Martinez Alvaro, M.; Paucar, Y.; Satué, K.; Blasco Mateu, A.; Hernández, P. (2018). Liver metabolism traits in two rabbit lines divergently selected for intramuscular fat. animal. 12(6):1217-1223. https://doi.org/10.1017/S1751731117002695

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

M. Martínez-Álvaro[1], Y. Paucar[1], K. Satué[2], A. Blasco[1], and P. Hernández[1]

[1] *Institute for Animal Science and Technology, Universitat Politècnica de València, 46022 Valencia, Spain.*

[2] *Department of Animal Medicine and Surgery, Universidad Cardenal Herrera, 46113 Valencia, Spain.*

Corresponding author: Pilar Hernández. E-mail: phernan@dca.upv.es

Short title: metabolism of intramuscular fat

Abstract

Intramuscular fat (**IMF**) has a large effect in the sensory properties of meat because it affects tenderness, juiciness and flavour. A divergent selection experiment for IMF in *Longissimus dorsi* (**LD**) muscle was performed in rabbits. Since liver is the major site of lipogenesis in rabbits, the objective of this work is to study the liver metabolism in the lines of the divergent selection experiment. Intramuscular fat content, perirenal fat weight, liver weight, liver lipogenic activities and plasma metabolites related to liver metabolism were measured in the eighth generation of selection. Direct response on IMF was 0.34 g /100 g of LD, which represented 2.7 SD of the trait, and selection showed a positive correlated response in the perirenal fat weight. High-IMF line

showed greater liver size and greater liver lipogenic activities of enzymes glucose-6-phosphate dehydrogenase and malic enzyme (**EM**). We did not find differences between lines for fatty acid synthase lipogenic activity. With regard to plasma metabolites, low-IMF line showed greater plasma concentration of triglycerides, cholesterol, bilirubin and alkaline phosphatase than high-IMF line, whereas high-IMF line showed greater albumin and alanine transaminase concentrations than low-IMF line. We did not observe differences between lines for glucose, total protein and plasma concentrations. Phenotypic correlations between fat (IMF and perirenal fat weight) and liver traits showed that liver lipogenesis affects fat deposition in both, muscle and carcass. The lipoproteins related to IMF variation should be profiled for a better understanding of the mechanisms of liver lipogenesis involved in IMF content.

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

Implications

Intramuscular fat (**IMF**) is a main factor in meat quality because it affects sensory properties of meat. Genetic selection for intramuscular fat in rabbits modifies liver size and lipogenic activity, particularly the activity of the enzyme glucose-6-phosphate dehydrogenase. Our study shows that liver plays a main role in the genetics of IMF deposition in rabbits.

Introduction

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

Liver tissue is a major site of lipogenesis in some species such as chickens (O`Hea and Leveille, 1969), rats (Ballard *et al*., 1969) and growing rabbits (Gondret *et al*., 1997). In these species, IMF deposition may depend not only on the metabolism of intramuscular adipocytes, but also on metabolic activity of liver. Differences on lipogenic activities in liver have been related to differences in IMF in chickens (Cui *et al*., 2012), and to differences in fat depots in rats (Turkenkopf *et al.*, 1980 and Smith *et al.*, 1980) and pigs (Muñoz *et al*., 2013). Our hypothesis is that the different IMF deposition in the divergent rabbit lines of our experiment would be related to different lipogenic activities in liver. To test this hypothesis, we propose to measure in both lines lipogenic enzyme activities in liver, and plasma metabolites that are related to

lipogenesis. The advantage of comparing divergent lines selected for IMF is that they only differ in this trait and in correlated traits; therefore differences between lines can be only attributed to differences in IMF deposition.

