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Describing backfat and *Semimembranosus* muscle fatty acid variability in heavy pigs: analysis of non–genetic factors

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Abstract

This study aimed to describe the multivariate structure of *Semimembranosus* muscle and backfat fatty acid (FA) composition in 798 Italian Large White heavy pigs and to investigate the effects of environmental factors and carcass characteristics on FA variations. The total FA variability in muscle and backfat was characterized by a negative correlation between saturated and polyunsaturated FAs, which strongly depended on the carcass adiposity. Slaughtering season was also relevant, with pigs slaughtered in autumn having more *n*-6 FAs and eicosadienoic acid in backfat, while pigs slaughtered in winter displayed more saturated FAs.

Regarding *Semimembranosus* muscle, pigs with heavier belly cuts and slaughtered in autumn had higher proportions of *cis*-vaccenic and palmitoleic acids, while those slaughtered in summer had more saturated FAs. Slaughtering season emerged as a relevant factor shaping both backfat and muscle FA composition, indicating that more studies and attention should be paid to environmental factors, which may have effects on FA metabolism and deposition in finishing pigs.

Keywords: fatty acid composition; subcutaneous fat quality; *Semimembranosus* muscle; swine; Principal Component Analysis.

1 Introduction

Global meat demand is expected to be 16% higher in 2025 over the 2013-2015 period, with poultry and pork production and demand leading the trend in developing countries (OECD, 2016). The demand for both fresh and processed meat products is expected to increase. Italy is a top producer of processed meat products, particularly of Protected Designation of Origin (PDO) products, contributing to about one-third of the European heritage meat product (Dalle Zotte, Brugiapaglia, & Cullere, 2017). Parma and San Daniele PDO hams accounted for more than half of the total turnover generated by the Italian PDO pork products in Italy in 2017 (ISMEA, 2019). These high-quality dry-cured hams are obtained from heavy pig hind legs, just salted, and ripened for a period that is generally not shorter than 13 months. Most of the Italian heavy pig production relies on animals slaughtered at a minimum age of 9 months and an average live weight of 160-170 kg. These pigs come from a specific selection scheme by the national herdbook, or from selection schemes with comparable selection goals (Consorzio del Prosciutto di Parma, 1992; Lo Fiego, Santoro, Macchioni, & De Leonibus, 2005; MIPAAF, 2007; Lo Fiego, Macchioni, Minelli, & Santoro, 2010).

The amount and quality of covering adipose tissue and intramuscular fat (IMF) are relevant for pigs used to produce seasoned meat products. The amount of subcutaneous, as well as IMF, strongly affects the technological yield of green hams limiting excessive seasoning losses (Bosi & Russo, 2004). Indeed, adipose tissue represents a barrier to water diffusion and salt penetration. Because of the inverse relationship of fat thickness with seasoning losses and salt content, leaner hams are expected to have a higher salt content (Čandek-Potokar, Monin, & Zlender, 2002), which is generally deemed negative for a human healthy diet. Furthermore, it has been reported that a suitable IMF content has a beneficial effect on juiciness (Ventanas, Ruiz, García, & Ventanas, 2007) and texture of dry-cured hams (Ruiz Carrascal et al., 2000). On the contrary, because of its influence on water loss and salt penetration dynamics, a high level of fat infiltration in the muscles was found to be associated with excessive softness and pastiness (Parolari, Rivaldi, Leonelli, Bellatti, & Bovis, 1988; Gou, Guerrero, & Arnau, 1995). Pigs with greater fat deposition tend to have a higher proportion of saturated fatty acids (SFAs; Tibau et al., 2002), which has positive effects on fat firmness and oxidative stability during the long maturation process of green hams (Virgili & Schivazappa, 2002; Bosi & Russo, 2004). A lower fat level in hams is associated with more polyunsaturated fatty acids (PUFAs; Bosi & Russo, 2004), mainly confined to phospholipids. Among PUFAs, *n*-3 are preferred by consumers for their positive effects on human health. However, PUFAs are also more prone to incur in lipolytic and oxidative processes causing rancidity, abnormal flavors, fat softness, and altered organoleptic properties of dry-cured hams (Wood et al., 2003; Juárez et al., 2011). On the other hand, meat fat content is important for the technological and sensory quality of dry-cured hams, because lipolysis and subsequent fat oxidation cause the development of volatile organic compounds determining the ham aroma (López et al., 1992; Pinna, Simoncini, Toscani, & Virgili, 2012). Different environmental, physiological, and molecular factors affect fat deposition and composition, contributing to the variability in the technological and sensory features of dry-cured hams and other meat products. For that reason, factors affecting fatty acid (FA) composition of different tissues have been under investigation for many years. FA composition showed in general high-to-moderate heritability estimates in pigs slaughtered at about 100 kg live weight, which were intended for fresh meat products (Suzuki et al., 2006; Sellier, Maignel, & Bidanel, 2010), and in Duroc pigs slaughtered at about 125 kg live weight (Ros-Freixedes, Reixach, Bosch, Tor, & Estany, 2014). Recent studies carried out on Italian Large White (ILW) heavy pigs (slaughtered at about 155 kg live weight) found that the FA composition of fat stored in muscle and backfat (BF) are the result of moderately heritable traits (Davoli et al., 2019; Zappaterra et al., 2020) and associated with genetic markers (Zappaterra, Ros-Freixedes, Estany & Davoli, 2018; Catillo et al., 2020). Diet has also a major role in the variability noticed in pork FA composition, as proved by the considerable literature produced over the years (Morgan, Noble, Cocchi, & McCartney, 1992; Leskanich, Matthews, Warkup, Noble, & Hazzledine, 1997; Carrapiso, Tejeda, Noguera, Ibáñez-Escriche, & González, 2020). However, except for the studies concerning the effects of genetics and diet on the FA metabolism and deposition, very few researchers have noted the role other factors play in determining FA composition in heavy pigs (Catillo, Zappaterra, Lo Fiego, Steri, & Davoli, 2021).

The purpose of this research was to describe and investigate the possible effects of environmental factors and carcass characteristics on the FA composition of *Semimembranosus* muscle (SM) and BF tissues in a population of ILW heavy pigs selected for the production of dry-cured hams. A multivariate approach was used to identify possible metabolic patterns explaining concentrations of individual FAs in different tissues and relate these patterns with environmental factors and carcass characteristics.

2. Material and methods

2.1 Animals and tissue samplings

A sample of 798 purebred ILW pigs was used in the present study. These samples were included in a previous work (Davoli et al., 2019). Briefly, the experimental pigs came from the sib-testing station of the Italian Pig Breeder National Association (Associazione Nazionale Allevatori Suini, ANAS, http://www.anas.it). Their sib-testing program is based on the performances of triplets of full sibs (two gilts and one barrow) reared in the same environmental conditions in a unique testing station. The experimental population came from 323 litters by 87 boars and 371 sows. Each group of siblings entered the sib-testing station located near Reggio Emilia (Italy) at the age of 30-45 days and the testing period lasted a maximum of 145 days, with an average final live weight of about 155 kg. During the testing period, siblings were allotted in a natural-ventilated facility and fed the same diets. The finishing diet (Supplementary Table S1) was fed from about 90-100 kg live weight until slaughter weight was reached at *a quasi ad libitum* feeding level (i.e. 60% of the pigs were able to ingest the whole ration). Pigs were slaughtered on 26 different dates between 2011 and 2012 at the same commercial abattoir. Each litter was slaughtered on at least two different dates. Handling and slaughtering of the animals used in this study were performed in compliance with European rules on the protection of animals during transport and at slaughtering (Council Regulation (EC) No. 1/2005 and Council Regulation (EC) No. 1099/2009). Sampling occurred with ANAS permission.

BF and SM tissues were sampled on the trimming line from the carcass left sides. BF samples were collected approximately between the fifth and the sixth lumbar vertebra, close to the point where the hind leg is separated from the rest of the carcass, at the level of BF maximum thickness. BF and SM samples were wrapped in aluminum foil, immediately put in vacuum-sealed bags, frozen in liquid nitrogen, and kept at -80°C for further use.

