Animal 15 (2021) 100073

Contents lists available at ScienceDirect

Animal
The international journal of animal biosciences

On-site visible–near IR prediction of iodine number and fatty acid composition of subcutaneous fat of raw hams as phenotypes for a heavy pig breeding program

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ARTICLE INFO

Article history:
Received 21 February 2020
Received in revised form 30 August 2020
Accepted 7 September 2020
Available online 15 December 2020

Keywords:
Fat composition
Genetic correlation
Ham
Heritability
IR spectroscopy

ABSTRACT

The quality of subcutaneous fat of raw hams is a trait of interest in selective breeding programs for pig lines used in dry-cured ham production, and rapid, non-invasive methods for its assessment are available. However, the efficacy of such methods to provide indicator traits for breeding programs needs to be proven. The study investigated the accuracy of on-site visible–near IR spectroscopy predictions of iodine number and fatty acid (**FA**) composition of raw ham subcutaneous fat, and it evaluated their effectiveness as indicator traits of ham fat quality in a pig breeding program. Prediction equations were developed using visible–near IR spectra acquired at the slaughterhouse from five sites in subcutaneous fat of raw hams of 1025 crossbred pigs. Pigs were raised, under standardized rearing and feeding conditions, in the sib-testing program of the Goland C21 boar line and slaughtered at nine months of age and average body weight of 166 ± 15 kg. Accuracy was generally relatively poor, but R^2 in external validation was > 0.7 for iodine number and concentration of C18:2n-6, polyunsaturated FAs and omega-6 FAs. To assess the effectiveness of the on-site predictions as indicator traits in a breeding program, (co)variance components of the measured traits (**OBS**) and of their predictions using in-lab (**in-lab-PR**) or on-site (**on-site-PR**) spectrometers were estimated. Available records for OBS were 6814 and 2048, for iodine number and FA composition, respectively. Predictions using in-lab were available for pigs slaughtered between 2006 and 2014, for a total of 10 153 records. Predictions using on-site were obtained from spectra collected since 2011, for a total of 10 296 records. The estimated heritabilities for the investigated traits ranged from 0.34 to 0.50 and were greater for on-site-PR than for OBS. Genetic correlations between OBS and in-lab-PR were very close to 1.00 for all the investigated traits, whereas those between OBS and on-site-PRED ranged from 0.86 to 0.94. On-site visible-IR predictions are accurate enough to support the use of this technique for large-scale phenotyping of raw ham fat quality, even when dealing with animals of a single genetic line raised in standardized conditions, and may be implemented as indicator traits in breeding programs.

Implications

The study investigated the accuracy of on-site visible–near infrared spectroscopy for the prediction of subcutaneous fat quality of raw hams in animals of a single genetic line raised in standardized conditions. On-site visible–near infrared predictions of raw ham fat quality are accurate enough to support the use of this technique in large-scale phenotyping initiatives for selective breeding purposes. Predictions showed high and positive genetic correlations with fat quality traits measured by reference methods and may be exploited in pig breeding programs as indicator traits of fat quality of hams processed into dry-cured products.

Introduction

Quality of subcutaneous fat is a key trait of raw hams processed into "protected denomination of origin" (**PDO**) dry-cured products. High concentration of unsaturated fatty acids (**FA**s) is detrimental for dry-cured ham quality, being associated with an increase in oiliness, softness and yellowish of subcutaneous fat and greater risk for oxidative rancidity (Bosi and Russo, 2004; Čandek-Potokar and Škrlep, 2012). Product specifications of PDO Italian dry-cured hams set thresholds for C18:2n-6 concentration and iodine number (**IOD**) of subcutaneous fat of raw hams to a maximum of 15% and 70, respectively

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https://doi.org/10.1016/j.animal.2020.100073

(Consorzio del Prosciutto di Parma, 1992; Consorzio del Prosciutto di San Daniele, 2007). As a consequence, the quality of subcutaneous fat of raw hams, as defined by the FA composition, is a trait of interest in selective breeding programs for pig lines used in dry-cured ham production.

For regular and large-scale phenotyping for fat quality, visible–near IR spectroscopy (**VIS–NIRS**) enables fast and simple assessment of the amount of many chemical compounds simultaneously and can effectively replace lengthy and expensive chemical analyses (Prieto et al., 2017). Several studies have focused on the development of prediction equations, also using IR portable instruments, with the aim of supplying the pork industry with new tools for the routine screening of fat quality (Kucha et al., 2018). The large majority of these studies were performed on Iberian pig (González-Martín et al., 2003; Pérez-Marín et al., 2009; Pérez-Juan et al., 2010; Zamora-Rojas et al., 2013).

The breeding companies providing breeding stocks to pig farmers would particularly benefit from the development of non-invasive, on-site methods for large-scale fat quality assessment. The potential of on-site IR predictions to serve as a large-scale phenotyping tool in selective breeding programs for the control of ham weight loss during dry-curing has been demonstrated recently (Bonfatti and Carnier, 2020), but the effectiveness of this technique for providing indicator traits of fat quality needs to be determined. Selective breeding focuses on pigs of a specific line, in which variation in the FA composition of fat is expected to be reduced because of the standardization of rearing conditions (e.g., feeding regime and diet) and the increased uniformity in the genetic characteristics of animals. For this reason, the development of accurate prediction models might be particularly challenging under these circumstances.

The aims of this study were to assess the accuracy of on-site NIRS when predicting IOD and FA composition of raw ham subcutaneous fat in a single pig line raised in standard conditions and to investigate the possible role of such predictions as indicator traits in a pig breeding program by estimating the genetic relationships with in-lab assessments of fat quality.