Material and methods

Animals

A divergent selection experiment for IMF in LD was performed in rabbits. A male and a female from the first parity of each doe were slaughtered at 9 wk of age and evaluated for IMF, and the average between these two values was calculated. Then, all dams were ranked according to this average, and selection for high or low IMF was performed on rabbits from the second parity. All females of the approximately 20% best dams were selected for next generation. As each sire was mated with five dams, only one male of its best dam was selected. This selection within male family was performed in order to reduce inbreeding. Normally, the first parity was used to collect the IMF data and the second parity to select the rabbits for next generation, although exceptionally some IMF measurements were made on the second or third parity. Lines selected for high-IMF and low-IMF were reared contemporary at the farm of the Universitat Politècnica de València. The housing had a constant photoperiod of 16:8 h and controlled ventilation. Litters were homogenized by performing adoptions at birth up to 9 kits per litter. From weaning to slaughter, rabbits

84 were reared collectively and fed *ad libitum*. More details of this experiment can be
85 found in Martínez-Álvaro *et al*. (2016).
86 This study was performed with 175 rabbits from the eighth generation of this
87 selection experiment, 83 from the high-IMF line and 92 from the low-IMF line. Body
88 weight was recorded at 9 wk of age. Then, all rabbits were fasted at least 19 h before
89 slaughtering by electrical stunning and exsanguination. Carcasses were prepared
90 according to the norms of the World Rabbit Science Association (Blasco and
91 Ouhayoun, 1996). Carcasses were chilled for 24 h at 4 ºC and the weight of the
92 chilled carcass was recorded. Perirenal fat depot was excised from the carcass and
93 weighed. Muscle LD was excised, minced, freeze-dried and scanned with Near
94 Infrarred Spectrophotometry (model 5000, FOSS NIRSystems INC., Hilleroed,
95 Denmark). Intramuscular fat was determined in g/100g of muscle applying the
96 calibration equations previously developed by Zomeño *et al*. (2011).
97 A subsample of 63 rabbits (30 from the high-IMF and 33 from the low-IMF line) was
98 taken to study the liver lipogenic activity and plasma metabolites. Animals were
99 slaughtered as described before. Blood samples were collected at slaughter from the
100 jugular vein in 1 ml lyophilized lithium heparin (0,04 mg/ml) tubes (TapVal Aquisel,
101 Barcelona, Spain) and plasma was prepared by centrifugation at 3000 rpm for 10 min
102 and then stored at -80ºC. Liver was dissected from the carcass and weighed

103 immediately after slaughter. A liver sample was frozen in liquid nitrogen, vacuum
104 packed and stored at -80ºC for lipogenic enzyme assays.
105 All experimental procedures involving animals were approved by the Universitat
106 Politècnica de València Research Ethics Committee, according to council directive
107 2010/63/EU (European Commission Directive, 2010).
108 *Lipogenic activities measurements*
109 Activity of enzymes G6PDH (EC 1.1.1.49), ME1(EC 1.1.1.40) and FASN (EC
110 2.3.1.85) were measured. For ME1and G6PDH measurements, 1 g of liver was
111 homogenized in 5 ml of ice-cold 0.25 M sucrose solution, whereas for FASN
112 measurement, 0.5 g of liver was homogenized in 2.5 ml of ice-cold 0.25 M sucrose
113 solution containing 1mM dithiothreitol and 1mM EDTA. Homogenates were
114 centrifuged at 12,000 g for 1 h at 4 ºC and supernatants were filtered through glass
115 wool and collected for enzyme assays. Lipogenic activities were assessed at 37 ºC
116 using a spectrophotometric analyzer Fluostar Galaxy (BMG Lab Technologies,
117 Offenburg, Germany) at 340 nm, according to the method described by Zomeño *et*
118 *al*. (2010) with some modifications. Enzyme activities were expressed in nmols of
119 NADH phosphate produced (G6PDH and EM) or oxidized (FAS) per minute and g of
120 fresh tissue. Soluble protein was determined in liver supernatant using the
121 bicinchoninic acid (BCA) Protein Assay Kit provided by Pierce (Rockford,IL), and
122 enzyme activities were also expressed in a soluble-protein basis.