2.2 Phenotyping

At slaughtering, hot carcass weight (kg) and optical measures (expressed in mm) of loin and BF thicknesses were taken by Fat-O-Meat’er (FOM - CrometecGmbh, Lünen, Germany) between the third and fourth last ribs, 8 cm off the carcass midline. These measures were used to estimate the percentage of carcass lean meat, which was then used for EUROP carcass grading following EU Decision 2001/468/CE of June 8th, 2001 (European Commission, 2001). BF thickness (BFT; expressed in mm) was also measured at the level of the *Gluteus medius* muscle by a caliper. Furthermore, on the left side, the weights (in kg) of belly and jowl cuts were also recorded.

Intramuscular fat content (IMF) was determined in the SM by extraction with petroleum ether from 1 g fresh sample using an XT15 Ankom apparatus (Macedon, NY, USA), according to Official procedure AOCS Am 5-04 (AOAC, 2005). IMF was determined in % as g of IMF per 100 g of tissue.

BF FA composition was determined as described in Catillo, Zappaterra, Lo Fiego, Steri, & Davoli (2021) and Serra et al. (2014), and was expressed as g FA per 100 g of total FA (i.e. percent FA composition). SM FA determination was described in Catillo et al. (2020). Briefly, the total muscle lipids destined for the gas-chromatographic analysis were extracted from SM 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 2N solution of potassium hydroxide (KOH) in methanol (CH_3OH) (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 an internal standard in SM FA determination. Intramuscular fatty acid methyl esters (FAMEs) were then submitted to gas-chromatographic analysis using TRACE™GC Ultra (Thermo Electron Corporation, Rodano, MI, Italy) equipped with a Flame Ionization Detector, a PVT injector, and a TR-FAME Column 30 m × 0.25 mm i.d., 0.2 µm film thickness (Thermo Scientific, Rodano, MI, Italy). The Chrom-Card software (vers.2.3.3, Thermo Electron Corporation, Rodano, MI, Italy) was used to record and integrate the peaks of FAMEs. Individual FAME were identified by comparing their retention times with the retention times of a standard FAME mixture prepared in-house with known quantities of each methyl ester (Larodan Fine Chemicals AB, Solna, Sweden). In order to present data in the same way as BF, the amount of each FA determined in SM was reported as g FA per 100 g of total FA (i.e. percent FA composition).

2.3 Statistical analysis

2.3.1 Data handling

The 26 slaughtering dates were grouped into a new variable with four levels corresponding to the four slaughtering seasons (i.e. six dates in spring; six in summer; nine in autumn; five in winter).

2.3.2 Multivariate analysis of the two tissues

In order to identify underlying structures in the dataset and patterns linking individual FAs, a Principal Component Analysis (PCA) was applied to the FA composition of BF and SM. Each tissue was independently analyzed with the aim of investigating the main non-genetic factors that could shape the variability of BF and SM FA composition. A PCA was run for each tissue including all the individual FAs. First, the projection of the samples in the Principal Components (PC) space (scores) was calculated. Samples with a high value for at least one of the distances within and orthogonal to the projection plane (Hubert, Rousseeuw, & Vanden Branden, 2005) were considered as outliers and not further included in the PCA analysis. A total of four and one outliers were removed for BF and SM tissues, respectively. After outlier removal, a PCA was run again for each of the considered tissues and PC scores were obtained. Each PC was determined by a specific combination of the original variables, which, based on their weight in each PC, contribute to explain total variance. The weights of individual FAs within each PC were then used to discuss possible metabolic pathways capable to explain the combinations found. To test whether the distribution of samples in the PCA scoreplot may have been influenced by major factors of variability, the distribution of samples on the projection plane was evaluated by plotting the variables of slaughtering season, sex, and EUROP carcass grading.

PCAs were performed using the *ropls* package (Thévenot, Roux, Xu, Ezan, & Junot, 2015) in the R environment (R Core Team, 2020).

2.3.3 Univariate models for the FA composition of the two tissues

2.3.3.1 Stepwise multiple regression model of the PC scores

The results of the multivariate approach (PCs) were further integrated by a univariate approach aimed to evaluate the effects of the categorical variables on the phenotypic variability noticed for each FA or FA class. The scores of the first two PCs obtained for each PCA were then included as dependent variables in backward stepwise multiple linear regression models. The initial model evaluated with the backward stepwise automatic elimination was the following:

$$y_{ijk} = \mu\ Ss_i + Sex_j + b_1(Age_k) + b_2(\text{hot carcass weight}_k) + b_3(\text{Carcass lean}_k) + b_4(BFT_k) + b_5(IMF_k) + b_6(\text{belly weight}_k) + b_7(\text{jowl weight}_k) + e_{ijk}$$

where: y_{ijk} was the vector of the scores of the first PCs identified with the PCAs; μ was the overall mean; Ss_i is the fixed effects of the i^th^ slaughter season (i=1 to 4) and Sex_j is the fixed effect of the sex (j=1,2); age at slaughtering, hot carcass weight, carcass lean %, BFT measured with a caliper, IMF percentage in SM, and the weights of belly and jowl were considered as covariates; b1, b2, b3, b4, b5, b6, b7 were the regression coefficients; e_{ijk} random residual effect for the k^th^ pigs.

Generalized Linear Models (GLMs) were performed with the *glm* function of the *stats* package (R Core Team, 2020) in the R environment. Backward stepwise multiple linear regression models were performed using the *step* function of the *stats* package (R Core Team, 2020) in the R environment. *Anova* function of *car* package in R environment (R Core Team, 2020) was used to adjust the results of the stepwise multiple linear regression models for the type III errors.

To complete the obtained results, the effect of the covariates for slaughtering season (4 levels) and sex of the animals (2 levels) were also tested on BFT, carcass lean % and IMF % with the *glm* function of the *stats* package, and *Anova* function of *car* package in the R environment (R Core Team, 2020). The results of the GLM for slaughtering season effects are reported as Least Squares Means (L.S.M.) and Standard Errors (S.E.), obtained with *lsmeans* function of *lsmeans* package in the R environment.

P-values < 0.05 were considered significant and the trend towards significance was set for *P*-values comprised between 0.10 and 0.05.

2.3.3.2 Multiple regression models for the individual FAs and FA classes

In order to highlight the effects of the independent categorical variables on each FA or FA category, the FA compositions of BF and SM were analyzed with a linear model in R environment (R Core Team, 2020). The linear models used for BF and SM FA composition were based on the results of the backward stepwise multiple linear regression models performed for the relative PCs. For each tissue, variables displaying a *P*-value less than or equal to 0.05 in at least one of the stepwise models were considered as independent variables in the linear model.

3. Results

3.1 BF FA composition

The PCA for BF FA composition identified two PCs, jointly explaining 52% of the total variance. Weights of individual FAs entering each PC are reported in Table 1 and the PCA scoreplot is displayed in Supplementary Figure 1. FAs showing the highest and lowest weights contributed the most in determining the variability of the PC they belonged to. The first PC (PC1), explaining 33% of the total variance, was mainly determined by the saturated FAs (SFA) stearic, arachidic and palmitic, while arachidonic, linoleic, dihomo-γ-linolenic, docosapentaenoic (DPA), heptadecenoic unsaturated FAs (UFAs), and lauric acid had negative loadings in PC1. Most of the total variance was thus determined by the antagonism shown by the animals located in the right side of the PCA scoreplot (characterized by more stearic, arachidic, and palmitic acids in BF) against those placed on the left side of Supplementary Figure S1 (with BF having greater proportions of arachidonic, linoleic, dihomo-γ-linolenic, DPA, heptadecenoic and lauric acids). The second PC (PC2), explaining 18% of the total variance, was mainly determined by the opposition between pigs having BF with greater proportions of palmitoleic acid and of the myristic, capric, palmitic, and lauric SFAs (pigs on the upper side of the PCA scoreplot), and animals displaying more eicosadienoic, gadoleic, and erucic acids in their BF tissue (on the bottom side of Supplementary Figure S1).

