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Martina Zappaterra[a]*, Gennaro Catillo[b], Domenico Pietro Lo Fiego[c,d], Giovanna Minelli[c,d], Barbara Padalino[a], Roberta Davoli[a]*

[a] Department of Agricultural and Food Sciences (DISTAL), University of Bologna, Viale G. Fanin 46, I-40127, Bologna, Italy.

[b] Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria (CREA) - Centro di ricerca Zootecnia e Acquacoltura (Research Centre for Animal Production and Aquaculture), Via Salaria 31, I-00015, Monterotondo, Italy.

[c] Department of Life Sciences (DSV), University of Modena and Reggio Emilia, Via G. Amendola 2, I-42122, Reggio Emilia, Italy.

[d] Interdepartmental Research Centre for Agri-Food Biological Resources Improvement and Valorization (BIOGEST-SITEIA), University of Modena and Reggio Emilia, P. le Europa 1, I-42124 Reggio Emilia, Italy.

*Corresponding authors: martina.zappaterra2@unibo.it; roberta.davoli@unibo.it

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Abstract

26 This study was conceived to evaluate the variations between backfat (BF) and *Semimembranosus*

27 (SM) muscle fatty acid (FA) composition in 789 heavy pigs and to estimate the genetic and

28 phenotypic correlations and the heritability values of these variations. Most FAs showed a common

29 genetic basis controlling their proportion in SM muscle and BF, while the n-6/n-3 ratio, α-linolenic

30 and erucic acids displayed a genetic control more oriented towards tissue-specific molecular

31 pathways. All variations between the FA composition of BF and SM muscle showed low-to-

32 medium heritability values, suggesting that there are also genetic mechanisms capable of

33 differentiating the deposition of FAs in BF from those in SM muscle. This result implies that a

34 better knowledge of the genes differentiating the FA composition of BF and SM muscle could

35 provide new tools allowing to select, in a partially independent manner, the FA composition of

36 muscle and subcutaneous fat.

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38

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

40

1 Introduction

42 The lipid content and fatty acid (FA) composition in pig tissues are of paramount importance both

43 from a technological and nutritional point of view (Raes, De Smet, & Demeyer, 2004; Wood et al.,

44 2008). This is true especially in the Italian heavy pig production chain, where the carcass cuts are

45 destined to long seasoning periods for Protected Denomination Origin (PDO) production (Catillo,

46 Zappaterra, Lo Fiego, Steri, & Davoli, 2021; Lo Fiego, Santoro, Macchioni, & De Leonibus, 2005;

47 Zappaterra et al., 2020). Italian PDO production is based on carcasses coming from heavy pigs

48 slaughtered at 9 months of age, with an average live body weight ranging from 160-170 kg and

49   belonging to genetic types under the control of the national selection or from commercial hybrids

50   considered as suitable for PDO products (Lo Fiego, Macchioni, Minelli, & Santoro, 2010).

51   The carcasses of heavy pigs have undergone significant changes in the last decades; the decrease of

52   carcass fat and the increase of lean meat resulted in a marked reduction in fat content in the depot fat

53   and an increase in polyunsaturated FA (PUFA) content (Wood & Enser, 1997; Andersen, 2000;

54   Piedrafita, Christian, & Lonergan, 2001; Gandemer, 2002; Lo Fiego, Macchioni, Minelli, & Santoro,

55   2010). Negative correlations were observed between the backfat thickness (BFT), carcass fatness,

56   and the content of intramuscular fat (IMF), with respect to the proportion of PUFAs, thus influencing

57   also meat susceptibility to oxidation (Daza, Olivares, Latorre, Rey, Callejo, & Lopez-Bote, 2017;

58   Maw, Fowler, Hamilton, & Petchey, 2003; Lo Fiego, Minelli, Volpelli, Ulrici, & Macchioni, 2016).

59   In fact, the increased level of unsaturation causes a reduction in the oxidative stability of pork

60   products (Sheard, Enser, Wood, Nute, Gill, & Richardson, 2000; Kouba, Enser, Whittington, Nute,

61   & Wood, 2003). As result, rancidity-related off-odors and flavors may occur (Rhee, Ziprin, Ordonez,

62   & Bohac, 1988) and shelf-life may be compromised (Amaral, da Silva, & Lannes, 2018). The FA

63   composition of pork influences the final quality of hams, with effects, in particular, during the

64   processing of dry-cured products, affecting their consistency, salt and water content, curing duration,

65   and lipid oxidation (Lopez-Bote, 2000; Lopez-Bote, Rey, & Menoyo, 2004).

66   In pigs, FA composition and deposition of adipose and muscle tissues can be affected by many factors

67   such as sex, weight and age at slaughter, livestock production system, genetics, and dietary

68   components (Mourot & Hermier, 2001; De Smet, Raes & Demeyer, 2004; Lopez-Bote, Rey, &

69   Menoyo, 2004; Wood et al., 2008; Duran-Montgé, Realini, Barroeta, Lizardo, & Esteve-Garcia,

70   2008). As far as fat deposition, adipose cells of muscle and subcutaneous fat tissue display significant

71   morphological, developmental, and metabolic variations (Gardan, Gondret, & Louveau, 2006;

72   Hausman et al., 2009; Komolka, Albrecht, Wimmers, Michal, & Maak, 2014). Marked differences

73   exist in the number, size, and metabolic properties of adipocytes between different porcine tissues,

74   and each fat depot shows specific growth, metabolic features, and genetic controls (Gardan, Gondret,

75 & Louveau, 2006; Komolka, Albrecht, Wimmers, Michal, & Maak, 2014). Furthermore, the

76 development of intramuscular and subcutaneous fat was proved to be controlled, at least in part, by

77 different factors (Kouba, Bonneau, & Noblet, 1999; Mourot & Kouba, 1999), and these two tissues

78 were found to respond differently to dietary treatments (Doran, Moule, Teye, Whittington, Hallett, &

79 Wood, 2006).

80 Among the pig fat depots, intramuscular adipose tissue is the latest developing adipose site, after

81 subcutaneous and intermuscular fat (Mourot & Kouba, 1999; Gardan, Gondret, & Louveau, 2006;

82 Gondret, Guitton, Guillerm-Regost, & Louveau, 2008; Hocquette, Gondret, Baéza, Médale, Jurie, &

83 Pethick, 2010). Intramuscular adipocytes have specific metabolic features and in pigs show much

84 lower capacity for synthesis and degradation of FAs compared to subcutaneous fat (Gardan, Gondret,

85 & Louveau, 2006). In fact, whatever the age of pigs (i.e., from 80 to 210 days), Gardan, Gondret, &

86 Louveau (2006) found that the expressions of genes involved in FA synthesis and lipogenesis (e.g.

87 *Fatty Acid Synthase*, *Malic Enzyme*), triglyceride hydrolysis (e.g., *Lipoprotein Lipase*, *Hormone-*

88 *Sensitive Lipase*), and transcriptional regulation of lipid metabolism (e.g., *Sterol Regulatory Element-*

89 *Binding Protein-1*, *Peroxisome Proliferator-Activated Receptor-Gamma*) were significantly lower in

90 intramuscular adipocytes than in subcutaneous tissue. Despite the lower rate of lipogenesis observed

91 in muscle in comparison with subcutaneous fat deposition, *in situ de novo* FA synthesis directly and

92 strongly contributes to the intramuscular FA composition (Corominas et al., 2013). Subcutaneous

93 adipose tissue tends instead to respond more quickly to environmental stimuli, such as diet

94 composition, (Nürnberg, Wegner, & Ender, 1998; Estany, Ros-Freixedes, Tor, & Pena, 2014) and is

95 reported to be more prone to remodeling than IMF, in particular when animals are fed diets with high

96 oleic acid, such as in Iberian heavy pigs feeding system (Segura et al., 2021).