123 *Plasma metabolites measurements*

124 Plasma concentrations (mg/dl) of glucose, total cholesterol and triglycerides were
125 determined by enzymatic colorimetric methods. Glucose was determined by the
126 Trinder glucose oxidase method, triglycerides were measured by the glycerol
127 phosphate dehydrogenase - peroxidase method and total cholesterol was measured
128 by the cholesterol oxidase - peroxidase method. Concentrations of bilirubin (mg/dl),
129 albumin (g/dl) and total protein (g/dl) were determined by dimethylsulfoxide,
130 Bromocresol Green and Biuret colorimetric methods, respectively. Finally, plasma
131 concentrations (UI/l) of enzymes aspartate transaminase (**AST**; EC 2.6.1.1), alanine
132 transaminase (**ALT**; EC 2.6.1.2) and alkaline phosphatase (**ALP**; EC 3.1.3.1.) were
133 measured by photometric methods. All the methods employed are described in
134 Kaplan *et al*., 2009. All the methodologies were integrated in an automatic chemistry
135 analyser model Spin 200E (Spinreact, Girona, Spain).

136 *Statistical analysis*

137 Descriptive statistics were estimated after correcting data by the fixed effects of line
138 and sex. Month-season and parity order fixed effects were additionally included for
139 IMF, BW, chilled carcass and perirenal fat weights analysis. Direct and correlated
140 responses to selection were estimated as the differences between high-IMF and low-
141 IMF lines. All the differences were estimated with a model including the fixed effects
142 of line, sex, month-season and parity order (as described before) and common litter

143 random effect. Phenotypic correlations of IMF and perirenal fat weight with liver
144 weight, liver lipogenic activities and plasma metabolites were estimated after
145 correcting data for line and sex.
146 Bayesian inference was used (Blasco, 2017). Common litter effect and residuals of
147 the models were assumed to be independently normally distributed. Bounded flat
148 priors were assumed for all fixed effects and variances. Marginal posterior
149 distributions were estimated using Gibbs sampling. Descriptive statistics and
150 differences between lines were performed with programme “Rabbit”, developed by
151 the Institute for Animal Science and Technology (Valencia, Spain). After some
152 exploratory analyses, results were based on Monte Carlo Markov chains runs
153 consisting of 60 000 iterations, with a burn-in period of 10 000, and only one of every
154 10 samples were saved for inferences. Phenotypic correlations were computed with
155 the software TM (Legarra *et al*., 2008). In this case, after some exploratory analyses
156 results were based on Monte Carlo Markov chains runs consisting of 1 000 000
157 iterations, with a burn-in period of 200 000, and only one of every 100 samples were
158 saved for inferences. Convergence was tested using the Z criterion of Geweke and
159 Monte-Carlo sampling errors were computed using time-series procedures.

160 The parameters obtained from the marginal posterior distributions of the differences
161 between lines and phenotypic correlations were: the median, the highest posterior
162 density region at 95% (**$HPD_{95\%}$**) and the probability of the difference or correlation

being greater than zero when the median is positive or lower than zero when the median is negative (**P_0**). Additionally, we considered 1/3 of the SD of a trait as a relevant value (**r**) and we calculated the probability of relevance (probability of the difference between lines being greater than R when the median is positive or lower than R when the median is negative) (**P_r**). A more detailed description of these features can be found in Blasco (2017).

Results

Response to selection and correlated responses in carcass traits

Table 1 shows descriptive statistics and differences between lines for IMF and carcass traits. Direct response to selection estimated as the difference between lines in the eight generation was 0.34 g /100 g of LD (P_r = 1.00) with a $HPD_{95\%}$ from 0.30 to 0.39. Expressed in units of SD, direct response was 2.7 SD of the trait. Selection for IMF showed a positive correlated response in the carcass adiposity. High-IMF line showed greater perirenal fat weight (P_0 = 1.00) than low-IMF line, and the difference between lines was relevant (P_r = 1.00). We did not find differences between lines in BW and chilled carcass weights.

Liver weight and lipogenic activities

Table 2 shows descriptive statistics and differences between lines for liver weight and liver lipogenic activities. The greatest lipogenic activity in liver was G6PDH. High-IMF line showed greater liver weight than low-IMF line (P_0 = 0.99) and the probability

183 of the difference between lines being relevant was P_r = 0.87. Besides, high-IMF line
184 showed greater G6PDH (P_0 = 1.00) and ME1activities (P_0 = 0.92) than low-IMF line.
185 The only relevant difference between lines was for G6PDH activity (P_r = 1.00),
186 showing a difference of 1182 nmol/ min and g, or 1.51 SD of the trait. We did not find
187 differences between lines for FASN activity. Results were similar when activities were
188 expressed in a soluble protein basis (data not shown).