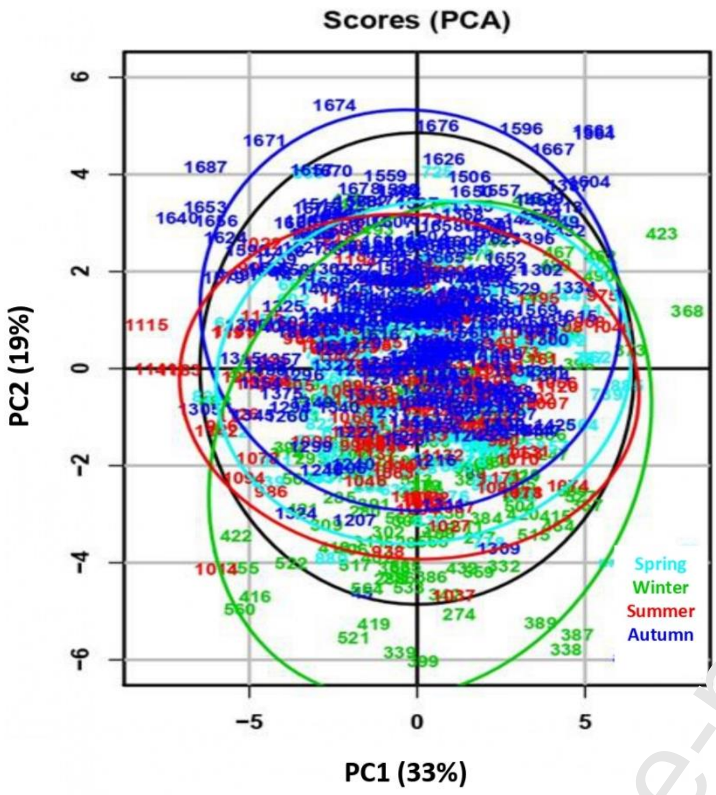
Table 1. Backfat (BF) individual fatty acids (FAs), identified by their shorthand notation and their common nomenclature between brackets, and the relative Principal Component (PC) loadings. The total variance explained by each PC is between brackets. Bold PC loadings indicate the lowest and highest PC loadings.

BF FAs (%)	**PC1 (33%)**	**PC2 (18%)**
C10:0 (capric acid)	-0.059	**-0.375**
C12:0 (lauric acid)	**-0.210**	**-0.285**
C14:0 (myristic acid)	-0.119	**-0.409**
C16:0 (palmitic acid)	**0.216**	**-0.335**
C16:1 *cis*-9 (palmitoleic acid)	-0.184	**-0.285**
C17:0 (margaric acid)	-0.215	0.071
C17:1 *cis*-9 (heptadecenoic acid)	**-0.277**	0.000
C18:0 (stearic acid)	**0.292**	0.066
C18:1 *cis*-9 (oleic acid)	0.021	0.180
C18:1 *cis*-11 (*cis*-vaccenic acid)	-0.205	0.036
C18:2 *cis*-9, *cis*-12 (linoleic acid)	**-0.317**	0.019
C18:3 *n*-3 (α-linolenic acid)	-0.152	-0.027
C20:0 (arachidic acid)	**0.269**	0.168
C20:1 *cis*-11 (gadoleic acid)	0.134	**0.259**
C20:2 *n*-6 (eicosadienoic acid)	-0.071	**0.404**
C20:3 *n*-6 (dihomo-γ-linolenic acid)	**-0.296**	0.105
C22:1 (erucic acid)	-0.157	**0.238**
C20:4 *n*-6 (arachidonic acid)	**-0.327**	0.023

C22:4 *n*-6 (adrenic acid)	**-0.235**	0.173
C22:5 *n*-3 (docosapentaenoic acid-DPA)	**-0.282**	0.120
C22:6 *n*-3 (docosahexaenoic acid-DHA)	-0.184	0.054

The samples in the scoreplot were then labeled with their levels for the independent variables of slaughtering season, sex, and EUROP carcass grading, in order to test whether these factors had a major role in the dataset variability. Samples in the scoreplot showed to be clustered based on slaughtering seasons, as pigs slaughtered in autumn showed positive PC2 loadings and those slaughtered in winter negative PC2 loadings (Figure 1). Therefore, the animals slaughtered in autumn had the highest contents of eicosadienoic, gadoleic, and erucic acids, while those slaughtered in winter had more myristic, capric, palmitic, palmitoleic, and lauric acids in BF.

Figure 1. Principal Component Analysis (PCA) scoreplot for backfat (BF) fatty acids (FAs) with the samples (plotted with their ID number) identified by different colors based on their slaughtering season.



No clear cluster in the scoreplot was observed for sex and EUROP carcass grading.

PC scores of the samples were then submitted to backward stepwise multiple linear regression and the results are reported in Table 2 and Table 3. For PC1 scores, the stepwise selection process retained BFT, the % of carcass lean meat content, and age in the final multiple linear regression model. Slaughtering season showed a trend towards significance and animal sex was also retained, but its *P*-value was above the threshold of 0.10. As can be noticed from Table 2, animals with negative PC1 scores have a thinner BF, are older, and have leaner carcasses.

Table 2. The covariates retained in the backward stepwise multiple linear regression model for PC1 scores obtained from the Principal Component Analysis (PCA) of backfat (BF) fatty acids (FAs). The estimate, *F*-value, and *P*-value are reported for each covariate.

Covariates		**Estimated effect on PC1 scores**		
Name	**Classes**	**Estimate**	***F*-value**	***P*-value (F)**

Sex	Barrows	Ref	2.260	0.133
	Gilts	0.324		
Age (days)	-	-0.026	4.514	0.034
BFT (mm)	-	0.121	23.354	<0.001
Carcass lean meat (%)	-	-0.129	8.020	0.005
Slaughtering season	Spring	Ref	2.260	0.061
	Summer	-0.099		
	Autumn	0.404		
	Winter	0.630		

Ref: reference class. The effect size of the other classes is expressed using the Ref class as a reference.

- indicates covariates with continuous values.

Table 3 shows the results of the stepwise selection process with the final multiple linear regression model obtained for PC2 scores. The strongest effect was found for slaughtering season, in agreement with the results reported in Figure 1. The estimate for slaughtering season confirmed that pigs slaughtered in autumn have positive PC2 scores and those slaughtered in winter tend to have negative PC2 scores. IMF, carcass lean meat %, jowl and belly weights were also significant, and a trend towards significance was observed for BFT. Pigs having higher contents of IMF in SM, leaner carcasses, and heavier jowl cuts are significantly associated with positive scores for PC2, while animals with heavier belly cuts are associated with negative PC2 scores.

Table 3. The covariates retained in the backward stepwise multiple linear regression model for PC2 scores obtained from the Principal Component Analysis (PCA) of backfat (BF) fatty acids (FAs). The estimate, *F*-value, and *P*-value are reported for each covariate.

Covariates		**Estimated effect on PC2 scores**		
Name	**Classes**	**Estimate**	***F*-value**	***P*-value (F)**

Slaughtering season	Spring	Ref	45.827	<0.001
	Summer	-0.339		
	Autumn	0.877		
	Winter	-1.383		
IMF (%)	-	0.162	7.306	0.007
Carcass lean meat (%)	-	0.070	5.778	0.016
BFT (mm)	-	-0.031	3.443	0.064
Jowl weight (kg)	-	0.394	6.725	0.010
Belly weight (kg)	-	-0.107	4.086	0.044

Ref: reference class. The effect size of the other classes is expressed using the Ref class as a reference.

- indicates covariates with continuous values.

The results of the multivariate approach (PCA) were further integrated by the univariate approach aimed to evaluate the effects of the categorical variables on the phenotypic variability noticed for each FA or FA class. Supplementary Table S2 displays the effects of slaughtering season, age, BFT, carcass lean meat %, belly weight, jowl weight, and IMF% on the individual FAs and FA categories in BF. The L.S.M. of individual FAs and FA categories estimated for the slaughtering seasons are reported in Supplementary Table S3. In accordance with the results identified by the multivariate approach, slaughtering season showed to affect the majority of the individual FAs and FA classes, followed by BFT, carcass lean meat %, and jowl weight. Belly weight was associated with changes in lauric, myristic, palmitoleic, margaric and *cis*-vaccenic acids, and age was significantly related to palmitic, stearic, linoleic, α-linolenic, gadoleic acids and the classes of SFAs and PUFAs.

