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# GENETIC ANALYSIS OF INTRAMUSCULAR FAT IN RABBITS

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# GENETIC ANALYSIS OF INTRAMUSCULAR FAT IN RABBITS

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By: Cristina Zomeño Segado

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*"Nuestra recompensa se encuentra en el esfuerzo y no en el resultado. Un esfuerzo total es una victoria completa."*

Mahatma Gandhi

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The aim of this Thesis is to examine the opportunities for genetic improvement of intramuscular fat (IMF) content by selection, and its consequences on other economically relevant traits. For this purpose, a divergent selection experiment on IMF content was performed in rabbits. Near infrared reflectance spectroscopy (NIRS) was used to measure IMF content, protein content and fatty acid composition during the selection process.

Accurate NIRS prediction equations were developed for measuring IMF content ($R^2_{CV}$=0.98 and SECV=0.07 g/100g muscle). Prediction models for saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), the main individual fatty acids of rabbit meat (C16:0, C18:1 n-9 and C18:2 n-6) and other minor fatty acids such as C14:0, C15:0, C16:1, C17:0, C18:0 and C18:3 n-3 were also accurate. Less accurate predictions were obtained for polyunsaturated fatty acids (PUFA), n-6, n-3, fatty acid ratios and protein content.

The application of NIRS in rabbit selection programmes for IMF content was studied. NIRS could be a proper technique to compare different groups or treatments because no differences were found when comparing IMF content measured by chemical analyses and that predicted by NIRS. However, regression statistics between chemical and predicted values of IMF showed that NIRS may not be accurate enough to predict individual genetic values for ranking animals, where a higher accuracy is needed. Nevertheless, NIRS could be applied in truncated selection. Intramuscular fat content of parents that would be selected by applying the chemical method was compared to that of parents that would be selected by applying NIRS, and no differences were found for both females and males. Thus, the response to selection using NIRS should be similar to the response using chemical methods.

A divergent selection experiment on IMF was carried out. Selection was based on the phenotypic value of IMF content measured in 2 full sibs of the first parity. Data were analyzed using Bayesian methodology. The difference between high and low lines for IMF content after 3 generations of selection was 0.09 g/100g of muscle. This difference represents a direct selection response of 9% of its mean. The estimated heritability for IMF content was 0.37 with a probability of 97% of being higher than 0.2. The selection response on IMF content was symmetrical, with values of 0.054 g/100g of muscle in

the high line and -0.051 g/100g of muscle in the low line in the third generation. The fitted animal model was validated, and results confirmed that this trait can be modified through selection in rabbits.

There was a correlated response on perirenal fat content, indicating that selection on IMF content may affect carcass quality. However, the estimated correlated response on this trait was not accurate. Meat quality was also modified by selection on IMF content. Muscle pH, SFA percentage, MUFA percentage and the n-6/n-3 ratio increased, while n-6 percentage, n-3 percentage and PUFA/SFA ratio decreased in the line with higher IMF content.

El objetivo de esta tesis ha sido examinar las oportunidades de mejorar genéticamente la grasa intramuscular (IMF) por selección y sus consecuencias en otros caracteres económicamente relevantes. Para ello, se ha llevado a cabo un experimento de selección divergente por el contenido de IMF en conejos. Se ha utilizado la espectroscopía de reflectancia en el infrarrojo cercano (NIRS) para medir el contenido de IMF, proteína y la composición de ácidos grasos durante el proceso de selección.

Se han obtenido ecuaciones de calibración precisas para medir el contenido de IMF ($R^2_{CV}$=0.98 and SECV=0.07 g/100g músculo). Las ecuaciones de predicción para el contenido de ácidos grasos saturados (SFA), monoinsaturados (MUFA), los ácidos grasos mayoritarios en la carne de conejo (C16:0, C18:1 n-9 y C18:2 n-6) y otros minoritarios (C14:0, C15:0, C16:1, C17:0, C18:0 y C18:3 n-3) han sido también precisas. Ecuaciones con menor precisión han sido obtenidas para el contenido de ácidos grasos poliinsaturados (PUFA), n-6, n-3, ratios y para proteína.

Se ha estudiado la aplicación de NIRS en programas de selección basados en IMF. NIRS es una técnica adecuada para la comparación de medias o de tratamientos ya que no se han encontrado diferencias al comparar el contenido de IMF medido por el método químico y el contenido predicho por NIRS. Sin embargo, los estadísticos de regresión muestran que NIRS no es suficientemente preciso para predecir valores genéticos individuales con los que establecer órdenes de animales, donde se necesita una precisión mayor. No obstante, NIRS podría ser utilizado en selección truncada. Se han comparado los valores de IMF de los padres que se hubieran seleccionado aplicando NIRS con los valores de IMF de los padres que se hubieran seleccionado aplicando el método químico y no se han encontrado diferencias ni para los padres ni para las madres. Por tanto, la respuesta obtenida utilizando NIRS sería similar a la respuesta obtenida utilizando el método químico.

Se ha llevado a cabo un experimento de selección divergente por IMF. La selección se ha basado en el valor fenotípico de IMF medido en 2 hermanos completos del primer parto. Se ha utilizado metodología Bayesiana para analizar los datos. La diferencia estimada entre las líneas alta y baja tras 3 generaciones de selección ha sido de 0.09 g/100g músculo. Esta diferencia representa una respuesta directa a la selección del 9% de la media del carácter. La heredabilidad estimada ha sido 0.37 con una probabilidad

del 97% de ser superior a 0.2. La respuesta ha sido simétrica, con valores de 0.054 g/100g músculo en la línea alta y -0.051 g/100g músculo en la línea baja en la tercera generación. El modelo animal utilizado ha sido validado, y los resultados confirman que este carácter puede ser modificado por selección en conejo.

Se ha observado una respuesta correlacionada a la selección por IMF en el contenido de grasa perirrenal. Esto indica que la selección podría afectar a la calidad de la canal. Sin embargo, la respuesta estimada no ha sido precisa. La calidad de la carne también se ha visto modificada por la selección. Se ha observado una respuesta correlacionada positiva en el pH, el porcentaje de SFA y de MUFA, así como el ratio n-6/n-3, y una respuesta correlacionada negativa en el porcentaje de n-6 y de n-3, y en el ratio PUFA/SFA.

L'objectiu d'aquesta tesi ha sigut estudiar les oportunitats de millorar genèticament el greix intramuscular (IMF) per selecció i les conseqüències en altres caràcters econòmicament rellevants. Per això, es va portar a terme un experiment de selecció divergent pel contingut de IMF en conills. Es va utilitzar l'espectroscòpia de reflectància en el infraroig proper (NIRS) per mesurar el contingut de IMF, proteïnes i la composició d'àcids grassos durant el procés de selecció. Es van obtindre equacions de calibratge precises per mesurar el contingut de IMF ($R^2_{CV}$=0.98 i SECV=0.07 g/100g múscul). Les equacions de predicció del contingut d'àcids grassos saturats (SFA), monoinsaturats (MUFA), els àcids grassos majoritaris en la carn de conill (C16:0, C18:1 n-9 i C18:2 n-6) i altres minoritaris (C14:0, C15:0, C16:1, C17:0, C18:0 i C18:3 n-3) també van ser precises. Per el contingut d'àcids grassos poliinsaturats (PUFA), n-3 i n-6 ràtios i proteïna, les equacions van ser menys precises.

Es va estudiar l'aplicació de NIRS en programes de selecció basats en IMF. NIRS és una tècnica adequada per a la comparació de mitjanes o de tractaments perquè no es trobaren diferències comparant el contingut de IMF mesurat per el mètode químic i el contingut mesurat per NIRS. No obstant això, les estadístiques de regressió mostren que NIRS no és prou precís per predir els valors genètics individuals amb el que s'estableixen ordres d'animals, on es necessita una major precisió. No obstant això, NIRS podria ser utilitzat en selecció truncada. Es van comparar els valors de IMF dels pares que s'haurien seleccionat aplicant NIRS amb el valors de IMF del pares que s'haurien seleccionat aplicant el mètode químic i no es trobaren diferències per a les mares o els pares. Per tant, la resposta obtinguda utilitzant NIRS seria semblant a la resposta obtinguda utilitzant el mètode químic.

Es va dur a terme un experiment de selecció divergent pel contingut de IMF. La selecció es va basar en el valor fenotípic de IMF mesurat en 2 germans complets del primer part. Es va utilitzar metodologia Bayesiana per analitzar les dades. La diferència entre les línies alta i baixa després de 3 generacions de selecció ha sigut de 0.09 g/ 100g de múscul. Aquesta diferència representa una resposta directa a la selecció d'un 9% de la mitjana del caràcter. L'heritabilitat estimada va ser de 0.37 amb una probabilitat del 97% de ser major de 0.2. La resposta ha sigut simètrica, amb valors de 0.054 g/100g múscul en la línia alta i -0.051 g/100g múscul en la línia baixa en la

tercera generació. El model animal utilitzat ha sigut validat i els resultats confirmen que aquest caràcter es pot modificar per selecció en conill.

Hi ha hagut una resposta correlacionada a la selecció per IMF en el contingut de greix perirrenal. Això indica que la selecció podria afectar la qualitat de la canal. No obstant això, la resposta prevista no ha estat precisa. La qualitat de la carn també s'ha modificat per la selecció. Es va produir una resposta correlacionada positiva al pH, el percentatge de SFA, MUFA i el ràtio n-6/n-3, així com una resposta correlacionada negativa el percentatge de n-6 i n-3 i el ràtio PUFA/SFA.

# GENERAL INTRODUCTION

Meat constitutes a substantial component of the human diet, and it is a source of high quality animal proteins, essential minerals, vitamins and fat (Warris, 2000). Nowadays, consumers of developed countries place a greater emphasis on the food quality. As a result, there is an increasing focus on improving meat quality in the meat industry. However, meat quality is a complex concept determined by multiple characteristics, making the overall improvement of meat quality difficult.

Intramuscular fat content plays an essential role in meat quality, largely determining eating quality and the nutritional value of the meat (reviewed by Wood et al., 2008). Therefore, strategies to increase IMF content can be considered as ways to improve meat quality. The moderate to high heritability (reviewed by Sellier, 1998) and the high variability of IMF content make it suitable for genetic improvement by selection methods. However, genetic programmes require the measurement of the traits of interest in a large number of animals. Moreover, IMF content is generally determined in slaughtered animals, and thus selection is based on data from full or half sibs. These disadvantages make the genetic improvement less optimal.

Rabbits serve as a good model for genetic studies in other livestock species due to their reduced generation interval, the low price of the carcasses and their small size. In addition, rabbits have some advantages over laboratory rodents because sensory studies can be performed with cooked rabbit meat, something which would not be possible with rodents.

Conventional chemical methods for measuring IMF content are time-consuming, expensive and destructive. In contrast, near infrared reflectance spectroscopy (NIRS) enables a faster measurement at relatively low cost. NIRS could be a suitable alternative to conventional methods for analyzing the high number of samples required in genetic programmes. Numerous studies have shown that NIRS is a good predictor of IMF in beef, pork and poultry meat (reviewed by Prieto et al., 2009). In rabbit meat, NIRS has also been applied for predicting fat content, but including both inter- and intramuscular fat content (Masoreo et al., 1994; Pla et al., 2004). Some studies have evaluated the possibilities of using NIRS for the prediction of meat chemical composition and fatty acid profile in selection programmes of pigs and beef

cattle (Zamora-Rojas et al., 2011; Cecchinato et al., 2012). However, there are no studies about the use of NIRS in rabbit selection programmes.

In spite of the fact that IMF content is suitable for selection, only few studies have focused on increasing it by selection (Sapp et al., 2002; Suzuki et al. 2005a; Schwab et al., 2009; Zhao et al., 2007) and none of them involved rabbits. These authors showed that this trait responds to selection.

Selection on IMF content may have consequences on other economically relevant traits that should be considered. However, there is little information on correlated responses on carcass and meat quality traits in pigs, beef cattle and poultry, and no information is available in rabbits. Schwab et al. (2009) showed that in pigs the positive response on IMF is accompanied by an increase in backfat content. However, the estimated genetic correlation between IMF and backfat content in pigs ranges from 0.04 to 0.60 (reviewed by Sellier, 1998), suggesting some genetic independence between fat depots. This indicates that the correlated response on carcass fat could be very variable. In fact, Sapp et al. (2002) obtained no correlated response on fat thickness in bulls selected for IMF. Intramuscular fat content has been genetically related to several meat quality traits such as water-holding capacity, reflectance, pH, tenderness, juiciness, overall acceptability, flavour, firmness of the meat and fatty acid composition (Sellier 1998; Suzuki et al., 2005b; Suzuki et al., 2006; Schwab et al., 2010; Gjerlaug-Enger et al., 2010). However, these correlations had large standard errors and showed a wide range of variation between studies due to the difficulty of having a large amount of data to estimate them.

Divergent selection experiments are used to examine the genetic determinism of the traits of interest faster than using a control population. Direct and correlated responses can be studied by comparing the means of high and low lines for different traits after selection, which requires fewer individuals to obtain reasonable accuracies. Correlated responses are less dependent of the model than the estimation of genetic correlations. They provide information about the sign and importance of the relationship, which is the aim of the estimation of genetic correlations. Due to the high inaccuracy of the estimated genetic correlations for meat traits, correlated responses give a better estimate of the consequences of selection.

**Literature cited**

Cecchinato, A., M. De Marchi, M. Penasa, J. Casellas, S. Schiavon, and G. Bittante. 2012. Genetic analysis of beef fatty acid composition predicted by near-infrared spectroscopy. Journal of Animal Science. 90:429-438.

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

Masoero, G., G. Xiccato, A. Dalle Zotte, R. Parigi-Bini, and G. Bergoglio. 1994. Analysis of freeze-dried rabbit meat by NIRS. Zootecnia Nutrizione Animale. 20:319-329.

Pla, M., M. Pascual, and B. Ariño. 2004. Protein, fat and moisture content of retail cuts of rabbit meat evaluated with the NIRS methodology. World Rabbit Science. 12:149-158.

Prieto, N., R. Roehe, P. Lavín, G. Batten, and S. Andrés. 2009. Application of near infrared reflectance spectroscopy to predict meat and meat products quality: A review. Meat Science. 83:175-186.

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. Journal of Animal Science. 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. Journal of Animal Science. 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. Journal of Animal Science. 88:69-79.

Sellier, P. 1998. Genetics of meat and carcass traits. In: M. F. Rothschild and A. Ruvinsky, editors, The Genetics of the Pig. CAB International, New York, USA. p. 463-510.

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

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

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

Warris, P. D. 2000. Meat Science. An Introductory Text. ed. CABI Publishing, Wallinford, Oxon, UK.

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 Science. 78:343–358.

Zamora-Rojas, E., A. Garrido-Varo, E. De Pedro-Sanz, J. E. Guerero-Ginel, and D. Pérez-Marín. 2011. Monitoring NIRS calibrations for use in routine meat analysis as part of Iberian pig-breeding programs. Food Chemistry. 129:1889-1897.

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. Poultry Science. 86:2309–2314.

# LITERATURE REVIEW

# 1. Intramuscular fat and meat quality

## 1.1. Intramuscular fat and other fat depots

In general, there are four major fat depots in the body: subcutaneous, internal organ-associated, intermuscular and intramuscular fat depots. There are substantial differences in fat distribution between and within species (reviewed by Gerbens, 2004). For example, the most important depot in pigs is subcutaneous fat, averaging 60 to 70% of total carcass fat. In cattle, the most abundant fat depot is intermuscular with 45% of total carcass fat; the internal organ-associated represents 37%. In rabbits, the most important depot is the internal perirenal fat depot, representing 50 to 60% of total carcass fat (Hernández et al., 2006; Pla et al., 2004). The distribution of fat between different fat depots also varies between breeds. In pigs, Candek-Potokar et al. (2002) reported that Duroc, Large White and Landrace crossbreds had similar backfat thickness, but the Duroc crossbred has more IMF.

Fat accumulation between different depots is partially controlled by common genes. This is shown by the reported genetic correlation between IMF content and backfat thickness in pigs (0.30; reviewed by Sellier, 1998) and cattle (0.26; Marshall, 1999). Nevertheless, these correlations are different from the unity, indicating a gene specific regulation in each depot.

Morphologically, intramuscular fat (IMF) is the total of lipids associated with all cells present in a meat sample, mainly myocytes and adipocytes. Chemically, intramuscular fat is mainly composed of phospholipids, triacylglycerols (TAG) and cholesterol. Phospholipids are the major class of membrane lipids. The content of phospholipids in muscle is almost constant; for example, in rabbits varies between 0.5 and 0.9 g/100g muscle depending on the muscle (Alasnier et al., 1996; Dalle Zotte, 2004). Triacylglycerols are the main component of IMF and an important source of energy for skeletal muscle during exercise. The content of TAG is more variable than the content of phospholipids, ranging between 0.5 to 3.9 g/100g muscle in different rabbit muscles (Alasnier et al., 1996). Within intramuscular adipose tissue, TAG are mainly stored in adipocytes, distributed between muscle fibres, and to a minor extent intramyocellularly, as droplets in myofiber cytoplasm (Pethick et al., 2004). The relative

contribution between intramyocellular and adipocyte lipid depots of intramuscular adipose tissue was studied by Gondret et al. (1998) during rabbit 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 after those two weeks.

Fat deposition in muscle results from the balance between uptake, synthesis and degradation of TAG, which involve several metabolic pathways in both intramuscular adipocytes and myocytes (Gondret et al., 2004). The synthesis of TAG in intramuscular adipocytes and myocytes proceeds from fatty acids synthesized *de novo* in this tissue or from fatty acids consumed in the diet and obtained from circulating TAG via adipose tissue lipoprotein lipase. Liver and adipose tissue are the major sites of *de novo* lipogenesis in mammals but the relative contribution varies between species. In ruminants (cattle, sheep and goats) and pigs, the adipose tissue is the principal site of fatty acid synthesis (reviewed by Dodson et al., 2010). In humans, the liver is the most important site, whereas in rat and mouse both tissues are contributing (reviewed by Gerbens, 2004). In growing rabbits, liver is the major site for *de novo* lipogenesis whereas adipose tissue became more relevant in adults (Gondret et al., 1997).

Marbling is a term used to refer to the appearance of white flecks of IMF between the bundles of muscle fibres. Marbling score is an important part of beef grading in the United States; the best grades has the highest marbling content. There is a high positive genetic correlation between IMF content and marbling in beef (0.81; Marshall, 1999).

## 1.2. Meat quality

### 1.2.1. Components of meat quality

Meat quality is a generic term used to describe properties and perceptions of meat. It consists of: sensory characteristics, such as colour, juiciness, tenderness and flavour; nutritional properties, such as appropriate proportions of bioactive compounds, proteins, lipids and their constituents; technological factors, such as pH, water-holding capacity and oxidative stability; microbiological and chemical safety; and ethical

aspects, such as acceptable husbandry of animals and the impact of animal production on the environment and on food safety.

Meat colour is one of the most important factors affecting consumer acceptance and purchasing decision. Tenderness, juiciness and flavour are the most important elements at the point of consumption (Maltin et al., 2003). Nowadays, consumers of developed countries also place emphasis on the nutritional aspects of meat as well as on the safety and the environmental impact of meat production. Besides, meat processors focus on adequate technological characteristics. As a result, improved meat quality is becoming an indispensable requirement for the meat industry. However, meat quality is a multi-factorial and complex concept, which makes the overall improvement of meat quality difficult.

### 1.2.2. Rabbit meat quality

Rabbit meat offers good nutritive and dietetic properties (reviewed by Hernández and Gondret, 2006 and Hernández and Dalle Zotte, 2010). It is a lean meat with high protein content. Meat from the *Longissimus* muscle and the hind leg have the highest protein content (22.1 and 21.2 g/100g edible meat, respectively), while the lowest value for protein content corresponds to the thoracic cage (18.7 g/100g edible meat) (Pla et al., 2004). Rabbit meat also contains high levels of essential amino acids, constituting proteins with a high biological value (Dalle Zotte, 2004). The fat content varies depending on the carcass portion considered. The *Longissimus* muscle has the lowest fat content (1.2 g/100g edible meat), whereas the fattest part is the thoracic cage (12.8 g/100g edible meat) (Pla et al., 2004). Fat of rabbit meat comprises mostly saturated (SFA) and polyunsaturated fatty acids (PUFA), with percentages around 38% and 36.5% of the total fatty acids in the *Longissimus* muscle. Monounsaturated fatty acids are present in a lower amount (26.7% of the total fatty acids in the *Longissimus* muscle). The amount of SFA in rabbit meat is similar than in pork (37%) and beef meat (39.5%), but the amount of PUFA is much higher than that found in these species (18.5% in pork and 9.5% in beef meat) (Combes, 2004). The ratio PUFA/SFA is high in rabbit meat, with values of 0.95 in the *Longissimus* muscle.

The most ubiquitous fatty acids are palmitic, oleic and linoleic acids, showing percentages higher than 20% of the total. Among the PUFA, linoleic and linolenic acids

are essential fatty acids because they are necessary precursors for the synthesis of other products, and they must be supplied by the diet. Linoleic acid is the precursor of omega-6 family of PUFA, while linolenic acid serves as the same function for the omega-3 family, especially for eicosapentanoic (EPA) and docosahexanoic (DHA) acids. Linoleic acid represents 24.8% of the total fatty acids in rabbit *Longissimus* muscle, while in pork represents 14.3% and beef meat 6.3% (Combes, 2004). Linolenic acid has a lower percentage than linoleic acid in rabbit meat (2.2% of total fatty acids), but this amount is higher than in pork (0.55%) and beef meat (0.91%) (Combes, 2004). The amounts of EPA and DHA are low in rabbit meat. Nevertheless, these amounts can be improved through the diet (reviewed by Hernández and Dalle Zotte, 2010). Rabbit meat has a high n-6/n-3 ratio, with values of 5.1 for the *Longissimus* muscle and 10 for the hind leg meat (Combes and Dalle Zotte, 2005).

The cholesterol level is lower in rabbits (45 mg/100g) than in meat of other species (61 in pork, 70 in beef and 81 mg/100g in poultry) (Dalle Zotte, 2004). Rabbit meat is also an important source of B vitamins (B2, B5, B6, B3 and B12) (Combes, 2004). The mineral fraction of rabbit meat is characterised by low contents in sodium and iron (37 and 1.1 g/100g muscle, respectively), whereas the phosphorous level is high (222 g/100g muscle) (Dalle Zotte, 2004).

The main sensory properties of meat are colour, juiciness, tenderness and flavour (Warris, 2000). Rabbit meat is considered to have positive sensory properties (Dalle Zotte, 2002). It is a pale meat with a low redness index (Hernández et al., 1997). Mean colour values of the *Longissimus* muscle are: L*=56-60, a*=2.6-3.4 and b*=4.0-5.0; this muscle has higher lightness (L*) and lower redness (a*) in rabbits than in pigs (L*=48-52; a*=8-11) or beef (L*=41-44; a*=20-21), while yellowness (b*) is similar to pigs and lower than beef (Dalle Zotte, 2004). Juiciness has an important role in meat quality. It is determined by the water-holding capacity (WHC) and the IMF content of meat. Meat WHC is defined as the ability of meat to retain its water content during the application of external forces as cutting, heating, grinding or pressing (Warris, 2000).

Rabbit meat was considered one of the most tender meats in a sensory study which evaluated meat from 15 species (Rødbotten et al., 2004). Meat tenderness mainly depends on the post mortem changes affecting myofibrillar proteins and the

connective tissue (collagen content and solubility) (Lawrie, 1998). Rabbit meat has lower collagen content and higher collagen solubility than meat from other species (Combes et al., 2003). Tenderness can be measured instrumentally by Warner-Brazler shear force testing (WBSF) and texture profile analysis. Values of WBSF are low in rabbit meat, averaging 3.57 for the *Longissimus* muscle (Ariño et al., 2006).

Rabbit meat was considered one of the meats with the least flavour intensity and flavour attributes such as sweet, liver and gamy (Rødbotten et al., 2004). However, the taste of wild game meat is sometimes perceived by the consumer and constitutes a cause of refusal of this meat (Dalle Zotte, 2002). Muscle pH influences eating and technological meat quality. The post mortem evolution of pH and the pH measured at 24 hours *post mortem* (pHu) affects lightness, WHC and toughness (Lawrie, 1998). In rabbits, pHu ranges between 5.4 and 6.4 depending on muscle (Hulot and Ouhayoun, 1999). To date, no case of pale, soft and exudative meat or acidic meat has been reported in rabbit meat. However, dark, firm and dry meat has been shown by Rodrígez-Calleja et al. (2005).

## 1.3. The role of intramuscular fat in meat quality

### 1.3.1. Eating quality

Intramuscular fat content is one of the important traits determining eating quality characteristics such as juiciness, flavour, tenderness and overall acceptability of meat in different species (reviewed by Warris, 2000 and Wood et al., 2008), although the relationship between IMF and eating quality varies between studies. It is generally agreed that low levels of IMF will lead to dry and less tasty meat. This is the case of young bulls from double-muscled cattle breeds such as Belgian Blue (Raes et al., 2001) or pig genotypes selected for lean content (Wood et al., 2004). However, relevant relationships with sensory traits were often observed only for high variations in IMF content.