97 The present research mainly deals with the study of the genetic and environmental aspects that

98 influence the FA composition of backfat (BF) and *Semimembranosus* (SM) muscle in a population

99 of Italian Large White (ILW) pigs. This study aimed at evaluating the differences of the individual

100 FAs and FA classes between the two tissues, and at estimating the genetic and phenotypic correlations

101 and the heritability values of these differences. The description of the genetic and non-genetic factors

102 involved in the variations between the FA composition of muscle and BF can contribute to a better

103 understanding of the major biological processes and the main environmental conditions

104 differentiating the development of fat deposition in the two considered tissues. The obtained results

105 allowed us to identify which factors affect the variations between FAs in SM muscle and BF tissues.

106 These results can be considered as a further element of deeper knowledge on the factors affecting FA

107 composition of pig SM muscle and BF tissue, also given the role played by these traits in the quality

108 of meat intended for PDO products.

109

110 **2. Material and methods**

111 **2.1 Animals and phenotypes**

112 A population of 789 sib-test ILW heavy pigs was utilized for the present study. The used animals

113 were already included in Zappaterra, Catillo, Lo Fiego, Belmonte, Padalino, & Davoli (2022). These

114 pigs were triplets of full sibs coming from 323 litters (87 boars x 371 sows) reared in a unique testing

115 station of the Italian Pig Breeders National Association (Associazione Nazionale Allevatori Suini,

116 ANAS, http://www.anas.it). During the testing period (lasting from 30-45 days of age to about 9

117 months of age), siblings were kept separated and fed the same diets. In the testing station, pigs are

118 fed the same growing and finishing diets in order to avoid any possible effects related to diet

119 composition. Finishing diet composition is reported in Supplementary Table S1. Pigs were

120 slaughtered at an average final weight of 155 ± 6 kg on 26 different dates between 2011 and 2012 at

121 the same commercial abattoir. The animals were handled and slaughtered in compliance with

122 European rules on the protection of animals during transport and at slaughtering (Council Regulation

123 (EC) No. 1/2005 and Council Regulation (EC) No. 1099/2009). As the used animals were Sib Test

124 individuals and were slaughtered during routine assessments performed for ANAS Sib Test selection

125 schemes, the present research did not need approval from a research ethics committee. All slaughter

126   procedures were monitored by the veterinary team appointed by the Italian Ministry of Health and

127   were performed within the ANAS routine assessments. Sampling occurred with ANAS permission.

128   BF and SM muscle tissues were sampled on the splitting line from carcasses left sides. BF samples

129   were collected at the level of BF maximum thickness, approximately between the fifth and the sixth

130   lumbar vertebra, close to the point where the hind leg is separated from the rest of the carcass,

131   wrapped in aluminum foil, immediately put in vacuum-sealed bags, frozen in liquid nitrogen, and

132   kept at -80°C for further use. SM muscle samples were gathered from the distal part of the muscle

133   and sampling was performed in the same location of the inner face of the thighs. Hot carcass weight

134   (kg) and optical measures (expressed in mm) of loin and BF thicknesses were taken by Fat-O-Meat'er

135   (FOM - CrometecGmbh, Lünen, Germany) between the third and fourth last ribs, 8 cm off the carcass

136   midline. The measures of BF and loin thickness were used to estimate the percentage of carcass lean

137   meat, and to subsequently grade carcasses in EUROP carcass grading, following EU Decision

138   2001/468/CE of June, 8th 2001 (European Commission, 2001). Furthermore, the weights (kg) of the

139   head of the animals and of the belly and jowl cuts measured on the carcass left side were also recorded.

140   As reported in our previous paper (Davoli et al., 2016), IMF content was determined in the SM muscle

141   by extraction with petroleum ether from 1 g fresh sample using an XT15 Ankom apparatus (Macedon,

142   NY, USA), according to Official procedure AOCS Am 5-04 (AOAC, 2005). IMF was determined in

143   % as g of IMF per 100 g of tissue.

144   For each pig, the FA compositions of BF and SM muscle tissues were determined as reported in our

145   previous papers (Catillo, Zappaterra, Lo Fiego, Steri, & Davoli, 2021; Zappaterra, Catillo, Lo Fiego,

146   Belmonte, Padalino & Davoli, 2022).

147   In details, the samples of BF, finely minced, were submitted to lipids extraction by

148   chloroform:methanol mixture (2:1, v/v) (Carlo Erba Reagents, Milan, Italy) according to Folch, Lees,

149   and Sloane Stanley (1957). Then 50 mg of extracted lipids were trans-esterified at room temperature

150   for 5 min with 0.5 ml of sodium methoxide (0.5 N) in methanol. FA methyl-esters, extracted with 1

151   ml of hexane, were injected in a Gas-Chromatograph apparatus (GC 2010 plus, Shimadzu, Columbia,

152 MD, USA) equipped with a flame ionization detector and a high polar capillary column (SP 2560 100

153 m × 0.25 mm, Supelco, Bellefonte, PA, US). FA methyl-esters were identified by comparison with a

154 commercial standard mix of FA methyl-esters (GLC-674 Nuchek, Elysian, MN, US) added with

155 missing FA standards (Nuchek, Elysian, MN, US; Larodan, Solna, Sweden), so obtaining a complete

156 standard set of 105 FA methyl-esters. For each FA methyl-ester, response factors to flame ionization

157 detector and inter- and intra-assay coefficients of variation were calculated by using a reference

158 standard butter (CRM 164, Community Bureau of Reference, Brussels, Belgium). Results were

159 expressed as g FA per 100 g of total FA (i.e., percent fatty acid composition).

160 For SM FA determination the total muscle lipids destined for the gas-chromatographic analysis were

161 extracted using a mixture of chloroform: methanol (2:1, v/v) (Carlo Erba Reagents, MI, Italy)

162 according to Folch, Lees, and Sloane Stanley (1957). Methylation was performed with a 2 N solution

163 of potassium hydroxide (KOH) in methanol (CH3OH) (Carlo Erba Reagents, Milan, Italy) according

164 to Ficarra, Lo Fiego, Minelli, & Antonelli (2010). Tridecanoic acid (C13:0) (Larodan Fine Chemicals

165 AB, Solna, Sweden) was used as internal standard in SM FA determination. Intramuscular fatty acid

166 methyl esters (FAMEs) were then submitted to gas-chromatographic analysis using TRACE™GC

167 Ultra (Thermo Electron Corporation, Rodano, MI, Italy) equipped with a Flame Ionization Detector,

168 a PVT injector, and a TR-FAME Column 30 m × 0.25 mm i.d., 0.2 μm film thickness (Thermo

169 Scientific, Rodano, MI, Italy). The Chrom-Card software (vers.2.3.3, Thermo Electron Corporation,

170 Rodano, MI, Italy) was used to record and integrate the peaks of FAMEs. Individual FAME were

171 identified by comparing their retention times with the retention times of a standard FAME mixture

172 prepared in-house with known quantities of each methyl ester (Larodan Fine Chemicals AB, Solna,

173 Sweden). In order to present data in the same way as BF, the amount of each FA determined in SM

174 was reported as g FA per 100 g of total FA (i.e., percent FA composition).

175

176

177 **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.