3.2 Muscle FA composition

The PCA for the muscle FA composition identified two PCs, jointly explaining 53% of the total variance. Weights of individual FAs entering each PC are reported in Table 4 and the PCA scoreplot is reported in Supplementary Figure S2. The first PC (PC1) explained 39% of the total variance noticed for SM: animals located in the right side of the PCA scoreplot were characterized by more oleic and myristic acids in SM, while those placed on the left side of Supplementary Figure 2 had SM with greater proportions of erucic, DPA, adrenic, dihomo-γ-linolenic, arachidonic, docosahexaenoic (DHA), and eicosadienoic acids. The second PC (PC2), explaining 14% of the total variance, was mainly determined by the opposition between pigs with greater proportions of *cis*-vaccenic and palmitoleic acids on one hand (pigs on the upper side of the PCA scoreplot), and animals displaying more stearic, palmitic, lauric, arachidic, and myristic acids in their SM tissue (on the bottom side of Supplementary Figure S2).

Table 4. Muscle individual fatty acids (FAs) in *Semimembranosus*, identified by their shorthand notation and their common nomenclature between brackets, and their Principal Component (PC) loadings. In brackets, the fraction of total variance explained by each PC. Bold PC loadings indicate the lowest and highest PC loadings.

Muscle FAs (%)	**PC1 (39%)**	**PC2 (14%)**
C10:0 (capric acid)	0.140	0.023
C12:0 (lauric acid)	0.113	**-0.256**
C14:0 (myristic acid)	**0.220**	**-0.210**
C16:0 (palmitic acid)	0.181	**-0.281**
C16:1 *cis*-9 (palmitoleic acid)	0.171	**0.387**
C17:0 (margaric acid)	-0.220	-0.175
C17:1 *cis*-9 (heptadecenoic acid)	-0.169	0.082
C18:0 (stearic acid)	-0.071	**-0.415**
C18:1 *cis*-9 (oleic acid)	**0.265**	0.204
C18:1 *cis*-11 (*cis*-vaccenic acid)	0.037	**0.509**

C18:2 *cis*-9, *cis*-12 (linoleic acid)	**-0.298**	-0.113
C18:3 *n*-3 (α-linolenic acid)	-0.131	-0.139
C20:0 (arachidic acid)	0.001	**-0.247**
C20:1 *cis*-11 (gadoleic acid)	0.143	0.026
C20:2 *n*-6 (eicosadienoic acid)	**-0.224**	-0.112
C20:3 *n*-6 (dihomo-γ-linolenic acid)	**-0.283**	0.075
C22:1 (erucic acid)	**-0.318**	0.107
C20:4 *n*-6 (arachidonic acid)	**-0.279**	0.104
C22:4 *n*-6 (adrenic acid)	**-0.312**	0.076
C22:5 *n*-3 (docosapentaenoic acid-DPA)	**-0.317**	0.079
C22:6 *n*-3 (docosahexaenoic acid-DHA)	**-0.265**	0.067

When plotting sample labels of IMF FA composition for the independent variables of slaughtering seasons, animal sex, and EUROP carcass grading, no cluster was observed in the muscle FA PCA scoreplot.

PC scores of the samples were then submitted to backward stepwise selection analysis. Table 5 reports the final multiple regression model for PC1 scores. The independent variables of slaughtering season, sex, age, EUROP carcass grading, BFT, hot carcass weight, belly weight, jowl weight, and IMF% were retained. In particular, IMF% was the covariate showing the strongest association with PC1 scores, as pigs with higher IMF deposited in SM were associated with positive PC1 scores. Animals with lower percentages of lean meat (i.e. U, R, and O carcasses *vs*. E carcasses) were also associated with positive PC1 scores. Animals with lower hot carcass weights, heavier jowl and belly weights, older, and with a thicker BF tend to have positive scores for the PC1. Also, winter and autumn as slaughtering seasons showed opposed effects, with autumn being associated with negative and winter with positive PC1 scores.

Table 5. The covariates retained in the backward stepwise multiple linear regression model for PC1 scores obtained from the Principal Component Analysis (PCA) of *Semimembranosus* muscle (SM) fatty acids (FAs). The estimate, *F*-value, and *P*-value are reported for each covariate.

Covariates		**Estimated effect on PC1 scores**		
Name	**Classes**	**Estimate**	***F*-value**	***P*-value (F)**
Slaughtering season	Spring	Ref	3.34	0.018
	Summer	0.550		
	Autumn	-0.267		
	Winter	0.381		
Sex	Barrows	Ref	10.59	0.001
	Gilts	-0.724		
Age (days)	-	0.027	4.64	0.031
EUROP carcass grading	E	Ref	3.85	0.009
	U	1.330		
	R	1.907		
	O	2.464		
BFT (mm)	-	0.050	3.85	0.050
Hot carcass weight (kg)	-	-0.052	8.75	0.003
Belly weight (kg)	-	0.399	6.03	0.014
Jowl weight (kg)	-	0.663	6.25	0.012
IMF (%)	-	0.718	58.06	<0.001

Ref: reference class. The effect size of the other classes is expressed using the Ref class as reference.

- indicates covariates with continuous values.

Table 6 shows the results of the backward stepwise selection process for the PC2 scores estimated for the samples. Two variables entered with strong significant effects in the model: slaughtering season, and belly weight. Pigs with heavier belly cuts and slaughtered in autumn had higher PC2 scores, while summer as slaughtering season was associated with negative scores for PC2.

Table 6. The covariates retained in the backward stepwise multiple linear regression model for PC2 scores obtained from the Principal Component Analysis (PCA) of *Semimembranosus* muscle (SM) fatty acids (FAs). The estimate, *F*-value, and *P*-value are reported for each covariate.

Covariates		**Estimated effect on PC2 scores**		
Name	**Classes**	**Estimate**	***F*-value**	***P*-value (F)**
Slaughtering season	Spring	Ref	7.68	<0.001
	Summer	-0.114		
	Autumn	0.625		
	Winter	0.213		
Belly weight (kg)	-	0.246	11.42	<0.001

The results of the multivariate approach were further integrated with the univariate approach. Supplementary Table S4 displays the effects of slaughtering season, age, sex, EUROP carcass grading, hot carcass weight, belly weight, jowl weight, and IMF % on the individual FAs and FA categories in SM. The L.S.M. of individual FAs and FA categories estimated for the slaughtering seasons are reported in Supplementary Table S5. In accordance with the results of the multivariate approach, slaughtering season, IMF content, sex, carcass weight and conformation (i.e. EUROP carcass grading, belly weight, and jowl weight) showed to affect the majority of the individual FAs and FA classes. Age was significantly related to palmitic, margaric, heptadecenoic, stearic, oleic, eicosadienoic, erucic, arachidonic, adrenic, DHA acids, and with the classes of SFAs and MUFAs. To gain a more complete view of the relationships occurring between the covariates considered, the effects of the slaughtering season and animals’ sex were also tested on BFT, SM IMF%, and carcass lean %. Gilts had significantly lower contents of IMF in SM ($P = 0.003$), thinner BFT ($P = 0.002$), and leaner carcasses ($P < 0.001$) when compared with barrows. Pigs slaughtered in spring had thicker BFT (L.S.M. ± S.E; 29.00 ± 0.36 mm), and lower carcass lean % (47.10 ± 0.20 %) compared with those slaughtered in autumn (25.40 ± 0.29 mm and 49.60 ± 0.16 %, respectively). The animals slaughtered in winter and spring had BFT and carcass lean % displaying values of L.S.M. intermediate between those observed in spring and autumn (27.90 ± 0.47 mm for BFT and 48.90 ± 0.26 % for carcass lean % in winter; 27.30 ± 0.37 mm for BFT and 48.50 ± 0.21 % for carcass lean % in summer). The slaughtering season did not affect IMF% in SM.