Material and methods

Animal population and rearing conditions

The data used in this study were from crossbred finishing pigs produced in the sib-testing program of the C21 Goland sire line (Gorzagri, Fonzaso, Italy). The breeding goal of the sire line includes, since 2001, traits related to the quality of dry-cured ham (Bonfatti and Carnier, 2020). In the program, C21 nucleus boars are mated to a group of crossbred sows in order to produce crossbred finishing pigs providing phenotypic data used to predict the genetic merit of purebred C21 breeding candidates (half-sibs of crossbred finishing pigs).

All crossbred pigs are born and fattened at the sib-testing program farm under standardized feeding conditions. Piglets are weaned at 28 d after birth and fed *ad libitum* up to 80 kg of BW. From 80 kg of BW onward, restricted feeding is used. From 80 kg of BW to slaughter weight, the diet has the following characteristics: 13.2 MJ/kg of metabolizable energy, 123 g/kg of CP, 46 g/kg of lipids, 35 g/kg of crude fiber, 460 g/kg of starch, 5.8 g/kg of total lysine, 4.9 g/kg of standardized ileal digestible lysine and a standardized ileal digestible methionine to lysine ratio equal to 0.33.

Pigs are slaughtered at the same abattoir (Montorsi, Correggio, Italy), after CO_2 stunning, in groups of about 70 animals each. Age at slaughter is constrained to a minimum of nine months by guidelines of Parma dry-cured ham production (Consorzio del Prosciutto di Parma, 1992). After slaughter, all carcasses are weighted and hams are removed from both halves and weighted. Left hams are then used for the assessment of raw ham quality traits.

Use of in-lab IR spectroscopy in the population

Routine assessment of IOD and periodical checks of FA composition for the crossbred pigs enrolled in the sib-testing program started in 2001. Since 2006, in-lab IR spectroscopy has replaced reference laboratory analyses to provide individual predictions of IOD, C18:0, C18:2n-6, polyunsaturated FA (**PUFA**), omega-6 FAs and monounsaturated (**MUFA**) to PUFA ratio (**MP**) for ham subcutaneous fat (Riovanto et al., 2011). Thereafter, such predictions have been routinely used as indicator traits of fat quality in the breeding program. The IR prediction models were built on a data set of 344 subcutaneous fat samples collected in different years to maximize the sample variation in fat quality. Models have been periodically updated with fat quality measures provided by reference methods of new samples until a total of 409 samples was obtained. Acquisition of the IR spectra was performed after fat sample homogenization, using an in-lab instrument (Foss NIRSystem model 5000, Foss NIRSystem, Silver Spring, MD, USA) with a wavelength range of 1100 to 2500 nm (NIR region). The accuracy of final prediction models was very high, with $R^2 > 0.85$ for most traits, as detailed in Riovanto et al. (2011).

Development of new on-site visible–near IR prediction models

The current study investigated the feasibility of replacing the in-lab predictions with a new, on-site prediction method. Development of on-site VIS–NIR prediction equations was based on data of 1025 crossbred pigs, slaughtered in 13 slaughter groups (from November 2010 to July 2011). At the slaughterhouse, immediately after ham trimming (24 h after slaughter), VIS–NIR spectra were acquired from left raw hams using a portable spectrometer LabSpec®5000 (ASD Inc., Boulder, Colorado, USA), working over the spectral range from 350 to 2500 nm (VIS–NIR region) and equipped with a 1.5 m fiber-optic contact probe with a quartz-halogen source. Data acquisition was carried out using the Indico™ Pro software (ASD Inc., Boulder, Colorado, USA). Spectra were acquired from the transversal section of the subcutaneous fat (including both the inner and outer fat layers) of each ham at the sites depicted in Fig. 1. The five sites were classified as 1 (cranio-medial), 2 (caudo-medial), 3 (caudo-lateral), 4 (lateral) and 5 (cranio-lateral). One spectrum was collected from each site. The temperature of the hams at spectra acquisition varied from 1 to 10 °C. Spectra absorbance was obtained as log(1/Reflectance).

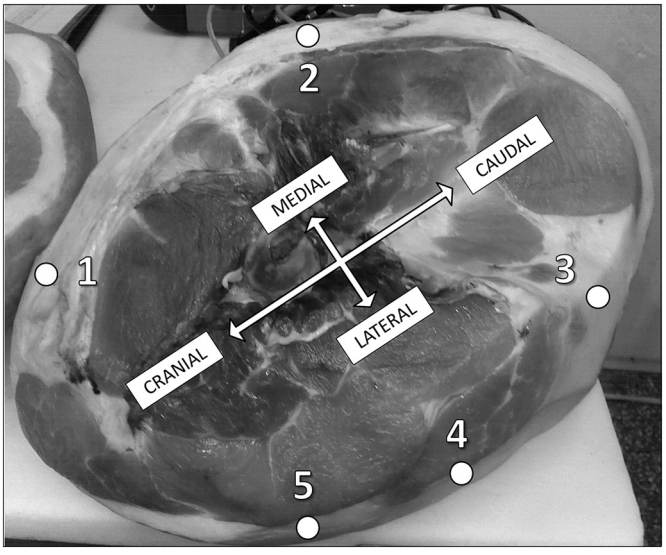

Fig. 1. Sites of acquisition of visible–near IR spectra in raw ham subcutaneous fat of heavy pigs.

