Effect of selection for intramuscular fat on the fatty acid composition of rabbit meat

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Short title: correlated responses to intramuscular fat selection

Abstract

Intramuscular fat (**IMF**) content and composition are relevant for the meat industry due to their effect on human health and meat organoleptic properties. A divergent selection experiment for IMF of *Longissimus dorsi* (**LD**) muscle was performed in rabbits during eight generations. The aim of this study is to estimate the correlated responses to selection for IMF on the fatty acid composition of LD. Response to selection for IMF was 0.34 g/100g of LD, representing 2.4 phenotypic SD of the trait. High-IMF line showed 9.20% more monounsaturated fatty acids (**MUFA**) and 0.39%, 9.97% and 10.3% less n-3, n-

6 and polyunsaturated fatty acids (**PUFA**) respectively, than low-IMF line. The main MUFA and PUFA individual fatty acids followed a similar pattern, except for C18:3n-3 that was greater in the high-IMF line. We did not observe differences between lines for the percentage of total saturated fatty acids (**SFA**), although high-IMF line showed greater C14:0 and C16:0 and lower C18:0 percentages than low-IMF line. Heritability estimates were generally high for all fatty acids percentages, ranging from 0.43 to 0.59 with a SD around 0.08, showing an important genetic component on these traits. Genetic correlations between IMF and LD fatty acid percentages were strong and positive for C14:0, C16:1, C18:1n-9, and MUFA, ranging from 0.88 to 0.97, and strong and negative for C18:0, C18:2n-6, C20:4n-6, n-6 and PUFA, ranging from -0.83 to -0.91. These correlations were accurately estimated, with SD ranging from 0.02 to 0.06. The genetic correlations between IMF and other fatty acids were estimated with lower accuracy. In general, phenotypic and genetic correlations were of the same order. Our experiment shows that selection for IMF strongly affects the fatty acid composition of meat, due the high heritabilities of fatty acids and their high genetic correlations with IMF.

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

Implications: Increasing intramuscular fat (**IMF**) by selection is a successful way for improving meat quality. However, this study shows that selection for IMF has important consequences in the fatty acid (**FA**) percentages, some of them negative. These results should be considered when selecting for IMF to improve meat quality.

Introduction

Increasing intramuscular fat (**IMF**) content of meat by genetic selection is an effective way to improve its tenderness (Zhao *et al*., 2007 in chickens) and flavor (Schwab *et al*., 2009 in pigs). However, selection for IMF could produce changes in the fatty acid composition that can influence nutritional, organoleptic and technological properties of meat. Great amounts of monounsaturated (**MUFA**) and saturated (**SFA**) fatty acids improve meat flavor (Carrapiso *et al*., 2003 and Burkett, 2009) but nutritional institutions recommend reducing the intake of SFA (World Health Organization, 2008). Polyunsaturated fatty acids (**PUFA**) are beneficial from a nutritional point of view, but can lead to undesirable flavors, to a decrease in the melting point of fat, and to a shortened shelf life of the meat (Wood *et al*., 2004).

Three previous selection experiments for IMF have been performed (Sapp *et al*., 2002 in cattle, Zhao *et al*., 2007 in chickens and Schwab *et al*., 2009 in pigs), but only the experiment in pigs reported correlated responses in the fatty acid composition of meat (Burkett, 2009). We have developed two experimental rabbit lines divergently selected during eight generations for IMF, to study the genetics and metabolism of IMF deposition (Martínez-Álvaro *et a*l., 2016a, b and 2017a, b). Rabbit is a good genetic model for other livestock species because permits having large samples at reasonable costs. Rabbit has also importance as livestock species in several countries (FAO-STAT, 2014).

The aim of this study is to estimate the correlated responses to selection for IMF on fatty acid composition and their genetic parameters. This is the first time that the genetics of IMF and meat fatty acid composition is studied in rabbits.

