. 1991. Relationship between *Longissimus* composition and the composition of other major muscles of the beef carcass. J.Anim.Sci. 69:631-640.

Burkett, J.L. 2009. The effect of selection for intramuscular fat on fatty acid composition in Duroc pigs. Graduate Theses and Dissertations. Paper 10539.

Ciobanu, D.C., S.M. Lonergan and E.J. Huff- Lonergan. 2011. Genetics of meat quality and carcass traits. In: M.F. Rothschild and A. Ruvinsky. The genetics of the pig. CAB Int. Oxfordshire (UK).pp. 355-389.

Dalle Zotte, A. 2002. Perception of rabbit meat quality and major factors influencing the rabbit carcass and meat quality. Livest. Prod. Sci. 75:11–32.

Delmas, D. and J. Ouhayoun. 1990. Technologie de l`abattage du lapin. I. Etude descriptive de la musculature. Viandes. Prod. Carnés 11:11-14.

Enser, M., K. Hallett, B. Hewitt, A.J. Fursey and J.D. Wood. 1996 Fatty acid content and composition of English beef, lamb and pork at retail. Meat Sci. 42:443-456.

European Economic Community. 1998. Council Directive 98/58/EC of 20 July 1998 concerning the protection of animals kept for farming purposes. Official Journal L, 221(8), 8.

European Commission Directive. 2010. Council, E. P. A. E. 2010/63/EU on the protection of animals used for scientific purposes. Institute for Health and Consumer Protection, Ispra, Italy, b7.

Gondret, F., J. Mourot and M. Bonneau. 1998. Comparison of intramuscular adipose tissue cellularity in muscles differing in their lipid content and fibre type composition during rabbit growth. Livest. Prod. Sci. 54:1-10.

Hernández, P., J.L. Navarro and F. Toldrá. 1998. Lipid composition and lipolytic enzyme activities in porcine skeletal muscles with different oxidative pattern. Meat Sci. 49:1-10.

Hernández, P., S. Aliaga, M. Pla and A. Blasco. 2004. The effect of selection for growth rate and slaughter age on carcass composition and meat quality traits in rabbits. J. Anim. Sci. 82:3138–3143.

Hernández, P., B. Ariño, A. Grimal and A. Blasco. 2006. Comparison of carcass and meat characteristics of three rabbit lines selected for litter size or growth rate. Meat Sci. 73: 645-650.

Lawrie, R.A. and D.A. Ledward. 2006. The structure and growth of muscle. In: Meat Science. 7th ed. Woodhead Publising limited, Cambrige, England. p. 41–73.

Legarra, A., L. Varona and E. López de Maturana. 2008. TM: Thershold model. http://snp.toulouse.inra.fr/~alegarra/manualtm.pdf.

Larzul, C., L. Lefaucheur, P. Ecolan, J. Gogué, A. Talmant, P. Sellier, P. Le Roy and G. Monin. 1997. Phenotypic and genetic parameters for *Longissimus* muscle fiber

characteristics in relation to growth, carcass and meat quality traits in large white pigs. J. Anim. Sci. 75:3126-3137.

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

Martínez-Álvaro, M., A. Blasco and P. Hernández. 2016. Divergent selection for intramuscular fat in rabbits: responses to selection and genetic parameters. J. Anim. Sci. 94: 4993-5003.

Mateescu, R.G. 2015. Genetics of meat quality. In: D.J. Garrick and A. Ruvinsky, editors. The genetics of cattle 2nd Edn. CAB Int, Oxfordshire, UK. pp. 544-570.

Pascual, M. de los D., M. Pla and A. Blasco. 2008. Effect of selection for growth rate on relative growth in rabbits. J. Anim. Sci. 86:3409-3417.

Pascual, M., de los D.E., W. Calle and A. Blasco. 2015. Comparison of degrees of maturity of rabbit lines selected for different traits. World Rabbit Sci. 23:155-161.

Quintanilla, R., R.N. Pena, D. Gallardo, A. Cánovas, O. Ramírez, I. Díaz, J.L. Noguera and M. Amilis. 2011 Porcine intramuscular fat content and composition are regulated by quantitative trait loci with muscle-specific effects. J. Anim. Sci. 89: 2963-2971.

Ros-Freixedes, R., J. Reixarch, L. Bosch, M. Tor and J. Estany. 2014. Genetic correlations of intramuscular fat content and fatty acid composition among muscles and with subcutaneous fat in Duroc pigs. J. Anim. Sci. 92:5417-5425.

Sapp, R.L., J.K. Bertrand, T.D. Pringle and D.E. Wilson. 2002. Effects of selection for ultrasound intramuscular fat percentage in Angus bulls on carcass traits of progeny. J. Anim. Sci. 80:2017–2022.

Schwab, C.R., T.J. Baas, K.J. Stalder and D. Nettleton. 2009. Results from six generations of selection for intramuscular fat in Duroc swine using real-time ultrasound. I. Direct and correlated phenotypic responses to selection. J. Anim. Sci. 87:2774–2780.

Wilson, B.R., A.M. Pearson and F.B. Shorland. 1976. Effect of total lipids and phospholipids on warmed-over flavour in red and white muscle from several species as measured by thiobarbituric acid analysis. J. Agric. Food Chem. 24:7-11.

Wood, J.D., R.I. Richardson, G.R. Nute, A.V. Fisher, M.M. Campo, E. Kasapidou, P.R. Sheard and M. Enser. 2004. Effects of fatty acids on meat quality: A review. Meat Sci. 66:21–32.

World Health Organization. 2008. Interim summary of conclusions and dietary recommendations on total fat and fatty acids. Joint FAO/WHO expert consultation on fats and fatty acids in human nutrition, 10-14.

Zhao, G.P., J. Wen, J.L. Chen, M.Q. Zheng and X. Li. 2006. Selection response and estimation of the genetic parameters for intramuscular fat in a quality chicken line. Acta Vet. Zootech Sinica 37: 870-873.

Zhao, G.P., J.L. Chen, M.Q. Zheng, J. Wen and Y. Zhang. 2007. Correlated responses to selection for increased intramuscular fat in a Chinese quality chicken line. Poult. Sci. 86:2309–2314.

Zomeño, C., P. Hernández and A. Blasco. 2011. Use of near infrared spectroscopy for intramuscular fat selection in rabbits. World Rabbit Sci. 19:203–208.

Zomeño, C., V. Juste and P. Hernández. 2012. Application of NIRS for predicting fatty acids in intramuscular fat of rabbit. Meat Sci. 91:155-159.

Zomeño, C., P. Hernández and A. Blasco. 2013. Divergent selection for intramuscular fat content in rabbits. I. Direct response to selection. J. Anim. Sci. 91: 4526-4531.

CHAPTER 4

Effect of divergent selection for intramuscular fat on sensory traits and instrumental texture in rabbit meat

The content of this chapter has been published in Journal of Animal Science. 94(12), 5137-5143.

ABSTRACT: Intramuscular fat (IMF) is one of the main parameters affecting meat quality. This work analyses the effect of selection for IMF on sensory attributes and instrumental texture parameters in rabbit meat. A total of 115 rabbits after six generations of divergent selection for IMF were slaughtered at 9 or 13 wk (57 and 58 animals, respectively). For each animal, left *Longissimus dorsi* (LD) muscle was analyzed by near infrared spectroscopy to measure IMF whereas right LD was used for the sensory or instrumental texture analysis. Sensory attributes measured were rabbit odor, liver odor, rabbit flavor, liver flavor, aniseed flavor, hardness, juiciness and fibrousness. The instrumental texture parameters maximum shear force, shear firmness and total work to cut the sample were measured by a Warner-Bratzler shear test. Ratios between lines (high IMF: low IMF line) and between ages (13 : 9 wk) were estimated. The line selected for high IMF showed 58% greater intramuscular fat than the line selected for low IMF. This divergence affected firmness that was 9.9% greater in the low-IMF line, although no effect was found for the other instrumental texture traits. No effect of selection was observed in any odor or flavor, except for aniseed flavor, that was greater in high-IMF line than in low-IMF line. Age had an effect on IMF, instrumental texture parameters and sensory attributes. Rabbits at 13 wk showed greater IMF, instrumental and sensory hardness, and more intense odor and flavor and lower juiciness than rabbits at 9 wk.

Key words: instrumental texture, intramuscular fat, rabbit, sensory analysis.

1. INTRODUCTION

Intramuscular fat (IMF) is one of the main traits affecting meat quality. Generally, IMF shows positive phenotypic correlations with sensory tenderness, juiciness and flavor (reviewed by Listrat et al., 2016) and negative phenotypic correlations with Warner-Bratzler shear force (Hocquette et al., 2010).

Intramuscular fat presents a high heritability in pigs (Ciobanu et al., 2011) and cattle (Mateescu, 2015) that favors its genetic selection. Only few experiments of selection for IMF have been published, in pigs (Schwab et al., 2009), chickens (Zhao et al., 2007) and cattle (Sapp et al., 2002). Selection for IMF did not change meat sensory attributes in the experiment in pigs (Schwab et al., 2009) but modified some

instrumental texture traits in the experiments in chickens (Zhao et al., 2007) and pigs (Schwab et al., 2009), and there is no information in the experiment in cattle. Currently, we are running a divergent selection experiment for IMF in rabbits. In the seventh generation of this experiment, the lines differed in 2.6 standard deviations of the trait (Martínez-Álvaro et al., 2016). Rabbit is a lean meat and this divergence in IMF by selection could modify its sensory quality. There are some studies comparing texture and sensory properties in rabbit breeds with different IMF (Gašperlin et al., 2006; Polak et al., 2006), but the genetic backgrounds of the groups compared in these studies are diverse. These breeds differ in a broad set of traits, which makes difficult to explain the observed differences. In our divergent selection experiment, sensory and instrumental texture traits can be compared in rabbit lines with the same genetic origin, only differing in IMF and correlated traits.

Intramuscular fat increases with age but also different biochemical changes in muscle occurs during growth (Lepetit, 2008). In rabbits, some studies compared commercial animals with minor differences in age, with no changes in instrumental texture or sensory properties (Xicatto et al., 1994; Gašperlin et al., 2006). Usually, commercial carcass of rabbits varies between countries from 9 to 13 wk of age. In our study we compare rabbit meat quality at these two commercial ages.

This work studies the effect of selection for IMF on sensory attributes and texture parameters in rabbit meat. Additionaly, we analysed the effect of age on these traits.

2. MATERIAL AND METHODS

2.1. Animals

This study was performed with rabbits from the sixth generation of a divergent selection experiment for IMF in *Longissimus dorsi* muscle (LD). Briefly, animals came from a synthetic rabbit line described in Zomeño et al., (2013). The base population consisted of 13 males and 83 females. Lines selected for high IMF and low IMF had approximately 8 males and 40 females per generation. Selection criterion was the average phenotypic value of IMF measured by near infrared reflectance spectroscopy (NIRS) in 2 full sibs of the candidate (a male and a female) at 9 wk of age, as described

in Zomeño et al., (2013). As a routine control, in each generation, around 20% of the total NIRS scanned samples were also chemically analysed for IMF (ether extraction with a previous acid hydrolysis) to confirm that NIRS predictions are in line with intramuscular fat chemical measurements. In six generations, a total of 2,137 rabbits were considered in the pedigree file, from which 1,184 were measured for IMF to estimate the selection response. In both lines, selection pressure on females was approximately 20% per generation. Males were chosen within male families to avoid inbreeding. Litters were homogeneized at birth up to 9 kits per litter. Rabbits were reared collectively from weaning to slaughter and were fed *ad libitum* with a commercial diet.

Fifty seven rabbits (29 from the high-IMF line and 28 from the low-IMF line) were slaughtered at 9 wk of age and 58 rabbits (29 per line) were slaughtered at 13 wk of age. All the rabbits came from the first parity. Live weights of the animals were 1,719 g and 2,148 g at 9 and 13 wk respectively. Animals were slaughtered using electrical stunning and exsanguination. After slaughter, carcasses were chilled for 24h at 4ºC. From each animal, both LD were excised. Right LD were vacuum packed and stored at -20ºC until instrumental texture or sensory analyses were performed, whereas left LD were reseved for IMF measurements.

All experimental procedures involving animals were approved by the Universitat Politècnica de València Research Ethics Committee, according to Council Directives 98/58/EC (European Economic Communityt, 1998) and 2010/63/EU (European Commision Directive, 2010).

2.2. Sensory analysis

A descriptive analysis (Lawless and Heymann, 2010) was carried out in 56 right LD, 14 for each selection line and age group. Muscles were thawed at 4ºC during 24 h in their plastic bag, cooked at 80ºC for 1h by immersion in a water bath with automatic temperature control (HS-B20, IKA Labortechnik, Staufen, Germany), as described by Ariño et al., (2007). Internal temperature of control samples was measured (72.9 ± 0.3 ºC) by penetration thermistor probes (Checktemp 1 Digital Thermometer - HI98509; Hanna Instruments, Vöhringen, Deutschland GmbH). Immediately after cooking, loins

were unpacked and cut lengthwise into four pieces (from caudal to cranial end), wrapped in aluminum foil and served hot using a heating equipment.

The sensory analysis was performed with 8 assessors trained in the evaluation of rabbit meat. Measured attributes are described in Table 1. Each assessor evaluated 4 samples per session (one per group of age and line) during 7 sessions, following a complete block design (Stell and Torrie, 1980). The samples were evaluated using a 10 cm unstructured continuous line, as recommended by the norm UNE-EN-ISO 4121:2006 (AENOR, 2006) and the distances (cm) from the left extreme to the evaluation mark were registered. Assesors tasted the samples with three-digit blinding codes under red colored lights to minimize bias, and the order of sample presentation was randomized (Macfie et al., 1989). Assessors were asked not to smoke, eat or drink anything except water for 1h before the evaluation sessions, and were provided with water and unsalted bread for cleansing their palate between samples. Evaluations were carried out in a standard laboratory according with the UNE 8589:2010 norm (AENOR, 2010).

Table 1. Definition of the eight sensory attributes included in the evaluation form.

Atribute	Definition
Rabbit odor	Intensity of characteristic odor of rabbit meat.
Liver odor	Characteristic odor of organs and blood of animals.
Rabbit flavor	The combination of taste, odor and tactile stimuli perceived retronasally during chewing – referring to the characteristic flavor of rabbit meat.
Liver flavor	The combination of taste, odor and tactile stimuli perceived retronasally during chewing – referring to the characteristic flavor of organs and blood of animals.
Aniseed flavor	The combination of taste, odor and tactile stimuli perceived retronasally during chewing – referring to the characteristic flavor of anise and grass.
Hardness	Force required to bite the meat sample with molar teeth during the firsts chewings.
Juiciness	Moisture perceived during chewing, from the moisture released by the sample and from the secreted saliva.
Fibrousness	Number and thickness of fibers perceived during chewing.

2.3. Instrumental texture analysis and cooking loss

Instrumental texture and cooking loss analyses were performed in 59 right LD, from rabbits slaughtered at 9 wk (15 from the high-IMF line and 14 from the low-IMF line) and from rabbits slaughtered at 13 wk (15 per line). Muscles were thawed and cooked as in the sensory analysis. After cooking, they were cooled at the fridge (4ºC) during 1h before the analysis. Muscles were weighed before and after cooking, and the cooking loss was calculated as the ratio (x100) of the difference in weight between the cooked and raw muscle relative to the weight of the raw muscle.

Instrumental texture was measured by a Warner-Bratzler shear test. Meat samples were obtained by cutting three prisms measuring 2 x 1 cm^2, with the muscle fiber parallel to the longitudinal axis. Maximum shear force (Moller, 1980), shear firmness (Brady and Hunecke, 1985) and total work to cut the sample were measured in a Texture Analyser model TA-XT2 (Stable Micro Systems, London, UK).

2.4. Intramuscular fat masurements by NIRS

Left LD muscles were ground, freeze-dried and scanned with NIRS (model 5000, FOSS NIRSystems INC., Hilleroed, Denmark) to measure IMF applying a calibration equation previously developed for measuring IMF content in rabbit meat (Zomeño et al., 2011, 2012). The calibration reported for IMF had a high precision and accuracy, according to the statistics R^2 (0.98) and residual predictive deviation (7.57).

2.5. Statistical analysis

To correct the assessor effect, sensory data were standardized subtracting the mean and dividing by the standard deviation of each assessor, as recommended by Næs et al., (2010). Descriptive statistics were performed after correcting data by fixed effects of their respective models, described below.