189 *Plasma metabolites related to liver*

190 Table 3 reports descriptive statistics and differences between IMF rabbit lines for
191 plasma metabolites related to liver. Low-IMF line showed greater plasma
192 concentration of triglycerides, cholesterol, bilirubin and ALP than high-IMF line and all
193 the differences between lines were relevant, except for cholesterol concentration, in
194 which P_r was very low. High-IMF line showed greater albumin and ALT
195 concentrations (P_0 = 1.00), and differences between lines were relevant. We did not
196 observe differences between lines for glucose, total protein and AST plasma
197 concentrations.

198 *Relationships between fat and liver traits*

199 Table 4 shows phenotypic correlations between fat traits (IMF and perirenal fat
200 weight) and liver traits (liver weight, lipogenic activities and plasma metabolites).
201 Intramuscular fat was positively correlated with liver weight and with G6PDH and
202 FASN activities, correlations went from 0.28 to 0.38. We do not have enough

evidence to state the sign of the correlation between IMF and ME1activity. Perirenal fat weight was positively correlated (0.34) with ME1activity. The correlations between perirenal fat weight and G6PDH and FASN activities and between perirenal fat and liver weights were also positive, but with lower evidence (P_0 between 0.88 and 0.89) and showing lower values (from 0.16 to 0.17).

Albumin concentration in plasma was positively correlated with IMF (0.27) and with perirenal fat weight (0.35) (Table 4). Total protein plasma concentration had a low positive correlation with IMF (0.21, P_0 = 0.94). Phenotypic correlations between IMF and perirenal fat weight and the other plasma metabolites measured were weak (data not shown).

Discussion

Divergent selection for IMF in rabbits was successful, as previously observed in Martínez-Álvaro *et al*., 2016. The genetic progress was approximately one third of the SD of the trait per generation. Selection for IMF showed a positive and relevant correlated response in perirenal fat weight, which is the main carcass fat depot in rabbits (Hernández *et al*., 2006). Other selection experiments for IMF also found a positive correlated response in the carcass adiposity (Schwab *et al*., 2009 in pigs and Zhao *et al*., 2007 in chickens), and the positive genetic correlation between intramuscular and carcass fat is widely documented (Martínez-Álvaro *et al*., 2016 in

223 rabbits and Ciobanu *et al*., 2011 in a pig review). High-IMF line showed greater liver
224 size than low-IMF line, which should be related to its greater fat deposition, since
225 liver is the tissue with the greatest lipogenic activity in growing rabbits (Gondret *et*
226 *al*., 1997).
227 Divergent selection for IMF allows studying the lipid metabolism strictly underlying
228 IMF deposition, since the selected lines have the same genetic background and only
229 differ in genes involved in IMF and correlated traits. Differences in the fat deposition
230 of the high-IMF and low-IMF lines can be explained by different G6PDH and
231 ME1lipogenic activities in liver. Differences between lines were particularly great
232 (1.51 SD) and relevant for G6PDH, which was the main lipogenic activity in rabbit
233 liver, in agreement with other studies in rabbits (Gondret *et al*., 1997 and Gondret *et*
234 *al*., 2004). We did not observe differences between lines for FASN activity, although
235 these results should be taken with caution because of large $HPD_{95\%}$. Both G6PDH
236 and ME1enzymes generate NADPH for the support of fatty acid and steroid
237 biosynthesis, G6PDH by the hexose monophosphate shunt and ME1by the citric acid
238 cycle. In a previous study of the lipogenic activities in LD, *Semimembranosus*
239 *proprius* muscle and perirenal fat of the lines, Martínez-Álvaro *et al*. (2017) observed
240 greater lipogenic activities in the high-IMF line at 13 wk, but not at 9 wk, in all tissues.
241 Moreover, differences between lines at 13 wk were particularly great in the G6PDH