203 Furthermore, the phenotypic and genetic correlations between the amount of each FA or FA class in

204 the two tissues were estimated by restricted maximum likelihood (REML) methodology using the

205 VCE software system version 6 (Groeneveld, Kovač, & Mielenz, 2010) and were carried out by

206 mixed bi-varied animal models, as follows:

207
$$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} X_1 & 0 \\ 0 & X_2 \end{bmatrix} \begin{bmatrix} b_1 \\ b_2 \end{bmatrix} + \begin{bmatrix} Z_1 & 0 \\ 0 & Z_2 \end{bmatrix} \begin{bmatrix} a_1 \\ a_1 \end{bmatrix} + \begin{bmatrix} W_1 & 0 \\ 0 & W_2 \end{bmatrix} \begin{bmatrix} d_1 \\ d_2 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \end{bmatrix}$$

208 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

209 vector of fixed effects (found to be significant for each trait): the fixed factor of slaughter season (i.e.,

210 4 levels); the fixed factor of sex with two levels (i.e. barrows and gilts); the fixed effect of the BFT

211 classes (4 levels); the fixed effect of IMF classes (4 levels); the fixed effect of EUROP carcass grading

212 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

213 the vectors of the random effect of litter for the two traits (323 levels), and e_1 and e_2 are the vectors

214 of random errors; X_1 and X_2 are the matrices of incidence of $n \times p$ order associating each observation

215 (n) to the pertinent level of slaughter season, sex, BFT classes, IMF classes, and EUROP grading (p);

216 W_1 and W_2 are the matrices of incidence of $n \times q$ order associating each observation (n) to the

217 pertaining litter level (q); Z_1 and Z_2 are the matrices of incidence of $n \times s$ order associating each

218 observation (n) to each animal (s).

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

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

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

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

223 It was assumed that:

224 $$\text{Var}\begin{bmatrix} a_1 \\ a_2 \\ d_1 \\ d_2 \\ e_1 \\ e_2 \end{bmatrix} = \begin{bmatrix} \mathbf{A}g_{11} & \mathbf{A}g_{12} & 0 & 0 & 0 & 0 \\ \mathbf{A}g_{21} & \mathbf{A}g_{22} & 0 & 0 & 0 & 0 \\ 0 & 0 & \mathbf{I}q_{11} & \mathbf{I}q_{12} & 0 & 0 \\ 0 & 0 & \mathbf{I}q_{21} & \mathbf{I}q_{22} & 0 & 0 \\ 0 & 0 & 0 & 0 & \mathbf{I}s_{11} & \mathbf{I}s_{12} \\ 0 & 0 & 0 & 0 & \mathbf{I}s_{21} & \mathbf{I}s_{22} \end{bmatrix}$$

225 Where: g_{11} was the additive genetic variance for the direct effect for trait 1; g_{12} was equal to g_{21} and

226 was the additive genetic covariance between the two traits; g_{22} was the additive genetic variance for

227 direct effect for trait 2; q_{11}, q_{12}, q_{21}, and q_{22} were the variance and covariance matrices for the litter

228 effect; s_{11}, s_{12}, s_{21} and s_{22} were the variance and covariance matrices for the residual effect; A was the

229 relationship matrix between all animals. Matrix A included information on a three-generation

230 pedigree for a total of 2318 animals, 623 males and 1695 females; I is identity matrix for litter and

231 residual variance and covariance.

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

233 $r_g = g_{12}/(\sqrt{g_{11}}*g_{22})$

234

235 2.2.3 Descriptive statistics and genetic parameters for the differences between

236 backfat and Semimembranosus muscle FA composition

237 The Δs (i.e., the differences for each% FA or FA class obtained subtracting their amount in SM

238 muscle from that in BF) were investigated with an Anova to identify the environmental parameters

239 affecting their variability. This analysis was performed with ANOVA procedure of SAS software

240 vers. 9.4, considering the fixed effects of season, BFT classes, IMF classes, EUROP classes, and

241 sex. The used model was the following:

242 $y = \mu + SEASON + SEX + BFT + IMF + EUROP + e$

243 Where y is the observation vector for the ith trait; μ was the overall mean; SEASON was the fixed

244 factor of the slaughter season (i.e., 4 levels); SEX is the fixed factor of sex with two levels (i.e.

245 barrows and gilts); BFT was the fixed effect of the BFT classes (4 levels); IMF was the fixed effect

246 of IMF classes (4 levels); EUROP was the fixed effect of carcass grading classes (4 levels); e was

247 the vector of random residuals. The association between each ΔFA and the fixed effects was

248 estimated with the F-value and the *P*-value. Orthogonal contrasts were constructed for each fixed

249 effect to compare the ΔFA mean values between the fixed effect classes. In particular, spring-

250 summer *vs*. autumn-winter were compared for the slaughter season; samples with a BFT $<$ 27 mm

251 *vs*. those with BFT $>$ 27 mm were compared for the BFT effect; samples with an IMF $<$ 1.72% *vs*.

252 those with IMF $>$ 1.72% mm were compared for the IMF effect; E and U carcasses *vs*. R and O

253 ones were compared for the EUROP carcass grading fixed effect. The ΔFA mean values were also

254 compared between gilts and barrows with Student's t-test.

255 The ΔFA data set was also used to estimate genetic parameters, such as variance

256 components,heritability and standard error (SE), and the genetic correlations and the relative SE

257 among ΔFA components. Estimates were calculated by REML methodology using the VCE

258 software system version 6 (Groeneveld, Kovač, & Mielenz, 2010) and were carried out by two

259 multiple trait animal models: one involving the measures recorded at slaughtering added with Δs of

260 individual FAs (number of dependent variables $=$ 27), the other involving the same measurements at

261 slaughtering (i.e., hot carcass weight, loin thickness, belly weight, jowl weight, head weight and

262 carcass lean meat percentage) added with Δs of FA classes (number of dependent variables $=$ 12).

263 The multiple trait animal models are mixed infinitesimal models where Δs of all individual FA or

264 FA classes were fitted together. The used model was the following:

265
$$\begin{bmatrix} y_1 \\ \vdots \\ y_i \end{bmatrix} = \begin{bmatrix} X_1 & \cdots & 0 \\ \vdots & \ddots & \vdots \\ 0 & \cdots & X_i \end{bmatrix} \begin{bmatrix} b_1 \\ \vdots \\ b_i \end{bmatrix} + \begin{bmatrix} Z_1 & \cdots & 0 \\ \vdots & \ddots & \vdots \\ 0 & \cdots & Z_i \end{bmatrix} \begin{bmatrix} a_1 \\ \vdots \\ a_i \end{bmatrix} + \begin{bmatrix} W_1 & \cdots & 0 \\ \vdots & \ddots & \vdots \\ 0 & \cdots & W_i \end{bmatrix} \begin{bmatrix} d_1 \\ \vdots \\ d_i \end{bmatrix} + \begin{bmatrix} e_1 \\ \vdots \\ e_i \end{bmatrix}$$

266 where: y_i is the vector of phenotypic observation for the *i*th trait ($i = 27$: measurements at slaughtering

267 added with Δs of FAs and $i = 12$: measurements at slaughtering added with Δs of FA classes); b_i was

268 the vector of fixed effects (found to be significant for each trait): the fixed factor of slaughter season

269 (i.e., 4 levels); the fixed factor of sex with two levels (i.e. barrows and gilts); the fixed factor of the

270 BFT classes (4 levels); the fixed factor of IMF classes (4 levels); the fixed factor of EUROP carcass

271 grading classes (4 levels); a_i was the vector of additive genetic effects, d_i was the vector of the random

272   effect of the litter for the ith trait (323 levels) and e_i was the vector of random error; X_i was the matrix

273   of incidence of $n \times p$ order associating each observation (n) to the pertinent level of slaughter season,

274   sex, BFT classes, IMF classes, and EUROP grading (p); W_i was the matrix of incidence of $n \times q$ order

275   associating each observation (n) to the pertaining litter level (q); Z_i was the matrix of incidence of n

276    $\times$ s order associating each observation (n) to each animal (s).