In the review of Wood et al. (2008), the correlation between IMF and juiciness was 0.31 in pork and 0.36 in lamb meat. In contrast, the correlation between IMF and tenderness was lower (0.13 in pork meat and between IMF and toughness -0.06 in lamb meat). In rabbits, the relationship between IMF and juiciness was 0.24, and between IMF and toughness was -0.16 (Hernández et al., 2000). Thus, juiciness seems

to be the trait most affected by increasing levels of IMF. This relationship may be explained by the fact that fat promotes the flow of saliva in the mouth. There is also an improvement in flavour which may derive from reactions of the fat during cooking (Warris, 2000). The effect of IMF on tenderness is mainly due to the increase of juiciness. In fact, Hernández et al. (2000) reported a high negative correlation between juiciness and toughness (-0.60). In the case of high variations in IMF content, there is a direct influence of IMF on tenderness. Intramuscular fat deposited between fibre bundles disrupts the structure of the endomysium, and that perimysium is separated into thinner collagen fibres. This disorganizes the structure of connective tissue and contributes to the increase of meat tenderness (Nishimura et al., 1999).

Results of studies trying to quantify the effect of IMF on eating quality were inconclusive. Relevant relationships with sensory traits were often observed only for high variations in IMF content. Thus, rather than trying to find a linear relationship with eating quality characteristics, some investigations aim at determining a level of IMF content that ensures an acceptable eating quality. There are no reliable studies concerning the optimal range of IMF required to ensure a satisfactory eating quality in meat. Nevertheless, Gerbens (2004) reported in his review that the recommended level of IMF is approximately 2.5-3.0% in pork meat. Hocquette et al. (2010) reported values of about 3.0-4.0% for beef and 5.0% for sheep meat to achieve acceptable consumer satisfaction. However, these values would correspond to adult animals, which have a lower acceptability in some markets.

### 1.3.2. Nutritional value

Intramuscular fat content and its fatty acid composition determine the nutritional value of the meat. High levels of fat and SFA have been reported to be associated with obesity, various cancers, and especially with coronary heart disease (Wood et al., 2003). High dietary saturated fat and cholesterol intakes led to increased plasma LDL-cholesterol concentrations and thus increase coronary heart disease risk (Sacks and Katan, 2002). A report of a joint WHO/FAO expert consultation (WHO, 2003) recommended a total dietary fat intake between 15-30% of daily energy intake for preventing cardiovascular diseases. The recommendation for SFA was to reduce the intake to less than 10% of daily energy intake. However, current epidemiologic studies

have shown that there is insufficient evidence for concluding that dietary saturated fats are associated with an increased risk of coronary heart disease or cardiovascular disease (Siri-Tanino et al., 2010). The link between high plasma cholesterol and heart disease is more complex than previously believed. Several factors have been identified to causes heart disease, but the precise mechanisms have not been fully elucidated (Willyard, 2013).

More recently, nutritionists have focused on PUFA and the balance in the diet between n-3 PUFA and n-6 PUFA. Polyunsaturated fatty acids have been shown to protect against coronary heart disease by lowering plasma LDL-cholesterol (Sacks and Katan, 2002). According to the ISSFAL (2004), an adequate diet for healthy adults should include an amount of 2% of linoleic acid and 0.7% of linolenic acid of the total energy intake. For cardiovascular health, a minimum intake of combined eicosapentanoic acid (EPA) and docosahexanoic (DHA) of 500 mg/day is also recommended (ISSFAL, 2004). The ratio of n-6/n-3 is also a risk factor in cancers and coronary disease. Current recommendations state that the ratio n-6/n-3 in human diets should be reduced to values of 5-10, although a value lower than 4 is recommended for reducing the risk of many chronic diseases (Simopoulos, 2002; Tres et al., 2008).

### 1.3.3. Technological quality

The fatty acid composition of muscle also determines some aspects of technological meat quality such as fat firmness and shelf life (reviewed by Wood et al., 2003 and 2008). The effect of fatty acids on fat firmness is due to the different melting points of the fatty acids. Unsaturated fatty acids have lower melting points than saturated. Variations in the structure of the molecule are also important. *Trans*- fatty acids have a higher melting point than their *cis*-isomers (Wood et al., 2003).

The effect on shelf life is due to the propensity of unsaturated fatty acids to oxidative breakdown (Warris, 2000). This affects the oxidative stability of meat during processing and retail display, and therefore its shelf life. Lipid oxidation products produce a rancid odour and taste. Meat colour can also be affected by the oxidation of red oxymioglobin to brown metmyoglobin (Wood et al., 2003).

### 1.4. Intramuscular fat as a way to improve meat quality

Nowadays, consumers are placing a great emphasis on aspects of meat quality and are demanding high quality products. As a result, there is an increasing focus on improving meat quality in the different livestock species. A possible way to improve meat quality is the increase of IMF content. In this sense, the goal should be to increase IMF content enough to satisfy eating quality demands, but avoiding an excess of fat that will impair human health and meat appearance.

Finally, the implementation of strategies for improving meat quality depends on the willingness of consumers to buy and pay more for meat of better quality. Consumers need to be able to discriminate between different categories of meat eating quality. Therefore, accurate systems of meat grading, branding and labelling should be provided for meat with improved eating quality. Different surveys among consumers, meat quality experts and the retail industry reported a positive acceptance, both by the consumer and the meat industry, of improved meat quality through optimizing IMF content (von Rohr et al., 1999; Gerbens, 2004). Some North American beef plants are grading carcasses on the basis of IMF content (Mermelstein, 2013). This way of grading is called quality grade and has been developed by researchers of the U. S. Meat Animal Research Center (USMARC) (www.ams.usda.gov). The quality grade is an estimate of the expected tenderness, juiciness and flavour, and is based on marbling within the rib eye and the age of the animal. There are four potential USDA quality grades: USDA Prime, the highest quality, has the highest amount of IMF content; USDA Choice, the most common grade sold at retail and in restaurants, has less IMF than USDA Prime; USDA Select, has less IMF than USDA Choice and is sold at a lower price than the higher grades, based on lower expected tenderness and flavour; USDA Standard has the least IMF content and is frequently sold as ungraded.

## 2. The use of Near Infrared Reflectance Spectroscopy to assess intramuscular fat content

Near infrared reflectance spectroscopy (NIRS) offers numerous advantages over chemical conventional methods based on fat extraction using different organic solvents. It is a non-destructive technique which provides a fast and accurate

measurement at relatively low cost, with minimal or no sample preparation. No reagents are required and no waste is produced. Simultaneous measurements of different attributes are possible with NIRS. Moreover, it can be applied under online conditions in the industry (reviewed by Prieto et al., 2009). The main disadvantages of NIRS are its dependence of the reference method, a weak sensitivity to minor constituents and a complex spectral data interpretation (reviewed by Prevolnik et al., 2004).

### 2.1. NIRS concepts

Near infrared reflectance spectroscopy is an analytical technique that uses a source producing light of known wavelength pattern (from 800 to 2500 nm), and that provides structural information related to the vibration of chemical bonds. When the sample is irradiated, the electromagnetic spectrum produced involves the response of the molecular bonds O-H, C-H, C-O and N-H.

The data acquired from NIRS contain information about the sample but also background information and noises. In order to obtain reliable, accurate and stable calibration models, it is necessary to pre-process spectral data and to eliminate the redundant information (Cen et al., 2007). At present, there are numerous pre-processing methods, such as smoothing, derivative, standard normal variate transformation (SNV), detrending (DT), and multiplicative scatter correction (MSC). After studying spectral data, calibration models have to be developed. There are also different statistical methods to develop calibrations such as multiple linear regressions, partial and modified partial least square (PLS), principal component (PCR), and more recently neural networks.

The prediction accuracy of the models can be assessed by two methods: cross-validation and external validation. Cross-validation is a method in which the sample set of the calibration is also used for prediction. The calibration set is divided into different groups and the prediction is carried out on every group with the calibration developed from the remaining groups. Thus, the error of cross-validation represents a true estimate of the prediction accuracy. The external validation uses a different group from the calibration set. The common statistical parameters used to assess the prediction accuracy are: the coefficient of determination of calibration ($R^2_{CA}$) or cross-

validation ($R^2_{CV}$) or prediction ($R^2_P$); the standard error of calibration (SEC) or cross-validation (SECV) or prediction (SEP); the residual predictive deviance (RPD), defined as the ratio between standard deviation of the reference data to the SEC or SECV or SEP; and the range error ratio (RER), defined as the ratio between the range of the reference data to the SEC or SECV or SEP.

### 2.2. Application of NIRS to predict IMF content

Numerous studies have shown that NIRS has a high potential to predict meat chemical composition in poultry, sheep, beef and pork meat (reviewed by Prevolnik et al., 2004 and Prieto et al., 2009). From these studies, the highest prediction accuracy is reported for IMF content; showing $R^2_{CA}$ from 0.79 to 0.99 and RPD from 1.83 to 8.3 in homogenised samples. The good performance of NIRS to predict IMF content is due to the absorption of the C-H bonds of fatty acids at 1100-1400 nm, 1700 nm and 2200-2400 nm.

Different prediction accuracies were found between studies. These differences should be attributed to several factors. Sample preparation has an important effect on NIRS prediction ability. Minced samples are more homogeneous and can be predicted more accurately than intact samples. For example, in the review of Prieto et al. (2009) the $R^2_{CA}$ was from 0.34 to 0.97 in intact meat. In these samples, the muscle fibres tend to conduct the light by means of internal reflections. In addition, intact muscle absorbs more light and then it gives less reflectance than homogenised samples. Freeze-drying of samples allows more homogeneous samples. Viljoen et al. (2005, 2007) reported accurate equations for IMF content in freeze-dried samples of ostrich ($R^2_{CA}$=0.99 and SEP=0.29%) and mutton meat ($R^2_{CA}$=1.00 and SEP=0.43%), respectively. Freeze-drying also avoids spectra regions with high absorption which have high levels of noise and samples are less affected by temperature fluctuations. The variability present within the calibration group is also a determining factor for developing accurate models. In addition, the success of NIRS depends on the reliability of the reference method; large errors in the reference data will be present in the model and will reduce it accuracy. Finally, NIRS equipments and the wavelength range studied are also variation factors.

There is only a small number of studies on rabbit meat. Masoero et al. (1994) developed prediction equations for measuring fat content in freeze-dried *Longissimus*

muscle and hind leg, obtaining good accuracy ($R^2_{CV}$=0.99 and $SE_{CV}$=0.26%). Bazar et al. (2007) also reported a good equation for measuring fat content in meat from freeze-dried hind leg ($R^2_{CV}$=0.99 and SEP=0.35%), but less accurate in meat from hind leg ($R^2_{CV}$=0.89 and SEP=0.83%). Fat content was also accurately predicted in several parts of rabbit carcasses (forelegs, thoracic cage, loin part and hind leg) by Pla et al. (2004), showing $R^2_{CV}$ of 0.98 and $SE_{CV}$=0.49%. Nevertheless, the meat of all these studies included inter- and intramuscular fat content.

NIRS could be a suitable alternative to chemical methods in genetic programmes, where numerous determinations are needed on a continual basis. Prevolnik et al. (2005) obtained accurate models to determine IMF content in pork and beef meat, and proposed their use in animal selection programmes. Additionally, Zamora-Rojas et al. (2011) showed the possibilities of using NIRS for classifying individual animals in breeding programmes on the basis of IMF content. Moreover, these authors proposed a method for producing and improving robust calibration models that ensures a reliable evaluation applicable in long-term breeding programmes.

### 2.3. Application of NIRS to predict other meat quality characteristics

With regard to chemical composition, literature results showed a good ability of NIRS to predict protein and moisture content, although the accuracy was somewhat lower than for IMF content (reviewed by Prevolnik et al., 2004 and Prieto et al., 2009). The predictive capacity of NIRS to asses water and protein content is explained by the specific absorbance of O-H bonds at 1450 and 1940 nm for water, and the absorption of the N-H bounds at 1460-1570 nm and 2000-2180 nm for protein. Some attempts were made for predicting other chemical components. The reported results for collagen content were less accurate (Prieto et al., 2006). This can be explained by the weak sensitivity of NIRS to minor constituents and the low reliability of the reference method. Similarly, myoglobin could not be successfully predicted by NIRS (Prieto et al., 2006). However, Ripoll et al. (2008) accurately predicted this pigment in beef meat by using visible and near infrared regions of the spectra.

The fatty acid content has also been studied by NIRS in meat of several species such as pork (González-Martín et al., 2005), beef (Prieto et al., 2011; Cecchinato et al., 2012), lamb (Guy et al., 2011), poultry (Berzaghi et al., 2005) and rabbit (Pla et al., 2007).

Nevertheless, this technique has a limited ability for estimating some individual fatty acids due to the similarity between their NIRS absorption patterns (Windham and Morrison, 1998).

NIRS has only limited ability for estimating technological and sensory attributes of meat (reviewed by Prevolnik et al., 2004 and Prieto et al., 2009). The main part of the studies on technological parameters aimed at predicting water holding capacity, colour and pH. Reported results showed a great variation between studies, and a less accurate prediction of these parameters compared to chemical composition traits. The prediction of meat sensory attributes such as tenderness, juiciness, firmness, flavour and acceptability by NIRS is difficult. These characteristics are related to the interactions of many chemical and physical properties. Moreover, they are complex traits measured by scores, a non-continuous type of measurement, and affected by the subjectivity of the assessors (Meilgaard et al., 1999).

NIRS is a suitable technique for categorisation of meat and meat products (reviewed by Prieto et al., 2009). Discriminant analysis enables the differentiation among meat from different species (Ding and Xu, 1999), between frozen and unfrozen beef (Thyholt and Isakson, 1997) and poultry meat (Ding et al., 1999), between conventional and organic rabbits (Pla et al., 2007). A very useful application of NIRS is the detection of fraudulent products in meat; for example, the detection of beef hamburger adulteration or illegal growth promoters in meat (reviewed by Prieto et al., 2009). More recently, the USMARC has been working with visible and near- infrared reflectance (VisNIR) spectroscopy to predict tenderness (Mermelstein, 2013). The researchers have developed a system which identifies the most tender beef carcasses or cuts. These carcasses and cuts are marketed accordingly their predicted tenderness. USMARC researchers are also working on measuring the colour stability of lean muscle using the same VisNir system, although the method is still being refined.

### 2.4. Other non-invasive methods to assess intramuscular fat content

There are other methods for measuring IMF content directly on carcass or meat in a non-invasive way. Some of these methods can also be applied in the live animal.

### 2.4.1. Real-time ultrasound scanning

Ultrasound technology is a non-invasive method that can be used for measuring carcass composition and also live animal composition. Ultrasonic waves are transmitted through biological tissues and, depending on the acoustical properties of each tissue, either penetrate further into the tissue or are reflected. Ultrasound units that produce real-time images are now the most commonly accepted ultrasonic instrumentation for use with livestock (Schulte, 2010). This technique has been widely used for measuring carcass characteristics such as fat thickness and *Longissimus* muscle area in pigs (reviewed by Moeller, 2002), beef cattle (reviewed by Williams, 2002) and sheep (Silva et al., 2005).

Intramuscular fat content has been successfully measured by using real-time ultrasound technology in conjunction with image analysis software in live pigs (Newcom et al., 2002) and cattle (Hassen et al., 2001; Aas et al., 2009). This method is simple and can be used on farm, on a large number of animals and at reasonable costs. Thus, it is an alternative to data collection in sibling selection. Examples of this application can be found in Sapp et al. (2002) and Schwab et al. (2009), who used real-time ultrasound analysis for assessing IMF content in selection experiments for this trait in beef cattle and pigs, respectively. In rabbits, ultrasound has been applied *in vivo* for determining body composition based on the measurement of the perirenal fat thickness (Pascual et al., 2000). The application of ultrasound on IMF has not been studied yet; the low IMF content of rabbit muscles (1.2 g/100 g in the *Longissimus* muscle) could make this application difficult.

### 2.4.2. Computed Tomography

Computed tomography (CT) scanning is another non-invasive method that provides very accurate *in vivo* predictions of body composition in pigs (Szabo et al., 1999), sheep (Macfarlane et al., 2006) and rabbits (Szendro et al., 1992). The size of CT scanners prevents the use of this methodology in live beef cattle, but it can be used to scan primal cuts and determine their composition (Navajas et al., 2010). This technique is based on the attenuation of X-rays through tissues and objects depending on their different densities. It provides more accurate information than ultrasound technology, since cross-sectional scans can be collected at many anatomical positions along the

body of the animal. However, CT scanning has some disadvantages. Firstly, it is much more expensive than ultrasounds. In addition, it is located at a fixed place with special building characteristics for X-rays, requiring the transport of animals, whereas ultrasound is portable.

The prediction of IMF content using CT scanning has been based on CT muscle density, which is expressed as the average pixel values of muscle tissue in the CT image. In sheep, Karamichou et al. (2006) showed that IMF content can be accurately predicted with CT measurements, the muscle density being the predominant prediction factor. Lambe et al. (2009) developed a more complete method to predict IMF content of the *Longissimus* muscle in sheep, including the whole tissue density range. In beef, IMF was successfully predicted by spiral computed tomography (Prieto et al., 2010). This CT technology involves movement in a helical pattern, which allows an increase of the image resolution. More recently, Font-i-Furnols et al. (2013) have shown that CT also has good potential for measuring IMF content in the *Longissimus* muscle of pigs. As for ultrasound scanning, this technology has not been applied in rabbits for measuring IMF content and the success of this application is doubtful.

### 2.4.3. Video Image Analysis

Video image analysis (VIA) is a non-invasive technology which allows the assessment of carcass and meat in an objective manner. It could be automated in the abattoir at line speed. VIA was developed in the USA for objective beef carcass evaluation in the early 1980s. Since the 1980s, VIA has been applied to several aspects of both carcass and meat quality evaluation (reviewed by Craigie et al., 2012). There are different VIA systems depending on the species and the application (www.eplusv.com). The instrument has a camera inside which is placed over the carcass and take different pictures of the carcass. Then, image processing and analyzing software are applied.

With regard to IMF content, VIA system has been applied in beef (Mermelstein, 2013) and pork meat (Faucitano et al., 2005). In the case of beef, the VIA instrument assesses the degree of marbling in the rib eye. Then, based on this measurement, the carcass is graded into 4 quality grades. This technology has not been applied in rabbits; as for the previous techniques, the application could be difficult due to the low IMF content of rabbits.

## 3. Genetic strategies to improve intramuscular fat content

The selection for increasing lean content developed in pigs during the last decades can lead to a decrease in backfat thickness, but also to a decrease in IMF content, with a consequent deterioration of meat quality (reviewed by Gerbens, 2004). For example, de Vries et al. (1994) estimated that for each extra percent of carcass leanness, IMF would be reduced by 0.07%. Schwab et al. (2006), comparing a control population with a commercial population selected on carcass leanness for the last 20 years, reported a negative correlated response on IMF content, instrumental texture and different sensory parameters (visual colour, pork flavour and off-flavour scores). However, they found no effect on sensory tenderness, juiciness, chewiness, or cooking loss. Cameron et al. (1999) reported that selection for lean growth rate changed some meat characteristics, but this change did not have consequences for meat acceptability, which was determined by a sensory panel.

As meat quality has become more important in the recent years, breeding programmes should consider meat quality traits. One way to improve meat quality can be to genetically optimise IMF content. Rabbits are good models for genetic studies due to their reduced generation interval, the low price of the carcasses and their small size. In addition, rabbits have some advantages over laboratory rodents because sensory studies can be performed with cooked rabbit meat, something which would not be possible with rodents.

### 3.1. Breed crosses

The use of breeds with higher IMF content in crosses can be a procedure for genetically improving IMF content. Duroc is generally considered to be the breed with the highest IMF levels in pigs. It is used as a terminal sire in breeding schemes to improve pork meat quality (Warris, 2000). Duroc pigs may have too high IMF levels (>3-4%) for consumer acceptability and for processing into cooked hams. Thus, crosses containing up to 50% of Duroc genes are normally used.

In cattle, there are some breeds with an extraordinary ability for fat deposition such as Japanese Black cattle. These breeds are used mainly in Japan, Australia and the United States to produce high-marbled meat. The IMF content can reach up to 20 to 30%

(Jurie et al., 2007). High amounts of fat are also accumulated in the carcass of these breeds, especially subcutaneous fat. Nevertheless, the development of fat in other depots of the carcass as per percent of IMF deposition is relatively lower than in European breeds (Gotoh et al., 2009). However, the use of breed crosses for improving IMF content is not as common as in pigs.

### 3.2. Selection for intramuscular fat content

Intramuscular fat content has a moderate to high heritability. In pigs, Sellier (1998) reported an average of 0.50 in his review, with values ranging between 0.26 and 0.86. More recent estimates are also within this range (Suzuki et al., 2005a; Solanes et al., 2009; Sellier et al., 2010). Marshall (1999) showed heritabilities for IMF in beef cattle between 0.26 and 0.93, with an average of 0.54. The moderate to high heritability and the high variability of IMF content make it suitable for genetic improvement by using selection methods. Suzuki et al. (2005b) carried out a selection experiment on daily gain, loin eye area, backfat thickness and IMF content in Duroc pigs. The desired gains for daily gain, loin eye area and backfat thickness were not achieved, but the estimated breeding value for IMF content increased after seven generations of selection. However, the inclusion of IMF as one of the different selection objectives makes the determination of the effect of selection on IMF content more difficult.

There is a small number of selection experiments that focuses only on IMF content. Sapp et al. (2002) and Schwab et al. (2009) reported an increase in IMF content after selection in beef cattle and pigs, respectively. However, selection on IMF content may have consequences on other economically important traits that should be considered. For example, Schwab et al. (2009) showed that in pigs the response on IMF is accompanied by an increase in backfat content. Nevertheless, the genetic correlation between intramuscular fat and backfat content is moderate (an average of 0.30) and has a wide range of variation (0.04-0.60 reviewed by Sellier, 1998), suggesting the possibility of improving IMF independently from carcass fat content. In beef cattle, the genetic correlation between IMF content (%) and fat thickness (usually measured between the $12^{th}$ and $13^{th}$ rib and used to estimate total carcass fat) averaged 0.26, ranging from -0.06 to 0.71 (Marshall, 1999). The correlation between marbling score and fat thickness averaged 0.10, ranging from -0.13 to 0.44 (Bertrand et al., 2001).

Sapp et al. (2002) obtained no correlated response on fat thickness in bulls selected for IMF content.

Intramuscular fat content has been genetically related to several meat quality traits in pigs, such as water-holding capacity, reflectance, pH, tenderness, juiciness, overall acceptability, flavour, firmness of the meat and fatty acid composition (Sellier 1998; Suzuki et al., 2005a; Suzuki et al., 2006; Schwab et al., 2010; Gjerlaug-Enger et al., 2010). Similarly, IMF has been genetically related to shear force, calpastatin activity, tenderness score and fatty acid composition in beef cattle (Marshall, 1999; Bertrand et al., 2001; Pitchford et al., 2002; Nogi et al., 2011). However, these correlations had large standard errors and showed a wide range of variation between studies due to the difficulty of having a large amount of data to estimate them. The most consistent and least variable correlation was that between IMF and overall acceptability in pigs, with an average of 0.61 and a range between 0.54 and 0.68 (Sellier, 1998). For the other traits, there was either a discrepancy between studies or an insufficient number of studies for showing clear relationships.

Additionally, the genetic correlations between IMF values from different muscles may not be necessarily positive or elevated. This could lead to an increase of IMF content in one muscle, while not affecting or even decreasing IMF content in other muscles. In fact, Moreno-Sánchez et al. (2006) reported a negative genetic correlation between IMF content of two different muscles. Moreover, Quintanilla et al. (2011) showed a lack of concordance between QTLs affecting IMF content of two muscles, suggesting the existence of muscle-specific genetic factors regulating IMF content and composition.

Thus, it would be important for future breeding programmes in rabbits, poultry, pigs and cattle to have a better knowledge about the genetic relationships between IMF in one muscle and that in another muscle as well as between IMF content and other economically relevant traits, such as carcass fat content, growth traits and other traits involving IMF composition.

Divergent selection experiments have some advantages for examining the genetic determinism of the traits of interest. Firstly, direct and correlated responses can be studied by comparing the means of high and low lines for different traits after

selection, which requires fewer individuals to obtain a reasonable precision in comparison to the estimation of genetic correlations. In addition, means comparison is less dependent on the model than genetic correlations. An unselected control population is not needed for eliminating environmental fluctuations, because each selection line acts as a control for the other. Finally, the effectiveness of the selection process is doubled because both lines are selected. Thus, the genetic differentiation between lines is faster than when using a control population, without changing the variance of the response. However, the response is not always symmetrical in both lines.

### 3.3. Genomic approach

The advances in molecular genetics and computational biology of the last decades opened some possibilities for dissecting and understanding the genetic factors influencing meat quality (Ciobanu et al., 2011). These new tools are of particular interest because they can be applied on selection candidates and because information can be available early in life.

The existence of genes with a substantial effect on IMF content has been proved by segregation analysis in pigs (Janss et al., 1997). These genes or genomic regions may be applied in breeding by marker-assisted selection (MAS) or by gene introgression. Marker-assisted selection can increase the selection response in traits with a low heritability and/or in carcass and meat quality traits such as IMF content (Meuwissen and Goddard, 1996). During the past 20 years, both genome scan and candidate gene approaches have been widely used to localise chromosome regions (QTL) or functional genes, both of which affect fat deposition and composition traits. With regard to IMF content, considerable efforts have been made to identify QTLs in pigs (reviewed by Gerbens, 2004 and Ciobanu et al., 2011), poultry (Jennen et al., 2005) and beef cattle (reviewed by Burrow et al., 2001). The implementation of marker-assisted selection for IMF in breeding schemes depends on the presence of QTLs for IMF and the absence of genes that could adversely affect other economically important traits in the QTL region affecting IMF content. The problem is that in pigs the aforementioned QTLs affecting IMF are close to other QTLs that affect backfat thickness (Gerbens, 2004). In beef cattle, QTL mapping for both marbling and subcutaneous fat depth showed that from

the known QTLs only few have a common genetic background (reviewed by Hausman et al., 2009). This would make the selection for marbling and against fat thickness easier for the beef industry.

