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Genetic parameters and analysis of factors affecting variations between backfat and *Semimembranosus* muscle fatty acid composition in heavy pigs

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Abstract

This study was conceived to evaluate the variations between backfat (BF) and *Semimembranosus* (SM) muscle fatty acid (FA) composition in 789 heavy pigs and to estimate the genetic and phenotypic correlations and the heritability values of these variations. Most FAs showed a common genetic basis controlling their proportion in SM muscle and BF, while the *n*-6/*n*-3 ratio, α-linolenic and erucic acids displayed a genetic control more oriented towards tissue-specific molecular pathways. All variations between the FA composition of BF and SM muscle showed low-to-medium heritability values, suggesting that there are also genetic mechanisms capable of differentiating the deposition of FAs in BF from those in SM muscle. This result implies that a better knowledge of the genes differentiating the FA composition of BF and SM muscle could provide new tools allowing to select, in a partially independent manner, the FA composition of muscle and subcutaneous fat.

Keywords: genetic correlations; swine; heritability; meat quality.

1 Introduction

The lipid content and fatty acid (FA) composition in pig tissues are of paramount importance both from a technological and nutritional point of view (Raes, De Smet, & Demeyer, 2004; Wood et al., 2008). This is true especially in the Italian heavy pig production chain, where the carcass cuts are destined to long seasoning periods for Protected Denomination Origin (PDO) production (Catillo, Zappaterra, Lo Fiego, Steri, & Davoli, 2021; Lo Fiego, Santoro, Macchioni, & De Leonibus, 2005; Zappaterra et al., 2020). Italian PDO production is based on carcasses coming from heavy pigs slaughtered at 9 months of age, with an average live body weight ranging from 160-170 kg and

belonging to genetic types under the control of the national selection or from commercial hybrids considered as suitable for PDO products (Lo Fiego, Macchioni, Minelli, & Santoro, 2010).

The carcasses of heavy pigs have undergone significant changes in the last decades; the decrease of carcass fat and the increase of lean meat resulted in a marked reduction in fat content in the depot fat and an increase in polyunsaturated FA (PUFA) content (Wood & Enser, 1997; Andersen, 2000; Piedrafita, Christian, & Lonergan, 2001; Gandemer, 2002; Lo Fiego, Macchioni, Minelli, & Santoro, 2010). Negative correlations were observed between the backfat thickness (BFT), carcass fatness, and the content of intramuscular fat (IMF), with respect to the proportion of PUFAs, thus influencing also meat susceptibility to oxidation (Daza, Olivares, Latorre, Rey, Callejo, & Lopez-Bote, 2017; Maw, Fowler, Hamilton, & Petchey, 2003; Lo Fiego, Minelli, Volpelli, Ulrici, & Macchioni, 2016). In fact, the increased level of unsaturation causes a reduction in the oxidative stability of pork products (Sheard, Enser, Wood, Nute, Gill, & Richardson, 2000; Kouba, Enser, Whittington, Nute, & Wood, 2003). As result, rancidity-related off-odors and flavors may occur (Rhee, Ziprin, Ordonez, & Bohac, 1988) and shelf-life may be compromised (Amaral, da Silva, & Lannes, 2018). The FA composition of pork influences the final quality of hams, with effects, in particular, during the processing of dry-cured products, affecting their consistency, salt and water content, curing duration, and lipid oxidation (Lopez-Bote, 2000; Lopez-Bote, Rey, & Menoyo, 2004).

In pigs, FA composition and deposition of adipose and muscle tissues can be affected by many factors such as sex, weight and age at slaughter, livestock production system, genetics, and dietary components (Mourot & Hermier, 2001; De Smet, Raes & Demeyer, 2004; Lopez-Bote, Rey, & Menoyo, 2004; Wood et al., 2008; Duran-Montgé, Realini, Barroeta, Lizardo, & Esteve-Garcia, 2008). As far as fat deposition, adipose cells of muscle and subcutaneous fat tissue display significant morphological, developmental, and metabolic variations (Gardan, Gondret, & Louveau, 2006; Hausman et al., 2009; Komolka, Albrecht, Wimmers, Michal, & Maak, 2014). Marked differences exist in the number, size, and metabolic properties of adipocytes between different porcine tissues, and each fat depot shows specific growth, metabolic features, and genetic controls (Gardan, Gondret,

& Louveau, 2006; Komolka, Albrecht, Wimmers, Michal, & Maak, 2014). Furthermore, the development of intramuscular and subcutaneous fat was proved to be controlled, at least in part, by different factors (Kouba, Bonneau, & Noblet, 1999; Mourot & Kouba, 1999), and these two tissues were found to respond differently to dietary treatments (Doran, Moule, Teye, Whittington, Hallett, & Wood, 2006).

Among the pig fat depots, intramuscular adipose tissue is the latest developing adipose site, after subcutaneous and intermuscular fat (Mourot & Kouba, 1999; Gardan, Gondret, & Louveau, 2006; Gondret, Guitton, Guillerm-Regost, & Louveau, 2008; Hocquette, Gondret, Baéza, Médale, Jurie, & Pethick, 2010). Intramuscular adipocytes have specific metabolic features and in pigs show much lower capacity for synthesis and degradation of FAs compared to subcutaneous fat (Gardan, Gondret, & Louveau, 2006). In fact, whatever the age of pigs (i.e., from 80 to 210 days), Gardan, Gondret, & Louveau (2006) found that the expressions of genes involved in FA synthesis and lipogenesis (e.g. *Fatty Acid Synthase*, *Malic Enzyme*), triglyceride hydrolysis (e.g., *Lipoprotein Lipase*, *Hormone-Sensitive Lipase*), and transcriptional regulation of lipid metabolism (e.g., *Sterol Regulatory Element-Binding Protein-1*, *Peroxisome Proliferator-Activated Receptor-Gamma*) were significantly lower in intramuscular adipocytes than in subcutaneous tissue. Despite the lower rate of lipogenesis observed in muscle in comparison with subcutaneous fat deposition, *in situ de novo* FA synthesis directly and strongly contributes to the intramuscular FA composition (Corominas et al., 2013). Subcutaneous adipose tissue tends instead to respond more quickly to environmental stimuli, such as diet composition, (Nürnberg, Wegner, & Ender, 1998; Estany, Ros-Freixedes, Tor, & Pena, 2014) and is reported to be more prone to remodeling than IMF, in particular when animals are fed diets with high oleic acid, such as in Iberian heavy pigs feeding system (Segura et al., 2021).

The present research mainly deals with the study of the genetic and environmental aspects that influence the FA composition of backfat (BF) and *Semimembranosus* (SM) muscle in a population of Italian Large White (ILW) pigs. This study aimed at evaluating the differences of the individual FAs and FA classes between the two tissues, and at estimating the genetic and phenotypic correlations

and the heritability values of these differences. The description of the genetic and non-genetic factors involved in the variations between the FA composition of muscle and BF can contribute to a better understanding of the major biological processes and the main environmental conditions differentiating the development of fat deposition in the two considered tissues. The obtained results allowed us to identify which factors affect the variations between FAs in SM muscle and BF tissues. These results can be considered as a further element of deeper knowledge on the factors affecting FA composition of pig SM muscle and BF tissue, also given the role played by these traits in the quality of meat intended for PDO products.

2. Material and methods

2.1 Animals and phenotypes

A population of 789 sib-test ILW heavy pigs was utilized for the present study. The used animals were already included in Zappaterra, Catillo, Lo Fiego, Belmonte, Padalino, & Davoli (2022). These pigs were triplets of full sibs coming from 323 litters (87 boars x 371 sows) reared in a unique testing station of the Italian Pig Breeders National Association (Associazione Nazionale Allevatori Suini, ANAS, http://www.anas.it). During the testing period (lasting from 30-45 days of age to about 9 months of age), siblings were kept separated and fed the same diets. In the testing station, pigs are fed the same growing and finishing diets in order to avoid any possible effects related to diet composition. Finishing diet composition is reported in Supplementary Table S1. Pigs were slaughtered at an average final weight of 155 ± 6 kg on 26 different dates between 2011 and 2012 at the same commercial abattoir. The animals were handled and slaughtered in compliance with European rules on the protection of animals during transport and at slaughtering (Council Regulation (EC) No. 1/2005 and Council Regulation (EC) No. 1099/2009). As the used animals were Sib Test individuals and were slaughtered during routine assessments performed for ANAS Sib Test selection schemes, the present research did not need approval from a research ethics committee. All slaughter

procedures were monitored by the veterinary team appointed by the Italian Ministry of Health and were performed within the ANAS routine assessments. Sampling occurred with ANAS permission.

BF and SM muscle tissues were sampled on the splitting line from carcasses left sides. BF samples were collected at the level of BF maximum thickness, approximately between the fifth and the sixth lumbar vertebra, close to the point where the hind leg is separated from the rest of the carcass, wrapped in aluminum foil, immediately put in vacuum-sealed bags, frozen in liquid nitrogen, and kept at -80°C for further use. SM muscle samples were gathered from the distal part of the muscle and sampling was performed in the same location of the inner face of the thighs. Hot carcass weight (kg) and optical measures (expressed in mm) of loin and BF thicknesses were taken by Fat-O-Meat'er (FOM - CrometecGmbh, Lünen, Germany) between the third and fourth last ribs, 8 cm off the carcass midline. The measures of BF and loin thickness were used to estimate the percentage of carcass lean meat, and to subsequently grade carcasses in EUROP carcass grading, following EU Decision 2001/468/CE of June, 8th 2001 (European Commission, 2001). Furthermore, the weights (kg) of the head of the animals and of the belly and jowl cuts measured on the carcass left side were also recorded.