Reference analyses of fat quality

Reference analyses were performed according to the methods used for the development and update of the in-lab prediction models. One trimmed slice of subcutaneous fat (10–20 cm long, 3–4 cm wide, 100–150 g) was collected from each thigh. Trimmed samples were taken from the lateral position (Fig. 1, site 4) and included both the inner and the outer fat layers. This is also the sampling site of the official screening procedures for assessing fat quality of raw hams processed into dry-cured PDO products (Istituto Parma Qualità, 2012). After removal of skin and muscular tissue residues, fat samples were vacuum packaged and preserved at −20 °C until homogenization. Frozen fat samples were finely minced using a Retsch Grindomix GM 200 laboratory mill (4000 rpm × 10 s; Retsch GmbH, Haan, Germany)

For the assessment of IOD, homogenized fat (30 g) was melted at 100 °C for 40 min and then filtered with a paper filter and poured with anhydrous sodium sulfate to remove residual moisture. Samples were then heated at 100 °C for 30 min. An aliquot of 0.4 g was used for the determination of IOD following the Wijs method (AOAC, 1980). Fatty acid composition was assessed by gas chromatography on an aliquot of 0.04 g of melted fat, as detailed by Riovanto et al. (2011). Results were expressed as the amount (g) of each FA or sum of FAs per 100 g of fat. Only the FAs or groups of FAs accounting for more than 1 g per 100 g of fat were considered for chemometric analyses. The variables investigated were: C16:0, C18:0, C18:1n-9, C18:2n-6, saturated fatty acids (**SFA**), MUFA, PUFA, omega-6 FAs, omega-3 FAs, unsaturation index (**UNS**, i.e. the ratio of unsaturated to total FAs) and MP.

Chemometrics

Preliminary prediction models were developed using: a) the VIS–NIR spectra acquired at each of the five sites in subcutaneous fat separately, b) the VIS–NIR spectrum averaged across sites 3, 4 and 5, and c) the spectrum averaged across the five sites. Data processing was performed using WinISI II software (Infrasoft International Inc., State College, PA). Before development of models, for each group of spectra in a), b), and c), detection of anomalous spectra was carried out using the standardized Mahalanobis (H) distance. Spectra with $H > 3$ were treated as outliers (H-outliers) and eliminated. These included mostly spectra that were erroneously acquired including portions of skin or muscle. For each acquisition site, the number of H-outliers ranged from 5 to 12. When models included the VIS–NIR spectrum averaged across sites 3, 4 and 5, or averaged across the five sites, only samples with complete spectral information were used. After the elimination of H-outliers, samples were partitioned on the basis of their H values in a modeling set, used for model development and internal cross-validation, and a validation set, used for external validation.

In order to maximize the variability in the modeling set and mimic the procedure used for the development of the original in-lab prediction models, the 500 samples with the greatest value for H were used for model development, whereas the remaining samples were used for external validation of prediction models. Increasing further the number of samples in calibration did not improve the accuracy of preliminary calibration models.

Based on accuracy of preliminary models, standard normal variate and detrend followed by first derivative, with a gap of 10 and a smooth of 5 (1, 10, 5, 1), were chosen as spectra pretreatment. The spectra regions from 350 to 400 contain noise, and the region above 2000 did not provide any improvement in model accuracy in preliminary analyses; hence, the spectral region between 400 and 2000 nm was selected for further chemometric analyses. Prediction equations were developed by modified partial least square regression procedures (Shenk and Westerhaus, 1991) with internal 4-fold cross-validation. Samples for which the difference between the observed and the predicted values was larger than 2.5 times the standard error of prediction were considered as T-statistic outliers (Shenk and Westerhaus, 1995) and removed. Two steps of outlier elimination were used. The proportion of T-outliers in each calibration-validation partition ranged from 5 to 5.4% of the total number of samples. T-outliers were samples with particularly high or low values for the FAs or groups of FAs investigated, with high leverage in the regression analyses.

The predictive ability of models was evaluated using the standard error in cross-validation ($\mathbf{SE_{CV}}$) and external validation ($\mathbf{SE_V}$) and the squared correlation in cross-validation ($\mathbf{R^2_{CV}}$) and external validation ($\mathbf{R^2_V}$). The Ratio of Performance to Deviation (**RPD**), i.e. the ratio between the standard deviation of the samples and the standard error in prediction, was also calculated.

Estimation of genetic parameters for measures and predictions of ham fat quality

To evaluate the effectiveness of the on-site predictions as indicator traits for fat quality in the pig breeding program, (co)variance components and genetic parameters were estimated for traits measured by reference methods (**OBS**) and their in-lab (**in-lab-PR**) and on-site (**on-site-PR**) predictions. The variables considered were IOD, C18:2n-6, PUFA, omega-6 FAs and MP because, for such traits, on-site-PR exhibited $R^2_{CV} > 0.75$, which is considered a good prediction accuracy (Minasny and McBratney, 2013). These also correspond to the traits routinely predicted in-lab since 2006. As the reference values and predictions of MP were not normally distributed, they were log-transformed before the analysis.

Distribution over time of the data available for the genetic analysis is depicted in Fig. 2. The assessment of fat quality for the Goland C21 sire line started in 2001. Due to budget constraints, the large majority of samples were analyzed only for IOD. At the end of 2005, reference samples for IOD and FA profile were used to build the in-lab NIRS prediction models. In 2010–2011, new samples were collected and analyzed for the development of the on-site VIS–NIRS models. The number of records available for the estimation of genetic parameters of OBS, in-lab-PR and on-site-PR for IOD and FA composition is reported in Table 1. Available records for OBS were 6814 and 2048, for IOD and FA composition, respectively, and included the samples used to develop on-site-PR models and all the samples that were analyzed since 2001. In-lab-PR for IOD and FA were available for all the crossbred pigs of the testing group slaughtered between 2006 and 2014, for a total of 10 153 records. On-site prediction for IOD and FA was obtained by applying the best prediction model (obtained averaging the spectra acquired at sites 3, 4 and 5) on all the VIS–NIR spectra acquired on-site since 2011, for a total of 10 296 records.