Materials and methods

Animals

A divergent selection experiment for IMF in rabbits was performed during 8 generations. Animals came from a synthetic rabbit line. The base population consisted of 13 males and 83 females, and then, lines selected for high and low IMF had approximately 8 males and 40 females per generation. Two full sibs (a male and a female) of the first parity of each doe were slaughtered at 9 wk of

age and their IMF content was measured in *Longissimus dorsi* (**LD**) muscle. All dams were ranked according to the average of the two phenotypic IMF values obtained from their offspring. The 20% best dams provided all females for the next generation. Each sire was mated with five does, and to reduce inbreeding only one male progeny of the sire, from highest ranked mate, was selected for breeding the next generation. Normally, the first parity was used to collect the IMF data and the second parity to provide the rabbits for next generation, although exceptionally some IMF measurements were made on the second or third parity. More details of this experiment can be found in Martínez-Álvaro *et al*. (2016a). A total of 2 713 rabbits were considered in the pedigree file, from which 1 511 were evaluated. A total of 173 rabbits from the eighth generation were used to study the correlated responses to selection on fatty acid composition of LD; 82 from the high-IMF line and 91 from the low-IMF line.

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

100 carcasses were chilled for 24h at 4ºC. All experimental procedures involving
101 animals were approved by the Universitat Politècnica de València Research
102 Ethics Committee, according to council directive 2010/63/EU (European
103 Commission Directive, 2010).
104
105 *Intramuscular fat and fatty acids measurements*
106 After refrigeration, LD was excised, minced, freeze-dried and scanned with near
107 infrared spectroscopy to measure IMF and fatty acid composition, applying the
108 calibration equations previously developed by Zomeño *et al*. (2012) with some
109 modifications. Intramuscular fat and fatty acid contents were obtained in g /100g
110 of LD muscle on a fresh basis. Fatty acids were expressed as percentage of
111 total fatty acids. Fatty acids studied were the major individual fatty acids C14:0,
112 C16:0, C16:1, C18:0, C18:1n-9, C18:2n-6, C18:3n-3 and C20:4n-6 and the
113 SFA, MUFA, n-3, n-6 and PUFA groups, which included the major fatty acids
114 cited above and all identified minor fatty acids (i.e. C15:0 and C17:0 for SFA,
115 C18:1n-7 for MUFA and C20:2n-6, C20:3n-6, C20:5n-3, C22:4n-6, C22:5n-3
116 and C22:6n-3 for PUFA).
117 *Statistical analysis*
118 Descriptive statistics and phenotypic correlations between IMF and fatty acid
119 percentages of LD were estimated with data from all generations, after

120 correcting data by line-generation-season, parity order and sex fixed effects.
121 Direct and correlated responses to selection were estimated as the phenotypic
122 differences between high and low-IMF lines at the eight generation of selection.
123 Phenotypic differences between lines were estimated with the model

124 $$\mathbf{y} = \mathbf{Xb} + \mathbf{Wc} + \mathbf{e}$$

125 Data were assumed to be conditionally distributed as

126 $$\mathbf{y} \mid \mathbf{b}, \mathbf{c}, \sigma_e^2 \sim N(\mathbf{Xb} + \mathbf{Wc}, \mathbf{I}\sigma_e^2)$$

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

131 $$\mathbf{c} \sim N(\mathbf{0}, \mathbf{I}\sigma_c^2)$$

132 in which σ_c^2 is the common litter variance.
133 Heritabilities and genetic correlations with IMF were estimated by fitting a
134 bivariate animal model, with the same effects for all traits