As reported in our previous paper (Davoli et al., 2016), IMF content was determined in the SM muscle by extraction with petroleum ether from 1 g fresh sample using an XT15 Ankom apparatus (Macedon, NY, USA), according to Official procedure AOCS Am 5-04 (AOAC, 2005). IMF was determined in % as g of IMF per 100 g of tissue.

For each pig, the FA compositions of BF and SM muscle tissues were determined as reported in our previous papers (Catillo, Zappaterra, Lo Fiego, Steri, & Davoli, 2021; Zappaterra, Catillo, Lo Fiego, Belmonte, Padalino & Davoli, 2022).

In details, the samples of BF, finely minced, were submitted to lipids extraction by chloroform:methanol mixture (2:1, v/v) (Carlo Erba Reagents, Milan, Italy) according to Folch, Lees, and Sloane Stanley (1957). Then 50 mg of extracted lipids were trans-esterified at room temperature for 5 min with 0.5 ml of sodium methoxide (0.5 N) in methanol. FA methyl-esters, extracted with 1 ml of hexane, were injected in a Gas-Chromatograph apparatus (GC 2010 plus, Shimadzu, Columbia,

MD, USA) equipped with a flame ionization detector and a high polar capillary column (SP 2560 100 m × 0.25 mm, Supelco, Bellefonte, PA, US). FA methyl-esters were identified by comparison with a commercial standard mix of FA methyl-esters (GLC-674 Nuchek, Elysian, MN, US) added with missing FA standards (Nuchek, Elysian, MN, US; Larodan, Solna, Sweden), so obtaining a complete standard set of 105 FA methyl-esters. For each FA methyl-ester, response factors to flame ionization detector and inter- and intra-assay coefficients of variation were calculated by using a reference standard butter (CRM 164, Community Bureau of Reference, Brussels, Belgium). Results were expressed as g FA per 100 g of total FA (i.e., percent fatty acid composition).

For SM FA determination the total muscle lipids destined for the gas-chromatographic analysis were extracted using a mixture of chloroform: methanol (2:1, v/v) (Carlo Erba Reagents, MI, Italy) according to Folch, Lees, and Sloane Stanley (1957). Methylation was performed with a 2 N solution of potassium hydroxide (KOH) in methanol (CH3OH) (Carlo Erba Reagents, Milan, Italy) according to Ficarra, Lo Fiego, Minelli, & Antonelli (2010). Tridecanoic acid (C13:0) (Larodan Fine Chemicals AB, Solna, Sweden) was used as internal standard in SM FA determination. Intramuscular fatty acid methyl esters (FAMEs) were then submitted to gas-chromatographic analysis using TRACE™GC Ultra (Thermo Electron Corporation, Rodano, MI, Italy) equipped with a Flame Ionization Detector, a PVT injector, and a TR-FAME Column 30 m × 0.25 mm i.d., 0.2 μm film thickness (Thermo Scientific, Rodano, MI, Italy). The Chrom-Card software (vers.2.3.3, Thermo Electron Corporation, Rodano, MI, Italy) was used to record and integrate the peaks of FAMEs. Individual FAME were identified by comparing their retention times with the retention times of a standard FAME mixture prepared in-house with known quantities of each methyl ester (Larodan Fine Chemicals AB, Solna, Sweden). In order to present data in the same way as BF, the amount of each FA determined in SM was reported as g FA per 100 g of total FA (i.e., percent FA composition).

2.2 Statistical analysis

2.2.1 Data handling

The continuous variables of BFT and IMF were divided into quartile classes using the UNIVARIATE procedure of SAS software vers. 9.4. Normality was tested for both traits with the Shapiro-Wilk test. For BFT the following classes were defined: carcasses with a BFT < 23 mm, from 23 to 27 mm, from 27 to 30 mm, and BFT > 30 mm. For IMF the quartiles were: samples with IMF < 1.24%, from 1.24% to 1.72%, from 1.72% to 2.63%, and with IMF > 2.63%. BFT and IMF quartiles were then used as independent variables in the statistical analyses.

The 22 slaughter days were grouped in four seasons (spring, summer, autumn, and winter) in order to verify the relative importance of seasonal effects over the effects of random variables (i.e. handling, personnel).

Furthermore, BF and SM muscle FA compositions were used to obtain a new set of variables calculated as the difference between each FA or FA class in BF (%) and the same FA or FA class in SM muscle tissue (%). For example, for each sample, the % of capric acid (C10:0) in SM muscle was subtracted from the % of C10:0 measured in BF tissue. From this point onward, these variables will be referred to as Δ followed by the name of the individual FA or FA class (e.g., ΔC10:0; ΔC12:0; etc…).

2.2.2 Descriptive statistics, phenotypic and genetic correlations between the two tissues

First, a linear Analysis of Variance (Anova) was used to compare the FA composition of each sample for both tissues. This analysis was performed with ANOVA procedure of SAS software vers. 9.4, with the tissue (i.e., BF and SM muscle) as fixed effect. The used model was the following:

$y_{ij} = \mu + T_i + e_{ij}$

Where: y_{ij} was the vector of the percentage of each FA or FA class; μ was the overall mean; T_i was the fixed effect of the tissue (i.e., BF or SM muscle), and e_{ij} was the vector of residual errors.

Furthermore, the phenotypic and genetic correlations between the amount of each FA or FA class in the two tissues were estimated by restricted maximum likelihood (REML) methodology using the VCE software system version 6 (Groeneveld, Kovač, & Mielenz, 2010) and were carried out by mixed bi-varied animal models, as follows:

$$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} X_1 & 0 \\ 0 & X_2 \end{bmatrix} \begin{bmatrix} b_1 \\ b_2 \end{bmatrix} + \begin{bmatrix} Z_1 & 0 \\ 0 & \mathrm{Z}_2 \end{bmatrix} \begin{bmatrix} a_1 \\ a_1 \end{bmatrix} + \begin{bmatrix} W_1 & 0 \\ 0 & W_2 \end{bmatrix} \begin{bmatrix} d_1 \\ d_2 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \end{bmatrix}$$

Where: y_1 and y_2 are the vectors of phenotypic observations for the traits 1 and 2; b_1 and b_2 are the vector of fixed effects (found to be significant for each trait): the fixed factor of slaughter season (i.e., 4 levels); the fixed factor of sex with two levels (i.e. barrows and gilts); the fixed effect of the BFT classes (4 levels); the fixed effect of IMF classes (4 levels); the fixed effect of EUROP carcass grading classes (4 levels); a_1 and a_2 are the vectors of additive genetic effects for the two traits; d_1 and d_2 are the vectors of the random effect of litter for the two traits (323 levels), and e_1 and e_2 are the vectors of random errors; X_1 and X_2 are the matrices of incidence of n × p order associating each observation (n) to the pertinent level of slaughter season, sex, BFT classes, IMF classes, and EUROP grading (p); W_1 and W_2 are the matrices of incidence of n × q order associating each observation (n) to the pertaining litter level (q); Z_1 and Z_2 are the matrices of incidence of n × s order associating each observation (n) to each animal (s).

Variance-covariance matrices between the amount of a FA in BF and SM were as follows:

Genetic: $\mathrm{Var}\begin{bmatrix} a_1 \\ a_2 \end{bmatrix} = \begin{bmatrix} \mathrm{A}g_{11} & \mathrm{A}g_{12} \\ \mathrm{A}g_{21} & \mathrm{A}g_{22} \end{bmatrix}$

Litter: $\mathrm{Var}\begin{bmatrix} d_1 \\ d_2 \end{bmatrix} = \begin{bmatrix} \mathrm{I}q_{11} & \mathrm{I}q_{12} \\ \mathrm{I}q_{21} & \mathrm{I}q_{22} \end{bmatrix}$

Residual: $\mathrm{Var}\begin{bmatrix} e_1 \\ e_2 \end{bmatrix} = \begin{bmatrix} \mathrm{I}s_{11} & \mathrm{I}s_{12} \\ \mathrm{I}s_{21} & \mathrm{I}s_{22} \end{bmatrix}$

It was assumed that:

$$\mathrm{Var}\begin{bmatrix} a_1 \\ a_2 \\ d_1 \\ d_2 \\ e_1 \\ e_2 \end{bmatrix} = \begin{bmatrix} \mathbf{A}g_{11} & \mathbf{A}g_{12} & 0 & 0 & 0 & 0 \\ \mathbf{A}g_{21} & \mathbf{A}g_{22} & 0 & 0 & 0 & 0 \\ 0 & 0 & \mathbf{I}q_{11} & \mathbf{I}q_{12} & 0 & 0 \\ 0 & 0 & \mathbf{I}q_{21} & \mathbf{I}q_{22} & 0 & 0 \\ 0 & 0 & 0 & 0 & \mathbf{I}s_{11} & \mathbf{I}s_{12} \\ 0 & 0 & 0 & 0 & \mathbf{I}s_{21} & \mathbf{I}s_{22} \end{bmatrix}$$

Where: g_{11} was the additive genetic variance for the direct effect for trait 1; g_{12} was equal to g_{21} and was the additive genetic covariance between the two traits; g_{22} was the additive genetic variance for direct effect for trait 2; q_{11}, q_{12}, q_{21}, and q_{22} were the variance and covariance matrices for the litter effect; s_{11}, s_{12}, s_{21} and s_{22} were the variance and covariance matrices for the residual effect; A was the relationship matrix between all animals. Matrix A included information on a three-generation pedigree for a total of 2318 animals, 623 males and 1695 females; I is identity matrix for litter and residual variance and covariance.