(Co)variance components for OBS, in-lab-PR and on-site-PR were estimated in a set of restricted maximum likelihood (**REML**) univariate and multivariate animal model analyses using VCE (version 6.0; Groeneveld et al., 2010). The univariate mixed model was:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e}$$

where $\mathbf{y}$ is the vector of OBS, in-lab-PR or on-site-PR for a given trait (IOD, C18:2n-6, PUFA, omega-6 FAs, or MP), $\mathbf{b}$, $\mathbf{a}$, and $\mathbf{e}$ are vectors of fixed non-genetic, random animal additive genetic and random residual effects, respectively, and $\mathbf{X}$ and $\mathbf{Z}$ are incidence matrices relating observations in $\mathbf{y}$ to effects in $\mathbf{b}$ and $\mathbf{a}$. The effects in $\mathbf{b}$ were sex (female or castrated male) and slaughter group (302 and 241 slaughter groups for IOD and FA, respectively).

For each fat quality trait, multivariate REML was based on a trivariate model analyzing OBS, in-lab-PR and on-site-PR. The effects included in the model were the same described for the univariate analyses. Conditional on the unknown parameters of the model, the data were assumed to be generated from this multivariate normal distribution:

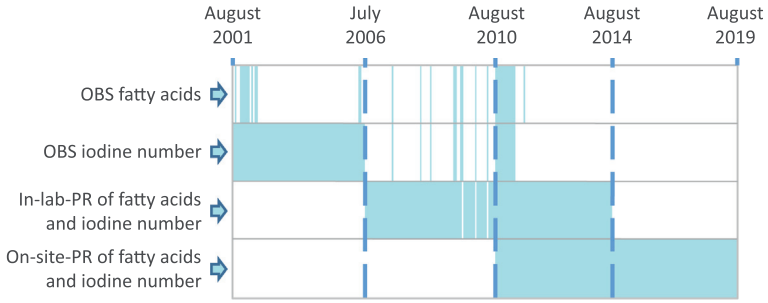

Fig. 2. Distribution over time (colored area) of reference-method measurements (OBS) of quality traits and of their predictions obtained using an in-lab (in-lab-PR) near IR spectrometer or an on-site visible–near IR spectrometer (on-site-PR) in subcutaneous fat of heavy pigs.

$$\mathbf{y}|\mathbf{b},\mathbf{a},\mathbf{R}\sim\mathrm{MVN}(\mathbf{Xb}+\mathbf{Za},\mathbf{R})$$

where **R** is a residual covariance matrix and MVN denotes a multivariate normal distribution. Animal additive genetic effects were assumed to follow a multivariate normal distribution:

$$(\mathbf{a}|\mathbf{K})\sim\mathrm{MVN}(\mathbf{0},\mathbf{K})$$

After sorting the data by individual, the animal (co)variance matrix **K** can be rewritten as **A**⊗**G**, where **G** is the (co)variance matrix for additive genetic effects of animals, and **A** is the numerator of Wright's relationship matrix. Additive genetic relationships between animals were traced back for as many generations as possible and were computed on the basis of at least 10 generations of known ancestors for the paternal line. Only the sire, the maternal grandsire and granddam were known for the dams of the crossbred pigs. Sires and dams of crossbred pigs were unrelated. The final pedigree included 25 684 animals.

Results

Pigs' average daily gain from the start of the growing phase (nearly 80 kg BW) to the end of fattening averaged 0.740 kg/d. In the same time interval, daily feed intake averaged 2.61 kg/d, and the resulting feed efficiency (gain to feed ratio) averaged 0.282 kg/kg, which corresponds to an average feed conversion ratio of 3.55.

Descriptive statistics for ham fat quality traits assessed by laboratory reference analyses are reported in Table 2. Fatty acid composition exhibited a limited variability, especially for SFA and MUFA. In particular, CV of IOD, C16:0, C18:1, SFA and MUFA was lower than 5%, being variation in FA profile mostly ascribed to variation in the concentration of PUFA.

Predictive ability of spectra acquired from single sites in ham subcutaneous fat

Prediction performance parameters of models exploiting on-site VIS–NIR spectra from single sites of ham subcutaneous fat are given in Supplementary Table S1, as well as the actual number of samples used in model development after outlier elimination. Models exploiting spectra collected at sites 1 and 2 had R^2_{CV} lower than 0.63 for all the investigated traits, whereas use of absorbances measured at sites 3, 4 and 5 ensured enhanced predictive ability. In general, the best predictions were obtained using the spectra collected at site 4.

The greatest R^2_{CV} obtained with site-specific prediction models for C16:0, C18:0 and SFA was 0.36, 0.48 and 0.61, respectively, whereas the highest R^2_{CV} obtained for C18:1n-9 and MUFA was 0.35 and 0.33, respectively. More encouraging results were obtained in the prediction of the PUFA. In particular, use of spectral information acquired from sites 3, 4 and 5 resulted in R^2_{CV} greater than 0.8 for IOD, C18:2n-6, PUFA and omega-6 FAs. A lower value of R^2_{CV} (0.64) was found for omega-3 FAs.

