Universitat de Lleida

Document downloaded from:

<http://hdl.handle.net/10459.1/70092>

The final publication is available at:

<https://doi.org/10.1016/j.meatsci.2020.108399>

Copyright

cc-by-nc-nd, (c) Elsevier, 2020

Està subjecte a una llicència de
[Reconeixement-NoComercial-SenseObraDerivada 3.0 de Creative Commons](https://creativecommons.org/licenses/by-nc-nd/3.0/)

**Leptin receptor and fatty acid desaturase-2 gene variants affect fat,
color and production profile of dry-cured hams**

Rafael Suárez-Mesa^{a,b}, Roger Ros-Freixedes^a, Marc Tor^a, Josep Reixach^c,
Ramona N. Pena^a, Joan Estany^{a*}

^a Department of Animal Science, University of Lleida-Agrotecnio Center, Av. Rovira
Roure 191, 25198 Lleida, Catalonia, Spain

^b Research Group of Immunobiology and Pathogenesis, University of Tolima, Barrio
Santa Helena Parte Alta, Ibagué, Colombia.

^c Selección Batallé S.A., 17421 Riudarenes, Catalonia, Spain.

* Corresponding author. Tel.: +34 973 70 2893; fax: +34 973 70 2874.

E-mail address: joan.estany@udl.cat (J. Estany).

ABSTRACT

The effects of *LEPR* (rs709596309C>T) and *FADS2* (rs321384923A>G) single nucleotide polymorphisms on production and quality attributes in purebred Duroc dry-cured hams were examined. As compared to *LEPR*-C– hams, the *LEPR*-TT hams had more intramuscular fat (+2.2% dry matter, $P<0.01$). As a result, they showed higher saturated (+1.54%, $P<0.01$) and lower polyunsaturated (-1.05%, $P<0.01$) fatty acids content and were brighter (L^* : +1.07, $P<0.05$) and yellower (b^* : +0.78, $P<0.01$). The *FADS2*-A allele enhanced the C20:4n-6 to C18:2n-6 ratio but did not affect either fat content or color coordinates. However, hams carrying the *FADS2*-A allele reached the target weight loss earlier, thereby spending less time in seasoning (-8.4 d, $P<0.01$). Thus, production batches could be arranged by genotype, with longer manufacturing times for fatter *LEPR*-TT and shorter times for *FADS2*-A– hams. These results confirm that genetic markers validated in raw pork are effective in dry-cured ham, but also stress that product-specific validations are still needed to unravel specific outcomes.

Key words: fatty acid composition; genetic marker; intramuscular fat; meat quality; pigs.

36 **HIGHLIGHTS**

- 37 • Recessive T allele at *LEPR* (rs709596309C>T) increases intramuscular fat in hams
- 38 • *LEPR*-induced fatness enhances the saturated to polyunsaturated fatty acid ratio
- 39 • *LEPR*-TT hams are prone to look brighter and yellower
- 40 • The A allele at *FADS2* (rs321384923A>G) enhances the C20:4n-6/C18:2n-6 ratio
- 41 • Hams carrying the *FADS2*-A allele reach target weight loss earlier

42

1. Introduction

Dry-cured ham production is of great economic importance in Southern Europe (Cruz, 2019) and increasingly worldwide (Rentfrow, Chaplin, & Suman, 2012; Xia et al., 2017). There is a constant increase of consumers who place enhanced emphasis on quality, which is largely determined by the characteristics of the raw material and the manufacturing conditions. Green hams for dry-curing are selected based on their weight and fat content, which in turn influence the duration of seasoning. In addition, intramuscular fat (IMF) content and fatty acid composition affect relevant quality features such as color, texture and aroma (Čandek-Potokar & Škrlep, 2012; Ruiz-Carrascal, Ventanas, Cava, Andrés, & García, 2000). These characteristics have a genetic basis, as evidenced by the existing variability between (Wood et al., 1996) and within (Ros-Freixedes, Reixach, Tor, & Estany, 2012) breed. This is the reason why Iberian and Duroc are among the most preferred breeds for producing premium dry-cured hams. However, selection for quality traits within breed is challenging because these traits are difficult and costly to phenotype. Thus, the use of genetic markers has been proposed as an alternative (Otto et al., 2007).

Several genetic markers associated with pork quality traits have already been described (Hong et al., 2015; Rohrer, Nonneman, Miller, Zerby, & Moeller, 2012), including some that have been specifically tested in green hams for use in production of dry-cured ham (Davoli et al., 2017; Ramos, Glenn, Serenius, Stalder, & Rothschild, 2008; Renaville et al., 2010). However, only a few genetic markers for fat content or fatty acid composition have been validated directly on dry-cured ham (Henríquez-Rodríguez, Tor, Pena, & Estany, 2015; Santé-Lhoutellier et al., 2012; Stalder, Rothschild, & Lonergan, 2005). Recently, we reported two polymorphisms that segregate in Duroc pigs: one in the leptin receptor gene (*LEPR*), which impacts fat

deposition (Ros-Freixedes et al., 2016), and another in the fatty acid desaturase-2 gene (*FADS2*), with effects on the n-6 fatty acid profile (Gol, Pena, Rothschild, Tor, & Estany, 2018). These effects have not been confirmed in dry-cured ham and other potential side-effects have not been investigated. Therefore, the aim of this research was to examine whether these two polymorphisms affect production and quality attributes of commercial dry-cured hams, including color, fat content and fatty acid composition.

2. Material and methods

2.1. Animals and samples

Data on 332 dry-cured hams from the same purebred Duroc line (272 barrows and 60 females) were used for the analyses (Ros-Freixedes et al., 2012). Pigs were raised in separate batches but followed a similar management. At about 75 d of age, piglets were moved to the fattening units, where they were penned by sex (8 to 12 pigs per pen) until they were slaughtered at around 210 d of age in the same commercial abattoir. During the fattening period pigs had *ad libitum* access to commercial diets. After slaughter, in a subsample of 264 pigs, the carcass was weighed and the carcass backfat thickness at 6 cm off the midline between the third and fourth last ribs was ultrasonically measured with an automatic carcass grading equipment (AutoFOM, SFK-Technology, Denmark). Then, after chilling for about 24 h at 2°C, each carcass was divided into primal cuts and the left green ham was weighed. Each ham was trimmed according to customary procedure used for manufacturing traditional dry-cured Spanish ham. Hams were dry-cured through a four-step process (Henríquez-Rodríguez et al., 2015). First, hams were covered with salt and kept for 10 to 14 d at 2°C. Then, the hams were dried for 3 months following a slow ramp of increasing temperatures (from 3°C to

10°C). After that, the hams were ripened for 6 months at 9°C to 14°C. Finally, in the last ripening step, the hams were allocated into seasoning batches and kept in room temperature (15°C to 20°C) until around one third of the initial weight was lost. At the end of this process, the total manufacturing time (from slaughter until the end of dry-curing), the final weight of the bone-in cured ham and the weight loss during the whole dry-curing process were recorded. A slice from the middle of the ham was taken (Fig. 1). Slices were individually vacuum-packaged and stored at -20°C for further determinations.