The genetic correlation for trait 1 vs 2 (the percentage of a FA in BF and in SM) was calculated as:

$r_g = g_{12}/(\sqrt{g_{11}} * g_{22})$

2.2.3 Descriptive statistics and genetic parameters for the differences between backfat and Semimembranosus muscle FA composition

The Δs (i.e., the differences for each% FA or FA class obtained subtracting their amount in SM muscle from that in BF) were investigated with an Anova to identify the environmental parameters affecting their variability. This analysis was performed with ANOVA procedure of SAS software vers. 9.4, considering the fixed effects of season, BFT classes, IMF classes, EUROP classes, and sex. The used model was the following:

$y = \mu + SEASON + SEX + BFT + IMF + EUROP + e$

Where y is the observation vector for the *i*th trait; μ was the overall mean; SEASON was the fixed factor of the slaughter season (i.e., 4 levels); SEX is the fixed factor of sex with two levels (i.e. barrows and gilts); BFT was the fixed effect of the BFT classes (4 levels); IMF was the fixed effect of IMF classes (4 levels); EUROP was the fixed effect of carcass grading classes (4 levels); *e* was

the vector of random residuals. The association between each ΔFA and the fixed effects was estimated with the F-value and the *P*-value. Orthogonal contrasts were constructed for each fixed effect to compare the ΔFA mean values between the fixed effect classes. In particular, spring-summer *vs*. autumn-winter were compared for the slaughter season; samples with a BFT < 27 mm *vs.* those with BFT > 27 mm were compared for the BFT effect; samples with an IMF < 1.72% *vs.* those with IMF > 1.72% mm were compared for the IMF effect; E and U carcasses *vs.* R and O ones were compared for the EUROP carcass grading fixed effect. The ΔFA mean values were also compared between gilts and barrows with Student's t-test.

The ΔFA data set was also used to estimate genetic parameters, such as variance components,heritability and standard error (SE), and the genetic correlations and the relative SE among ΔFA components. Estimates were calculated by REML methodology using the VCE software system version 6 (Groeneveld, Kovač, & Mielenz, 2010) and were carried out by two multiple trait animal models: one involving the measures recorded at slaughtering added with Δs of individual FAs (number of dependent variables = 27), the other involving the same measurements at slaughtering (i.e., hot carcass weight, loin thickness, belly weight, jowl weight, head weight and carcass lean meat percentage) added with Δs of FA classes (number of dependent variables = 12). The multiple trait animal models are mixed infinitesimal models where Δs of all individual FA or FA classes were fitted together. The used model was the following:

$$\begin{bmatrix} y_1 \\ \vdots \\ y_i \end{bmatrix} = \begin{bmatrix} X_1 & \cdots & 0 \\ \vdots & \ddots & \vdots \\ 0 & \cdots & X_i \end{bmatrix} \begin{bmatrix} b_1 \\ \vdots \\ b_i \end{bmatrix} + \begin{bmatrix} Z_1 & \cdots & 0 \\ \vdots & \ddots & \vdots \\ 0 & \cdots & Z_i \end{bmatrix} \begin{bmatrix} a_1 \\ \vdots \\ a_i \end{bmatrix} + \begin{bmatrix} W_1 & \cdots & 0 \\ \vdots & \ddots & \vdots \\ 0 & \cdots & W_i \end{bmatrix} \begin{bmatrix} d_1 \\ \vdots \\ d_i \end{bmatrix} + \begin{bmatrix} e_1 \\ \vdots \\ e_i \end{bmatrix}$$

where: y_i is the vector of phenotypic observation for the *i*th trait (*i* = 27: measurements at slaughtering added with Δs of FAs and *i* = 12: measurements at slaughtering added with Δs of FA classes); b_i was the vector of fixed effects (found to be significant for each trait): the fixed factor of slaughter season (i.e., 4 levels); the fixed factor of sex with two levels (i.e. barrows and gilts); the fixed factor of the BFT classes (4 levels); the fixed factor of IMF classes (4 levels); the fixed factor of EUROP carcass grading classes (4 levels); a_i was the vector of additive genetic effects, d_i was the vector of the random

effect of the litter for the *i*th trait (323 levels) and e_i was the vector of random error; X_i was the matrix of incidence of n × p order associating each observation (n) to the pertinent level of slaughter season, sex, BFT classes, IMF classes, and EUROP grading (p); W_i was the matrix of incidence of n × q order associating each observation (n) to the pertaining litter level (q); Z_i was the matrix of incidence of n × s order associating each observation (n) to each animal (s).

Variance-covariance matrices between the Δs were as follows:

Genetic: $\mathrm{Var}\begin{bmatrix} a_1 \\ \cdot \\ \cdot \\ a_i \end{bmatrix} = \begin{bmatrix} \mathrm{A}g_{11} \,..\, \mathrm{A}g_{ij} \\ \cdot \\ \cdot \\ \mathrm{A}g_{ji} \,..\, \mathrm{A}g_{ii} \end{bmatrix}$, with $g_{ij} = g_{ji}$

Litter: $\mathrm{Var}\begin{bmatrix} d_1 \\ \cdot \\ \cdot \\ d_i \end{bmatrix} = \begin{bmatrix} \mathrm{I}q_{11} \,..\, \mathrm{I}q_{ij} \\ \cdot \\ \cdot \\ \mathrm{I}q_{ji} \,..\, \mathrm{I}q_{ii} \end{bmatrix}$, with $q_{ij} = q_{ji}$

Residual: $\mathrm{Var}\begin{bmatrix} e_1 \\ \cdot \\ \cdot \\ e_i \end{bmatrix} = \begin{bmatrix} \mathrm{I}s_{11} \,..\, \mathrm{I}s_{ij} \\ \cdot \\ \cdot \\ \mathrm{I}s_{ji} \,..\, \mathrm{I}s_{ii} \end{bmatrix}$, with $s_{ij} = s_{ji}$

It was assumed that:

$$\mathrm{Var}\begin{bmatrix} a_1 \\ \vdots \\ a_i \\ d_1 \\ \vdots \\ d_i \\ e_1 \\ \vdots \\ e_i \end{bmatrix} = \begin{bmatrix} \mathrm{A}g_{11} & \cdots & \mathrm{A}g_{ij} & 0 & \cdots & 0 & 0 & \cdots & 0 \\ \vdots & \cdots & \vdots & \vdots & \cdots & \vdots & \vdots & \cdots & \vdots \\ \mathrm{A}g_{ji} & \cdots & \mathrm{A}g_{ii} & 0 & \cdots & 0 & 0 & \cdots & 0 \\ 0 & \cdots & 0 & \mathrm{I}q_{11} & \cdots & \mathrm{I}q_{ij} & 0 & \cdots & 0 \\ \vdots & \cdots & \vdots & \vdots & \cdots & \vdots & \vdots & \cdots & \vdots \\ 0 & \cdots & 0 & \mathrm{I}q_{ji} & \cdots & \mathrm{I}q_{ii} & 0 & \cdots & 0 \\ 0 & \cdots & 0 & 0 & \cdots & 0 & \mathrm{I}s_{11} & \cdots & \mathrm{I}s_{ij} \\ \vdots & \cdots & \vdots & \vdots & \cdots & \vdots & \vdots & \cdots & \vdots \\ 0 & \cdots & 0 & 0 & \cdots & 0 & \mathrm{I}s_{ji} & \cdots & \mathrm{I}s_{ii} \end{bmatrix}$$

where: g_{11}, g_{ij}, g_{ji}, and g_{ii} were elements of G, the additive genetic variance and covariance matrix for the animal effect; q_{11}, q_{ij}, q_{ji}, and q_{ii} were elements of Q, the variance and covarince matrix for the litter effect; s_{11}, s_{ij}, s_{ji}, and s_{ii} were the elements of S, the variance and covariance matrix for the residual effect; A was the relationship matrix as previously defined; I was the identity matrix for litter and residual variance and covariance.

Genetic correlation for all traits were estimated as follows: $r_g = g_{ij}/(\sqrt{g_{ii}}*g_{jj})$

The heritability value for each ΔFA or ΔFA class was estimated as the ratio of the genetic variance to the phenotypic variance (genetic + environmental): $h^2 = g_{ii}/(g_{ii}+s_{ii})$.

For both models (bivariate and multivariate), the VCE software concluded the process with a state equal to 1, so the first derivative is effectively equal to zero, which indicates that convergence has been met and the estimated components are optimal.

A *P*-value less than 0.05 was chosen as the threshold for statistical significance.