Intramuscular fat, instrumental texture parameters and cooking loss were analyzed fitting a model including line, age and sex as fixed effects and common litter as random effect, normally independently distributed. Sensory data were analyzed using a model including the same effects plus muscle location and session fixed effects. Residuals were assumed to be independently normally distributed.

A bayesian analysis was performed. Bayesian techniques have some advantages in comparison with classical methods. In a Bayesian context, the objective is to describe the uncertainty about the true value of some parameter, for example, the difference between two treatments, using probability as a measure of this uncertainty, allowing to give an exact value of the probability of the difference between treatments, for example 92%, which could be enough evidence for the researcher, without the need of using P-values and significance (Blasco, 2005).

Bayesian inference is based in probabilities, which gives a great flexibility to construct all kinds of confidence intervals. This allows us to ask questions that we could not ask within the classical inference approach. For example, in some cases it may be important to know how big the difference between treatments is, with a chosen probability. We can state that the difference between treatments takes at least a value k, with a probability of 80%, 95%, or the one we choose. This value k is a sort of 'guaranteed value' with a determined probability. It seems logical to use a value lower than 95% for two reasons, one is that k it is a "limit" value, not our estimate of the difference between treatments, and the other one is that an uncertainty of 80% seems to be good enough for the 'guaranteed values' of sensory analyses.

Bounded flat priors were assumed for all unknowns. Marginal posterior distributions were estimated using Gibbs Sampling and convergence was tested for each chain using the Z criterion of Geweke and Monte Carlo sampling errors were computed using time-series procedures (Sorensen and Gianola, 2002). Chains of 60, 000 samples with a burn-in period of 10, 000 were used and one sample each 10 was saved to avoid high correlations between consecutive samples. Rabbit programme, developed in the Insitute for Animal Science and Technolgy (Valencia, Spain) was used to solve the models.

To study the effects of selection and age, ratios between lines (high-IMF line: low-IMF line ratio) and age levels (13 wk : 9 wk ratio) were analyzed. This is easily done from the results of the Gibbs sampling chains and allows us to express the superiority of one treatment over another as a percentage. The features of the marginal posterior distributions calculated were: median of the ratio between levels; high posterior density interval at 95% of probability ($HPD_{95\%}$); probability (P_0) of the ratio > 1 when

the median is greater than one or < 1 when the median is lower than one; and the guaranteed value of a ratio with a probability of 80% ($k_{80\%}$), i.e. limit of the interval [k, +∞) when the median is greater than 1 or the limit of the interval [0, k] when the median is lower than 1. More details of these features can be found in Blasco (2005).

3. RESULTS AND DISCUSSION

3.1. Descriptive statistics

Table 2 shows descriptive statistics of the traits. The sensory attributes included in this study are commonly used in the sensory evaluation of rabbit meat (Hernández et al., 2005; Gašperlin et al., 2006; Polak et al., 2006). Other authors reported similar values for instrumental texture parameters (Ramírez et al., 2004; Ariño et al., 2006) and cooking loss (Bataglini et al., 1994) in rabbit loin.

Table 2. Descriptive statistics of intramuscular fat, instrumental texture parameters, cooking loss and sensory attributes of rabbit meat.

	n	Mean	CV x 100
Intramuscular fat, g/100g	115	1.19	15.5
Instrumental texture parameters			
Shear Force, kg	59	3.27	18.2
Firmness, kg/s	59	1.58	16.2
Total work, kg*s	59	4.88	24.3
Cooking loss, %	58	28.2	10.2
Sensory attributes			
Rabbit odor	56	4.53	21.6
Liver odor	56	1.67	58.0
Rabbit flavor	56	4.15	23.4
Liver flavor	56	2.17	46.1
Aniseed flavor	56	0.80	132.6
Hardness	56	4.50	20.0
Juiciness	56	3.60	24.2
Fibrousness	56	4.50	20.1

Cooking in plastic bags in water bath at 80ºC is a method widely used in rabbit meat to evaluate sensory traits as well as instrumental texture parameters, as pointed out by Combes et al., (2003). These authors studied the effect of cooking temperature

and cooking time on Warner-Bratzler tenderness measurement and collagen content in rabbit meat. They found no differences in Warner-Bratzler tenderness measurement (stress and total energy) between 20 and 60 minutes at 80ºC. Our shear force values (Table 2) are in line with the stress values found for these authors.

3.2. Effect of selection for IMF

Table 3 shows the effect of selection on instrumental texture parameters, cooking loss, sensory attributes and IMF.

Table 3. Features of the marginal posterior distributions of the ratio between the high-intramuscular fat (IMF) line and the low-IMF line for instrumental texture parameters, cooking loss and sensory attributes.

Traits	Median[1]	$HPD_{95\%}$[2]	P_0[3]	$k_{80\%}$[4]
Intramuscular fat	1.58	1.47, 1.69	1.00	1.53
Instrumental texture parameters				
Shear Force, kg	0.95	0.80, 1.10	0.76	
Firmness, kg/s	0.91	0.81, 1.00	0.97	0.95
Total work, kg*s	0.97	0.77, 1.18	0.62	
Cooking Loss	1.00	0.92, 1.09	0.51	
Sensory attributes				
Rabbit odor	1.02	0.96, 1.08	0.71	
Liver odor	0.99	0.82, 1.15	0.54	
Rabbit flavor	1.02	0.94, 1.09	0.67	
Liver flavor	1.01	0.87, 1.16	0.55	
Aniseed flavor	1.21	0.82, 1.65	0.85	1.03
Hardness	0.99	0.94, 1.05	0.59	
Juiciness	1.00	0.94, 1.07	0.55	
Fibrousness	1.01	0.95, 1.06	0.59	

[1]Median = median or the ratio between the high-IMF line and the low-IMF line; [2]$HPD_{95\%}$ = highest posterior density interval at a 95% of probability; [3] P_0 = probability of the high-IMF line: low-IMF line ratio > 1 when the median is greater than one or < 1 when the median is lower than 1; [4]$k_{80\%}$ = limit of the interval [k, +∞) when the median is greater than 1 and [0, k] when the median is lower than 1 at 80% of probability. It is only displayed when the median and $k_{80\%}$ are greater or lower than 1.

The high-IMF line showed 58% greater IMF than the low-IMF line. In Bayesian analysis, several confidence intervals can be easily estimated; for instance, we can state that the high-IMF line showed at least 53% greater IMF than the low-IMF line with 80% probability ($k_{80\%}$) (Blasco, 2005). Currently, there is no other experiment of selection for IMF in rabbits. In other species, experiments of selection for IMF showed high responses (Sapp et al., 2002 in cattle; Zhao et al., 2007 in chickens and Schwab et al., 2009 in pigs).

Firmness was 9.9% greater in the low-IMF than in the high-IMF line (P_0 = 0.97) whereas other instrumental and sensory texture parameters and cooking loss were similar in both lines. No effect of selection was observed in any odor or flavor, except for aniseed flavor, in which there is some evidence that this was greater in the high-IMF line than in the low-IMF line (P_0 = 0.85), although this is not a relevant attribute because all the scores were low. Our results are in agreement with other experiments of selection for IMF. Zhao et al., (2007) in chickens and Schwab et al. (2009) in pigs, observed lower instrumental toughness in the line selected for high IMF than in the control line. Besides, Schwab et al., (2009) in pigs, observed similar sensory hardness, juiciness and cooking loss and only greater pork flavor in the line selected for high IMF than in the control line. The experiment of selection for IMF in cattle did not report instrumental texture or sensory measurements (Sapp et al., 2002).

It is known that IMF increases meat tenderness because it is infiltrated in the connective tissue, weakening cross-linkages between collagen fibers (Essén-Gustavsson et al., 1994 in pigs and Nishimura et al., 1999 in cattle). Juiciness increases with IMF due to the stimulation of salivation during chewing of meat lipids (Lawrie and Ledward, 2006) and flavor is affected by IMF by the generation of volatile compounds from fat during cooking (Warris, 2010). However, these sensory effects are associated only to high levels of IMF (reviewed by Hocquette et al., 2010 and Listrat et al., 2016). Rabbit is a lean meat in comparison with other species (Valsta et al., 2005 in a review) and no wide ranges of variation for IMF can be found. Our results indicate that the IMF divergence between our lines affects firmness but did not lead to any change in the rest of instrumental texture and sensory attributes.

3.3. Effect of age

Table 4 shows the effect of age on measured traits. Rabbits slaughtered at 13 wk presented 30% greater IMF than rabbits slaughtered at 9 wk. In previous studies, older rabbits showed greater IMF, in line with our results (Gondret et al., 1998; Polak et al., 2006).

Table 4. Features of the marginal posterior distributions of the ratio between 13 wk and 9 wk of age (13:9wk) for intramuscular fat content, instrumental texture parameters, cooking loss and sensory attributes.

Traits	Median[1]	$HPD_{95\%}$[2]	P_0[3]	$k_{80\%}$[4]
Intramuscular fat	1.30	1.23, 1.37	1.00	1.27
Instrumental texture parameters				
Shear Force, kg	1.13	1.03, 1.22	1.00	1.09
Firmness, kg/s	1.00	0.92, 1.09	0.51	
Total work, kg*s	1.32	1.18, 1.48	1.00	1.26
Cooking Loss	0.93	0.88, 0.98	1.00	0.94
Sensory attributes				
Rabbit odor	1.09	1.03, 1.15	1.00	1.06
Liver odor	1.16	0.98, 1.35	0.96	1.08
Rabbit flavor	1.09	1.02, 1.15	1.00	1.06
Liver flavor	0.96	0.84, 1.08	0.76	
Aniseed flavor	2.21	1.43, 3.46	1.00	1.87
Hardness	1.11	1.06, 1.18	1.00	1.09
Juiciness	0.80	0.75, 0.85	1.00	0.83
Fibrousness	1.12	1.06, 1.19	1.00	1.10

[1]Median = median or the ratio between 13 wk and 9 wk of age; [2]$HPD_{95\%}$ = highest posterior density interval at a 95% of probability; [3] P_0 = probability of 13 : 9 wk ratio > 1 when the median is greater than one or < 1 when the median is lower than 1; [4]$k_{80\%}$ = limit of the interval [k, +∞) when the median is greater than 1 and [0, k] when the median is lower than 1 at 80% of probability. It is only displayed when the median and $k_{80\%}$ are greater or lower than 1.

Age had an effect on instrumental texture parameters and sensory attributes (Table 4). Thirteen wk old animals showed 13% greater shear force and 32% greater total work than 9 wk old animals (P_0 = 1.00), whereas firmness was similar at both

ages. Results in instrumental and sensory texture parameters were consistent. Rabbits at 13 wk of age were 11% harder, 12% more fibrous and 25% less juicy than rabbits at 9 wk of age (P_0 = 1.00). Regarding odor and flavor traits, older rabbits showed 9% greater intensity of rabbit odor and flavor and 16% greater liver odor than younger rabbits ($P_0 \geq 0.97$), whereas liver flavor was similar at both ages. Aniseed flavor was more than two times greater at 13 wk than at 9 wk, although scores were low in both cases.

In rabbits, Jehl and Juin (1999) observed more tenderness and juiciness and less meat odor in hind legs of younger animals when comparing 10 to 14 wk rabbits, in agreement with our results. In general, literature in other species shows that age increases meat toughness (Čandek-Potokar et al., 1999 in pigs and Baéza et al., 2012 in poultry) and decreases juiciness (Schönfeldt and Strydom, 2011 in beef and Pannier et al., 2014 in lamb). The effect of age on odor and flavor of meat does not present a general trend in literature, and there are studies reporting an increase (Madruga et al., 2000 in goat), decrease (Schönfeldt and Strydom, 2011 in beef) or no age effect (Baéza et al., 2012 in chickens and Shackelford et al., 1995 in beef) on these attributes.

The cooking loss was 7.5 % greater in 9 wk compared with 13 wk rabbits (P_0 = 1.00, Table 4). In agreement with our results, Rudolph and Fischer, (1979) observed greater cooking loss in 12 wk rabbits than in 14 wk rabbits, however the majority studies in rabbit did not find an effect of age in cooking loss (Osman, 1991; Bataglini et al., 1994; Xicatto et al., 1994; Trocino et al., 2015). In the literature from other species, results vary partly explained by the variability between studies in the cooking procedure and in the size and surface area of the meat sample.

4. CONCLUSIONS

High-IMF line showed greater intramuscular fat than the low-IMF line. This divergence affects firmness but did not lead to any change in the rest of instrumental texture and sensory attributes. Rabbits at 13 wk showed greater IMF, instrumental and sensory hardness, odor and flavor and lower juiciness than rabbits at 9 wk.

5. LITERATURE CITED

AENOR, Asociación Española de Normalización y Certificación. 2010. Norm UNE-EN ISO 8589:2010. Sensory analysis. General guidance for the design of test rooms (ISO 8589:2007). Asociación Española de Normalización y Certificación (AENOR) editors, Madrid, Spain.

AENOR, Asociación Española de Normalización y Certificación. 2006. Norm UNE-EN ISO 4121:2006. Sensory analysis. Guidelines for the use of quantitative response scales (ISO 4121:2003). Asociación Española de Normalización y Certificación (AENOR) editors, Madrid, Spain.

Ariño, B., P. Hernández, and A. Blasco. 2006. Comparison of texture and biochemical characteristics of three rabbit lines selected for litter size or growth rate. Meat Sci. 73:687–692.

Ariño, B., P. Hernández, M. Pla and A. Blasco. 2007. Comparison between rabbit lines for sensory meat quality. Meat Sci. 75:494–498.

Baéza, E., C. Arnould, M. Jlali, P. Chartrin, V. Gigaud, F. Mercerand, C. Durand, K. Méteau, E.Le Bihan-Duval, and C. Berri. 2012. Influence of increasing slaughter age of chickens on meat quality, welfare, and technical and economic results. J. Anim. Sci. 90:2003–2013.

Bataglini, B.M., C. Castellini, and P. Lattaioli. 1994. Rabbit carcass and meat quality: effect of strain, rabbitry and age. Ital. J. Food Sci. 2:157–166.

Blasco, A. 2005. The use of Bayesian statistics in meat quality analyses: A review. Meat Sci. 69:115-122.

Brady, P.L., and M.E. Hunecke. 1985. Correlations and instrumental evaluations of roast beef texture. J. Food Sci. 50:300–303.

Čandek-Potokar, M., L. Lefaucheur, B. Žlender, and M. Bonneau. 1999. Effect of slaughter weight and/or age on histological characteristics of pig *Longissimus dorsi* muscle as related to meat quality. Meat Sci. 52:195–203.

Ciobanu, D.C., S. M. Lonergan and E.J. Huff- Lonergan. 2011. Genetics of meat quality and carcass traits. In: M.F. Rothschild and A. Ruvinsky. The genetics of the pig. CAB Int. Oxfordshire (UK).pp. 355-389.

Combes, S., J. Lepetit, B. Darche and F. Lebas. 2003. Effect of cooking temperature and cooking time on Warner-Bratzler tenderness measurement and collagen content in rabbit meat. Meat Sci. 66:91-96.

Essén-Gustavsson, B., A. Karlsson, K. Lundström, and A.C. Enfält. 1994. Intramuscular fat and muscle fibre lipid contents in halothane-gene-free pigs fed high or low protein diets and its relation to meat quality. Meat Sci. 38:269–77.

European Economic Community. 1998. Council Directive 98/58/EC of 20 July 1998 concerning the protection of animals kept for farming purposes. Official Journal L, 221(8), 8.

European Commission Directive. 2010. Council, E. P. A. E. 2010/63/EU on the protection of animals used for scientific purposes. Institute for Health and Consumer Protection, Ispra, Italy, b7.

Gašperlin, L., T. Polak, A. Rajar, M. Skvarèa, and B. Lender. 2006. Effect of genotype, age at slaughter and sex on chemical composition and sensory profile of rabbit meat. World Rabbit Sci. 14:157–166.

Gondret, F., H. Juin, J. Mourot, and M. Bonneau. 1998. Effect of age at slaughter on chemical traits and sensory quality of *Longissimus lumborum* muscle in the rabbit. Meat Sci. 48:181–187.

Hernández, P., L. Guerrero, J.A. Ramírez, W. Mekkawy, M. Pla, B. Ariño, N. Ibáñez, and A. Blasco. 2005. A Bayesian approach to the effect of selection for growth rate on sensory meat quality of rabbit. Meat Sci. 69:123–127.

