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

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

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21 Declarations of interest: Roberta Davoli declares to be a member of the Meat Science journal

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**Abstract**

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

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

**1 Introduction**

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

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, Guillermin-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  $155 \pm 6$  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, 8<sup>th</sup> 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,

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

For SM FA determination the total muscle lipids destined for the gas-chromatographic analysis were extracted using a mixture of chloroform: methanol (2:1, v/v) (Carlo Erba Reagents, MI, Italy) according to Folch, Lees, and Sloane Stanley (1957). Methylation was performed with a 2 N solution of potassium hydroxide (KOH) in methanol (CH<sub>3</sub>OH) (Carlo Erba Reagents, Milan, Italy) according to Ficarra, Lo Fiego, Minelli, & Antonelli (2010). Tridecanoic acid (C13:0) (Larodan Fine Chemicals AB, Solna, Sweden) was used as internal standard in SM FA determination. Intramuscular fatty acid methyl esters (FAMES) were then submitted to gas-chromatographic analysis using TRACE<sup>TM</sup>GC Ultra (Thermo Electron Corporation, Rodano, MI, Italy) equipped with a Flame Ionization Detector, a PVT injector, and a TR-FAME Column 30 m  $\times$  0.25 mm i.d., 0.2  $\mu$ m film thickness (Thermo Scientific, Rodano, MI, Italy). The Chrom-Card software (vers.2.3.3, Thermo Electron Corporation, Rodano, MI, Italy) was used to record and integrate the peaks of FAMES. Individual FAME were identified by comparing their retention times with the retention times of a standard FAME mixture prepared in-house with known quantities of each methyl ester (Larodan Fine Chemicals AB, Solna, Sweden). In order to present data in the same way as BF, the amount of each FA determined in SM was reported as g FA per 100 g of total FA (i.e., percent FA composition).

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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  $\Delta$  followed by the name of the individual FA or FA class (e.g.,  $\Delta$ C10:0;  $\Delta$ 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:

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

201 Where:  $y_{ij}$  was the vector of the percentage of each FA or FA class;  $\mu$  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:

$$\text{Var} \begin{bmatrix} a_1 \\ a_2 \\ d_1 \\ d_2 \\ e_1 \\ e_2 \end{bmatrix} = \begin{bmatrix} Ag_{11} & Ag_{12} & 0 & 0 & 0 & 0 \\ Ag_{21} & Ag_{22} & 0 & 0 & 0 & 0 \\ 0 & 0 & Iq_{11} & Iq_{12} & 0 & 0 \\ 0 & 0 & Iq_{21} & Iq_{22} & 0 & 0 \\ 0 & 0 & 0 & 0 & Is_{11} & Is_{12} \\ 0 & 0 & 0 & 0 & Is_{21} & Is_{22} \end{bmatrix}$$

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

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

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

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

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

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

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

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

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

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

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

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  $\Delta$ s were as follows:

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

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

280 Residual:  $\text{Var} \begin{bmatrix} e_1 \\ \vdots \\ e_i \end{bmatrix} = \begin{bmatrix} Is_{11} & \dots & Is_{ij} \\ \vdots & & \vdots \\ Is_{ji} & \dots & Is_{ii} \end{bmatrix}$ , 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  $\Delta FA$  or  $\Delta FA$  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- $\gamma$ -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 ( $\alpha$ -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- $\gamma$ -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



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

321

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

FAs (%)	Phenotypic correlations			Genetic correlations		
	$r$	SE	$P$ -value	$r_g$	SE	$P$ -value
C10:0 (capric acid)	0.099	0.035	0.005	-0.154	0.035	<.0001
C12:0 (lauric acid)	0.213	0.035	<.0001	0.963	0.010	<.0001
C14:0 (myristic acid)	0.310	0.034	<.0001	0.581	0.029	<.0001
C16:0 (palmitic acid)	0.461	0.032	<.0001	0.651	0.027	<.0001
C17:0 (margaric acid)	0.419	0.032	<.0001	0.835	0.020	<.0001
C18:0 (stearic acid)	0.502	0.031	<.0001	0.927	0.013	<.0001
C20:0 (arachidic acid)	-0.048	0.036	0.174	0.249	0.035	<.0001
C16:1 <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 ( $\alpha$ -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- $\gamma$ -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