4 Discussion

The results obtained in the present study allowed the characterization of the environmental factors and carcass features associated with changes in BF and SM FA composition in ILW heavy pigs fed the same diet. To the best of our knowledge, this is the first study that has used a multivariate approach (PCA) to reach this objective. PCA is a dimensionality reduction technique that is used to uncover hidden structures in multidimensional data (Simmons et al., 2015), and provide key insights on the relationships linking the variables. For these reasons, PCA has been used in the present study to characterize the patterns linking the proportions of FAs in the BF and SM tissues. The characterization of the metabolic profile of a tissue produces high-dimensional data, where variables are often interconnected in metabolic patterns and share portions of their variances. Similarly, the FA composition of a tissue is determined by a complex of metabolic processes regulating the fluxes of *de novo* FA biosynthesis, lipolysis, and FA deposition. Previous studies have used PCA or other multivariate statistics to investigate changes in the multidimensional structure of FA composition in porcine tissues in relation to breed (Aboagye et al., 2020), divergent levels of boar taint compounds (Mörlein & Tholen, 2015; Liu et al., 2017), and different diets (Bermúdez, Franco, Franco, Carballo, & Lorenzo, 2012). PCA has also been used in this work, but with a different purpose. This statistical analysis has indeed been selected to highlight possible metabolic patterns linking FAs in BF and SM of ILW purebred heavy pigs. The obtained new variables (PCs) have been investigated to test which factors may influence the variability of the linearly dependent FAs included in each PC; the identification of these factors may be useful to better understand whether some environmental factors can affect the organoleptic and nutritional qualities of the final pork products.

The PCA for BF FAs was able to capture the negative relation linking SFAs and PUFAs, which had opposite loadings in the first two PCs. The variability of the first PC for BF was determined on one hand by the SFAs stearic, arachidic, and palmitic, with positive PC loadings, and on the other hand by arachidonic, linoleic, adrenic, and DPA, having negative loadings in PC1. These latter FAs are mainly PUFAs participating in the endogenous synthesis of *n*-6 FAs. Linoleic acid is, indeed, one of the essential FAs, and is used as a substrate for further elongation and desaturation steps. The proportion of *n*-6 PUFAs in tissues is dependent on diet and complex enzymatic systems, consisting of desaturases and elongases, responsible for the conversion of linoleic acid into longer chain *n*-6 PUFAs (Brenner, 1989; Raes, De Smet & Demeyer, 2004). Linoleic acid may undergo subsequent desaturation and elongation steps to produce dihomo-γ-linolenic, arachidonic, and adrenic acids (Brenner, 1989; Raes, De Smet & Demeyer, 2004), which in this study were all related by negative PC1 loadings. These negative weights in PC1 may thus be linked to the fact that linoleic, arachidonic, adrenic, and DPA share a large covariance amount, as they are all linked to the endogenous synthesis of *n*-6 PUFAs. Hence, PC1 possibly captured this shared variability linking the amounts of these *n*-6 PUFAs in BF. Together with those FAs, DPA showed also a negative loading in PC1, indicating that also the variation of this metabolite is partly determined by the same sources of variability of the *n*-6 PUFAs synthesized from linoleic acid. This result may be related to the fact that DPA can be synthesized from α-linolenic acid (C18:3 *n*-3) through desaturation and elongation steps controlled by the same enzymes catalyzing the elongation/desaturation steps required for the transformation of linoleic acid into longer *n*-6 PUFAs (Brenner, 1989; Raes, De Smet & Demeyer, 2004). In humans and several other animal species, these steps are controlled by the enzymes encoded by the genes *Fatty acid desaturase 1* (*FADS1*), *FADS2*, *ELOVL elongase 2*

(*ELOVL2*), and *ELOVL5* (Castro, Tocher & Monroig, 2016; Gol, Pena, Rothshild, Tor & Estany, 2018). In particular, as reported in the literature, *FADS1* and *FADS2* display markers associated with the amounts of MUFAs and PUFAs in porcine BF tissue of crossbred pigs (Crespo-Piazuelo et al., 2020) and in IMF and BF of Duroc pigs (Gol, Pena, Rothshild, Tor & Estany, 2018). Furthermore, some studies conducted in different pig breeds indicated that arachidonic acid contents in BF and muscle were positively correlated with carcass lean mass (Gol, Pena, Rothshild, Tor & Estany, 2018; Davoli et al., 2019; Zappaterra et al., 2020). In agreement with those studies, the present research indicated that pigs with leaner carcasses tended to have negative PC1 scores for BF, and thus were characterized by higher contents of arachidonic, linoleic, adrenic, and DPA FAs. The linoleic acid percentage in BF is of great importance for ham quality and covering fat stability during ham processing, as a percentage of linoleic acid above 15% of total FA is associated with a content of PUFAs that can increase the oxidability of ham fat (Bosi & Russo, 2004). Hence, PDO ham production rules set threshold values for the linoleic acid percentage that must not exceed 15% (Consorzio del Prosciutto di Parma, 1992; MIPAAF, 2007). Leaner carcasses may therefore have an amount of linoleic acid above the permitted amount, making those thighs unsuitable for PDO ham production. On the other hand, individuals displaying high BFT were significantly associated with positive PC1 scores. These animals had, thus, higher contents of palmitic, stearic, and arachidic acids. These three FAs originate from subsequent elongation steps in the endogenous biosynthesis of the SFAs: in mammals, palmitic acid may, indeed, undergo elongation steps and can be transformed into stearic and arachidic acids (Miyazaki & Ntambi, 2008). The first PC for BF FAs thus captured the negative correlation linking SFAs and PUFAs, and their association with carcass composition; higher fat depots are mainly determined by triacylglycerols, the main neutral lipids used to store energy, which mainly consists of SFAs and MUFAs (De Smet, Raes & Demeyer, 2004). Fatter animals are therefore characterized by increased proportions of SFAs and MUFAs deposited in tissues, causing a decrease in the relative amount of PUFAs on the total FAs (De Smet, Raes & Demeyer, 2004; Lo Fiego, Santoro, Macchioni, & De Leonibus, 2005; Matthews, 2011). On the other hand, it is well known that lower amounts of stored fat are associated with lower depositions of SFAs and total FAs, which in turn cause an increase in the relative amount of PUFAs (Monziols, Bonneau, Davenel, & Kouba, 2007; Matthews, 2011). As suggested in the literature, this increased proportion of PUFAs stored in tissues of leaner animals is not due to a rise in PUFA synthesis, but rather in a higher percentage of PUFAs on the reduced amount of total FAs (Matthews, 2011). While SFAs are, indeed, quite fluctuating in tissues as they depend on the nutritional state of the animal, the amount of PUFAs deposited in tissues tends to be highly dependent on dietary *n*-6 and *n*-3 PUFAs contents. Therefore, in individuals fed the same diet, PUFA content tends to remain more stable than SFAs, as UFAs play essential roles in membrane flexibility, inflammation control, eicosanoid production, plasma triacylglycerol synthesis, and gene expression (reviewed in Fernandez & West, 2005). Because the pigs used in the present study were fed the same diets, it is possible to hypothesize that the higher proportion of PUFAs characterizing some of the studied pigs might be due to their lower adiposity and thus lower amount of total FAs stored in their BF.