Some candidate genes have also been identified affecting IMF deposition. Genes involved in intracellular fatty acid transport within skeletal muscles have been studied. The adipocyte fatty-acid binding protein (FABP4) gene has been associated with IMF content variations in pigs (Gerbens et al., 1998) and cattle (Jurie et al., 2007). Candidate gene analysis showed that the leptin receptor gene (LEPR) is also associated with IMF content in pigs (Óvilo et al., 2002). Lipogenic genes have also been postulated as good markers for IMF content or its composition, such as fatty acid synthase, sterol regulatory element binding protein-1 (SREBP1) and stearoyl-CoA desaturase-1 (SCD1) (reviewed by Hocquette et al., 2010).

However, the associations between these genetic markers for IMF content are not always positive and consistent, as they are dependent on the species, the breed or the trait studied (IMF content or marbling score). Besides, they depend on the presence of QTLs of large or at least medium effect. This problem may be solved by genomic selection (Meuwissen et al., 2001). This approach is based on markers that cover the whole genome, and has become feasible thanks to the large number of single nucleotide polymorphisms (SNP) discovered by genome sequencing and new methods to efficiently genotype large numbers of those SNP. As the whole genome is scanned, the presence of major genes affecting the trait is no needed. Nevertheless, this type of approach needs powerful SNP panels and moderately large phenotypic databases for discovery and validation. Moreover, the relationships between SNPs and genes are being lost and should be re-estimated each three or four generations (see for example Ibáñez-Escriche and Blasco, 2011).

## 4. Literature cited.

Aass, L., C. G. Fristedt, and J. D. Gresham. 2009. Ultrasound prediction of intramuscular fat content in lean cattle. Livestock Science. 125:177–186.

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

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 Science. 73:687-692.

Bázár G. Y., Z. Princz, G. Jekkel, L. Locsmándi, G. Andrássy-Baka, G. Y. Kövér, Z. S. Szendrő, and R. Romvári. 2007. NIRS prediction for protein and intramuscular fat content of rabbit hind leg meat. Agriculture. 13:155-158.

Bertrand, J. K., R. D. Green, W. O. Herring, and D. W. Moser. 2001. Genetic evaluation for beef carcass traits. Journal of Animal Science. 79(Suppl.):E190-E200.

Berzaghi, P., A. Dalle Zotte, L. M. Jansson, and I. Andrighetto. 2005. Near-infrared reflectance spectroscopy as a method to predict chemical composition of breast meat and discriminate between different n-3 feeding sources. Poultry Science. 84:128–136.

Burrow, H. M., S. S. Moore, D. J. Johnston, W. Barense, and B. M. Bindon. 2001. Quantitative and molecular genetic influences on properties of beef: a review. Australian Journal of Experimental Agriculture. 41:893–919.

Cameron, N. O., G. R. Nute, S. N. Brown, M. Enser, and J. O. Wood. 1999. Meat quality of Large White pig genotypes selected for components of efficient lean growth rate. Animal Science. 68: 115-127

Candek-Potokar, M., G. Monin, and B. Zlender. 2002. Pork quality, processing, and sensory characteristics of dry-cured hams as influenced by Duroc crossing and sex. Journal of Animal Science. 80:988–996.

Cen, H., and Y. He. 2007. Theory and application of near infrared reflectance spectroscopy in determination of food quality. Trends in Food Science and Technology. 18:72-83.

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, editors, The Genetics of the Pig, 2nd Edition. CAB International, Cambridge, USA. p. 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 Science. 66:91–96.

Combes, S. 2004. Valeur nutritionnelle de la viande de lapin. *INRA Production Animales* 17, 373–383.

Combes, S., and A. Dalle Zotte, 2005. La viande de lapin: valeur nutritionnelle et particularites technologiques. In: Proceedings of 11èmes Journées de la Recherche Cunicole. Paris, France. p. 167–180.

Craigie, C. R., E. A. Navajas, R. W. Purchas, C. A. Maltin, L. Bünger, S. O. Hoskin, D. W. Ross, S. T. Morris, R. Roehe. 2012. A review of the development and use of video image analysis (VIA) for beef carcass evaluation as an alternative to the current EUROP system and other subjective systems. Meat Science 92: 307–318.

Dalle Zotte, A. 2002. Perception of rabbit meat quality and major factors influencing the rabbit carcass and meat quality. Livestock Production Science. 75:11–32.

Dalle Zotte, A. 2004. Avantage dietetiques. Le lapin doit apprivoiser le consommateur. Viandes Produits Carnés. 23:1–7.

de Vries, A. G., P. G. Van der Wal, .T. Long, G. Eihelenboom, and J. W. M. Merks. 1994. Genetic parameters of pork quality and production traits in Yorkshire populations. Livestock Production Science. 40:277-289.

Ding, H. B., and R. J. Xu. 1999. Differentiation of beef and kangaroo meat by visible/near-infrared reflectance spectroscopy. Journal of Food Science. 64:814–817.

Ding, H., R. J. Xu, and D. K. O. Chan.1999. Identification of broiler chicken meat using a visible/near-infrared spectroscopic technique. Journal of the Science of Food and Agriculture. 79:1382–1388.

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. International Journal of Biological Sciences. 6:691-699.

Faucitano, L., P. Huff, F. Teuscher, C. Gariepy, and J. Wegner. 2005. Application of computer image analysis to measure pork marbling characteristics. Meat Science. 69:537–543.

Font-i-Furnols, M., A. Brun, N. Tous, and M. Gispert. 2013. Use of linear regression and partial least square regression to predict intramuscular fat of pig loin computed tomography images. Chemometrics and Intelligent Laboratory Systems. 122:58–64.

Gerbens, F., A. Jansen, A. J. M. Van Erp, F. Harders, T. H. E. Meuwissen, G. Rettenberger, J. H. Veerkamp, and M. F. W. Te Pas. 1998. The adipocyte fatty acid-binding protein locus: characterization and association with intramuscular fat content in pigs. Mammalian Genome. 9:1022–1026.

Gerbens, F. 2004. Genetic Control of Intramuscular Fat Accretion. In: M. F. W. te Pas, M. E. Everts, H. P. Haagsman, editors, Muscle Development of Livestock Animals. CAB International. Wallingford, UK. p. 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*). Comparative Biochemistry Physiology. 117B:259-265.

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. Livestock Production Science. 54:1-10.

Gondret, F., J. F. Hocquette, and P. Herpin. 2004. Age-related relationship between muscle fat content and metabolic traits in growing rabbits. Reproduction Nutrition Development. 44:1–16.

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

Gotoh, T., E. Albrecht, F. Teuscher, K. Kawabata, K. Sakashita, H. Iwamoto, J. Wegner. 2009. Differences in muscle and fat accretion in Japanese Black and European cattle. Meat Science. 82:300–308.

Guy, F., A. Prache, D. Thomas, D. Bauchart, and D. Andueza. 2011. Prediction of lamb mat fatty acid composition using near-infrared reflectance spectroscopy (NIRS). Food Chemistry. 127:1280-1286.

Hassen, A., D. E. Wilson, V. Z. Amin, G. H. Rouse, and C. L. Hays. 2001. Predicting percentage of intramuscular fat using two types of real-time ultrasound equipment. Journal of Animal Science. 79:11–18.

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. The biology and regulation of preadipocytes and adipocytes in meat animals. Journal of Animals Science. 87:1218-1246.

Hernández, P., M. Pla, and A. Blasco. 1997. Relationships of Meat Characteristics of Two Lines of Rabbits Selected for Litter Size and Growth Rate. Journal of Animal Science. 75:2936–2941.

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 Science, 55:379-384.

Hernández, P., and F. Gondret. 2006. Rabbit meat quality and safety. In: L. Maertens and P. Coudert, editors, Recent Advances in Rabbit Sciences. ILVO, Melle, Belgium. p. 267-290

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

Hernández, P., and A. Dalle Zotte. 2010. Influence of diet on rabbit meat quality. In: C. de Blas, and J. Wiseman, editors, Nutrition of the rabbit. CAB International. Wallinford, Oxon, UK. p. 163-178.

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.

Hulot, F., and J. Ouhayoun. 1999. Muscular pH and related traits in rabbits: a review. World Rabbit Science. 7:15–36.

Ibáñez-Escriche, N., and A. Blasco. 2011. Modifying growth curve parameters by multitrait genomic selection. Journal of Animal Science. 89:661-668.

ISSFAL, 2004. 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

Janss, L. L., J. A. Van Arendonk, and E. W. Brascamp. 1997. Bayesian statistical analyses for presence of single genes affecting meat quality traits in a crossed pig population. Genetics. 145:395–408.

Jennen D. G., A. L. Vereijken, H. Bovenhuis, R. M. Crooijmans, J. J. van der Poel and M. A. Groenen. 2005. Confirmation of quantitative trait loci affecting fatness in chickens. Genetics Selection Evolution. 37:215–228.

Jurie, C., I. Cassar-Malek, M. Bonnet, C. Leroux, D. Bauchart, P. Boulesteix, D. W. Pethick, and J. F. Hocquette. 2007. Adipocyte fatty acid-binding protein and mitochondrial enzyme activities in muscles as relevant indicators of marbling in cattle. Journal of Animal Science. 85:2660–2669.

Karamichou, E., R. I. Richardson, G. R. Nute, K. A. McLean, and S. C. Bishop. 2006. Genetic analyses of carcass composition, as assessed by X-ray computer tomography and meat quality traits in Scottish Blackface sheep. Animal Science. 82:151−162.

Lambe, N. R., N. B. Jopson, E. A. Navajas, K. McLean, P. L. Johnson, and L. Bünger. 2009. In-vivo prediction of meat quality in lambs using CT scanning. In: Proceedings of the 2nd workshop on the use of computer tomography (CT) in pig carcass classification. Other CT applications: Live animals and meat technology. Monells (Girona), Spain. http://www.recercat.net/handle/2072/39299?locale=es

Lawrie, R. A. 1998. Lawrie´s Meat Science. Woodhead Publishing Limited. Cambridge, England.

Macfarlane, J. M., G. E. Emmans, R. M. Lewis, M. J. Young, and G. Simm. 2006. Predicting carcass composition of terminal sire sheep using X-ray computed tomography. Animal Science. 82: 289–300.

Maltin C., D. Balcerzak, R. Tilley and M. Delday. 2003. Determinants of meat quality: tenderness. In: Proceedings of the Nutrition Society. 62:337–347.

Marshall, D. M. 1999. Genetics of Meat Quality. In: R. Fries and A. Ruvinsky, editors, The genetics of cattle. CAB International. New York, USA. p. 605-636.

Masoero, G., G. Xiccato, A. Dalle Zotte, R. Parigi-Bini, and G. Bergoglio. 1994. Analysis of freeze-dried rabbit meat by NIRS. Zootecnia Nutrizione Animale. 20:319-329.

Meilgaard, M., G. V. Civille, and B. T. Carr. 1999. Sensory evaluation Techniques. CRC Press LLC. Florida, USA.

Mermelstein, N. H. 2013. Instrumental Grading of Meat. Food Tecnology. 03-13:67-69.

Meuwissen, T. H. E., and M. E. Goddard. 1996. The use of marker haplotypes in animal breeding shcemes. Genetic Selection Evolution. 28:161-176.

Meuwissen, T. H. E., B. J. Hayes, and M. E. Goddard. 2001. Prediction of total genetic value using genome-wide dense marker maps. Genetics. 157:1819–1829.

Moeller, S. J. 2002. Evolution and use of ultrasonic technology in the swine industry. 80:E19-E27.

Moreno-Sánchez, N., A. Moreno, M. J. Carabaño, M. T. Fernández, M. García and C. Díaz. 2006. Genetic correlations for meat quality traits measured in two different muscles in the Avileña Negra Ibérica breed. In: Proceedings of the 8th World Congress on Genetics Applied to Livestock Production. Belorizonte, Brasil.

Navajas, E., A., C. A. Glasbey, A. V. Fisher, D. W. Ross, J.J. Hyslop, R. I. Richardson, G. Simm, and R. Roehe. 2010. Assessing beef carcass tissue weights using computed tomography spirals of primal cuts. Meat Science. 84:30-38.

Newcom, D. W., T. J. Baas, and J. F. Lampe. 2002. Prediction of intramuscular fat percentage in live swine using real-time ultrasound. Journal of Animal Science. 80:3046-3052.

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. Journal of Animal Science. 77:93–104.

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. Journal of Animal Science. 89:615–621.

Óvilo, C., A. Oliver, J. L. Noguera, A. Clop, C. Barragán, L. Varona, C. Rodríguez, M. Toro, A. Sánchez, M. Pérez-Enciso, and L. Silió. 2002. Test for positional candidate genes for body composition on pig chromosome 6. Genetics, Selection, Evolution.34:465-479.

Pascual, J. J., F. Castella, C. Cervera, E. Blas, and J. Fernández-Carmona. 2000. The use of ultrasound measurement of perirenal fat thickness to estimate changes in body composition of young female rabbits. Animal Science. 70:435-442.

Pethick, D. W., G. S. Harper, and V. H. Oddy. 2004. Growth, development and nutritional manipulation of marbling in cattle: a review. Australian Journal of Experimental Agriculture. 44:705-715.

Pitchford, W. S., M. P. B. Deland, B. D. Siebert, A. E. O. Malau-Aduli, and C. D. K. Bottema. 2002. Genetic variation in fatness and fatty acid composition of crossbred cattle. Journal of Animal Science. 80:2825–2832.

Pla, M., M. Pascual, and B. Ariño. 2004. Protein, fat and moisture content of retail cuts of rabbit meat evaluated with the NIRS methodology. World Rabbit Science. 12:149-158.

Pla, M., P. Hernández, B. Ariño, J. A. Ramírez, and I. Díaz. 2007. Prediction of fatty acid content in rabbit meat and discrimination between conventional and organic production systems by NIRS methodology. Food Chemistry. 100:165-170.

Prevolnik, M., M. Candek-Potokar, and D. Skorjanc. 2004. Ability of NIR spectroscopy to predict meat chemical composition and quality- a review. Czech Journal of Animal Science. 49:500-510.

Prevolnik, M., M. Čandek-Potokar, D. Škorjanc, Š. Velikonja-Bolta, M. Škrlep, T. Žnidaršič, and D. Babnik. 2005. Predicting intramuscular fat content in pork and beef by near infrared spectroscopy. Journal of Near Infrared Spectroscopy. 13:77-86.

Prieto, N., S. Andres, F. J. Giraldez, A. R. Mantecon, and P. Lavin. 2006. Potential use of near infrared reflectance spectroscopy (NIRS) for the estimation of chemical composition of oxen meat samples. Meat Science. 74:487–496.

Prieto, N., R. Roehe, P. Lavín, G. Batten, and S. Andrés. 2009. Application of near infrared reflectance spectroscopy to predict meat and meat products quality: A review. Meat Science. 83:175-186.

Prieto, N., E. A. Navajas, R. I. Richardson, D. W. Ross, J. J. Hyslop, G. Simm, and R. Roehe. 2010. Predicting beef cuts composition, fatty acids and meat quality characteristics by spiral computed tomography. Meat Science. 86:770-779.

Prieto, N., D. W. Ross, E. A. Navajas, R. I. Richardson, J. J. Hyslop, G. Simm, and R. Roehe. 2011. Online prediction of fatty acid profile in crossbred Limousin and Aberdeen Angus beef cattle using near infrared reflectance spectroscopy. Animal. 5:155-16.

Quintanilla, R., R. N. Pena, D. Gallardo, A. Cánovas, O. Ramírez, I. Díaz, J. L. Noguera, and M. Amills. 2011. Porcine intramuscular fat content and composition are

regulated by quantitative trait loci with muscle-specific effects. Journal of Animal Science. 89:2963-2971.

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

Ripoll, G., P. Alberti, B. Panea, J. L. Olleta, and C. Sañudo. 2008. Near-infrared reflectance spectroscopy for predicting chemical, instrumental and sensory quality of beef. Meat Science. 80:697–702.

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 Science. 68:137–144.

Rodríguez-Calleja, J. M., M. L. Garcia-Lopez, J. A. Santos, and A. Otero. 2005. Development of the aerobic spoilage flora of chilled rabbit meat. Meat Science. 70:389–394.

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. Journal of Animal Science. 80:2017-2022.

Sacks, F. M., and M. Katan. 2002. Randomized clinical trials on the effects of dietary fat and carbohydrate on plasma lipoproteins and cardiovascular disease. The American Journal of Medicine. 113, Suppl. 9B, 13S–24S.

Schwab, C. R., T. J. Baas, K. J. Stalder, and J. W. Mabry. 2006. Effect of long-term selection for increased leanness on meat and eating quality traits in Duroc swine. Journal of Animal Science. 84:1577-1583.

Schwab, 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. Journal of Animal Science. 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. Journal of Animal Science. 88:69-79.

Schulte, K. J. 2010. An evaluation of equipment and procedures for the prediction of intramuscular fat in live swine. Graduate Theses and Dissertations. Paper 11774. Iowa State University.

Sellier, P. 1998. Genetics of meat and carcass traits. In: M. F. Rothschild and A. Ruvinsky, editors, The Genetics of the Pig. CAB International, New York, USA. p. 463-510.

Sellier, P., L. Maignel, and 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.

Silva, S. R., M. J. Gomes, A. Dias-da-Silva, L. F. Gil, and J. M. T. Azevedo. 2005. Estimation in vivo of the body and carcass chemical composition of growing lambs by real-time ultrasonography. Journal of Animal Science. 83:350-357

Simopoulos, A. P. 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomedical Pharmacotherapy. 56:365–379.

Siri-Tarino, P. W., Q. Sun, F. B. Hu, R. M. Krauss. 2010. Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease. The American Journal of Clinical Nutrition. 91:535–546.

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

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

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

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

Szabo, C. S., L. Babinszky, M. W. A. Verstegen, O. Vangen, A. J. M. Jansman, and E. Kanis. 1999. The application of digital imaging techniques in the in vivo estimation of the body composition of pigs: A review. Livestock Production Science. 60:1–11.

Szendro, Z. S., P., Horn, G. Y. Kövér, E. Berényi, I. Radnai, and E. Biró-Németh. 1992. In vivo measurement of the carcass traits of meat type rabbits by X-ray computerised tomography. Journal of Applied Rabbit Research. 15:799-809.

Thyholt, K., and T. Isaksson. 1997. Differentiation of frozen and unfrozen beef using near-infrared spectroscopy. Journal of the Science of Food and Agriculture. 73: 525–532.

Tres, A., R. Bou, R. Codony, F. Guardiola. Influence of different dietary doses of n-3- or n-6-rich vegetable fats and alpha-tocopheryl acetate supplementation on raw and cooked rabbit meat composition and oxidative stability. Journal of Agricultural and Food Chemistry. 56:7243–7253.

Viljoen, M., L. C. Hoffman, and T. S. Brand. 2005. Prediction of the chemical composition of freeze dried ostrich meat with near infrared reflectance spectroscopy. Meat Science. 69:255–261.

Viljoen, M., L. C. Hoffman, and T. S. Brand. 2007. Prediction of the chemical composition of mutton with near infrared reflectance spectroscopy. Small Ruminant Research. 69:88–94.

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

Warris P. D., 2000. Meat Quality. Meat Science. An Introductory Text. CABI Publishing, Wallinford, Oxon, UK.

WHO. 2003. Diet, Nutrition and the Prevention of Chronic Diseases. Report of a Joint WHO/FAO Expert Consultation. WHO Technical Report Series 916, Geneva.

Williams, A. R. 2002. Ultrasound applications in beef cattle carcass research and management. Journal of Animal Science. 80:E183-E188.

Willyard, C. 2013. At the heart of the problem. Nature. 493:S11-S10.

Windham, W. R., and W. H. Morrison. 1998. Prediction of fatty acid content in beef neck lean by near infrared reflectance analysis. Journal of Near Infrared Spectroscopy. 6:229-234.

Wood, J. D., R. I. Richardson, G. R. Nute, A. V. Fisher, M. M. Campo, E. Kasapidou, P. R. Sheard, and M. Enser. 2003. Effects of fatty acids on meat quality: a review. Meat Science. 66:21–32.

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

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 Science. 78:343–358.

Zamora-Rojas, E., A. Garrido-Varo, E. De Pedro-Sanz, J. E. Guerero-Ginel, and D. Pérez-Marín. 2011. Monitoring NIRS calibrations for use in routine meat analysis as part of Iberian pig-breeding programs. Food Chemistry. 129:1889-1897.

# OBJECTIVES

Intramuscular fat content is important for meat quality. It can be improved through genetic strategies, but its genetic relationships with other relevant traits are still unclear. This Thesis studies the opportunities for genetic improvement of intramuscular fat content by selection in rabbits, and its consequences on other economically relevant traits.

The specific objectives of this Thesis are:

1. To develop NIRS calibrations equations for predicting intramuscular fat content, fatty acid composition and protein content in the *Longissimus* muscle of rabbits.

2. To evaluate the application of NIRS in rabbit selection programmes focused on intramuscular fat content.

3. To perform a divergent selection experiment on intramuscular fat content based on NIRS measurements in rabbits, and to examine the direct selection response on intramuscular fat content and the correlated response on carcass and meat quality characteristics after 3 generations of divergent selection.

# CHAPTER 1

## Application of NIRS for predicting fatty acids in intramuscular fat of rabbit

**The content of this chapter has been published in Meat Science. 91: 155-159. 2012**

**ABSTRACT**: The aim of this study was to evaluate the use of near infrared reflectance spectroscopy (NIRS) for predicting fatty acid content in intramuscular fat (IMF) to be applied in rabbit selection programmes. One hundred and forty three freeze-dried *Longissimus* muscles (LM) were scanned by NIRS (1100-2498 nm). Modified Partial Least Squares models were obtained. Equations were selected according to standard error of cross validation (SECV) and coefficient of determination of cross validation ($R^2_{CV}$). Residual predictive deviation of cross validation ($RPD_{CV}$) was also studied. Accurate predictions were reported for IMF ($R^2_{CV}$=0.98; $RPD_{CV}$=7.57), saturated ($R^2_{CV}$=0.96; $RPD_{CV}$=5.08) and monounsaturated fatty acid content ($R^2_{CV}$=0.98; $RPD_{CV}$=6.68). Lower accuracy was obtained for polyunsaturated fatty acid content ($R^2_{CV}$=0.83; $RPD_{CV}$=2.40). Several individual fatty acids were accurately predicted such as C14:0, C15:0, C16:0, C16:1, C17:0, C18:0, C18:1 n-9, C18:2 n-6 and C18:3 n-3 ($R^2_{CV}$=0.91-0.97; $RPD_{CV}$>3). Long chain polyunsaturated fatty acids and C18:1 n-7 presented less accurate prediction equations ($R^2_{CV}$=0.12-0.82; $RPD_{CV}$<3).

**Keywords*:*** fatty acids, intramuscular fat, NIRS, rabbit.

## 1. INTRODUCTION

Rabbit meat offers good nutritive and dietetic properties because it has lower fat and higher polyunsaturated fatty acid (PUFA) content than other meats (Hernández and Gondret, 2006). The most ubiquitous fatty acids (FA) are palmitic (C16:0), oleic (C18:1 n-9) and linoleic (C18:2 n-6) acids, showing percentages higher than 20% of total FA. Rabbit meat also contains high protein content and high levels of essential amino acids compared to other meats (Hernández and Dalle Zote, 2010).

Conventional methods used to determine meat chemical composition are laborious, expensive, time-consuming and destructive. In contrast, near infrared reflectance spectroscopy (NIRS) is a fast, accurate and cheap analytical technique. Moreover, it enables to measure simultaneous attributes with a simple sample preparation. Therefore, it could be a suitable alternative to chemical conventional methods. Previous studies have showed that NIRS technique is a good predictor of intramuscular fat (IMF) and protein content in meat with higher prediction accuracy for IMF (Prevolnik et al., 2004; Prieto et al., 2009). Fatty acid content has also been predicted

by NIRS; nevertheless, this technique has a limited ability for estimating some individual FA due to the similarity between their NIR absorption patterns (Windham and Morrison, 1998). In rabbit, NIRS has been applied for predicting fat and protein content in meat (Masoero et al., 1994; Pla et al., 2004). To our knowledge, there are not studies about NIRS predictions of FA profile in IMF in rabbits, since Pla et al. (2007) studied FA in hind leg meat, including inter- and intramuscular fat.

Rabbit selection programmes focused on IMF are being conducted in our institute. These programmes need a substantial amount of data, thus fast and accurate methods are required. NIRS could be a suitable alternative to study IMF and possible changes in FA profile in all individuals involved in genetic programmes.

The objective of this study was to evaluate the potential use of NIRS for predicting FA and IMF content in the *Longissimus* muscle to be applied in rabbit selection programmes.

## 2. MATERIALS AND METHODS

### 2.1. Animals and meat samples

All experimental procedures involving animals were approved by the Research Ethics Committee of the Universitat Politècnica de València. A total of 143 rabbits (61 females and 82 males) were used in this experiment. To ensure enough variability in the samples analysed, rabbits were from three different synthetic lines and were slaughtered between 5 and 61 weeks of age.

Animals were reared at the experimental farm of the Universitat Politècnica de València. From weaning (4 weeks of age) to 9 weeks of age, rabbits were reared collectively and were fed *ad libitum* with a commercial diet formulated for growing rabbits (15.7% crude protein, 16.4% crude fiber, 3.0% fat). During the subsequent experimental period, rabbits were housed in individual cages and received a restricted feed with a diet formulated for adults (17% crude protein, 15.8% crude fiber, 3.5% fat). The amount of feed was 135 grams/day and was distributed once daily.

