CHANGES IN FATTY ACID COMPOSITION DUE TO SELECTION FOR INTRAMUSCULAR FAT

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ABSTRACT

Two rabbit lines divergently selected for intramuscular fat (IMF) in the *longissimus thoracis et lumborum* muscle (LM) were studied to evaluate the relationship between IMF content and the fatty acid composition. Data from the ninth generation were analysed using Bayesian methodology. Response to selection was estimated as the phenotypic difference between high-IMF and low-IMF lines. The direct response to selection for IMF was 0.51g/100 g of muscle, representing 3.3 phenotypic standard deviation. Moreover, selection for IMF content affected clearly the fatty acid composition. The correlated response to selection was positive for saturated (SFA) and monounsaturated (MUFA) fatty acids percentages, 1.71% and 3.24 %, respectively, with greater values in the high-IMF line. In contrast, it was negative for polyunsaturated fatty acids (PUFA) percentage (-4.96%), with greater values for low-IMF line. The divergent lines were clearly separated by the projection on latent structures discriminant analysis (R^2 = 84%). High-IMF line was mainly influenced by linolenic acid and SFA percentages, whereas low-IMF line was influenced by stearic acid and PUFA percentages. Thus, selection for IMF changes the meat fatty acid composition and affects its quality.

Key words: Intramuscular fat, fatty acids, divergent selection, discriminant analysis.

INTRODUCTION

Intramuscular fat (IMF) is a key trait influencing nutritional, organoleptic and technological meat quality (Hernández and Dalle Zotte, 2010). Three selection experiments for IMF were performed in cattle (Sapp et al., 2002), chickens (Zhao et al., 2007) and pigs (Schwab et al., 2009). However, only in pigs, Burkett (2009) reported a correlated response to selection on the fatty acid composition. There is scarce information about the correlated response on the fatty acid composition due to selection for IMF in rabbits. A divergent selection experiment was carried out at the Universitat Politècnica de València, during nine generations to assess the genetic determinism of IMF and evaluate these correlated responses. The present study aimed to examine the relationship between IMF content and the fatty acids profile of *longissimus thoracis et lumborum* muscle (LM).

MATERIAL AND METHODS

Animals, intramuscular fat and fatty acid composition

The study was carried out on two rabbit lines divergently selected for IMF during nine generations. The base population consisted of 13 sires and 83 does. Details of the experiment were reported by Martínez-Álvaro et al. (2018). The present study was performed on 475 individuals from the ninth generation of selection (high-IMF line: 239 and low-IMF line: 236).

IMF content was measured in *longissimus thoracis et lumborum* muscle (LM) by near-infrared reflectance spectroscopy (NIRS), using the calibration equation developed by Zomeño et al. (2012). As a routine control, 20% of the total NIRS scanned samples were also chemically analysed (ether extraction with a previous acid hydrolysis). For the fatty acid composition, we used the chemical method described by O'Fallon et al. (2007) to obtain fatty acid methyl esters (FAME). After that, we

determined the fatty acid composition by gas chromatography (FOCUS, Thermo, Milan, Italy). The individual fatty acids were identified by comparing their retention times with standards of FAME supplied by Supelco (PA, USA) and quantified by using C13:0 as internal standard.
Fatty acid contents were obtained in g/100 g of LM, and then expressed in percentage of total fatty acids. 23 fatty acids were analysed, namely, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C16:1, C18:1n9, C18:1n7, C20:1, C22:1n9, C18:2n6, C18:3n6, C18:3n3, C20:2n6, C20:3n6, C20:3n3, C20:4n6, C20:5n3, C22:4n6, C22:5n3, C22:6n3.The SFA, MUFA, PUFA groups, and their related ratios were calculated.

Statistical analysis
The model used for analysing IMF and fatty acid composition was:

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

where **b** is the vector of the fixed effects (line, sex, parity order, season) and **c** is the vector of the random effect (common litter). **X** and **W** are incidence matrices. **e** is the residual term. Common litter random effects and residuals were assumed to be distributed as $\mathbf{c} \sim N(0, \mathbf{I}\sigma_c^2)$ and $\mathbf{e} \sim N(0, \mathbf{I}\sigma_e^2)$, respectively. **I** is the identity matrix, σ_c^2 is the common litter variance, and σ_e^2 is the residual variance. The effects were assumed to be independent between them. The fixed effects and variances priors were bounded uniform.