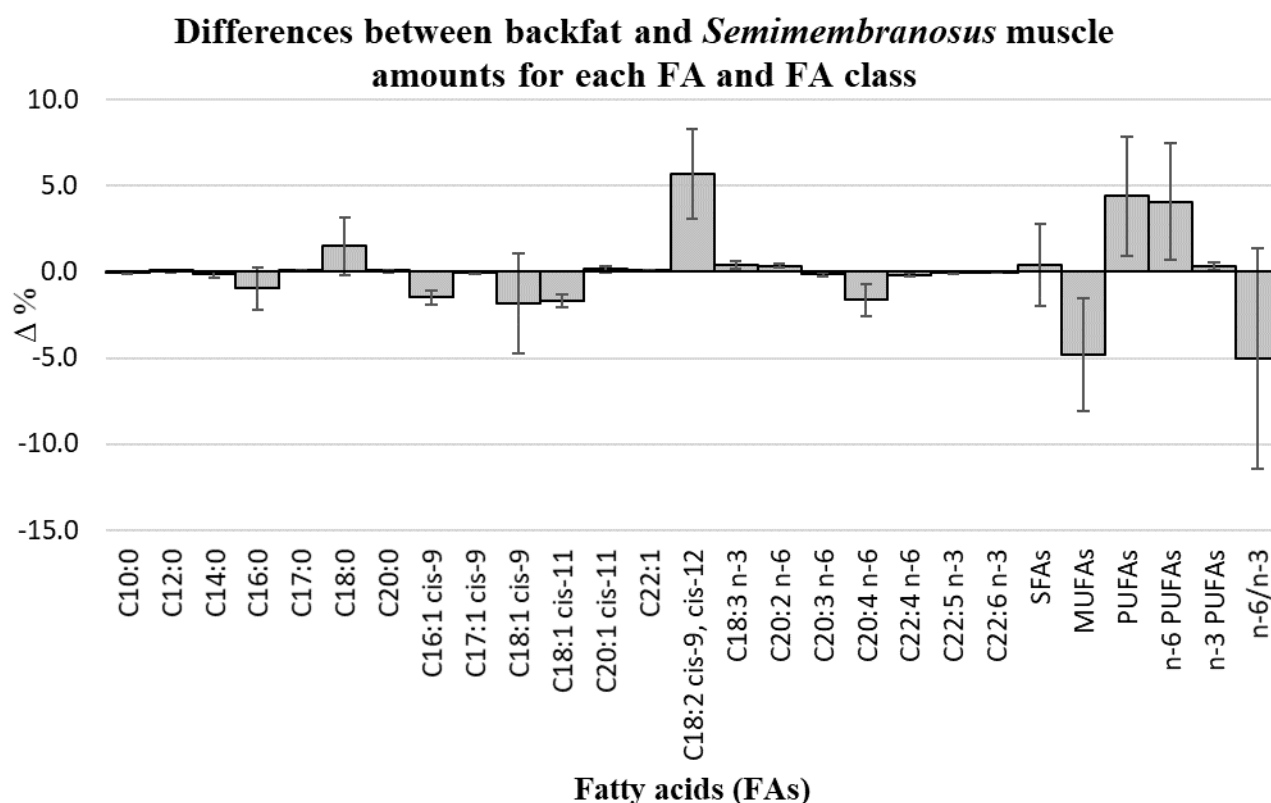
<i>n</i> -3 PUFAs ( <i>n</i> -3	0.038	0.036	0.2821	0.305	0.034	<.0001
Polyunsaturated fatty acids)						
<i>n</i> -6/ <i>n</i> -3	-0.048	0.036	0.1746	-0.588	0.029	<.0001