135 $$\begin{pmatrix} \mathbf{y}_1 \\ \mathbf{y}_2 \end{pmatrix} = \begin{pmatrix} \mathbf{X} & \mathbf{0} \\ \mathbf{0} & \mathbf{X} \end{pmatrix}\begin{pmatrix} \mathbf{b}_1 \\ \mathbf{b}_2 \end{pmatrix} + \begin{pmatrix} \mathbf{Z} & \mathbf{0} \\ \mathbf{0} & \mathbf{Z} \end{pmatrix}\begin{pmatrix} \mathbf{u}_1 \\ \mathbf{u}_2 \end{pmatrix} + \begin{pmatrix} \mathbf{W} & \mathbf{0} \\ \mathbf{0} & \mathbf{W} \end{pmatrix}\begin{pmatrix} \mathbf{c}_1 \\ \mathbf{c}_2 \end{pmatrix} + \begin{pmatrix} \mathbf{e}_1 \\ \mathbf{e}_2 \end{pmatrix}$$

136 Data were assumed to be conditionally distributed as

137 $$\begin{pmatrix} \mathbf{y}_1 \\ \mathbf{y}_2 \end{pmatrix} \mid \mathbf{b}_1, \mathbf{b}_2, \mathbf{u}_1, \mathbf{u}_2, \mathbf{c}_1, \mathbf{c}_2, \mathbf{R} \sim N\left(\begin{bmatrix} \mathbf{X} & \mathbf{0} \\ \mathbf{0} & \mathbf{X} \end{bmatrix}\begin{bmatrix} \mathbf{b}_1 \\ \mathbf{b}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{Z} & \mathbf{0} \\ \mathbf{0} & \mathbf{Z} \end{bmatrix}\begin{bmatrix} \mathbf{u}_1 \\ \mathbf{u}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{W} & \mathbf{0} \\ \mathbf{0} & \mathbf{W} \end{bmatrix}\begin{bmatrix} \mathbf{c}_1 \\ \mathbf{c}_2 \end{bmatrix}, \mathbf{R}\right)$$

in which $\mathbf{b}_1$, $\mathbf{b}_2$ are the vectors of fixed effects (month, sex and parity order); $\mathbf{u}_1$, $\mathbf{u}_2$ are the vectors of additive genetic effects; $\mathbf{c}_1$, $\mathbf{c}_2$ are the vectors of common litter effects; **X**, **Z** and **W** are known incidence matrices, and **R** is the residual co (variance) matrix between the two traits.

Sorting data by individuals, additive effects were distributed as

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

common litter effects were distributed as

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

and residuals were distributed as

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

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

Bayesian inference was used, with bounded flat priors for all unknowns. Marginal posterior distributions were estimated using Gibbs sampling (see Blasco, 2001, 2017). Descriptive statistics and phenotypic differences between lines were computed with the programme Rabbit developed by the Institute for

158 Animal Science and Technology (Valencia, Spain). After some exploratory
159 analyses, results were based on Monte Carlo Markov chains consisting of 60
160 000 iterations, with a burn-in period of 10 000, and only one of every 10
161 samples were saved for inferences. Phenotypic correlations and genetic
162 analyses were computed with the software TM (Legarra *et al*., 2008). After
163 some exploratory analyses results were based on Monte Carlo Markov chains
164 consisting of 1 000 000 iterations, with a burn-in period of 200 000; only one of
165 every 100 samples were saved for inferences. In all analyses, convergence was
166 tested using the Z criterion of Geweke and Monte Carlo sampling errors were
167 computed using time-series procedures included in the Rabbit and TM
168 programs. In all cases, Monte Carlo standard errors were small and lack of
169 convergence was not detected by the Geweke test.
170 The parameters obtained from the marginal posterior distributions of the
171 phenotypic differences between high and low-IMF lines were the median of the
172 difference (**D**), the highest posterior density region at 95% ($\mathbf{HPD_{95\%}}$), and the
173 probability of the difference being greater than zero when D > 0 or lower than
174 zero when D < 0 ($\mathbf{P_0}$). We considered 1/3 of the phenotypic standard deviation
175 of a trait as a relevant value (**r**), and we calculated the probability of relevance
176 (probability of the difference being greater than r when D > 0 or lower than r
177 when D < 0) ($\mathbf{P_r}$). For heritabilities, we estimated the median of each marginal

