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

4

5 Martina Zappaterra^{a*}, Gennaro Catillo^b, Domenico Pietro Lo Fiego^{c,d}, Giovanna Minelli^{c,d}, Barbara
6 Padalino^a, Roberta Davoli^{a*}

7

8 ^a Department of Agricultural and Food Sciences (DISTAL), University of Bologna, Viale G. Fanin
9 46, I-40127, Bologna, Italy.

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

13 ^c Department of Life Sciences (DSV), University of Modena and Reggio Emilia, Via G. Amendola
14 2, I-42122, Reggio Emilia, Italy.

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

18

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

20

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23

24

25 **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.

37

38

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

40

41 **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**

178 **2.2.1 Data handling**

179 The continuous variables of BFT and IMF were divided into quartile classes using the UNIVARIATE
180 procedure of SAS software vers. 9.4. Normality was tested for both traits with the Shapiro-Wilk test.
181 For BFT the following classes were defined: carcasses with a BFT < 23 mm, from 23 to 27 mm, from
182 27 to 30 mm, and BFT > 30 mm. For IMF the quartiles were: samples with IMF < 1.24%, from 1.24%
183 to 1.72%, from 1.72% to 2.63%, and with IMF > 2.63%. BFT and IMF quartiles were then used as
184 independent variables in the statistical analyses.

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

188 Furthermore, BF and SM muscle FA compositions were used to obtain a new set of variables
189 calculated as the difference between each FA or FA class in BF (%) and the same FA or FA class in
190 SM muscle tissue (%). For example, for each sample, the % of capric acid (C10:0) in SM muscle was
191 subtracted from the % of C10:0 measured in BF tissue. From this point onward, these variables will
192 be referred to as Δ followed by the name of the individual FA or FA class (e.g., Δ C10:0; Δ C12:0;
193 etc...).

194

195 **2.2.2 Descriptive statistics, phenotypic and genetic correlations between the two** 196 **tissues**

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

$$200 y_{ij} = \mu + T_i + e_{ij}$$

201 Where: y_{ij} was the vector of the percentage of each FA or FA class; μ was the overall mean; T_i was
202 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:

$$207 \quad \begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} X_1 & 0 \\ 0 & X_2 \end{bmatrix} \begin{bmatrix} b_1 \\ b_2 \end{bmatrix} + \begin{bmatrix} Z_1 & 0 \\ 0 & Z_2 \end{bmatrix} \begin{bmatrix} a_1 \\ a_2 \end{bmatrix} + \begin{bmatrix} W_1 & 0 \\ 0 & W_2 \end{bmatrix} \begin{bmatrix} d_1 \\ d_2 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \end{bmatrix}$$

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:

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

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

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

223 It was assumed that:

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

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:

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

243 Where y is the observation vector for the i th 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 Δ FA 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 Δ FA 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 Δ FA mean values were also
 254 compared between gilts and barrows with Student's *t*-test.

255 The Δ FA 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 Δ FA 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 Δ s 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 Δ s of FA classes (number of dependent variables = 12).

263 The multiple trait animal models are mixed infinitesimal models where Δ s 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 Δ s of FAs and *i* = 12: measurements at slaughtering added with Δ s 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 i th 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 Δ s were as follows:

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

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

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

281 It was assumed that:

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

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 covariance 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 Δ FA or Δ FA 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			<i>P</i> -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 <i>cis</i> -9 (palmitoleic acid)	1.453 ± 0.254	0.818	2.393	2.928 ± 0.486	1.481	4.472	<.0001
C17:1 <i>cis</i> -9 (heptadecenoic acid)	0.153 ± 0.034	0.070	0.308	0.232 ± 0.054	0.117	0.479	<.0001
C18:1 <i>cis</i> -9 (oleic acid)	38.884 ± 1.586	30.528	43.824	40.722 ± 2.892	27.895	48.691	<.0001
C18:1 <i>cis</i> -11 (<i>cis</i> -vaccenic acid)	2.262 ± 0.235	1.575	2.998	3.932 ± 0.440	2.265	5.269	<.0001
C20:1 <i>cis</i> -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 <i>cis</i> -9, <i>cis</i> -12 (linoleic acid)	16.528 ± 2.037	11.539	24.146	10.830 ± 2.093	6.278	21.133	<.0001
C18:3 <i>n</i> -3 (α -linolenic acid)	0.762 ± 0.179	0.383	1.720	0.373 ± 0.086	0.023	0.687	<.0001
C20:2 <i>n</i> -6 (eicosadienoic acid)	0.803 ± 0.101	0.525	1.242	0.450 ± 0.075	0.275	0.693	<.0001
C20:3 <i>n</i> -6 (dihomo- γ -linolenic acid)	0.087 ± 0.016	0.045	0.171	0.228 ± 0.102	0.000	0.706	<.0001
C20:4 <i>n</i> -6 (arachidonic acid)	0.230 ± 0.047	0.084	0.495	1.875 ± 0.928	0.461	7.763	<.0001
C22:4 <i>n</i> -6 (adrenic acid)	0.097 ± 0.020	0.048	0.385	0.289 ± 0.115	0.094	0.888	<.0001
C22:5 <i>n</i> -3 (docosapentaenoic acid-DPA)	0.055 ± 0.012	0.008	0.101	0.109 ± 0.045	0.027	0.613	<.0001
C22:6 <i>n</i> -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
<i>n</i> -6 PUFAs (<i>n</i> -6 Polyunsaturated fatty acids)	17.745 ± 2.117	12.588	25.593	13.672 ± 3.034	7.287	30.870	<.0001
<i>n</i> -3 PUFAs (<i>n</i> -3 Polyunsaturated fatty acids)	0.831 ± 0.184	0.428	1.763	0.510 ± 0.114	0.177	1.184	<.0001
<i>n</i> -6/ <i>n</i> -3	22.121 ± 3.966	7.815	45.605	27.160 ± 4.811	13.912	85.262	<.0001

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 <i>cis</i> -9 (palmitoleic acid)	0.461	0.032	<.0001	0.855	0.018	<.0001
C17:1 <i>cis</i> -9 (heptadecenoic acid)	0.216	0.035	<.0001	0.917	0.014	<.0001
C18:1 <i>cis</i> -9 (oleic acid)	0.261	0.034	<.0001	0.824	0.020	<.0001

C18:1 <i>cis</i> -11 (<i>cis</i> -vaccenic acid)	0.247	0.035	<.0001	0.975	0.008	<.0001
C20:1 <i>cis</i> -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 <i>cis</i> -9, <i>cis</i> -12 (linoleic acid)	0.474	0.031	<.0001	0.442	0.032	<.0001
C18:3 <i>n</i> -3 (α -linolenic acid)	0.374	0.033	<.0001	-0.018	0.036	0.500
C20:2 <i>n</i> -6 (eicosadienoic acid)	0.016	0.036	0.654	0.574	0.029	<.0001
C20:3 <i>n</i> -6 (dihomo- γ -linolenic acid)	0.363	0.033	<.0001	0.578	0.029	<.0001
C20:4 <i>n</i> -6 (arachidonic acid)	0.130	0.035	0.0002	0.999	0.002	<.0001
C22:4 <i>n</i> -6 (adrenic acid)	0.128	0.035	0.0003	0.684	0.026	<.0001
C22:5 <i>n</i> -3 (docosapentaenoic acid-DPA)	0.048	0.036	0.179	0.999	0.002	<.0001
C22:6 <i>n</i> -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
<i>n</i> -6 PUFAs (<i>n</i> -6 Polyunsaturated fatty acids)	0.173	0.035	<.0001	0.462	0.032	<.0001