Rabbits were slaughtered by electrical stunning and exsanguination at the abattoir on the farm. No fasting was applied. After the slaughter, the carcasses were stored at 4°C

during 24 hours and then the *Longissimus* muscles (LM) were excised from the carcass. Meat obtained from LM was ground, freeze-dried, vacuum-packed and stored at 80°C until analyses.

### 2.2. Intramuscular fat analysis

Total lipids were determined by ether extraction (Soxtec 2055, Tecator, Höganäs, Sweden) with a previous acid hydrolysis (Soxcap 2022, Tecator, Höganäs, Sweden) (ISO-R-1443) in triplicate from freeze-dried LM in 143 samples. Lipid content was expressed as grams per 100 grams of fresh tissue, this value was obtained taking into account the dry matter content determined from the weight of minced LM before and after freeze-drying.

### 2.3. Protein analysis

Determination of protein content was based on Total Nitrogen content by Kjeldahl procedure (ISO-R-937) using a Kjeltec 2300 Analyzer (Tecator, Höganäs, Sweden). Protein content was quantified in triplicate from freeze-dried LM in 122 samples. Results were expressed as grams per 100 grams of fresh tissue, this value was obtained taking into account the dry matter content determined from the weight of minced LM before and after freeze-drying.

### 2.4. Fatty acid analysis

Fatty acid profile of freeze-dried LM was determined in 123 samples. Fatty acid methyl esters (Fame) were prepared as described by O'Fallon et al. (2007) and were analysed in a Focus Gas Chromatograph (Thermo, Milan, Italy) equipped with a split/splitless injector and a flame ionization detector. The separation of methyl esters was performed in a fused silica capillary column SPTM 2560 (Supelco, PA, USA) (100 m x 0.25 mm x 0.2 µm film thickness). The carrier gas was Helium at a linear velocity of 20 cm/sec. The samples were injected with a split ratio of 1/100. The initial oven temperature was set at 140°C held for 5 min and increased to 240 at 4°C/min and finally maintained at that temperature for 30 min. Both detector and injector temperatures were set at 260°C. The individual FA were identified by comparing their retention times with standards of Fame supplied by Supelco (PA, USA) and quantified by using C21:0 as internal standard.

Fatty acids were expressed as milligrams per 100 grams of fresh tissue, this value was obtained taking into account the dry matter content determined from the weight of minced LM before and after freeze-drying.

### 2.5. NIRS analysis

*Spectral data collection*

Once freeze-dried muscle samples reached room temperature, they were scanned between 1100 and 2498 nm with a monochromator (model 5000, NIRSystem INC., Silver Spring, MD, USA) equipped with a transport module using ISI software, version 3.10 from Infrasoft International (Infrasoft International LLC, State College, PA, USA). Absorbance data were recorded at 2nm and stored as log (1/reflectance). Sample measurements were taken in circular cups with quartz windows of 3.8 cm diameter. A sample cup was filled, placed in NIRS unit and two spectra, rotating 90 degrees the sample cup, were obtained. The sample cup was refilled with the same sample and the procedure was repeated in order to obtain four spectra of each sample. The similarity between the four reflectance spectra was studied by using Root Mean Squared (RMS) statistic. Then, the four spectra were averaged.

*Pre-treatment of spectral data*

All chemometric analyses were performed using WinISI-4 version 1.60 from Infrasoft International and Foss (Infrasoft International LLC, State College, PA, USA and FOSS, Höganäs, Sweden). Spectral anomalous were identified using the Mahalanobis distance to the center of the population (GH). Samples with a GH value higher than 3 were considered spectral outliers (Shenk and Westerhaus, 1996) and were eliminated from the population. Spectral data pre-treatments such as Standard Normal Variate (SNV) and Detrending (DT) and first or second derivative mathematical treatments were applied.

*Calibration development*

Once spectral outliers were removed, calibrations were performed using the WinISI-4 software version 1.60 (Infrasoft International LLC, State College, PA, USA and FOSS, Höganäs, Sweden). Prediction equations were obtained using Modified Partial Least Squares (MPLS) as regression method (Shenk and Westerhaus, 1996) for IMF, protein,

FA groups, FA ratios and individual FA. Cross-validation was performed in order to select the optimal number of factors and avoid overfitting. Concentration outliers were identified by using T-statistic, which indicates the difference between the reference and the predicted value. Samples with a T-value higher than 2.5 were considered as concentration outliers (Shenk and Westerhaus, 1996), and the reference chemical analysis of was repeated. Just enough passes were performed to detect outliers. Critical value for GH outliers was set at 10 in this step. The cross-validation operated with 5 groups. Regression equations were obtained using different combinations of scatter correction (no correction, SNV, SNV+DT) and mathematical treatments: (1,4,4,1), (2,4,4,1), (1,5,5,1) and (2,5,5,1), where the first digit is the order of the derivative, the second is the gap over which the derivative is calculated, the third is the number of data points used in the first smoothing, and the fourth is the number of data points used in the second smoothing. The best equation for each parameter was selected attending to Standard Error of Cross-Validation (SECV) and Determination Coefficient of Cross Validation ($R^2_{CV}$). Moreover, the Residual Predictive Deviation ($RPD_{CV}$), defined as the ratio between standard deviation of reference data to the SECV, was used to evaluate the predictive ability of the calibration models (Williams, 2001). This author suggested RPD values of 1.6 to 2.0 for very rough screening, 2.1 to 2.5 for rough screening, 2.6 to 3.0 for screening purposes and higher than 3.0 for suitable prediction models.

### 2.6. Statistical analysis

Descriptive statistics of reference chemical data for IMF, protein, FA profile (groups and individual) and FA ratios were computed by the SAS statistical package (SAS Institute Inc. Cary, USA, 2002).

## 3. RESULTS AND DISCUSSION

### 3.1. Chemical data

Descriptive statistics for IMF, protein, FA groups and FA ratios of LM used in the calibration are summarized in Table 1. LM showed a low fat (1.32 g/100g muscle) and high protein content (22.5 g/100g muscle) since it is the leanest muscle of the carcass. Similar values for fat and protein content were found by Pla et al. (2004) in this muscle.

Intramuscular fat showed a wide range of variability (CVx100=40.2), which is essential to obtain successful prediction equations. As expected, protein content had a lower variation (CVx100=4.8).

**Table 1.** Descriptive statistics for intramuscular fat (IMF) (n=139), protein (n=120), fatty acid groups and fatty acid ratios (n=119) in rabbit *Longissimus* muscle of the calibration set

| Parameter | Mean | SD[1] | Min[2] | Max[3] | CVx100[4] |
|---|---|---|---|---|---|
| **IMF (g/100 g muscle)** | 1.32 | 0.53 | 0.75 | 3.25 | 40.2 |
| **Protein (g/100 g muscle)** | 22.5 | 1.1 | 18.1 | 26.3 | 4.8 |
| ***Groups* (mg/100 g muscle)** | | | | | |
| **SFA[5]** | 352 | 164 | 162 | 858 | 46.5 |
| **MUFA[6]** | 266 | 162 | 92 | 778 | 61.0 |
| **PUFA[7]** | 319 | 89 | 143 | 568 | 28.0 |
| **n-6 PUFA[8]** | 264 | 80 | 110 | 493 | 30.2 |
| **n-3 PUFA[9]** | 54.3 | 11.4 | 23.6 | 82.2 | 21.1 |
| ***Ratios* (mg/100 g muscle)** | | | | | |
| **PUFA/SFA** | 0.98 | 0.21 | 0.51 | 1.61 | 21.4 |
| **n-6/n-3** | 4.87 | 0.87 | 2.94 | 7.27 | 17.9 |

[1]SD, standard deviation; [2]Min, minimum; [3]Max, maximum; [4]CV, coefficient of variation; [5]SFA, saturated fatty acids=C14:0+C15:0+C16:0+C17:0+C18:0; [6]MUFA, monounsaturated fatty acids=C16:1+C18:1n-9+C18:1n-7; [7]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; [8]n-6 PUFA=C18:2n-6+C20:2n-6+C20:3n-6+C20:4n-6+C22:4n-6; [9]n-3 PUFA=C18:3n-3+C20:5n-3+C22:5n-3+C22:6n-3.

The main FA in rabbit LM were saturated (SFA) and polyunsaturated (PUFA), with percentages around 38% and 34% of total FA, respectively. Monounsaturated (MUFA) FA represented lower percentage (28%). Among PUFA, n-6 FA were the most abundant with percentages of 28%, while n-3 FA were less represented (6%). PUFA/SFA and n-6/n-3 ratios, used to evaluate the nutritional quality of fat, showed values close to the nutritional recommendations (higher than 0.45 for PUFA/SFA and lower than 4 for n-6/n-3) (reviewed by Hernández and Dalle Zotte, 2010). SFA and MUFA content had a high variability (CVx100 of 46.5 and 61.0, respectively), but PUFA as well n-6, n-3 and FA ratios showed a lower variability (CVx100 between 17.9 and 30.2).

**Table 2.** Descriptive statistics for individual fatty acids in rabbit *Longissimus* muscle of the calibration set (n=119)

| Parameter (mg/100 g muscle) | Mean | SD[1] | Min[2] | Max[3] | CVx100[4] |
|---|---|---|---|---|---|
| **C14:0 (myristic)** | 18.3 | 13.4 | 3.71 | 62.5 | 73.5 |
| **C15:0 (pentadecanoic)** | 4.43 | 2.18 | 0.31 | 10.8 | 49.2 |
| **C16:0 (palmitic)** | 251 | 122 | 113 | 621 | 48.9 |
| **C16:1 (palmitoleic)** | 28.2 | 27.1 | 3.41 | 120 | 95.9 |
| **C17:0 (heptadecanoic)** | 6.18 | 2.70 | 2.08 | 15.0 | 43.7 |
| **C18:0 (stearic)** | 72.7 | 24.8 | 39.4 | 153 | 34.1 |
| **C18:1 n-7 (vaccenic)** | 13.8 | 6.8 | 3.79 | 38.1 | 49.4 |
| **C18:1 n-9 (oleic)** | 224 | 130 | 78.1 | 620 | 58.3 |
| **C18:2 n-6 (linoleic)** | 194 | 73.4 | 52.9 | 419 | 37.9 |
| **C18:3 n-3 (linolenic)** | 14.8 | 8.9 | 1.37 | 41.8 | 59.7 |
| **C20:2 n-6 (eicosadienoic)** | 2.33 | 0.80 | 0.45 | 4.80 | 34.2 |
| **C20:3 n-6 (eicosatrienoic)** | 4.05 | 0.81 | 2.21 | 6.47 | 20.0 |
| **C20:4 n-6 (arachidonic)** | 48.1 | 9.2 | 32.4 | 71.5 | 19.1 |
| **C20:5 n-3 (eicosapentanoic)** | 11.9 | 4.2 | 0.79 | 22.2 | 35.3 |
| **C22:4 n-6 (docosatetraenoic)** | 15.9 | 2.6 | 10.4 | 23.3 | 16.2 |
| **C22:5 n-3 (docosapentanoic)** | 7.11 | 1.86 | 4.39 | 12.3 | 26.1 |
| **C22:6 n-3 (docosahexanoic)** | 20.5 | 6.6 | 8.52 | 42.3 | 32.2 |

[1]SD, standard deviation; [2]Min, minimum; [3]Max, maximum; [4]CV, coefficient of variation.

Table 2 shows descriptive statistics for individual FA of rabbits used in the calibration. The most ubiquitous FA in LM were palmitic (C16:0), oleic (C18:1 n-9) and linoleic (C18:2 n-6) acids, showing percentages of 27%, 24% and 21%, respectively. Stearic (C18:0) and arachidonic acids (C20:4 n-6) were also important with percentages around 8% and 5%, repectively. Linolenic acid (C18:3 n-3) and some long chain PUFA (i.e. C20:5 n-3, C22:4 n-6 and C22:6 n-3) were also present in rabbit meat although at a lower content. The FA composition of rabbit LM was in agreement with that observed in previous studies (reviewed by Hernández and Gondret, 2006). Most individual FA showed a wide range of variation, mainly C14:0, C16:1, C18:1 n-9 and C18:3 n-3 (CVx100 between 58.3 and 95.9). The variability among calibration set found in this study was in similar range as in other works in beef (Sierra et al., 2008) and lamb (Guy et al., 2011) meat for the most of parameters.

### 3.2. NIRS calibrations

Calibration statistics for IMF, protein, FA groups and FA ratios are reported in Table 3. The parameters corresponding to IMF calibration indicated good prediction ability ($R^2_{CV}$=0.98 and $RPD_{CV}$=7.57). Accurate NIRS calibrations for IMF have also been reported in poultry, beef and pork meat (reviewed by Prieto et al., 2009). In rabbit meat, Masoero et al. (1994) and Pla et al. (2004) also obtained good prediction equations for fat content which included inter- and intramuscular fat. The calibration model for protein content had a low $R^2_{CV}$ (0.77) and $RPD_{CV}$ (2.07), but it could be adequate for rough screening (Williams, 2001). A lower accuracy of protein prediction had been previously observed in beef, pork and poultry meat (reviewed by Prieto et al., 2009). These authors proposed as possible causes a narrow range on variability within the calibration set and analytical differences between Kjeldahl and NIRS methodology, which is in accordance with our results (CVx100 of protein=4.8).

Equations for SFA and MUFA content (Table 3) showed good accuracy ($R^2_{CV}$ of 0.96 and 0.98 and $RPD_{CV}$ of 5.09 and 6.69, respectively). Prediction models for PUFA and n-6 FA content were less accurate ($R^2_{CV}$=0.83 and 0.87, $RPD_{CV}$=2.40 and 2.82, respectively), indicating suitable predictions for screening. Results for n-3 FA content indicated no accurate predictions ($R^2_{CV}$=0.50 and $RPD_{CV}$=1.41). The higher accuracies for SFA and MUFA compared to PUFA content found in this study are in line with findings of other authors (Sierra et al., 2008; Guy et al., 2011), and might be related to the narrow range of variability in PUFA content (Table 1) and a less ability of NIRS to detect the higher double bonds presents in PUFA. Regarding n-6 and n-3 FA content predictions, a similar pattern was observed by Pla et al. (2007); the lower accuracy for n-3 FA prediction might be due to a lower presence and variability of n-3 FA in rabbit meat. Equations for FA ratios had low accuracies ($R^2_{CV}$=0.81 for PUFA/SFA ratio and $R^2_{CV}$=0.64 for n-6/n-3 ratio), only suitable for rough screening. No information was found about prediction of ratios in the literature using NIRS. Nevertheless, FA ratios predictions could be interesting from the point of view of nutritional quality of meat. NIRS technology provides a direct prediction of the ratio with its prediction error. This is more suitable than obtaining two parameters separately and calculating its ratio, since its standard error cannot be estimated directly.

**Table 3.** Statistical parameters of equations for near infrared reflectance spectroscopy calibrations of intramuscular fat (IMF), protein, fatty acid content and fatty acid ratios in rabbit *Longissimus* muscle

| Parameter | N[1] | Mean | SD[2] | Interval | $R^2_{CV}$ [3] | SECV[4] | $RPD_{CV}$ [5] |
|---|---|---|---|---|---|---|---|
| **IMF (g/100 g muscle)** | 137 | 1.32 | 0.53 | 0.75 - 3.25 | 0.98 | 0.07 | 7.57 |
| **Protein (g/100 g muscle)** | 106 | 22.5 | 0.85 | 20.4 - 24.3 | 0.77 | 0.41 | 2.07 |
| ***Groups* (mg/100 g muscle)** | | | | | | | |
| **SFA[6]** | 119 | 352 | 164 | 162 - 858 | 0.96 | 32.2 | 5.08 |
| **MUFA[7]** | 116 | 263 | 162 | 91.7 - 778 | 0.98 | 24.2 | 6.68 |
| **PUFA[8]** | 119 | 319 | 89 | 143- 568 | 0.83 | 37.2 | 2.40 |
| **n-6 PUFA[9]** | 117 | 262 | 78 | 110 - 493 | 0.87 | 27.8 | 2.82 |
| **n-3 PUFA[10]** | 117 | 54.4 | 11.1 | 31.3- 82.2 | 0.50 | 7.87 | 1.40 |
| ***Ratios* (mg/100 g muscle)** | | | | | | | |
| **PUFA/SFA** | 116 | 0.97 | 0.20 | 0.51 - 1.44 | 0.81 | 0.09 | 2.25 |
| **n-6/n-3** | 116 | 4.84 | 0.82 | 3.15 - 6.59 | 0.64 | 0.49 | 1.66 |

[1]N, number of samples, [2]SD, standard deviation, [3] $R^2_{CV}$, coefficient of determination of cross-validation, [4]SECV, standard error of cross validation, [5]$RPD_{CV}$, SD/SECV. [6]SFA, saturated fatty acids= C14:0+C15:0+C16:0+C17:0+C18:0; [7]MUFA, monounsaturated fatty acids= C16:1+C18:1n-9+C18:1n-7; [8]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; [9]n-6= C18:2n-6+C20:2n-6+C20:3n-6+C20:4n-6+C22:4n-6; [10]n-3= C18:3n-3+C20:5n-3+C22:5n-3+C22:6n-3.

Calibration equation results for individual FA content are shown in Table 4. The best calibration equations were found for C18:1 n-9, C16:0 and C18:3 n-3 with $R^2_{CV}$ higher than 0.95. Accurate equations were also obtained for C14:0, C15:0, C16:1, C17:0, C18:0 and C18:2 n-6 with $R^2_{CV}$ between 0.91 and 0.94. $RPD_{CV}$ statistics of these equations showed values higher than those recommended in literature for suitable prediction models (Williams, 2001). Other minor FA as C18:1 n-7 presented less accurate predictions ($R^2_{CV}$=0.82 and $RPD_{CV}$=2.37), only adequate for rough screening. Inferior results were obtained for long chain PUFA such as C20:2 n-6, C20:4 n-6, C20:5 n-3 and C22:5 n-3 ($R^2_{CV}$ between 0.61 and 0.73 and $RPD_{CV}$ between 1.60 and 1.95) indicating proper models for very rough screening. Finally, C20:3 n-6, C22:4 n-6 and C22:6 n-3 models were unacceptable for predictions ($R^2_{CV}$ between 0.12 and 0.57 and $RPD_{CV}$ between 1.06 and 1.52).

**Table 4.** Statistical parameters of equations for near infrared reflectance spectroscopy calibrations of individual fatty acid content in rabbit *Longissimus* muscle

| Parameter (mg/100 g muscle) | N[1] | Mean | SD[2] | Interval | $R^2_{CV}$ [3] | SECV[4] | $RPD_{CV}$ [5] |
|---|---|---|---|---|---|---|---|
| **C14:0** | 116 | 17.5 | 12.5 | 3.71- 59.0 | 0.94 | 2.96 | 4.23 |
| **C15:0** | 116 | 4.42 | 2.14 | 1.25 - 10.8 | 0.91 | 0.63 | 3.38 |
| **C16:0** | 118 | 249 | 121 | 113 -621 | 0.96 | 24.5 | 4.93 |
| **C16:1** | 115 | 27.1 | 26.6 | 3.41 - 120 | 0.92 | 7.42 | 3.59 |
| **C17:0** | 113 | 5.91 | 2.43 | 2.08 - 13.6 | 0.91 | 0.75 | 3.24 |
| **C18:0** | 116 | 71.3 | 23.4 | 39.4 - 153 | 0.90 | 7.33 | 3.20 |
| **C18:1 n-7** | 117 | 13.8 | 6.9 | 3.79 - 38.1 | 0.82 | 2.90 | 2.37 |
| **C18:1 n-9** | 116 | 221 | 130 | 78.1 - 620 | 0.97 | 21.3 | 6.10 |
| **C18:2 n-6** | 115 | 190 | 71 | 52.9 - 419 | 0.91 | 21.3 | 3.33 |
| **C18:3 n-3** | 113 | 14.3 | 8.6 | 1.37 - 41.8 | 0.95 | 2.18 | 3.93 |
| **C20:2 n-6** | 114 | 2.30 | 0.75 | 0.58 - 4.80 | 0.72 | 0.39 | 1.92 |
| **C20:3 n-6** | 115 | 3.99 | 0.76 | 2.21 - 5.87 | 0.57 | 0.50 | 1.52 |
| **C20:4 n-6** | 117 | 47.8 | 9.0 | 32.4 -68.5 | 0.61 | 5.60 | 1.60 |
| **C20:5 n-3** | 115 | 12.0 | 3.9 | 3.61 - 20.5 | 0.73 | 2.01 | 1.95 |
| **C22:4 n-6** | 118 | 15.8 | 2.5 | 10.4 -21.9 | 0.12 | 2.34 | 1.06 |
| **C22:5 n-3** | 115 | 7.04 | 1.84 | 4.39 - 12.3 | 0.73 | 0.95 | 1.94 |
| **C22:6 n-3** | 117 | 20.2 | 6.25 | 8.52 - 36.5 | 0.38 | 4.95 | 1.26 |

[1]N, number of samples, [2]SD, standard deviation, [3] $R^2_{CV}$, coefficient of determination of cross-validation, [4]SECV, standard error of cross validation, [5]$RPD_{CV}$, SD/SECV.

Comparisons between studies are difficult due to the use of different equipment, wavelength range, sample preparation and chemical analyses. Fatty acid data were commonly expressed as percentage of the total FA when used for prediction by NIRS in previous studies in rabbit (Pla et al., 2007), beef (Windham and Morrison, 1998) and pork meat (González-Martín et al., 2005). However, more recently studies in beef (Sierra et al., 2008; Prieto et al., 2011) and lamb meat (Guy et al., 2011) used reference data expressed as concentration (mg or g) in the muscle and obtained higher accuracies of prediction. This work is the first analysis of FA content of IMF by NIRS in rabbit and FA content was expressed as concentration (mg) in the muscle. Calibrations for the main FA content in rabbit LM were similar to those obtained by Guy et al.

(2011) in ground lamb meat. These authors found accurate prediction models for C16:0, C18:0, C18:1 n-9 FA; however, we obtained higher accuracies for C18:2 n-6 and C18:3 n-3 predictions. Higher errors for the prediction of several long chain PUFA, present at al lower amount in rabbit meat, were also found in other works (Sierra et al., 2008; Guy et al., 2011). The prediction accuracies obtained in this study were higher than those obtained by Pla et al. (2007) in hind leg meat for most individual FA except for C18:2 n-6, C20:4 n-6 and 20:3 n-6, which were similar.

## 4. CONCLUSIONS

Accurate predictions were obtained for IMF, SFA and MUFA content as well as for the main individual FA content in rabbit meat (C16:0, C18:1 n-9 and C18:2 n-6) and other FA found in lower amounts (C14:0, C15:0, C16:1, C17:0, C18:0 and C18:3 n-3). Less accurate predictions were obtained for protein content, PUFA, n-6, n-3, FA ratios as well as C18:1 n-7 and long chain PUFA. NIRS can be a suitable alternative to chemical conventional methods to predict IMF and its FA content in rabbit meat for being used in genetic programmes. The models developed in this study will be applied in rabbit selection programmes of our institute.

## LITERATURE CITED

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

Guy, F., A. Prache, D. Thomas, D. Bauchart, and D. Andueza. 2011. Prediction of lamb meat fatty acid composition using near-infrared reflectance spectroscopy (NIRS). Food Chemistry. 127:1280-1286.

Hernández, P., and F. Gondret. 2006. Rabbit meat quality and safety. In: L. Maertens and P. Coudert, editors, Recent Advances in Rabbit Sciences. ILVO, Melle, Belgium. p. 267-290

Hernández, P., and A. Dalle Zotte. 2010. Influence of diet on rabbit meat quality. In: C. de Blas, and J. Wiseman, editors, Nutrition of the rabbit. CAB International. Wallinford, Oxon, UK. p. 163-178.

Masoero, G., G. Xiccato, A. Dalle Zotte, R. Parigi-Bini, and G. Bergoglio. 1994. Analysis of freeze-dried rabbit meat by NIRS. Zootecnia Nutrizione Animale. 20:319-329.

O'Fallon, J. V., J. R. Busboom, M. L. Nelson, and C. T. Gaskins. 2007. A direct method for fatty acid methyl ester synthesis: Application to wet meat tissue, oils, and feedstuffs. Journal of Animal Science. 85:1511-1521.

Pla, M., M. Pascual, and B. Ariño. 2004. Protein, fat and moisture content of retail cuts of rabbit meat evaluated with the NIRS methodology. World Rabbit Science. 12:149-158.

Pla, M., P. Hernández, B. Ariño, J. A. Ramírez, and I. Díaz. 2007. Prediction of fatty acid content in rabbit meat and discrimination between conventional and organic production systems by NIRS methodology. Food Chemistry. 100:165-170.

Prevolnik, M., M. Candek-Potokar, and D. Skorjanc. 2004. Ability of NIR spectroscopy to predict meat chemical composition and quality- a review. Czech Journal of Animal Science. 49: 500-510.

Prieto, N., R. Roehe, P. Lavín, G. Batten, and S. Andrés. 2009. Application of near infrared reflectance spectroscopy to predict meat and meat products quality: A review. Meat Science. 83:175-186.

Prieto, N., D. W. Ross, E. A.Navajas, R. I. Richardson, J. J. Hyslop, G. Simm, and R. Roehe. 2011. Online prediction of fatty acid profile in crossbred Limousin and Aberdeen Angus beef cattle using near infrared reflectance spectroscopy. Animal. 5:155-16.