Predictive ability of models exploiting multiple on-site visible–near IR spectra

Prediction performance of models obtained using the averaged spectra of site 3, 4 and 5 or of all acquisition sites is reported in Table 3, as well as the actual number of samples used in model development after outlier elimination. Averaging enhanced substantially all prediction performance parameters. Values of R^2_{CV} obtained when using the average spectra of sites 3, 4 and 5 were 0.85, 0.88, 0.88, 0.88, 0.73 and 0.83 for IOD, C18:2n-6, PUFA, omega-6 FAs, omega-3 FAs and MP, respectively. Further improvement in model performance was not achieved when the average included also spectra from sites 1 and 2.

In general, despite the use of spectra from different sites, models had a limited ability to predict the concentration of SFA and MUFA, even

Table 1
Number of records available for ham fat quality traits measured by reference methods (OBS) and predicted by in-lab (in-lab-PR) or on-site (on-site-PR) IR spectroscopy in heavy pigs[1].

Method	Trait					
	Iodine number			Fatty acid composition		
	OBS	In-lab-PR	On-site-PR	OBS	In-lab-PR	On-site-PR
OBS	6 814	1 095	820	2 048	1 128	856
in-lab-PR		10 153	3 936		10 153	3 900
on-site-PR			10 296			10 296

[1] Diagonal: total number of records for a trait, off-diagonal: number of common records for a pair of traits.

Table 2
Descriptive statistics of ham fat quality traits assessed in subcutaneous fat of heavy pigs by reference methods for the samples used in the development and validation of on-site IR prediction models[1].

Trait	n	Mean	SD	CV (%)	1st percentile	99th percentile
IOD	1 006	67.74	3.13	4.62	59.29	74.93
C16:0 (g/100 g fat)	1 025	21.53	0.97	4.51	18.89	23.57
C18:0 (g/100 g fat)	1 025	10.63	1.15	10.78	8.16	13.60
C18:1n-9 (g/100 g fat)	1 025	42.17	1.71	4.06	37.82	46.69
C18:2n-6 (g/100 g fat)	1 025	13.39	1.69	12.63	8.80	17.99
SFA (g/100 g fat)	1 025	33.98	1.69	4.98	29.75	37.88
MUFA (g/100 g fat)	1 025	48.89	1.94	3.96	43.82	53.70
PUFA (g/100 g fat)	1 025	15.56	1.91	12.26	10.43	20.70
n-6 (g/100 g fat)	1 025	13.73	1.73	12.57	9.11	18.42
n-3 (g/100 g fat)	1 025	1.02	0.15	15.27	0.60	1.33
UNS	1 025	1.90	0.14	7.34	1.61	2.27
MP	1 025	3.20	0.53	16.41	2.25	5.13

[1] IOD, iodine number; C16:0, palmitic acid; C18:0, stearic acid; C18:1n-9, oleic acid; C18:2n-6, linoleic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n-6, concentration of omega-6 fatty acids (C18:2n-6, C18:3n-6, C20:3n-6, C20:4n-6); n-3, concentration of omega-3 fatty acids (C18:3n-3, C20:3n-3); UNS, unsaturation index = (MUFA+PUFA)/SFA; MP = MUFA/PUFA.

Table 3
Performance parameters for models exploiting average spectral information of different sites in the trans-section of raw ham subcutaneous fat of heavy pigs[1,2,3].

Trait[4]	Average spectrum of site 3, 4 and 5						Average spectrum of all sites					
	Calibration			Validation			Calibration			Validation		
	n	SE_{CV}	R^2_{CV}	n	SE_V	R^2_V	n	SE_{CV}	R^2_{CV}	n	SE_V	R^2_V
IOD	479	1.33	0.85	454	1.36	0.72	479	1.44	0.83	460	1.55	0.61
C16:0	484	0.72	0.40	473	0.85	0.19	490	0.75	0.35	479	0.85	0.24
C18:0	484	0.74	0.58	473	0.69	0.63	486	0.71	0.59	479	0.75	0.58
C18:1n-9	478	1.12	0.58	473	1.17	0.46	488	1.10	0.55	479	1.16	0.40
C18:2n-6	477	0.66	0.88	473	0.66	0.77	478	0.72	0.86	479	0.71	0.71
SFA	485	1.04	0.64	473	1.00	0.61	485	1.07	0.60	479	1.04	0.58
MUFA	487	1.32	0.56	473	1.16	0.58	488	0.40	0.52	479	1.21	0.49
PUFA	480	0.76	0.88	473	0.75	0.76	477	0.80	0.86	479	0.82	0.70
n-6	475	0.67	0.88	473	0.68	0.76	479	0.74	0.85	479	0.73	0.70
n-3	474	0.08	0.73	475	0.11	0.49	479	0.09	0.71	478	0.11	0.44
UNS	491	0.09	0.59	473	0.08	0.63	487	0.09	0.58	479	0.09	0.56
MP	477	0.23	0.83	473	0.22	0.73	476	0.24	0.81	479	0.22	0.66

[1] Spectra were acquired from the subcutaneous fat of the raw ham at 5 five sites: 1 (cranio-medial), 2 (caudo-medial), 3 (caudo-lateral), 4 (lateral) and 5 (cranio-lateral).
[2] The number of samples reported for each subset is the number of samples after outlier elimination.
[3] SE_{CV}: standard error of cross-validation; R^2_{CV}: R^2 in cross-validation; SE_V: standard error of external validation; R^2_V: R^2 in external validation.
[4] C16:0, palmitic acid; C18:0, stearic acid; C18:1n-9, oleic acid; C18:2n-6, linoleic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n-6, concentration of omega-6 fatty acids (C18:2n-6, C18:3n-6, C20:3n-6, C20:4n-6); n-3, concentration of omega-3 fatty acids (C18:3n-3, C20:3n-3); UNS, unsaturation index = (MUFA + PUFA)/SFA; MP = MUFA/PUFA.