3. Results

3.1 Descriptive statistics and genetic correlations between the FA composition of the two tissues

The descriptive statistics of the FAs and FA classes measured in the BF and SM muscle tissues of the 789 ILW pigs are reported in Table 1. As can be noticed, the most abundant FAs in both tissues were oleic, palmitic, stearic, and linoleic acids. Except for lauric acid, all FAs and FA classes showed significant differences in their amounts between the two tissues. SM muscle tissue showed greater amounts of capric (C10:0), myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1 *cis*-9), heptadecenoic (C17:1 *cis*-9), oleic (C18:1 *cis*-9), *cis*-vaccenic (C18:1 *cis*-11), dihomo-γ-linolenic (C20:3 *n*-6), arachidonic (C20:4 *n*-6), adrenic (C22:4 *n*-6), docosapentaenoic (DPA; C22:5 *n*-3), and docosahexaenoic (C22:6 *n*-3; DHA) acids. These differences resulted in a higher proportion of MUFAs and in lower proportions of SFAs and PUFAs (both *n*-6 and *n*-3 PUFAs) in muscle. The *n*-6/*n*-3 ratio was instead higher in SM compared to BF.

Table 1. Descriptive statistics for the percentages of fatty acids (FAs) in backfat (BF) and *Semimembranosus* (SM) muscle. For each tissue are presented the mean, standard deviation (S.D.), minimum (Min), maximum (Max), and the ANOVA *P*-value of the comparison for the % of each FA or FA classes between the two tissues.

	BF			**SM**			
FA (%)	**Mean ± S.D.**	**Min**	**Max**	**Mean ± S.D.**	**Min**	**Max**	***P-value***
C10:0 (capric acid)	0.057 ± 0.014	0.028	0.135	0.130 ± 0.027	0.067	0.296	<.0001
C12:0 (lauric acid)	0.105 ± 0.033	0.043	0.237	0.104 ± 0.020	0.054	0.191	0.375
C14:0 (myristic acid)	1.221 ± 0.160	0.836	1.720	1.358 ± 0.152	0.867	1.856	<.0001
C16:0 (palmitic acid)	22.546 ± 1.211	18.407	27.232	23.512 ± 1.139	20.085	26.744	<.0001
C17:0 (margaric acid)	0.200 ± 0.042	0.107	0.371	0.155 ± 0.031	0.086	0.274	<.0001
C18:0 (stearic acid)	13.383 ± 1.767	8.594	18.946	11.885 ± 1.192	8.556	18.823	<.0001
C20:0 (arachidic acid)	0.191 ± 0.040	0.076	0.380	0.156 ± 0.034	0.014	0.346	<.0001
C16:1 *cis*-9 (palmitoleic acid)	1.453 ± 0.254	0.818	2.393	2.928 ± 0.486	1.481	4.472	<.0001
C17:1 *cis*-9 (heptadecenoic acid)	0.153 ± 0.034	0.070	0.308	0.232 ± 0.054	0.117	0.479	<.0001
C18:1 *cis*-9 (oleic acid)	38.884 ± 1.586	30.528	43.824	40.722 ± 2.892	27.895	48.691	<.0001
C18:1 *cis*-11 (*cis*-vaccenic acid)	2.262 ± 0.235	1.575	2.998	3.932 ± 0.440	2.265	5.269	<.0001
C20:1 *cis*-11 (gadoleic acid)	0.839 ± 0.218	0.398	2.042	0.685 ± 0.101	0.022	1.148	<.0001

C22:1 (erucic acid)	0.128 ± 0.021	0.068	0.261	0.019 ± 0.011	0.000	0.107	<.0001
C18:2 *cis*-9, *cis*-12 (linoleic acid)	16.528 ± 2.037	11.539	24.146	10.830 ± 2.093	6.278	21.133	<.0001
C18:3 *n*-3 (α-linolenic acid)	0.762 ± 0.179	0.383	1.720	0.373 ± 0.086	0.023	0.687	<.0001
C20:2 *n*-6 (eicosadienoic acid)	0.803 ± 0.101	0.525	1.242	0.450 ± 0.075	0.275	0.693	<.0001
C20:3 *n*-6 (dihomo-γ-linolenic acid)	0.087 ± 0.016	0.045	0.171	0.228 ± 0.102	0.000	0.706	<.0001
C20:4 *n*-6 (arachidonic acid)	0.230 ± 0.047	0.084	0.495	1.875 ± 0.928	0.461	7.763	<.0001
C22:4 *n*-6 (adrenic acid)	0.097 ± 0.020	0.048	0.385	0.289 ± 0.115	0.094	0.888	<.0001
C22:5 *n*-3 (docosapentaenoic acid-DPA)	0.055 ± 0.012	0.008	0.101	0.109 ± 0.045	0.027	0.613	<.0001
C22:6 *n*-3 (docosahexaenoic acid-DHA)	0.014 ± 0.007	0.003	0.111	0.029 ± 0.015	0.001	0.228	<.0001
SFAs (Saturated fatty acids)	37.704 ± 2.542	30.417	44.605	37.300 ± 2.031	31.940	47.893	<.0005
MUFAs (Monounsaturated fatty acids)	43.720 ± 1.831	33.962	49.167	48.518 ± 3.288	34.086	56.823	<.0001
PUFAs (Polyunsaturated fatty acids)	18.576 ± 2.187	13.344	26.812	14.182 ± 3.120	7.581	32.054	<.0001
n-6 PUFAs (*n*-6 Polyunsaturated fatty acids)	17.745 ± 2.117	12.588	25.593	13.672 ± 3.034	7.287	30.870	<.0001
n-3 PUFAs (*n*-3 Polyunsaturated fatty acids)	0.831 ± 0.184	0.428	1.763	0.510 ± 0.114	0.177	1.184	<.0001
n-6/*n*-3	22.121 ± 3.966	7.815	45.605	27.160 ± 4.811	13.912	85.262	<.0001

Table 2 shows the phenotypic and genetic correlations between the amounts of FAs or FA classes in BF and in SM muscle. Significant genetic correlations between the two tissues were noticed for all FAs and FA classes except for α-linolenic acid. The genetic correlations were generally higher and more significant than the phenotypic ones, with lauric (C12:0), margaric (C17:0), stearic (C18:0), palmitoleic, heptadecenoic, oleic (C18:1 *cis*-9), *cis*-vaccenic, gadoleic (C20:1 *cis*-11), erucic (C22:1), arachidonic, DPA, and DHA acids showing genetic correlation coefficients (r_g) > 0.80 and *P*-values < 0.0001. The strongest negative genetic correlation was noticed for the *n*-6/*n*-3 ratio, followed by erucic and capric acids.

Table 2. Phenotypic (r) and genetic (r_g) correlations with the relative standard errors (SE) between fatty acids (FAs) and FA classes in the backfat and *Semimembranosus* muscle tissues.

FAs (%)	**Phenotypic correlations**			**Genetic correlations**		
	r	**SE**	***P-value***	**r_g**	**SE**	***P-value***
C10:0 (capric acid)	0.099	0.035	0.005	-0.154	0.035	<.0001
C12:0 (lauric acid)	0.213	0.035	<.0001	0.963	0.010	<.0001
C14:0 (myristic acid)	0.310	0.034	<.0001	0.581	0.029	<.0001
C16:0 (palmitic acid)	0.461	0.032	<.0001	0.651	0.027	<.0001
C17:0 (margaric acid)	0.419	0.032	<.0001	0.835	0.020	<.0001
C18:0 (stearic acid)	0.502	0.031	<.0001	0.927	0.013	<.0001
C20:0 (arachidic acid)	-0.048	0.036	0.174	0.249	0.035	<.0001
C16:1 *cis*-9 (palmitoleic acid)	0.461	0.032	<.0001	0.855	0.018	<.0001
C17:1 *cis*-9 (heptadecenoic acid)	0.216	0.035	<.0001	0.917	0.014	<.0001
C18:1 *cis*-9 (oleic acid)	0.261	0.034	<.0001	0.824	0.020	<.0001