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

324

325

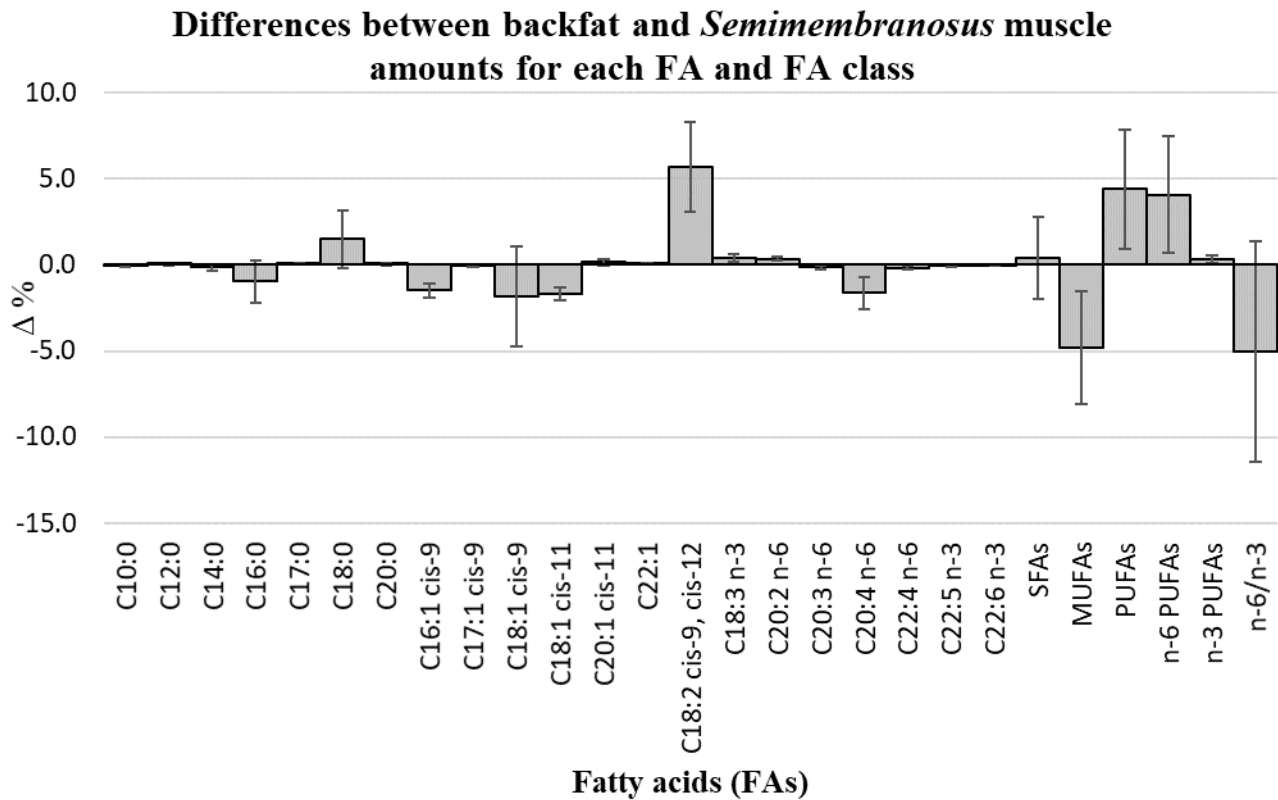
326 **3.2 Descriptive statistics and genetic parameters for the differences between**

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

328 The means, standard deviations, minimum and maximum values for the obtained Δ s are reported in
329 Supplementary Table S2. Means and standard deviations for the Δ s are graphically displayed in
330 Figure 1. As can be noticed from Figure 1, Δ s 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 ($5.70 \pm 2.61\%$), PUFAs ($4.39 \pm 3.48\%$), and *n*-6
333 PUFAs ($4.07 \pm 3.39\%$), while the lowest Δ s were observed for *n*-6/*n*-3 ratio ($-5.04 \pm 6.38\%$),
334 MUFAs ($-4.80 \pm 3.29\%$), and oleic acid ($-1.84 \pm 2.93\%$). Comparing Δ s 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 ($\Delta\%$) between the fatty
340 acids (FAs) and FA classes in backfat (BF) and *Semimembranosus* (SM) muscle.