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

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

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



341

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

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

347 season showed to affect almost all  $\Delta$ s, except those for margaric, erucic, dihomog- $\gamma$ -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  $\Delta$  values for lauric, myristic, heptadecenoic acids, and  $n$ -6/ $n$ -3 ratio, while those slaughtered in

351 spring-summer had higher  $\Delta$  values for stearic, gadoleic,  $\alpha$ -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

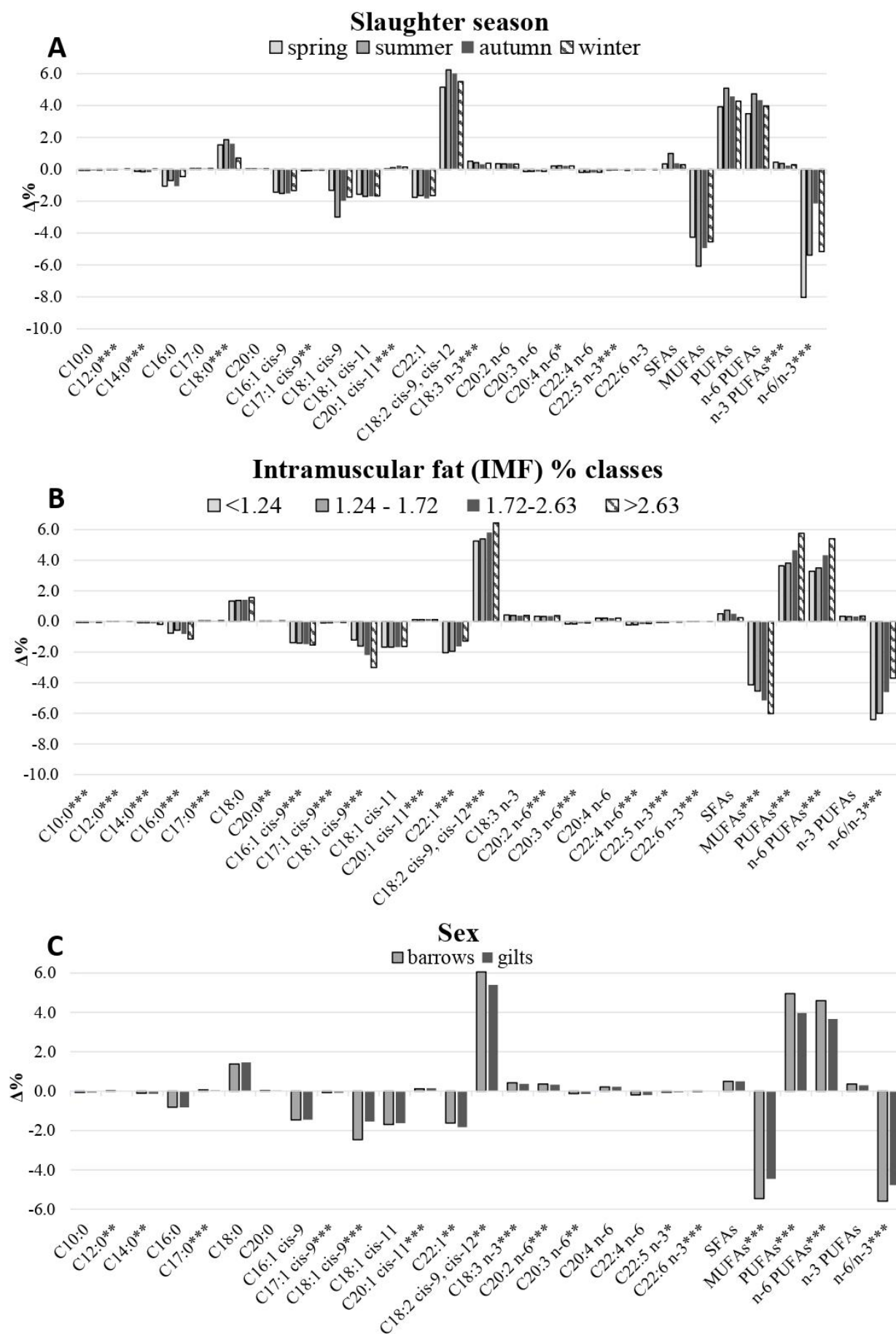
353 almost all  $\Delta$ s, except those for stearic, *cis*-vaccenic,  $\alpha$ -linolenic, arachidonic acids, SFAs and  $n$ -3