The variability noticed for BF PC2 scores was strongly associated with the slaughtering season, with pigs slaughtered in winter being characterized by greater proportions of capric, lauric, myristic, palmitic, and palmitoleic acids, and those slaughtered in autumn showing higher contents of eicosadienoic, gadoleic, and erucic acids. BF is one of the first fat depots to develop in pigs, while IMF develops later, particularly in the muscles of the hind leg (Kouba & Bonneau, 2009). In heavy pigs, BFT and FA composition are mainly determined by the diet and environmental conditions applied during the finishing period, which lasts from 110-120 kg live weight to slaughtering at about 160 kg. The finishing period takes about three months in Italian heavy pigs and has the main objective to improve meat quality. FA composition of IMF and subcutaneous fat depots are thought to take a long time to vary, so that different fattening period lengths did not affect BF and IMF FA composition in extensively reared Iberian pigs (Ayuso, González, Peña, Hernández-García & Izquierdo, 2020). Given that changes in the FA composition of tissues occur slowly, it is reasonable to assume that the association found in the present study between PC2 scores and slaughtering season may reflect the consequence of the whole finishing period on the BF FA composition found at slaughter. The studied animals were fed the same diets and were reared in the same genetic station located in Po Valley (Italy), a geographical region characterized by a hot and highly humid weather during the late spring and summer. Prolonged periods with high temperature humidity indices cause heat stress in pigs, which lack functional sweat glands and poorly dissipate heat (White et al., 2008). Increasing temperatures and humidity have been indicated as factors affecting the performance of growing-finishing pigs as heat stress was proved to affect growth, feed intake, and caloric and feed efficiency (Renaudeau, Gourdine, & St-Pierre, 2011; Kellner, Baumgard, Prusa, Gabler, & Patience, 2016). In the present study, pigs slaughtered in autumn (and particularly in early autumn) spent their finishing period in the hottest months. These environmental conditions may have led pigs slaughtered in early autumn to have thinner BFT when compared to those slaughtered in winter and spring. In those animals, a reduction in BFT and therefore in SFAs may explain why their BF was characterized by higher proportions of eicosadienoic acid, an *n*-6 PUFA. On the other hand, pigs slaughtered in winter (and in particular in late winter) may have not experienced a hot and muggy environment during the finishing period, which may have caused higher BFT and thus greater proportions of SFAs being stored in subcutaneous fat. Therefore, taking into account these suggestions, it might not be so surprising that the two most different seasons were autumn and winter. Stearic acid, instead, did not follow the same pattern evidenced in PC2 for the other SFAs. This FA entered with a high weight in PC1, and its content in BF tissue was higher in pigs slaughtered in summer and autumn. Several studies suggest the role of stearic acid and its monounsaturated counterpart (i.e. oleic acid) in the regulation of cellular membrane fluidity in animals living at different environmental temperatures (Roy, Das & Ghosh, 1997; Malekar et al., 2018). Changes in oleic and stearic acid contents are particularly visible in poikilothermic animals, such as fish (Roy, Das & Ghosh, 1997; Malekar et al., 2018), with increased stearic acid incorporation in membranes as environmental temperatures rise (Malekar et al., 2018). Accordingly, the enzyme catalyzing the unsaturation of stearic to oleic acid (i.e. Stearoyl Co-A desaturase, SCD) has been suggested as an important regulator of cellular endoplasmic reticulum membrane fluidity in mammals and fat globule fluidity in cow milk (Timmen & Patton, 1988). Stearic acid has a melting point higher than the body temperature of animal species (69.6°C), and its increased incorporation permits the maintenance of cellular membrane characteristics also during high-temperature seasons. The higher content of stearic acid in the BF of pigs slaughtered in summer and autumn may therefore reflect the attempt of the adipocyte membranes to maintain membrane integrity by incorporating higher contents of this SFA, and consequently increasing their resistance to high-temperature environments.

Similar to what was observed for BF, the first PC for SM FAs was able to capture the negative relation linking SFAs and MUFAs with PUFAs. Unlike BF, however, the results of the multiple regression models for the PCs of SM indicated that the effect of slaughtering season (and thus of the finishing period season) on muscle FA composition was mediated by other factors, which strongly influenced the muscle FA patterns. Together with slaughtering season, SM IMF% and animals' sex were highly significant for PC1 variability. Pigs displaying higher IMF % had indeed increased contents of oleic and myristic acids, and lower amounts of erucic, DPA, adrenic, dihomo-γ-linolenic, arachidonic, DHA, and eicosadienoic acids. This observation is in agreement with the positive relation linking IMF deposition, and the amounts of SFAs and MUFAs found in muscle fat depots (Bosch, Tor, Reixach, & Estany, 2012). In particular, oleic acid has been found to share a consistent proportion of genetic variance with IMF deposition in different muscles of Duroc pigs (Ros-Freixedes, Reixach, Bosch, Tor, & Estany, 2014), and a moderate positive genetic correlation with SM IMF% in ILW pigs (Zappaterra et al., 2020). An association was also identified between pigs' age and muscle PC1 scores, with older animals having higher contents of oleic and myristic acids in SM. This is consistent with the fact that IMF increases with age and IMF saturation level is enhanced by greater IMF deposition (Bosch, Tor, Reixach, & Estany, 2012). Pigs slaughtered at later ages are therefore expected to have more IMF, SFAs and MUFAs in muscles, as SFA and

MUFA amounts increase with lipid deposition in porcine muscles (Bosch, Tor, Reixach, & Estany, 2012; Ros-Freixedes, Reixach, Bosch, Tor, & Estany, 2014).

The variability noticed in SM PC2 scores was mainly determined by the antagonism shown by *cis*-vaccenic and palmitoleic MUFAs against major SFAs (i.e. lauric, myristic, palmitic, stearic, and arachidic). Pigs slaughtered in summer showed higher proportions of SFAs in SM IMF. This positive association between the summer as slaughtering season and SFAs may originate from the attempt of the muscle-interspersed adipocytes to maintain membrane integrity by incorporating higher contents of SFAs, which increase membrane resistance to a high-temperature environment. However, unlike BF, slaughter season did not determine changes in IMF%, suggesting that different environmental conditions may affect FA metabolism and deposition, but they do not change the amount of fat deposited in muscle. Based on these results, further studies proving the effects of different environmental temperatures on lipid and energy metabolism in heavy pigs may be of interest.

Conclusions

The multivariate approach applied to the FA composition of porcine BF and SM allowed the identification of patterns in the FA deposition shaping the variability in the FA composition of the two studied tissues. An inverse relationship of the deposition of SFAs with PUFAs resulted to be among the major patterns characterizing both BF and SM. The overall variability in the FAs deposited in subcutaneous fat and muscle showed to be strongly related to the slaughtering season and carcass features. In agreement with the literature, leaner carcasses were associated with higher proportions of PUFAs, confirming that carcasses with high lean mass deposition may have FA composition unsuitable for the processing into PDO dry-cured hams. Remarkably, slaughtering seasons emerged as relevant factors shaping both BF and muscle FA composition. More efforts should be applied to understand the effect that high environmental temperatures may have on FA metabolism and deposition in finishing heavy pigs.

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Author statement

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Supplementary data

Supplementary material

References

Aboagye, G., Zappaterra, M., Pasini, F., Dall'Olio, S., Davoli, R., Nanni Costa, L. (2020). Fatty acid composition of the intramuscular fat in the longissimus thoracis muscle of Apulo-Calabrese and crossbreed pigs. *Livestock Science*, *232*, 103878. https://doi.org/10.1016/j.livsci.2019.103878

Association of Official Analytical Chemists (AOAC). (2005). Official methods of analysis, method 920.39, fat (crude) or ether extract in animal feed, 18th edition. AOAC, Gaithersburg, MD, USA.

Ayuso, D., González, A., Peña, F., Hernández-García, F. I., & Izquierdo, M. (2020). Effect of Fattening Period Length on Intramuscular and Subcutaneous Fatty Acid Profiles in Iberian Pigs Finished in the Montanera Sustainable System. *Sustainability*, 12, Article 7937. https://doi.org/10.3390/su12197937

Bermúdez, R., Franco, I., Franco, D., Carballo, J., Lorenzo, J. M. (2012). Influence of inclusion of chestnut in the finishing diet on fatty acid profile of dry-cured ham from Celta pig breed. *Meat Science, 92,* 394-399. https://doi.org/10.1016/j.meatsci.2012.05.001

Bosch, L., Tor, M., Reixach, J., & Estany, J. (2012). Age-related changes in intramuscular and subcutaneous fat content and fatty acid composition in growing pigs using longitudinal data. *Meat Science, 91*, 358-363. https://doi.org/10.1016/j.meatsci.2012.02.019

Bosi, P., & Russo, V. (2004). The production of the heavy pig for high quality processed products. *Italian Journal of Animal Science, 4,* 309–321. https://doi.org/10.4081/ijas.2004.309

Brenner, R.R. (1989). Factors influencing fatty acid chain elongation and desaturation. In: Vergroesen, A.J., Crawford, M. (Eds.), The Role of Fats in Human Nutrition. Academic Press, California, USA, pp. 45–80.