The response to selection was estimated as the phenotypic difference between high-IMF and low-IMF lines. Marginal posterior distributions were estimated by Gibbs sampling (Blasco, 2017). We used Monte Carlo Markov chains of 60,000 samples, with a burn-in period of 10,000, and a lag of 10. Bayesian inference was made from the marginal posterior distributions of the differences. It provided the median of the difference (D), HPD (95%), the probability (P_0) of D being greater than zero when $D > 0$ or lower than zero when $D < 0$, and the probability of relevance (Pr) defined as the probability of D being greater than a relevant value (R). The relevant value was assumed to be one-third of the phenotypic standard deviation of each trait. In all analyses, the Z criterion of Geweke did not detect a lack of convergence. Bayesian inference was performed with the Rabbit program developed by the Institute for Animal Science and Technology (Valencia, Spain).

For each individual, all data are non-negative and sum up to a constant (100%); thus, our data are compositional. We transformed data using the centred logratio transformation (CLR) (Greenacre, 2018). Thereby, each variable was referred to the geometric mean of all the variables. Furthermore, we performed a projection on latent structures discriminant analysis (PLS-DA) on the CLR transformed data using SIMCA software (Umetrics, Umea, Sweden). We considered the categorical variable, the line, as the response, and all the fatty acids as predictors. The objective of this analysis was to assess the relationships between the lines and all the fatty acids simultaneously.

RESULTS AND DISCUSSION

Descriptive statistics of the main individual fatty acids, the SFA, MUFA, PUFA groups, and the ratios are displayed in Table 1. The mean and residual standard deviation of IMF were 1.06 and 0.15 g/100g of LM respectively. The direct response to selection for the ninth generation was 0.51g/100 g of LM, representing 3.3 phenotypic standard deviation. Percentages of SFA and PUFA were 36.4% and 39.2%, respectively, while MUFA had a lower percentage (24.4%). The most abundant fatty acids were linoleic (C18:2n6), palmitic (C16:0) and oleic (C18:1n9) acids, representing 27.66%, 25.68% and 20.31% of total fatty acids, respectively. Ratios PUFA/SFA and MUFA/SFA were 1.08 and 0.67, respectively. These results are in line with previous results by (Martinez-Alvaro et al., 2018).

Phenotypic differences between high-IMF and low-IMF lines were relevant (Pr=1) for almost all the traits (Table 1). High-IMF line showed greater percentages of SFA and MUFA than low-IMF line, whereas PUFA percentage was greater in the low-IMF line. Conversely, α-linolenic (C18:3n3) percentage was greater in high-IMF line. Differences between lines for linolenic acid (C18:2n6) were not relevant. The former differences were expected, since the high-IMF line contains greater

proportion of triglycerides respect to phospholipids than the low-IMF line. In general, triglycerides fraction is rich in both MUFA and SFA, whereas phospholipid fraction is rich in PUFA. De Smet et al. (2004) also reported an increase of MUFA and SFA compared to PUFA with the increases of fatness.

Table 1: Descriptive statistics and differences between high-IMF and low-IMF lines for IMF (g/100g of muscle) and fatty acids (% of total fatty acids) of *longissimus thoracis et lumborum* muscle in the ninth generation.

Trait	Mean	SD	CV (%)	D	HPD (95%)	P_0	R	Pr
IMF	1.06	0.15	14.37	0.51	0.48 (0.55)	1.00	0.05	1
C16:0	25.68	1.17	4.57	2.90	2.65 (3.18)	1	0.39	1
C18:0	8.34	0.66	7.93	-2.29	-2.43 (-2.14)	1	0.22	1
SFA	36.39	0.94	2.59	1.71	1.50 (1.92)	1	0.31	1
C18:1n9c	20.31	0.88	4.31	2.15	1.95 (2.35)	1	0.29	1
MUFA	24.42	1.27	5.21	3.24	2.95 (3.54)	1	0.42	1
C18:2n6c	27.66	1.18	4.27	0.32	0.04 (0.57)	0.99	0.39	0.3
C18:3n3	1.16	0.14	12.03	0.60	0.57 (0.63)	1	0.05	1
C20:4n6	7.45	1.05	14.11	-4.50	-4.74 (-4.27)	1	0.35	1
PUFA	39.19	1.83	4.67	-4.96	-5.38 (-4.53)	1	0.61	1
PUFA/SFA	1.08	0.07	6.71	-0.19	-0.20 (-0.17)	1	0.02	1
MUFA/SFA	0.67	0.03	4.92	0.06	0.05 (0.07)	1	0.01	1