2.2. Color measurements

Color measurements were done in a subsample of 190 hams (last six seasoning batches). Ham slices were defrosted and placed on a polystyrene white tray. Seven color measurements were made directly on the exposed surface of the slice, six in different muscle sites (ST: *m. semitendinosus*; BFA: *m. biceps femoris caudal*; BFR: *m. biceps femoris cranial*; SMA: *m. semimembranosus caudal*; SMR: *m. semimembranosus cranial*; and RF: *m. rectus femoris*) and one in the outer subcutaneous fat (Fig. 1), following the procedures described by Álvarez-Rodríguez et al. (2018). Color readings were taken with a Konica Minolta CM-700d spectrophotometer (Konica Minolta Sensing Inc., Osaka, Japan) in the CIELAB space (Commission Internationale de l'Éclairage, CIE, 1986) with a measured area diameter of 8 mm, including specular component and a 0%-ultraviolet, standard illuminant D65, which simulates daylight (color temperature 6504 K), observer angle 10° and white calibration. In each of the measurement sites, color-space values for lightness (L^*), redness (a^* ; from green [a^*-] to red [a^*+]), and yellowness (b^* ; from blue [b^*-] to yellow [b^*+]) were recorded as the average of three readings. The hue angle ($h^* = \arctan(b^*/a^*)$), expressed in degrees;

from dark to white) and chroma ($C^* = \sqrt{a^{*2} - b^{*2}}$; from dull to vivid intensity) variables were calculated.

2.3. Chemical analyses

The *biceps femoris* muscle was dissected out from the ham slice (Fig. 1) and minced. A sample of at least 20 g of the minced muscle was used to determine dry matter (DM) by drying for 12 to 18 h at 104°C in an air oven and ash content by drying for 3 h at 550 °C. The rest of the sample was freeze-dried and pulverized. Representative aliquots from the freeze-dried muscle were used for crude protein and fat analysis. Crude protein content was determined by the Kjeldhal method (AOAC, 2005) and IMF content and fatty acid composition were determined in duplicate by quantitative determination of the individual fatty acids by gas chromatography (Bosch, Tor, Reixach, & Estany, 2009). Fatty acid methyl esters were obtained by transesterification with 20% boron trifluoride in methanol (Rule, 1997) and were analyzed by gas chromatography using a 30 m × 0.25 mm capillary column (Agilent DB-23, Agilent Technologies, Santa Clara, CA, USA) and a flame ionization detector with helium as the carrier gas at 2 mL/min. The oven temperature program increased from 150°C to 220°C at 8°C/min, and injector and detector temperatures were both 250°C. The quantification was carried out through area normalization with an external mixture of fatty acid methyl esters (Supelco® 37 Component FAME Mix, Sigma, Tres Cantos, Madrid). The internal standard was 1,2,3-tripentadecanoylglycerol. Then, fatty acid composition was expressed as the percentage of each individual fatty acid relative to total fatty acids. The complete profile for each sample included saturated (SFA: C14:0, C16:0, C18:0, and C20:0); monounsaturated (MUFA: C16:1n-9, C18:1n-7, C18:1n-9 and C20:1n-9); and polyunsaturated (PUFA; 18:2n-6, C18:3n-3, C20:2n-6,

and C20:4n-6) fatty acids (Henríquez-Rodríguez et al., 2015). The IMF content in each muscle was calculated as the sum of the individual fatty acids expressed as triglyceride equivalents (AOAC, 2000).

2.4. Genotyping

The isolation of genomic DNA was carried out from muscle samples using a standard protocol. All 332 hams were genotyped for *LEPR* (rs709596309 C>T; on SSC6) and *FADS2* (rs321384923A>G; on SSC2) single nucleotide polymorphisms (SNP). The *LEPR* SNP was genotyped by real time qPCR (QuantStudio3, Applied Biosystems) with High Resolution Melt analysis (Luminaris Color HRM Master Mix, Thermo Scientific) as described in Ros-Freixedes et al. (2016). The RFLP-PCR genotyping protocol detailed in Gol et al. (2018) was used to genotype the *FADS2* SNP. To account for potential biases, all hams were also genotyped for the stearyl-CoA desaturase (*SCD*) gene SNP on SSC14 (rs80912566 T>C), which is known to influence fatty acid composition in Duroc. *SCD* SNP genotyping was performed by allelic discrimination assay with primers and probes indicated in Estany, Ros-Freixedes, Tor, & Pena (2014).

2.5. Statistical analyses

The effect of *LEPR* and *FADS2* genotypes on ham production traits was estimated using a fixed effects model that included the slaughter batch by sex (7 levels, each level corresponding to contemporaneous pigs of the same sex slaughtered at the same time) and the genotype for *LEPR* (CC, CT, and TT), *FADS2* (AA, AG, and GG) and *SCD* (TT, CT and CC). A similar model was used to estimate the effect of the genotype on chemical composition traits but here the slaughter batch was replaced by the seasoning batch by sex (10 levels, each level corresponding to hams of the same sex

in the same seasoning batch) and the weight of the green ham was added as a covariate. Color traits measured in different muscles were jointly analyzed using a mixed model including the seasoning batch, the genotypes of the three markers, the muscle (BFA, BFR, SMA, SMR, ST and RF) and the weight of the green ham as fixed effects and the ham as a random effect. The variance of the ham and the residual variance were estimated by restricted maximum likelihood. As a result, the intraclass correlation between muscles was calculated dividing the variance of the ham by the total variance. Color traits in subcutaneous fat were analyzed after removing the muscle and the ham from the model. To assess the effect of fat content on fatty acid composition and color traits, IMF was added as a covariate to the corresponding models. Fixed effects were tested following an F-test. Multiple pairwise comparisons between genotypes and muscles were tested by a Tukey test. All the analyses were performed using the statistical package JMP Pro 14 (SAS Institute Inc., Cary, NC).