Hocquette, J.F., F. Gondret, E. Baéza, F. Médale, C. Jurie, and D. W. Pethick. 2010. Intramuscular fat content in meat-producing animals: development, genetic and nutritional control, and identification of putative markers. Animal 4:303-319.

Jehl, N., and H. Juin. 1999. Effet de l'âge d'abattage sur les qualités sensorielles de la viande de lapin. Cuniculture 148:171–174.

Lawless, H.T., and H. Heymann. 2010. Sensory evaluation of food – Principles and practices. Springer Science Business Media. New York. (USA).

Lawrie, R.A. and D.A. Ledward. 2006. The eating of meat quality. Chapter 10. Meat Science. Editors Lawrie, R.A. and Leward, D.A. 7th ed. Wooding Publishing Series in Food Science, Technology and Nutrition editor. Cambrige, England.

Lepetit, J. 2008. Collagen contribution to meat toughness: Theoretical aspects. Meat Sci. 80:960–967.

Listrat, A., B. Lebret, I. Louveau, T. Astruc, M. Bonnet, L. Lefaucheur, B. Picard, and J. Bugeon. 2016. Review article: How Muscle Structure and Composition Influence Meat and Flesh Quality. The Scientific World Journal. 2016: 1-14.

Macfie, H., N. Bratchell, K. Greenhoff, and L.Vallis. 1989. Designs to balance the effects of order of presentation and first-order carry-over effects in hall test. J. Sens. Stud. 4:129–148.

Madruga, M., S.G. Arruda, N. Narain, and J.Souza. 2000. Castration and slaughter age effects on panel assessment and aroma compounds of the “mestiço” goat meat. Meat Sci. 56:117–125.

Martínez-Álvaro, M., P. Hernández and A. Blasco. 2016. Selection on intramuscular fat in rabbits: direct and correlated responses. In: Proc. 67th EAAP Annual meeting. Belfast, UK. 29 Aug – 2 Sept 2016.p 489.

Mateescu, R.G. 2015. Genetics of meat quality. In: D.J. Garrick and A. Ruvinsky, editors. The genetics of cattle 2nd Edn. CAB Int, Oxfordshire, UK. p. 544-570.

Moller, A. J. 1980. Analysis of Warner-Bratzler shear pattern with regard to myofibrillar and connective tissue components of tenderness. Meat Sci. 5:247–260.

Næs, T., P. Brockhoff, and O. Tomic. 2010. Correction methods and other remedies for improving sensory profile data. In: John Wiley and Sons Publishers, Statistics for sensory and consumer science.Editors Næs, T., P. Brockhoff, and O. Tomic. 1st ed. West Sussex, United Kingdom. pp. 39–46.

Nishimura, T., A. Hattori, and K. Takahashi. 1999. Structural changes in intramuscular connective tissue during the fattening of Japanese Black cattle: Effect of marbling on beef tenderization. J. Anim. Sci. 77:93–104.

Osman, A.M.A. 1991. Effect of reducing feeding time on the growth performance, carcass traits and meat quality of growing rabbits. Arch. Geflügelk 55:196–200.

Pannier, L., G.E. Gardner, K.L. Pearce, M. McDonagh, A.J. Ball, R.H. Jacob, and D.W. Pethick. 2014. Associations of sire estimated breeding values and objective meat quality measurements with sensory scores in Australian lamb. Meat Sci. 96:1076–87.

Polak, T., L. Gašperlin, A. Rajar, and B. Žlender. 2006. Influence of genotype lines, age at slaughter and sexes on the composition of rabbit meat. Food Technol. Biotechnol. 44:65–73.

Ramírez, J.A., M.A. Oliver, M. Pla, L. Guerrero, B. Ariño, A. Blasco, M. Pascual, and M. Gil. 2004. Effect of selection for growth rate on biochemical, quality and texture characteristics of meat from rabbits. Meat Sci. 67:617–624.

Rudolph, W., and W. Fischer. 1979. Carcass quality of broiler rabbits aged 86 and 100 days. Arch. fur Tierzucht. 22:201–207.

Sapp, R.L., J.K. Bertrand, T.D. Pringle, and D.E. Wilson. 2002. Effects of selection for ultrasound intramuscular fat percentage in Angus bulls on carcass traits of progeny. J. Anim. Sci. 80:2017–2022.

Schönfeldt, H.C., and P.E. Strydom. 2011. Effect of age and cut on cooking loss, juiciness and flavour of South African beef. Meat Sci. 87:180–90.

Schwab, C.R., T.J. Baas, K.J. Stalder, and D. Nettleton. 2009. Results from six generations of selection for intramuscular fat in Duroc swine using real-time ultrasound. I. Direct and correlated phenotypic responses to selection. J. Anim. Sci. 87:2774–2780.

Shackelford, S.D., M. Koohmaraie, and T.L. Wheeler. 1995. Effects of slaughter age on meat tenderness and USDA carcass maturity scores of beef females. J. Anim. Sci. 73:3304–3309.

Sorensen, D. and D. Gianola. 2002. Likelihood, Bayesian, and MCMC methods in quantitative genetics. Springer, New York, NY.

Stell, R.G., and J.H. Torrie. 1980. Principles and procedures of statistics. McGraw-Hill, New York.

Trocino, A., E. Filiou, M. Tazzoli, M. Birolo, A. Zuffellato, and G. Xiccato. 2015. Effects of floor type, stocking density, slaughter age and gender on productive and qualitative traits of rabbits reared in collective pens. Animal 9:855–861.

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

Warris, P.D. 2010. Meat science: an introductory text. 2nd edition. CABI Publishing. Cambrige, England.

Xicatto, G., R. Parigi-Bini, A. Dalle Zotte, and A. Carazzolo. 1994. Effect of sex, age and trasportation on the composition and sensory prperties of rabbit meat. In: 40th Int. Cong. Meat Sci. Technol, The Hague, The Netherlands.pp 52.

Zhao, G.P., J.L. Chen, M. Q. Zheng, J. Wen, and Y. Zhang. 2007. Correlated responses to selection for increased intramuscular fat in a Chinese quality chicken line. Poult. Sci. 86:2309–2314.

Zomeño, C., P. Hernández, and A. Blasco. 2011. Use of near infrared spectroscopy for intramuscular fat selection in rabbits. World Rabbit Sci. 19:203–208

Zomeño, C., V. Juste and P. Hernández. 2012. Application of NIRS for predicting fatty acids in intramuscular fat of rabbit. Meat Sci. 91:155-159.

Zomeño, C., P. Hernández, and A. Blasco. 2013. Divergent selection for intramuscular fat content in rabbits. I. Direct response to selection. J. Anim. Sci. 91:4526–4531.

CHAPTER 5

Lipid metabolism in two rabbit lines divergently selected for intramuscular fat

I. Muscle metabolism in two rabbit lines divergently selected for intramuscular fat

II. Liver metabolism traits in two rabbit lines divergently selected for intramuscular fat

I. Muscle metabolism in two rabbit lines divergently selected for intramuscular fat

The content of the first part of this chapter has been accepted in *Journal of Animal Science*

ABSTRACT: A divergent selection experiment for intramuscular fat (IMF) of *Longissismus dorsi* muscle at 9 wk of age was performed in rabbits. The objective of this work was to compare the lipid metabolism in muscles and fat tissues of the high-IMF and low-IMF lines. Lipogenic, catabolic and lipolytic activities were studied in two muscles with different oxidative patterns (*Longissimus dorsi* and *Semimembranosus proprius*) and in perirenal fat depot at two ages, 9 wk and 13 wk. In addition, adipocytes were characterized in perirenal fat. In the fifth generation, direct response to selection was 0.26 g of IMF/100 g of muscle. Lines showed differences in their lipogenic activities of muscles and fat tissues at 13 wk, but not at 9 wk. High-IMF line showed greater glucose-6-P dehydrogenase (G6PDH), malic enzyme (EM) and fatty acid synthase (FAS) activities in *Longissimus dorsi* than low-IMF line with probabilities P_0 = 1.00, 0.93 and 0.90, respectively. Differences between lines were particularly great for G6PDH activity, representing 1.13 SD. High-IMF line also showed greater G6PDH and FAS activities in *Semimembranosus proprius* (P_0 = 0.98 for G6PDH and 0.95 for FAS) and perirenal fat (P_0 = 0.91 for G6PDH and 0.96 for FAS). However, in perirenal fat, EM activity was greater in the low-IMF line (P_0 = 0.90). No differences between lines were found almost in any catabolic or lipolytic activities of muscles. Regarding adipocyte characteristics, high-IMF line showed larger adipocytes in perirenal fat depot (P_0 = 0.97) tissue compared to the low-IMF line, but no differences between lines were observed in the number of adipocytes. This study cast light on the metabolic activities involved in the genetic differentiation on lipid deposition in rabbits. This study shows that lipogenic activities in muscles and fat tissues, in particular G6PDH in *Longissimus dorsi*, are involved in the lipid accumulation in muscle and adipose tissues.

Key words: intramuscular fat, lipid metabolism, rabbits.

1. INTRODUCTION

Intramuscular fat (IMF) plays an essential role in meat quality, affecting sensory and technologic meat properties (Wood et al., 2008). Intramuscular fat content can be easily modified by genetic selection due to its adequate variability and high heritability (Ciobanu et al., 2011 in pigs, Mateescu, 2015 in cattle and Martínez-Álvaro et al., 2016 in rabbits). However, only three selection experiments for IMF have been published

(Sapp et al., 2002 in cattle, Zhao et al., 2007 in chickens and Schwab et al., 2009 in pigs). In the Universitat Politècnica de València we are performing a divergent selection experiment for IMF of *Longissimus dorsi* muscle in rabbits (Zomeño et al., 2013 and Martínez-Álvaro et al., 2016). Generally, commercial carcass of rabbits varies between countries from 9 wk to 13 wk of age, showing greater IMF content at the elder age.

An increased IMF content has been related to greater lipogenic rate in muscle and changes in catabolic activities in several species, including rabbits (Hernández et al., 2008 and Zomeño et al., 2010), pigs (Mourot and Kouba, 1998, 1999 and Gondret and Lebret, 2007) and cattle (Bonnet et al., 2007 and Hocquette et al., 2012). In addition, differences in the amount of fat have been ascribed to differences in the size and/or number of adipocytes (Steele et al., 1973 and 1974 in pigs). In our divergent selection experiment lipid metabolism can be compared in rabbit lines with the same genetic origin and environment, differing only in IMF and correlated traits (such as perirenal fat weight, or IMF in several muscles, reported in Martínez-Álvaro et al., 2015 and 2016). None of the previous selection experiments for IMF analyzed the lipid metabolism of their lines.

Longissimus dorsi is a predominantly white fast twitch muscle. Selection for IMF on this muscle could affect in different ways the lipid metabolism of muscles having other oxidative patterns (as pure oxidative *Semimembranosus proprius* muscle) and adipose tissues. In this line, Quintanilla et al., (2011) suggested that IMF content is regulated by QTL´s with different muscle-specific effects. The objective of this work was to compare the lipid metabolism and adipocytes cellularity of muscles with diverse oxidative patterns and fat tissues at 9 and 13 wk of age in two rabbit lines divergently selected for IMF.

2. MATERIAL AND METHODS

2.1. Animals

All experimental procedures involving animals were approved by the Universitat Politècnica de València Research Ethics Committee, according to Council

Directives 98/58/EC (European Economic Community, 1998) and 2010/63/EU (European Commision Directive, 2010).

This study was performed with rabbits from the fifth generation of a divergent selection experiment for IMF of *Longissimus dorsi* muscle at 9 wk of age. Animals came from a synthetic rabbit line. The base population consisted in 13 males and 83 females. Lines selected for high and low IMF had 8 males and 40 females per generation. Intramuscular fat content was evaluated in two rabbits (a male and a female) from the first parity of each doe, and the average between these two phenotypic values was calculated. Then, all dams were ranked according to this average, and selection for high or low IMF was performed on rabbits from the second parity. All females of the approximately 20% best dams were selected for next generation. Each sire was mated with five does, and to reduce inbreeding only one male progeny of the sire, from highest ranked mate, was selected for breeding the next generation. This selection within male family was performed in order to reduce inbreeding. Normally, the first parity was used to collect the IMF data and the second parity to select the rabbits for next generation, although some exceptions were made resulting in some IMF measurement collection during the second or third parity. More details of this experiment can be found in Zomeño et al. (2013) and Martínez-Álvaro et al. (2016). Litters were homogenised at birth up to 9 kits per litter. Rabbits were reared collectively from weaning to slaughter and were fed *ad libitum* with a commercial diet. The housing had a constant photoperiod of 16:8 h and controlled ventilation.

In the fifth generation, direct response to selection was estimated using 202 rabbits (100 from the high-IMF line and 102 from the low-IMF line). Between them, 160 came from the first parity, 24 from the second parity and 18 from the third parity. Rabbits were slaughtered at 9 wk using electrical stunning and exsanguination. After slaughter, carcasses were chilled for 24h at 4ºC. Then, *Longissimus dorsi* muscle was excised, minced, freeze-dried and scanned with Near Infrarred Spectrophotometry (model 5000, FOSS NIRSystems INC., Hilleroed, Denmark) to measure IMF using the prediction equations developed by Zomeño et al. (2011 and 2012). Intramuscular fat was expressed as g/100 g of muscle on a fresh basis.

Enzyme activities were measured in an additional sample of 110 rabbits, 62 slaughtered at 9 wk (32 from high-IMF and 30 from low-IMF line) and 48 slaughtered at 13 wk (24 per line). All of these rabbits came from the first parity. Adipocytes were characterized in another additional sample of 45 rabbits, 23 slaughtered at 9 wk (10 from high-IMF and 13 from low-IMF line) and 22 slaughtered at 13 wk (10 from high-IMF and 12 from low-IMF line), all of them from the third parity. Animals used for enzymatic assays and adipocytes measurements were slaughtered as described above. Immediately after slaughter, hot carcass weight was registered according to the norms proposed by the World Rabbit Science Association (Blasco and Ouhayoun, 1996), and *Longissimus dorsi* and *Semimembranosus proprius* muscles and perirenal fat depot were excised and weighed. For enzymatic assays, samples of the three tissues were frozen in liquid nitrogen and stored at −80°C. For adipocytes measurements, samples of 0.5 g of perirenal fat were collected immediately after slaughter and placed in tubes with 10 mL of Tyrode's solution with a of pH 7.6 (0.15 M NaCl; 6 mM KCl; 2mM $CaCl_2$; 6 mM glucose; 2 mM $NaHCO_3$) at 39 ºC.

2.2. Measurement of enzyme activities

Activities of lipogenic enzymes glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme (EM) and fatty acid synthase (FAS) were measured in *Longissimus dorsi* and *Semimembranosus proprius* muscles and perirenal fat. A quantity of tissue (1 g for *Longissimus dorsi* and perirenal fat and 0.5 g for *Semimembranosus proprius*) was homogenized in a volume of ice-cold 0.25 M sucrose solution containing 1mM dithiothreitol and 1mM EDTA (2.5 mL for muscles and 5 mL for perirenal fat). Homogenates were centrifuged at 12,000 x *g* for 1 h at 4 ºC and supernatants were filtered through glass wool and collected for enzyme assays. Activities were assessed at 37 ºC using a spectrophotometer (model UV-1601, Shimadzu Co, Tokyo, Japan) at 340 nm according to the methods described by Fitch et al. (1959) for G6PDH, Hsu and Lardy (1969) for EM and Chang et al. (1967) for FAS. Enzyme activities were expressed in nmol of NADPH produced for G6PDH and EM or oxidized for FAS/min and g of fresh tissue.

Activities of catabolic enzymes β-hydroxyacyl-CoA dehydrogenase (HAD), citrate synthase (CS) and lactate dehydrogenase (LDH) were determined in *Longissimus dorsi* and *Semimembranosus proprius* muscles. Samples of 0.2 g of muscle tissue were homogeneized in 50 vol (wt/vol) of ice-cold 0.1 M phosphate buffer (pH 7.5) and 2mM EDTA. Homogenates were centrifuged at 6,000 x *g* for 15 min at 4 ºC and the resulting supernatants were filtered as described above. Enzymatic activities were assessed at 30 ºC in a spectrophotometric analyzer (Fluostar Galaxy, BMG Lab Technologies, Offenburg, Germany) at 340 nm for HAD and LDH and at 405 nm for CS according to the methods described by Bass et al. (1969); Srere (1969) and Bergmeyer and Bernt (1974), respectively. Enzyme activities were expressed as µmol of NADH for HAD and LDH or of mercaptide ion for CS released/min and g of fresh tissue.