341

342 The results of the ANOVA performed on Δ s 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 Δ s. The Least Squares Means (L.S.M.) of Δ s for

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

347 season showed to affect almost all Δ s, 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 Δ s, except those for stearic, *cis*-vaccenic, α -linolenic, arachidonic acids, SFAs and *n*-3

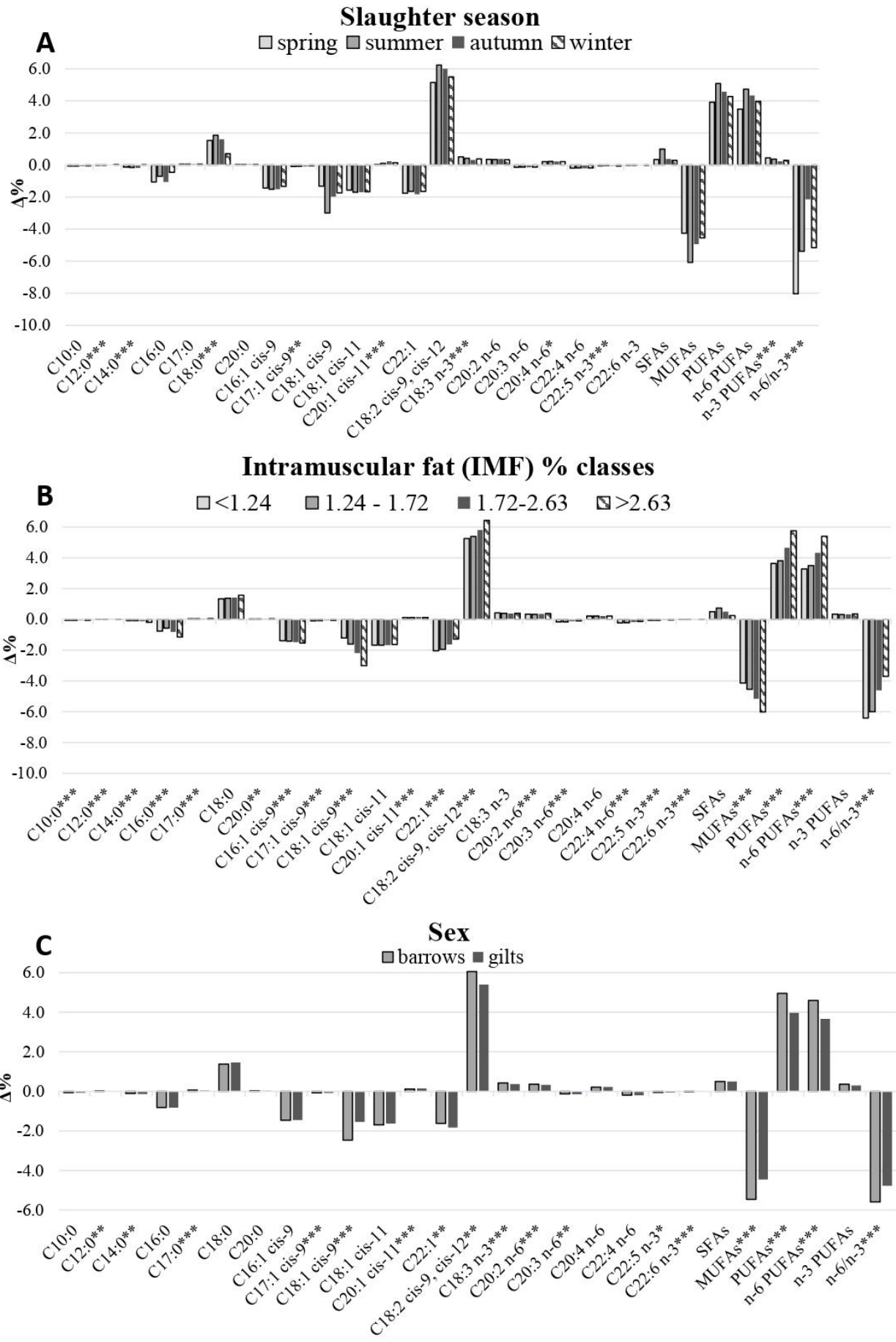
354 PUFAs (Figure 2B and Supplementary Table S3). Supplementary Table S5 shows the results of the

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

356 values of L.S.M. for the Δ s 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 Δ s 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 Δ s 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 Δ s in barrows and gilts. When compared with
365 barrows, gilts had lower Δ s 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 ($\Delta\%$) 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).