3. Results and discussion

3.1. Effects on ham production traits

The main effects of the investigated markers on production traits were the effect of *LEPR* on carcass backfat thickness and ham weight loss rate and the effect of *FADS2* on manufacturing time (Table 1). In line with previous research in this Duroc line, albeit in fresh pork, (Henríquez-Rodríguez, Bosch, Tor, Pena, & Estany, 2016; Ros-Freixedes et al., 2016), homozygous *LEPR*-TT pigs had higher carcass backfat thickness (2.9 ± 0.9 mm, $P < 0.01$) compared to the other two *LEPR* genotypes (C–), which did not differ between them. Ros-Freixedes et al. (2016) already showed that the T allele behaves recessively while Henríquez-Rodríguez et al. (2016) demonstrated that its effect on

backfat thickness is observed throughout the finishing period. The effect of this polymorphism on backfat thickness has also been observed in other Duroc lines (Uemoto et al., 2012) and in Iberian crossbreds (Muñoz et al., 2009). The greater fatness of *LEPR*-TT pigs was reflected in their lower weight loss rate in relation to the other two *LEPR* genotypes (-0.09 ± 0.03 kg/year, $P < 0.01$).

We observed a negative effect of *FADS2* genotype on carcass and ham weight that was associated to changes in weight loss and, consequently, manufacturing time. The effects of the *FADS2*-AA genotype on carcass and ham weight should be taken with caution due to the low number of hams with this genotype and because Gol et al. (2018) did not observe any effect of *FADS2* on carcass weight with a much bigger sample size. The *FADS2* gene encodes for the delta-6-desaturase, which is involved in the biosynthesis of arachidonic acid (C20:4n-6) from linoleic acid (C18:2n-6). In a previous work in fresh pork (Gol et al., 2018), we have shown that the A allele in this polymorphism behaves additively by increasing C20:4n-6 as well as the ratio of C20:4n-6 to C18:2n-6 in muscle. Because C20:4n-6 is inversely related with fat content, a first hypothesis for this finding was that hams from A- pigs, which display higher values for C20:4n-6 than GG pigs, are leaner and therefore needed shorter curing time. However, this was not the case (Table 1). Manufacturing time, determined as the time to reach the targeted weight loss, was more influenced by green ham weight (5.7 ± 1.4 d/kg, $P < 0.01$) than by backfat thickness (0.4 ± 0.3 d/mm, $P = 0.16$). This could explain the differences between *FADS2*-AA and GG hams, but not between *FADS2*-AG and GG hams. Whereas *FADS2*-AA hams, which were lighter than *FADS2*-GG hams, had a shorter manufacturing period and higher weight loss, *FADS2*-AG hams, which had a similar weight to that of *FADS2*-GG hams, had a shorter manufacturing period than *FADS2*-GG hams despite similar weight loss (Table 1). On the other hand, the effect of

FADS2 on manufacturing time cannot be attributed to differences in muscle pH (results not shown). C20:4n-6 has a wide range of biological implications and it is known to contribute to maintain membrane fluidity and cell signaling (Martin, Brash, & Murphy, 2016). Further research is needed to confirm and elucidate whether C20:4n-6 biological functions can explain this effect, but the *FADS2* association described here points to genetic variation in manufacturing time that is independent from fat content and the weight of the green ham. The sensory quality of dry-cured hams depends on the biochemical changes that occur during ripening, so ideally there is an optimum duration of processing for each ham within a given production scenario. In commercial practice, however, aging is mostly performed in batches, so genetic markers such as *LEPR* and *FADS2* may help to introduce individual criteria other than weight in order to arrange more homogeneous seasoning batches.

3.2. Effects on intramuscular fat content and fatty acid composition

The *LEPR* SNP had a relevant impact on IMF content and fatty acid composition (Table 2). The *LEPR*-TT hams showed greater IMF than *LEPR*-C- hams, whether expressed on a fresh ($+1.4 \pm 0.3\%$ fresh matter, $P < 0.01$; Fig. 2) or dry matter ($+2.2 \pm 0.5\%$ DM, $P < 0.01$; Table 2) basis. The positive effect of *LEPR*-TT on IMF was of similar relative magnitude to that observed in raw muscle (Ros-Freixedes et al., 2016) and opposite to that on protein content ($-2.8 \pm 0.8\%$ DM, $P < 0.01$). In accordance with these results, *LEPR*-TT hams had greater DM than *LEPR*-C- hams ($+1.3 \pm 0.5\%$, $P < 0.01$). No difference was detected between *LEPR* genotypes for ash content.

Intramuscular fat content has a determinant role in premium dry-cured ham production. Depending on the gene frequency of the T allele and the crossbreeding scheme used, the investigated *LEPR* SNP can explain a relevant proportion of variation

in IMF. Here, with the frequencies indicated in Table 1, the *LEPR* SNP explained 6.7% of the phenotypic variance of IMF, a value that could almost double in some Duroc x Iberian hams, where the genotypic frequency of *LEPR*-TT can be close to 50%. The *FADS2* SNP did not affect the chemical composition of dry-cured *biceps femoris* muscle (Table 2).

Fatty acid composition is also of concern in premium dry-cured ham production. It is known that fatty acid composition changes with fat content, increasing both SFA and MUFA at the expense of PUFA (Bosch, Tor, Reixach, & Estany, 2012). Following this trend, fatter *LEPR*-TT hams showed higher SFA ($+1.54 \pm 0.51\%$, $P < 0.01$) and lower PUFA ($-1.05 \pm 0.25\%$, $P < 0.01$) than *LEPR*-C- hams. In contrast, the *LEPR* SNP did not modify MUFA ($-0.49 \pm 0.43\%$, $P = 0.25$), in agreement with what we have previously seen in raw muscle (Henríquez-Rodríguez et al., 2016; Ros-Freixedes et al., 2016). Thus, *LEPR* mostly affected the SFA to PUFA ratio (Table 3), with *LEPR*-TT hams showing greater values than *LEPR*-C- hams ($+0.57 \pm 0.13$, $P < 0.01$). However, after adjusting for IMF, the effects of *LEPR*-TT on fatty acid composition vanished, except for the ratio SFA to PUFA ($+0.21 \pm 0.11$, $P = 0.05$). This result indicates that the *LEPR*-T allele enhances endogenous biosynthesis of SFA at the expense of PUFA deposition beyond what it would be expected from a linear increase in IMF.