Čandek-Potokar, M., Monin, G., & Zlender, B. (2002). Pork quality, processing, and sensory characteristics of dry-cured hams as influenced by Duroc crossing and sex. *Journal of Animal Science*, *80*, 988–996. https://doi.org/10.2527/2002.804988x

Carrapiso, A. I., Tejeda, J. F., Noguera, J. L., Ibáñez-Escriche, N., & González, E. (2020). Effect of the genetic line and oleic acid-enriched mixed diets on the subcutaneous fatty acid composition and sensory characteristics of dry-cured shoulders from Iberian pig. *Meat Science*, *159*, 107933. https://doi.org/10.1016/j.meatsci.2019.107933

Castro, L. F. C., Tocher, D. R., & Monroig, O. (2016). Long-chain polyunsaturated fatty acid biosynthesis in chordates: Insights into the evolution of Fads and Elovl gene repertoire. *Progress in Lipid Research*, *62*, 25-40. https://doi.org/10.1016/j.plipres.2016.01.001

Catillo, G., Zappaterra, M., Lo Fiego, D.P., Steri, R., & Davoli, R. (2021). Relationships between EUROP carcass grading and backfat fatty acid composition in Italian Large White heavy pigs. *Meat Science*, *171*, 108291. https://doi.org/10.1016/j.meatsci.2020.108291

Catillo, G., Zappaterra, M., Zambonelli, P., Buttazzoni, L., Steri, R., Minelli, G., & Davoli, R. (2020). Genome-wide association study identifies quantitative trait loci regions involved in muscle acidic profile in Large White heavy pigs. *Animal*, *14*, 1342-1350. https://doi.org/10.1017/S1751731120000099

Consorzio del Prosciutto di Parma, 1992. Prosciutto di Parma Denominazione di Origine Protetta - *Disciplinare Generale e Dossier di cui all'articolo 4 del Regolamento (CEE) n°2081/92 del Consiglio del 14 luglio 1992*, Consorzio del Prosciutto di Parma (Ed.), Parma, Italy.

Crespo-Piazuelo, D., Criado-Mesas, L., Revilla, M., Castelló, A., Noguera, J. L., Fernández, A. I., Ballester, M., & Folch, J. M. (2020). Identification of strong candidate genes for backfat and intramuscular fatty acid composition in three crosses based on the Iberian pig. *Scientific Reports*, *10*, Article 13962. https://doi.org/10.1038/s41598-020-70894-2

Dalle Zotte, A., Brugiapaglia, A., & Cullere, M. (2017). What is meat in Italy? *Animal Frontiers*, *7*, 63–70. https://doi.org/10.2527/af.2017.0448

Davoli, R., Catillo, G., Serra, A., Zappaterra, M., Zambonelli, P., Meo Zilio, D., Steri, R., Mele, M., Buttazzoni, L., & Russo, V. (2019). Genetic parameters of backfat fatty acids and carcass traits in Large White pigs. *Animal*, *13*(5), 924-932. https://doi.org/10.1017/S1751731118002082

De Smet, S., Raes, K., & Demeyer, D. (2004). Meat fatty acid composition as affected by fatness and genetic factors: A review. *Animal Research, 53*, 81-98. https://doi.org/10.1051/animres:2004003

European Commission (2001). Commission Decision of 8 June 2001 authorising methods for grading pig carcasses in Italy. *Official Journal of the European Communities*, 163, 31-33. Available on https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32001D0468&from=it

Fernandez, M. L., West, K. L. (2005). Mechanisms by which dietary fatty acids modulate plasma lipids. *The Journal of Nutrition, 135*, 2075-2078. https://doi.org/10.1093/jn/135.9.2075

Ficarra, A., Lo Fiego, D. P., Minelli, G., & Antonelli, A. (2010). Ultra fast analysis of subcutaneous pork fat. *Food Chemistry, 121*, 809-814. https://doi.org/10.1016/j.foodchem.2010.01.003

Folch, J. M., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, 226, 497-509. https://doi.org/10.1016/S0021-9258(18)64849-5

Gol, S., Pena, R. N., Rothschild, M. F., Tor, M., & Estany, J. (2018). A polymorphism in the fatty acid desaturase-2 gene is associated with the arachidonic acid metabolism in pigs. *Scientific Reports, 8*, 14336. https://doi.org/10.1038/s41598-018-32710-w

Gou, P., Guerrero, L., & Arnau, J. (1995). Sex and crossbreed effects on the characteristics of dry-cured ham. *Meat Science*, *40*, 21-31. https://doi.org/10.1016/0309-1740(94)00021-X

Hubert, M., Rousseeuw, P.J., & Vanden Branden, K. (2005). ROBPCA: A New Approach to Robust Principal Component Analysis. *Technometrics, 47*(1), 64-79. https://doi.org/10.1198/004017004000000563

ISMEA (2019). Settore suinicolo. Scheda di settore. http://www.ismeamercati.it/carni/carne-suina-salumi

Juárez, M., Dugan, M. E. R., Aldai, N., Aalhus, J. L., Patience, J. F., Zijlstra, R. T., & Beaulieu, A. D. (2011). Increasing omega-3 levels through dietary co-extruded flaxseed supplementation negatively affects pork palatability. *Food Chemistry*, *126*, 1716-1723. https://doi.org/10.1016/j.foodchem.2010.12.065

Kellner, T. A., Baumgard, L. H., Prusa, K. J., Gabler, N. K., & Patience, J. F. (2016). Does heat stress alter the pig's response to dietary fat? *Journal of Animal Science*, *94*, 4688-4703. https://doi.org/10.2527/jas.2016-0756

Kouba, M., & Bonneau, M. (2009). Compared development of intermuscular and subcutaneous fat in carcass and primal cuts of growing pigs from 30 to 140kg body weight. *Meat Science*, *81*, 270-274. https://doi.org/10.1016/j.meatsci.2008.08.001

Leskanich, C. O., Matthews, K. R., Warkup, C. C., Noble, R. C., & Hazzledine, M. (1997). The effect of dietary oil containing (n-3) fatty acids on the fatty acid, physicochemical, and organoleptic characteristics of pig meat and fat. *Journal of Animal Science*, *75*, 673–683. https://doi.org/10.2527/1997.753673x

Liu, X., Trautmann, J., Wigger, R., Zhou, G., Mörlein, D. (2017). Fatty acid composition and its association with chemical and sensory analysis of boar taint. *Food Chemistry*, *231*, 301-308. https://doi.org/10.1016/j.foodchem.2017.03.112

Lo Fiego, D. P., Macchioni, P., Minelli, G., & Santoro, P. (2010). Lipid composition of covering and intramuscular fat in pigs at different slaughtering age. *Italian Journal of Animal Science*, *9*, 200-205. https://doi.org/10.4081/ijas.2010.e39

Lo Fiego, D. P., Santoro, P., Macchioni, P., & De Leonibus, E. (2005). Influence of genetic type, live weight at slaughtering and carcass fatness on fatty acid composition of subcutaneous adipose tissue of raw ham in the heavy pig. *Meat Science 69*, 107-114. https://doi.org/10.1016/j.meatsci.2004.06.010

López, M. O., de la Hoz, L., Cambero, M. I., Gallardo, E., Reglero, G., Ordóñez, J. A. (1992). Volatile compounds of dry hams from Iberian pigs. *Meat Science*, *31*, 267-277. https://doi.org/10.1016/0309-1740(92)90057-B

Malekar, V. C., Morton, J. D., Hider, R. N., Cruickshank, R. H., Hodge, S., & Metcalf, V. J. (2018). Effect of elevated temperature on membrane lipid saturation in Antarctic notothenioid fish. *PeerJ*, *6,* Article e4765. https://doi.org/10.7717/peerj.4765

Matthews, K. R. (2011). Saturated fat reduction in butchered meat, In: Reducing saturated fats in food, (Ed.) G. Talbot, Woodhead Publishing Limited, Cambridge, pp. 195-209.