Lipolytic enzyme activities of acid lipase (AL), neutral lipase (NL) and acid phospholipase (APL) were assayed on *Longissimus dorsi* muscle, according to the method described by Hernández et al. (1999) using 4-methylumbelliferyloleate as the fluorescent substrate. Lipolytic activity was measured only in *Longissimus dorsi* because of the lack of *Semimembranosus proprius* sample remaining after other analyses. A sample of 4 g of *Longissimus dorsi* muscle was homogenized in 20 mL of 50 mM phosphate buffer (pH 7.5) containing 5 mM ethylene glycol tetraacetic acid. The homogenate was centrifuged at a 10,000 x *g* for 20 min at 4 ºC, and the resulting supernatant was filtered as described above. Reaction mixtures of lipase assays with fluometric substrates were incubated at 37 ºC for 20 min. The fluorescence was measured at an excitation wavelength of 460 nm using a Fluostar Galaxy fluometer (BMG Lab Technologies, Offenburg, Germany). Enzyme activities were expressed as µmol of substrate hydrolyzed/h and g of fresh tissue.

Soluble protein was determined from supernatant in muscles *Longissimus dorsi* and *Semimembranosus proprius* using the bicinchoninic acid (BCA) Protein Assay Kit (Smith et al., 1985) provided by Pierce (Rockford, Illinois, United States), and enzyme activities were also expressed in a soluble-protein content basis.

2.3. Charcaterization of adipocytes

Perirenal fat samples underwent digestion with collagenase (Robdell, 1964). Approximately 100 mg of each sample was digested with 0.1 mg of collagenase type 2, 4 mg of bovine serum albumin and 100 µl of T199 medium at 39 °C for 1 h. After digestion, a drop of the superficial phase was taken to prepare slides for microscope examination. Images obtained with the microscope were digitized and analyzed using the image analysis software ImageJ (U.S. National Institutes of Health, Bethesda, Maryland, USA). Adipocytes diameter was determined from 300 cells and the average diameter was calculated per sample. Adipocytes with diameters under 20 µm were excluded from the count. Lipid content was determined in the resting tissue by ether extraction (Soxtec 1043 extraction unit, Tecator, Höganäs, Sweden) and was expressed as g/100 g of fresh tissue. Number of adipocytes per g of tissue was calculated by dividing the lipid content in a g of sample by the lipid content of one adipocyte (assuming a lipid density value of 0.915 g/mL and cells to be spherical in shape). Total number of adipocytes was calculated by multiplying the number of adipocytes per g of tissue by the weight of the tissue.

2.4. Statistical analysis

Descriptive statistics of the traits at 9 and 13 wk of age were performed after correcting data by line, sex and parity order effects. Since data of the selection trait was collected during a long period of time, the effect of month-season was included in the analysis. For enzymatic activities and adipocyte characteristics the effect of parity order was not included in the analysis since animals came from the same parity. For adipocyte characteristics and lipid content, records at 9 and 13 wk were analyzed together, correcting data by the effect of age.

The effect of selection was estimated as the differences between high-IMF and low-IMF lines at 9 and 13 wk. Models included fixed effects of line, sex, month-season and parity order as indicated before, and common litter as a random effect. For adipocyte characteristics, differences between lines were estimated with all data, including the additional fixed effect of age. We also estimated the differences between ages were for enzymatic activities using all data and including the effect of age.

A Bayesian analysis was performed. Common litter effect and residuals of the models were assumed to be independently normally distributed. Bounded flat priors were assumed for all fixed effects and variances (Blasco, 2001, 2005 and 2017). Marginal posterior distributions were estimated using Gibbs sampling, testing the convergence for each chain with the Z criterion of Geweke, and Monte Carlo sampling errors were computed using time-series procedures (Sorensen and Gianola, 2002). Chains of 60,000 samples with a burn-in of 10,000 were used. The programme "Rabbit" developed by the Institute for Animal Science and Technology (Valencia, Spain) was used for the analysis. The parameters obtained from the marginal posterior distributions of the differences were the median (D), the highest posterior density region at 95% ($HPD_{95\%}$) and the probability of the lines being different, calculated as the probability of the line difference being greater than zero given the absolute value of D (P_0). More details of these Bayesian parameters can be found in Blasco (2001, 2005).

3. RESULTS

3.1. Direct response to selection and correlated responses in carcass traits

In the fifth generation, the mean of the selection trait, IMF of *Longissimus dorsi* muscle at 9 wk, was 1.03 g of IMF/100 g of muscle with a SD of 0.15. Direct response to selection estimated as the differences between high and low-IMF lines was 0.26 g of IMF/100 g of muscle (P_0 = 1.00) with a $HPD_{95\%}$ from 0.21 to 0.31.

Table 1 shows descriptive statistics and differences between lines in carcass traits at 9 and 13 wk of age. Perirenal fat weight represented a low percentage of the hot carcass weight (0.77% at 9 wk and 1.41% at 13 wk). Perirenal fat weight was greater in high-IMF than in low-IMF line at 13 wk of age (P_0 = 1.00) but no difference was observed between lines at 9 wk. The difference between lines at 13 wk represented a 0.43% of the hot carcass weight. No differences between lines were observed in hot carcass and muscle weights at any age, except for hot carcass weight at 13 wk which was greater in the low-IMF than in the high-IMF line (P_0 = 0.91).

Table 1. Descriptive statistics and differences between lines in carcass traits (g) at 9 and 13 wk of age.

	9 wk						13 wk					
Trait[1]	**Mean**	**SD**	**D**[2]	**HPD$_{95\%}$**[3]		**P$_0$**[4]	**Mean**	**SD**	**D**[2]	**HPD$_{95\%}$**[3]		**P$_0$**[4]
HCW	1022	90.5	-10.1	-74.9,	53.6	0.62	1645	137	-43.6	-110,	17.9	0.91
LDW	84.7	10.2	1.85	-6.59,	10.5	0.67	166	15.7	-4.07	-12.7,	5.53	0.81
SPW	2.06	0.27	0.00	-0.21,	0.24	0.50	3.72	0.47	-0.13	-0.35,	0.09	0.88
PF	7.91	3.18	0.80	-3.22,	4.01	0.67	23.3	8.57	6.62	2.96,	10.2	1.00

[1]HCW = hot carcass weight; LDW = *Longissimus dorsi* weight; SPW = *Semimembranosus proprius* weight; PF = perirenal fat weight; [2]D = median of the marginal posterior distribution of the difference between high-intramuscular fat (IMF) and low-IMF lines; [3]HPD$_{95\%}$ = highest posterior density region at 95% of probability; [4]P$_0$ = probability of the difference being greater than zero when D > 0 or lower than zero when D < 0.

3.2. Lipogenic enzyme activities in muscles and perirenal fat

Table 2 shows descriptive statistics and differences between lines in lipogenic activities in muscles and perirenal fat at 9 and 13 wk in nmol/min*g of tissue. *Semimembranosus proprius* muscle showed greater G6PDH and FAS activities and less EM activity compared to *Longissimus dorsi* at both 9 and 13 wk. However, EM activity was greater in *Semimembranosus proprius* when the results were expressed in a soluble-protein basis, due to the low protein content of this muscle (data not shown). Perirenal fat tissue showed greater G6PDH and FAS activities and lower EM activity than muscles at both ages.

All the lipogenic activities were greater at 13 than 9 wk in the three tissues. The differences between 13 and 9 wk of age in nmol/min*g of tissue were 11.6 for

Table 2. Descriptive statistics and differences between lines in lipogenic[1] activities measured in several tissues at 9 and 13 wk of age.

		9 wk					13 wk				
Tissue	**Trait**	**Mean**	**SD**	**D[2]**	**HPD$_{95\%}$[3]**	**P$_0$[4]**	**Mean**	**SD**	**D[2]**	**HPD$_{95\%}$[3]**	**P$_0$[4]**
***Longissimus dorsi* muscle**	**G6PDH**	119	32.0	-5.81	-24.5, 10.6	0.74	130	29.7	33.4	12.1, 52.8	1.00
	EM	484	167	-42.3	-170, 83.3	0.76	740	205	96.8	-39.0, 219	0.93
	FAS	12.7	5.66	-1.26	-6.53, 4.61	0.68	16.9	6.60	3.56	-2.53, 8.77	0.90
***Semimembranosus proprius* muscle**	**G6PDH**	287	84.4	-37.8	-105, 26.7	0.88	425	123	76.6	2.62, 153	0.98
	EM	378	87.6	-58.6	-118, -0.03	0.98	414	99.4	28.3	-35.1, 89.3	0.82
	FAS	63.2	18.4	8.12	-8.02, 23.9	0.85	87.9	25.5	15.7	-2.49, 33.0	0.95
Perirenal fat depot	**G6PDH**	765	224	35.1	-132, 217	0.66	940	322	138	-70.3, 336	0.91
	EM	175	67.6	-9.98	-58.5, 35.1	0.67	251	83.7	-34.1	-84.7, 18.6	0.90
	FAS	265	72.8	-14.6	-79.2, 64.0	0.65	314	85.0	56.4	-6.25, 117	0.96

[1]Activities of the lipogenic enzymes glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme (EM) and fatty acid synthase (FAS) in nmol/min*g of tissue; [2]D = median of the marginal posterior distribution of the difference between high-intramuscular fat (IMF) and low- IMF lines; [3]$HPD_{95\%}$ = highest posterior density region at 95% of probability; [4]P_0 = probability of the difference being greater than zero when $D > 0$ or lower than zero when $D < 0$.

Table 3. Descriptive statistics and differences between lines in catabolic[1] activities measured in muscles at 9 and 13 wk of age.

		9 wk					13 wk				
Tissue	**Trait**	**Mean**	**SD**	**D[2]**	**HPD$_{95\%}$[3]**	**P$_0$[4]**	**Mean**	**SD**	**D[2]**	**HPD$_{95\%}$[3]**	**P$_0$[4]**
***Longissimus dorsi* muscle**	**HAD**	1.47	0.34	0.25	-0.05, 0.53	0.96	1.43	0.38	0.13	-0.14, 0.39	0.83
	CS	3.46	0.67	0.44	-0.29, 1.25	0.88	3.39	0.85	-0.13	-0.83, 0.49	0.66
	LDH	857	182	40.7	-122, 194	0.69	1139	255	-38.0	-192, 120	0.70
	AL	0.65	0.10	0.01	-0.06, 0.07	0.58	0.54	0.08	-0.01	-0.07, 0.06	0.57
	NL	4.04	0.87	0.22	-0.28, 0.68	0.81	3.18	0.58	0.13	-0.33, 0.65	0.71
	APL	0.38	0.07	0.01	-0.03, 0.06	0.72	0.31	0.07	0.00	-0.05, 0.04	0.57
***Semimembranosus proprius* muscle**	**HAD**	2.80	0.63	0.28	-0.21, 0.82	0.86	2.65	0.59	-0.15	-0.64, 0.31	0.72
	CS	5.43	1.05	0.14	-0.58, 0.85	0.65	5.10	0.98	-0.48	-1.17, 0.27	0.90
	LDH	47.7	13.2	0.30	-9.01, 8.49	0.52	49.9	13.1	1.06	-7.46, 9.95	0.60

[1]Activities of the enzymes β-hydroxyacyl-CoA dehydrogenase (HAD), citrate synthase (CS) and lactate dehydrogenase (LDH) in µmol/min*g of tissue. Acid lipase activity (AL), neutral lipase activity (NL) and acid phospholipase activity (APL) in µmol/h*g of tissue; [2]D = median of the marginal posterior distribution of the difference between high-intramuscular fat (IMF) and low-IMF lines; [3]HPD$_{95\%}$ = highest posterior density region at 95% of probability; [4]P$_0$ = probability of the difference being greater than zero when D > 0 or lower than zero when D < 0.

G6PDH (P_0 = 0.96), 265 for EM (P_0 = 1.00) and 4.77 for FAS (P_0 = 0.99) in *Longissimus dorsi* muscle; 139 for G6PDH (P_0 = 1.00), 38.2 for EM (P_0 = 0.96) and 24.8 for FAS (P_0 = 1.00) in *Semimembranosus proprius* muscle and 175 for G6PDH (P_0 = 1.00), 72.5 for EM (P_0 = 1.00) and 52.6 for FAS (P_0 = 0.99) in perirenal fat.

Lines showed differences in their lipogenic activities at 13 wk but not at 9 wk of age. At 13 wk, high-IMF line showed greater G6PDH (P_0 = 1.00), EM (P_0 = 0.93) and FAS (P_0 = 0.90) activities in *Longissimus dorsi*, and greater G6PDH (P_0 = 0.98) and FAS (P_0 = 0.95) activities in *Semimembranosus proprius* than the low-IMF line, whereas there was less evidence for the difference between lines in EM activity in this muscle (P_0 = 0.82). Similar results were obtained when lipogenic activities were expressed in a soluble-protein basis for muscles tissues (data not shown). In perirenal fat, high-IMF had greater G6PDH (P_0 = 0.91) and FAS (P_0 = 0.96) activities, but lower EM activity than the low-IMF line (P_0 = 0.90). At 9 wk, we did not observe differences between lines in lipogenic activities, except for EM activity in *Semimembranosus proprius* that was greater in the low-IMF line than in the high-IMF line (P_0 = 0.98).

3.3. Catabolic enzyme activities in muscles

Table 3 shows descriptive statistics and differences between lines in oxidative and glycolytic (expressed in µmol/min*g of tissue) and lipolytic (in µmol/h*g of tissue) activities in *Longissimus dorsi* and *Semimembranosus proprius* muscles at 9 and 13 wk of age. *Longissimus dorsi* muscle had greater LDH activity, whereas *Semimembranosus proprius* showed greater HAD and CS activities. Similar results were obtained when activities were expressed in a soluble-protein basis (data not shown). Oxidative and glycolytic activities were similar at both ages, except for HAD activity in *Semimembranosus proprius* that decreased with age; the difference between ages (13 wk minus 9 wk) in HAD activity was -0.22 µmol/min*g of tissue (P_0 = 0.93). Lipolytic activities in *Longissimus dorsi* decreased with age (P_0 = 1.00); the differences between ages (13 wk minus 9 wk) in µmol/h*g of tissue were -0.11 for AL (P_0 = 1.00), -0.89 for NL (P_0 = 1.00) and -0.07 for APL (P_0 = 1.00).

We almost did not find differences between lines in the catabolic activities of muscles. Few differences between lines were observed in oxidative activities, although

results were not consistent between muscles or ages. High-IMF line showed greater HAD activity in *Longissimus dorsi* only at 9 wk (P_0 = 0.96) compared to the low-IMF line which showed greater CS activity in *Semimembranosus proprius* only at 13 wk (P_0 = 0.90). We did not observe differences between lines in LDH or lipolytic activities. Results did not change when activities were expressed in a soluble-protein basis (data not shown).

3.4. Adipocytes characteristics in perirenal fat

Table 4 shows descriptive statistics and differences between lines in adipocyte characteristics of perirenal fat. The diameter of adipocytes was greater in the high-IMF than in the low-IMF line (P_0 = 0.97). In contrast, we did not find differences between lines in the number of adipocytes.

Table 4. Descriptive statistics and differences between lines in adipocyte characteristics in perirenal fat depot.

Trait	Mean	SD	D[1]	HPD$_{95\%}$[2]	P_0[3]
Adipocyte diameter, µm	96.5	13.4	11.0	-0.37, 23.3	0.97
Total nº adipocytes (x10^6)	29.3	12.2	-2.35	-14.7, 8.50	0.67

[1]D = median of the marginal posterior distribution of the difference between high-intramuscular fat (IMF) and low-IMF lines; [2]HPD$_{95\%}$ = highest posterior density region at 95% of probability; [3]P_0 = probability of the difference between lines being greater than zero when D > 0 or lower than zero when D < 0.

4. DISCUSSION

Direct response to selection represented 1.7 SD of the trait, 24.5% of the mean, and a genetic progress of approximately 5% of the mean per generation. Other selection experiments for IMF in pigs (Schwab et al., 2009), chickens (Zhao et al., 2007) and cattle (Sapp et al., 2002) also obtained great direct responses to selection. Selection for IMF showed a positive correlated response in perirenal fat weight at 13 wk of age, but we did not find differences at 9 wk. This result could be a sampling

effect; when considering the animals of the whole generation (n = 202), perirenal fat weight was 2.39 g greater in high-IMF line compared to low-IMF line (P_0 = 1.00), with 95% confidence interval [1.25, 3.47]. A positive genetic correlation between IMF and perirenal fat was estimated with data from these selection lines (0.33, Martínez-Álvaro et al., 2016). A positive correlated response to selection for IMF in carcass fat has been observed in pigs (Schwab et al., 2009) and chickens (Zhao et al., 2007), but not in the selection experiment in cattle (Sapp et al., 2002) where selection was performed only during one generation.