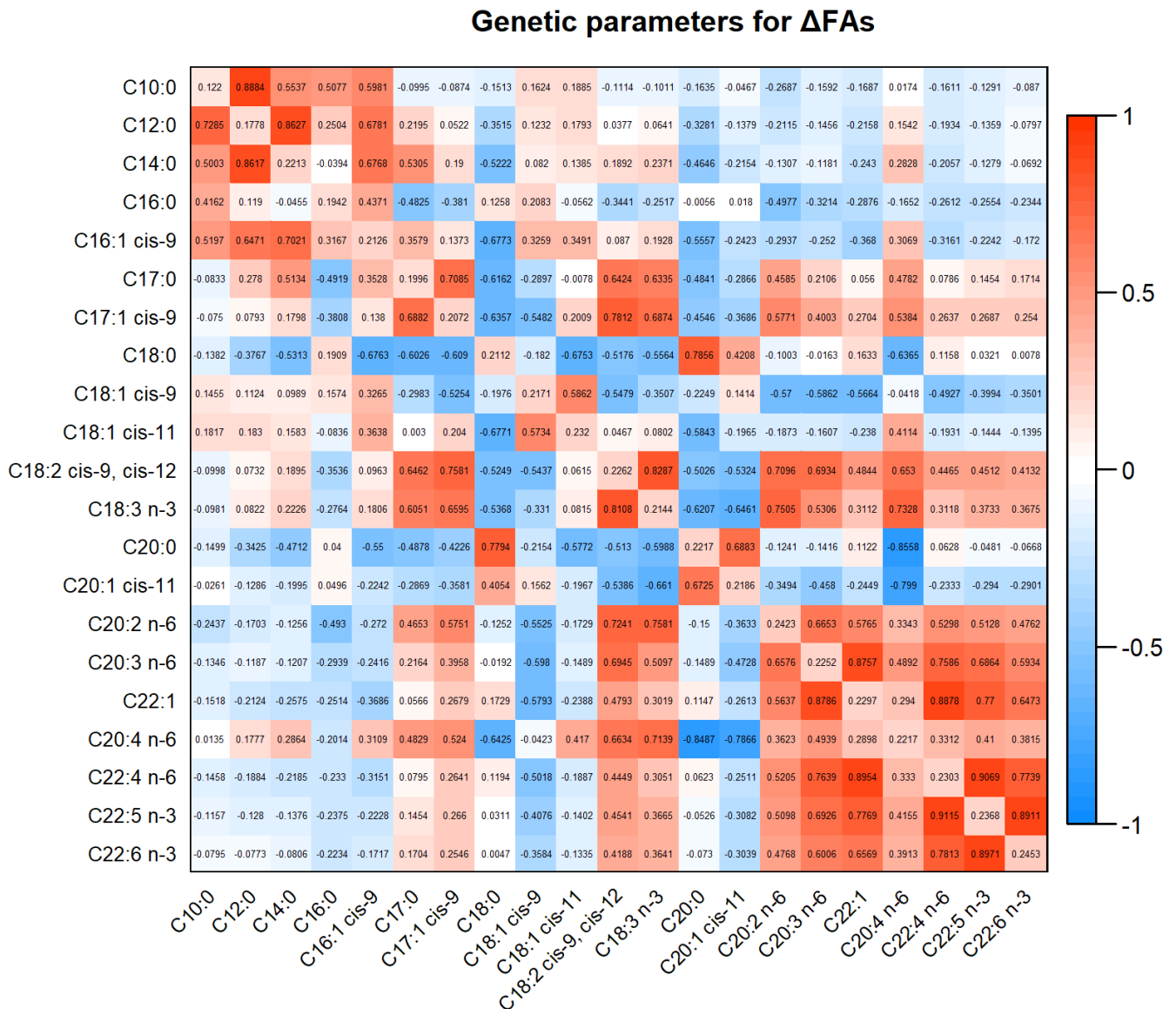
375 BFT classes and EUROP carcass grading showed to affect to a lesser extent the Δ s, with BFT being
376 associated with changes in Δ s for stearic, palmitoleic, *cis*-vaccenic, linoleic, arachidonic acids, and
377 SFAs, and EUROP carcass grading was only associated with arachidonic acid (Δ C20:4 *n*-6;
378 Supplementary Table S3). In particular, the orthogonal contrasts reported in Supplementary Table
379 S7 that BFT classes greater than 27 mm were associated with lower Δ s for palmitoleic, *cis*-
380 vaccenic, arachidonic acids, and *n*-6/*n*-3 ratio, suggesting that pigs with a thicker BF layer had
381 lower amounts of those MUFAs and arachidonic, thus decreasing the *n*-6/*n*-3 ratio.
382 No significant differences were observed for the orthogonal contrasts between EUROP carcass
383 classes.