MIPAAF (2007). Disciplinare di produzione della denominazione d'origine protetta "Prosciutto di San Daniele". *Gazzetta Ufficiale della Repubblica Italiana, supplemento ordinario alla Gazzetta Ufficiale N. 77 del 2 aprile 2007*. Istituto Poligrafico e Zecca dello Stato, Roma.

Miyazaki, M., Ntambi, J. M. (2008). CHAPTER 7 - Fatty acid desaturation and chain elongation in mammals, In: Biochemistry of Lipids, Lipoproteins and Membranes. Editor(s): Dennis E. Vance, Jean E. Vance, Fifth Edition, Elsevier, 191-211.

Monziols, M., Bonneau, M., Davenel, A., & Kouba, M. (2007). Comparison of the lipid content and fatty acid composition of intermuscular and subcutaneous adipose tissues in pig carcasses. *Meat Science*, *76*, 54-60. https://doi.org/10.1016/j.meatsci.2006.10.013

Mörlein, D., & Tholen, E. (2015). Fatty acid composition of subcutaneous adipose tissue from entire male pigs with extremely divergent levels of boar taint compounds — An exploratory study. *Meat Science*, *99*, 1-7. https://doi.org/10.1016/j.meatsci.2014.08.002

Morgan, C. A., Noble, R. C., Cocchi, M. & McCartney, R. (1992). Manipulation of the fatty acid composition of pig meat lipids by dietary means. *Journal of the Science of Food and Agriculture*, *58*, 357-368. https://doi.org/10.1002/jsfa.2740580310

OECD (2016). "Meat", in OECD-FAO Agricultural Outlook 2016-2025, OECD Publishing, Paris, https://doi.org/10.1787/agr_outlook-2016-10-en.

Parolari, G., Rivaldi, R., Leonelli, C., Bellatti, M., & Bovis, N. (1988). Colore e consistenza del prosciutto crudo in rapporto alla material prima e alla tecnica di stagionatura. *Industria Conserve*, 63, 45-49.

Pinna, A., Simoncini, N., Toscani, T., Virgili, R. (2012). Volatile organic compounds of Parma dry-cured ham as markers of ageing time and aged ham aroma. *Italian Journal of Food Science: IJFS, 24*, 321-331.

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/.

Renaudeau, D., Gourdine, J. L., & St-Pierre, N. R. (2011). A meta-analysis of the effects of high ambient temperature on growth performance of growing-finishing pigs. *Journal of Animal Science*, *89*, 2220–2230. https://doi.org/10.2527/jas.2010-3329

Roy, R., Das, A. B., Ghosh, D. (1997). Regulation of membrane lipid bilayer structure during seasonal variation: a study on the brain membranes of *Clarias batrachus*. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, 1323, 65-74. https://doi.org/10.1016/S0005-2736(96)00176-9

Ros-Freixedes, R., Reixach, J., Bosch, L., Tor, M., & Estany, J. (2014). Genetic correlations of intramuscular fat content and fatty acid composition among muscles and with subcutaneous fat in Duroc pigs. *Journal of Animal Science, 92*, 5417-5425. https://doi.org/10.2527/jas.2014-8202

Ruiz Carrascal, J., Ventanas, J., Cava, R., Andres, A. I., & Garcia, C. (2000). Texture and appearance of dry cured ham as affected by fat content and fatty acid composition. *Food Research International, 33*, 91-95. https://doi.org/10.1016/S0963-9969(99)00153-2

Sellier, P., Maignel, L., & Bidanel, J. (2010). Genetic parameters for tissue and fatty acid composition of backfat, perirenal fat and longissimus muscle in Large White and Landrace pigs. *Animal*, *4*, 497-504. https://doi.org/10.1017/S1751731109991261

Serra, A., Buccioni, A., Rodriguez-Estrada, M. T., Conte, G., Cappucci, A., & Mele, M. (2014). Fatty acid composition, oxidation status and volatile organic compounds in “Colonnata” lard from Large White or Cinta Senese pigs as affected by curing time. *Meat Science, 97*, 504–512. https://doi.org/10.1016/j.meatsci.2014.03.002

Simmons, S., Peng, J., Bienkowska, J., Berger, B. (2015). Discovering what dimensionality reduction really tells us about RNA-Seq data. *Journal of Computational Biology*, *22*, 715-28. https://doi.org/10.1089/cmb.2015.0085

Suzuki, K., Ishida, M., Kadowaki, H., Shibata, T., Uchida, H., & Nishida, A. (2006). Genetic correlations among fatty acid compositions in different sites of fat tissues, meat production, and meat quality traits in Duroc pigs. *Journal of Animal Science*, *84*, 2026–2034. https://doi.org/10.2527/jas.2005-660

Thévenot, E. A., Roux, A., Xu, Y., Ezan, E., & Junot, C. (2015). Analysis of the human adult urinary metabolome variations with age, body mass index and gender by implementing a comprehensive workflow for univariate and OPLS statistical analyses. *Journal of Proteome Research*, *14*, 3322-3335. https://doi.org/10.1021/acs.jproteome.5b00354

Tibau, J., González, J., Soler, J., Gispert, M., Lizardo, R., & Mourot, J. (2002). Influence du poids a l'abattage du porc entre 25 et 140 kg de poids vif sur la composition chimique de la carcasse: effets du genotype et du sexe. *Journées de la Recherche Porcine en France*, *34*, 121-127. http://journees-recherche-porcine.com/texte/2002/02txtAlim/a0210.pdf

Timmen, H., & Patton, S. (1988). Milk fat globules: Fatty acid composition, size and in vivo regulation of fat liquidity. *Lipids*, *23*, 685-689. https://doi.org/10.1007/BF02535669

Virgili, R., & Schivazappa, C. (2002). Muscle traits for long matured dried meats. *Meat Science*, *62*, 331–343. https://doi.org/10.1016/S0309-1740(02)00118-3

Ventanas, S., Ruiz, J., García, C., & Ventanas, J. (2007). Preference and juiciness of Iberian dry-cured loin as affected by intramuscular fat content, crossbreeding and rearing system. *Meat Science*, *77*, 324-330. https://doi.org/10.1016/j.meatsci.2007.04.001.

White, H. M., Richert, B. T., Schinckel, A. P., Burgess, J. R., Donkin, S. S., & Latour, M. A. (2008). Effects of temperature stress on growth performance and bacon quality in grow-finish pigs housed at two densities. *Journal of Animal Science*, *86*, 1789–1798. https://doi.org/10.2527/jas.2007-0801

Wood, J.D., Richardson, R.I., Nute, G.R., Fisher, A.V., Campo, M.M., Kasapidou, E., Sheard, P.R., & Enser, M. (2003). Effects of fatty acids on meat quality: a review. *Meat Science*, *66,* 21-32. https://doi.org/10.1016/S0309-1740(03)00022-6

Raes, K., De Smet, S., & Demeyer, D. (2004). Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat: a review. *Animal Feed Science and Technology*, *113*, 199–221. https://doi.org/10.1016/j.anifeedsci.2003.09.001

Zappaterra, M., Catillo, G., Belmonte, A. M., Lo Fiego, D. P., Zambonelli, P., Steri, R., Buttazzoni, L., & Davoli, R. (2020). Genetic parameters of muscle fatty acid profile in a purebred Large White heavy pig population. *Meat Science, 163,* Article 108057. https://doi.org/10.1016/j.meatsci.2020.108057

Zappaterra, M., Ros-Freixedes, R., Estany, J., & Davoli, R. (2018). Association study highlights the influence of ELOVL fatty acid elongase 6 gene region on backfat fatty acid composition in Large White pig breed. *Animal*, *12,* 2443-2452. https://doi.org/10.1017/S1751731118000484

Highlights

- Various intrinsic and extrinsic factors affect muscle and backfat fatty acids.
- The multivariate structure of pig muscle and backfat fatty acids was investigated.
- The antagonism of saturated vs. *n*-6 fatty acids was the main relation identified.
- The dataset structure was associated with slaughtering season and carcass traits.
- Pigs had more vaccenic and palmitoleic acids in muscle when slaughtered in autumn.