posterior distribution, the $HPD_{95\%,}$ and the limit k of the interval [k, 1] with 80% probability, i.e. the guaranteed value with probability of 80% (**$k_{80\%}$**). For genetic and phenotypic correlations, we estimated the median of each marginal posterior distribution, the $HPD_{95\%}$**,** the probability of being greater than 0 when the median is positive or lower than 0 when the median is negative (P_0), and the guaranteed value with probability of 80%; i. e., the limit k of the interval [k, 1] when the median is positive or [-1, k] when the median is negative with 80% probability. A more detailed description of these features can be found in Blasco, (2017).

Results and discussion

Intramuscular fat composition

Table 1 shows descriptive statistics of IMF content and fatty acid composition of LD. On average, IMF was 1.04 g/100g of LD. Percentages of SFA and PUFA were similar (38.2% and 41.8%, respectively), while MUFA percentage was lower (24.8%). Polyunsaturated fatty acids were mainly composed by n-6 (39.5%), whereas n-3 represented a lower percentage (2.92%). Linoleic (C18:2n-6), palmitic (C16:0), and oleic (C18:1n-9) acids were the most abundant fatty acids in rabbit meat, according to the fatty acid composition of the diet. They were followed by stearic (C18:0) and arachidonic acids (C20:4n-

198 6), whereas other fatty acids (C14:0, C16:1 and C18:3n-3) represented minor
199 percentages. These results are in agreement with other studies in rabbits
200 (reviewed by Dalle Zotte, 2002).
201 Ratios PUFA/SFA, n-6/n-3 and MUFA/SFA are healthy indicators of meat. In
202 our experiment, PUFA/SFA ratio was 1.09, above the minimum of 0.60
203 recommended by the World Health Organization (2008). However, the n-6/n-3
204 ratio was not favorable (13.5), it was higher than the nutritional
205 recommendations for human diets of 5-10 (World Health Organization, 2008)
206 due to the high amount of C18:2n-6 in rabbit meat. The ratio MUFA/SFA was
207 0.65. We did not find a nutritional recommendation range for this ratio, although,
208 in general, it is recommended to replace the intake of SFA by unsaturated fatty
209 acids (World Health Organization, 2008). In comparison with other species,
210 rabbit meat shows greater PUFA, C18:2n-6 and C18:3n-3 percentages and
211 PUFA/SFA ratio than pig, cattle and sheep, and lower n-6/n-3 ratio than pig,
212 cattle and chicken (reviewed by Dalle Zotte, 2002). However, MUFA/SFA ratio
213 in rabbit meat is lower than in other species (Dalle Zotte, 2002). Overall, the
214 fatty acid composition of rabbit meat makes it a high quality meat from a
215 nutritional point of view.
216

217 *Response to selection and correlated responses in fatty acid composition of LD*

218 Table 2 shows the direct response to selection for IMF and correlated
219 responses in fatty acid composition of LD estimated as differences between
220 high-IMF and low-IMF lines. Comparisons between lines should be done at the
221 same stage of maturity, and our lines were approximately at the same stage
222 (Pascual *et al*., 2015). Response to selection for IMF was 0.34 g/100g of LD,
223 representing 2.4 phenotypic SD of the trait, with a probability of the difference
224 between lines being relevant $P_r = 1.00$. Other authors obtained great responses
225 to selection for IMF in cattle (Sapp *et al*., 2002), chickens (Zhao *et al*., 2007)
226 and pigs (Schwab *et al*., 2009), in line with our results.