SAS 2002. SAS/STAT User's guide (Release 9.1) *Statistical Analysis System Institute Inc, Cary, NC, USA.*

Shenk, J. S., and M. O. Westerhaus. 1996. Calibration the ISI way. In: A.M.C. Davies, and P. Williams, editors, Near Infrared Spectroscopy: The Future Waves. NIR Publications. Chichester, UK. p. 198-202.

Sierra, V., N. Aldai, P. Castro, K. Osoro, A. Coto-Montes, and M. Olivan. 2008. Prediction of the fatty acid composition of beef by near infrared transmittance spectroscopy. Meat Science. 78:248-255.

Williams, P.C. 2001. Implementation of Near-Infrared Technology. In P. C. Williams and K. Norris, editors, Near-Infrared Technology in the Agricultural and Food Industries. American Association of Cereal Chemists. St. Paul, Minnesota, USA. p.145-169.

Windham, W. R., and W. H. Morrison. 1998. Prediction of fatty acid content in beef neck lean by near infrared reflectance analysis. Journal of Near Infrared Spectroscopy. 6:229-234.

# CHAPTER 2

# Use of NIRS for intramuscular fat selection in rabbits

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**ABSTRACT**: The potential use of near infrared reflectance spectroscopy (NIRS) for the determination of intramuscular fat (IMF) content in rabbit selection programmes was evaluated. One hundred and thirty seven rabbits from three different synthetic lines slaughtered between 5 and 61 weeks of age were used for NIRS calibration. *Longissimus* muscles (LM) were homogenised, freeze-dried, scanned by NIRS and total lipid content was chemically analysed. Parameters of calibration equation reported appropriate results for IMF (standard error of cross validation, SECV=0.07g/100g muscle; and coefficient of determination of cross-validation, $R^2_{CV}$=0.98). Another 88 rabbits were used to study the suitability of NIRS in selection programmes. Intramuscular fat was measured in LM using chemical and NIRS analyses. Descriptive statistics showed that NIRS could be a proper technique to average comparison, but regression analyses ($R^2$=0.92) and rank correlation measures, especially Kendall's tau-b correlation coefficient (0.83), indicated that NIRS may not be accurate enough to predict genetic individual values and produce ranking of animals. However, NIRS technique could be applied in truncated selection where the efficiency of the method is measured by the response to selection. The IMF values of the animals that would be selected by applying the chemical method were similar than that of the animals that would be selected by applying NIRS. Thus, response to selection using NIRS should be similar to direct response using chemical analysis values. Results of the present experiment confirmed the potential of NIRS for the determination of IMF content in rabbit selection programmes instead of using laborious chemical methods.

**Key words:** intramuscular fat, NIRS, rabbit, selection

## 1. INTRODUCTION

Intramuscular fat (IMF) content is an important meat trait because it is related to human nutrition and health and sensory meat properties (Wood et al., 2008). IMF can be improved by selection due to its high heritability and variability (Sellier 1998; Suzuki et al., 2005). Rabbit meat has a lower fat content, thus selection for increasing IMF could improve its meat quality. Rabbits are an excellent experimental material for genetic studies due to its reduced generation interval and the low cost of the carcasses.

The chemical conventional methods to determine IMF content are laborious, expensive and time-consuming. Near infrared reflectance spectroscopy (NIRS) could be a suitable alternative to these conventional methods. Many studies have confirmed the ability of NIRS to predict the content of IMF in several species (Prieto et al., 2009), although a few studies have been carried out in rabbits (Masoero et al., 1994; Pla et al., 2004; Bázár et al., 2007). Genetic programmes need a substantial amount of data, thus it is necessary to have a fast, accurate and cheap analytical technique to estimate IMF in all individuals. However, to our knowledge there are no studies about the application of NIRS in rabbit selection.

The objective of this study was to evaluate the possibility of use NIRS instead of the chemical methods for the determination of IMF content in rabbit selection programmes.

## 2. MATERIAL AND METHODS

### 2.1. Animals and samples

A total of 137 rabbits (58 females and 79 males) was used for NIRS calibration. To ensure variability in the samples analysed, rabbits were from three different synthetic lines and were slaughtered between 5 and 61 weeks of age.

Other 88 rabbits (44 females and 44 males) were used to study the application of NIRS in selection programmes. These rabbits came from one of the synthetic lines mentioned before and were slaughtered at 9 weeks of age.

All rabbits were slaughtered by electrical stunning and exsanguination. After slaughter, the carcasses were stored at 3-5°C during 24 hours and then the *Longissimus* muscles (LM) were excised from the carcass. Meat obtained from LM was ground by a mincer, freeze-dried, vacuum packed and stored at -80°C until analyses.

### 2.2. Chemical analyses

Total lipids were determined by ether extraction (Soxtec 2055 extraction unit, Tecator, Höganäs, Sweden) (ISO-R-1443) with a previous acid hydrolysis (Soxcap 2022, Tecator Höganäs, Sweden) in triplicate from freeze-dried LM. Lipid content was expressed as grams per 100 g of fresh tissue, this value was obtained taking into account the dry

matter content determined from the weight of minced LM before and after freeze-drying.

Throughout the paper we will consider IMF values those obtained by the chemical method.

### 2.3. NIRS calibration for intramuscular fat

The 137 samples of *Longissimus* muscle were scanned between 1100 and 2498 nm with a monochromator (model 5000, NIRSystem INC., Silver Spring, MD, USA) equipped with a transport module. Two round sample cups with quartz windows of 3.8 cm diameter were filled with each sample and two spectra, rotating 90 degrees each cup were recorded. The 4 reflectance spectra of each sample were averaged.

Calibrations were calculated using the chemometric software WINISI-4 ver. 1.60 (Infrasoft International, LLC and FOSS). Prediction equations were obtained using MPLS (Modified Partial Least Squares) as regression method (Shenk and Weasterhaus, 1996). Cross-validation, with 5 groups, was performed in order to select the optimal number of factors and avoid overfitting. Just enough passes were performed to detect outliers. Critical values for "T" outliers (actual versus predicted) was set at 2.5 and for "H" outliers (spectral distance from the population mean) was set at 10. Regression equations were obtained using several mathematical treatments by combining different derivative orders, different number of data points in the segment used to calculate the derivative, different number of data points over which running average smoothing was conducted and with or without scatter correction. The best equation was selected attending to determination coefficient of cross-validation ($R^2_{CV}$), residual predictive deviation of cross-validation ($RPD_{CV}$=SD/SECV), and range error ratio of cross-validation ($RER_{CV}$=range/SECV), SD being the standard deviation of the reference data and SECV the standard error of cross-validation.

### 2.4. Statistical analyses

Descriptive statistics, regression and nonparametric measures of association (Spearman rank-order and Kendall's tau-b correlation) were computed by the statistical package SAS (SAS, 2002).

## 3. RESULTS AND DISCUSSION

### 3.1. NIRS calibration for intramuscular fat

The parameters corresponding to the NIRS calibration (Table 1) indicate good prediction ability for IMF. Williams and Sobering (1996) considered that the residual predictive deviation (RPD) should ideally be at least 3 and the range error ratio (RER) at least 10. In our case, RPD and RER were higher than those limits. Results on the accuracy of calibration equation for predicting IMF are similar to previous works in rabbit (Masoero et al., 1994; Pla et al., 2004), poultry (Abeni and Bergoglio, 2001), beef and pork meat (Prevolnik et al., 2005).

**Table 1.** Descriptive statistics of intramuscular fat (IMF) content in rabbits used in calibration and statistical parameters of the best calibration equation to predict IMF by near infrared reflectance spectroscopy**.**

| Parameter | N[1] | Mean | SD[2] | Min-Max | SECV[3] | $R^2_{CV}$[4] | $RPD_{CV}$[5] | $RER_{CV}$[6] |
|---|---|---|---|---|---|---|---|---|
| **IMF (g/100g muscle)** | 129 | 1.29 | 0.53 | 0.75-3.25 | 0.07 | 0.98 | 7.57 | 35.71 |

[1]N, number of samples; [2]SD, standard deviation; [3]SECV, standard error of cross-validation; [4]$R^2_{CV}$, coefficient of determination of cross-validation; [5]$RPD_{CV}$, residual predictive deviation=SD/SECV; [6]$RER_{CV}$, range error ratio=range/SECV.

### 3.2. Selection for increased intramuscular fat using NIRS

Table 2 shows descriptive statistics of IMF content measured by chemical analysis and predicted by NIRS. No significant differences were found between two methods of analyses ($P<0.05$) or between males and females ($P<0.05$). Mean, standard deviation and coefficient of variation were similar for both methods, but the range was slightly different. These results show that NIRS could be a proper technique to compare different treatments or different populations.

**Table 2.** Descriptive statistics of intramuscular fat (IMF) content (g/100 g muscle) measured by chemical analysis and predicted by near infrared reflectance spectroscopy (NIRS)

| Method | N[1] | Mean | SE[2] | SD[3] | Min-Max | CVx100[4] |
|---|---|---|---|---|---|---|
| **Chemical** | 88 | 1.19 | 0.02 | 0.17 | 0.87-1.73 | 14.3 |
| **NIRS** | 88 | 1.22 | 0.02 | 0.16 | 0.93-1.67 | 13.1 |

[1]N, number of samples; [2]SE, standard error; [3]SD, standard deviation; [4]CV, coefficient of variation.

The relationship between chemical and NIRS values for IMF can be observed in Fig. 1. Regression statistics indicate that NIRS may not be an accurate enough technique to predict individual values ($R^2$=0.92).

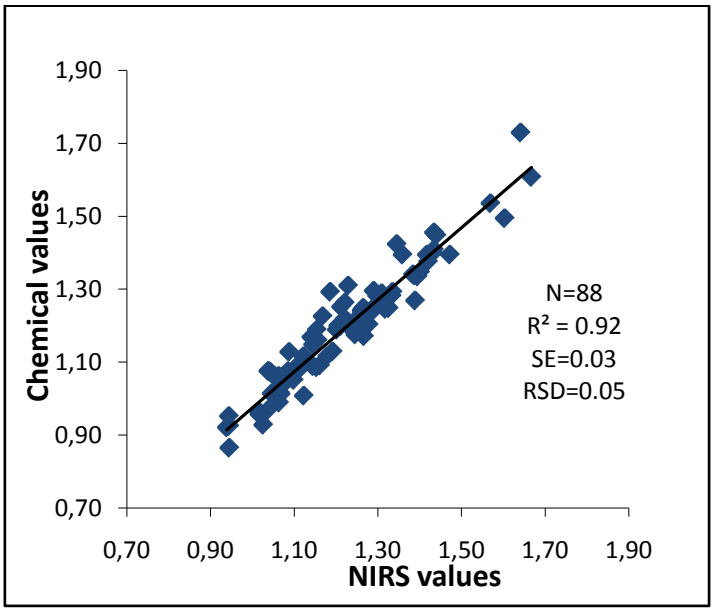


**Figure 1.** Regression between intramuscular fat (IMF) content in rabbits measured by chemical analysis and predicted by near infrared reflectance spectroscopy (NIRS). N: number of samples; SE: standard error of slope; RSD: residual standard deviation.

Predicted individual values can be used in genetic evaluation systems to produce ranking of animals in which the order is related to their economic value, and a high accuracy in estimating genetic differences is required (Goddard and Wiggans, 1999). Therefore, the application of NIRS to rank individuals needs a complete concordance between rankings based on chemically-determined and NIRS-predicted IMF values. Two measures of rank correlation have been calculated to examine the agreement among chemical and NIRS rankings. Spearman rank correlation was proposed earlier as

the ordinary correlation coefficient between the ranked values and it is widely used because it is easy to compute. Kendall's tau-b correlation coefficient was proposed later as an alternative procedure for measuring rank correlation, although it is more difficult to compute. Kendall's tau-b correlation measures the accuracy of one of the rankings when considering the other one as the correct order classification (Snedecor and Cockran, 1980). According to Kendall (1952), Kendall's tau-b correlation coefficient offers a satisfactory measure of the compatibility of two rankings and has certain statistical advantages. In our case, there is not a complete concordance between rankings, as showed by Spearman rank and especially by Kendall's tau-b correlation coefficients (Table 3). Therefore, we concluded that rankings of animals based on IMF content will not be the same if NIRS or chemical analyses are used.

**Table 3.** Spearman rank and Kendall's tau-b correlation coefficients between intramuscular fat rank values measured by chemical analysis (chemical rank) and predicted by near infrared reflectance spectroscopy (NIRS rank)

| | **Spearman rank correlation** | **Kendall's tau-b correlation** |
|---|---|---|
| | **NIRS rank** | **NIRS rank** |
| **Chemical rank** | 0.95** (n=88) | 0.83** (n=88) |

**Significant correlation at P<0.001.

However, NIRS technique could be successfully used in truncated selection. This procedure is widely applied in rabbit, pig and poultry breeding. The population is divided at a point of truncation and all individuals above this value are selected. The relevant parameter is selection differential which determines the response to selection (Falconer and Mackay, 1996). Thus, the possible application of NIRS in selection for IMF will depend on the response to selection using NIRS-predicted value which is similar to the response obtained using chemically-determined values.

In selection experiments for IMF, the data cannot be normally measured on the same individuals that will be used as parents and they have to be measured on relatives, then selection is based on the values of relatives. Selection can be made on the second parities using the IMF value of 2 full sibs (a male and a female) of the first parities. Table 4 offers descriptive statistics of IMF content of the best 10 females and 5 males

that could be selected either by appling the chemical method or by appling NIRS. No differences between the means of the 2 selection criteria were found for the IMF ($P<0.05$ for females and males). Applying the same selection pressure, the average IMF content should be only 0.6% higher (1.408 g/100 g vs. 1.416 g/100 g) in parents that would be selected on the base of chemically-determined values than that of parents that would be selected on the base of NIRS-predicted values.

**Table 4.** Descriptive statistics of intramuscular fat (IMF) content (g/100 g muscle) in parents selected using IMF values measured by chemical analysis and predicted by near infrared reflectance spectroscopy (NIRS)

| | | NIRS | Chemical |
|---|---|---|---|
| **Females** | **N** | 10 | 10 |
| | **Mean** | 1.374 | 1.385 |
| | **SE**[1] | 0.035 | 0.033 |
| | **CVx100**[2] | 8.2 | 7.6 |
| **Males** | **N** | 5 | 5 |
| | **Mean** | 1.442 | 1.447 |
| | **SE**[1] | 0.057 | 0.056 |
| | **CVx100**[2] | 8.9 | 8.6 |

[1]SE, standard error; [2]CV, coefficient of variation.

## 4. CONCLUSIONS

A good NIRS calibration for predicting IMF in rabbit meat was obtained. NIRS is a suitable technique for average comparisons, but it may not be accurate enough to predict genetic individual values for ranking animals, where a higher accuracy is required. Chemical IMF values of animals that would be selected on the base of NIRS-predicted values were similar to that values obtained when chemical criterion was applied. Thus, response to selection using NIRS should be similar to direct response using chemical analysis values. NIRS is a reasonable alternative for the determination of IMF content in rabbit selection programmes.

## LITERATURE CITED

Abeni, F., and G. Bergoglio. 2001. Characterization of different strains of broiler chicken by carcass measurements, chemical and physical parameters and NIRS on breast muscle. Meat Science. 57:133-137.

Bázár, G. Y., Z. Princz, G. Jekkel, L. Locsmándi, G. Andrássy-Baka, G. Y. Kövér, Z. S. Szendrő, and R. Romvári. 2007. NIRS prediction for protein and intramuscular fat content of rabbit hind leg meat. Agriculture. 13:155-158.

Falconer, D. S., and T. F. C. Mackay. 1996. Selection: I. The response and its prediction. In: Introduction to quantitative genetics. Longman, England. p. 184-207.

Goddard, M. E., and G. R. Wiggans. 1999. Genetic improvement of dairy cattle. In: R. Fries and A. Ruvinsky, editors, The genetics of cattle. CAB Int., New York, USA. p. 511-538.

Kendall, M.G. 1952. Rank correlation. In: The advanced theory of statistics. Charles Griffin & CO., LTD., London, UK. p. 388-437.

Masoero, G., G. Xiccato, A. Dalle Zotte, R. Parigi Bini, and G. Bergoglio. 1994. Analysis of freeze-dried rabbit meat by NIRS. Zootecnia e Nutrizione Animale. 20:319-329.

Pla, M., M. Pascual, B. Ariño. 2004. Protein, fat and moisture content of retail cuts of rabbit meat evaluated with the NIRS methodology. World Rabbit Science. 12:149-158.

Prevolnik, M., M. Čandek-Potokar, D. Škorjanc, Š. Velikonja-Bolta, M. Škrlep, T. Žnidaršič, and D. Babnik. 2005. Predicting intramuscular fat content in pork and beef by near infrared spectroscopy. Journal of Near Infrared Spectroscopy. 13:77-86.

Prieto, N., R. Roehe, P. Lavín, G. Batten, and S. Andrés. 2009. Application of near infrared reflectance spectroscopy to predict meat and meat products quality: A review. Meat Science. 83:175-186.

SAS 2002. SAS/STAT User's guide (Release 9.1). *Statistical Analysis System Institute Inc., Cary NC, USA.*

Sellier, P. 1998. Genetics of meat and carcass traits. In: M.F. Rothschild and A. Ruvinsky, editors, The Genetics of the Pig. CAB International. New York, USA. p. 463-510.

Shenk, J. S., and M. O. Westerhaus. 1996. Calibration the ISI way. In: A. M. C. Davis and P. Williams, editors, Near Infrared Spectroscopy: The future waves. NIR Publications. Chichester, UK. p. 198-202.

Snedecor, G. W. and W. C. Cochran. 1980. Correlation. In: Statistical methods. The Iowa State University Press. Ames, USA. p. 175-193.

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

Williams, P., and D. Sobering. 1996. How do we it: brief summary of the methods we use in developing near infrared calibration. In: A. M. C. Davis and P. Williams, editors, Near Infrared Spectroscopy: The future waves. NIR Publications. Chichester, UK. p. 185-188.

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 Science. 78:343–358.

# CHAPTER 3

## Divergent selection for intramuscular fat content in rabbits

### I. Direct response to selection

### II. Correlated responses on carcass and meat quality traits

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# Divergent selection for intramuscular fat content in rabbits. I. Direct response to selection.

**ABSTRACT**: The aim of this study was to evaluate the selection response on intramuscular fat (IMF) content of the *Longissimus* muscle after 3 generations of divergent selection. Heritability and genetic means for IMF content were also analysed. Selection was based on the phenotypic value of IMF content measured in 2 full sibs of the first parity. Selection pressure on females was 13% in the base generation and 26% for the following generations. Males were selected within sire families in order to reduce inbreeding. Line size was 13 males and 83 females in the base population and approximately 8 males and 40 females for high (H) and low (L) lines in the following generations. A total of 668 records were used to estimate the selection response on IMF. The pedigree file used to estimate heritability and genetic means contained 1332 animals. Data were analysed using Bayesian methodology. Differences between lines for IMF were 0.08, 0.10 and 0.09 g/100g muscle in the 1st, 2nd and 3rd generation, respectively. These differences represent a direct and cumulative selection response of 9% of the mean, of which 6.8% was obtained in the first generation. Heritability of IMF content was moderate to high (0.37) with a probability of 97% of being greater than 0.20. The response to selection estimated using an animal model was 0.033, 0.052 and 0.054 g/100g muscle in line H, and -0.032, -0.046 and -0.051 g/100g muscle in line L in the 1st, 2nd and 3rd generation, respectively. Results of the present experiment confirmed that IMF content can be improved through selection in rabbits.

**Key words:** Bayesian inference, intramuscular fat, rabbits, response to selection

## 1. INTRODUCTION

Intramuscular fat (IMF) plays an essential role in meat quality, determining mainly organoleptic properties and the nutritional value of meat (Wood et al., 2008). However, an excessive deposition of fat could lead to profit loss.

To our knowledge, there are no estimates of genetic parameters for IMF in rabbits but most of the published studies in pigs (Sellier, 1998; Suzuki et al., 2005a; Sellier et al., 2010) and beef cattle (Bertrand et al., 2001) report moderate to high estimates of

heritability. Few studies have focused on increasing IMF by selection (Sapp et al., 2002; Suzuki et al. 2005b; Schwab et al., 2009) showing that genetic improvement of this trait is feasible, but none of them involved rabbits.

Divergent selection experiments are used to examine the genetic determinism of the traits of interest. Direct and correlated responses can be studied by comparing the means of high and low lines for the same and for different traits after selection.

Rabbits serve as an excellent model for genetic studies in other species due to their reduced generation interval and the low cost of producing the carcasses. In addition, sensory studies can be performed with cooked rabbit meat. On the other hand, rabbit meat has a relatively low IMF content, so selection for increasing IMF could improve its meat quality.

The aim of this study is to evaluate the selection response on IMF content of the *Longissimus* muscle (LM) after 3 generations of divergent selection for IMF in rabbits and to estimate heritability and genetic means for IMF content.

## 2. MATERIALS AND METHODS

### 2.1. Animals and experimental design

All management and experimental procedures involving animals were approved by the Research Ethics Committee of the Universitat Politècnica de València.

Data from 3 generations of a divergent selection experiment for IMF content of LM were used in this study. Animals were from a synthetic line formerly selected for ovulation rate for 10 generations (Laborda et al., 2011), and then selection was relaxed for 2 generations. The base population consisted of 13 males and 83 females. High (H) and low (L) lines had approximately 8 males and 40 females per generation. Selection was performed on rabbits from the second parity based on the phenotypic value of IMF content measured in 2 full sibs mostly of the first parity. Some IMF measurements were made on the second or third parity. Selection pressure on females was on average 13% for the first generation and 26% for the second and third generations. Males were selected within sire families in order to reduce inbreeding. The pedigree file used for the estimation of IMF heritability and genetic means contained 1332

rabbits (447 males and 885 females), from which 668 were measured for IMF to estimate the selection response.

Animals were housed at the experimental farm of the Universitat Politècnica de València. Litters were homogenized at birth, with 9 rabbits per litter. From weaning to 9 weeks of age, rabbits were reared collectively and were fed *ad libitum* with a commercial diet formulated for growing rabbits with an average composition of 15.7% crude protein, 16.4% crude fibre and 3.0% fat. High and L lines were contemporarily raised. Two rabbits (a male and a female) of the first parity of each doe were slaughtered at 9 weeks of age. After chilling for 24 h at 4°C, LM muscles were excised from the carcass. Meat obtained from LM was ground, freeze-dried and scanned with near infrared reflectance spectroscopy (model 5000, FOSS NIRSystems INC., Hilleroed, Denmark). Intramuscular fat content of LM was determined applying calibration equations previously developed (Zomeño et al., 2012) and was expressed as g/100g of muscle on a fresh basis.

Number of animals, means, standard deviations and applied effective selection differential for each generation of selection are provided in Table 1.

**Table 1.** Raw mean, standard deviation and effective selection differential in 3 generations of divergent selection for intramuscular fat content (g/100g muscle)

| G[1] | Line | Mean | SD | S[2] | |
|---|---|---|---|---|---|
| | | | | Males | Females |
| 0 (n[3]=168) | H[4] | 1.21 | 0.13 | 0.13 | 0.17 |
| | L[5] | 1.21 | 0.13 | -0.15 | -0.15 |
| 1(n[3]=168) | H[4] | 1.24 | 0.16 | 0.18 | 0.14 |
| | L[5] | 1.17 | 0.16 | -0.11 | -0.13 |
| 2 (n[3]=158) | H[4] | 1.17 | 0.10 | 0.11 | 0.08 |
| | L[5] | 1.07 | 0.08 | -0.05 | -0.08 |
| 3 (n[3]=174) | H[4] | 1.07 | 0.10 | - | - |
| | L[5] | 0.98 | 0.11 | - | - |

[1]G, generation number; [2]S, effective selection differential; [3]n, sample size;[4]H, high line; [5]L, low line.

Each generation was 8-10 months long. In this period of time, IMF analyses were conducted in approximately 3-4 months. The effective selection differentials were calculated by weighting the IMF content deviations of the selected animals according to the number of their offspring in the next generation.

### 2.2. Statistical analyses

Selection response on IMF was estimated by comparing H and L lines at the same generation of selection. Differences between lines for IMF content were estimated by fitting the following model:

$$y_{ijkl} = LG_i + S_j + PO_k + p_{ikl} + e_{ijkl}$$

where $LG_i$ is the effect of line-generation (7 levels: base population, H line-1st generation, L line-1st generation, H line-2nd generation, L line-2nd generation, H line-3rd generation and L line-3rd generation), $S_j$ is the effect of sex (2 levels), $PO_k$ is the effect of parity order (3 levels), $p_{ikl}$ is the effect of common litter (334 levels) and $e_{ijkl}$ is the residual of the model.

Bayesian inference was used. Intramuscular fat was assumed to be conditionally distributed as follows:

$$\mathbf{y} \mid \mathbf{b}, \mathbf{p}, \sigma^2_e \sim N(\mathbf{Xb} + \mathbf{Wp}, \mathbf{I}\sigma^2_e)$$

where **b** is the vector including LG, S and PO effects; **p** is the vector of common litter effects including genetic and environmental effects of the litter; $\sigma^2_e$ is the residual variance; **X** and **W** are the known incidence matrices and **I** an identity matrix of appropriate order. Bounded uniform priors were used to represent vague previous knowledge of **b**. Prior knowledge concerning common litter effects was represented by assuming that they were normally distributed, conditionally on the associated variance component, as follows:

$$\mathbf{p} \mid \sigma^2_p \sim N(\mathbf{0}, \mathbf{I}\sigma^2_p)$$

where $\sigma^2 p$ is the common litter variance and **I** an identity matrix of the same order as the number of levels of common litter effects. Bounded uniform priors were used for residual and common litter variances.