277   Variance-covariance matrices between the Δs were as follows:

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

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

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

281   It was assumed that:

282    $\mathrm{Var}\begin{bmatrix} a_1 \\ \vdots \\ a_i \\ d_1 \\ \vdots \\ d_i \\ e_1 \\ \vdots \\ e_i \end{bmatrix} = \begin{bmatrix} Ag_{11} & .. & Ag_{ij} & 0 & .. & 0 & 0 & .. & 0 \\ . & .. & . & . & .. & . & . & .. & . \\ Ag_{ji} & .. & Ag_{ii} & 0 & .. & 0 & 0 & .. & 0 \\ 0 & .. & 0 & Iq_{11} & .. & Iq_{ij} & 0 & .. & 0 \\ . & .. & . & . & .. & . & . & .. & . \\ 0 & .. & 0 & Iq_{ji} & .. & Iq_{ii} & 0 & .. & 0 \\ 0 & .. & 0 & 0 & .. & 0 & Is_{11} & .. & Is_{ij} \\ . & .. & . & . & .. & . & . & .. & . \\ 0 & .. & 0 & 0 & .. & 0 & Is_{ji} & .. & Is_{ii} \end{bmatrix}$

283   where: g_{11}, g_{ij}, g_{ji}, and g_{ii} were elements of G, the additive genetic variance and covariance matrix for

284   the animal effect; q_{11}, q_{ij}, q_{ji}, and q_{ii} were elements of Q, the variance and covarince matrix for the

285   litter effect; s_{11}, s_{ij}, s_{ji}, and s_{ii} were the elements of S, the variance and covariance matrix for the

286   residual effect; A was the relationship matrix as previously defined; I was the identity matrix for litter

287   and residual variance and covariance.

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

289 The heritability value for each ΔFA or ΔFA class was estimated as the ratio of the genetic variance

290 to the phenotypic variance (genetic + environmental): $h^2 = g_{ii}/(g_{ii}+s_{ii})$.

291 For both models (bivariate and multivariate), the VCE software concluded the process with a state

292 equal to 1, so the first derivative is effectively equal to zero, which indicates that convergence has

293 been met and the estimated components are optimal.

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

295

296 **3. Results**

297 **3.1 Descriptive statistics and genetic correlations between the FA composition of**

298 **the two tissues**

299 The descriptive statistics of the FAs and FA classes measured in the BF and SM muscle tissues of

300 the 789 ILW pigs are reported in Table 1. As can be noticed, the most abundant FAs in both tissues

301 were oleic, palmitic, stearic, and linoleic acids. Except for lauric acid, all FAs and FA classes

302 showed significant differences in their amounts between the two tissues. SM muscle tissue showed

303 greater amounts of capric (C10:0), myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1 *cis*-9),

304 heptadecenoic (C17:1 *cis*-9), oleic (C18:1 *cis*-9), *cis*-vaccenic (C18:1 *cis*-11), dihomo-γ-linolenic

305 (C20:3 *n*-6), arachidonic (C20:4 *n*-6), adrenic (C22:4 *n*-6), docosapentaenoic (DPA; C22:5 *n*-3),

306 and docosahexaenoic (C22:6 *n*-3; DHA) acids. These differences resulted in a higher proportion of

307 MUFAs and in lower proportions of SFAs and PUFAs (both *n*-6 and *n*-3 PUFAs) in muscle. The *n*-

308 6/*n*-3 ratio was instead higher in SM compared to BF.

309 **Table 1.** Descriptive statistics for the percentages of fatty acids (FAs) in backfat (BF) and *Semimembranosus* (SM) muscle. For each tissue are

310 presented the mean, standard deviation (S.D.), minimum (Min), maximum (Max), and the ANOVA *P*-value of the comparison for the % of each FA

311 or FA classes between the two tissues.

FA (%)	BF			SM			*P-value*
	Mean ± S.D.	**Min**	**Max**	**Mean ± S.D.**	**Min**	**Max**	
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

312

313 Table 2 shows the phenotypic and genetic correlations between the amounts of FAs or FA classes in

314 BF and in SM muscle. Significant genetic correlations between the two tissues were noticed for all

315 FAs and FA classes except for α-linolenic acid. The genetic correlations were generally higher and

316 more significant than the phenotypic ones, with lauric (C12:0), margaric (C17:0), stearic (C18:0),

317 palmitoleic, heptadecenoic, oleic (C18:1 *cis*-9), *cis*-vaccenic, gadoleic (C20:1 *cis*-11), erucic

318 (C22:1), arachidonic, DPA, and DHA acids showing genetic correlation coefficients (r_g) > 0.80 and

319 *P*-values < 0.0001. The strongest negative genetic correlation was noticed for the *n*-6/*n*-3 ratio,

320 followed by erucic and capric acids.

321

322 **Table 2.** Phenotypic (r) and genetic (r_g) correlations with the relative standard errors (SE) between

323 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

324

325

326 **3.2 Descriptive statistics and genetic parameters for the differences between**

327 **backfat and IMF FAs and FA classes**

328 The means, standard deviations, minimum and maximum values for the obtained Δs are reported in

329 Supplementary Table S2. Means and standard deviations for the Δs are graphically displayed in

330 Figure 1. As can be noticed from Figure 1, Δs were on average positive for lauric, margaric, stearic,

331 arachidic, gadoleic, erucic, linoleic, α-linolenic, eicosadienoic, SFAs, and PUFAs. In particular, the

332 highest mean values were noticed for linoleic acid (5.70 ± 2.61%), PUFAs (4.39 ± 3.48%), and *n*-6

333 PUFAs (4.07 ± 3.39%), while the lowest Δs were observed for *n*-6/*n*-3 ratio (-5.04 ± 6.38%),

334 MUFAs (-4.80 ± 3.29%), and oleic acid (-1.84 ± 2.93%). Comparing Δs with the average

335 percentages of the relative FAs found in the two tissues, the FAs that showed the greatest difference

336 in relative terms was erucic acid, which had a five-times higher amount in BF when compared with

337 SM, followed by α-linolenic acid, with a percentage in BF that was double the percentage in SM.

338

339 **Figure 1.** Means and standard deviations (error bars) of percent differences (Δ%) between the fatty

340 acids (FAs) and FA classes in backfat (BF) and *Semimembranosus* (SM) muscle.