The main effects of the *FADS2* SNP were on the fatty acids involved in the biosynthesis of C20:4n-6 from C18:2n-6, and particularly on the C20:4n-6 to C18:2n-6 ratio, which increased with the A allele (Table 3). As an enzyme, FADS2 desaturates C18:2n-6 to C18:3n-6, which is then elongated to C20:3n-6 and desaturated to C20:4n-6. However, FADS2 also intervenes in a surrogate reaction where C20:2n-6, as a product of C18:2n-6 elongation, is desaturated via FADS2 to C20:3n-6 and then to C20:4n-6 (Stoffel et al., 2014). Thus, the C20:4n-6 to C18:2n-6 ratio can be interpreted

as the overall efficiency of transforming C18:2n-6 to C20:4n-6 through both paths. As expected, the effects of *FADS2* SNP were independent of IMF content. After adjusting for IMF, the C20:4n-6 to C18:2n-6 ratio was 11.7% greater in the *FADS2*-AA than in the *FADS2*-GG genotype, a value that is in line with the observed in raw *gluteus medius* (12.0%) and *longissimus thoracis* (11.2%) muscles (Gol et al., 2018). Likewise, the C20:4n-6 to C20:2n-6 ratio was also greater for the *FADS2*-AA than for the *FADS2*-GG genotype, showing a similar relative value (16.4%) to that found in raw muscle. Individual fatty acid content changes were consistent with increased *FADS2* enzyme activity in pigs carrying the *FADS2*-A allele. However, contrarily to what was observed in raw *gluteus medius* and *longissimus thoracis* muscles, the effect of *FADS2*-A in dry-cured *biceps femoris* was more evident for C18:2n-6 (additive value of $-0.21 \pm 0.09\%$, $P < 0.05$) than for C20:4n-6 (additive value of $+0.04 \pm 0.03\%$, $P < 0.10$). Differences among genotypes for C20:4n-6 could have been mitigated during seasoning as a result of its greater susceptibility to autoxidation compared to C18:2n-6 (Hanna & Hafez, 2018). Although changes in fatty acid composition due to *FADS2* are small, correlative effects beyond them cannot be discarded. Ultimately, the results obtained indicate that genetic markers with minor but contrasted effects in raw meat are still effective after a long dry-curing period.

3.3 Effects on color profile

The effects of *LEPR* and *FADS2* genetic markers on instrumental color coordinates across muscles are given in Table 4. The most remarkable effect was that of *LEPR* on muscle lightness and yellowness. No effect was detected of *LEPR* on the color of the subcutaneous fat. Hams with the *LEPR*-TT genotype had greater L^* ($+1.07 \pm 0.44$, $P < 0.05$), b^* ($+0.78 \pm 0.24$, $P < 0.01$) and h^* ($+3.49 \pm 0.94$, $P < 0.01$) than hams with the

LEPR-C- genotype. Lightness increases with IMF (Nollet & Toldrá, 2010; Ruiz-Carrascal et al., 2000) and moisture (Ferrini, Comaposada, Arnau, & Gou, 2012). Our results indicate that IMF exerted a greater impact on L^* (0.25 ± 0.06 units/%DM, $P < 0.01$) than moisture (0.08 ± 0.07 units/%, $P = 0.29$). Thus, after adjusting for IMF, *LEPR*-TT and *LEPR*-C- genotypes did not differ for L^* ($+0.53 \pm 0.44$, $P = 0.23$), thereby indicating that the positive effect of *LEPR*-TT on L^* is mostly due to increased IMF. Fat oxidation could be behind the positive effect of the *LEPR*-TT on b^* . Since the *SCD*-T allele, which enhances MUFA but not IMF (Estany et al., 2014), also had a positive impact on b^* (TT vs. CC: $+0.63 \pm 0.23$, $P < 0.01$), total MUFA rather than IMF may better contribute to explain the effect of *LEPR*-T on yellowness. This interpretation is consistent with previous findings showing that yellowness is associated with fats of lower solid fat content (Gandemer, 2002), as occurs in Iberian hams with a higher MUFA profile (Carrapiso & García, 2005). In dry-cured hams, fat with a golden hue is generally well-accepted because it may reflect traditional ripening, although this apparently positive effect has not been corroborated in sensory evaluations (Carrapiso & García, 2008). As it could be also the case for *LEPR* genotypes, color differences were likely not enough to be perceived by the panelists (Carrapiso & García, 2005, 2008). The *FADS2* SNP was not associated with changes in the color coordinates (Table 4).

Color coordinates were within the range of reported values (Carrapiso & García, 2005; Stalder et al., 2005; Yim, Hong, & Chung, 2016), but with a great variation across muscles and even between locations within muscle (Table 5). The average correlation of values taken in a slice was low (0.12 - 0.20 , $P < 0.01$), confirming that color outcome is highly dependent on the site of measurement. Despite this variation, the effect of *LEPR* for L^* and b^* was consistent across muscles. In particular, the difference between *LEPR* TT and C- genotypes ranged from 0.55 ± 0.76 (BFA; $P = 0.47$) to 2.22 ± 0.95 (ST; $P < 0.05$),

for L^* , and from 0.52 ± 0.54 (SMR; $P=0.33$) to 1.35 (BFA; $P<0.01$), for b^* . Evidences for the effect of *LEPR* and *SCD* on a^* across muscles were mixed. Even though *LEPR*-TT hams generally had greater values of a^* than *LEPR*-C- (1.04 ± 0.45 , for SMA; $P<0.05$), this was not always the case (-0.66 ± 0.36 , for ST; $P<0.10$). This result highlights the need for validation of color effects across muscles.

4. Conclusions

This work provides evidence that the *LEPR* (rs709596309C>T) SNP affects fat content, fatty acid composition and color development in dry-cured ham. The *LEPR*-TT genotype can be used to identify hams with increased fat content and lower weight loss rate. Because of this, *LEPR*-TT hams present a higher ratio of SFA to PUFA and are more prone to look bright and yellow than *LEPR*-C- hams. The *FADS2* (rs321384923A>G) SNP has little impact over fat and color attributes, with the major effect being the enhancement of the C18:2n-6 to C20:4n-6 ratio, but hams carrying the *FADS2*-A allele show reduced manufacturing time. Thus, while *LEPR*-TT hams can be more suitable for longer seasonings, *FADS2*-A- hams can be more adequate for shorter seasonings. The two genetic markers can contribute to arrange more homogeneous ham seasoning batches and, particularly the *LEPR* SNP, have potential for use in marker-assisted selective breeding in pigs. The results obtained demonstrate that contrasted genetic markers in raw pork are also effective in dry-cured products.

Declaration of competing interest

The authors declare no conflict of interests.

Acknowledgments

The authors acknowledge the financial support from the Spanish Ministry of Science, Innovation & Universities and the EU Regional Development Funds (grant RTI2018-101346-B-I00). RS-M acknowledges the support from the Administrative Department of Science, Technology and Innovation (COLCIENCIAS, announcement 755 for the formation of high-level human capital for Tolima). We thank the staff of Selección Batallé for their cooperation in the experiment.