Marginal posterior distributions for all unknowns were estimated using Gibbs sampling. The programme, Rabbit, developed by Institute for Animal Science and Technology (Valencia, Spain), was used for all procedures. Chains of 60,000 samples with a burn-in period of 10,000 were used. One sample each 10 was saved in order to avoid high correlations between consecutive samples. Convergence was tested using the Z-criterion of Geweke (Geweke, 1992). Details of the procedure can be found in Blasco (2001). The following parameters were obtained from the marginal posterior distributions of the differences between lines: the median of the marginal posterior distribution of the difference ($D_{H-L}$) in each generation; the highest posterior density region at 95% ($HPD_{95\%}$); the probability of lines being different (probability of the difference between H and L lines being greater than zero when this difference is greater than zero, or the probability of the difference being lower than zero when this difference is lower than zero) (*P*); the guaranteed value of a difference with a probability of 95% or 80% (limit of the interval [k, $+\infty$) when the difference is greater than zero or the limit of the interval ($-\infty$, k] when the difference is lower than zero); and the probability of relevance (probability of the difference being greater than a relevant value) ($P_R$). A relevant value is a minimum amount having biological or economical significance and it is the difference to be detected in experimental designs.

To estimate the heritability of IMF content and genetic means, the following univariate animal model was fitted:

$$y_{ijklm} = G_i + S_j + PO_k + c_{ikl} + a_{ijklm} + e_{ijklm},$$

where $G_i$ is the effect of generation (4 levels: zero, 1st, 2nd and 3rd), $S_j$ is the effect of sex (2 levels), $PO_k$ is the effect of parity order (3 levels), $c_{ikl}$ is the effect of common environment litter (334 levels), $a_{ijklm}$ is the additive value of the animal and $e_{ijklm}$ is the residual of the model. Generation effect only includes environmental effects.

Bayesian inference was used. Intramuscular fat was assumed to be conditionally distributed as follows:

$$\mathbf{y} \mid \mathbf{b}, \mathbf{a}, \mathbf{c}, \sigma^2_e \sim N(\mathbf{Xb} + \mathbf{Za} + \mathbf{Wc}, \mathbf{I}\sigma^2_e),$$

where **b** is the vector including SG, S and PO effects; **a** is the vector of additive genetic effects; **c** is the vector of common environment litter effects; $\sigma^2_e$ is the residual

variance; **X**, **Z** and **W** are the known incidence matrices and **I** is an identity matrix of appropriate order. Bounded uniform priors were used to represent vague previous knowledge of **b**. Prior knowledge concerning additive and common environment effects was represented by assuming that they were normally distributed, conditionally on the associated variance component, as follows:

$$\mathbf{a} \mid \mathbf{A}\sigma^2_a \sim N(\mathbf{0}, \mathbf{A}\sigma^2_a),$$

where **A** is the known additive genetic relationship matrix including all the animals involved in the selection process, and the parents and grandparents of the base generation, and $\sigma^2_a$ the genetic variance.

$$\mathbf{c} \mid \sigma^2_c \sim N(\mathbf{0}, \mathbf{I}\sigma^2_c),$$

where $\sigma^2_c$ is the common environment litter variance and **I** is an identity matrix of the same order as the number of levels of common environment liter effects. Bounded uniform priors were used for residual, additive and common environment variances.

Marginal posterior distributions for all unknowns were estimated using Gibbs sampling. The programme TM by Legarra et al. (2008) was used for all Gibbs sampling procedures. Chains of 1,000,000 samples with a burn-in period of 200,000 were used. One sample each 100 was saved in order to avoid high correlations between consecutive samples. Convergence was tested using the Z-criterion of Geweke.

Marginal posterior distributions of the estimated genetic means by the animal model per generation were computed, and the following parameters were determined: the median of the marginal posterior distribution and the highest posterior density region at 95%. In addition, marginal posterior distributions of the differences between estimated genetic means by the animal model for H and L lines were obtained. The following parameters were determined: the median of the marginal posterior distribution of the difference; the highest posterior density region at 95%; the probability of lines being different; the guaranteed value of a difference with a probability of 95% and 80%; and the probability of relevance.

## 3. RESULTS AND DISCUSSION

### 3.1. Response to selection on intramuscular fat content

In divergent selection experiments, on the assumption that environmental differences affect both lines alike, the difference between lines estimates the genetic improvement made by selection (Falconer and Mackay, 1996). The effectiveness of the selection process is doubled because both lines are selected. Thus, the genetic differentiation between lines is faster than using a control population, without changing the variance of the response.

Table 2 shows the features of the estimated marginal posterior distributions of the differences between H and L lines for IMF. Line H was 7% greater than line L in the first and 9% in the second and third generations. The differences between lines were at least 0.06, 0.08 and 0.07 g/100g muscle with a probability of 80% ($k_{80\%}$) in the 1st, 2nd and 3rd generations, respectively.

**Table 2.** Features of the estimated marginal posterior distributions of the differences between high and low lines for intramuscular fat content (g/100g muscle) in 3 generations of divergent selection

| G[1] | Mean[2] | $D_{H-L}$[3] | $HPD_{95\%}$[4] | | $P$[5] | $k_{95\%}$[6] | $k_{80\%}$[7] | $P_R$[8] |
|---|---|---|---|---|---|---|---|---|
| **1** | 1.18 | 0.08 | 0.03 | 0.14 | 1.00 | 0.03 | 0.06 | 0.85 |
| **2** | 1.10 | 0.10 | 0.04 | 0.16 | 1.00 | 0.05 | 0.08 | 0.96 |
| **3** | 1.00 | 0.09 | 0.04 | 0.15 | 1.00 | 0.04 | 0.07 | 0.92 |

[1]G, generation number; [2]Mean, average of the medians of the marginal posterior distributions for intramuscular fat content of high and low lines in each generation; [3]$D_{H-L}$, median of the marginal posterior distribution of the difference; [4]$HPD_{95\%}$, highest posterior density region at 95% of probability; [5]$P$, probability of the difference being greater than zero; [6]$k_{95\%}$, limit of the interval [k, +∞) at 95% of probability; [7]$k_{80\%}$, limit of the interval [k, +∞) at 80% of probability; [8]$P_R$, probability of relevance (probability of the difference being greater than 0.05 g/100g muscle).

In practice, we are interested not only in finding whether the lines are different but whether this difference is relevant (Blasco, 2005). A difference can be relevant or important from either an economic or biological point of view. A fraction of the standard deviation of the trait can also be considered as a relevant difference,

generally between one half and one third of the standard deviation. In the present experiment, we considered an amount of 0.05g/100g muscle as a relevant difference between lines (R), which is one third of the standard deviation of the trait and represents that line H is 5% greater than L. The probability of being greater than R was moderate in the first generation ($P_R$=0.85) and high in the second and third generations ($P_R$=0.96 and 0.92, respectively).

Differences between lines represented a direct and cumulative selection response on IMF of 9% of the mean. A total of 6.8% was obtained in the first generation, 2.2% in the second generation and no response was obtained in the third. The reduction in the response can be related to the reduction of the effective selection differential (Table 1) and larger sampling errors.

To our knowledge, no previous selection studies have been conducted to increase IMF content in rabbits. Some selection experiments have been conducted in pigs (Suzuki et al., 2005b; Schwab et al., 2009) and cattle (Sapp et al., 2002), with an improvement of IMF content in line selected for increased IMF.

### 3.2. Heritability and genetic means for intramuscular fat content

Heritability for IMF content of LM was moderate to high (0.37; Table 3) but the accuracy of this estimation was low ($HPD_{95\%}$ between 0.19 and 0.59). However, this heritability was at least 0.29 with a probability of 80% and the probability of being greater than 0.20 was 97% ($P_{0.20}$).

**Table 3.** Features of the estimated marginal posterior distribution of the heritability of intramuscular fat content

| Mean[1] | Median[2] | $HPD_{95\%}$[3] | | $k_{95\%}$[4] | $k_{80\%}$[5] | $P_{0.20}$[6] |
|---|---|---|---|---|---|---|
| 0.38 | 0.37 | 0.19 | 0.59 | 0.22 | 0.29 | 0.97 |

[1]Mean, mean of the marginal posterior distribution of the heritability; [2]Median, median of the marginal posterior distribution of the heritability; [3]$HPD_{95\%}$, highest posterior density region at 95% of probability; [4]$k_{95\%}$, limit of the interval [k, +1] at 95% of probability; [5]$k_{80\%}$, limit of the interval [k, +1] at 80% of probability; [6]$P_{0.20}$, probability of the heritability being greater than 0.20.

To our knowledge, no estimates of heritability for IMF content of rabbit are available in the literature. Most of the published studies evaluating genetic parameters of IMF have been conducted in pigs and beef cattle. The heritability estimated in this study is in line with reported values found in the literature which are moderate to high. In pigs, for example, Sellier (1998) reported values from 19 studies ranging from 0.26 to 0.86. More recent studies reported estimates within this range (Solanes et al., 2009; Sellier et al., 2010; Ros-Freixedes et al., 2012). In beef cattle, reported heritability estimates for IMF are also moderate to high, with values ranging from 0.15 to 0.93 (reviewed by Bertrand et al., 2001). Cecchinato et al. (2012) obtained a lower heritability for IMF (0.18) using a Bayesian analysis, but also with a large highest posterior density region at 95%. The ratio of common environment litter variation to total variation was moderate (0.24).

A limitation of divergent selection experiments is that response can be asymmetrical. The first model does not indicate the response in each line. Moreover, the precision of the estimation of the response by using the first model is lower than that obtained by estimating genetic means applying an animal model. Figure 1 plots the medians and standard deviations of the marginal posterior distributions of the estimated genetic means per generation. These distributions were approximately normal. Selection response on IMF was 0.033, 0.052 and 0.054 g/100g muscle for line H, and -0.032, -0.046 and -0.051 g/100g for line L in the 1st, 2nd and 3rd generations, respectively. As showed in Table 2, most of the response took place in the first and second generations of selection, and response was symmetrical. The accuracy of the estimated genetic means per generation of H and L lines was also calculated. Highest posterior density regions at 95% of probability were between -0.03 and 0.10 g/100g muscle in the first generation, and between -0.01 and 0.12 g/100g muscle in the second and third generations for H line. For L line, $HPD_{95\%}$ were between -0.09 and 0.03 g/100g muscle in the first generation, and between -0.11 and 0.02 g/100g muscle in the second and third generations. Although zero was included in these intervals, the probabilities of genetic means being greater than zero were between 0.91 and 0.95 in the second and third generations for H and L lines.

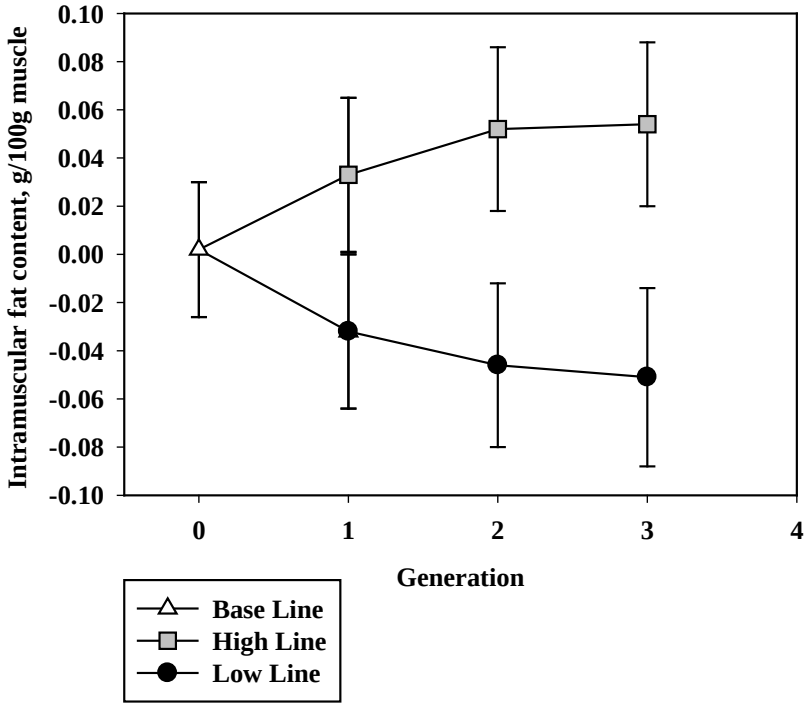


**Figure 1.** Medians and SD of the marginal posterior distributions of estimated genetic means for intramuscular fat per generation.

Moreover, the probability of the differences between genetic means of H and L lines being greater than zero was calculated and was 100% in the 3 generations (Table 4), which supports that selection response was realized.

**Table 4.** Features of the estimated marginal posterior distributions of the differences between estimated genetic means of high and low lines for intramuscular fat content (g/100g muscle) in each generation of divergent selection

| G[1] | $D_{H-L}$[2] | $HPD_{95\%}$[3] | | $P$[4] | $k_{95\%}$[5] | $k_{80\%}$[6] | $P_R$[7] |
|---|---|---|---|---|---|---|---|
| **1** | 0.07 | 0.03 | 0.09 | 1.00 | 0.04 | 0.05 | 0.83 |
| **2** | 0.10 | 0.07 | 0.13 | 1.00 | 0.07 | 0.08 | 1.00 |
| **3** | 0.11 | 0.07 | 0.14 | 1.00 | 0.08 | 0.09 | 1.00 |

[1]G, generation number; [2]$D_{H-L}$, median of the marginal posterior distribution of the difference between genetic means of high and low lines; [3]$HPD_{95\%}$, highest posterior density region at 95% of probability; [4]*P*, probability of the difference being greater than zero; [5]$k_{95\%}$, limit of the interval [k, +∞) at 95% of probability; [6]$k_{80\%}$, limit of the interval [k, +∞) at 80% of probability; [7]$P_R$, probability of relevance (probability of the difference being greater than 0.05 g/100 g muscle).

Differences between lines increased each generation and were at least 0.05, 0.08 and 0.09 g/100g muscle with 80% of probability ($k_{80\%}$) in the 1st, 2nd and 3rd generation, respectively (Table 4). The probability of being greater than 0.05 g/100g muscle, considered as a relevant value, also increased each generation being 100% in the second and third generations. Differences between lines provided in Table 4 were similar to those shown in Table 2, supporting the model applied.

Genetic means depend on the heritability considered. In order to validate our results, genetic means were estimated again using an estimate of heritability with a probability of 95% (0.22; Table 3), and values are provide in Fig. 2. Selection response for IMF was very similar to that shown in Fig. 1.

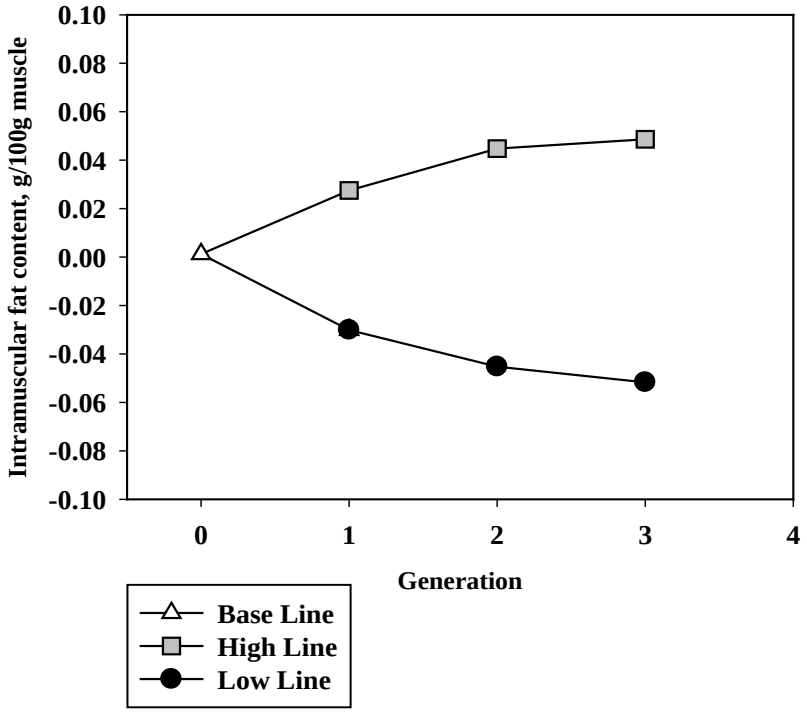


**Figure 2.** Estimated genetic means for intramuscular fat per generation using an estimate of heritability with a probability of 95% (heritability=0.22).

Environmental means were computed and were almost null and constant for both lines during the 3 generations of selection. Thus, the change of IMF content in the lines could be assigned entirely to the selection process.

## 4. CONCLUSIONS

Results from this study showed a direct selection response on IMF after 3 generations of selection, with a total cumulative difference between lines of 0.09 g/100g muscle. The estimated heritability for IMF was moderately high with a probability of 97% of being greater than 0.2. Response to selection estimated using an animal model was 0.054 g/100g muscle in the H line and -0.051 g/100g muscle in the L line after 3 generations of selection. We can consider that the animal model fitted in this study has been validated and results confirmed that this trait can be improved through selection in rabbits.

## LITERATURE CITED

Bertrand, J. K., R. D. Green, W. O. Herring, and D. W. Moser. 2001. Genetic evaluation for beef carcass traits. Journal of Animal Science. 79(Suppl.):E190-E200.

Blasco, A. 2001. The Bayesian controversy in animal breeding. Journal of Animal Science. 79:2023-2046.

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

Cecchinato, A., M. De Marchi, M. Penasa, J. Casellas, S. Schiavon, and G. Bittante. 2012. Genetic analysis of beef fatty acid composition predicted by near-infrared spectroscopy. Journal of Animal Science. 90:429-438.

Geweke. 1992. Evaluating the accuracy of sampling-based approaches to the calculation of posterior moments. In: J. M. Bernardo, J. O. Berger, A. P. Dawid, and A. F. M. Smith, editors, Bayesian Statistics 4. Oxford University Press, Oxford, UK. p. 169–193.

Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to Quantitative Genetics. 4th ed. Longman Group Limited, Essex, UK.

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. Journal of Animal Science. 89:2981-2987.

Legarra, A., L. Varona, and E. López de Maturana. 2008. TM Threshold Model. Available at http://snp.toulouse.inra.fr/~alegarra/ manualtm.pdf [accessed Aug. 3, 2011; verified January 7, 2013]. GenoToul Bioinformatics, Toulouse, France.

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

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. Journal of Animal Science. 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. Journal of Animal Science. 87:2774-2780.

Sellier, P. 1998. Genetics of meat and carcass traits. In: M. F. Rothschild and A. Ruvinsky, editors, The Genetics of the Pig. CAB International, New York, USA. p. 463-510.

Sellier, P., L. Maignel, and 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.

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

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

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

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 Science. 78:343–358.

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

# Divergent selection for intramuscular fat content in rabbits. II. Correlated responses on carcass and meat quality traits.

**ABSTRACT**: Correlated responses on carcass and meat quality characteristics after 3 generations of divergent selection for intramuscular fat (IMF) content were assessed by comparing the high (H) and low (L) lines. Selection was based on the phenotypic value of IMF content of the *Longissimus* muscle (LM), measured in 2 full sibs of the first parity. Traits measured were: body weight (BW), hot carcass weight (HCW), commercial carcass weight (CCW), reference carcass weight (RCW), scapular (SF) and perirenal fat (PF) content, meat to bone ratio (M/B) of the hind leg, pH of the LM, colour (lightness, L*; redness, a*; and yellowness, b*) of the carcass and of a LM section, protein content and fatty acid (FA) composition of the LM. A total of 174 records was used to estimate the correlated selection response. Data were analysed using the Bayesian methodology. We considered one third of the phenotypic standard deviation of a trait as a relevant value for the difference between lines. Then, the probability of the difference being greater than a relevant value ($P_R$) was calculated. A low $P_R$ implies that the lines compared are similar. Carcass weights ($P_R$ between 0.24 and 0.31) and the M/B of the hind leg ($P_R$=0.15) were not modified by selection for IMF content. There was a slight negative correlated response for BW, although the evidence of its relevance was low ($P_R$=0.48). Scapular fat content was similar between lines ($P_R$=0.03). However, there were differences for PF content, although there was low evidence for showing its relevance ($P_R$=0.47). Colour parameters of the carcass were not affected by selection ($P_R$ between 0.04 and 0.30). In muscle, L* was also similar between lines ($P_R$=0.26). However, there were differences for a* and b*, although there was low evidence of their relevance ($P_R$=0.35 and 0.40, respectively). There was a positive correlated response on muscle pH, and differences could be relevant ($P_R$=0.77). Protein content of the LM was similar between lines ($P_R$=0.13), whereas FA composition was affected by selection. There were relevant differences between lines for monounsaturated FA ($P_R$=0.99), n-3 ($P_R$=0.95) and n-6 ($P_R$=0.98) percentages. For individual FA, differences were relevant for C18:1 n-9 ($P_R$=0.97) and

C20:5 n-3 ($P_R$=0.98). In conclusion, selection for IMF content may modify carcass quality by increasing PF content. Moreover, it led to some modifications in pH and FA composition of the LM.

**Key words:** Bayesian inference, carcass quality, intramuscular fat, meat quality, rabbits, response to selection

## 1. INTRODUCTION

The importance of intramuscular fat (IMF) content on meat quality is well established (Warriss 2000; Wood et al., 2008). The selection experiments developed in pigs (Suzuki et al. 2005a; Schwab et al., 2009) and cattle (Sapp et al., 2002) to increase IMF content showed that this trait responds to selection. A companion paper has reported that IMF can also be enhanced through selection in rabbits (C. Zomeño, unpublished data). However, there is little information about correlated responses on carcass and meat quality traits in pigs and cattle, and no information is available about rabbits.

Selection for IMF content may have consequences on other traits that should be considered. Intramuscular fat content is unfavourably genetically correlated to carcass fatness as well as to carcass leanness (reviewed by Sellier, 1998), although these correlations showed a wide range of variation across studies. Estimates of genetic correlations between IMF and some meat quality traits have been reported (Suzuki et al., 2005b; Schwab et al., 2010; Gjerlaug-Enger et al., 2010). In rabbits, the number of publications reporting genetic parameters of meat quality traits is scarce (reviewed by Hernández and Gondret, 2006), and the genetic association between IMF and carcass and meat quality characteristics has not been investigated yet. Genetic correlations involving meat quality traits have high standard errors due to the difficulty of having a large amount of data to estimate them. The estimation of correlated response is a more accurate method to examine the consequences of selection for IMF on other traits.

The aim of this study is to assess the correlated responses on carcass and meat quality characteristics after 3 generations of divergent selection for IMF in rabbits.

## 2. MATERIALS AND METHODS

### 2.1. Animals and experimental design

All management and experimental procedures involving animals were approved by the Research Ethics Committee of the Universitat Politècnica de València.

Data from the third generation of a divergent selection experiment for IMF content of the *Longissimus* muscle (LM) were used in this study. Animals came from a synthetic line formerly selected for ovulation rate for 10 generations (Laborda et al., 2011), and then selection was relaxed for 2 generations. The base population consisted of 13 males and 83 females, obtained after the selection for ovulation rate was relaxed for 2 generations. High (**H**), selected for high IMF, and low (**L**), selected for low IMF, lines had approximately 8 males and 40 females per generation. Selection was performed on the second parity based on the phenotypic value of IMF content measured in 2 full sibs mostly of the first parity. Some IMF measurements were made on the second or third parity. Selection pressure on females was on average 13% for the first generation and 26% for the second and third generations. Males were selected within sire families in order to reduce inbreeding. A total of 174 rabbits was used to estimate the correlated response on carcass and meat quality characteristics.

Animals were housed at the experimental farm of the Universitat Politècnica de València. Litters were homogenized at birth, with 9 rabbits per litter. From weaning to 9 weeks of age, rabbits were reared collectively and were fed *ad libitum* with a commercial diet formulated for growing rabbits with an average composition of 15.7% crude protein, 16.4% crude fibre and 3.0% fat. High and L lines were contemporarily raised. Feed intake was measured collectively during the growing period. Two rabbits (a male and a female) of the first parity of each doe were slaughtered at 9 wk of age.

### 2.2. Carcass quality traits

Before slaughter, the body weight (BW) of the rabbits was recorded. After slaughter, hot carcasses were weighted (HCW) and were chilled for 24 h at 4°C. The weight of the chilled carcasses was recorded (CCW). These carcasses contained the head, liver, lungs, thymus, esophagus, heart, and kidneys, which were removed to obtain the reference carcass. The weight of the reference carcasses (RCW) was measured, containing only

meat, fat, and bone. Scapular (SF) and perirenal fat (PF) were excised from the carcass and were weighted. From the hind part of the carcass, the left leg was dissected to separate bone from edible meat, and then meat to bone ratio (M/B) was calculated. Colour (lightness, L*; redness, a*; and yellowness, b*) of the carcasses was measured on the surface of the fourth lumbar vertebra of the right side using a CR300 Minolta Chromameter (Minolta Camera, Osaka, Japan).

### 2.3. Meat quality traits

The muscle pH was measured at 24 hours *post mortem* in the LM muscle at the level of the fifth lumbar vertebra of the left side and recorded with a Crison pH-meter Basic 20+ (Crison Instruments, Barcelona, Spain). The *Longissimus* muscles were excised from the carcass. Meat colour was measured at the seventh lumbar vertebra transversal section of the right LM muscle. The parameters L*, a*, and b* were recorded as previously indicated. Meat obtained from the LM was ground, freeze-dried and scanned with near infrared reflectance spectroscopy (NIRS) (model 5000, FOSS NIRSystems INC., Hilleroed, Denmark). Protein content and fatty acid (FA) composition of the LM was determined applying calibration equations previously developed (Zomeño et al., 2012). Protein content was expressed as g/100g of muscle in a fresh basis and FA composition as a percentage of total FA.