Differences between backfat and *Semimembranosus* muscle amounts for each FA and FA class

Fatty acids (FAs)
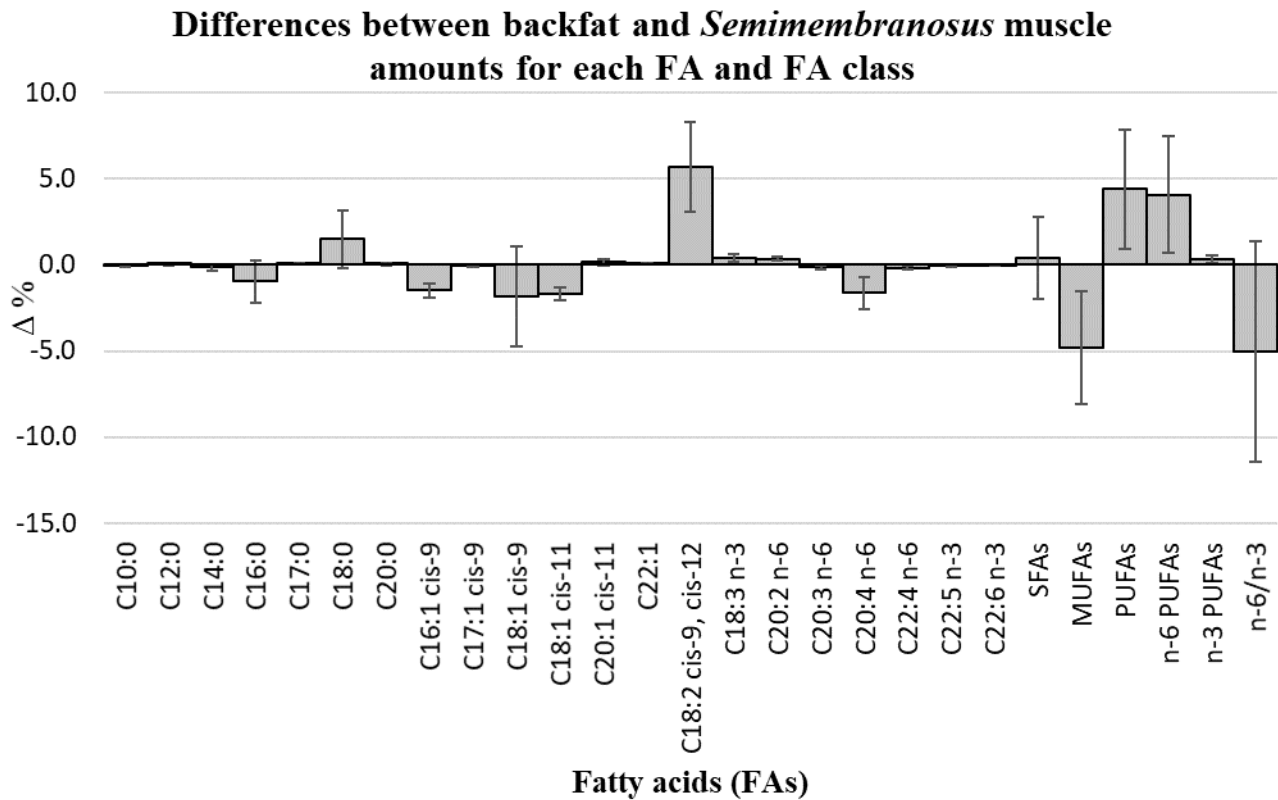
341

342 The results of the ANOVA performed on Δs to investigate the effects of slaughter season, BFT

343 classes, IMF classes, EUROP carcass grading, and animals' sex are reported in Supplementary

344 Table S3. Slaughter season, IMF classes, and animals' sex showed to be the variables affecting the

345 most the variability noticed among the samples for Δs. The Least Squares Means (L.S.M.) of Δs for

346 slaughter season, IMF classes, and animals' sex are graphically presented in Figure 2. Slaughter

347 season showed to affect almost all Δs, except those for margaric, erucic, dihomo-γ-linolenic, and

348 adrenic acids (Supplementary Table S3). Supplementary Table S4 shows the results of the

349 orthogonal contrasts for slaughter season. The animals slaughtered during autumn-winter had higher

350 Δ values for lauric, myristic, heptadecenoic acids, and *n*-6/*n*-3 ratio, while those slaughtered in

351 spring-summer had higher Δ values for stearic, gadoleic, α-linolenic (C18:3 *n*-3), DPA acids and *n*-

352 3 PUFAs (Figure 2A and Supplementary Table S4). IMF classes were associated with changes in

353 almost all Δs, except those for stearic, *cis*-vaccenic, α-linolenic, arachidonic acids, SFAs and *n*-3
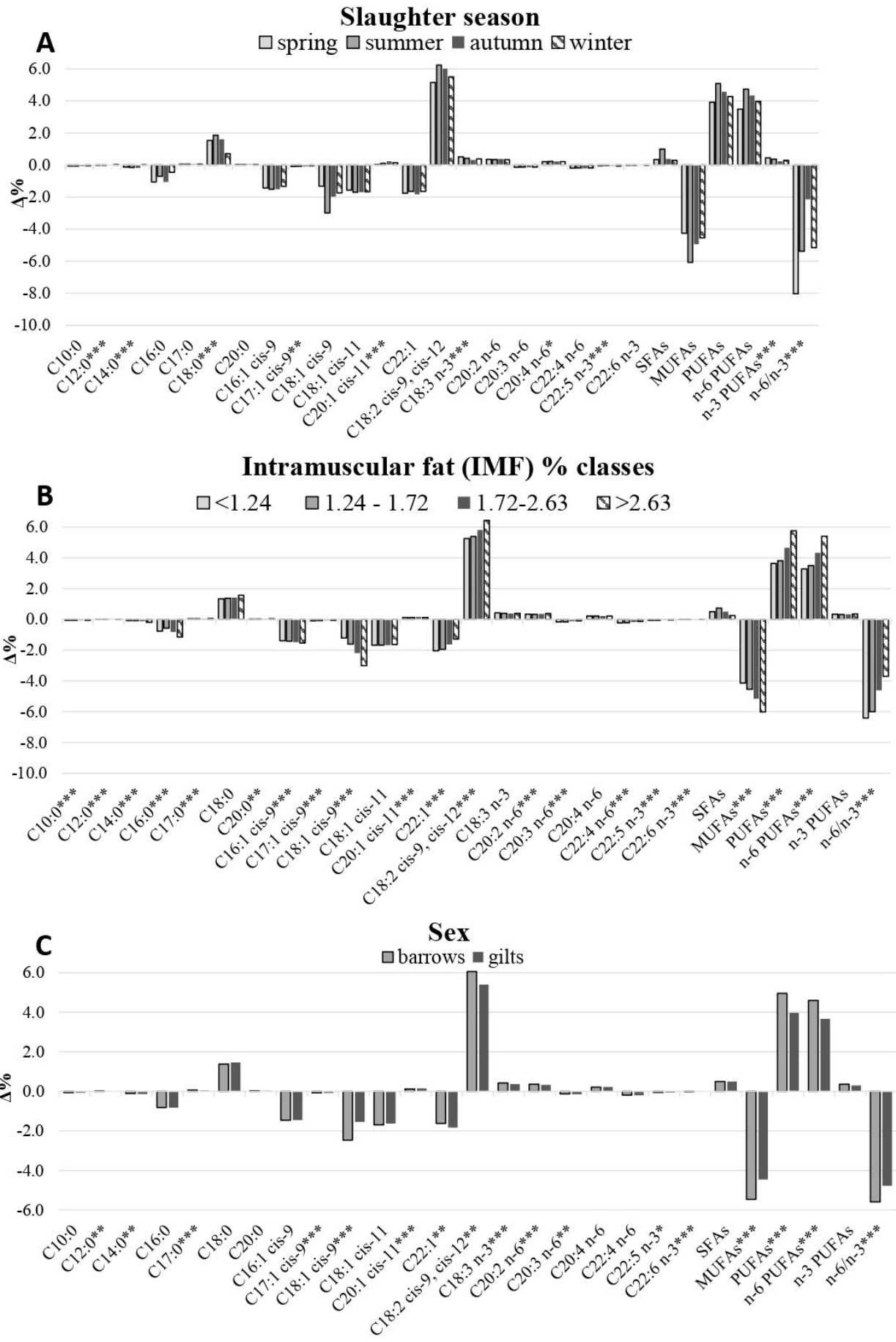
354 PUFAs (Figure 2B and Supplementary Table S3). Supplementary Table S5 shows the results of the