242 activity of LD. Results after selection for IMF reveal the important role of G6PDH
243 activity in the genetic variability on fat deposition in rabbits.
244 Liver lipogenic activities have been previously measured in breeds with different IMF;
245 however, this is the first work that studies liver lipogenic activities in animals with the
246 same genetic origin, divergently selected for IMF. Greater FAS gene expression in
247 liver has been related to greater IMF in a comparison between two chicken breeds
248 (Cui *et al*., 2012). However, breeds can differ in a wide set of traits, which made
249 difficult to attribute the causes of the differences in IMF. Several studies show that
250 animals with greater carcass fat deposition have greater liver weight (Wise *et al*.,
251 1993 and Pond *et al*., 1992 in pigs divergently selected for plasma total cholesterol)
252 and greater G6PDH, ME1and FAS activities in liver (Turkenkopf *et al*., 1980 and
253 Smith *et al*., 1980 in fat genotyped Zucker rats). In pigs, Muñoz *et al*. (2013)
254 observed that selection for decreased backfat thickness at constant IMF was
255 accompanied by a reduction of FAS expression in liver, suggesting that hepatic
256 lipogenesis might affect fat partitioning in pigs (Muñoz *et al*., 2013).
257 Our lines showed normal concentrations of all plasma metabolites except for ALP, in
258 which both lines showed concentrations above normal levels for rabbits (Washington
259 and Van Hoosier, 2012). However, Melillo, (2007) suggested that high plasma
260 concentration of ALP in healthy rabbits is a common finding, since ALP is the sum of
261 three different isoenzimes (two isoenzimes produced in the liver and one in the

intestine) with a wide range of variation. Besides, growing rabbits show particularly high ALP concentrations caused by its high osteoblastic activity, since ALP is involved in the precipitation of calcium phosphate in bones (Melillo, 2007). To our knowledge, our results are the first reports of plasma metabolites in animals selected for IMF.

Circulating plasma concentrations of glucose, triglycerides and cholesterol are the result of the production and uptake by lipogenic tissues. We did not find differences between lines for glucose concentration, which is a primary energy source in rabbits (Melillo, 2007), although the $HPD_{95\%}$ of the difference between lines was large. Low-IMF line had greater plasma triglycerides and cholesterol concentrations than high-IMF line in spite of its lower liver lipogenic activity. A study in rats observed that high plasma concentrations of triglyceride-rich lipoproteins played a regulation role inhibiting hepatic fatty acid synthesis (Lakshmanan *et al*., 1977). In animals selected for different criterions, it has been observed a negative relationship between plasma lipids and carcass fat deposition (Bakke, 1975 selecting for BW gain and carcass leanness and Pond *et al*., 1992 selecting for plasma cholesterol, both in pigs). The lower fat deposition of the low-IMF line suggests that its increased concentration of lipids in plasma is not taken up by muscles and fat depots in a similar rate than in the high-IMF line. The release of plasma lipids to muscle and fat tissues are limited by the activity of the enzyme lipoprotein lipase, which has been suggested as a good

282 indicator of lipid deposition in pigs (Allen *et al*., 1976). Further studies would be
283 necessary to examine the lipoprotein lipase activity of the IMF lines.
284 Bilirubin is a subproduct of hemolysis and it is taken up from plasma by the liver
285 (Wang *et al*., 2006). Low-IMF line showed relevantly greater plasma concentration of
286 bilirubin than the high-IMF line. In healthy humans, greater body fat percentage is
287 related with lower plasma concentration of bilirubin (Jenko-Praznikar *et al*., 2013).
288 This is explained because obesity is associated with an increased oxidative stress
289 and inflammation states, and bilirubin, which has antioxidant and anti-inflammatory
290 properties, is greatly consumed in obese individuals (Jenko-Praznikar *et al*., 2013).
291 Albumin is synthetized in liver and represents the main part of the total protein
292 concentration in plasma (Washington and Van Hoosier, 2012). It transports many
293 plasma metabolites, including bilirubin and free fatty acids. High-IMF line showed
294 relevantly greater albumin concentration than low-IMF line, which can indicate
295 greater transport fluxes of these metabolites in plasma. Although we did not find
296 difference between lines in total protein, this result was estimated with a large
297 $HPD_{95\%}$.
298 Plasma concentrations of ALT, AST and ALP enzymes are used clinically as
299 indicators of liver damage, which was not the case of none of our lines. High-IMF line
300 showed relevant greater ALT concentration than the low-IMF line. This enzyme is
301 involved in the amino acids metabolism (Frayn, 1998). By other side, plasma