227 Selection for IMF led to great modifications in the fatty acid composition of LD
228 (Table 2). The high-IMF line showed greater MUFA and lower PUFA
229 percentages than the low-IMF line. The differences between high-IMF and low-
230 IMF lines for these fatty acid groups were both relevant ($P_r = 1.00$) and of
231 similar magnitude. Within PUFA, both n-6 and n-3 were relevantly lower in the
232 high-IMF line ($P_r = 1.00$), being the differences between lines greater for n-6
233 (3.8 phenotypic SD) than for n-3 (1.4 phenotypic SD). We did not observe
234 differences between lines for the SFA percentage. High-IMF line showed
235 relevantly greater amounts of SFA, MUFA, n-6, n-3 and PUFA groups and of all
236 individual fatty acids in absolute terms (g / 100 g of LD) respect to the low-IMF

237 line (data not shown), due to its greater amount of IMF. Differences in the fatty
238 acid percentages between lines are the consequence of a greater proportion of
239 triglycerides (stored in adipocytes) respect to phospholipids (located in cells
240 membranes) in the high-IMF than in the low-IMF line. In general, phospholipid
241 fraction shows greater percentages of all individual PUFA whereas triglycerides
242 fraction is richer in all MUFA and SFA. The faster increase of MUFA and SFA
243 respect to PUFA when fatness increases is well documented (reviewed by De
244 Smet *et al*., 2004 and Wood *et al*., 2008 in several farm species).
245 We have studied the differences between lines on the fatty acid ratios
246 MUFA/SFA, PUFA/SFA and n-6/n-3 in order to evaluate the effect of selection
247 for IMF from a nutritional point of view. On one hand, high-IMF line showed
248 greater MUFA/SFA ratio (0.57) respect to the low-IMF line (0.33), which implies
249 an improvement of meat quality in the high-IMF line. The mean for the
250 MUFA/SFA ratio in the eight generation was 0.45, differing from the mean of the
251 whole selection experiment showed in Table 1. The n-6/n-3 ratio was also more
252 favorable in the high-IMF (11.6) than in the low-IMF line (13.1), due to the
253 greater differences between lines in n-6 than in n-3. In contrast, selection for
254 high IMF led to a detriment of the PUFA/SFA ratio respect to selection for low
255 IMF, which was 0.98 in the high-IMF line and 1.23 in the low-IMF line. However,

256 in both cases, PUFA/SFA ratio was above the minimum of 0.60 recommended
257 by the World Health Organization (2008).
258 Modifications in the IMF content and in its fatty acid composition could also
259 affect organoleptic and technological meat quality traits such as flavor, fat
260 consistence and shelf life (Wood *et al*., 2004). However, in a previous study we
261 did not find differences between our rabbit lines in organoleptic properties
262 (Martínez-Álvaro *et al*., 2016b).
263 In general, monounsaturated and polyunsaturated individual fatty acids showed
264 similar patterns as their groups in correlated responses (Table 2), but this was
265 not observed for individual SFA. High-IMF line showed greater percentages of
266 individual SFA C14:0 and C16:0 but lower percentage of C18:0 than low-IMF
267 line, and differences between lines were relevant. Rabbits, and mammals in
268 general, are able to synthetize SFA and MUFA from glucose through
269 lipogenesis *de novo*, which produces primarily C16:0. In contrast, PUFA are
270 entirely derived from the diet. Previous studies observed that high-IMF line
271 showed greater lipogenic activities than low-IMF line in several tissues such as
272 LD, perirenal fat and liver (Martínez-Álvaro *et al*., 2017a and b). These findings
273 explain the greater proportion of C14:0 and C16:0 individual SFA and total
274 MUFA observed in the high-IMF line in comparison to the low-IMF line, and
275 consequently, its lower proportion of PUFA. However, high-IMF line showed