IMF= Intramuscular fat, SFA: Saturated fatty acids = C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0, MUFA: Monounsaturated fatty acids = C16:1 + C18:1n9c + C18:1n7 + C20:1 + C22:1n9, PUFA : Polyunsaturated fatty acids = C18:3n3 + C20:3n3 + C20:5n3 + C22:5n3 + C22:6n3+ C18:2n6c + C18:3n6 + C20:2n6 + C20:3n6 + C20:4n6 + C22:4n6, SD: standard deviation, CV: coefficient of variation, D: median of the marginal posterior distribution of the difference between high-IMF and low-IMF lines, HPD (0.95): highest posterior density region at 95%, P_0: probability of the difference being greater than zero when D>0 or lower than zero when D<0, R: Relevant value, proposed as 1/3 of the standard deviation of the trait, Pr: probability of relevance, probability of the difference being > R when D>0 or being < R when D<0.

The first latent variable (t_1), defined as a linear combination of the fatty acids, clearly separated the two lines (R^2=82%) (Figure 1). The PLS-DA score plot showed the projection of data on the two latent variables (t_1 and t_2) (R^2=84%). This projection explained 83% of the predicted variance from cross validation (Q^2). Besides, the loading plot showed how the variables relate to each other (figure 2). SFA and PUFA are negatively correlated. In addition, individual fatty acids within each group (SFA and PUFA) are related to each other. Most of the individual SFA were close to high-IMF line, and distant from low-IMF line, except the C18:0. In contrast, the individual PUFA were close to the low-IMF line and distant from high-IMF line, except the C18:3 that was close to the high-IMF line. These exceptions were expected, since in rabbits, C18:0 is more abundant in phospholipids than in triglycerides, unlike the other individual SFA, and C18:3 is greater in triglycerides than in phospholipids (Alasnier et al., 1996).

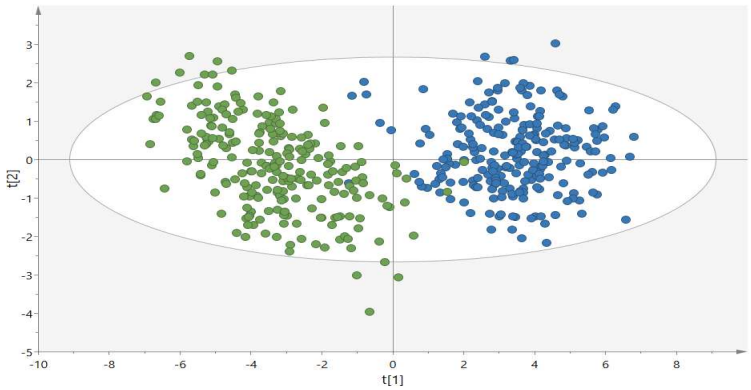

Figure 1: Score plot of PLS-DA. Projection of the observations in the plane defined by the latent variables (t_1, t_2). Each circle represents an observation. Colours: green, high-IMF line; blue, low-IMF line.

Figure 2: Loading plot of PLS-DA. Colours: green, predictors (fatty acids); red, response (line).

CONCLUSIONS

Selection for IMF content had substantial effects on the fatty acid composition. The increase of IMF content was associated with an increase of SFA and MUFA, and consequently the decrease of PUFA. The divergent lines were clearly separated by the PLS-DA. High-IMF line was mainly influenced by linolenic acid and SFA, whereas low-IMF line was influenced by stearic acid and PUFA.

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