when performing the prediction of SFA or MUFA total amount ($R^2_{CV} < 0.70$). As a consequence, also UNS was poorly predicted. However, regardless of the limited variability of our data and the on-site spectra collection on intact raw hams, in external validation, our models ensured R^2_V values >0.7 for IOD, C18:2n-6, PUFA and omega-6 FAs. The value of R^2_V was lower (< 0.5) for omega-3 FAs. Values of SE_{CV} ranged from 39 to 74% (for omega-6 FAs and C16:0, respectively) of the SD of the traits. While values of R^2_V were generally lower than the corresponding R^2_{CV}, values of SE_V were in line with the corresponding SE_{CV}, with the exception of C16:0 and omega-3 FAs, which SE_V were 18 and 38% higher than the SE_{CV}.

Effectiveness of on-site predictions as indicator traits in pig breeding programs

Additive genetic variance, residual variance and heritability ($\mathbf{h^2}$) estimates of OBS, on-site-PR and in-lab-PR for traits that were predicted on-site with $R^2_V > 0.7$ are reported in Table 4. The estimated h^2 of OBS for the investigated traits ranged from 0.34 to 0.47. Estimates of h^2 for on-site-PR and in-lab-PR were similar and ranging from 0.41 to 0.50. The estimated h^2 for the predicted traits was greater than that of OBS due to low residual variance and high or equal additive genetic variance estimates relative to OBS. For OBS, values of h^2 estimated from multivariate analyses were from 9 to 20% greater than those estimated in univariate analyses, while their SE were from 33 to 50% lower. For the predicted traits, differences in h^2 and SE between univariate and multivariate models were small as a consequence of the increased amount of information available to the estimation process in comparison with OBS.

Additive genetic correlations between OBS and predicted traits are reported in Table 5. As a result of the very high accuracy of the in-lab-PR models, genetic correlations between OBS and in-lab-PR were very close to 1.00 for all the investigated traits. Correlations between OBS and on-site-PR were very high, ranging from 0.86 (for MP) to 0.94 (for IOD), and for the traits related to the amount of unsaturated FAs (i.e., the traits that are included in the breeding goal of the C21 pig sire line), genetic correlations were >0.90. As a consequence of the very tight relationship between OBS and in-lab-PR, the correlations between on-site-PR and in-lab-PR were consistent with those estimated between OBS and on-site-PR.

Discussion

The fatty acid composition found in our data was limited in comparison with other studies (González-Martín et al., 2003; Pérez-Marín et al., 2009; Pérez-Juan et al., 2010; Zamora-Rojas et al.,

Table 4
Estimates of additive genetic variance (σ^2_a), residual variance (σ^2_e) and heritability (h^2) ± SE for ham fat quality traits measured by reference methods (OBS) and predicted by in-lab (in-lab-PR) and on-site (on-site-PR) IR spectroscopy in heavy pigs.

Trait[1]	Analysis[2]	OBS			in-lab-PR			on-site-PR		
		σ^2_a	σ^2_e	$h^2 \pm SE$	σ^2_a	σ^2_e	$h^2 \pm SE$	σ^2_a	σ^2_e	$h^2 \pm SE$
IOD	Univariate	2.18	4.28	0.34 ± 0.03	2.52	2.54	0.50 ± 0.03	2.65	2.78	0.49 ± 0.03
	Multivariate	2.46	4.13	0.37 ± 0.02	2.83	2.82	0.50 ± 0.02	2.23	2.77	0.45 ± 0.02
C18:2n-6	Univariate	0.63	0.93	0.40 ± 0.06	0.73	0.83	0.47 ± 0.03	0.68	0.75	0.47 ± 0.03
	Multivariate	0.78	0.90	0.46 ± 0.03	0.80	0.87	0.48 ± 0.02	0.60	0.76	0.44 ± 0.02
PUFA	Univariate	0.77	1.20	0.39 ± 0.06	0.93	1.05	0.47 ± 0.03	0.88	0.97	0.47 ± 0.03
	Multivariate	1.01	1.16	0.47 ± 0.03	1.01	1.10	0.48 ± 0.02	0.92	0.97	0.44 ± 0.02
n-6	Univariate	0.65	1.01	0.39 ± 0.06	0.76	0.88	0.46 ± 0.03	0.70	0.78	0.47 ± 0.03
	Multivariate	0.84	0.96	0.47 ± 0.03	0.83	0.92	0.47 ± 0.02	0.62	0.79	0.44 ± 0.02
logMP	Univariate	$0.49\ 10^{-2}$	$0.70\ 10^{-2}$	0.41 ± 0.06	$0.57\ 10^{-2}$	$0.69\ 10^{-2}$	0.45 ± 0.03	$0.34\ 10^{-2}$	$0.46\ 10^{-2}$	0.42 ± 0.03
	Multivariate	$0.53\ 10^{-2}$	$0.61\ 10^{-2}$	0.47 ± 0.03	$0.59\ 10^{-2}$	$0.71\ 10^{-2}$	0.45 ± 0.02	$0.33\ 10^{-2}$	$0.47\ 10^{-2}$	0.41 ± 0.02

[1] IOD, iodine number; C18:2n-6, linoleic acid; PUFA, polyunsaturated fatty acids; n-6, concentration of omega-6 fatty acids (C18:2n-6, C18:3n-6, C20:3n-6, C20:4n-6); logMP = log (MUFA/PUFA), where log denotes the natural logarithm.
[2] For each trait, multivariate restricted maximum likelihood analysis included OBS, in-lab-PR and on-site-PR.