### 2.4. Statistical analyses

Correlated responses on carcass and meat quality traits were estimated by comparing the H and L lines at the third generation of selection. Differences between lines for each trait were estimated by fitting the following univariate model:

$$y_{ijkl} = LG_i + S_j + PO_k + p_{ikl} + e_{ijkl}$$

where $LG_i$ is the effect of line-generation (7 levels: base population, H line-1st generation, L line-1st generation, H line-2nd generation, L line-2nd generation, H line-3rd generation and L line-3rd generation), $S_j$ is the effect of sex (2 levels), $PO_k$ is the effect of parity order (3 levels), $p_{ikl}$ is the effect of common litter (334 levels) and $e_{ijkl}$ is the residual of the model.

Bayesian inference was used. Each trait was assumed to be conditionally distributed as follows:

$$\mathbf{y} \mid \mathbf{b}, \mathbf{p}, \sigma^2_e \sim N(\mathbf{Xb} + \mathbf{Wp}, \mathbf{I}\sigma^2_e)$$

where **b** is the vector including LG, S and PO effects; **p** is the vector of common litter effects including genetic and environmental effects of the litter; **X** and **W** are the known incidence matrices; $\sigma^2_e$ is the residual variance and **I** an identity matrix of appropriate order. Bounded uniform priors were used to represent vague previous knowledge of **b**. Prior knowledge concerning common litter effects was represented by assuming that they were normally distributed, conditionally on the associated variance component, as follows:

$$\mathbf{p} \mid \sigma^2_p \sim N(\mathbf{0}, \mathbf{I}\sigma^2_p)$$

where $\sigma^2$p is the common litter variance and **I** is an identity matrix of the same order as the number of levels of common litter effects. Bounded uniform priors were used for residual and common litter variances.

Marginal posterior distributions for all unknowns were estimated using Gibbs sampling. The programme Rabbit developed by Institute for Animal Science and Technology (Valencia, Spain) was used for all procedures. Chains of 60,000 samples with a burn-in period of 10,000 were used. One sample each 10 was saved in order to avoid high correlations between consecutive samples. Convergence was tested using the Z-criterion of Geweke. Details of the procedure can be found in Blasco (2001). The following parameters were obtained from the marginal posterior distributions of the differences between lines: the median of the marginal posterior distribution of the difference ($D_{H-L}$); the probability of lines being different (probability of the difference between H and L lines being greater than zero when this difference is greater than zero, or the probability of the difference being lower than zero when this difference is lower than zero) (*P*); the highest posterior density region at 95% ($HPD_{95\%}$); the guaranteed value of a difference (k) with a probability of 80% (limit of the interval [k, $+\infty$) when the difference is greater than zero or the limit of the interval ($-\infty$, k] when the difference is lower than zero) and the probability of relevance (probability of the difference being greater than a relevant value) ($P_R$). The use of Bayesian inference in meat quality analyses was proposed by Blasco (2005). A relevant value (R) is a minimum amount having biological or economical significance and it is the difference to be detected in experimental designs. For most productive traits, the economical

importance of a difference is relevant when it ranges between one half and one third of the standard deviation of the trait. Thus, we considered one third of the phenotypic standard deviation of the trait as a relevant value for the differences between lines. The standard deviation was calculated using all data of the selection experiment (668 rabbits). The k values are displayed in the tables only when $D_{H-L}$ and k value have the same sign.

## 3. RESULTS

Descriptive statistics of BW and carcass composition are presented in Table 1. Table 2 shows descriptive statistics for carcass and meat quality measurements.

**Table 1.** Descriptive statistics of BW (g) and carcass composition (g) of rabbits

| Trait | Mean | SD | CVx100 | No. of animals |
|---|---|---|---|---|
| **BW** | 1,651 | 138 | 8.4 | 174 |
| **HCW**[1] | 983 | 83 | 8.4 | 174 |
| **CCW**[2] | 943 | 82 | 8.7 | 174 |
| **RCW**[3] | 748 | 68 | 9.1 | 174 |
| **SF**[4] | 3.33 | 0.83 | 25.0 | 173 |
| **PF**[5] | 6.66 | 2.18 | 32.7 | 174 |

[1]HCW, hot carcass weight;[2]CCW, chilled carcass weight;[3]RCW, reference carcass weight;[4]SF, scapular fat content;[5]PF, perirenal fat content.

**Table 2.** Descriptive statistics of carcass and meat quality measurements of rabbits

| Trait | Mean | SD | CVx100 | No. of animals |
|---|---|---|---|---|
| **M/B**[1] | 4.30 | 0.55 | 12.8 | 114 |
| **C L***[2] | 55.2 | 2.4 | 4.3 | 173 |
| **C a***[3] | 3.43 | 0.92 | 26.7 | 174 |
| **C b***[4] | -0.66 | 1.88 | -285 | 167 |
| **LM pH**[5] | 5.59 | 0.10 | 1.7 | 174 |
| **LM L***[6] | 53.4 | 2.6 | 4.8 | 173 |
| **LM a***[7] | 3.96 | 1.11 | 28.0 | 174 |
| **LM b***[8] | 0.91 | 0.83 | 91.4 | 173 |

[1]M/B, meat to bone ratio of the hind leg; [2]C L*, lightness of the carcass surface; [3]C a*, redness of the carcass surface; [4]C b*, yellowness of the carcass surface; [5]LM pH, pH of the *Longissimus*

muscle; [6]LM L*, lightness of the *Longissimus* muscle; [7]LM a*, redness of the *Longissimus* muscle; [8]LM b*, yellowness of the *Longissimus* muscle.

Tables 3 and 4 present descriptive statistics for protein content and FA composition. For all the traits analyzed, Monte Carlo standard errors were very small. Therefore, they are not displayed in the tables. The Geweke test did not detect lack of convergence in any case.

**Table 3.** Descriptive statistics of protein content (g/100g muscle) and fatty acid composition (percentage of total fatty acids) of the *Longissimus* muscle

| Trait | Mean | SD | CVx100 | No. of animals |
|---|---|---|---|---|
| **Protein** | 21.7 | 0.4 | 2.0 | 174 |
| **SFA[1]** | 35.7 | 2.1 | 6.0 | 174 |
| **MUFA[2]** | 23.7 | 2.2 | 9.1 | 174 |
| **PUFA[3]** | 39.8 | 3.6 | 9.1 | 174 |
| **n-3[4]** | 7.07 | 0.86 | 12.2 | 174 |
| **n-6[5]** | 33.4 | 2.4 | 7.1 | 174 |
| **n-6/n-3** | 4.64 | 0.39 | 8.4 | 170 |
| **PUFA/SFA** | 1.16 | 0.06 | 5.6 | 174 |

[1]SFA = C14:0+C15:0+C16:0+C17:0+C18:0;[2]MUFA = C16:1+C18:1n-9+C18:1n-7;[3]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;[4]n-3 = C18:3n-3+C20:5n-3+C22:5n-3+C22:6n-3;[5]n-6 = C18:2n-6+C20:2n-6+C20:3n-6+C20:4n-6+C22:4n-6.

**Table 4.** Descriptive statistics of individual fatty acid composition (percentage of total fatty acids) of the *Longissimus* muscle of rabbits

| Trait | Mean | SD | CVx100 | No. of animals |
|---|---|---|---|---|
| **C14:0** | 1.36 | 0.32 | 23.8 | 174 |
| **C15:0** | 0.48 | 0.02 | 4.2 | 174 |
| **C16:0** | 21.7 | 1.5 | 7.1 | 174 |
| **C16:1** | 0.89 | 0.55 | 62.1 | 150 |
| **C17:0** | 0.70 | 0.04 | 5.7 | 174 |
| **C18:0** | 8.79 | 0.63 | 7.2 | 174 |
| **C18:1 n-7** | 1.67 | 0.12 | 7.0 | 173 |
| **C18:1 n-9** | 20.0 | 1.7 | 8.6 | 174 |

| | | | | |
|---|---|---|---|---|
| **C18:2 n-6** | 21.5 | 1.8 | 8.6 | 174 |
| **C18:3 n-3** | 1.25 | 0.30 | 24.0 | 172 |
| **C20:2 n-6** | 0.32 | 0.04 | 11.1 | 169 |
| **C20:3 n-6** | 0.62 | 0.08 | 13.7 | 168 |
| **C20:4 n-6** | 6.48 | 1.00 | 15.5 | 169 |
| **C20:5 n-3** | 1.89 | 0.36 | 18.9 | 171 |
| **C22:4 n-6** | 2.37 | 0.37 | 15.8 | 174 |
| **C22:5 n-3** | 0.98 | 0.19 | 19.3 | 169 |
| **C22:6 n-3** | 3.34 | 0.70 | 21.0 | 167 |

### 3.1. Correlated response on body weight and carcass measurements

Features of the marginal posterior distributions of the differences between H and L lines for BW and carcass composition in the third generation of selection are shown in Table 5.

**Table 5.** Features of the estimated marginal posterior distributions of the differences between high and low lines for BW (g) and carcass composition (g) in the third generation of selection

| Trait | $D_{H-L}$[1] | $P$[2] | $HPD_{95\%}$[3] | | $k_{80\%}$[4] | R[5] | $P_R$[6] |
|---|---|---|---|---|---|---|---|
| **BW** | -48.8 | 0.96 | -109 | 4.45 | -25.8 | -50 | 0.48 |
| **HCW**[7] | -21.0 | 0.88 | -56.1 | 14.8 | -6.06 | -30 | 0.31 |
| **CCW**[8] | -17.4 | 0.83 | -54.9 | 16.2 | -2.17 | -30 | 0.24 |
| **RCW**[9] | -15.4 | 0.85 | -44.0 | 14.9 | -3.20 | -25 | 0.27 |
| **SF**[10] | -0.05 | 0.60 | -0.39 | 0.36 | - | -0.40 | 0.03 |
| **PF**[11] | 1.06 | 0.95 | -0.11 | 2.34 | 0.53 | 1.10 | 0.47 |

[1]$D_{H-L}$, median of the marginal posterior distribution of the difference; [2]$P$, probability of the difference being greater than zero when $D_{H-L}>0$ and probability of the difference being lower than zero when $D_{H-L}<0$; [3]$HPD_{95\%}$, highest posterior density region at 95% of probability; [4]$k_{80\%}$, limit of the interval $[k, +\infty)$ when $D_{H-L}>0$ and $(-\infty, k]$ when $D_{H-L}<0$ at 80% of probability. It is displayed in the table only when $D_{H-L}$ and $k_{80\%}$ have the same sign; [5]R, relevant value defined as one third of the SD of the trait; [6]$P_R$, probability of relevance (probability of the difference being greater than R); [7]HCW, hot carcass weight; [8]CCW, chilled carcass weight; [9]RCW, reference carcass weight; [10]SF, scapular fat content; [11]PF, perirenal fat content.

Body weight and carcass weights were higher in line L than in line H (*P* between 0.83 and 0.96). However, the estimated differences represent a low percentage of the mean (2.95% for BW, 2.14% for HCW, 1.85% for CCW and 2.06% for RCW), and the guaranteed values of the differences with a probability of 80% ($k_{80\%}$) were very small. In some cases, we are also interested in knowing which will be the minimum difference between lines with a determined probability. When using estimates of the difference in the discussion, we only guarantee a minimum value with a probability of 50%. Thus, a minimum value with a probability of 80% improves the discussion. Notice than although zero was included in some $HPD_{95\%}$, the *P* was higher than 0.95. This is because the right tail of the distribution is considered in *P* but not in $HPD_{95\%}$, see Blasco (2005). We considered one third of the phenotypic standard deviation of a trait as a relevant difference between lines. The probability of the difference being greater than R was intermediate for BW ($P_R$=0.48), indicating low evidence for showing whether differences were relevant or not. For carcass weights, differences were not relevant, as indicated by the low $P_R$ (between 0.24 and 0.31). For SF content, differences between lines were irrelevant ($P_R$=0.03; Table 5). However, for PF content, line H had a greater value than line L (*P*=0.95), the difference being 1.06 g. This represents a correlated response of 15.9% of its mean. This difference was at least 0.53 g with a probability of 80%. Considering 1.10 g as a relevant value, there was low evidence for showing whether differences were relevant or not ($P_R$=0.47).

### 3.2. Correlated response on carcass and meat quality traits

Table 6 shows the features of estimated marginal posterior distributions of the differences between H and L lines for carcass and meat quality traits in the third generation of selection. The M/B ratio of the hind leg was not affected by the selection process *(*$P_R$=0.15). Line L showed greater values for L* (*P*=0.87) and b* (*P*=0.96) of the carcass. However, differences were not relevant ($P_R$=0.28 and 0.30, respectively). Differences for a* of the carcass were also irrelevant ($P_R$=0.04). Muscle pH was greater in line H than in line L (*P*=0.99), with a difference between lines of 0.04. This difference was at least 0.03 with a probability of 80%. The $P_R$ was moderately high for this trait (0.77), indicating that the difference could have some consequences on meat quality. Colour parameters of the muscle (L*, a* and b*) were greater in line L (*P* between 0.88

and 0.97), although differences were not relevant for L* ($P_R$=0.26). For a* and b*, the intermediate $P_R$ (0.35 and 0.40, respectively) showed low precision for inferring on the relevance of the differences.

**Table 6.** Features of the estimated marginal posterior distributions of the differences between high and low lines for carcass and meat quality measurements in the third generation of selection

| Trait | $D_{H-L}$[1] | $P$[2] | $HPD_{95\%}$[3] | | $k_{80\%}$[4] | R[5] | $P_R$[6] |
|---|---|---|---|---|---|---|---|
| **M/B**[7] | 0.07 | 0.72 | -0.18 | 0.31 | - | 0.20 | 0.15 |
| **C L***[8] | -0.53 | 0.87 | -1.48 | 0.35 | -0.14 | -0.80 | 0.28 |
| **C a***[9] | -0.04 | 0.60 | -0.33 | 0.27 | - | -0.30 | 0.04 |
| **C b***[10] | -0.47 | 0.96 | -1.03 | 0.05 | -0.24 | -0.60 | 0.30 |
| **LM pH**[11] | 0.04 | 0.99 | 0.01 | 0.08 | 0.03 | 0.03 | 0.77 |
| **LM L***[12] | -0.51 | 0.88 | -1.41 | 0.32 | -0.15 | -0.80 | 0.26 |
| **LM a***[13] | -0.28 | 0.95 | -0.63 | 0.05 | -0.14 | -0.35 | 0.35 |
| **LM b***[14] | -0.26 | 0.97 | -0.54 | 0.01 | -0.14 | -0.30 | 0.40 |

[1]$D_{H-L}$, median of the marginal posterior distribution of the difference; [2]$P$, probability of the difference being greater than zero when $D_{H-L}>0$ and probability of the difference being lower than zero when $D_{H-L}<0$; [3]$HPD_{95\%}$, highest posterior density region at 95% of probability; [4]$k_{80\%}$, limit of the interval [k, $+\infty$) when $D_{H-L}>0$ and ($-\infty$, k] when $D_{H-L}<0$ at 80% of probability. It is displayed in the table only when $D_{H-L}$ and $k_{80\%}$ have the same sign; [5]R, relevant value defined as one third of the SD of the trait; [6]$P_R$, probability of relevance (probability of the difference being greater than R); [7]M/B, meat to bone ratio of the hind leg; [8]C L*, lightness of the carcass surface; [9]C a*, redness of the carcass surface; [10]C b*, yellowness of the carcass surface; [11]LM pH, pH of the *Longissimus* muscle; [12]LM L*, lightness of the *Longissimus* muscle; [13]LM a*, redness of the *Longissimus* muscle; [14]LM b*, yellowness of the *Longissimus* muscle.

Features of estimated marginal posterior distributions of the differences between H and L lines for protein content and FA composition of the LM in the third generation of selection are presented in Tables 7 and 8. Differences for protein content were irrelevant ($P_R$=0.13). However, there were differences for FA composition. Saturated FA and MUFA percentages were greater in line H than in line L ($P$=0.98 and 1.00, respectively). Conversely, PUFA ($P$=0.99), n-3 and n-6 ($P$=1.00) percentages were greater in line L. Favourable values for FA ratios were found for L line, exhibiting lower n-6/n-3 values and greater PUFA/SFA values than line H ($P$=0.99 and 1.00,

respectively). Differences for MUFA, n-3 and n-6 percentages were relevant ($P_R$ between 0.95 and 0.99). For SFA and PUFA percentages as well as for n-6/n-3 and PUFA/SFA ratios, there was a moderate to high $P_R$ ($P_R$ between 0.64 and 0.80) indicating that the differences might be relevant.

**Table 7.** Features of the estimated marginal posterior distributions of the differences between high and low lines for protein content (g/100g muscle) and fatty acid composition (percentage of total fatty acids) of the *Longissimus* muscle in the third generation of selection

| Trait | $D_{H-L}$[1] | $P$[2] | $HPD_{95\%}$[3] | | $k_{80\%}$[4] | R[5] | $P_R$[6] |
|---|---|---|---|---|---|---|---|
| **Protein** | -0.05 | 0.72 | -0.23 | 0.12 | - | -0.15 | 0.13 |
| **SFA**[7] | 0.86 | 0.98 | 0.00 | 1.65 | 0.50 | 0.70 | 0.64 |
| **MUFA**[8] | 1.58 | 1.00 | 0.85 | 2.33 | 1.27 | 0.70 | 0.99 |
| **PUFA**[9] | -1.57 | 0.99 | -2.85 | -0.32 | -1.02 | -1.10 | 0.79 |
| **n-3**[10] | -0.47 | 1.00 | -0.75 | -0.22 | -0.36 | -0.25 | 0.95 |
| **n-6**[11] | -1.87 | 1.00 | -2.79 | -0.91 | -1.47 | -0.90 | 0.98 |
| **n-6/n-3** | 0.19 | 0.99 | 0.02 | 0.34 | 0.12 | 0.15 | 0.67 |
| **PUFA/SFA** | -0.04 | 1.00 | -0.07 | -0.01 | -0.03 | -0.03 | 0.80 |

[1]$D_{H-L}$, median of the marginal posterior distribution of the difference; [2]*P*, probability of the difference being greater than zero when $D_{H-L}>0$ and probability of the difference being lower than zero when $D_{H-L}<0$; [3]$HPD_{95\%}$, highest posterior density region at 95% of probability; [4]$k_{80\%}$, limit of the interval [k, $+\infty$) when $D_{H-L}>0$ and ($-\infty$, k] when $D_{H-L}<0$ at 80% of probability. It is displayed in the table only when $D_{H-L}$ and $k_{80\%}$ have the same sign; [5]R, relevant value defined as one third of the SD of the trait; [6]$P_R$, probability of relevance (probability of the difference being greater than R); [7]SFA=C14:0+C15:0+C16:0+C17:0+C18:0; [8]MUFA=C16:1+C18:1n-9+C18:1n-7; [9]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; [10]n-3=C18:3n-3+C20:5n-3+C22:5n-3+C22:6n-3; [11]n-6=C18:2n-6+C20:2n-6+C20:3n-6+C20:4n-6+C22:4n-6

Line H showed greater values for C14:0, C15:0, C16:0, C16:1, C18:1 n-9 and C18:3 n-3 percentages (*P* between 0.87 and 1.00; Table 8). Line L had greater percentages of C17:0, C18:0, C18:1 n-7, C18:2 n-6 and long chain PUFA (*P* between 0.82 and 1.00; Table 8). Differences for C18:1 n-9, C20:5 n-3 and C22:4 n-6 were relevant ($P_R$ between 0.92 and 0.98). The $P_R$ ranged from moderate to high for C14:0, C18:0, C20:3n-6 and C20:4 n-6 ($P_R$ between 0.80 and 0.88) showing that differences can be relevant. For C16:0, C16:1 and C22:6 n-3, $P_R$ was intermediate (between 0.56 and 0.65) indicating

that there was low evidence of its relevance. The differences for C15:0, C17:0, C18:1 n-7 and C20:2 n-6 percentages were very small. They represent a low percentage of the mean (between 1.79 and 3.13%) and were not relevant ($P_R$ between 0.19 and 0.33). Similarly, differences for C18:2 n-6, C18:3 n-3 and C22:5 n-3 were not relevant ($P_R$ between 0.07 and 0.25).

**Table 8.** Features of the estimated marginal posterior distributions of the differences between high and low lines for individual fatty acid composition (percentage of total fatty acids) of the *Longissimus* muscle in the third generation of selection

| Trait | $D_{H-L}$[1] | $P$[2] | $HPD_{95\%}$[3] | | $k_{80\%}$[4] | R[5] | $P_R$[6] |
|---|---|---|---|---|---|---|---|
| **C14:0** | 0.16 | 1.00 | 0.04 | 0.28 | 0.11 | 0.10 | 0.85 |
| **C15:0** | 0.01 | 0.96 | 0.00 | 0.01 | 0.01 | 0.01 | 0.19 |
| **C16:0** | 0.65 | 0.98 | 0.05 | 1.23 | 0.39 | 0.60 | 0.56 |
| **C16:1** | 0.29 | 1.00 | 0.07 | 0.51 | 0.19 | 0.20 | 0.65 |
| **C17:0** | -0.02 | 0.97 | -0.03 | 0.00 | -0.01 | -0.02 | 0.33 |
| **C18:0** | -0.33 | 1.00 | -0.53 | -0.12 | -0.24 | -0.20 | 0.88 |
| **C18:1 n-7** | -0.03 | 0.86 | -0.07 | 0.02 | -0.01 | -0.04 | 0.27 |
| **C18:1 n-9** | 1.16 | 1.00 | 0.59 | 1.73 | 0.92 | 0.50 | 0.97 |
| **C18:2 n-6** | -0.34 | 0.82 | -1.12 | 0.34 | -0.04 | -0.60 | 0.25 |
| **C18:3 n-3** | 0.06 | 0.87 | -0.04 | 0.15 | 0.02 | 0.10 | 0.10 |
| **C20:2 n-6** | -0.01 | 0.94 | -0.03 | 0.00 | -0.01 | -0.02 | 0.28 |
| **C20:3 n-6** | -0.06 | 1.00 | -0.10 | -0.02 | -0.05 | -0.04 | 0.88 |
| **C20:4 n-6** | -0.45 | 0.99 | -0.81 | -0.13 | -0.31 | -0.30 | 0.80 |
| **C20:5 n-3** | -0.24 | 1.00 | -0.36 | -0.10 | -0.18 | -0.10 | 0.98 |
| **C22:4 n-6** | -0.19 | 1.00 | -0.31 | -0.06 | -0.14 | -0.10 | 0.92 |
| **C22:5 n-3** | -0.03 | 0.84 | -0.10 | 0.03 | 0.00 | -0.06 | 0.07 |
| **C22:6 n-3** | -0.23 | 0.97 | -0.48 | 0.01 | -0.12 | -0.20 | 0.60 |

[1] $D_{H-L}$, median of the marginal posterior distribution of the difference; [2] $P$, probability of the difference being greater than zero when $D_{H-L}>0$ and probability of the difference being lower than zero when $D_{H-L}<0$; [3] $HPD_{95\%}$, highest posterior density region at 95% of probability; [4] $k_{80\%}$, limit of the interval $[k, +\infty)$ when $D_{H-L}>0$ and $(-\infty, k]$ when $D_{H-L}<0$ at 80% of probability. It is displayed in the table only when $D_{H-L}$ and $k_{80\%}$ have the same sign; [5] R, relevant value defined as one third of the SD of the trait; [6] $P_R$, probability of relevance (probability of the difference being greater than R).

## 4. DISCUSSION

Rabbit carcasses have a small percentage of dissectible fat (Pla et al., 1996). Scapular and perirenal fat tissues are two of the main carcass fat depots and, on average, account for 65% of the carcass dissectible fat in the rabbit (Hernández et al., 2006). In this study, SF and PF represented a very small percentage of the chilled carcass (1.06%), which is in line with previous studies (Hernández et al., 2006; Zomeño et al., 2010).

Values for carcass and meat colour variables and muscle pH were similar to those obtained in previous studies (Hernández et al., 2004; Hernández et al., 2006). The M/B ratio of the hind leg, used as an indicator of the M/B ratio of the carcass (Hernández et al., 1996), was lower than in other studies. Results for protein content and FA profile obtained in this study were close to the values reported for the *Longissimus* muscle of rabbits (reviewed by Hernández and Dalle Zotte, 2010).

### 4.1. Correlated response on body weight and carcass measurements

After 3 generations of divergent selection for IMF, the difference between H and L lines for IMF content was 0.09 g/100g of muscle (C. Zomeño, unpublished data). This difference represents a direct selection response of 9% of its mean. Selection for IMF content resulted in a slightly negative correlated response on BW. However, carcass weight and M/B ratio, which determine carcass yield, were not affected by selection for IMF. Regarding adipose tissues of the carcass, SF content was not modified, but there was a positive correlated response on PF content. These findings are in agreement with Schwab et al. (2009) who obtained no correlated responses in growth performance traits and higher values of backfat measurements (mm) in a pig line selected for increased IMF. Genetic correlations between IMF content and backfat have been studied in pigs showing a wide range from 0.04 (reviewed by Sellier, 1998) to 0.64 (Solanes et al., 2009). These estimates suggest that part of the genetic variation of IMF is independent of the genetic variation in overall lipid content of the carcass. In fact, Sapp et al. (2002) obtained no correlated response on fat thickness in bulls selected for IMF content, indicating that selection for increased IMF can be achieved without increasing carcass fat.