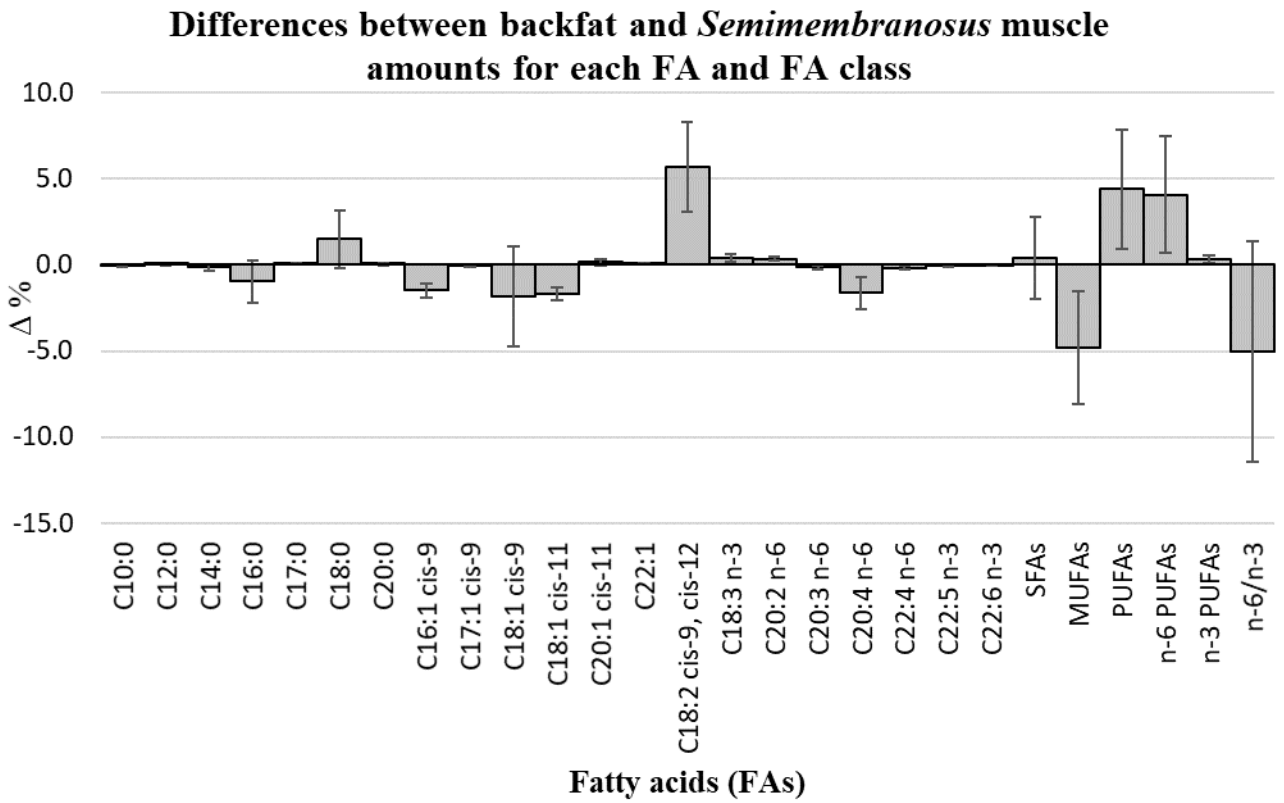
C18:1 *cis*-11 (*cis*-vaccenic acid)	0.247	0.035	<.0001	0.975	0.008	<.0001
C20:1 *cis*-11 (gadoleic acid)	0.200	0.035	<.0001	0.912	0.015	<.0001
C22:1 (erucic acid)	0.067	0.036	0.061	-0.225	0.035	<.0001
C18:2 *cis*-9, *cis*-12 (linoleic acid)	0.474	0.031	<.0001	0.442	0.032	<.0001
C18:3 *n*-3 (α-linolenic acid)	0.374	0.033	<.0001	-0.018	0.036	0.500
C20:2 *n*-6 (eicosadienoic acid)	0.016	0.036	0.654	0.574	0.029	<.0001
C20:3 *n*-6 (dihomo-γ-linolenic acid)	0.363	0.033	<.0001	0.578	0.029	<.0001
C20:4 *n*-6 (arachidonic acid)	0.130	0.035	0.0002	0.999	0.002	<.0001
C22:4 *n*-6 (adrenic acid)	0.128	0.035	0.0003	0.684	0.026	<.0001
C22:5 *n*-3 (docosapentaenoic acid-DPA)	0.048	0.036	0.179	0.999	0.002	<.0001
C22:6 *n*-3 (docosahexaenoic acid-DHA)	0.200	0.035	<.0001	0.999	0.002	<.0001
SFAs (Saturated fatty acids)	0.467	0.032	<.0001	0.893	0.016	<.0001
MUFAs (Monounsaturated fatty acids)	0.279	0.034	<.0001	0.837	0.020	<.0001
PUFAs (Polyunsaturated fatty acids)	0.176	0.035	<.0001	0.495	0.031	<.0001
n-6 PUFAs (*n*-6 Polyunsaturated fatty acids)	0.173	0.035	<.0001	0.462	0.032	<.0001

n-3 PUFAs (*n*-3 Polyunsaturated fatty acids)	0.038	0.036	0.2821	0.305	0.034	<.0001
n-6/*n*-3	-0.048	0.036	0.1746	-0.588	0.029	<.0001

3.2 Descriptive statistics and genetic parameters for the differences between backfat and IMF FAs and FA classes

The means, standard deviations, minimum and maximum values for the obtained Δs are reported in Supplementary Table S2. Means and standard deviations for the Δs are graphically displayed in Figure 1. As can be noticed from Figure 1, Δs were on average positive for lauric, margaric, stearic, arachidic, gadoleic, erucic, linoleic, α-linolenic, eicosadienoic, SFAs, and PUFAs. In particular, the highest mean values were noticed for linoleic acid (5.70 ± 2.61%), PUFAs (4.39 ± 3.48%), and *n*-6 PUFAs (4.07 ± 3.39%), while the lowest Δs were observed for *n*-6/*n*-3 ratio (-5.04 ± 6.38%), MUFAs (-4.80 ± 3.29%), and oleic acid (-1.84 ± 2.93%). Comparing Δs with the average percentages of the relative FAs found in the two tissues, the FAs that showed the greatest difference in relative terms was erucic acid, which had a five-times higher amount in BF when compared with SM, followed by α-linolenic acid, with a percentage in BF that was double the percentage in SM.

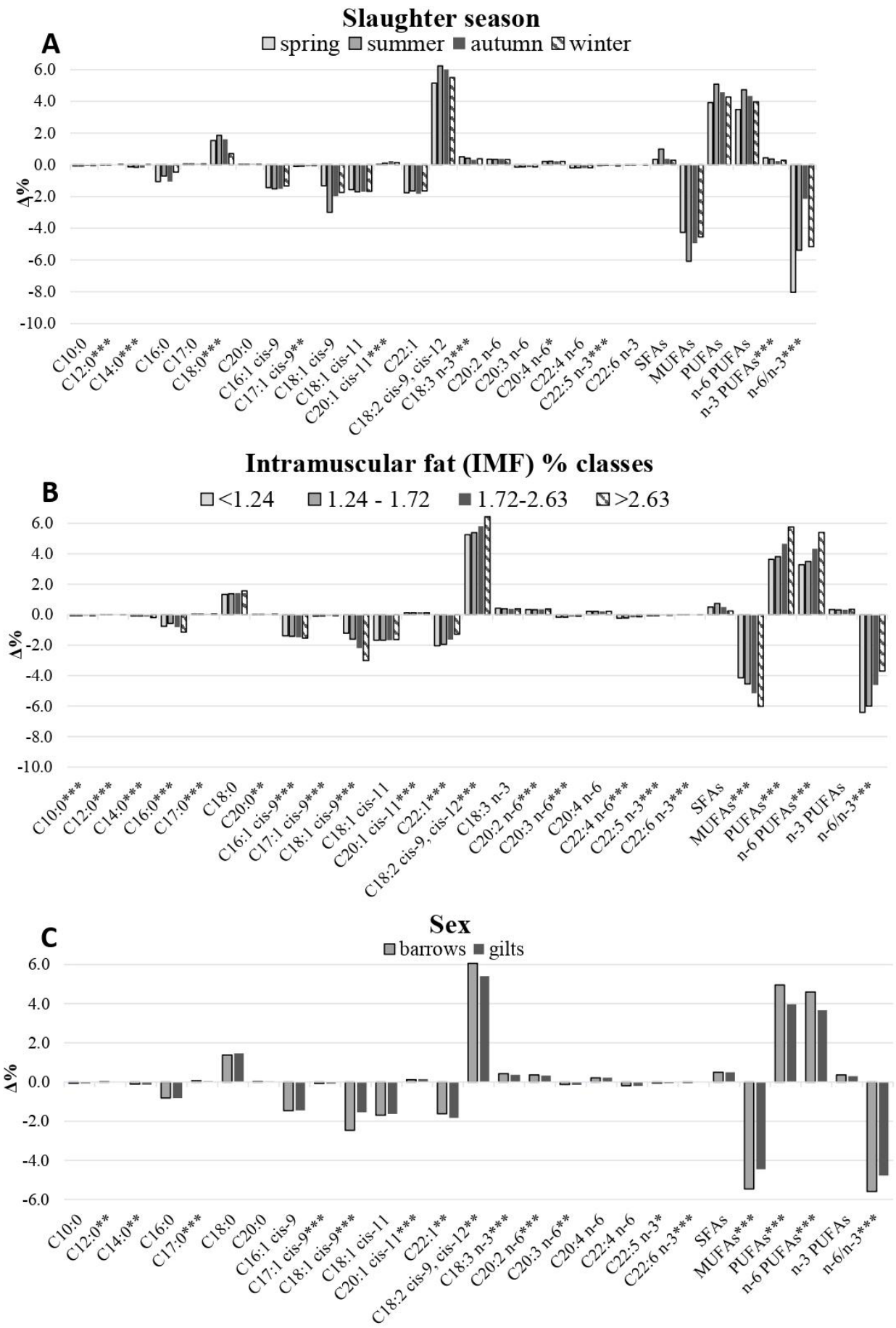
Figure 1. Means and standard deviations (error bars) of percent differences (Δ%) between the fatty acids (FAs) and FA classes in backfat (BF) and *Semimembranosus* (SM) muscle.