In the fifth generation, the difference between lines for IMF at 9 wk in *Longissimus dorsi* was 0.26 g of IMF/100 g muscle. Additionally, a previous study within these selection lines showed a positive correlated response in the IMF of *Semimembranosus proprius* at 9 wk of age (Martínez-Álvaro et al., 2015). However, differences between lines in IMF at 9 wk were not explained by differences in lipogenic activities in muscle tissues with different oxidative patterns. The lack of lines differences in several enzymatic activities should be taken with caution because of large $HPD_{95\%}$. Gondret et al. (1997) pointed out that intramuscular adipose tissue displays a slower rate of development compared to other adipose tissues in rabbits. Differences in IMF at 9 wk of age could be related to lipogenic activity in liver, which is the major lipogenic tissue in growing rabbits (Leung and Bauman, 1975 and Vézinhet and Nouguès, 1977).

At 13 wk of age the IMF differences between lines were greater than at 9 wk in *Longissimus dorsi* and *Semimembranosus proprius* (Martínez-Álvaro et al., 2015), which corresponds well with the lines differences observed in lipogenic activities. Differences expressed in units of SD allow comparing their magnitude. Lines especially differed in the G6PDH activity in *Longissimus dorsi*. In this muscle, differences between high and low-IMF lines were 1.13 SD for G6PDH, 0.47 SD for EM and 0.54 SD for FAS. The enzyme G6PDH is involved in the pentose phosphate pathway and produces NADPH required for *de novo* synthesis of fatty acids. Our results indicate that G6PDH activity must be explaining at least part of the genetic variability on IMF deposition in rabbits. This enzyme has also been related to marbling in cattle (Bonnet et al., 2007). In *Semimembranosus proprius* muscle, G6PDH and FAS activities were affected by

selection in a similar way with differences between lines at 13 wk of 0.62 SD for G6PDH and FAS activities.

In perirenal fat, differences between high and low-IMF lines at 13 wk were 0.43 SD for G6PDH, -0.41 SD for EM and 0.67 SD for FAS. In this tissue, the greater G6PDH and FAS activities in the high-IMF line were consistent with its greater perirenal fat weight at 13 wk. Malic enzyme activity was greater in the low-IMF than in the high-IMF line. However, perirenal fat showed greater G6PDH than EM activity, suggesting that G6PDH is the main supplier of NADPH to this tissue in rabbits (Table 2), as previously observed in cattle (Bonnet et al., 2007) and pigs (Ramírez et al., 2007). At 9 wk, we did not find differences between lines in perirenal fat weight in this sample, which could explain the lack of differences observed in their lipogenic activities.

This was the first work that studied the lipogenic activities of muscles and fat depots in animals divergently selected for IMF. Steele et al., 1972 studied the effect of divergent selection for backfat thickness in pigs and found greater EM and G6PDH activities in the subcutaneous fat tissue of the fat line. Several studies have related greater lipogenic activities or lipogenic gene expressions to greater IMF in rabbits (Zomeño et al., 2010), pigs (Mourot and Kouba, 1998, 1999 and Ramírez et al., 2007) and cattle (Bonnet et al., 2007 and Ward et al., 2010). In addition, greater lipogenic activities have been also related to greater carcass fat depots in pigs (Hood and Allen et al., 1973; Mourot et al., 1996 and Ramírez et al., 2007) and cattle (Bonnet et al., 2007). However, all these studies compared breeds with different genetic backgrounds, whereas in our study animals shared the same genetic origin, only differing in IMF and correlated traits.

Longissimus dorsi and *Semimembranosus proprius* muscles showed different oxidative patterns (Table 3). In this experiment, catabolic activities did not explain the differences between lines in IMF in either muscle studied. In other selection experiments for IMF, catabolic activities of muscles were not measured. In several species, greater fatty acid oxidation has been related to lower IMF (Kim et al., 2000 in humans, Young et al., 2002 in rats, Gondret and Lebret, 2007 in pigs, Zomeño et al., 2010 in rabbits and Hocquette et al., 2012 in cattle). The LDH enzyme is involved in the

ATP production from glucose in muscle. This study did not show any relationship between IMF and LDH activity. Previous studies in rabbits (Zomeño et al., 2010) and cattle (Hocquette et al., 2012) also did not find any relationship between LDH activity and IMF. On the other side, lipolytic enzymes in muscle degrade IMF releasing free fatty acids for metabolism requirements (Zechner et al., 2012). However, in this study lipolytic activities were not related to IMF. The relationship between lipolytic activity in muscle and IMF is not clear. Some studies have related greater IMF deposition to greater lipolytic activities (Hernández et al., 2008 in rabbits) or greater expression of genes involved in the lipolysis (Cánovas et al., 2010 in pigs), while other studies have related it to lower lipolytic activities (Cava et al., 2004 in pigs, Zomeño et al., 2010 in rabbits) or lower lipolysis genes expressions (Jeong et al., 2012 in cattle). In two lines of pigs divergently selected for backfat thickness, lipolysis could not explain the different fat deposition between lines (Mersmann et al., 1985). However, all these studies are developed with animals from different genetic origins, selected for different criterions or subjected to different feeding treatments, but none of them have evaluated animals that differ exclusively in IMF and correlated traits. Thus, comparisons with our results should be taken with caution.

Due to the large variability of the adipocytes measurements and to the low number of samples per each age-line group (n = 10), the $HPD_{95\%}$ of the differences between lines at 9 and 13 wk were very large and we were not able to make any statement (data not shown). Analyzing the effect of selection pooling samples from both ages (n = 20) led to higher accurate estimations of the differences between lines (Table 4). The greater carcass fat deposition of the high-IMF line can be ascribed to larger adipocytes in perirenal fat tissues with respect to the low-IMF line. Larger adipocytes can be related with the greater lipogenic activity on this tissue in the high-IMF line. A greater adipocyte volume has been closely related to greater lipogenic activities in pigs (Lee and Kauffman, 1974) and to greater lipogenic genes expression in cattle (Baik et al., 2014). We did not observe differences between lines in the number of adipocytes; however, this trait showed a great variation in comparison with adipocytes diameter, and differences were estimated with low accuracy (i.e., large $HPD_{95\%}$). Previous selection experiments for IMF did not study the adipocyte

characteristics of their lines. In the divergent selection experiment of backfat thickness in pigs, the fat line had greater size and number of adipocytes than the lean line in fat depots in animals slaughtered at 100 days (Steele et al., 1974); although adipocyte size was concluded to be more related to total carcass fat (Steele et al., 1973).

This study casts light on the metabolic activities involved in the genetic differentiation on lipid deposition in rabbits. Differences between lines after five generations of selection for IMF were partially explained by differences in the lipogenic activities in muscles with diverse oxidative pattern at 13 wk, but not at the selection age (9 wk). Particularly, lines showed a greater difference in the G6PDH activity of *Longissimus dorsi* muscle at 13 wk. In contrast, the different IMF deposition of the lines was not explained by different catabolic activities in muscles. For perirenal fat weight, differences between lines at 13 wk correspond to differences in G6PDH and FAS activities in this tissue. The greater perirenal fat weight of the high-IMF with respect to the low-IMF line appeared to be the result of larger adipocytes, whereas we did not find differences between lines in the number of adipocytes.

5. LITERATURE CITED

Baik, M., J.Y. Jeong, T.T.T. Vu, M.Y. Piao and H.J. Kang. 2014. Effects of castration on the adiposity and expression of lipid metabolism genes in various fat depots of Korean cattle. Livest. Sci. 168:168-176.

Bass, A., D. Brdiczka, P. Eyer, S. Hofer and D. Pette. 1969. Metabolic differentiation of distinct muscle types at the level of enzymatic organization. Eur. J. Biochem. 10:198–206.

Bergmeyer, H.U. and E. Bernt. 1974. Lactate dehydrogenase: UV-assay with pyruvate and NADH. In: N. Academic Press, New York, editor. Methods of Enzymatic Analysis. pp. 574–579.

Blasco, A. and J. Ouhayoun. 1996. Harmonization of Criteria and Terminology in Rabbit Meat Research. Revised Proposal. World Rabbit Sci. 4:93–99.

Blasco, A. 2001. The Bayesian controversy in animal breeding. J. Anim. Sci. 79:2023-2046.

Blasco, A. 2005. The use of Bayesian statistics in meat quality analyses: A review. Meat Sci. 69:115-122.

Bonnet, M., Y. Faulconnier, C. Leroux, C. Jurie, I. Cassar-Malek, D. Bauchart, P. Boulesteix, D. Pethick, J. F. Hocquette and Y. Chilliard. 2007. Glucose-6-phosphate dehydrogenase and leptin are related to marbling differences among Limousin and Angus or Japanese Black x Angus steers. J. Anim. Sci. 85:2882–2894.

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

Cava, R., J.M. Ferrer, M. Estévez, D. Morcuende and F. Toldrá. 2004. Composition and proteolytic and lipolytic enzyme activities in muscle *Longissimus dorsi* from Iberian pigs and industrial genotype pigs. Food Chem. 88: 25-33.

Chang, H.C., I. Seidman, G. Teebor and M.D. Lane. 1967. Liver acetyl CoA carboxylase and fatty acid synthetase: Relative activities in the normal state and in hereditary obesity. Biochem. Biophys. Res. Commun. 28:682–686.

Ciobanu, D.C., S. M. Lonergan and E.J. Huff- Lonergan. 2011. Genetics of meat quality and carcass traits. In: M.F. Rothschild and A. Ruvinsky. The genetics of the pig. CAB Int. Oxfordshire (UK).pp. 355-389.

European Economic Community. 1998. Council Directive 98/58/EC of 20 July 1998 concerning the protection of animals kept for farming purposes. Official Journal L, 221(8), 8.

European Commission Directive. 2010. Council, E. P. A. E. 2010/63/EU on the protection of animals used for scientific purposes. Institute for Health and Consumer Protection, Ispra, Italy, b7.

Fitch, W.M., R. Hill and I.L. Chaikoff. 1959. The effect of fructose feeding on glycolytic enzyme activities of the normal rat liver. J. Biol. Chem. 234:1048–1051.

Gondret, F., J. Mourot and M. Bonneau. 1997. Developmental changes in lipogenic enzymes in muscle compared to liver and extramuscular adipose tissues in the rabbit (Oryctolagus cuniculus). Comp. Bioch. Physiol. B Biochem. Mol. Biol. 117: 259-265.

Gondret, F. and B. Lebret. 2007. Does feed restriction and re-alimentation differently affect lipid content and metabolism according to muscle type in pigs (Sus scrofa)? Comp. Bioch. Physiol. A: Mol. Int. Phys., 147:375-382.

Hernández, P., J.L. Navarro and F. Toldrá. 1999. Effect of frozen storage on lipids and lipolytic activities in the *Longissimus dorsi* muscle of the pig. Zeitschrift Leb. und forsch. A 208:110–115.

Hernández, P., V. Cesari, and A. Blasco. 2008. Effect of genetic rabbit lines on lipid content, lipolytic activities and fatty acid composition of hind leg meat and perirenal fat. Meat Sci. 78:485–491.

Hocquette, J.F., I. Cassar-Malek, C. Jurie, D. Bauchart, B. Picard and G. Renand. 2012. Relationships between muscle growth potential, intramuscular fat content and different indicators of muscle fibre types in young Charolais bulls. Anim. Sci. J. 83, 750-758.

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

Hsu, R.Y., and H.A. Lardy. 1969. Malic enzyme. In: N.Y.N. Academic Press, editor. Methods in enzymology. p. 230–235.

Jeong, J., E.G. Kwon, S.K. Im, K.S. Seo, and M. Baik. 2012. Expression of fat deposition and fat removal genes is associated with intramuscular fat content in *Longissimus dorsi* muscle of Korean cattle steers. J. Anim. Sci. 90:2044-2053.

Kim, J.Y., R.C. Hickner, R.L. Cortright, G.L. Dohm and J. Houmard. 2000. Lipid oxidation is reduced in obese human skeletal muscle. Am. J. Physiol. Endocrinol. Metab. 279:E1039-E1044.

Leung, T.T. and D.E. Bauman. 1975 In vivo studies of the site of fatty acid synthesis in the rabbit. Int. J. Biochem. 6:801-805.

Martínez-Álvaro, M., V. Juste, A. Blasco and P. Hernández. 2015. Response to selection for intramuscular fat content and correlated responses in several muscles in rabbits. Proc. 66th Ann. Meet. of EAAP. Warsaw (Poland), 31 Aug. - 4 Sept.

Martínez-Álvaro, M., A. Blasco and P. Hernández. 2016. Divergent selection for intramuscular fat in rabbits: responses to selection and genetic parameters. J. Anim. Sci. 94: 4993-5003.

Mateescu, R.G. 2015. Genetics of meat quality. In: D.J. Garrick and A. Ruvinsky, editors. The genetics of cattle 2nd Edn. CAB Int, Oxfordshire, UK. pp. 544-570.

Mersmann, H.J. 1985. Adipose tissue lipolytic rate in genetically obese and lean swine. J. Anim. Sci. 60(1):131-135.

Mourot, J., M. Kouba and M. Bonneau. 1996. Comparative study of in vitro lipogenesis in various adipose tissues in the growing meishan pig: comparison with the large white pig (Sus domesticus). Comp. Biochem. Physiol. Part B Biochem. Mol. Biol. 115:383–388.

Mourot, J. and Kouba, M. 1998. Lipogenic enzyme activities in muscle of growing Large White and Meishang pigs. Livest. Prod. Sci. 55:127-133.

Mourot, J. and M. Kouba. 1999. Development of intra- and intermuscular adipose tissue in growing Large White and Meishang pigs. Reprod. Nutr. Dev. 39: 125-132.

Ramírez, M. R., D. Morcuende and R. Cava. 2007. Fatty acid composition and adipogenic enzyme activity of muscle and adipose tissue, as affected by Iberian×Duroc pig genotype. Food Chem. 104:500–509.

Robdell, M. 1964. Metabolism of isolated fat cells: I. Effects of hormones on glucose metabolism and lipolysis. J. Biol. Chem. 239:375–80.

Sapp, R. L., J. K. Bertrand, T. D. Pringle, and D. E. Wilson. 2002. Effects of selection for ultrasound intramuscular fat percentage in Angus bulls on carcass traits of progeny. J. Anim. Sci. 80:2017–2022.

Schwab, C. R., T. J. Baas, K. J. Stalder, and D. Nettleton. 2009. Results from six generations of selection for intramuscular fat in Duroc swine using real-time ultrasound. I. Direct and correlated phenotypic responses to selection. J. Anim. Sci. 87:2774–2780.

Smith, P. K., R. I. Krohn, G. T. Hermanson, A. K. Mallia, F. H. Gartner, M. D. Provenzano, E. K. Fujimoto, N. M. Goeke, B. J. Olson, and D. C. Klenk. 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. 150:76–85.

Sorensen, D.A. and D. Gianola. 2002. Likelihood, Bayesian, and MCMC methods in quantitative genetics. Springer, New York, NY.

Srere, P. A. 1969. Citrate synthase. In: N. Academic Press, New York, editor. Methods in Enzymology. Vol. 13. Academic Press. 3–11.

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

Steele, N.C., L.T. Frobish and M. Keeney. 1973. Adiposity and metabolic activity in adipose tissue of swine selected for backfat thickness: age constant. J. Anim. Sci. 37:367-367.

Steele, N.C., L.T. Frobish and M. Keeney. 1974. Lipogenesis and cellularity of adipose tissue from genetically lean and obese swine. J. Anim. Sci. 39(4):712-719.

Vézinhet, A. and J. Nouguès. 1977. Evolution postnatale de la lipogenèse dans le tissu adipeux et le foie du mouton et du lapin. Ann. Biol. Anim. Bioch. Biophys. 5B:851-863.

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

Wood, J.D., M. Enser, A.V. Fisher, G.R. Nute, P.R. Sheard, R.I. Richardson, S.I. Hughes and F.M. Whittington. 2008. Fat deposition, fatty acid composition and meat quality: a review. Meat Sci. 78:343-358.