Table 5
Estimates of additive genetic correlations (±SE) between measures of ham fat quality traits (OBS) and their predictions obtained using in-lab (in-lab-PR) and on-site (on-site-PR) IR spectroscopy in heavy pigs.

Trait[1]	Additive genetic correlation		
	OBS with in-lab-PR	OBS with on-site-PR	in-lab-PR with on-site-PR
IOD	$1.00 \pm 0.5\ 10^{-2}$	0.94 ± 0.02	0.93 ± 0.01
C18:2n-6	$0.99 \pm 0.5\ 10^{-2}$	0.91 ± 0.02	0.92 ± 0.01
PUFA	$0.99 \pm 0.4\ 10^{-2}$	0.92 ± 0.02	0.92 ± 0.01
n-6	$0.99 \pm 0.4\ 10^{-2}$	0.91 ± 0.02	0.91 ± 0.01
logMP	$1.00 \pm 0.5\ 10^{-2}$	0.86 ± 0.03	0.88 ± 0.02

[1] IOD, iodine number; C18:2n-6, linoleic acid; PUFA, polyunsaturated fatty acids; n-6, concentration of omega-6 fatty acids (C18:2n-6, C18:3n-6, C20:3n-6, C20:4n-6); logMP = log(MUFA/PUFA), where log denotes the natural logarithm.

2013). As the pigs involved in this study were from the same farm, feeding regime and genetic line, little variation in fat composition was to some extent expected. However, a high level of variability is important to develop robust calibration equations (González-Martín et al., 2003).

Predictive ability of spectra acquired from ham subcutaneous fat

Consistent with screening procedures operated by the major PDO dry-cured ham consortium (Istituto Parma Qualità, 2012), the fat sample used in laboratory reference analyses for fat quality was collected in correspondence of the subcutaneous fat region surrounding site 4 and this can explain the superior ability of spectra collected at site 4 to predict FA composition. Subcutaneous fat of the medial side of the ham, which comprises sites 1 and 2, was thinner and of lower firmness than that located in the lateral side of the thigh. Differences in depth, firmness, oiliness and color of subcutaneous fat are associated with variation in FA composition (Čandek-Potokar and Škrlep, 2012). Hence, the detected differences across predictive models exploiting site-specific spectral information may be explained by variations in FA composition across sites in ham subcutaneous fat.

Values of R^2_V may be underestimated due to the reduced spectra variability of the validation set induced by including the samples with the highest spectral distance in the modeling set. Conversely, values of SE_V, which are not affected by the variability of the validation set, were generally very similar to the corresponding SE_{CV}.

Despite the generally moderate accuracy of models, predictions of single or groups of PUFA, which are the most relevant fat quality traits for product specifications of PDO Italian dry-cured hams, can be considered satisfactory as their R^2_{CV} was > 0.75 and their RPD, which is considered an indicator of the practical validity of models, was > 2. As described by Minasny and McBratney (2013) and confirmed by Bonfatti et al. (2016), for a normally distributed variable and a large sample size, the relationship between RPD and R^2_{CV} is $RPD = (1 - R^2_{CV})^{-0.5}$. When $R^2_{CV} = 0.75$, RPD is equal to 2, which is the threshold upon which a calibration model is considered having a good predictive ability (Minasny and McBratney, 2013).

Although, as confirmed by the poor prediction accuracy of omega-3 FAs, the ability of VIS–NIRS models to predict the amount of a specific FA is, to a certain extent, related to its amount in the sample (Kucha et al., 2018), predictions were very poor for SFA and MUFA, whereas they were more accurate for PUFA.

None of the poorly predicted FA groups (SFA, MUFA and omega-3 FAs) are mentioned among the requirements for ensuring compliance with the PDO product specifications (Consorzio del Prosciutto di Parma, 1992; Consorzio del Prosciutto di San Daniele, 2007) and, for this reason, from a purely technological point of view, they have trivial importance for dry-cured ham production process. As a consequence of the reduced ability of models to predict MUFA and SFA, also UNS, which largely depends on these traits, could not be accurately predicted.

In the literature, values of R^2_{CV} obtained in the prediction of FA composition of intact samples of pork fat are generally higher than those of this study. Previous studies have been conducted mostly on Iberian pigs (De Pedro et al., 2007; Pérez-Marín et al., 2009; Zamora-Rojas et al., 2013), which include three different productive types (i.e., "Bellota", "Recebo" and "Cebo") based on different feeding regimes. As a consequence, fat from these pigs exhibits a largely variable FA composition. In studies involving other pig breeds (Pérez-Juan et al., 2010), animals were fed different diets which were responsible for a large variation in FA composition of subcutaneous fat. Feeding techniques employed in pig farming for the production of Italian dry-cured hams are less variable (Bosi and Russo, 2004) and not comparable to those used in Iberian pig production. Moreover, as the main aim of the study was to evaluate the ability of the on-site VIS–NIR technique to serve as a phenotyping tool in a breeding program of a specific pig genetic line raised in standard conditions, a low variation in the investigated traits relative to the one detected in literature studies was a key feature to deal with.