References

- Álvarez-Rodríguez, J., Ros-Freixedes, R., Gol, S., Henríquez-Rodríguez, E., Pena, R. N., Bosch, L., Estany, J., Vilaró, F & Tor, M. (2018). Carcass lean-yield effects on the fatty acid and amino acid composition of Duroc pork and its technological quality after vacuum-aging. *Animal Production Science*, 58(12), 2335–2343. <https://doi.org/10.1071/AN17070>
- AOAC. (2000). *Official method 960.39 fat (crude) or ether extract in meat*. In *Official Methods of Analysis*. (A. International, Ed.), Association of Official Agricultural Chemists (17th ed.). Arlington, VA. Retrieved from <http://www.eoma.aoac.org/methods/info.asp?ID=16128>
- AOAC. (2005). *Official method 992.15 Crude Protein in Meat and Meat Products Including Pet Foods In Official Methods of Analysis*. Association of Official Agricultural Chemists (18th ed.). Gaithersburg, USA. Retrieved from <http://www.eoma.aoac.org/methods/info.asp?ID=16519>
- Bosch, L., Tor, M., Reixach, J., & Estany, J. (2009). Estimating intramuscular fat content and fatty acid composition in live and post-mortem samples in pigs. *Meat Science*, 82(4), 432–437. <https://doi.org/10.1016/j.meatsci.2009.02.013>
- 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(3), 358–363. <https://doi.org/10.1016/j.meatsci.2012.02.019>
- Čandek-Potokar, M., & Škrlep, M. (2012). Factors in pig production that impact the quality of dry-cured ham: A review. *Animal*, 6(2), 327–338. <https://doi.org/10.1017/S1751731111001625>
- Carrapiso, A. I., & García, C. (2005). Instrumental colour of Iberian ham subcutaneous fat and lean (biceps femoris): Influence of crossbreeding and rearing system. *Meat Science*, 71(2), 284–290. <https://doi.org/10.1016/j.meatsci.2005.03.024>
- Carrapiso, A. I., & García, C. (2008). Effect of the Iberian pig line on dry-cured ham characteristics. *Meat Science*, 80(2), 529–534. <https://doi.org/10.1016/j.meatsci.2008.02.004>
- Cruz, J. (2019). El sector jamonero mantiene la senda del crecimiento en producción, consumo y exportaciones. *Eurocarne*, 279, 93–108.

- Davoli, R., Schivazappa, C., Zambonelli, P., Braglia, S., Rossi, A., & Virgili, R. (2017). Association study between single nucleotide polymorphisms in porcine genes and pork quality traits for fresh consumption and processing into Italian dry-cured ham. *Meat Science*, 126, 73–81. <https://doi.org/10.1016/j.meatsci.2016.11.018>
- Estany, J., Ros-Freixedes, R., Tor, M., & Pena, R. N. (2014). A functional variant in the stearoyl-CoA desaturase gene promoter enhances fatty acid desaturation in pork. *PLoS ONE*, 9(1), 1–11. <https://doi.org/10.1371/journal.pone.0086177>
- Ferrini, G., Comaposada, J., Arnau, J., & Gou, P. (2012). Colour modification in a cured meat model dried by Quick-Dry-Slice process® and high pressure processed as a function of NaCl, KCl, K-lactate and water contents. *Innovative Food Science and Emerging Technologies*, 13(JANUARY), 69–74. <https://doi.org/10.1016/j.ifset.2011.09.005>
- Gandemer, G. (2002). Lipids in muscles and adipose tissues, changes during processing and sensory properties of meat products. *Meat Science*, 62(3), 309–321. [https://doi.org/10.1016/S0309-1740\(02\)00128-6](https://doi.org/10.1016/S0309-1740(02)00128-6)
- 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(1), 1–9. <https://doi.org/10.1038/s41598-018-32710-w>
- Hanna, V. S., & Hafez, E. A. A. (2018). Synopsis of arachidonic acid metabolism: A review. *Journal of Advanced Research*, 11(March 2018), 23–32. <https://doi.org/10.1016/j.jare.2018.03.005>
- Henríquez-Rodríguez, E., Bosch, L., Tor, M., Pena, R. N., & Estany, J. (2016). The effect of SCD and LEPR genetic polymorphisms on fat content and composition is maintained throughout fattening in Duroc pigs. *Meat Science*, 121(March 2001), 33–39. <https://doi.org/10.1016/j.meatsci.2016.05.012>
- Henríquez-Rodríguez, E., Tor, M., Pena, R. N., & Estany, J. (2015). A polymorphism in the stearoyl-CoA desaturase gene promoter increases monounsaturated fatty acid content in dry-cured ham. *Meat Science*, 106, 38–43. <https://doi.org/10.1016/j.meatsci.2015.03.019>
- Hong, J., Kim, D., Cho, K., Sa, S., Choi, S., Kim, Y., ... Chung, H. (2015). Effects of genetic variants for the swine FABP3, HMGA1, MC4R, IGF2, and FABP4 genes on fatty acid composition. *Meat Science*, 110, 46–51. <https://doi.org/10.1016/j.meatsci.2015.06.011>
- Martin, S. A., Brash, A. R., & Murphy, R. C. (2016). Thematic Review Series: Living History of Lipids: The Discovery and Early Structural Studies of Arachidonic Acid. *Journal of Lipid Research*, 57(7), 1126–1132.
- Muñoz, G., Ovilo, C., Silló, L., Tomás, A., Noguera, J. L., & Rodríguez, M. C. (2009). Single- And joint-population analyses of two experimental pig crosses to confirm quantitative trait loci on Sus scrofa chromosome 6 and leptin receptor effects on fatness and growth traits. *Journal of Animal Science*, 87(2), 459–468. <https://doi.org/10.2527/jas.2008-1127>
- Nollet, L. M. L., & Toldrá, F. (2010). *Sensory analysis of foods of animal origin. Sensory Analysis of Foods of Animal Origin*. <https://doi.org/10.1201/b10822>
- Otto, G., Roehe, R., Looft, H., Thoelking, L., Knap, P. W., Rothschild, M. F., ... Kalm, E. (2007). Associations of DNA markers with meat quality traits in pigs with

emphasis on drip loss. *Meat Science*, 75(2), 185–195.
<https://doi.org/10.1016/j.meatsci.2006.03.022>

Ramos, A. M., Glenn, K. L., Serenius, T. V., Stalder, K. J., & Rothschild, M. F. (2008). Genetic markers for the production of US country hams. *Journal of Animal Breeding and Genetics*, 125(4), 248–257. <https://doi.org/10.1111/j.1439-0388.2007.00710.x>

Renaville, B., Piasentier, E., Fan, B., Vitale, M., Prandi, A., & Rothschild, M. F. (2010). Candidate gene markers involved in San Daniele ham quality. *Meat Science*, 85(3), 441–445. <https://doi.org/10.1016/j.meatsci.2010.02.013>

Rentfrow, G., Chaplin, R., & Suman, S. P. (2012). Technology of dry-cured ham production: Science enhancing art. *Animal Frontiers*, 2(4), 26–31. <https://doi.org/10.2527/af.2012-0059>