355 orthogonal contrasts for IMF classes. The classes of IMF > 1.72% showed significantly lower

356 values of L.S.M. for the Δs of capric, lauric, myristic, palmitic, palmitoleic, oleic acids, and

357 MUFAs when compared with IMF < 1.72% classes (Supplementary Table S5). The differences

358 noticed for the Δs between pigs with IMF > 1.72% and animals with IMF < 1.72% indicate that

359 higher contents of IMF are associated with an increase of capric, lauric, myristic, palmitic,

360 palmitoleic, oleic acids, and MUFAs in muscle but not in backfat. Also, animals' sex showed to be

361 an important factor affecting Δs for lauric, myristic, margaric, heptadecenoic, oleic, erucic, linoleic

362 (C18:2 *cis*-9, *cis*-12), α-linolenic acid, eicosadienoic (C20:2 *n*-6), dihomo-γ-linolenic, DPA, DHA

363 acids, MUFAs, and PUFAs (Supplementary Table S3). Supplementary Table S6 shows the results

364 of the comparisons between the L.S.M. of the Δs in barrows and gilts. When compared with

365 barrows, gilts had lower Δs for lauric, myristic, margaric, heptadecenoic, erucic, linoleic, α-

366 linolenic acid, eicosadienoic, dihomo-γ-linolenic, DPA, DHA acids, and PUFAs (Supplementary

367 Table S6), indicating that gilts tend to store higher % of these FAs in muscle but not in backfat.

368

369 **Figure 2.** Least Squares Means (L.S.M.) of percent differences (Δ%) between the fatty acids (FAs)

370 and FA classes in backfat (BF) and *Semimembranosus* (SM) muscle for slaughter season (A),

371 intramuscular fat (IMF) content % classes (B), and sex (C). FAs with stars are those showing

372 significant differences for the comparisons (i.e., * means *P*-value < 0.05; ** *P*-value < 0.01; *** *P*-

373 value < 0.001).

Slaughter season

□ spring □ summer ■ autumn ▨ winter

A

Intramuscular fat (IMF) % classes

□ <1.24 □ 1.24 - 1.72 ■ 1.72-2.63 ▨ >2.63

B

Sex

□ barrows ■ gilts

C

374

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

401 (FAs) in backfat (BF) and *Semimembranosus* (SM) muscle. Positive correlation coefficients are

402 displayed in red, and negative correlation coefficients in blue.

Genetic parameters for ΔFAs
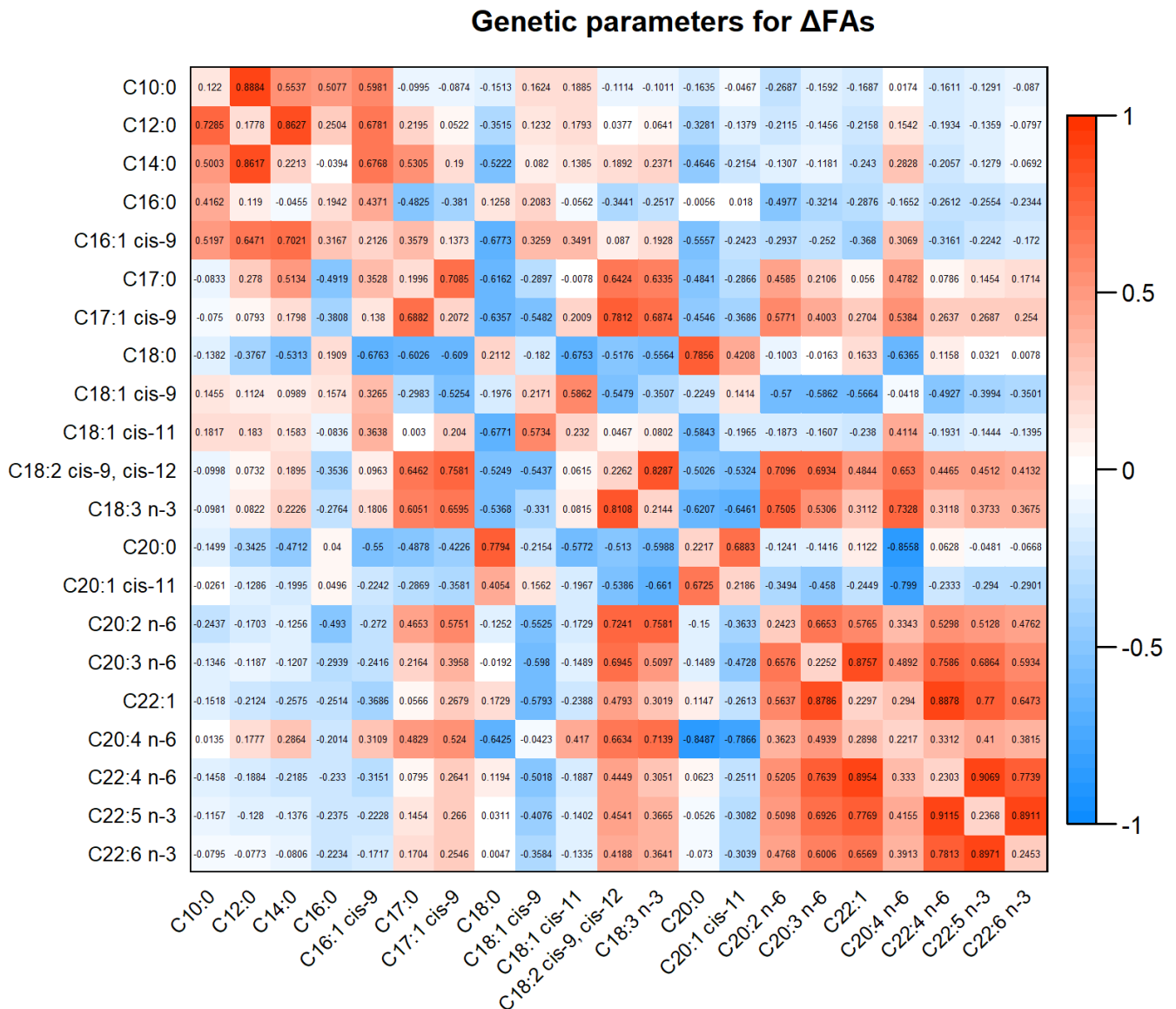
403

404 Table 3 reports the heritability values, phenotypic, and genetic correlations for the Δs of the FA

405 classes. The heritability values estimated for the ΔFA classes were all similar and of moderate

406 magnitude, ranging from 0.225 for ΔSFAs, to 0.232 for ΔPUFAs and Δ*n*-6 PUFAs (Table 3). The

407 estimated genetic and phenotypic correlations were all significant. The differences for SFAs and

408 MUFAs showed similar genetic correlation patterns, as both these classes had differences

409 displaying negative genetic correlations with ΔPUFAs, Δ*n*-6 PUFAs, and Δ*n*-3 PUFAs.