In comparison with this study, Pérez-Marín et al. (2009) reported higher ability of predicting the FA profile of Iberian pigs using spectra collected on the transversal section of subcutaneous fat with R^2_{CV} of 0.93, 0.84, 0.90 and 0.64 for C16:0, C18:0, C18:1 and C18:2n-6, respectively. Results were similar when spectra were acquired directly from live animals or carcasses. The variation in the concentration of C16:0 and C18:1, as measured by the SD, reported by those authors ranged from two to three times the one detected in our study, but for C18:2n-6, the SD was 50% lower. Prediction models for C18:2n-6 displayed poor predictive ability relative to the one of our study, and the authors suggested that the calibration set was not enough variable to lead to the development of a robust prediction equation for linoleic acid. Results obtained in previous laboratory studies conducted on intact subcutaneous fat samples from Iberian pigs using a fiber-optic probe (González-Martín et al., 2003; De Pedro et al., 2007) were consistent with those reported by Pérez-Marín et al. (2009).

Differences in the predictive ability of models between the current study and the literature can also be partly attributed to different working conditions. Unlike the large majority of previous studies, which were carried out in a laboratory even when a portable spectrometer was used, in this study, the spectra were acquired on-site from hams at the slaughterhouse. The low working temperature was responsible for a thin layer of moisture in the inner side of the probe, likely interfering with reflectance measurements. Also variation in sample temperature when acquiring the spectra might have affected the predictive ability. Spectra acquisition was always performed 24 h after slaughter, but temperature of the hams at spectra collection varied across slaughter groups, according to slaughterhouse operations and activities, and it was not possible to standardize it. All these factors contribute additional variation that is not under control at the slaughterhouse, but can be easily overcome in a laboratory. Zamora-Rojas et al. (2013) reported R^2 values in validation between 0.78 and 0.94 for C16:0, C18:0, C18:1 and C18:2n-6, using spectral information collected in-situ on intact carcasses. However, again, the variability of the dataset used by those authors was much higher than the one of our study.

According to literature results (Kucha et al., 2018), the efficiency in predicting the chemical composition of minced samples is greater than the one of intact samples. Prevolnik et al. (2004) reported that also grinding size affected the accuracy of prediction, which increased when increasing the grinding intensity. Reliable predictions of FA composition have been reported by several authors using NIRS on melted fat (Kucha et al., 2018). Despite lower predictive abilities, the on-site spectra acquisition has the advantage of being instantaneous and of allowing in-field inspection of individual thighs without interfering with the processing procedure at the slaughter plant. Homogenization or melting of fat requires a traceability system for samples during ham trimming and

additional processing, before spectra acquisition, for fat isolation from skin and meat residues.

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The estimated h^2 of OBS for the investigated traits was in agreement with estimates reported in other studies for the same traits in other pig populations (Fernandez et al., 2003; Sellier et al., 2010). In the literature, variations in the proportion of residual and additive genetic variances between measured and IR-predicted traits have been reported for other traits and species (Bonfatti et al., 2017).

The very high correlation between OBS and in-lab-PR indicate that in-lab NIRS can effectively replace the more expensive and time-consuming laboratory reference analyses for ham fat quality to provide breeding programs of the C21 Goland sire line with large-scale phenotypes. Being the on-site-PR less accurate than the in-lab-PR, lower genetic correlations with OBS were expected. However, the high genetic correlations between OBS and on-site-PR indicate that, despite the low accuracy obtained for on-site VIS-NIRS applied to intact ham subcutaneous fat relative to in-lab NIRS on homogenized fat samples, the loss in magnitude of the genetic correlation between OBS and predictions is small. A very high genetic correlation (0.88) was also found on the same population for measures of ham weight loss at the end of the dry-curing process (12 months) and their on-site VIS-NIR predictions obtained from spectra collected on the raw hams (Bonfatti and Carnier, 2020).

These results provide evidence that, even under the peculiar circumstances of a testing procedure used in selective breeding, where animals of a single genetic line are raised in standardized conditions to minimize the residual variation, on-site VIS-NIRS predictions of raw ham fat quality have the potential to be accurate enough to be used for routine large-scale phenotyping for breeding purposes.

Although the predictive ability of NIRS increases after in-lab homogenization of fat samples, this difference is not as such as to justify the complication of the operational procedures arising from fat sampling and laboratory procedures. Based on positive and high additive genetic relationships with OBS or in-lab-PR and greater heritabilities than OBS, on-site-PR may be implemented as indicator traits of ham fat quality in the breeding programs of the C21 Goland sire line addressed to the enhancement of IOD and C18:2n-6 concentration, key traits for thighs processed into dry-cured hams.

Supplementary materials

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2020.100073

Ethics approval

Animal Care and Use Committee approval was not needed because animals were subjected to standard production and slaughter conditions, and no additional measurements were taken. Observations used in this study were from the genetic evaluation program of the C21 Goland sire line (Gorzagri, Fonzaso, Italy) and were registered at the farm where the program is being carried out since 1998. The farm operates in compliance with regulations of the European and Italian law concerning animal protection and welfare.

Data and model availability statement

None of the data were deposited in an official repository.

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Valentina Bonfatti: Investigation, methodology, formal analysis, writing-original draft preparation. **Elena Boschi**: Writing-reviewing and editing. **Luigi Gallo**: Writing-reviewing and editing. **Paolo Carnier**: Conceptualization, supervision, writing-reviewing and editing.

Declaration of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors are indebted with Gorzagri s.s. (Fonzaso, Italy) for having provided animals and assistance during sampling procedures. The assistance by the staff of the Veronesi group (Verona, Italy) in operations at the slaughter plant is also gratefully acknowledged.

Financial support statement

This research received no specific grant from any funding agency, commercial or not-for-profit section.

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