276 lower percentage of C18:0. This is explained because C18:0 percentage is
277 greater in phospholipids than in the triglycerides fraction in rabbits (Alasnier *et*
278 *al*., 1996; Cambero *et al*., 1991a and b; Otake *et al*., 1971). This particularity is
279 not observed in other species (Leseigneur-Meynier and Gandemer, 1991 and
280 Burkett, 2009 in pigs and Wood *et al*., 2004 in a review including pigs, lambs
281 and cattle).
282 Concerning individual MUFA, high-IMF line had relevantly greater C18:1n-9 and
283 C16:1 percentages (P_r = 1.00, Table 2). The ratio between C18:1n-9 and C18:0
284 is a common indicator of the stearoyl-CoA desaturase (**SCD**) activity (Attie *et*
285 *al*., 2002), enzyme responsible for the synthesis of main MUFA from their SFA
286 forms. This ratio was 1.98 for high-IMF line and 1.09 for low-IMF line, indicating
287 greater SCD activity in the high-IMF line. Within PUFA, high–IMF line showed
288 lower C18:2n-6 and C20:4n-6 percentages than low-IMF line (P_r = 1.00), but
289 greater percentage of C18:3n-3 (P_r = 1.00). In rabbits, C18:3n-3 percentage is
290 much greater in triglycerides than in phospholipids (Otake *et al* 1971 and
291 Alasnier *et al*., 1996). In other species such as pig, C18:3n-3 is similar in both
292 fractions (reviewed by De Smet *et al*., 2004, Wood *et al*., 2004 and 2008), or it
293 is only slightly greater in triglycerides (Burkett, 2009).
294 Our results are in close agreement with findings of the selection experiment for
295 IMF in pigs (Burkett, 2009). The line of pigs selected for high IMF showed more

296 MUFA percentages and lower PUFA percentages respect to a control line, with
297 the exception of C18:3n-3 (Burkett, 2009), and no significant differences for
298 SFA percentage. In a simulation study in pigs, Ros-Freixedes *et al*., (2012)
299 expected a positive response to selection in C18:1n-9 percentage when
300 selecting by IMF and other traits, including IMF. Some studies compared the
301 fatty acid composition in several genetic rabbit lines differing in their IMF, but
302 they did not show any common pattern (Gašperlin *et al*., 2006 and Hernández
303 *et al*., 2008).
304
305 *Heritabilities of the traits*
306 For all traits, the differences between the genetic means of the high-IMF and
307 low-IMF lines estimated with the animal model matched with the phenotypic
308 differences between lines (Table 2), which corroborates the model used for the
309 genetic analysis and then, the estimated parameters.
310 Table 3 shows the heritabilities (**h^2**) of fatty acid composition of LD. In general,
311 fatty acid composition showed high heritabilities. Percentages of MUFA, n-6 and
312 PUFA groups displayed the greatest h^2 estimates (from 0.56 to 0.59), showing
313 guaranteed values from 0.50 to 0.52 with 80% of probability. Their major fatty
314 acids, C18:1n-9 and C16:1 for MUFA and C18:2n-6 and C20:4n-6 for n-6 and
315 PUFA, also showed great h^2 estimates (from 0.43 to 0.53). However, n-3 group

316 and its major fatty acid C18:3n-3, showed lower estimates (0.15 to 0.18).
317 Percentage of SFA group showed a low h^2 (0.12 with a $k_{80\%}$ = 0.07) because of
318 the low h^2 of its main component, C16:0, although other important individual
319 SFA percentages such as C18:0 and C14:0 displayed high heritabilities.
320 Heritability estimates were high for the major fatty acids (except for C18:3n-3
321 and C16:0). Even though C18:2n-6 should come from diet, our results show that
322 there is an important genetic control for all these fatty acids accumulation in
323 IMF, whereas this was not observed for C18:3n-3 (Table 3). Several studies
324 report high to moderate h^2 estimates for IMF fatty acid percentages (Burkett *et*
325 *al*., 2009; Sellier *et al*., 2010 and Ibáñez-Escriche *et al*., 2016 in pigs and Nogi
326 *et al*., 2016 in cattle), with some exceptions. The h^2 estimate of C18:3n-3
327 percentage reported by Ibáñez-Escriche *et al*. (2016) was moderate (0.22) and
328 those reported by Sellier *et al*. (2010) and Nogi *et al*. (2011) were null. In these
329 three cases, h^2 of C18:3n-3 was lower than h^2 of the other fatty acids, which is
330 in line with our results in rabbits. Only Burkett *et al*. (2009) reported a low h^2 for
331 C16:0, near 0.
332
333 *Correlations between IMF and fatty acid composition*
334 Table 4 shows the phenotypic and genetic correlations ($\mathbf{r_g}$) between IMF and
335 fatty acid composition of LD. To our knowledge, there are no previous reports of