410 Contrariwise, ΔSFAs had a mild positive genetic correlation with Δ*n*-6/*n*-3, while ΔMUFAs had a

411 negative genetic correlation with the Δ*n*-6/*n*-3 (Table 3).

412   **Table 3.** Heritability estimates (diagonal, in bold), phenotypic (below the diagonal) and genetic correlations (above the diagonal) ± Standard Errors

413   (SE) for the differences (Δ) between the fatty acid (FA) classes in backfat (BF) and *Semimembranosus* (SM) muscle. * stands for *P*-value < 0.05; **

414   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	-0.562*** ± 0.029	-0.628*** ± 0.028	0.769*** ± 0.023	0.775*** ± 0.022	**0.228 ± 0.004**	-0.234*** ± 0.066
acids)						
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**

415

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

417 and positive genetic correlations with ΔPUFAs.

418 The genetic correlations between ΔFAs and carcass traits were almost negligible and most of them

419 did not reach the significance threshold. Hot carcass weight displayed a positive genetic correlation

420 with the Δ for palmitic acid ($r_g = 0.137$; $P < 0.001$), and negative genetic correlations with the Δs

421 for lauric ($r_g = -0.070$; $P < 0.05$) and eicosadienoic acids ($r_g = -0.098$; $P < 0.01$). Loin thickness

422 showed a positive genetic correlation with the Δ for palmitic acid ($r_g = 0.118$; $P < 0.001$), and a

423 negative genetic correlation with the Δ for eicosadienoic acid ($r_g = -0.074$; $P < 0.05$). Finally, a

424 positive genetic correlation was noticed between BFT measured with a caliper and the Δ for capric

425 acid ($r_g = 0.079$; $P < 0.05$). No significant genetic correlations were noticed between carcass traits

426 and the Δs for FA classes.

427

428 **4. Discussion**

429 Meat has a relevant role in the human diet as it provides important nutrients, such as high-value

430 proteins, vitamins, minerals, and essential FAs (Schmid, 2010; Pereira & Vicente, 2013). Porcine

431 fat depots and their FA composition influence pork technological quality and its sensory attributes

432 such as tenderness, juiciness, and aroma (Wood et al., 2008). Subcutaneous fat and IMF FA

433 compositions are also of great importance in determining meat nutritional features, supporting the

434 need for a better characterization of meat FA composition and of the variables affecting FA

435 deposition and composition of the different fat depots in pig carcasses. This study evaluated in

436 heavy pigs the differences of the individual FAs and FA classes between BF and SM muscle

437 tissues, allowing for the estimation of their genetic parameters and for the identification of their

438 associations with slaughter season, gender, EUROP carcass classification, BF thickness and IMF %.

439

440 The comparison of the FA composition highlighted significant differences between the two tissues

441 for all FAs and FA classes investigated, except for lauric acid. Lauric acid provides a fast energy

442 supply to cells as this FA is one of the most efficient substrates for β-oxidation (Leyton, Drury, &

443 Crawford, 1987; Lyudinina, Ivankova, & Bojko, 2018). This major role of lauric acid in tissue

444 energy metabolism may therefore explain why, in the present study, its proportion remains stable

445 between SM muscle and BF tissue. The marked compositional differences noticed for the other FAs

446 and FA classes are instead in agreement with the large body of literature proving intramuscular

447 adipocytes display different metabolic roles when compared with subcutaneous adipocytes (Gardan,

448 Gondret, & Louveau, 2006; Zhang et al., 2014). In particular, among the considered FAs, some

449 differences between the two tissues were noticed for stearic and oleic acids, two of the major

450 components of animal tissues FA composition (Wood et al., 2008). In the present study, stearic acid

451 was more associated with BF tissue, while oleic was present in higher proportions in IMF. The

452 proportions of these two FAs were however quite variable among the tested pigs, as suggested by

453 the high standard error bars in Figure 1. Variations in stearic acid Δs showed to be highly associated

454 with slaughter season, with pigs slaughtered in winter having lower stearic acid Δs when compared

455 to those slaughtered in summer, autumn, and spring. In a previous study performed on the same

456 animals, we analyzed the two tissues separately, and we evidenced a decreased storage of stearic

457 acid in the BF tissue of pigs slaughtered in winter (Zappaterra, Catillo, Lo Fiego, Belmonte,

458 Padalino, & Davoli, 2022). These changes were not noticed in SM, suggesting that the lowest

459 values of stearic acid Δs noticed in winter in the present study depend on the decreased proportion

460 of stearic acid deposited in BF during this season. It is widely accepted in the scientific literature

461 that in mammals the composition of the fat in the different anatomical regions is determined by the

462 temperature to which the tissues are subjected (Fawcett and Lyman, 1954). In humans, white

463 adipocytes in the subcutaneous tissue were proved to undergo metabolic rearrangements associated

464 with the seasons (Kern et al., 2014). Similarly, pigs subjected to heat stress were found to have an

465 increased expression of genes related to lipogenic activity in subcutaneous fat tissue (Qu, &

466 Ajuwon, 2018). Our results are therefore in agreement with these studies, confirming that

467 subcutaneous fat may be more prone to changes related to temperature and thus season compared to

468 other fat depots, such as IMF. In accordance with the scientific literature, the highest content of

469 stearic acid identified in the BF tissue could indicate an attempt of the subcutaneous adipocytes to

470 maintain membrane integrity by incorporating higher contents of this SFA, increasing membrane

471 resistance to high-temperature environments (Roy, Das, & Ghosh, 1997; Malekar, Morton, Hider,

472 Cruickshank, Hodge, & Metcalf, 2018). The fluctuations in the stearic acid stored in IMF were less

473 evident, suggesting that FA metabolism is more dynamic in porcine BF tissue and responds more

474 widely to changes in environmental conditions than IMF adipocytes. This finding is in line with the

475 results reported in cattle adipocytes, where subcutaneous fat was more responsive to external

476 stimuli and fast physiological changes when compared with IMF adipocytes (Smith, Lin, Wilson,

477 Lunt, & Cross, 1998). Furthermore, the stearic and oleic Δs were neither genetically nor

478 phenotypically correlated in the present study. The deposition of these two FAs in the two tissues is

479 therefore highly variable, but that variability in heavy pigs mostly relies on genetic and

480 environmental factors that are, at least in part, different between SM and BF tissues. This

481 observation agrees with the findings reported in a recent study investigating the gene expression

482 networks associated with the deposition of oleic and stearic acid contents in ILW SM muscle

483 (Zappaterra, Gioiosa, Chillemi, Zambonelli, & Davoli, 2021). These FAs showed indeed to be

484 linked to different gene expression networks, suggesting that the complex molecular cascades

485 regulating their deposition in SM muscle may differ (Zappaterra, Gioiosa, Chillemi, Zambonelli, &

486 Davoli, 2021).

487

488 The significant differences observed for Δs among pigs with the different IMF classes and between

489 barrows and gilts (Figure 1) were instead mainly determined by fluctuations in IMF FA

490 composition, as reported in Zappaterra, Catillo, Lo Fiego, Belmonte, Padalino, & Davoli (2022).

491 Sex was already found to be a factor influencing in porcine muscle the mRNA and protein

492 expression levels of genes involved in FA biosynthesis (Braglia, Zappaterra, Zambonelli, Comella,

493 Dall'Olio, & Davoli, 2014; Zappaterra, Deserti, Mazza, Braglia, Zambonelli, & Davoli, 2016;

494   Revilla et al., 2018). This evidence supports the hypothesis that sex and hormonal signals may have

495   a stronger impact on muscle fat deposition and composition, while BF FA composition seems to be

496   more dependent on environmental conditions, such as slaughter season. Among FAs showing

497   differences related to muscle IMF deposition, oleic acid Δ decreased as IMF% increased. This trend

498   suggests that as IMF deposition enhances, the percentage of oleic acid does not increase

499   proportionally in muscle and backfat, with oleic acid deposition being more sustained in muscle.