The results of the ANOVA performed on Δs to investigate the effects of slaughter season, BFT classes, IMF classes, EUROP carcass grading, and animals' sex are reported in Supplementary Table S3. Slaughter season, IMF classes, and animals' sex showed to be the variables affecting the most the variability noticed among the samples for Δs. The Least Squares Means (L.S.M.) of Δs for slaughter season, IMF classes, and animals' sex are graphically presented in Figure 2. Slaughter season showed to affect almost all Δs, except those for margaric, erucic, dihomo-γ-linolenic, and adrenic acids (Supplementary Table S3). Supplementary Table S4 shows the results of the orthogonal contrasts for slaughter season. The animals slaughtered during autumn-winter had higher Δ values for lauric, myristic, heptadecenoic acids, and *n*-6/*n*-3 ratio, while those slaughtered in spring-summer had higher Δ values for stearic, gadoleic, α-linolenic (C18:3 *n*-3), DPA acids and *n*-3 PUFAs (Figure 2A and Supplementary Table S4). IMF classes were associated with changes in almost all Δs, except those for stearic, *cis*-vaccenic, α-linolenic, arachidonic acids, SFAs and *n*-3 PUFAs (Figure 2B and Supplementary Table S3). Supplementary Table S5 shows the results of the orthogonal contrasts for IMF classes. The classes of IMF > 1.72% showed significantly lower

values of L.S.M. for the Δs of capric, lauric, myristic, palmitic, palmitoleic, oleic acids, and MUFAs when compared with IMF < 1.72% classes (Supplementary Table S5). The differences noticed for the Δs between pigs with IMF > 1.72% and animals with IMF < 1.72% indicate that higher contents of IMF are associated with an increase of capric, lauric, myristic, palmitic, palmitoleic, oleic acids, and MUFAs in muscle but not in backfat. Also, animals' sex showed to be an important factor affecting Δs for lauric, myristic, margaric, heptadecenoic, oleic, erucic, linoleic (C18:2 *cis*-9, *cis*-12), α-linolenic acid, eicosadienoic (C20:2 *n*-6), dihomo-γ-linolenic, DPA, DHA acids, MUFAs, and PUFAs (Supplementary Table S3). Supplementary Table S6 shows the results of the comparisons between the L.S.M. of the Δs in barrows and gilts. When compared with barrows, gilts had lower Δs for lauric, myristic, margaric, heptadecenoic, erucic, linoleic, α-linolenic acid, eicosadienoic, dihomo-γ-linolenic, DPA, DHA acids, and PUFAs (Supplementary Table S6), indicating that gilts tend to store higher % of these FAs in muscle but not in backfat.

Figure 2. Least Squares Means (L.S.M.) of percent differences (Δ%) between the fatty acids (FAs) and FA classes in backfat (BF) and *Semimembranosus* (SM) muscle for slaughter season (A), intramuscular fat (IMF) content % classes (B), and sex (C). FAs with stars are those showing significant differences for the comparisons (i.e., * means *P*-value < 0.05; ** *P*-value < 0.01; *** *P*-value < 0.001).


Slaughter season
A
spring
summer
autumn
winter
Δ%
6.0
4.0
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
C10:0
C12:0***
C14:0***
C16:0
C17:0
C18:0***
C20:0
C16:1 cis-9
C17:1 cis-9**
C18:1 cis-9
C18:1 cis-11
C20:1 cis-11***
C22:1
C18:2 cis-9, cis-12
C18:3 n-3***
C20:2 n-6
C20:3 n-6
C20:4 n-6*
C22:4 n-6
C22:5 n-3***
C22:6 n-3
SFAs
MUFAs
PUFAs
n-6 PUFAs
n-3 PUFAs***
n-6/n-3***
Intramuscular fat (IMF) % classes
B
<1.24
1.24 - 1.72
1.72-2.63
>2.63
Δ%
6.0
4.0
2.0
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
C10:0***
C12:0***
C14:0***
C16:0***
C17:0***
C18:0
C20:0**
C16:1 cis-9***
C17:1 cis-9***
C18:1 cis-9***
C18:1 cis-11
C20:1 cis-11***
C22:1***
C18:2 cis-9, cis-12***
C18:3 n-3
C20:2 n-6***
C20:3 n-6***
C20:4 n-6
C22:4 n-6***
C22:5 n-3***
C22:6 n-3***
SFAs
MUFAs***
PUFAs***
n-6 PUFAs***
n-3 PUFAs
n-6/n-3***
Sex
C
barrows
gilts
Δ%
6.0
4.0
2.0
0.0
-2.0
-4.0
-6.0
C10:0
C12:0**
C14:0**
C16:0
C17:0***
C18:0
C20:0
C16:1 cis-9
C17:1 cis-9***
C18:1 cis-9***
C18:1 cis-11
C20:1 cis-11***
C22:1**
C18:2 cis-9, cis-12**
C18:3 n-3***
C20:2 n-6***
C20:3 n-6**
C20:4 n-6
C22:4 n-6
C22:5 n-3*
C22:6 n-3***
SFAs
MUFAs***
PUFAs***
n-6 PUFAs***
n-3 PUFAs
n-6/n-3***

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BFT classes and EUROP carcass grading showed to affect to a lesser extent the Δs, with BFT being associated with changes in Δs for stearic, palmitoleic, *cis*-vaccenic, linoleic, arachidonic acids, and SFAs, and EUROP carcass grading was only associated with arachidonic acid (ΔC20:4 *n*-6; Supplementary Table S3). In particular, the orthogonal contrasts reported in Supplementary Table S7 that BFT classes greater than 27 mm were associated with lower Δs for palmitoleic, *cis*-vaccenic, arachidonic acids, and *n*-6/*n*-3 ratio, suggesting that pigs with a thicker BF layer had lower amounts of those MUFAs and arachidonic, thus decreasing the *n*-6/*n*-3 ratio.

No significant differences were observed for the orthogonal contrasts between EUROP carcass classes.

Figure 3 shows the heritability estimates, phenotypic, and genetic correlations for the Δs of the individual FAs. Darker colors indicate stronger correlation coefficients. Supplementary Table S8 reports the SE for the phenotypic and genetic correlation coefficients for the ΔFAs reported in Figure 3. The heritability values estimated for the ΔFAs were of low-to-moderate magnitude, ranging from 0.122 for the difference between capric acid percentages (ΔC10:0) to 0.245 for the difference between DHA percentages (ΔC22:6 *n*-3; Figure 3 and Supplementary Table S6). On the whole, almost all the genetic and phenotypic correlations between ΔFAs were significant. As can be noticed from Figure 3, the heatmap is characterized by two blocks of positive (red cells) genetic and phenotypic correlations. These two blocks are on the opposite sides of the diagonal and indicate, on one side, the positive genetic and phenotypic correlations relating the differences between short and medium SFAs and palmitoleic acid (i.e., ΔC10:0, ΔC12:0, ΔC14:0, ΔC16:0, and ΔC16:1 *cis*-9), on the other side the positive genetic and phenotypic correlations relating the differences between *n*-6 and *n*-3 PUFAs (i.e., ΔC18:2 *cis*-9, *cis*-12, ΔC18:3 *n*-3, ΔC20:2 *n*-6, ΔC20:3 *n*-6, ΔC20:4 *n*-6, ΔC22:4 *n*-6, ΔC22:5 *n*-3, ΔC22:6 *n*-3).

Figure 3. Heatmap with the heritability values (diagonal), phenotypic (below the diagonal) and genetic correlations (above the diagonal) for the differences (Δ) between the individual fatty acids

(FAs) in backfat (BF) and *Semimembranosus* (SM) muscle. Positive correlation coefficients are displayed in red, and negative correlation coefficients in blue.