336 r_g among IMF and fatty acid composition of meat in rabbits. Estimates of r_g
337 between IMF and fatty acid percentages of LD were strong and positive for
338 C14:0, C16:1, C18:1n-9 and MUFA (0.88 to 0.97), and strong and negative for
339 C18:0, C18:2n-6, C20:4n-6, n-6 and PUFA (-0.83 to -0.91). Because of the
340 high values of these correlations, 1 511 animals were enough to obtain quite
341 accurate estimates (Table 4). Phenotypic correlations between IMF and these
342 fatty acids were of the same order or slightly lower than r_g (Table 4). The r_g of
343 IMF with n-3 was negative, whereas with C18:3n-3 was positive. The r_g of IMF
344 with C16:0 and SFA percentages were low (0.48 and 0.30 respectively) with
345 wide $HPD_{95\%}$, but we can still say that they were positive with high probability
346 (P_0 = 0.99 and 0.94, respectively). Phenotypic correlations between IMF and
347 percentages of n-3, C18:3n-3, C16 and SFA showed the same sign as their
348 corresponding r_g (P_0=1.00) but their medians were lower. Our study reports
349 strong r_g between IMF and most of the major fatty acids percentages (except for
350 C16:0 and C18:3n-3), suggesting that, as IMF increases, there is a rapid
351 dilution of PUFA in MUFA and in C14:0. This dilution is due to the difference in
352 fatty acid composition between the muscle phospholipids and triglycerides (De
353 Smet *et al*., 2004). In pigs, the genetic correlations of IMF with PUFA and
354 C18:2n-6 percentages were negative and strong (-0.80 and -0.84, respectively)
355 in line with our estimates, and the genetic correlation between IMF and C14:0

356 was 0.50, lower than ours (Burkett *et al*., 2009). Their other genetic correlations
357 were estimated with very low accuracy due to their low amount of data (n =
358 663). In general, our results showed stronger correlations between IMF and
359 fatty acid composition of meat than the correlations reported in other studies in
360 pigs (Suzuki *et al*., 2006 and Ros-Freixedes *et al*., 2014) and cattle (Nogi *et al*.,
361 2011 and Buchanan *et al*., 2015). A couple of genes (*ELOVL6* and *SCD*)
362 affecting MUFA and SFA content without modifying IMF have been detected in
363 pigs (reviewed by Estany *et al*., 2016). However, the strong genetic correlations
364 between IMF and most fatty acids estimated in rabbits leave few options to
365 change the fatty acid composition of LD without varying IMF.
366
367 **Conclusions**
368 Our study shows substantial changes in the fatty acid composition of LD when
369 selecting for IMF. High-IMF line showed increased MUFA and decreased n-6, n-
370 3 and PUFA percentages in comparison to low-IMF line, and percentages of the
371 main MUFA and PUFA individual fatty acids followed a similar pattern as
372 groups, except for C18:3n-3 that was greater in the high-IMF line. We did not
373 observe differences between lines for the percentage of SFA group, but we
374 found greater C14:0 and C16:0 percentages in the high-IMF and lower
375 percentage of C18:0. Thus, the increase of IMF content by selection could

impair nutritional quality of the meat. The high heritabilities estimated for most of the fatty acids, together with the high genetic correlations with IMF, explain the great effect of selection for IMF on the fatty acid composition of meat.