302 concentration of ALP was relevantly greater in the low than in the high-IMF line. We
303 did not find information about the relationship of IMF with ALT, AST and ALP plasma
304 concentrations, but pigs with higher carcass adiposity showed greater ALT, AST and
305 lower ALP plasma concentrations with respect to leaner pigs, in a selection
306 experiment for plasma cholesterol (Pond *et al*., 1997).
307 Intramuscular fat and perirenal fat weight were both positively correlated with liver
308 weight and lipogenic activities although the correlations were low. These results
309 suggest that fat deposition in rabbits, both in muscle and carcass, is partially
310 explained by the liver lipogenic activity. However all the correlation estimates showed
311 a wide $HPD_{95\%}$ and we cannot make precise statements about their actual values. To
312 our knowledge, there is no literature about the correlations between intramuscular
313 and carcass fat and liver lipogenic activities.
314 Correlations between IMF and plasma metabolites may have a particular interest in
315 meat production, because they could be used as potential biomarkers of IMF.
316 However, we did not find any strong correlation between IMF and studied plasma
317 metabolites. Plasma metabolites have been previously studied as blood indicators of
318 IMF in pigs (Muñoz *et al*., 2012) and cattle (Adachi *et al*., 1999) with no significant
319 results. These findings suggest the complex biological mechanisms involved in the
320 regulation of IMF deposition, making difficult to find one specific biomarker strongly
321 correlated to IMF.

Conclusions

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

Acknowledgements

This work was supported by project AGL2014-55921-C2-01-P from the Spanish National Research Plan. M. Martínez-Álvaro acknowledges a FPI grant (BES-2012-052655) from the Economy Ministry of Spain. The authors thank Federico Pardo for their technical assistance.

References

Adachi K, Kawano H, Tsuno K, Nomura Y, Yamamoto N, Arikawa A, Tsuji A, Adachi M, Onimaru T and Ohwada K 1999. Relationship between serum biochemical values and

343 marbling scores in Japanese Black steers. Journal of Veterinary Medical Science 61, 961-
344 964.
345 Allen CE, Beitz DC, Cramer DA and Kauffman RG 1976. Biology of fat in meat animals.
346 North Central Regional Research Publication No 234. Univ. of Wisconsin, Madison, USA.
347 Bakke H 1975. Serum levels of cholesterol in lines of pigs selected for rate of gain and
348 thickness of backfat. Acta Agriculture Scandinavica 25, 14-16.
349 Ballard FJ, Hanson RW and Kronfield DS 1969. Gluconeogenesis and lipogenesis in tissue
350 from ruminant and non-ruminant animals. Federation Proceedings 28, 218-231.
351 Blasco A 2017. Bayesian analysis for animal scientists. Springer, NY, USA
352 Blasco A, Ouhayoun J 1996. Harmonization of criteria and terminology in rabbit meat
353 research. Revised proposal. World Rabbit Sci. 4, 93- 99.
354 Ciobanu DC, Lonergan SM and Huff- Lonergan EJ 2011. Genetics of meat quality and
355 carcass traits. In The genetics of the pig (ed. MF Rothschild and A Ruvinsky) pp. 355-389.
356 CABI Publishing, Oxfordshire (UK).
357 Cui HX, Zheng MQ, Liu RR, Zhao GP, Chen JL and Wen J 2012. Liver dominant expression
358 of fatty acid synthase (FAS) gene in two chicken breeds during intramuscular-fat
359 development. Molecular biology reports 39, 3479-3484.
360 European Commission Directive 2010. Council, E. P. A. E. 2010/63/ EU on the protection of
361 animals used for scientific purposes. Institute for Health and Consumer Protection, Ispra,
362 Italy, b7.
363 Frayn KN 1998. Regulación del metabolismo: una perspectiva humana. Omega, Barcelona,
364 Spain.