500   Similar results were already reported in our previous study (Zappaterra et al., 2020) and in Duroc

501   pigs, where the variability noticed in the oleic acid content in muscle had a positive genetic

502   correlation with SM IMF% while its amount in BF was not correlated with the variability noticed in

503   IMF (Ros-Freixedes, Reixach, Tor, & Estany, 2012; Ros-Freixedes, Reixach, Bosch, Tor, & Estany,

504   2014). In addition, in the present study, as SM IMF % enhanced, its content of linoleic acid

505   decreased. Similar results were also found in Duroc pigs, with linoleic acid in *Gluteus medius*

506   muscle having a negative genetic correlation (-0.66) with the IMF deposition in the same tissue

507   (Gol et al., 2019). The same Authors found arachidonic acid follows trends that are similar to what

508   is observed for linoleic acid (Gol et al., 2019). These two FAs are part of the same biosynthetic

509   process, as linoleic acid may be subjected to subsequent desaturation and elongation steps leading

510   to the formation of arachidonic acid (Nakamura & Nara, 2004). However, we were not able to

511   highlight similar patterns in the present study, as arachidonic acid Δs were neither associated with

512   SM IMF %, nor with animal sex. On the contrary, sex was an extremely important variable

513   determining significant fluctuations in the Δs of oleic and linoleic acids, with barrows having lower

514   oleic acid Δs and higher linoleic acid Δs when compared with gilts. This result agrees with the fact

515   that barrows tend to store more IMF than gilts (Ntawubizi, Raes, Buys, & De Smet, 2009;

516   Zappaterra et al., 2020), leading to an increased deposition of oleic acid in SM and a decreased

517   deposition of linoleic. As oleic and linoleic acids are two of the major components of MUFA and

518   PUFA classes, respectively, the observed trends in their Δs returned with similar fluctuations also in

519   the Δs for MUFAs and *n*-6 PUFAs (Figure 2B and 2C), with significant differences in those Δs

520   related to IMF % classes and animal sex.

521

522   Despite the discussed differences, the high and positive genetic correlations estimated between the

523   proportions of FAs and FA classes in SM muscle and BF tissues suggest that the FA composition in

524   SM muscle and BF shares a common genetic basis. Arachidonic, docosapentaenoic,

525   docosahexaenoic, *cis*-vaccenic, lauric, and stearic acids showed the highest genetic correlations,

526   which even corresponded to r_g values above 0.9, suggesting that their variability depended greatly

527   on the same genes and molecular patterns. Also, most of the other FAs and FA classes displayed

528   positive and medium-to-high genetic correlations between the two tissues, except for the *n*-6/*n*-3

529   ratio, capric, and erucic acids. The latter showed, indeed, negative genetic correlations, suggesting

530   that the genetic patterns controlling their deposition in one tissue may exert an opposite effect in

531   cells located in other body districts. Despite the genetic mechanisms underlying FA synthesis and

532   deposition seem to be mostly conserved between BF and SM muscle, the reason for the negative

533   genetic correlations linking *n*-6/*n*-3 ratio, capric, and erucic acids have yet to be elucidated. The

534   scientific literature lacks studies that suggest possible explanations for these negative genetic

535   correlations. However, our results are in accordance with results identified in mice, where the

536   deletion of the *Ceramide Synthase 1* (*CerS1*) gene determined an increased accumulation of erucic

537   acid in skeletal muscle sphingolipids, but no effect was noticed in white adipose tissue cells

538   (Turpin-Nolan et al., 2019). Among the FAs showing negative genetic correlations between their

539   proportions in BF and SM muscle is the *n*-6/*n*-3 ratio. This negative and highly significant genetic

540   correlation may be of great interest for further discussion, as it implies that some sort of difference

541   exists between muscle and subcutaneous fat tissues in the incorporation of *n*-3 and *n*-6 PUFAs. In

542   previous studies investigating the FA compositions of BF and SM individually, the amount of *n*-6

543   and *n*-3 PUFAs deposited in BF showed different genetic correlations when compared with the

544   same FA classes in SM muscle (Davoli et al., 2019; Zappaterra et al., 2020). While the selection for

545   a thicker BF was suggested to determine a decreased amount of both *n*-3 and *n*-6 PUFAs deposited

546   in BF (Davoli et al., 2019), an increased IMF % was correlated with a reduction in SM muscle *n*-6

547   PUFAs without exerting effects on *n*-3 PUFAs (Zappaterra et al., 2020). A different molecular

548   control regulating PUFAs deposition in SM muscle and BF is supported by the genetic correlations

549   estimated in the present study, where the proportion of *n*-3 PUFAs in the two tissues had a lower

550   genetic correlation when compared with the r_g value estimated for *n*-6 PUFAs. This difference may

551   be due by the fact that endogenous *n*-3 PUFAs derive from the elongation of α-linolenic acid

552   (Sinclair, Attar-Bashi, & Li, 2002). In the present study, this FA was among the FAs with the

553   greatest relative variations between BF and SM muscle and was the only one that did not display

554   genetic correlations between the two tissues. Together with linoleic acid, α-linolenic is considered

555   an essential FA, and thus it can not be *de novo* synthesized in mammals and must be introduced

556   with the diet (Sinclair, Attar-Bashi, & Li, 2002). Its variability is therefore mainly controlled by the

557   digestion and absorption of dietary α-linolenic acid. In the present study, however, the pigs were all

558   fed the same diet, and therefore the variations observed between pigs can not be linked to variations

559   in the FA composition of the diet.

560   Concerning the genetic parameters estimated for the variations between FA composition of BF and

561   SM muscle, all Δs showed low-to-medium heritability values, in line with the heritability values

562   estimated in ILW pigs for the FAs in SM muscle (Zappaterra et al., 2020) and BF (Davoli et al.,

563   2019). The fact that these differences are moderately heritable suggests that there are also genetic

564   mechanisms capable of differentiating the deposition of FAs in BF from those in SM muscle. This

565   result implies that a better knowledge of the genes differentiating the FA composition of BF and

566   SM muscle could provide new tools allowing to select, in a partially independent manner, the FA

567   composition of muscle and subcutaneous fat.

568

569   **5. Conclusion**

570 The study of genetic and non-genetic factors involved in variations between the FA composition of

571 SM muscle and BF tissue evidenced that common genetic control exists between the two porcine

572 tissues for most of the studied FAs. Some FAs and the n-6/n-3 ratio displayed a genetic control that

573 is more oriented towards tissue-specific molecular pathways, indicating that fluctuations in the

574 proportions of those FAs in one tissue would affect to a lesser extent the proportion of the same FA

575 or FA class in the other tissue. Furthermore, our results suggested that variations in the FA

576 composition of SM muscle and BF tissue can be modulated through genetic selection, and

577 confirmed the importance of non-genetic and physiological factors on SM muscle and BF tissue FA

578 composition. On the whole, these results can be useful for increasing the knowledge of factors

579 affecting FA composition in pig muscle and BF. Further studies are needed to identify the

580 molecular pathways and genes involved in the variations between BF and SM muscle FA

581 composition, also given the role played by these traits in the quality of meat intended for PDO

582 products.

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