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485 **Table 1** *Descriptive statistics of intramuscular fat (g/100g of muscle) and fatty*
486 *acid composition of Longissimus dorsi muscle (% of total fatty acids) of rabbits*
487 *from the whole experiment (n = 1 511).*

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

488 [1]IMF = intramuscular fat; SFA = saturated fatty acids, C14:0 + C15:0 + C16:0 + C17:0
489 + C18:0; MUFA = monounsaturated fatty acids, C16:1 + C18:1n-7 + C18:1n-9; n-3 =
490 C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3; n-6 = C18:2n-6 + C20:2n-6 + C20:3n-6 +
491 C20:4n-6 + C22:4n-6; PUFA = polyunsaturated fatty acids, C18:2n-6 + C18:3n-3 +
492 C20:2n-6 + C20:3n-6 + C20:4n-6 + C20:5n-3 + C22:4n-6 + C22:5n-3 + C22:6n-3.

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503 **Table 2** *Differences between high and low intramuscular fat (IMF) rabbit lines*
504 *for IMF (g/100g of muscle) and fatty acid composition of Longissimus dorsi*
505 *muscle (% of total fatty acids) in the eighth generation (n = 173).*

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

506 [1]SFA = saturated fatty acids, C14:0 + C15:0 + C16:0 + C17:0 + C18:0; MUFA =
507 monounsaturated fatty acids, C16:1 + C18:1n-7 + C18:1n-9; n-3 = C18:3n-3 + C20:5n-
508 3 + C22:5n-3 + C22:6n-3; n-6 = C18:2n-6 + C20:2n-6 + C20:3n-6 + C20:4n-6 +
509 C22:4n-6; PUFA = polyunsaturated fatty acids, C18:2n-6 + C18:3n-3 + C20:2n-6 +
510 C20:3n-6 + C20:4n-6 + C20:5n-3 + C22:4n-6 + C22:5n-3 + C22:6n-3.

511 [2]D = median of the marginal posterior distribution of the difference between high and
512 low-IMF lines.

513 [3]$HPD_{95\%}$ = highest posterior density region at 95% of probability.

514 [4]P_0 = probability of the difference being greater than zero when D >0 or lower than zero
515 when D <0.

516 [5]r = relevant value, proposed as 1/3 of the standard deviation of the trait.

517 [6]P_r = probability of relevance (probability of the difference being greater than r when D
518 >0 or lower than r when D <0).

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522 **Table 3** *Heritabilities of fatty acid composition of Longissimus dorsi muscle (in*

523 *% of total fatty acids) in rabbits.*

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

524 [1]SFA = saturated fatty acids, C14:0 + C15:0 + C16:0 + C17:0 + C18:0; MUFA =
525 monounsaturated fatty acids, C16:1 + C18:1n-7 + C18:1n-9; n-3 = C18:3n-3 + C20:5n-
526 3 + C22:5n-3 + C22:6n-3; n-6 = C18:2n-6 + C20:2n-6 + C20:3n-6 + C20:4n-6 +
527 C22:4n-6; PUFA = polyunsaturated fatty acids, C18:2n-6 + C18:3n-3 + C20:2n-6 +
528 C20:3n-6 + C20:4n-6 + C20:5n-3 + C22:4n-6 + C22:5n-3 + C22:6n-3.

529 [2]Median = median of the marginal posterior distribution of the heritability.

530 [3]$HPD_{95\%}$ = highest posterior density region at 95% of probability.

531 [4]$k_{80\%}$ = limit of the interval [k, 1] at 80% of probability.

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

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

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

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

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

[4]P_0 = probability of the correlation being greater than 0 when the median is positive, or lower than 0 when the median is negative.

[5]$k_{80\%}$ = limit of the interval [k, 1] at 80% of probability.