Rohrer, G. A., Nonneman, D. J., Miller, R. K., Zerby, H., & Moeller, S. J. (2012). Association of single nucleotide polymorphism (SNP) markers in candidate genes and QTL regions with pork quality traits in commercial pigs. *Meat Science*, 92(4), 511–518. <https://doi.org/10.1016/j.meatsci.2012.05.020>

Ros-Freixedes, R., Gol, S., Pena, R., Tor, M., Ibáñez-Escriche, N., Dekkers, J., & Estany, J. (2016). Genome-wide association study singles out SCD and LEPR as the two main loci influencing intramuscular fat content and fatty acid composition in duroc pigs. *PLoS ONE*, 11(3), 1–18. <https://doi.org/10.1371/journal.pone.0152496>

Ros-Freixedes, R., Reixach, J., Tor, M., & Estany, J. (2012). Expected genetic response for oleic acid content in pork. *Journal of Animal Science*, 90(12), 4230–4238. <https://doi.org/10.2527/jas.2011-5063>

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

Rule, D. C. (1997). Direct transesterification of total fatty acids of adipose tissue, and of freeze-dried muscle and liver with boron-trifluoride in methanol. *Meat Science*, 46(1), 23–32. [https://doi.org/10.1016/S0309-1740\(97\)00008-9](https://doi.org/10.1016/S0309-1740(97)00008-9)

Santé-Lhoutellier, V., Robert, N., Martin, J. F., Gou, P., Hortós, M., Arnau, J., ... Candek-Potokar, M. (2012). PRKAG3 and CAST genetic polymorphisms and quality traits of dry-cured hams-II. Associations in French dry-cured ham Jambon de Bayonne and their dependence on salt reduction. *Meat Science*, 92(4), 354–359. <https://doi.org/10.1016/j.meatsci.2012.06.022>

Stalder, K. J., Rothschild, M. F., & Lonergan, S. M. (2005). Associations between two gene markers and indicator traits affecting fresh and dry-cured ham processing quality. *Meat Science*, 69(3), 451–457. <https://doi.org/10.1016/j.meatsci.2004.08.014>

Stoffel, W., Hammels, I., Jenke, B., Binczek, E., Schmidt-Soltan, I., Brodesser, S., ... Thevis, M. (2014). Obesity resistance and deregulation of lipogenesis in $\Delta 6$ -fatty acid desaturase (FADS2) deficiency. *EMBO Reports*, 15(1), 110–120. <https://doi.org/10.1002/embr.201338041>

Uemoto, Y., Kikuchi, T., Nakano, H., Sato, S., Shibata, T., Kadowaki, H., ... Suzuki, K. (2012). Effects of porcine leptin receptor gene polymorphisms on backfat

474 thickness, fat area ratios by image analysis, and serum leptin concentrations in a
475 Duroc purebred population. *Animal Science Journal*, 83(5), 375–385.
476 <https://doi.org/10.1111/j.1740-0929.2011.00963.x>

477 Wood, J. D., Brown, S. N., Nute, G. R., Whittington, F. M., Perry, A. M., Johnson, S.
478 P., & Enser, M. (1996). Effects of breed, feed level and conditioning time on the
479 tenderness of pork. *Meat Science*, 44(1–2), 105–112.
480 [https://doi.org/10.1016/S0309-1740\(96\)00044-7](https://doi.org/10.1016/S0309-1740(96)00044-7)

481 Xia, D., Zhang, D. N., Gao, S. T., Chen, L., Li, N., Zheng, F. P., & Liu, Y. (2017).
482 Categorization of Chinese Dry-Cured Ham Based on Three Sticks' Method by
483 Multiple Sensory Technologies. *Journal of Food Quality*, 2017.
484 <https://doi.org/10.1155/2017/1701756>

485 Yim, D. G., Hong, D. Il, & Chung, K. Y. (2016). Quality characteristics of dry-cured
486 ham made from two different three-way crossbred pigs. *Asian-Australasian*
487 *Journal of Animal Sciences*, 29(2), 257–262. <https://doi.org/10.5713/ajas.15.0189>

488

489 **Table 1.** Means ($\pm$ SE) for ham production traits by *LEPR* and *FADS2* genotype.

Trait	<i>LEPR</i> genotype			<i>FADS2</i> genotype		
	TT	CT	CC	AA	AG	GG
No. of hams	49	117	98	11	98	155
Slaughter age, d	208.8 $\pm$ 0.4 ^a	207.6 $\pm$ 0.4 ^b	208.4 $\pm$ 0.4 ^{ab}	207.9 $\pm$ 0.8	208.4 $\pm$ 0.3	208.5 $\pm$ 0.2
Carcass weight, kg	97.2 $\pm$ 1.3	94.6 $\pm$ 1.1	95.5 $\pm$ 1.1	91.2 $\pm$ 2.4 ^b	97.8 $\pm$ 0.9 ^a	98.3 $\pm$ 0.7 ^a
Carcass backfat thickness, mm	32.1 $\pm$ 0.8 ^a	29.4 $\pm$ 0.7 ^b	28.9 $\pm$ 0.7 ^b	28.8 $\pm$ 1.6	30.9 $\pm$ 0.6	30.7 $\pm$ 0.5
Green ham weight, kg	12.1 $\pm$ 0.2	12.0 $\pm$ 0.1	12.2 $\pm$ 0.1	11.7 $\pm$ 0.3 ^b	12.2 $\pm$ 0.1 ^{ab}	12.4 $\pm$ 0.1 ^a
Cured ham weight, kg	7.9 $\pm$ 0.1	7.8 $\pm$ 0.1	8.0 $\pm$ 0.1	7.5 $\pm$ 0.2 ^b	8.0 $\pm$ 0.1 ^{ab}	8.2 $\pm$ 0.1 ^a
Manufacturing time, d	733.7 $\pm$ 3.5	736.0 $\pm$ 3.0	738.8 $\pm$ 3.1	733.6 $\pm$ 6.6 ^{ab}	733.2 $\pm$ 2.3 ^b	741.7 $\pm$ 1.9 ^a
Weight loss, %	34.7 $\pm$ 0.4	35.7 $\pm$ 0.3	35.6 $\pm$ 0.4	36.8 $\pm$ 0.7 ^a	34.6 $\pm$ 0.3 ^b	34.6 $\pm$ 0.2 ^b
Weight loss rate, kg/year	2.09 $\pm$ 0.03 ^b	2.16 $\pm$ 0.03 ^{ab}	2.20 $\pm$ 0.03 ^a	2.16 $\pm$ 0.06	2.14 $\pm$ 0.02	2.15 $\pm$ 0.02

490 ^{a,b} Within marker and trait, means with different superscripts differ significantly (P<0.05).