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

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

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



375 BFT classes and EUROP carcass grading showed to affect to a lesser extent the  $\Delta$ s, with BFT being  
 376 associated with changes in  $\Delta$ s for stearic, palmitoleic, *cis*-vaccenic, linoleic, arachidonic acids, and  
 377 SFAs, and EUROP carcass grading was only associated with arachidonic acid ( $\Delta$ 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  $\Delta$ 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  $\Delta$ 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  $\Delta$ FAs reported in  
 387 Figure 3. The heritability values estimated for the  $\Delta$ FAs were of low-to-moderate magnitude,  
 388 ranging from 0.122 for the difference between capric acid percentages ( $\Delta$ C10:0) to 0.245 for the  
 389 difference between DHA percentages ( $\Delta$ C22:6 *n*-3; Figure 3 and Supplementary Table S6). On the  
 390 whole, almost all the genetic and phenotypic correlations between  $\Delta$ 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.,  $\Delta$ C10:0,  $\Delta$ C12:0,  $\Delta$ C14:0,  $\Delta$ C16:0, and  $\Delta$ 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.,  $\Delta$ C18:2 *cis*-9, *cis*-12,  $\Delta$ C18:3 *n*-3,  $\Delta$ C20:2 *n*-6,  $\Delta$ C20:3 *n*-6,  $\Delta$ C20:4 *n*-6,  
 397  $\Delta$ C22:4 *n*-6,  $\Delta$ C22:5 *n*-3,  $\Delta$ 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 ( $\Delta$ ) between the individual fatty acids