Young, M.E., P.H. Guthrie, P. Razeghi, B. Leighton, S. Abbasi, S. Patil, K.A. Youker and H. Teagtmeyer. 2002 Impaired long-chain fatty acid oxidation and contractile dysfunction in the obese zucker rat heart. Diabetes 51:2587-2595.

Zechner, R., R. Zimmermann, T.O. Eichmann, S.D. Kohlwein, G. Haemmerle, A. Lass and F. Madeo. 2012. Fat signals- lipases and lipolysis in lipid metabolism and signaling. Cell Metab. 15:279-291.

Zhao, G.P., J.L. Chen, M.Q. Zheng, J. Wen and Y. Zhang. 2007. Correlated responses to selection for increased intramuscular fat in a chinese quality chicken line. Poult. Sci. 86:2309–2314.

Zomeño, C., A. Blasco and P. Hernández. 2010. Influence of genetic line on lipid metabolism traits of rabbit muscle. J. Anim. Sci. 88:3419–3427.

Zomeño, C., P. Hernández and A. Blasco. 2011. Use of near infrared spectroscopy for intramuscular fat selection in rabbits. World Rabbit Sci. 19:203–208.

Zomeño, C., V. Juste and P. Hernández. 2012. Application of NIRS for predicting fatty acids in intramuscular fat of rabbit. Meat Sci. 91:155-159.

Zomeño, C., P. Hernández and A. Blasco. 2013. Divergent selection for intramuscular fat content in rabbits. I. Direct response to selection. J. Anim. Sci. 91: 4526-4531.

II. Liver metabolism traits in two rabbit lines divergently selected for intramuscular fat

---

The content of the second part of this chapter has been submitted to *Animal.*

ABSTRACT: Intramuscular fat (IMF) has a large effect in the sensory properties of meat because affects tenderness, juiciness and flavor. A divergent selection experiment for IMF in *Longissimus dorsi* (LD) muscle was performed in rabbits. As liver is the major site of lipogenesis in rabbits, the objective of this work is to study the liver metabolism in the two rabbit lines selected for high and low IMF. Intramuscular fat content, perirenal fat weight, liver weight, liver lipogenic activities and plasma parameters related to liver metabolism were measured in the eight generation of selection. Direct response on IMF was 0.34 g /100 g of LD, which represented 2.7 SD of the trait, and selection showed a positive correlated response in the perirenal fat weight. High-IMF line showed greater liver size and greater liver lipogenic activities of enzymes glucose-6-phosphate dehydrogenase (G6PDH) and malic enzyme (EM), although the difference between lines for EM activity was not relevant, whereas we did not find differences between lines for fatty acid synthase (FAS) lipogenic activity. With regard to plasma parameters, low-IMF line showed greater plasma concentration of triglycerides, cholesterol, bilirubin and alkaline phosphatase than high-IMF line, whereas high-IMF line showed greater albumin and alanine transaminase concentrations, and all the differences between lines were relevant except for cholesterol concentration. We did not observe differences between lines for glucose, total protein and plasma concentrations. Phenotypic correlations between fat (IMF and perirenal fat weight) and liver traits suggested that liver lipogenesis affects fat deposition in both, muscle and carcass. None of the plasma parameters measured was strongly correlated with IMF.

Keywords*:* intramuscular fat, liver, metabolism, genetic selection, rabbits.

1. INTRODUCTION

Intramuscular fat (**IMF**) has a large effect in the sensory properties of meat. A high IMF content is associated with tender, juicy and flavorful meat (Wood et al., 2008). Intramuscular fat can be easily modified by genetic selection due to its high heritability (Martínez-Álvaro et al., 2016) and variability. However, there are only three selection experiments for IMF (Schwab et al., 2009 in pigs, Sapp et al., 2002 in cattle and Zhao et al., 2007 in chickens). In the Universitat Politècnica de València we are

performing a divergent selection experiment for IMF in *Longissimus dorsi* (LD) muscle in rabbits at 9 wk of age (Martínez-Álvaro et al., 2016). Rabbit is an excellent animal model for genetic experiments due to its short generation interval and the low cost of its carcasses.

Lipid deposition in muscle depends on a reciprocal balance between lipogenic and catabolic fatty acid fluxes. Lipogenic enzymes glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme (EM) and fatty acid synthase (FAS) play a key role in fatty acid biosynthesis. Enzyme FAS catalyzes the conversion of acetyl- and malonyl-coenzyme-A to palmitate in presence of NADPH, generated by EM and G6PDH (Hsu et al., 1965). Liver tissue is a major site of lipogenesis in some species as rabbits (Leung and Bauman, 1975), chickens (O`Hea and Leveille, 1969) and rats (Ballard et al., 1969). Differences on lipogenic activities in liver could lead to differences in IMF, as previously observed in chickens (Cui et al., 2012), and to differences in fat depots, as previously observed in rats (Turkenkopf et al., 1980 and Smith et al., 1980) and pigs (Muñoz et al., 2013). Circulating plasma concentrations of glucose, lipids, proteins or bilirubin give an overall view of the liver function, and enzymes as transaminases and alkaline phosphatase are indicators of damage in hepatocytes (Ghany and Hoofnagle, 2012). Some of these plasma parameters result from the lipogenic metabolism, and could be related with IMF deposition.

The objective of this work is to study the liver lipogenic activities and plasma parameters related to liver in two rabbit lines divergently selected for IMF.

2. MATERIALS AND METHODS

2.2. Animals

A divergent selection experiment for IMF in LD was performed in rabbits. A male and a female from the first parity of each doe were slaughtered at 9 wk of age and evaluated for IMF, and the average between these two values was calculated. Then, all dams were ranked according to this average, and selection for high or low IMF was performed on rabbits from the second parity. All females of the approximately 20% best dams were selected for next generation. As each sire was

mated with five dams, only one male of its best dam was selected. This selection within male family was performed in order to reduce inbreeding. Normally, the first parity was used to collect the IMF data and the second parity to select the rabbits for next generation, although exceptionally some IMF measurements were made on the second or third parity. Lines selected for high-IMF and low-IMF were reared contemporary at the farm of the Universitat Politècnica de València. The housing had a constant photoperiod of 16:8 h and controlled ventilation. Litters were homogenized by performing adoptions at birth up to 9 kits per litter. From weaning to slaughter, rabbits were reared collectively and fed *ad libitum*. More details of this experiment can be found in Martínez-Álvaro et al. (2016).

This study was performed with 175 rabbits from the eighth generation of this selection experiment, 83 from the high-IMF line and 92 from the low-IMF line. Body weight (BW) was recorded at 9 wk of age. Then, all rabbits fasted at least 19 h before slaughtering by electrical stunning and exsanguination. Carcasses were chilled for 24 h at 4 ºC and the weight of the chilled carcass was recorded. Perirenal fat depot was excised from the carcass and weighed. Muscle LD was excised, minced, freeze-dried and scanned with Near Infrarred Spectrophotometry (model 5000, FOSS NIRSystems INC., Hilleroed, Denmark). Intramuscular fat was determined in g/100g of muscle applying the calibration equations previously developed by Zomeño et al. (2011, 2012).

A subsample of 63 rabbits (30 from the high-IMF and 33 from the low-IMF line) was taken to study the liver lipogenic activity and plasma parameters. Animals were slaughtered as described before. Blood samples were collected at slaughter from the jugular vein in 1 ml lyophilized lithium heparin (0, 04 mg/ml) tubes (TapVal Aquisel, Barcelona, Spain) and plasma was prepared by centrifugation at 3000 rpm for 10 min and then stored at -80ºC. Liver was dissected from the carcass and weighed immediately after slaughter. A liver sample was frozen in liquid nitrogen, vacumm packed and stored at -80ºC for lipogenic enzyme assays.

All experimental procedures involving animals were approved by the Universitat Politècnica de València Research Ethics Committee, according to council directives 98/58/EC (European Economic Community, 1998) and 2010/63/EU (European Commission Directive, 2010).

2.3. Lipogenic activities measurements

Activity of enzymes G6PDH (EC 1.1.1.49), EM (EC 1.1.1.40) and FAS (EC 2.3.1.85) were measured. For EM and G6PDH measurements, 1 g of liver was homogenized in 5 ml of ice-cold 0.25 M sucrose solution, whereas for FAS measurement, 0.5 g of liver was homogenized in 2.5 ml of ice-cold 0.25 M sucrose solution containing 1mM dithiothreitol and 1mM EDTA. Homogenates were centrifuged at 12,000 g for 1 h at 4 ºC and supernatants were filtered through glass wool and collected for enzyme assays. Lipogenic activities were assessed at 37 ºC using a spectrophotometric analyzer Fluostar Galaxy (BMG Lab Technologies, Offenburg, Germany) at 340 nm, according to the method described by Fitch et al. (1959) for G6PDH, Hsu and Lardy (1969) for EM and Chang et al. (1967) for FAS. Enzyme activities were expressed in nmols of NADH phosphate produced (G6PDH and EM) or oxidized (FAS) per minute and g of fresh tissue.

Soluble protein was determined in liver supernatant using the bicinchoninic acid (BCA) Protein Assay Kit (Smith et al., 1985) provided by Pierce (Rockford, IL), and enzyme activities were also expressed in a soluble-protein basis.

2.4. Plasma parameters measurements

Plasma concentrations (mg/dl) of glucose, total cholesterol and triglycerides were determined by enzymatic colorimetric methods. Glucose was determined by the Trinder glucose oxidase method (Kaplan, 1984a), triglycerides were measured by the glycerol phosphate dehydrogenase - peroxidase method (Kaplan, 1984b) and total cholesterol was measured by the cholesterol oxidase - peroxidase method (Naito et al., 1984). Concentrations of bilirubin (mg/dl), albumin (g/dl) and total protein (g/dl) were determined by dimethylsulfoxide (Kaplan, 1984c), Bromocresol Green (Gendler et al., 1984) and Biuret (Koller, 1984) colorimetric methods, respectively. Finally, plasma concentrations (UI/l) of enzymes aspartate transaminase (AST; EC 2.6.1.1), alanine transaminase (ALT; EC 2.6.1.2) and alkaline phosphatase (ALP; EC 3.1.3.1.) were measured by photometric methods following the procedures described by Murray et al. (1984a, b) and Wenger et al. (1984), respectively. All the methodologies were integrated in an automatic chemistry analyser model Spin 200E (Spinreact, Girona, Spain).

2.5. Statistical analysis

Descriptive statistics were estimated after correcting data by the fixed effects of line and sex. Month-season and parity order fixed effects were additionally included for IMF, BW, chilled carcass and perirenal fat weights analysis. Direct and correlated responses to selection were estimated as the differences between high-IMF and low-IMF lines. All the differences were estimated with a model including the fixed effects of line, sex, month-season and parity order (as described before) and common litter random effect. Phenotypic correlations of IMF and perirenal fat weight with liver weight, liver lipogenic activities and plasma parameters were estimated after correcting data for line and sex.

Bayesian inference was used (Blasco, 2005). Common litter effect and residuals of the models were assumed to be independently normally distributed. Bounded flat priors were assumed for all fixed effects and variances. Marginal posterior distributions were estimated using Gibbs sampling. Descriptive statistics and differences between lines were performed with programme “Rabbit”, developed by the Institute for Animal Science and Technology (Valencia, Spain). After some exploratory analyses, results were based on Monte Carlo Markov chains runs consisting of 60,000 iterations, with a burn-in period of 10,000, and only one of every 10 samples were saved for inferences. Phenotypic correlations were computed with the software TM (Legarra et al., 2008). In this case, after some exploratory analyses results were based on Monte Carlo Markov chains runs consisting of 1, 000,000 iterations, with a burn-in period of 200,000, and only one of every 100 samples were saved for inferences. Convergence was tested using the Z criterion of Geweke and Monte-Carlo sampling errors were computed using time-series procedures (Sorensen and Gianola, 2002).

The parameters obtained from the marginal posterior distributions of the differences between lines and phenotypic correlations were: the median, the highest posterior density region at 95% ($HPD_{95\%}$) and the probability of the difference or correlation being greater than zero when the median is positive or lower than zero when the median is negative (P_0). Additionally, we considered 1/3 of the SD of a trait as a relevant value (r) and we calculated the probability of relevance (probability of the

difference between lines being greater than r when the median is positive or lower than r when the median is negative) (P_r). A more detailed description of these features can be found in Blasco (2005).

3. RESULTS AND DISCUSSION

3.1. Response to selection and correlated responses in carcass traits

Table 1 shows descriptive statistics and differences between lines for IMF and carcass traits.

Table 1. Descriptive statistics and differences between lines in intramuscular fat (IMF) of *Longissimus dorsi* (g/100g of muscle) and carcass traits (g) in the eighth generation of selection (n = 175).

Trait	Mean	SD	D[1]	$HPD_{95\%}$[2]	P_0[3]	r[4]	P_r[5]
Intramuscular fat	0.99	0.13	0.34	0.30, 0.39	1.00	4.36	1.00
BW	1,750	112	7.50	-33.2, 47.9	0.64	2.13	0.07
Chilled carcass weight	974	80.3	12.5	-22.2, 47.9	0.75	2.75	0.20
Perirenal fat weight	7.77	2.36	3.19	2.35, 4.05	1.00	10.1	1.00

[1]D = median of the marginal posterior distribution of the difference between high-IMF and low-IMF lines; [2]$HPD_{95\%}$ = highest posterior density region at 95% of probability; [3]P_0 = probability of the difference being greater than zero when D > 0 or lower than zero when D < 0; [4]r = relevant value, proposed as 1/3 of the standard deviation of the trait; [5]P_r = probability of relevance (probability of the difference being greater than r when D > 0 or lower than r when D < 0.

Divergent selection for IMF was successful (Martínez-Álvaro et al., 2016). Direct response to selection estimated as the difference between lines in the eight generation was 0.34 g /100 g of LD (P_r = 1.00) with a $HPD_{95\%}$ from 0.30 to 0.39. Expressed in units of SD, direct response represented 2.7 SD of the trait. Selection for IMF showed a positive correlated response in the carcass adiposity. High-IMF line showed greater perirenal fat weight (P_0 = 1.00) than low-IMF line, which is the main carcass fat depot in rabbits (Hernández et al., 2006), and differences between lines

were relevant (P_r = 1.00). Other selection experiments for IMF also found a positive correlated response in the carcass adiposity (Schwab et al., 2009 in pigs and Zhao et al., 2007 in chickens), and the positive genetic correlation between intramuscular and carcass fat is widely documented (Martínez-Álvaro et al., 2016 in rabbits, Ciobanu et al., 2011 in a pig review and Mateescu, 2015 in a cattle review). We did not find differences between lines in BW and chilled carcass weight.

3.2. Liver weight and lipogenic activities

Table 2 shows descriptive statistics and differences between lines for liver weight and liver lipogenic activities.

Table 2. Descriptive statistics and differences between lines in liver weight and liver lipogenic activities[1] in a subsample of the eight generation of selection (n = 63).

Trait	Mean	SD	D[2]	HPD$_{95\%}$[3]	P_0[4]	r[5]	P_r[6]
Liver weight, g	42.8	3.71	2.39	0.47, 4.50	0.99	2.88	0.87
G6PDH	4,383	817	1,182	698, 1,660	1.00	272	1.00
EM	416	102	44.8	-17.3, 108	0.92	33.8	0.64
FAS	686	83.0	9.60	-38.2, 56.9	0.65	27.7	0.22

[1]Activities of the lipogenic enzymes glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme (EM) and fatty acid synthase (FAS) are expressed in nmol/min and g of tissue; [2]D = median of the marginal posterior distribution of the difference between high-intramuscular fat (IMF) and low-IMF lines; [3]$HPD_{95\%}$ = highest posterior density region at 95% of probability; [4]P_0 = probability of the difference being greater than zero when D > 0 or lower than zero when D < 0; [5]r = relevant value, proposed as 1/3 of the standard deviation of the trait; [6]P_r = probability of relevance (probability of the difference being greater than r when D > 0 or lower than r when D < 0.

High-IMF line showed greater liver weight than low-IMF line (P_0 = 0.99) and the probability of the difference between lines being relevant was P_r = 0.87. This result should be related to the greater fat deposition in the high-IMF line, since liver is the tissue with the greatest lipogenic activity in growing rabbits (Leung and Bauman, 1975, Veizhnet and Nouguès, 1977, Gondret et al., 1997). The greatest lipogenic activity in

liver was G6PDH compared with EM and FAS (Table 2), in agreement with other studies in rabbits (Gondret et al., 1997; Gondret et al., 1998 and Gondret et al., 2004). High-IMF line showed greater G6PDH (P_0 = 1.00) and EM activities (P_0 = 0.92) in liver than low-IMF line, although differences between lines were relevant and great only for G6PDH activity (P_r = 1.00), in which, lines showed a difference of 1,182 nmol/min and g, or 1.51 SD of the trait. We did not find differences between lines for FAS activity, although these results should be taken with caution because of large $HPD_{95\%}$. Results were similar when activities were expressed in a soluble protein basis (data not shown).