365 Gondret F, Mourot J and Bonneau M 1997. Developmental changes in lipogenic enzymes in
366 muscle compared to liver and extramuscular adipose tissues in the rabbit (oryctolagus
367 cuniculus). Biochemistry and Molecular Biology117B, 259-265.
368 Gondret F, Hocquette JF and Herpin P. 2004. Age-related relationships between muscle fat
369 content and metabolic traits in growing rabbits. Reproduction Nutrition Development 44, 1-
370 16.
371 Hernández P, Ariño B, Grimal A and Blasco A 2006. Comparison of carcass and meat
372 characteristics of three rabbit lines selected for litter size or growth rate. Meat Science 73,
373 645-650.
374 Jenko-Praznikar Z, Petelin A, Jurdana M and Ziberna L 2013. Serum bilirubin levels are
375 lower in overweight asymptomatic middle-aged adults: an early indicator of metabolic
376 syndrome? Metabolism clinical and experimental 62, 976-985.
377 Kaplan LA, Pesce AJ and Kazmierczak SC 2009. Clinical chemistry: theory, analysis,
378 correlation, 5th edition C.V. Mosby, Toronto, Canada.
379 Lakshmanan MR., Muesing RA, Cook GA and Veech RL 1977. Regulation of lipogenesis in
380 isolated hepatocytes by triglyceride-rich lipoproteins. Journal of Biological Chemistry 252,
381 6581-6584.
382 Legarra A, Varona L and López de Maturana E 2008. TM: Thershold model. GenoToul
383 Bioinformatics, Tolouse, France. Accessed on May 7, 2017 from
384 http://genoweb.toulouse.inra.fr/~alegarra/tm_folder/

385 Martínez-Álvaro M, Hernández P and Blasco A 2016. Divergent selection on intramuscular
386 fat in rabbits: responses to selection and genetic parameters. Journal of Animal Science 94,
387 4993-5003.
388 Martínez-Álvaro M, Agha S, Blasco A and Hernández P 2017. Muscle lipid metabolism in two
389 rabbit lines divergently selected for intramuscular fat. Journal of Animal Science. doi:
390 10.2527/jas2017.1371.
391 Melillo A 2007. Rabbit Clinical Pathology. Journal of Exotic Pet Medicine 16, 135-145.
392 Muñoz R, Tor M, and Estany J. 2012. Relationship between blood lipid indicators and fat
393 content and composition in Duroc pigs. Livestock Science 148, 95–102.
394 Muñoz R, Estany J, Tor M and Doran O 2013. Hepatic lipogenic enzyme expression in pigs
395 affected by selection for decreased backfat thickness at constant intramuscular fat content.
396 Meat Science 93, 746-751.
397 O' Heau EK and Leveille, GA 1969. Lipid biosynthesis and transport in the domestic chick
398 (Gallus domesticus). Comparative Biochemistry and Physiology 30,149-159.
399 Pond WG, Insull W, Mersmann HJ, Wong WW, Harris KB, Cross HR, Smith EO, Heath JP
400 and Kömüves LG 1992. Effect of dietary fat and cholesterol level on growing pigs selected
401 for three generations for high or low serum cholesterol at age 56 days. Journal of Animal
402 Science 70, 2462-2470.
403 Pond WG, Su DR and Mersmann HJ 1997. Divergent concentrations of plasma metabolites
404 in swine selected for seven generations for high or low plasma total cholesterol. Journal of
405 Animal Science 75, 311-316.