384 Figure 3 shows the heritability estimates, phenotypic, and genetic correlations for the Δ s of the
385 individual FAs. Darker colors indicate stronger correlation coefficients. Supplementary Table S8
386 reports the SE for the phenotypic and genetic correlation coefficients for the Δ FAs reported in
387 Figure 3. The heritability values estimated for the Δ FAs were of low-to-moderate magnitude,
388 ranging from 0.122 for the difference between capric acid percentages (Δ C10:0) to 0.245 for the
389 difference between DHA percentages (Δ C22:6 *n*-3; Figure 3 and Supplementary Table S6). On the
390 whole, almost all the genetic and phenotypic correlations between Δ FAs were significant. As can be
391 noticed from Figure 3, the heatmap is characterized by two blocks of positive (red cells) genetic and
392 phenotypic correlations. These two blocks are on the opposite sides of the diagonal and indicate, on
393 one side, the positive genetic and phenotypic correlations relating the differences between short and
394 medium SFAs and palmitoleic acid (i.e., Δ C10:0, Δ C12:0, Δ C14:0, Δ C16:0, and Δ C16:1 *cis*-9), on
395 the other side the positive genetic and phenotypic correlations relating the differences between *n*-6
396 and *n*-3 PUFAs (i.e., Δ C18:2 *cis*-9, *cis*-12, Δ C18:3 *n*-3, Δ C20:2 *n*-6, Δ C20:3 *n*-6, Δ C20:4 *n*-6,
397 Δ C22:4 *n*-6, Δ C22:5 *n*-3, Δ C22:6 *n*-3).

398

399 **Figure 3.** Heatmap with the heritability values (diagonal), phenotypic (below the diagonal) and
400 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.



403
 404 Table 3 reports the heritability values, phenotypic, and genetic correlations for the Δ s of the FA
 405 classes. The heritability values estimated for the Δ FA classes were all similar and of moderate
 406 magnitude, ranging from 0.225 for Δ SFAs, to 0.232 for Δ PUFAs and $\Delta n-6$ 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 Δ PUFAs, $\Delta n-6$ PUFAs, and $\Delta n-3$ PUFAs.
 410 Contrariwise, Δ SFAs had a mild positive genetic correlation with $\Delta n-6/n-3$, while Δ MUFAs had a
 411 negative genetic correlation with the $\Delta n-6/n-3$ (Table 3).

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

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

$\Delta n-3$ PUFAs (*n-3*

Polyunsaturated fatty acids)	-0.562*** \pm 0.029	-0.628*** \pm 0.028	0.769*** \pm 0.023	0.775*** \pm 0.022	0.228 \pm 0.004	-0.234*** \pm 0.066
$\Delta n-6/n-3$	0.099** \pm 0.035	-0.323*** \pm 0.034	0.198*** \pm 0.035	0.282*** \pm 0.034	-0.256*** \pm 0.034	0.231 \pm 0.026

416 Both Δ s for *n*-6 and *n*-3 PUFAs showed negative genetic correlations with Δ SFAs and Δ MUFAs,
417 and positive genetic correlations with Δ PUFAs.
418 The genetic correlations between Δ FAs 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 Δ s
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 Δ s 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 Δ s showed to be highly associated
454 with slaughter season, with pigs slaughtered in winter having lower stearic acid Δ s 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 Δ s 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 Δ s 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 Δ s of oleic and linoleic acids, with barrows having lower
514 oleic acid Δ s and higher linoleic acid Δ s 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 Δ s returned with similar fluctuations also in

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

583

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