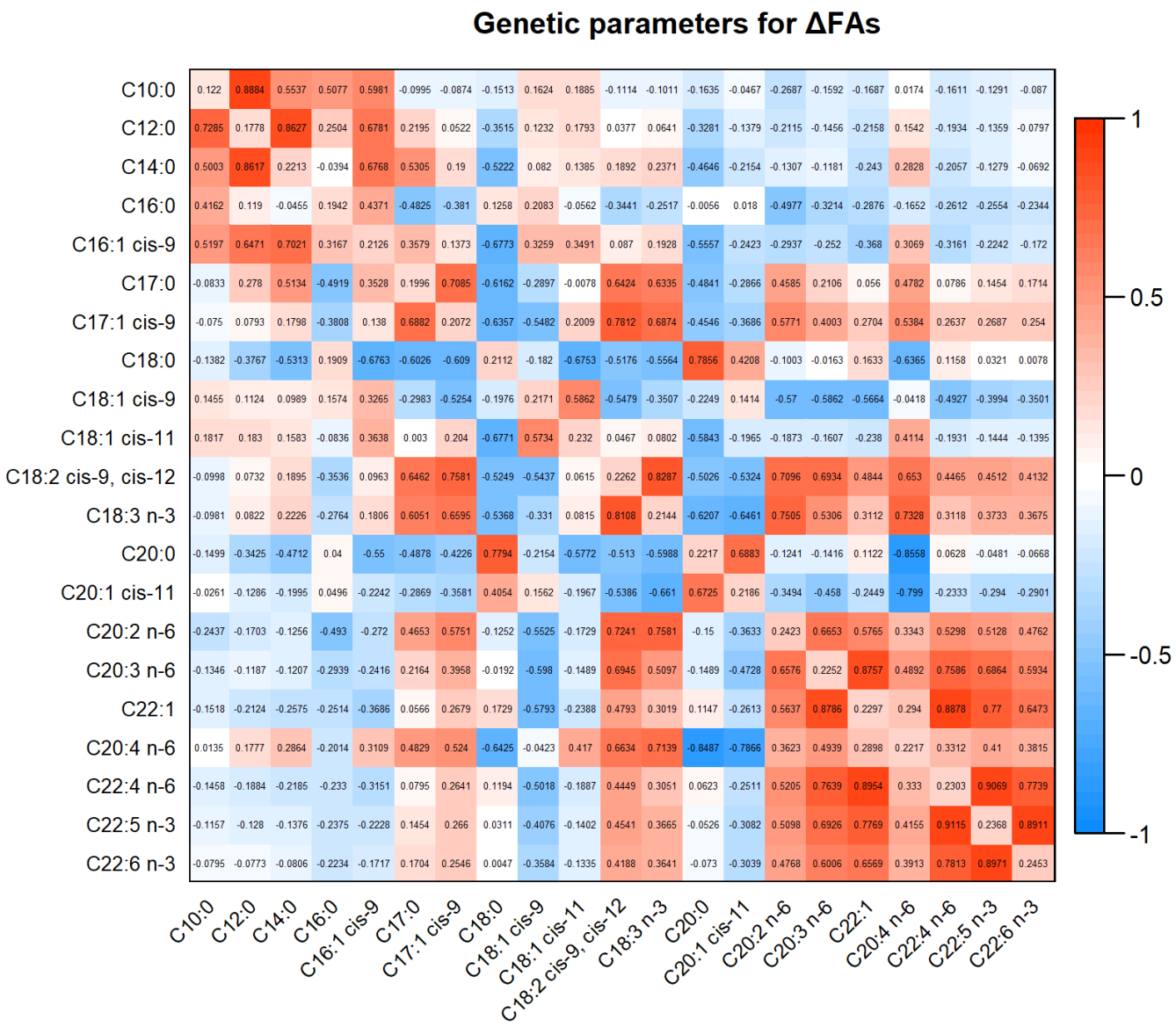

Table 3 reports the heritability values, phenotypic, and genetic correlations for the Δs of the FA classes. The heritability values estimated for the ΔFA classes were all similar and of moderate magnitude, ranging from 0.225 for ΔSFAs, to 0.232 for ΔPUFAs and Δ*n*-6 PUFAs (Table 3). The estimated genetic and phenotypic correlations were all significant. The differences for SFAs and MUFAs showed similar genetic correlation patterns, as both these classes had differences displaying negative genetic correlations with ΔPUFAs, Δ*n*-6 PUFAs, and Δ*n*-3 PUFAs. Contrariwise, ΔSFAs had a mild positive genetic correlation with Δ*n*-6/*n*-3, while ΔMUFAs had a negative genetic correlation with the Δ*n*-6/*n*-3 (Table 3).

Table 3. Heritability estimates (diagonal, in bold), phenotypic (below the diagonal) and genetic correlations (above the diagonal) ± Standard Errors (SE) for the differences (Δ) between the fatty acid (FA) classes in backfat (BF) and *Semimembranosus* (SM) muscle. * stands for *P*-value < 0.05; ** for *P*-values < 0.01; *** for *P*-values < 0.001.

ΔFAs % (BF FA% - SM FA%)	**ΔSFAs**	**ΔMUFAs**	**ΔPUFAs**	**Δ*n*-6 PUFAs**	**Δ*n*-3 PUFAs**	**Δ*n*-6/*n*-3**
ΔSFAs (Saturated fatty acids)	**0.225 ± 0.002**	0.089* ± 0.030	-0.589*** ± 0.009	-0.549*** ± 0.004	-0.564*** ± 0.009	0.084* ± 0.045
ΔMUFAs (Monounsaturated fatty acids)	0.075* ± 0.035	**0.231 ± 0.115**	-0.811*** ± 0.367	-0.794*** ± 0.256	-0.634*** ± 0.205	-0.328*** ± 0.112
ΔPUFAs (Polyunsaturated fatty acids)	-0.586*** ± 0.029	-0.808*** ± 0.021	**0.232 ± 0.004**	0.882*** ± 0.011	0.768*** ± 0.009	0.208*** ± 0.032
Δ*n*-6 PUFAs (*n*-6 Polyunsaturated fatty acids)	-0.548*** ± 0.030	-0.791*** ± 0.022	0.885*** ± 0.017	**0.232 ± 0.001**	0.774*** ± 0.009	0.292*** ± 0.022

Δ*n*-3 PUFAs (*n*-3 Polyunsaturated fatty acids)	-0.562*** ± 0.029	-0.628*** ± 0.028	0.769*** ± 0.023	0.775*** ± 0.022	**0.228 ± 0.004**	-0.234*** ± 0.066
Δ*n*-6/*n*-3	0.099** ± 0.035	-0.323*** ± 0.034	0.198*** ± 0.035	0.282*** ± 0.034	-0.256*** ± 0.034	**0.231 ± 0.026**

Both Δs for *n*-6 and *n*-3 PUFAs showed negative genetic correlations with ΔSFAs and ΔMUFAs, and positive genetic correlations with ΔPUFAs.

The genetic correlations between ΔFAs and carcass traits were almost negligible and most of them did not reach the significance threshold. Hot carcass weight displayed a positive genetic correlation with the Δ for palmitic acid (r_g = 0.137; $P < 0.001$), and negative genetic correlations with the Δs for lauric (r_g = -0.070; $P < 0.05$) and eicosadienoic acids (r_g = -0.098; $P < 0.01$). Loin thickness showed a positive genetic correlation with the Δ for palmitic acid (r_g = 0.118; $P < 0.001$), and a negative genetic correlation with the Δ for eicosadienoic acid (r_g = -0.074; $P < 0.05$). Finally, a positive genetic correlation was noticed between BFT measured with a caliper and the Δ for capric acid (r_g = 0.079; $P < 0.05$). No significant genetic correlations were noticed between carcass traits and the Δs for FA classes.

4. Discussion

Meat has a relevant role in the human diet as it provides important nutrients, such as high-value proteins, vitamins, minerals, and essential FAs (Schmid, 2010; Pereira & Vicente, 2013). Porcine fat depots and their FA composition influence pork technological quality and its sensory attributes such as tenderness, juiciness, and aroma (Wood et al., 2008). Subcutaneous fat and IMF FA compositions are also of great importance in determining meat nutritional features, supporting the need for a better characterization of meat FA composition and of the variables affecting FA deposition and composition of the different fat depots in pig carcasses. This study evaluated in heavy pigs the differences of the individual FAs and FA classes between BF and SM muscle tissues, allowing for the estimation of their genetic parameters and for the identification of their associations with slaughter season, gender, EUROP carcass classification, BF thickness and IMF %.

The comparison of the FA composition highlighted significant differences between the two tissues for all FAs and FA classes investigated, except for lauric acid. Lauric acid provides a fast energy

supply to cells as this FA is one of the most efficient substrates for β-oxidation (Leyton, Drury, & Crawford, 1987; Lyudinina, Ivankova, & Bojko, 2018). This major role of lauric acid in tissue energy metabolism may therefore explain why, in the present study, its proportion remains stable between SM muscle and BF tissue. The marked compositional differences noticed for the other FAs and FA classes are instead in agreement with the large body of literature proving intramuscular adipocytes display different metabolic roles when compared with subcutaneous adipocytes (Gardan, Gondret, & Louveau, 2006; Zhang et al., 2014). In particular, among the considered FAs, some differences between the two tissues were noticed for stearic and oleic acids, two of the major components of animal tissues FA composition (Wood et al., 2008). In the present study, stearic acid was more associated with BF tissue, while oleic was present in higher proportions in IMF. The proportions of these two FAs were however quite variable among the tested pigs, as suggested by the high standard error bars in Figure 1. Variations in stearic acid Δs showed to be highly associated with slaughter season, with pigs slaughtered in winter having lower stearic acid Δs when compared to those slaughtered in summer, autumn, and spring. In a previous study performed on the same animals, we analyzed the two tissues separately, and we evidenced a decreased storage of stearic acid in the BF tissue of pigs slaughtered in winter (Zappaterra, Catillo, Lo Fiego, Belmonte, Padalino, & Davoli, 2022). These changes were not noticed in SM, suggesting that the lowest values of stearic acid Δs noticed in winter in the present study depend on the decreased proportion of stearic acid deposited in BF during this season. It is widely accepted in the scientific literature that in mammals the composition of the fat in the different anatomical regions is determined by the temperature to which the tissues are subjected (Fawcett and Lyman, 1954). In humans, white adipocytes in the subcutaneous tissue were proved to undergo metabolic rearrangements associated with the seasons (Kern et al., 2014). Similarly, pigs subjected to heat stress were found to have an increased expression of genes related to lipogenic activity in subcutaneous fat tissue (Qu, & Ajuwon, 2018). Our results are therefore in agreement with these studies, confirming that subcutaneous fat may be more prone to changes related to temperature and thus season compared to