Divergent selection for IMF allows studying the lipid metabolism strictly underlying IMF deposition, since the selected lines have the same genetic background and only differ in genes involved in IMF and correlated traits. Differences in the fat deposition of the high-IMF and low-IMF lines can be explained by different G6PDH and EM lipogenic activities in liver, particularly for G6PDH. Both G6PDH and EM enzymes generate NADPH for the support of fatty acid and steroid biosynthesis, G6PDH by the hexose monophosphate shunt and EM by the citric acid cycle. In a previous study of the lipogenic activities in muscles and perirenal fat of the lines, Martínez-Álvaro et al., (2017) observed greater lipogenic activities in the high-IMF line at 13 wk, but not at 9 wk, in all tissues. Moreover, differences between lines at 13 wk were particularly great in the G6PDH activity of LD. Results after genetic selection for IMF reveal the important role of G6PDH activity in the genetic variability on fat deposition in rabbits.

Liver lipogenic activities have been previously measured in breeds with different IMF, however, this is the first work that studies liver lipogenic activities in animals with the same genetic origin divergently selected for IMF. Greater FAS gene expression in liver has been related to greater IMF in a comparison between two chicken breeds (Cui et al., 2012). However, breeds can differ in a wide set of traits, which made difficult to attribute the causes of the differences in IMF. Several studies show that animals with greater carcass fat deposition have greater liver weight (Wise et al., 1993 and Pond et al., 1992 in pigs divergently selected for plasma total cholesterol) and greater G6PDH, EM and FAS activities in liver (Turkenkopf et al., 1980 and Smith et al., 1980 in fat genotyped Zucker rats). In pigs, Muñoz et al. (2013)

observed that selection for decreasing backfat thickness at constant IMF was accompanied by a reduction of FAS expression in liver, suggesting that hepatic lipogenesis might affect fat partitioning in pigs (Muñoz et al., 2013).

3.3. Plasma parameters related to liver

Table 3 reports descriptive statistics and differences between lines for plasma parameters related to liver.

Table 3. Descriptive statistics and differences between lines in plasma parameters related to liver in a subsample of the eight generation of selection (n = 63).

Trait	Mean	SD	D[1]	$HPD_{95\%}$[2]	P_0[3]	r[4]	P_r[5]
Glucose, mg/dl	141	10.2	-0.90	-6.61, 4.47	0.63	3.38	0.20
Triglycerides, mg/dl	130	58.6	-43.6	-79.3, -6.86	0.99	19.5	0.91
Cholesterol, mg/dl	78.4	16.4	-6.78	-16.1, 2.64	0.93	5.47	0.61
Bilirubin, mg/dl	0.20	0.11	-0.12	-0.18, -0.06	1.00	0.04	0.99
Total protein, g/dl	6.81	0.54	0.00	-0.28, 0.31	0.51	0.18	0.12
Albumin, g/dl	4.36	0.26	0.23	0.07, 0.37	1.00	0.09	0.96
AST[6], UI/l	40.6	9.48	1.59	-4.13, 7.23	0.72	3.16	0.29
ALT[7], UI/l	69.4	19.6	15.05	3.99, 25.9	1.00	6.52	0.93
ALP[8], UI/l	616	111	-99.8	-165, -40.3	1.00	37.1	0.97

[1]D = median of the marginal posterior distribution of the difference between high and low-intramuscular fat lines; [2]$HPD_{95\%}$ = highest posterior density region at 95% of probability; [3]P_0 = probability of the difference being greater than zero when D > 0 or lower than zero when D < 0; [4]r = relevant value, proposed as 1/3 of the standard deviation of the trait; [5]P_r = probability of relevance (probability of the difference being greater than r when D > 0 or lower than r when D < 0; [6]AST = aspartate transaminase; [7]ALT = alanine transaminase; [8]ALP = alkaline phosphatase.

Our lines showed normal concentrations of all plasma parameters except for ALP, in which both lines showed concentrations above normal levels for rabbits (Nowland et al., 2015; Washington and Van Hoosier, 2012). However, Melillo, (2007) suggested that high plasma concentration of ALP in healthy rabbits is a common finding, since ALP is the sum of three different isoenzimes (two produced in the liver

and one in the intestine) with a wide range of variation. Besides, growing rabbits show particularly high ALP concentrations caused by its high osteoblastic activity, since ALP is involved in the precipitation of calcium phosphate in bones (Melillo, 2007).

Low-IMF line showed greater plasma concentration of triglycerides (P_0 = 0.99), cholesterol (P_0 = 0.93), bilirubin (P_0 = 1.00) and ALP (P_0 = 1.00) than high-IMF line, and all the differences between lines were relevant (P_r = 0.91 for triglycerides, P_r = 0.99 for bilirubin and P_r = 0.97 for ALP), except for cholesterol concentration, in which P_r was very low. High-IMF line showed greater albumin (P_0 = 1.00) and ALT (P_0 = 1.00) concentrations, and differences between lines were relevant with P_r = 0.96 and 0.93, respectively. We did not observe differences between lines for glucose, total protein and AST plasma concentrations, although their $HPD_{95\%}$ were large. To our knowledge, our results are the first reports of plasma parameters in animals selected for IMF.

Circulating plasma concentrations of glucose, triglycerides and cholesterol are the result of the production and uptake by lipogenic tissues. We did not find differences between lines for glucose concentration, which is a primary energy source in rabbits (Melillo, 2007). Low-IMF line had greater plasma triglycerides and cholesterol concentrations than high-IMF line (although differences in cholesterol were not relevant), in spite of its lower liver lipogenic activity. A study in rats observed that high plasma concentrations of triglyceride-rich lipoproteins played a regulation role inhibiting hepatic fatty acid synthesis (Lakshmanan et al., 1977). In animals selected for different criterions, it has been observed a negative relationship between plasma lipids and carcass fat deposition (Bakke, 1975 selecting for BW gain and carcass leanness and Pond et al., 1992 selecting for plasma cholesterol, both in pigs). The lower fat deposition of the low-IMF line suggests that its increased concentration of lipids in plasma is not taken up by muscles and fat depots in a similar rate than in the high-IMF line. The release of plasma lipids to muscle and fat tissues are limited by the activity of the enzyme lipoprotein lipase, which has been suggested as a good indicator of lipid deposition in pigs (Allen et al., 1976). Further studies would be necessary to examine the lipoprotein lipase activity of the IMF lines.

Bilirubin is a subproduct of hemolysis and it is taken up from plasma by the liver (Wang et al., 2006). Low-IMF line showed relevantly greater plasma concentration of

bilirubin than the high-IMF line (Table 3). In healthy humans, greater body fat percentage is related with lower plasma concentration of bilirubin (Jenko-Praznikar et al., 2013). This is explained because obesity is associated with an increased oxidative stress and inflammation states, and bilirubin, which has antioxidant and anti-inflammatory properties, is greatly consumed in obese individuals (Jenko-Praznikar et al., 2013).

Albumin is synthetized in liver and represents the main part of the total protein concentration in plasma (Washington and Van Hoosier, 2012). It transports many plasma metabolites, including bilirubin and free fatty acids. High-IMF line showed relevantly greater albumin concentration than low-IMF line, which can indicate a greater transport fluxes of these metabolites in plasma.

Plasma concentrations of ALT, AST and ALP enzymes are used clinically as indicators of liver damage, which was not the case of none of our lines. High-IMF line showed relevant greater ALT concentration than the low-IMF line. This enzyme is involved in the amino acids metabolism (Frayn, 1998). By other side, plasma concentration of ALP was relevantly greater in the low than in the high-IMF line. We did not find literature about the relationship of IMF with ALT, AST and ALP plasma concentrations. Pigs with higher carcass adipossity showed greater ALT, AST and lower ALP plasma concentrations respect leaner pigs, in a selection experiment for plasma cholesterol (Pond et al., 1997).

3.4. Relationships between fat and liver traits

Table 4 shows phenotypic correlations between fat traits (IMF and perirenal fat weight) and liver traits (liver weight, lipogenic activities and plasma parameters). Intramuscular fat was positively correlated with liver weight and with G6PDH and FAS activities with probabilities (P_0) of 0.98, 0.97 and 1.00, respectively, and the medians of these correlations went from 0.28 to 0.38. We do not have enough evidence to state the sign of the correlation between IMF and EM activity. Perirenal fat weight was positively correlated with EM activity with a high probability (P_0 = 0.99), and the median of the correlation was 0.34. The correlations between perirenal fat weight and G6PDH and FAS activities and between perirenal fat and liver weights were also positive, but with lower evidence (P_0 between 0.88 and 0.89) and showing lower

medians (from 0.16 to 0.17). Our results suggest that fat deposition in rabbits, both in muscle and carcass, is partially explained by the liver lipogenic activity. However, due to the low amount of data (n = 63) all the correlation estimates showed a wide $HPD_{95\%}$ and we cannot make precise statements about their actual values. To our knowledge, there are no literature about the correlations between intramuscular and carcass fat and liver lipogenic activities.

Table 4. Phenotypic correlations between intramuscular fat and perirenal fat weight and liver weight, lipogenic[1] activities and plasma parameters concentrations related to liver.

	Intramuscular fat				**Perirenal fat weight**			
Trait	**r_p[2]**	**$HPD_{95\%}$[3]**		**P_0[4]**	**r_p[2]**	**$HPD_{95\%}$[3]**		**P_0[4]**
Liver weight	0.28	0.04,	0.51	0.98	0.16	-0.08,	0.42	0.89
G6PDH	0.28	0.02,	0.51	0.97	0.16	-0.11,	0.40	0.88
EM	-0.05	-0.33,	0.24	0.62	0.34	0.08,	0.57	0.99
FAS	0.38	0.14,	0.60	1.00	0.17	-0.09,	0.43	0.89
Albumin	0.27	0.01,	0.51	0.98	0.35	0.12,	0.57	1.00
Total protein	0.21	-0.06,	0.46	0.94	0.12	-0.14	0.37	0.82

[1]Activities of the lipogenic enzymes glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme (EM) and fatty acid synthase (FAS) measured in nmol/min and g of tissue; [2]r_p = median of marginal posterior distribution of the phenotypic correlation; [3]$HPD_{95\%}$ = highest posterior density region at 95% of probability; [4]P_0 = probability of the phenotypic correlation of being greater than zero when $r_p > 0$ or lower than zero when $r_p < 0$.

Correlations between IMF and plasma parameters may have a particular interest in meat production, because they could be used as potential biomarkers of IMF. However, we did not find any strong correlation between IMF and studied plasma parameters. Albumin concentration in plasma was positively correlated with IMF (P_0= 0.98) and with perirenal fat weight (P_0 = 1.00); the medians of the phenotypic correlations were 0.27 and 0.35, respectively (Table 4). Total protein plasma concentration was positively correlated to IMF (P_0 = 0.94). However, the median of its correlation with IMF was low (0. 21). Phenotypic correlations between IMF and

perirenal fat weight and the other plasma parameters measured were weak (data not shown). Plasma parameters have been previously studied as blood indicators of IMF in pigs (Muñoz et al., 2012) and cattle (Adachi et al., 1999) with no significant results. These findings suggest the complex biological mecanisms involved in the regulation of IMF deposition, making difficult to find one specific biomarker strongly correlated to IMF.

4. CONCLUSIONS

In summary, liver plays an important role in the fat deposition of the lines divergently selected for IMF, high-IMF line showing greater liver weight and liver lipogenic activities (G6PDH and EM), particularly for G6PDH. Liver size and liver lipogenic activities were positively correlated to fat deposition in muscle (except for EM) and carcass, although phenotypic correlations were estimated with low accuracy. Selection for IMF affected some plasma parameters related to liver metabolism, low-IMF line showing greater concentration of triglycerides, cholesterol, bilirubin and ALP but lower concentrations of albumin and ALT than high-IMF line. However, none of this plasma parameters showed a strong correlation with IMF.

5. LITERATURE

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

Allen, C.E., D.C. Beitz, D.A. Cramer and R.G. Kauffman. 1976. Biology of fat in meat animals. North Central Regional Research Publication No 234. Univ. of Wisconsin, Madison.

Bakke, H. 1975. Serum levels of cholesterol in lines of pigs selected for rate of gain and thickness of backfat. Acta Agric. Scand. 25:14-16.

Ballard, F.J., R.W. Hanson and D.S. Kronfield. 1969. Gluconeogenesis and lipogenesis in tissue from ruminant and non-ruminant animals. Fed. Proc.28: 218.

Blasco, A. 2005. The use of Bayesian statistics in meat quality analyses: A review. Meat Sci. 69:115–122.

Ciobanu, D.C., S.M. Lonergan and E.J. Huff- Lonergan. 2011. Genetics of meat quality and carcass traits. In: M.F. Rothschild and A. Ruvinsky. The genetics of the pig. CAB Int. Oxfordshire (UK).pp. 355-389.

Chang, H.C., I. Seidman, G. Teebor and M. D. Lane. 1967. Liver acetyl CoA carboxylase and fatty acid synthetase: Relative activities in the normal state and in hereditary obesity. Biochem. Biophys. Res. Commun. 28:682–686.

Cui, H.X., M.Q. Zheng, R.R. Liu, G.P. Zhao, J.L. Chen and J. Wen. 2012. Liver dominant expression of fatty acid synthase (FAS) gene in two chicken breeds during intramuscular-fat development. Mol. Biol. Rep. 39:3479-3484.

European Economic Community. 1998. Council Directive 98/58/EC of 20 July 1998 concerning the protection of animals kept for farming purposes. Official Journal L, 221(8), 8.

European Commission Directive. 2010. Council, E. P. A. E. 2010/63/EU on the protection of animals used for scientific purposes. Institute for Health and Consumer Protection, Ispra, Italy, b7.

Fitch, W.M., R. Hill and I.L. Chaikoff. 1959. The effect of fructose feeding on glycolytic enzyme activities of the normal rat liver. J. Biol. Chem. 234:1048–1051.

Frayn, K.N. 1998. Regulación del metabolismo: una perspectiva humana. Ed. Omega, Barcela pp. 137.

Gendler S., 1984 Uric acid. In: Kaplan LA, Pesce, AJ. (ed). Clinical Chemistry: Theory, Analysis, Correlation. 1st ed. The C.V. Mosby Co. St Louis. Toronto. Princeton; pp. 1268-1273 and 425.

Ghany, M. and J.H. Hoofnagle. 2012. Estudio del paciente con enfermedad hepática. Harrison. Principios de Medicina Interna. 18ªEd. Mc Graw-Hill-Interamericana de España, Madrid, pp. 2520-2526.

Gondret, F., J. Mourot and M. Bonneau. 1997. Developmental Changes in Lipogenic enzymes in muscle Compared to Liver and extramuscular adipose tissues in the rabbit (*Oryctolagus cuniculus*). Comp. Biochem. Physiol., 117B : 259-265.

Gondret, F., J. Mourot, F. Lebas and M. Bonneau. 1998. Effects of dietary fatty acids on lipogenesis and lipid traits in muscle, adipose tissue and liver of growing rabbits. Anim. Sci. 66:483-489.

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

Hernández, P., B. Ariño, A. Grimal and A. Blasco. 2006. Comparison of carcass and meat characteristics of three rabbit lines selected for litter size or growth rate. Meat Sci. 73:645-650.

Hsu, R.Y., G. Wasson and J.W. Porter, J. W. 1965. The purification and properties of the fatty acid synthetase of pigeon liver. J. Biol. Chem. 240:3736-3746.

Hsu, R.Y. and H.A. Lardy. 1969. Malic enzyme. In: N. Y. N. Academic Press, editor. Methods in enzymology. p. 230–235.

Jenko-Praznikar, Z., A. Petelin, M. Jurdana and L. Ziberna. 2013. Serum bilirubin levels are lower in overweight asymptomatic middle-aged adults: an early indicator of metaboli syndrome? Metabolism, 62: 976-985.

Kaplan, A. 1984a Glucose. In: Kaplan LA, Pesce, AJ. (ed). Clinical Chemistry: Theory, Analysis, Correlation. 1st ed. The C.V. Mosby Co. St Louis. Toronto. Princeton, pp. 1032-1036.