491

492

493

494
495

Table 2. Means ($\pm$ SE) for intramuscular fat content and fatty acid composition in the *biceps femoris* muscle by *LEPR* and *FADS2* genotype.

Trait ^A	<i>LEPR</i> genotype			<i>FADS2</i> genotype		
	TT	CT	CC	AA	AG	GG
No. of hams	55	151	126	17	135	180
DM, %	51.9 $\pm$ 0.5 ^a	50.8 $\pm$ 0.3 ^{ab}	50.4 $\pm$ 0.4 ^b	51.6 $\pm$ 0.8	50.8 $\pm$ 0.3	50.6 $\pm$ 0.2
IMF, % DM	16.9 $\pm$ 0.5 ^a	14.7 $\pm$ 0.4 ^b	14.6 $\pm$ 0.4 ^b	15.1 $\pm$ 0.9	15.5 $\pm$ 0.3	15.6 $\pm$ 0.3
C14:0, % FA	1.53 $\pm$ 0.02	1.52 $\pm$ 0.02	1.52 $\pm$ 0.02	1.58 $\pm$ 0.04	1.50 $\pm$ 0.01	1.50 $\pm$ 0.01
C16:0, % FA	24.66 $\pm$ 0.30 ^a	23.94 $\pm$ 0.25 ^{ab}	23.51 $\pm$ 0.26 ^b	23.99 $\pm$ 0.55	24.24 $\pm$ 0.20	23.88 $\pm$ 0.16
C18:0, % FA	11.08 $\pm$ 0.23 ^a	10.66 $\pm$ 0.20 ^{ab}	10.28 $\pm$ 0.20 ^b	10.54 $\pm$ 0.43	10.91 $\pm$ 0.16	10.58 $\pm$ 0.13
C20:0, % FA	0.18 $\pm$ 0.01 ^b	0.20 $\pm$ 0.01 ^a	0.19 $\pm$ 0.01 ^{ab}	0.18 $\pm$ 0.01	0.20 $\pm$ 0.00	0.20 $\pm$ 0.00
SFA, % FA	37.46 $\pm$ 0.52 ^a	36.33 $\pm$ 0.44 ^{ab}	35.50 $\pm$ 0.44 ^b	36.29 $\pm$ 0.95	36.84 $\pm$ 0.35	36.16 $\pm$ 0.28
C16:1n-9, % FA	3.60 $\pm$ 0.06	3.67 $\pm$ 0.05	3.77 $\pm$ 0.05	3.85 $\pm$ 0.12	3.59 $\pm$ 0.04	3.60 $\pm$ 0.03
C18:1n-9, % FA	44.45 $\pm$ 0.36	44.57 $\pm$ 0.31	44.83 $\pm$ 0.31	44.49 $\pm$ 0.67	44.49 $\pm$ 0.25	44.87 $\pm$ 0.19
C18:1n-7, % FA	4.36 $\pm$ 0.05 ^b	4.44 $\pm$ 0.05 ^{ab}	4.55 $\pm$ 0.05 ^a	4.49 $\pm$ 0.10	4.41 $\pm$ 0.04	4.46 $\pm$ 0.03
C20:1n-9, % FA	0.81 $\pm$ 0.01	0.80 $\pm$ 0.01	0.80 $\pm$ 0.01	0.79 $\pm$ 0.03	0.80 $\pm$ 0.01	0.82 $\pm$ 0.01
MUFA, % FA	53.22 $\pm$ 0.43	53.49 $\pm$ 0.37	53.95 $\pm$ 0.37	53.62 $\pm$ 0.80	53.30 $\pm$ 0.29	53.74 $\pm$ 0.23
C18:2n-6, % FA	7.43 $\pm$ 0.18 ^b	8.06 $\pm$ 0.15 ^a	8.31 $\pm$ 0.15 ^a	7.93 $\pm$ 0.33	7.81 $\pm$ 0.12	8.07 $\pm$ 0.10
C18:3n-3, % FA	0.41 $\pm$ 0.01 ^b	0.44 $\pm$ 0.01 ^a	0.44 $\pm$ 0.01 ^a	0.42 $\pm$ 0.02	0.42 $\pm$ 0.01	0.43 $\pm$ 0.00
C20:2n-6, % FA	0.36 $\pm$ 0.01 ^b	0.38 $\pm$ 0.01 ^a	0.39 $\pm$ 0.01 ^a	0.37 $\pm$ 0.01	0.38 $\pm$ 0.01	0.39 $\pm$ 0.00
C20:4n-6, % FA	1.12 $\pm$ 0.06 ^b	1.30 $\pm$ 0.05 ^a	1.41 $\pm$ 0.05 ^a	1.37 $\pm$ 0.12	1.26 $\pm$ 0.04	1.21 $\pm$ 0.03
PUFA, % FA	9.32 $\pm$ 0.25 ^b	10.18 $\pm$ 0.21 ^a	10.56 $\pm$ 0.21 ^a	10.09 $\pm$ 0.46	9.87 $\pm$ 0.17	10.11 $\pm$ 0.13

496
497
498
499

^A DM: dry matter; IMF: intramuscular fat content (in % DM); SFA: saturated fatty acids (C14:0+C16:0+C18:0+C20:0); MUFA: monounsaturated fatty acids (C16:1n-9+C18:1n-9+ C18:1n-7+ C20:1n-9); PUFA: polyunsaturated fatty acids (C18:2n-6 + C18:3n-3 + C20:2n-6 + C20:4n-6).
^{a,b} Within marker and trait, means with different superscripts differ significantly (P<0.05).

500 **Table 3.** Means ($\pm$ SE) for fatty acid ratios in the *biceps femoris* muscle by *LEPR* and *FADS2* genotype.
501

Ratio ^A	<i>LEPR</i> genotype			<i>FADS2</i> genotype		
	TT	CT	CC	AA	AG	GG
MUFA/SFA	1.45 $\pm$ 0.04 ^b	1.50 $\pm$ 0.03 ^{ab}	1.55 $\pm$ 0.03 ^b	1.50 $\pm$ 0.07	1.48 $\pm$ 0.02	1.52 $\pm$ 0.02
MUFA/PUFA	5.83 $\pm$ 0.14 ^a	5.35 $\pm$ 0.12 ^b	5.25 $\pm$ 0.12 ^b	5.47 $\pm$ 0.26	5.51 $\pm$ 0.10	5.44 $\pm$ 0.08
SFA/PUFA	4.13 $\pm$ 0.13 ^a	3.65 $\pm$ 0.11 ^b	3.46 $\pm$ 0.11 ^b	3.72 $\pm$ 0.24	3.83 $\pm$ 0.09	3.69 $\pm$ 0.07
C20:4n-6/C18:2n-6 (x10)	1.50 $\pm$ 0.05 ^b	1.59 $\pm$ 0.04 ^{ab}	1.68 $\pm$ 0.04 ^a	1.69 $\pm$ 0.09 ^{ab}	1.59 $\pm$ 0.03 ^a	1.48 $\pm$ 0.03 ^b
C20:2n-6/C18:2n-6 (x100)	4.89 $\pm$ 0.05	4.77 $\pm$ 0.05	4.76 $\pm$ 0.05	4.71 $\pm$ 0.10	4.84 $\pm$ 0.04	4.87 $\pm$ 0.03
C20:4n-6/C20:2n-6	3.11 $\pm$ 0.12 ^b	3.38 $\pm$ 0.10 ^{ab}	3.57 $\pm$ 0.11 ^a	3.68 $\pm$ 0.23 ^a	3.31 $\pm$ 0.08 ^a	3.07 $\pm$ 0.07 ^b