other fat depots, such as IMF. In accordance with the scientific literature, the highest content of stearic acid identified in the BF tissue could indicate an attempt of the subcutaneous adipocytes to maintain membrane integrity by incorporating higher contents of this SFA, increasing membrane resistance to high-temperature environments (Roy, Das, & Ghosh, 1997; Malekar, Morton, Hider, Cruickshank, Hodge, & Metcalf, 2018). The fluctuations in the stearic acid stored in IMF were less evident, suggesting that FA metabolism is more dynamic in porcine BF tissue and responds more widely to changes in environmental conditions than IMF adipocytes. This finding is in line with the results reported in cattle adipocytes, where subcutaneous fat was more responsive to external stimuli and fast physiological changes when compared with IMF adipocytes (Smith, Lin, Wilson, Lunt, & Cross, 1998). Furthermore, the stearic and oleic Δs were neither genetically nor phenotypically correlated in the present study. The deposition of these two FAs in the two tissues is therefore highly variable, but that variability in heavy pigs mostly relies on genetic and environmental factors that are, at least in part, different between SM and BF tissues. This observation agrees with the findings reported in a recent study investigating the gene expression networks associated with the deposition of oleic and stearic acid contents in ILW SM muscle (Zappaterra, Gioiosa, Chillemi, Zambonelli, & Davoli, 2021). These FAs showed indeed to be linked to different gene expression networks, suggesting that the complex molecular cascades regulating their deposition in SM muscle may differ (Zappaterra, Gioiosa, Chillemi, Zambonelli, & Davoli, 2021).

The significant differences observed for Δs among pigs with the different IMF classes and between barrows and gilts (Figure 1) were instead mainly determined by fluctuations in IMF FA composition, as reported in Zappaterra, Catillo, Lo Fiego, Belmonte, Padalino, & Davoli (2022). Sex was already found to be a factor influencing in porcine muscle the mRNA and protein expression levels of genes involved in FA biosynthesis (Braglia, Zappaterra, Zambonelli, Comella, Dall'Olio, & Davoli, 2014; Zappaterra, Deserti, Mazza, Braglia, Zambonelli, & Davoli, 2016;

Revilla et al., 2018). This evidence supports the hypothesis that sex and hormonal signals may have a stronger impact on muscle fat deposition and composition, while BF FA composition seems to be more dependent on environmental conditions, such as slaughter season. Among FAs showing differences related to muscle IMF deposition, oleic acid Δ decreased as IMF% increased. This trend suggests that as IMF deposition enhances, the percentage of oleic acid does not increase proportionally in muscle and backfat, with oleic acid deposition being more sustained in muscle. Similar results were already reported in our previous study (Zappaterra et al., 2020) and in Duroc pigs, where the variability noticed in the oleic acid content in muscle had a positive genetic correlation with SM IMF% while its amount in BF was not correlated with the variability noticed in IMF (Ros-Freixedes, Reixach, Tor, & Estany, 2012; Ros-Freixedes, Reixach, Bosch, Tor, & Estany, 2014). In addition, in the present study, as SM IMF % enhanced, its content of linoleic acid decreased. Similar results were also found in Duroc pigs, with linoleic acid in *Gluteus medius* muscle having a negative genetic correlation (-0.66) with the IMF deposition in the same tissue (Gol et al., 2019). The same Authors found arachidonic acid follows trends that are similar to what is observed for linoleic acid (Gol et al., 2019). These two FAs are part of the same biosynthetic process, as linoleic acid may be subjected to subsequent desaturation and elongation steps leading to the formation of arachidonic acid (Nakamura & Nara, 2004). However, we were not able to highlight similar patterns in the present study, as arachidonic acid Δs were neither associated with SM IMF %, nor with animal sex. On the contrary, sex was an extremely important variable determining significant fluctuations in the Δs of oleic and linoleic acids, with barrows having lower oleic acid Δs and higher linoleic acid Δs when compared with gilts. This result agrees with the fact that barrows tend to store more IMF than gilts (Ntawubizi, Raes, Buys, & De Smet, 2009; Zappaterra et al., 2020), leading to an increased deposition of oleic acid in SM and a decreased deposition of linoleic. As oleic and linoleic acids are two of the major components of MUFA and PUFA classes, respectively, the observed trends in their Δs returned with similar fluctuations also in

the Δs for MUFAs and *n*-6 PUFAs (Figure 2B and 2C), with significant differences in those Δs related to IMF % classes and animal sex.

Despite the discussed differences, the high and positive genetic correlations estimated between the proportions of FAs and FA classes in SM muscle and BF tissues suggest that the FA composition in SM muscle and BF shares a common genetic basis. Arachidonic, docosapentaenoic, docosahexaenoic, *cis*-vaccenic, lauric, and stearic acids showed the highest genetic correlations, which even corresponded to r_g values above 0.9, suggesting that their variability depended greatly on the same genes and molecular patterns. Also, most of the other FAs and FA classes displayed positive and medium-to-high genetic correlations between the two tissues, except for the *n*-6/*n*-3 ratio, capric, and erucic acids. The latter showed, indeed, negative genetic correlations, suggesting that the genetic patterns controlling their deposition in one tissue may exert an opposite effect in cells located in other body districts. Despite the genetic mechanisms underlying FA synthesis and deposition seem to be mostly conserved between BF and SM muscle, the reason for the negative genetic correlations linking *n*-6/*n*-3 ratio, capric, and erucic acids have yet to be elucidated. The scientific literature lacks studies that suggest possible explanations for these negative genetic correlations. However, our results are in accordance with results identified in mice, where the deletion of the *Ceramide Synthase 1* (*CerS1*) gene determined an increased accumulation of erucic acid in skeletal muscle sphingolipids, but no effect was noticed in white adipose tissue cells (Turpin-Nolan et al., 2019). Among the FAs showing negative genetic correlations between their proportions in BF and SM muscle is the *n*-6/*n*-3 ratio. This negative and highly significant genetic correlation may be of great interest for further discussion, as it implies that some sort of difference exists between muscle and subcutaneous fat tissues in the incorporation of *n*-3 and *n*-6 PUFAs. In previous studies investigating the FA compositions of BF and SM individually, the amount of *n*-6 and *n*-3 PUFAs deposited in BF showed different genetic correlations when compared with the same FA classes in SM muscle (Davoli et al., 2019; Zappaterra et al., 2020). While the selection for

a thicker BF was suggested to determine a decreased amount of both *n*-3 and *n*-6 PUFAs deposited in BF (Davoli et al., 2019), an increased IMF % was correlated with a reduction in SM muscle *n*-6 PUFAs without exerting effects on *n*-3 PUFAs (Zappaterra et al., 2020). A different molecular control regulating PUFAs deposition in SM muscle and BF is supported by the genetic correlations estimated in the present study, where the proportion of *n*-3 PUFAs in the two tissues had a lower genetic correlation when compared with the r_g value estimated for *n*-6 PUFAs. This difference may be due by the fact that endogenous *n*-3 PUFAs derive from the elongation of α-linolenic acid (Sinclair, Attar-Bashi, & Li, 2002). In the present study, this FA was among the FAs with the greatest relative variations between BF and SM muscle and was the only one that did not display genetic correlations between the two tissues. Together with linoleic acid, α-linolenic is considered an essential FA, and thus it can not be *de novo* synthesized in mammals and must be introduced with the diet (Sinclair, Attar-Bashi, & Li, 2002). Its variability is therefore mainly controlled by the digestion and absorption of dietary α-linolenic acid. In the present study, however, the pigs were all fed the same diet, and therefore the variations observed between pigs can not be linked to variations in the FA composition of the diet.

Concerning the genetic parameters estimated for the variations between FA composition of BF and SM muscle, all Δs showed low-to-medium heritability values, in line with the heritability values estimated in ILW pigs for the FAs in SM muscle (Zappaterra et al., 2020) and BF (Davoli et al., 2019). The fact that these differences are moderately heritable suggests that there are also genetic mechanisms capable of differentiating the deposition of FAs in BF from those in SM muscle. This result implies that a better knowledge of the genes differentiating the FA composition of BF and SM muscle could provide new tools allowing to select, in a partially independent manner, the FA composition of muscle and subcutaneous fat.

5. Conclusion

The study of genetic and non-genetic factors involved in variations between the FA composition of SM muscle and BF tissue evidenced that common genetic control exists between the two porcine tissues for most of the studied FAs. Some FAs and the *n*-6/*n*-3 ratio displayed a genetic control that is more oriented towards tissue-specific molecular pathways, indicating that fluctuations in the proportions of those FAs in one tissue would affect to a lesser extent the proportion of the same FA or FA class in the other tissue. Furthermore, our results suggested that variations in the FA composition of SM muscle and BF tissue can be modulated through genetic selection, and confirmed the importance of non-genetic and physiological factors on SM muscle and BF tissue FA composition. On the whole, these results can be useful for increasing the knowledge of factors affecting FA composition in pig muscle and BF. Further studies are needed to identify the molecular pathways and genes involved in the variations between BF and SM muscle FA composition, also given the role played by these traits in the quality of meat intended for PDO products.

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