Kaplan, A. 1984b. Tryglycerides. In: In: Kaplan LA, Pesce, AJ. (ed). Clinical Chemistry: Theory, Analysis, Correlation. 1st ed. The C.V. Mosby Co. St Louis. Toronto. Princeton, pp. 1194-1206.

Kaplan, A. 1984c Bilirubin. In: Kaplan LA, Pesce, AJ. (ed). Clinical Chemistry: Theory, Analysis, Correlation. 1st ed. The C.V. Mosby Co. St Louis. Toronto. Princeton, pp. 1238-1241.

Koller A. 1984. Proteins., Pesce AJ, editors. Clinical Chemistry: theory, analysis and correlation. C.V.Mosby; Toranto, Canada, pp. 1268–1327.

Lakshmanan, M.R., R.A. Muesing, G.A. Cook and R.L. Veech. 1977. Regulation of lipogenesis in isolated hepatocytes by triglyceride-rich lipoproteins. J. Biol. Chem., 252:6581-6584.

Legarra, A., L. Varona and E. López de Maturana. 2008. TM: Thershold model. http://snp.toulouse.inra.fr/~alegarra/manualtm.pdf.

Leung, T.T. and D.E. Bauman. 1975 In vivo studies of the site of fatty acid synthesis in the rabbit. Int. J. Biochem 6: 801-805.

Martínez-Álvaro, M., P. Hernández, and A. Blasco. 2016a. Divergent selection on intramuscular fat in rabbits: Responses to selection and genetic parameters. J. Anim. Sci. 94:4993-5003.

Martínez-Álvaro, M., S. Agha, A. Blasco, P. Hernández 2017. Muscle lipid metabolism in two rabbit lines divergently selected for intramuscular fat. J. Anim. Sci. (accepted).

Mateescu, R.G. 2015. Genetics of meat quality. In: D.J. Garrick and A. Ruvinsky, editors. The genetics of cattle 2nd Edn. CAB Int, Oxfordshire, UK. pp. 544-570.

Melillo, A. 2007. Rabbit Clinical Pathology. J. Exotic Pet Med. 16:135-145.

Muñoz, R., M. Tor and J. Estany. 2012. Relationship between blood lipid indicators and fat content and composition in Duroc pigs. Livest. Sci. 148:95–102.

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

Murray, R. 1984a. Aspartate aminotransferase. In: Kaplan LA, Pesce, AJ. (ed). Clinical Chemistry: Theory, Analysis, Correlation. 1st ed. The C.V. Mosby Co. St Louis. Toronto. Princeton,pp: 1112-116

Murray, R. 1984b Alanine aminotransferase. In: Kaplan LA, Pesce, AJ. (ed). Clinical Chemistry: Theory, Analysis, Correlation. 1st ed. The C.V. Mosby Co. St Louis. Toronto. Princeton, pp: 1088-1090.

Naito, H.K. 1984. Cholesterol. In: Kaplan LA, Pesce, AJ. (ed). Clinical Chemistry: Theory, Analysis, Correlation. 1st ed. The C.V. Mosby Co. St Louis. Toronto. Princeton, pp. 1194-11206.

Nowland, M.H., D.W. Bramer, A. García and H.G. Rush. 2015. Biology and Diseases of Rabbits. In Laboratory Animal Medicine. pp. 411–461.

O' Heau, E.K. and G.A. Leveille. 1969. Lipid biosynthesis and transport in the domestic chick (*Gallus domesticus*). Comp. Biochem. Physiol. 30:149.

Pond, W.G., W. Insull, H.J. Mersmann, W.W. Wong, K.B. Harris, H.R. Cross, E.O. Smith, J.P. Heath and L.G. Kömüves. 1992. Effect of dietary fat and cholesterol level on growing pigs selected for three generations for high or low serum cholesterol at age 56 days. J. Anim. Sci, 70: 2462-2470.

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

Quintanilla, R., R.N. Pena, D. Gallardo, A. Cánovas, O. Ramírez, I. Díaz, J.L. Noguera and M. Amilis. 2011 Porcine intramuscular fat content and composition are regulated by quantitative trait loci with muscle-specific effects. J. Anim. Sci. 89: 2963-2971.

Sapp, R.L., J.K. Bertrand, T.D. Pringle and D. E. Wilson. 2002. Effects of selection for ultrasound intramuscular fat percentage in Angus bulls on carcass traits of progeny. J. Anim. Sci. 80:2017–2022.

Schwab, C.R., T.J. Baas, K.J. Stalder and D. Nettleton. 2009. Results from six generations of selection for intramuscular fat in Duroc swine using real-time ultrasound. I. Direct and correlated phenotypic responses to selection. J. Anim. Sci. 87:2774–2780.

Smith, P.A., and M.L. Kaplan. 1980. Development of hepatic and adipose tissue lipogenesis in the fa/fa rat. Int. J. Biochem. 11:217-228.

Smith, P.K., R.I. Krohn, G.T. Hermanson, A.K. Mallia, F.H. Gartner, M.D. Provenzano, E.K. Fujimoto, N.M. Goeke, B.J. Olson, and D.C. Klenk. 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. 150:76–85.

Sorensen, D. and D. Gianola. 2002. Likelihood, Bayesian, and MCMC methods in quantitative genetics. Springer, New York, NY.

Turkenkopf, I.J., J.L. Olsen, L. Moray, M.R.C. Greenwood and P.R. Johnson. 1980. Hepatic lipogenesis in preobese Zucker rat (40910). Proc. of the Society for experimental Biology and Medicine, 164:530-533.

Veizhnet, A. and J. Nouguès. 1977. Evolution postnatale de la lipogenèse dans le tissu adipeux et le foie du mouton et du lapin. Ann. Biol. Anim. Bioch. Biophys., 5B:851-863.

Wang, X., J. Roy Chowdhury and N. Roy Chowdhury. 2006. Bilirubin metabolism: applied physiology. Current paediatrics, 16, 70-74.

Washington, I.M. and G.V. Van Hoosier. 2012. Chapter 3. Clinical Biochemistry and Hematology. In M. A. Suckow, K. A. Stevens, and R. P. Wilson (Eds.), The Laboratory Rabbit, Guinea Pig, Hamster, and Other Rodents. pp. 57–116. Blackwell Publishing Professional, Iowa, USA.

Wenger C., A. Kaplan, F.F. Rubatelli, C. Hammerman. 1984. Alkaline phosphatase. . In: Kaplan LA, Pesce, AJ. (ed). Clinical Chemistry: Theory, Analysis, Correlation. 1st ed. The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; pp. 1094-1098.

Wise, T., Young, D.L. and Pond, W.G. 1993. Reproductive, endocrine and organ weight differences of swine selected for high or low serum cholesterol. J. Anim. Sci ,71: 2732-2738.

Wood, J.D., Enser, M., Fisher, A.V, Nute, G.R., Sheard, P.R., Richardson, R.I., Hughes, S.I. and Whittington, F.M. 2008. Fat deposition, fatty acid composition and meat quality: A review. Meat Sci. 78, 343–358.

Zhao, G.P., J.L. Chen, M.Q. Zheng, J. Wen, and Y. Zhang. 2007. Correlated responses to selection for increased intramuscular fat in a Chinese quality chicken line. Poult. Sci. 86:2309–2314.

Zomeño, C., Hernández, P. and Blasco, A. 2011. Use of near infrared spectroscopy for intramuscular fat selection in rabbits. World Rabbit Sci. 19:203–208

Zomeño, C., Juste V. and Hernández, P. 2012. Application of NIRS for predicting fatty acids in intramuscular fat of rabbit. Meat Sci. 91:155–159.

GENERAL DISCUSSION

This thesis is focused on the responses obtained in a divergent selection experiment for intramuscular fat (IMF) of *Longissimus dorsi* muscle in rabbits at 9 wk of age. Intramuscular fat is a main factor in meat quality, because has a strong effect in the sensory, technological and nutritional properties of meat. Rabbits are a good model for genetic studies in other livestock species due to their short generation interval and low cost of the carcasses. Rabbit has also importance as livestock species in several countries such as Mediterranean countries or China, (FAO-STAT, 2014) and allows performing sensory studies on its meat.

Selection for IMF during eight generations has greatly differentiated the high and low-IMF lines. Response to selection obtained was 2.4 SD of the trait, and both lines showed symmetrical genetic trends. Simultaneously, selection for IMF in muscle *Longissimus dorsi* has affected in the same direction other muscles independently of their metabolic profile, such as *Biceps femoris, Supraspinatus* and *Semimembranosus proprius*.

Selection for IMF has produced positive and negative consequences in the nutritional properties of meat. First, selection for high IMF has increased the percentage of MUFA but no effect was observed on SFA percentage, which is favourable from a nutritional point of view, since it is recommended to reduce the intake of SFA by unsaturated fatty acids (World Health Organization, 2008). However, selection for high IMF has decreased the percentage of PUFA and then the PUFA:SFA ratio has been reduced, which is unfavourable. Within PUFA, the n-6:n-3 ratio was more favourable in the high-IMF than in the low-IMF line, due to a lower decrease in the n-3 than in the n-6 fatty acids. We did not found differences between lines for SFA percentage, but we found greater C14:0 and C16:0 in the high-IMF line and greater C18:0 in the low line. Individual MUFA and PUFA percentages showed the same correlated responses as their groups, except for C18:3n-3. Differences in the fatty acid percentages between lines are explained to a rapid increase of triglycerides (rich in almost all SFA and MUFA) respect to phospholipids (rich in almost all PUFA) when fat increases. The opposite results observed for C18:0 and C18:3n-3 percentages are in line with the greater C18:0 in phospholipid fraction and greater C18:3n-3 in triglycerides fraction reported in rabbits (Alasnier et al., 1996). Besides, triglycerides

are stored in adipocytes and high-IMF line showed larger adipocytes than low-IMF line in perirenal fat. The faster increase of SFA and MUFA in the high than in the low-IMF line is due to its greater lipogenic activity, observed in several tissues such as *Longissimus dorsi* and *Semimembranosus proprius* muscles, perirenal fat and liver. Differences between lines were particularly great for glucose-6-phosphate dehydrogenase activity in *Longissimus dorsi* muscle and liver. Liver is the main lipogenic tissue in growing rabbits and selection for high-IMF showed a positive correlated response in the liver size. Besides, plasma parameters related to liver metabolism have been also affected by selection for IMF.

We did not find important changes after selection in the technological properties of meat, such us pH in several muscles, cooking loss or texture instrumental parameters, except for firmness that was greater in the low-IMF line. We did not find differences between lines in color parameters of fresh meat.

The changes in IMF content and fatty acid percentages produced by the genetic selection did not affect the sensory properties of rabbit meat. Lines showed similar values on their sensory attributes after their evaluation by a trained sensory panel. In literature, the effect of IMF on the sensory properties of meat is observed only with high levels of IMF (reviewed by Hocquette et al., 2010). Rabbit is a lean meat and it is difficult to obtain wide ranges of IMF variation.

Selection for high IMF showed a positive correlated response on carcass fat depots. High-IMF line showed greater perirenal fat weight than low-IMF line, which is the main fat depot in rabbits (Hernández et al., 2006). Scapular fat weight was also greater in the high-IMF than in the low-IMF line although differences were not relevant. Considering the low dissectible fat percentage of rabbit carcass, this correlated responses has no economic importance, but should be considered if these results are extrapolated to other species. We did not find differences between lines in other carcass traits (carcass weights, meat to bone ratio, color of the carcass surface).

Information about the genetic parameters of IMF and its genetic correlations with other commercial traits is a critical point before considering IMF as a breeding goal in meat industry. Estimating these parameters with a minimal precision requires a substantial amount of data. However, correlated responses obtained after some

generations of selection for IMF can corroborate the heritabilities and genetic correlations estimated. We have used carcass and meat quality data recorded during whole the selection experiment (n = 1,300 – 1,500) to estimate the genetic parameters of IMF and carcass and meat quality traits. The correlated responses to selection observed corroborated our estimates. Most of the genetic parameters reported in this thesis were never reported before in rabbits.

Although there are other selection experiments for IMF in other species (in pigs Schwab et al., 2009; chickens Zhao et al., 2007 and cattle Sapp et al., 2002), none of them performed a correlated responses study as wide as ours. Besides, none of them studied the lipid metabolism of the lines. Measuring meat and carcass quality and lipid metabolism traits simultaneously has permitted us to explain some of the causes of the changes in fat composition due to selection, since a metabolic point of view.

The short generation interval of rabbits allowed us to obtain two rabbit lines with the same genetic origin, only greatly differentiated in the genetic background of IMF, in a relative short period of time. Now, these two rabbit lines are an exceptional material for further studies of the whole intermediate steps between IMF genotype to IMF phenotype (genome, transcriptome, metabolome, microbiome, metagenome). Relationships between these intermediate phenotypes can bring substantial information about the metabolic routes that control the fat deposition in muscle. Relevant topics in animal production, such us the different metabolic routes that control the fat deposition in muscle or in carcass, could be approached.

Literature cited

Alasnier, C., H. Rémington and G. Gandemer. 1996. Lipid characteristics associated with oxidative and glycolytic fibres in rabbit muscles. Meat Sci. 43:213-224.

FAO Stat. 2014. Food and Agriculture Organization. http://www.fao.org/faostat/en

Hernández, P., B. Ariño, A. Grimal and A. Blasco. 2006. Comparison of carcass and meat characteristics of three rabbit lines selected for litter size or growth rate. Meat Sci. 73: 645-650.

Hocquette, J.F., F. Gondret, E. Baéza, F. Médale, C. Jurie, and D. W. Pethick. 2010. Intramuscular fat content in meat-producing animals: development, genetic and nutritional control, and identification of putative markers. Animal. 4:303-319.

Saap, R.L., J.K. Bertrand, T.D. Pringle and D.E. Wilson 2002. Effect of selection for ultrasound intramuscular fat percentage in Angus bulls on carcass traits of progeny. J. Anim. Sci. 80:2017-2022.

Schwab, C.R., T.J. Baas, K.J. Stalder and D. Nettleton. 2009. Results from six generations of selection for intramuscular fat in Duroc swine using real-time ultrasound. I. Direct and correlated phenotypic responses to selection. J. Anim. Sci. 87:2774-2780.

World Health Organization. 2008. Interim summary of conclusions and dietary recommendations on total fat and fatty acids. Joint FAO/WHO expert consultation on fats and fatty acids in human nutrition, pp. 10-14.

Zhao, G.P., J.L. Chen, M.Q. Zheng, J. Wen, and Y. Zhang. 2007. Correlated responses to selection for increased intramuscular fat in a Chinese quality chicken line. Poult. Sci. 86:2309–2314.

CONCLUSIONS

1. Divergent selection for intramuscular fat (IMF) of *Longissimus dorsi* muscle was successful. Direct response to selection after eight generations was 2.4 SD of the trait, which represents a genetic progress of 5% of the mean per generation. High and low-IMF lines showed symmetrical trends and the estimate of IMF heritability was high (0.54).
2. Selection for IMF showed a positive correlated response in carcass fat depots, and both traits showed a positive but low genetic correlation. We did not find differences between lines in other carcass quality traits.
3. Selection for IMF showed important modifications in the fatty acid percentages of meat. High-IMF line showed greater MUFA and lower PUFA than low-IMF line, and in general, individual fatty acids showed a similar pattern as for the groups, with the exception of C18:3n-3 that was greater in the high IMF line. Percentages of fatty acids C14:0 and C16:0 were greater in the high-IMF whereas C18:0 was greater in the low-IMF line. We did not find differneces between lines in SFA percentage.
4. A positive correlated response to selection was observed in the IMF content of other muscles with diverse oxidative pattern (*Biceps femoris, Supraspinatus* and *Semimembranosus proprius*), and their correlated responses in fatty acid composition were similar as for the ones observed in *Longissimus dorsi*.
5. We did not find differences between lines in meat color, pH, instrumental texture parameters, cooking loss and sensory attributes of meat.
6. Differences in the fat deposition of the lines can be explained by differences in their lipogenic activities in muscle, perirenal fat and liver tissues, particularly for the activity of glucose-6-phosphate dehydrogenase. Catabolic activities involved in the lipid metabolism did not explain the differences between lines for IMF in muscle. High-IMF line showed a greater size of the liver, and lines showed differences in plasma parameters related with liver metabolism. Our results show that liver plays an important role in the different fat deposition of the lines.