502
503 ^A See Table 2 for SFA, MUFA and PUFA abbreviations.
504 ^{a,b} Within marker and trait, means with different superscripts differ significantly (P<0.05).

505 **Table 4.** Means ($\pm$ SE) for instrumental color in muscle by *LEPR* and *FADS2* genotypes.

Trait ^A	<i>LEPR</i> genotype			<i>FADS2</i> genotype		
	TT	CT	CC	AA	AG	GG
No. of hams	34	93	63	5	71	114
L*	46.97 $\pm$ 0.44 ^a	46.03 $\pm$ 0.43 ^{ab}	45.77 $\pm$ 0.44 ^b	46.56 $\pm$ 1.00	46.23 $\pm$ 0.28	45.98 $\pm$ 0.22
a*	11.22 $\pm$ 0.21	11.02 $\pm$ 0.21	11.04 $\pm$ 0.21	11.47 $\pm$ 0.48	10.97 $\pm$ 0.13	10.84 $\pm$ 0.10
b*	5.70 $\pm$ 0.24 ^a	5.08 $\pm$ 0.23 ^b	4.76 $\pm$ 0.24 ^b	5.06 $\pm$ 0.54	5.33 $\pm$ 0.15	5.15 $\pm$ 0.12
h*	24.94 $\pm$ 0.93 ^a	22.01 $\pm$ 0.91 ^b	20.89 $\pm$ 0.93 ^b	21.28 $\pm$ 2.12	23.60 $\pm$ 0.59	22.96 $\pm$ 0.46
C*	12.99 $\pm$ 0.25	12.55 $\pm$ 0.25	12.38 $\pm$ 0.25	12.86 $\pm$ 0.57	12.63 $\pm$ 0.16	12.43 $\pm$ 0.12

506
507 ^A CIELAB coordinates: L* (lightness), a* (redness), b* (yellowness), h* (hue angle) and C* (chroma).

508
509 ^{a,b} Within marker and trait, means with different superscripts differ significantly (P<0.05).

Table 5. Means ($\pm$ SE) for instrumental color traits by muscle and subcutaneous fat.

Muscle ^A	Trait ^B				
	L*	a*	b*	h*	C*
BFA	51.36 $\pm$ 0.42 ^a	11.06 $\pm$ 0.21 ^c	7.04 $\pm$ 0.23 ^b	32.04 $\pm$ 0.93 ^b	13.25 $\pm$ 0.24 ^b
BFR	47.76 $\pm$ 0.41 ^b	11.63 $\pm$ 0.20 ^{ab}	6.15 $\pm$ 0.22 ^c	27.44 $\pm$ 0.91 ^c	13.27 $\pm$ 0.23 ^b
ST	52.35 $\pm$ 0.45 ^a	11.32 $\pm$ 0.21 ^{bc}	9.52 $\pm$ 0.23 ^a	40.13 $\pm$ 0.93 ^a	14.92 $\pm$ 0.24 ^a
SMA	44.58 $\pm$ 0.42 ^c	12.07 $\pm$ 0.21 ^a	5.43 $\pm$ 0.23 ^d	23.35 $\pm$ 0.93 ^d	13.39 $\pm$ 0.24 ^b
SMR	39.68 $\pm$ 0.42 ^e	9.98 $\pm$ 0.21 ^e	0.96 $\pm$ 0.23 ^f	3.45 $\pm$ 0.93 ^f	10.15 $\pm$ 0.24 ^d
RF	41.79 $\pm$ 0.42 ^d	10.51 $\pm$ 0.21 ^d	1.98 $\pm$ 0.23 ^e	9.26 $\pm$ 0.93 ^e	10.87 $\pm$ 0.24 ^c
SF	72.73 $\pm$ 0.18	2.71 $\pm$ 0.07	11.10 $\pm$ 0.12	76.10 $\pm$ 0.40	11.49 $\pm$ 0.11
r	0.18 ^g	0.17 ^g	0.15 ^g	0.12 ^g	0.20 ^g
b ^M _{weight}	0.67 $\pm$ 0.17 ^g	-0.05 $\pm$ 0.08	0.30 $\pm$ 0.09 ^g	1.71 $\pm$ 0.35 ^g	0.07 $\pm$ 0.09
b ^{SF} _{weight}	0.16 $\pm$ 0.17	0.11 $\pm$ 0.07	-0.28 $\pm$ 0.11 ^g	-0.79 $\pm$ 0.37 ^g	-0.24 $\pm$ 0.11 ^g

^A See Figure 1 for abbreviations; r: intraclass correlation adjusted for muscle and genotype; b^M_{weight} (b^{SF}_{weight}): linear regression coefficient of color traits in muscle (subcutaneous fat) on green ham weight (units/kg).

^B CIELAB coordinates: L (lightness), a* (redness), b* (yellowness), h* (hue angle) and C* (chroma).

^{a-f} Within muscle and trait, means with different superscripts differ significantly (P<0.05).

^g P<0.05

Figure 1. Dry-cured ham section (A) before dissection (ST: *m. semitendinosus*; BFA: *m. biceps femoris caudal*; BFR: *m. biceps femoris cranial*; SMA: *m. semimembranosus caudal*; SMR: *m. semimembranosus cranial*; RF: *m. rectus femoris*; SF: subcutaneous fat) and (B) by *LEPR* genotype.

Figure 2. Intramuscular fat content (% fresh matter) in the *biceps femoris* muscle by *LEPR* (n, TT=55; CT=151; CC=126) and *FADS2* (n, AA=17; AG=135; GG=180) genotype. Columns with different superscripts within marker differ significantly ($P<0.05$). Intramuscular fat content was higher in *LEPR*-TT than in *LEPR*-C– muscles ($8.9\pm0.3\%$ vs $7.5\pm0.2\%$, $P<0.01$).

Figure 1

571

572 **Figure 2**

573

574