### 4.2. Correlated response on carcass and meat quality traits

Meat colour is related to the appearance of the product and consequently it is crucial for the consumer. Rabbit carcasses are usually commercialized as a whole, but retail cuts are increasing in importance. Hence, carcass and muscle colour are essential quality traits. Colour traits of the carcass were not affected by selection for IMF. In muscle, L* was also similar between lines. However, there was a response on a* and b*, with low evidence of its relevance. Schwab et al. (2009) observed an increased muscle light reflectance and Hunter L* in a pig line selected for increased IMF. Different values of genetic correlations between lightness and IMF have been published. In pigs, Sellier (1998) reported a null genetic correlation whereas Suzuki et al. (2005b) and Schwab et al. (2010) estimated moderate positive genetic correlations (0.42 and 0.52, respectively), and Gjerlaug-Enger et al. (2010) reported a moderately low negative genetic correlation (-0.20).

Selection for IMF resulted in a positive correlated response on muscle pH. This finding is consistent with the relationship between ultimate pH and the *post mortem* metabolism of muscle. Therefore, high levels of IMF can lead to an increase in the oxidative metabolism, and consequently to an increased *post mortem* pH (Gjerlaug-Enger et al., 2010). To our knowledge, only one study investigated the correlated response on muscle pH (Schwab et al., 2009 in pigs), but no effect of selection for IMF was detected on this trait. A wide range of estimates of genetic correlations between IMF and muscle pH in pigs have been published. Suzuki et al. (2005b) reported a moderate negative relationship (-0.51), Schwab et al. (2010) found no relevant association (0.01), and Gjerlaug-Enger et al. (2010) estimated small (0.11) or moderate (0.42) positive genetic correlations between both traits.

Rabbit meat is characterized by its lower fat content, higher protein and favourable FA composition compared to meat of other (Hernández and Gondret, 2006). Results of this experiment showed no correlated response on protein content. Previous selection experiments for IMF content did not focus on correlated response on protein content. To our knowledge, there is only one study reporting genetic correlations between protein and IMF content (Gjerlaug-Enger et al., 2010 in pigs), in which moderate negative genetic correlations were estimated.

The positive correlated responses on SFA and MUFA percentages and negative correlated responses on PUFA percentage and PUFA/SFA ratio are related to the direct selection response on IMF content. Lines were contemporarily raised and fed with the same diet. The feed intake during the growing period was also similar for both lines (1.68 and 1.66 kg of feed/kg of BW in H and L lines, respectively). Thus, correlated response on fatty acid composition should be attributed to the selection process for IMF. De Smet et al. (2004) highlighted an increase in SFA and MUFA content and a decrease in PUFA with increasing fat content, resulting in a decrease of the relative proportion of PUFA and a reduction in the PUFA/SFA ratio. According to these authors, the relationship between fat content and FA composition is explained by the different FA composition of the two major lipid fractions, phospholipids and triacylglycerols, and by the relative contribution of these fractions to total lipids. Similarly, Sellier et al. (2010) reported a negative genetic correlation between IMF and PUFA/SFA ratio (-0.39) as well as between PUFA and SFA (-0.83) and MUFA (-0.73) percentages of the *Longissimus* muscle in pigs. A positive relationship was also found by Nogi et al. (2011) between IMF and MUFA (0.23) in beef cattle.

The PUFA/SFA and n-6/n-3 ratios are used to evaluate the nutritional quality of the meat. Our results showed values close to the recommendations (higher than 0.45 for PUFA/SFA and lower than 4 for n-6/n-3) (Hernández and Dalle Zotte, 2010), and were more favourable for line L, with greater PUFA/SFA and lower n-6/n-3 values, than for line H.

The correlated response on the individual FA percentages showed a similar pattern as for the FA groups, with an increase in SFA (C14:0 and C16:0) and MUFA (C16:1 and C18:1 n-9), and a decrease in PUFA (C20:3 n-6, C20:4 n-6, C20:5 n-3, C22:4 n-6 and C22:6 n-3) in the line selected for increased IMF; although there was low evidence for showing the relevance of the response on C16:0, C16:1 and C22:6 n-3. When measuring C20:3 n-6, C22:4 n-6 and C22:6 n-3 with NIRS, we obtained a very low accuracy (Zomeño et al., 2012). Thus, estimated differences between lines for these fatty acids should be treated with caution. Suzuki et al. (2006) reported positive genetic correlations between IMF and C14:0 (0.21) and C16:0 (0.49) in pigs. Pitchford et al. (2002) also obtained a positive genetic association between IMF and C16:0 (0.43)

in beef cattle. A moderately high genetic correlation between IMF and C18:1 n-9 (0.47) in pigs was reported by Ros-Freixedes et al. (2012). In beef cattle, small but also positive genetic correlations were reported by Nogi et al. (2011) between IMF and C18:1n-9 (0.19). These authors also obtained a negative genetic correlation between IMF and C18:0 (-0.27), which is in line with the negative correlated response on this FA found in our study.

Several thousands of data are needed to estimate genetic correlations accurately. Due to the difficulty of having a large amount of data in meat quality experiments, genetic correlations of the studies mentioned above have high standard errors. Results of the present experiment, based on a comparison between lines, confirm reported correlations and display a greater accuracy.

## 5. CONCLUSIONS

In conclusion, carcass quality may be affected by selection for IMF content, producing an increase in PF content. However, there was low evidence of the relevance of the response on this trait. Selection for IMF content also led to some modifications in muscle pH and FA composition.

## LITERATURE CITED

Blasco, A. 2001. The Bayesian controversy in animal breeding. Journal of Animal Science. 79:2023-2046.

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

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

Gjerlaug-Enger, E., L. Aass, J. Odegard 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. Pla, and A. Blasco. 1996. Prediction of carcass composition in the rabbit. Meat Science. 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. Journal of Animal Science. 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 Science. 73:645-650.

Hernández, P., and F. Gondret. 2006. Rabbit meat quality and safety. In: L. Maertens and P. Coudert, editors, Recent Advances in Rabbit Sciences. ILVO, Melle, Belgium. p. 267-290

Hernández, P., and A. Dalle Zotte. 2010. Influence of diet on rabbit meat quality. In: C. de Blas, and J. Wiseman, editors, Nutrition of the rabbit. CAB International. Wallinford, Oxon, UK. p. 163-178.

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. Journal of Animal Science. 89:2981-2987.

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. Journal of Animal Science. 89:615–621.

Pitchford, W. S., M. P. B. Deland, B. D. Siebert, A. E. O. Malau-Aduli, and C. D. K. Bottema. 2002. Genetic variation in fatness and fatty acid composition of crossbred cattle. Journal of Animal Science. 80:2825–2832.

Pla, M., P. Hernández, and A. Blasco. 1996. Carcass composition and meat characteristics of two rabbit breeds of different degrees of maturity. Meat Science. 44:85-92.

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

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. Journal of Animal Science. 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. Journal of Animal Science. 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. Journal of Animal Science. 88:69-79.

Sellier, P. 1998. Genetics of meat and carcass traits. In: M. F. Rothschild and A. Ruvinsky, editors, The Genetics of the Pig. CAB International, New York, USA. p. 463-510.

Sellier, P., L. Maignel, and 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.

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

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

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

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

Warris, P. D. 2000. Meat Science. An Introductory Text. ed. CABI Publishing, Wallinford, Oxon, UK.

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 Science. 78:343–358.

Zomeño, C., A. Blasco, and P. Hernández. 2010. Influence of genetic line on lipid metabolism traits of rabbit muscle. Journal of Animal Science. 88:3419-3427.

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

# GENERAL DISCUSSION

A divergent selection experiment on intramuscular fat (IMF) content has been performed in this Thesis. Near infrared reflectance spectroscopy (NIRS) was used to measure IMF content, protein content and fatty acid composition during the selection process. This technique is a suitable alternative to chemical methods because of its speed, accuracy and relatively low cost. First, NIRS calibrations equations for predicting IMF content, fatty acid composition and protein content were developed. Then, the application of NIRS in rabbit selection programmes was studied. Finally, direct response on IMF content and correlated response on carcass and meat quality traits were examined after 3 generations of divergent selection on IMF content.

## 1. NIRS calibration models and their application in rabbit selection programmes

The use of NIRS for predicting IMF, fatty acid and protein content in the *Longissimus* muscle, and its application in rabbit selection programmes have been evaluated. A model with high accuracy was obtained for IMF content ($R^2_{CV}$=0.98 and SECV=0.07 g/100g muscle). Accurate NIRS calibrations for IMF have also been reported in beef, pork and poultry meat (reviewed by Prieto et al., 2009). In rabbit meat, Masoero et al. (1994) and Pla et al. (2004) also obtained good equations for fat content, although the meat used in these studies included inter- and intramuscular fat.

This study is the first analysis of fatty acid content of IMF by NIRS in rabbits, and fatty acid content was expressed as concentration (mg) in the muscle. Previous studies in beef (Windham and Morrison, 1998), pigs (González-Martín et al., 2005) and rabbits (Pla et al., 2007) expressed fatty acids as a percentage of the total. In contrast, more recent studies in beef (Sierra et al., 2008; Prieto et al., 2011) and lamb meat (Guy et al., 2011) used data expressed as concentration (mg or g), obtaining higher accuracies. NIRS absorbance is based on the amount of molecular bonds in the organic matrix. Therefore, correlating NIRS data with fatty acids expressed as concentration should be more accurate than on a percentage basis. Prediction models for SFA and MUFA content obtained in this Thesis showed good accuracy. Equations for PUFA and n-6 content were less accurate, although they were sufficient for our purposes as we have seen in chapter one. For n-3 content, the model was insufficient for accurate predictions. The higher accuracies for SFA and MUFA compared to PUFA, n-6 and n-3

are in line with findings of other authors (Pla et al., 2007; Sierra et al., 2008; Guy et al., 2011), and could be related to the lower variability in PUFA, n-6 and n-3, and a low ability of NIRS to detect the higher double bonds present in these fatty acids. Prediction models for PUFA/SFA and n-6/n-3 ratios were also developed, although the equations had low accuracy.

Among individual fatty acids, accurate predictions were obtained for the main fatty acids (C16:0, C18:1 n-9 and C18:2 n-6) and for other fatty acids found in lower amounts (C14:0, C15:0, C16:1, C17:0, C18:0 and C18:3 n-3). Less accurate predictions were obtained for other minor fatty acids such as C18:1 n-7 and several long chain PUFA. Finally, equations for C20:3 n-6, C22:4 n-6, and C22:6 n-3 were not acceptable for predictions. Calibrations for the main fatty acid content in the *Longissimus* muscle were similar to those obtained by Guy et al. (2011) in lamb meat. Higher errors for the prediction of several long chain PUFA were also reported by other studies (Sierra et al., 2008; Guy et al., 2011).

The prediction model obtained for protein content had lower accuracy than for IMF content, but it is sufficient for our predictions. Similarly, Prieto et al. (2009) reported a lower accuracy of protein prediction in beef, pork and poultry meat. This should be attributed to the low variability for this parameter and to analytical differences between Kjeldahl and NIRS methodologies.

Some studies have shown the possibilities of using NIRS for the prediction of meat chemical composition and fatty acid profile in selection programmes of pigs and beef cattle (Zamora-Rojas et al., 2011; Cecchinato et al., 2012). However, there are no studies about the application of NIRS in rabbit selection. This Thesis showed no differences between IMF content measured by chemical analyses and that predicted by NIRS. Thus, NIRS could be a proper technique to compare different groups or treatments. However, regression statistics between chemical and predicted values of IMF showed that NIRS may not be accurate enough to predict individual genetic values. Predicted individual values can be used in the genetic evaluation systems requiring rankings of animals, in which the order is related to the economic value of the animal and therefore a high accuracy is needed. Two measures of rank correlation,

Spearman rank and Kendall's tau-b, were calculated and results indicated that there was no complete concordance between chemical and NIRS rankings of animals.

Nevertheless, NIRS technique could be successfully used in truncated selection. This procedure is widely applied in rabbit, pig and poultry breeding. The population is divided at a point of truncation and all individuals above this value are selected. Therefore, the ranking of the animals is not considered. Moreover, as the point of truncation is usually placed in a zone of the distribution with considerably high probability, the animals selected by applying the chemical method would have similar genetic values to those animals actually selected by applying NIRS. Intramuscular fat content of parents that would be selected by applying chemical method was compared to that of parents that would be selected by applying NIRS, and no differences were found for both females and males. Thus, the response to selection using NIRS should be similar to the response using chemical methods.

## 2. Divergent selection on IMF content

After developing accurate equations and confirming that NIRS can be used in rabbit selection programmes, we carried out a divergent selection experiment on IMF content. The difference between high and low lines for IMF content after 3 generations of selection was 0.09 g/100g of muscle. This difference represents a direct cumulative selection response of 9% of its mean. To our knowledge, this is the first study focused on the improvement of IMF content by selection in rabbits. Previous selection experiments conducted in pigs (Suzuki et al., 2005a; Schwab et al., 2009), beef cattle (Sapp et al., 2002) and poultry (Zhao et al., 2007) also showed a positive response on IMF content.

Differences in feed intake can lead to differences in IMF deposition in growing rabbits (Gondret et al., 2000). For this reason, we measured 518 animals for feed intake during the growing period and we found that it was similar for both lines (1.684 kg of feed/kg of body weight in line H and 1.660 kg of feed/kg of body weight in line L). Therefore, differences should be attributed to the selection process instead of differences in feed intake.

Response to selection on IMF content was also estimated by computing genetic means from an animal model. They showed a symmetrical response after 3 generations of divergent selection: 0.054 g/100g of muscle in the high line and -0.051 g/100g of muscle in the low line. The probabilities of genetic means being greater than zero were high (between 0.91 and 0.95) in the second and third generations for both lines. Moreover, the differences between genetic means of high and low lines had a probability of 100% of being greater than zero in the 3 generations, supporting that selection response has been obtained. Genetic means depend on the heritability estimated. In this Thesis, the estimated heritability for IMF was moderately high (0.37) with 97% of probability of being higher than 0.2. This value could be underestimated because, as mentioned above, NIRS may not be accurate enough to predict individual genetic values. To our knowledge, no estimates of heritability for IMF are available in rabbits. Our result is in line with reported values in pigs and cattle, which are moderate to high (Sellier, 1998; Bertrand et al., 2001). In order to validate the estimated genetic means, they were computed again using a guaranteed value (0.22) with 95% of probability for the heritability. Selection response for IMF was very similar to that reported with a heritability of 0.37. Moreover, environmental means were computed and were null for the 3 generations of selection. Thus, the change of IMF content should be entirely assigned to the selection process.

Selection on IMF content may have consequences on other traits that should be considered. Genetic correlations involving meat quality traits have usually high standard errors due to the difficulty of having a large amount of data to estimate them. The estimation of correlated responses is a more accurate method for examining the consequences of selection on IMF, since it only involves the comparison of means. In this study, carcass weight, meat to bone ratio and scapular fat content were not modified by selection for IMF. There was a positive correlated response on perirenal fat content, although with little evidence of its relevance. Similarly, Schwab et al. (2009) obtained no correlated responses in growth performance traits, but they obtained a positive correlated response on backfat content in a pig line selected for increasing IMF. In a review, genetic correlations between IMF content and backfat in pigs ranged from 0.04 to 0.60 (Sellier, 1998); and Solanes et al. (2009) reported a

slightly higher value of 0.64. In beef cattle, the genetic correlation between IMF content (%) and fat thickness ranges from -0.06 to 0.71 (Marshall, 1999), and between marbling score and carcass fat thickness ranges from -0.13 to 0.44 (Bertrand et al., 2001). Few estimates of genetic associations between IMF and carcass fat have been published in poultry and sheep. As in the previous cases, available genetic correlations in these species have large standard errors and show a wide range of variation. For example, Zerehdaran et al. (2004) reported a null genetic correlation (0.02) between IMF and abdominal fat in poultry, whereas Chen et al. (2008) estimated a positive relationship (0.66). The magnitudes of these correlations suggest that part of the genetic variation of IMF is independent of the genetic variation in overall fat content of the carcass. In fact, Sapp et al. (2002) obtained no correlated response on fat thickness in bulls selected for IMF content. Likewise, Zhao et al. (2007) found no correlated response on abdominal fat percentage in a chicken line selected for increased IMF. These findings indicate that selection for increased IMF can be achieved without increasing carcass fat. Moreover, Hernández-Sánchez et al. (2013) have recently reported different genomic architectures between IMF and backfat in pigs.

Colour parameters of carcass and muscle were almost not affected by selection on IMF. However, there was a positive correlated response on muscle pH, which is in line with the relationship between pH and the *post mortem* metabolism of muscle. To our knowledge, a correlated response on muscle pH was only studied by Schwab et al. (2009) in pigs, but no effect on this trait was found. Genetic correlations between IMF and muscle pH have been published by several authors, showing very different values ranging from -0.51 to 0.42 (Suzuki et al., 2005b; Gjerlaug-Enger et al., 2010; Schwab et al., 2010).

Fatty acid composition was modified by the selection process. There was an increase in SFA (C14:0 and C16:0) and MUFA (C16:1 and C18:1 n-9) percentages, and a decrease in PUFA (C20:3 n-6, C20:4 n-6, C20:5 n-3, C22:4 n-6 and C22:6 n-3) percentage, although there was low evidence of the relevance of the response on C16:0, C16:1 and C22:6 n-3. These modifications agree well with the direct selection response on IMF content; the change in fatty acid composition is explained by the relative increase of the triacylglycerol fraction, which is rich in SFA and MUFA, compared to the phospholipid

fraction, which is rich in PUFA, in the line with higher IMF content (De Smet et al., 2004). Similarly, positive genetic correlations between IMF and C14:0 and C16:0 were reported (Pitchford et al., 2002; Suzuki et al., 2006). Nogi et al. (2011) found a positive relationship between marbling score and MUFA, and Ros-Freixedes et al. (2012) between IMF and C18:1 n-9. The fatty acid ratios were more favourable for the low line, with greater PUFA/SFA and lower n-6/n-3 values. Sellier et al. (2010) reported a negative genetic correlation between IMF and PUFA/SFA ratio. The unfavourable fatty acid ratios and the reduction of some individual PUFA associated with healthy meat such as C20:5 n-3 and C22:6 n-3 in the line selected for increased IMF should be taken into account. Nevertheless, there was no correlated response on C20:5 n-3 and C22:6 n-3 expressed as concentration (mg/100g of fresh muscle). Thus, the absolute intake of fatty acids associated with a healthy diet in humans was not affected by selection for IMF content.

This Thesis showed no correlated response on protein content. Previous selection experiments for IMF content in other species did not focus on correlated response on protein content. To our knowledge, there is only one study reporting genetic correlations between protein and IMF content (Gjerlaug-Enger et al., 2010 in pigs), in which moderate negative genetic correlations were found.

Differences between lines obtained after 3 generations of selection were satisfactory to estimate the selection response. Nevertheless, the selection experiment will continue in order to guarantee an adequate difference between lines, allowing us to study other traits involving lipid deposition and composition. More generations of selection are needed to examine the long-term consequences of selecting for IMF content on other relevant traits.

The results of this Thesis can be valid for other livestock species, but some precautions should be taken. Rabbits have lower fat content than other species. In Spain, rabbits are usually slaughtered at 9 weeks of age in order to obtain light carcasses. This causes a lower degree of maturity at slaughter compared to pigs and cattle. However, in France and Italy, rabbits are slaughtered at 11 and 13 weeks of age respectively, obtaining heavy carcasses. At these ages, the IMF content is higher and more similar to IMF content in pigs. In the case of broiler chickens, they are also slaughtered at a lower

degree of maturity and IMF content of the breast is low, as in 9 weeks-old rabbits. In addition, we should consider the differences in the lipid metabolism between species, particularly between ruminant and non-ruminant animals. Nevertheless, the correlated responses on carcass fat, IMF and fatty acid composition obtained in the present study are in agreement with genetic correlations studied in other species. Thus, taken these aspects into account, rabbits can be considered a suitable animal model for other livestock species.

**Literature cited**

Bertrand, J. K., R. D. Green, W. O. Herring, and D. W. Moser. 2001. Genetic evaluation for beef carcass traits. Journal of Animal Science. 79(Suppl.):E190-E200.

Cecchinato, A., M. De Marchi, M. Penasa, J. Casellas, S. Schiavon, and G. Bittante. 2012. Genetic analysis of beef fatty acid composition predicted by near-infrared spectroscopy. Journal of Animal Science. 90:429-438.

Chen, J. L., G. P. Zhao, M. Q. Zheng, J. Wen, and N. Yang. 2008. Estimation of Genetic Parameters for Contents of Intramuscular Fat and Inosine-5-Monophosphate and Carcass Traits in Chinese Beijing-You Chickens. Poultry Science. 87:1098–1104.

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

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

Gondret, F., F. Lebas, and M. Bonneau. 2000. Restricted feed intake during fattening reduces intramuscular lipid deposition without modifying fiber characteristics in rabbits. Journal of Nutrition. 130:228–233.

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

Guy, F., A. Prache, D. Thomas, D. Bauchart, and D. Andueza. 2011. Prediction of lamb mat fatty acid composition using near-infrared reflectance spectroscopy (NIRS). Food Chemistry. 127:1280-1286.

Hernández-Sánchez, J., M. Amills, R. N. Pena, A. Mercadé, A. Manunza, and R. Quintanilla. 2013. Genomic architecture of heritability and genetic correlations for intramuscular and back fat contents in Duroc pigs. J. Anim. Sci. 91:623-632.

Marshall, D. M. 1999. Genetics of Meat Quality. In: R. Fries and A. Ruvinsky, editors, The genetics of cattle. CAB International. New York, USA. p. 605-636.

Masoero, G., G. Xiccato, A. Dalle Zotte, R. Parigi-Bini, and G. Bergoglio. 1994. Analysis of freeze-dried rabbit meat by NIRS. Zootecnia Nutrizione Animale. 20:319-329.

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. Journal of Animal Science. 89:615–621.

Pitchford, W. S., M. P. B. Deland, B. D. Siebert, A. E. O. Malau-Aduli, and C. D. K. Bottema. 2002. Genetic variation in fatness and fatty acid composition of crossbred cattle. Journal of Animal Science. 80:2825–2832.

Pla, M., M. Pascual, and B. Ariño. 2004. Protein, fat and moisture content of retail cuts of rabbit meat evaluated with the NIRS methodology. World Rabbit Science. 12:149-158.

Pla, M., P. Hernández, B. Ariño, J. A. Ramírez, and I. Díaz. 2007. Prediction of fatty acid content in rabbit meat and discrimination between conventional and organic production systems by NIRS methodology. Food Chemistry. 100:165-170.

Prieto, N., R. Roehe, P. Lavín, G. Batten, and S. Andrés. 2009. Application of near infrared reflectance spectroscopy to predict meat and meat products quality: A review. Meat Science. 83:175-186.

Prieto, N., D. W. Ross, E. A. Navajas, R. I. Richardson, J. J. Hyslop, G. Simm, and R. Roehe. 2011. Online prediction of fatty acid profile in crossbred Limousin and Aberdeen Angus beef cattle using near infrared reflectance spectroscopy. Animal. 5:155-16.

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

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. Journal of Animal Science. 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. Journal of Animal Science. 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. Journal of Animal Science. 88:69-79.

Sellier, P. 1998. Genetics of meat and carcass traits. In: M.F. Rothschild and A. Ruvinsky, editors, The Genetics of the Pig. CAB International, New York, USA. p. 463-510.

Sellier, P., L. Maignel, and 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.

Sierra, V., N. Aldai, P. Castro, K. Osoro, A. Coto-Montes, and M. Olivan. 2008. Prediction of the fatty acid composition of beef by near infrared transmittance spectroscopy. Meat Science. 78:248-255.

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

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

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

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

Windham, W. R., and W. H. Morrison. 1998. Prediction of fatty acid content in beef neck lean by near infrared reflectance analysis. Journal of Near Infrared Spectroscopy. 6:229-234.

Zamora-Rojas, E., A. Garrido-Varo, E. De Pedro-Sanz, J. E. Guerero-Ginel, and D. Pérez-Marín. 2011. Monitoring NIRS calibrations for use in routine meat analysis as part of Iberian pig-breeding programs. Food Chemistry. 129:1889-1897.

Zerehdaran, S., A. L. J. Vereijken, J. A. M. van Arendonk, and E. H. van der Waaij. 2004. Estimation of Genetic Parameters for Fat Deposition and Carcass Traits in Broilers. Poultry Science. 83:521–525.

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. Poultry Science. 86:2309–2314.

# CONCLUSIONS

1. NIRS is a reasonable alternative to chemical methods for the prediction of intramuscular fat content, protein content and fatty acid composition in rabbit selection programmes. Intramuscular fat content, SFA, MUFA as well as the main individual fatty acids of rabbit meat (C16:0, C18:1 n-9 and C18:2 n-6) and other minor fatty acids (C14:0, C15:0, C16:1, C17:0, C18:0 and C18:3 n-3) can be accurately predicted by NIRS. Less accurate predictions are obtained for PUFA, n-6, n-3, fatty acid ratios and protein content.

2. A selection response on intramuscular fat content was obtained after 3 generations of selection. The difference between high and low lines was 0.09 g/100g of muscle, corresponding to a direct selection response of 9% of its mean. The response was symmetrical in both lines. The estimated heritability for intramuscular fat content was moderately high (0.37) with a probability of 97% of being greater than 0.2.

3. There was a correlated response on perirenal fat content, indicating that carcass quality may be affected by selection on intramuscular fat content. However, there was low evidence of the relevance of the response on this trait. Meat quality was also modified by selection on IMF content. Muscle pH, SFA percentage, MUFA percentage and the n-6/n-3 ratio increased, while n-6 percentage, n-3 percentage and PUFA/SFA ratio decreased in the line with higher IMF content.

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