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

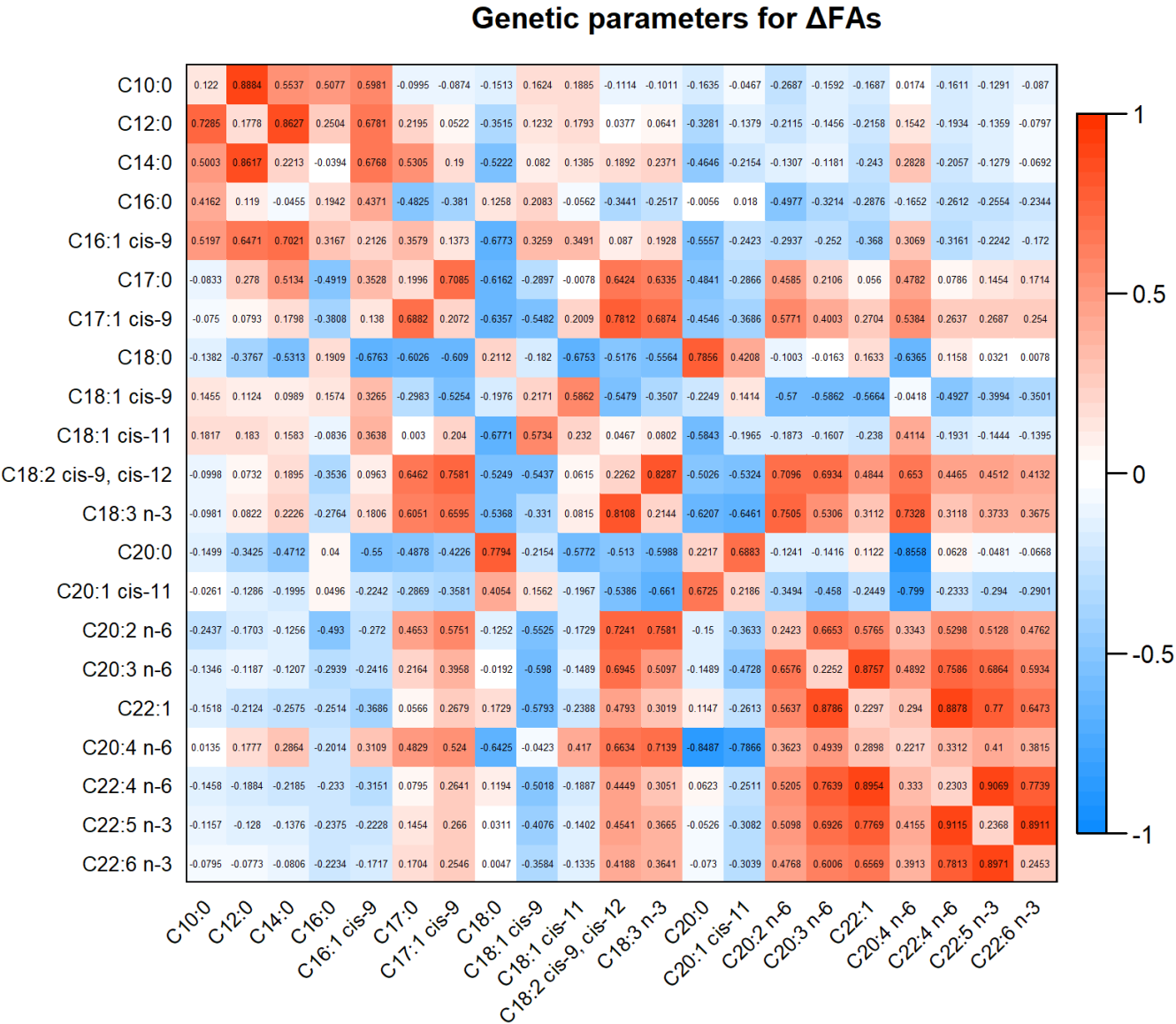


Table 3 reports the heritability values, phenotypic, and genetic correlations for the  $\Delta$ s of the FA classes. The heritability values estimated for the  $\Delta$ FA classes were all similar and of moderate magnitude, ranging from 0.225 for  $\Delta$ SFAs, to 0.232 for  $\Delta$ PUFAs and  $\Delta$ n-6 PUFAs (Table 3). The estimated genetic and phenotypic correlations were all significant. The differences for SFAs and MUFAs showed similar genetic correlation patterns, as both these classes had differences displaying negative genetic correlations with  $\Delta$ PUFAs,  $\Delta$ n-6 PUFAs, and  $\Delta$ n-3 PUFAs. Contrariwise,  $\Delta$ SFAs had a mild positive genetic correlation with  $\Delta$ n-6/n-3, while  $\Delta$ MUFAs had a 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 ( $\Delta$ ) 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  $< 0.001$ .

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

$\Delta n$ -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	<b>0.228 <math>\pm</math> 0.004</b>	-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	<b>0.231 <math>\pm</math> 0.026</b>

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Both  $\Delta$ s for *n*-6 and *n*-3 PUFAs showed negative genetic correlations with  $\Delta$ SFAs and  $\Delta$ MUFAs, and positive genetic correlations with  $\Delta$ PUFAs.

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

427

#### 4. Discussion

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

439

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

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

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

487

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

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  $\Delta$  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  $\Delta$ 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  $\Delta$ s of oleic and linoleic acids, with barrows having lower  
514 oleic acid  $\Delta$ s and higher linoleic acid  $\Delta$ 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  $\Delta$ s returned with similar fluctuations also in

519 the  $\Delta$ s for MUFAs and  $n$ -6 PUFAs (Figure 2B and 2C), with significant differences in those  $\Delta$ 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  $\alpha$ -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,  $\alpha$ -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  $\alpha$ -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  $\Delta$ 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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