406 Sapp RL, Bertrand JK, Pringle TD and Wilson DE 2002. Effects of selection for ultrasound
407 intramuscular fat percentage in Angus bulls on carcass traits of progeny. Journal of Animal
408 Science 80, 2017–2022.
409 Schwab CR, Baas TJ, Stalder KJ and Nettleton D 2009. Results from six generations of
410 selection for intramuscular fat in Duroc swine using real-time ultrasound. I. Direct and
411 correlated phenotypic responses to selection. Journal of Animal Science 87, 2774–2780.
412 Smith PA and Kaplan ML 1980. Development of hepatic and adipose tissue lipogenesis in
413 the fa/fa rat. International Journal of Biochemistry 11, 217-228.
414 Turkenkopf IJ, Olsen JL, Moray L, Greenwood MRC and Johnson PR 1980. Hepatic
415 lipogenesis in preobese Zucker rat (40910). Proceedings of the Society for experimental
416 Biology and Medicine 164, 530-533.
417 Wang X, Roy Chowdhury J and Roy Chowdhury N 2006. Bilirubin metabolism: applied
418 physiology. Current paediatrics 16, 70-74.
419 Washington IM and Van Hoosier GV. 2012. Clinical Biochemistry and Hematology. In The
420 Laboratory Rabbit, Guinea Pig, Hamster, and Other Rodents (ed. MA Suckow, KA Stevens
421 and RP Wilson) pp.57–116. Blackwell Publishing Professional, IA, USA.
422 Wise T, Young DL and Pond WG 1993. Reproductive, endocrine and organ weight
423 differences of swine selected for high or low serum cholesterol. Journal of Animal Science
424 71, 2732-2738.
425 Wood JD, Enser M, Fisher AV, Nute GR, Sheard PR, Richardson RI, Hughes SI and
426 Whittington FM 2008. Fat deposition, fatty acid composition and meat quality: a review. Meat
427 Science 78, 343–358.

Zhao GP, Chen JL, Zheng MQ, Wen J and Zhang Y 2007. Correlated responses to selection for increased intramuscular fat in a Chinese quality chicken line. Poultry Science 86, 2309-2314.

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

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

436

437 **Table 1** *Descriptive statistics and differences between high and low intramuscular fat*

438 *(IMF) rabbit lines in IMF and carcass traits (g).*

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

439 [1]D = median of the marginal posterior distribution of the difference between high-IMF and
440 low-IMF lines.
441 [2]HPD $_{95\%}$ = highest posterior density region at 95% of probability.
442 [3]P_0 = probability of the difference being greater than zero when D> 0 or lower than zero when
443 D< 0.
444 [4]r = relevant value, proposed as 1/3 of the standard deviation of the trait.
445 [5]P_r = probability of relevance (probability of the difference being greater than R when D> 0 or
446 lower than R when D< 0.
447
448
449
450
451

Table 2 *Descriptive statistics and differences between high and low intramuscular fat (IMF) rabbit lines in liver weight and liver lipogenic[1] activities.*

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

[1]Activities of the lipogenic enzymes glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme (EM) and fatty acid synthase (FAS) are expressed in nmol / min and g of tissue.

[2]D = median of the marginal posterior distribution of the difference between high-IMF and low-IMF lines.

[3]$HPD_{95\%}$ = highest posterior density region at 95% of probability.

[4]P_0 = probability of the difference being greater than zero when D> 0 or lower than zero when D< 0.

[5]r = relevant value, proposed as 1/3 of the standard deviation of the trait.

[6]P_r = probability of relevance (probability of the difference being greater than R when D> 0 or lower than R when D< 0.

Table 3 *Descriptive statistics and differences between high and low intramuscular fat (IMF) rabbit lines in plasma metabolites related to liver.*

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

[1]D = median of the marginal posterior distribution of the difference between high and low-intramuscular fat lines.

[2]$HPD_{95\%}$ = highest posterior density region at 95% of probability.

[3]P_0 = probability of the difference being greater than zero when D>0 or lower than zero when D<0.

[4]r = relevant value, proposed as 1/3 of the standard deviation of the trait.

[5]P_r = probability of relevance (probability of the difference being greater than R when D>0 or lower than R when D<0.

[6]AST = aspartate transaminase.

[7]ALT = alanine transaminase.

[8]ALP = alkaline phosphatase.

Table 4 *Phenotypic correlations of intramuscular fat and perirenal fat weight with liver weight, lipogenic[1] activities and plasma metabolites concentrations related to liver in rabbits.*

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

[1]Activities of the lipogenic enzymes glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme (EM) and fatty acid synthase (FAS) measured in nmol / min and g of tissue.

[2]r_p = median of marginal posterior distribution of the phenotypic correlation.

[3]$HPD_{95\%}$ = highest posterior density region at 95% of probability.

[4]P_0 = probability of the phenotypic correlation of being greater than zero when $r_p > 0$ or lower than zero when $r_p < 0$.