

Effect of *SCD* and *LEPR* gene polymorphisms on fat content and composition of Duroc premium pork

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Abstract –The effects of the stearoyl-CoA desaturase (*SCD*; AY487830:g.2228T>C) and leptin receptor (*LEPR*; NM_001024587:g.1987C>T) polymorphisms on fat content and fatty acid (FA) composition were investigated throughout fattening. Samples of *Longissimus thoracis* (LT) and subcutaneous fat (SF) from 214 Duroc barrows were collected from 160 days to 210 days using a longitudinal design. The results obtained indicate that the positive effect of the T allele at the *SCD* gene on monounsaturated FA and of the T allele at the *LEPR* gene on saturated FA are maintained throughout the finishing period, both in LT and SF. There is limited evidence of genotype by age interaction. These findings confirmed that the combined selection for the *SCD* T and *LEPR* C alleles can be useful to increase MUFA content regardless of the age at slaughter.

Key Words – age, fatty acids, meat quality.

I. INTRODUCTION

Fat content and its fatty acid profile affect meat quality; hence genes involved in lipid metabolism are an important target of research in pig breeding. The leptin receptor (*LEPR*) and the stearoyl-CoA desaturase (*SCD*) are two of these genes. *LEPR*, as a mediator of the satiety effect of the leptin hormone, influences overall fatness [1], while *SCD*, the rate-limiting enzyme required for the biosynthesis of monounsaturated (MUFA) from saturated (SFA) fatty acids (FA), affects fatty acid composition [2]. In pigs, an exonic polymorphism in the *LEPR* gene has been reported to be strongly associated with fatness [3,4]. Similarly, a polymorphism in the promoter region of the *SCD* gene affecting MUFA in both intramuscular (IMF) and subcutaneous fat (SF) has been reported [5,6]. Previous studies have shown that the fatty acid profile of muscle and SF changes with age [7,8]. Therefore the objective of this study was to assess whether the effects of the *SCD* and *LEPR*

polymorphisms on fat content and composition is modified throughout the fattening period.

II. MATERIALS AND METHODS

Experimental procedures and laboratory analyses:

All experimental procedures were approved by the Ethics Committee for Animal Experimentation of the University of Lleida. Samples from 214 pigs of the purebred Duroc barrows referred in Bosch *et al.* [7] were used for this experiment. The animals were raised up to slaughter in three separate batches in a commercial farm and were given *ad libitum* access to feed. A pelleted growing and finishing diet were given from 110 to 160 days and from 160 to 220 days, respectively. Pigs were subjected to repeated sampling throughout the finishing period. A biopsy of m. *Longissimus thoracis* (LT) and of SF was taken in 191 pigs at around 185 days (183.0, SD 4.3). Additionally, samples of both tissues were taken at 160 days (158.0, SD 6.9; n=81) and at 210 days (207.9, SD 3.0; n=60). At each age, the live body weight was measured and the backfat thickness (BT) and loin thickness at 5 cm of the midline between the third and fourth last ribs were ultrasonically recorded. Biopsies were taken 5 cm deep at the same location where BT was measured and they were extracted using 8-mm cannula inserted into spring-loaded biopsy device (PPB-U Biotech, Nitra, Slovakia) [9]. All the necessary measures were taken to prevent animal discomfort during and after the process [10]. Muscle and fat samples were trimmed from skin and separately frozen in liquid nitrogen until analysis. The fat content and FA composition of each sample were determined in duplicate as described in Bosch *et al.* [11]. The IMF content was expressed as percentage of dry matter and the saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) FA content

as their percentage relative to total FA. All pigs in the study were genotyped for the *SCD* AY487830:g.2228T>C [5] and *LEPR* NM_001024587:g.1987C>T [4] single nucleotide polymorphisms (SNP), which were used as tag SNPs for capturing the variance associated to *SCD* and *LEPR* genes, respectively.

Statistical analyses: The effect of the *SCD* and *LEPR* genotypes by age were estimated using a linear mixed model which included the batch (3 levels), the age at measurement (160, 185, and 210 days), the *SCD* genotype (TT, CT and CC), the *LEPR* genotype (CC, CT and TT) and the interaction of each genotype by age at measurement as fixed effects and the pig as a random effect. The effects of *SCD* and *LEPR* were tested with an F-test while multiple pairwise comparisons between genotypes at a given gene were done using the Tukey test. All the analyses were performed using the statistical package JMP 8 (SAS Institute Inc., Cary, NC).

III. RESULTS AND DISCUSSION

The average effects of the *SCD* and *LEPR* genotypes on body weight, BT, loin thickness, IMF and FA composition in loin and SF during the finishing period are shown in Tables 1 and 2, respectively. The interaction of genotype with age was significant ($P<0.05$) for BT and PUFA, for *SCD*, and for SFA in LT, for *LEPR*. However, they only showed minor effects, which were limited to small changes in magnitude. The effect of both genotypes was consistent across tissues and throughout the finishing period. The modifications of the fatty acid profile throughout fattening, in LT and SF, followed the same pattern reported in previous works [7], with MUFA increasing and PUFA decreasing.

The *SCD* genotype did not affect production traits and fat content, but pigs carrying the T allele at *SCD* had increased MUFA in both LT and SF, in line with previous reports [5,6]. The T allele at *LEPR* increased BT as well as SFA content in muscle and SF.

Table 1. Least square means ($\pm$ SE) for production traits, intramuscular fat content (IMF) and fatty acid composition in m. *Longissimus thoracis* and subcutaneous fat by *SCD* genotype during the finishing period (from 160 to 210 days of age)^A

	TT	CT	CC
Body weight, kg	111.2 $\pm$ 1.7	113.0 $\pm$ 0.9	115.3 $\pm$ 1.2
BT, mm	19.2 $\pm$ 0.6	20.3 $\pm$ 0.3	19.8 $\pm$ 0.4
Loin thickness, mm	43.2 $\pm$ 0.6	44.1 $\pm$ 0.3	44.8 $\pm$ 0.4
m. <i>Longissimus thoracis</i>			
IMF, % DM	16.8 $\pm$ 0.9	17.4 $\pm$ 0.5	16.8 $\pm$ 0.7
SFA, mg/g FA	404.6 $\pm$ 3.7 ^b	415.2 $\pm$ 2.0 ^a	421.3 $\pm$ 2.7 ^a
MUFA, mg/g FA	452.0 $\pm$ 3.5 ^a	443.2 $\pm$ 1.9 ^a	428.9 $\pm$ 2.6 ^b
PUFA, mg/g FA	142.9 $\pm$ 4.1	141.5 $\pm$ 2.3	149.9 $\pm$ 3.0
MUFA/SFA	1.12 $\pm$ 0.01 ^a	1.07 $\pm$ 0.01 ^b	1.02 $\pm$ 0.01 ^c
Subcutaneous fat			
SFA, mg/g FA	406.0 $\pm$ 4.2 ^b	408.2 $\pm$ 2.3 ^b	424.3 $\pm$ 3.2 ^a
MUFA, mg/g FA	414.3 $\pm$ 3.1 ^a	410.4 $\pm$ 1.7 ^a	400.7 $\pm$ 2.3 ^b
PUFA, mg/g FA	181.5 $\pm$ 3.5	181.1 $\pm$ 1.9	175.1 $\pm$ 2.6
MUFA/SFA	1.02 $\pm$ 0.02 ^a	1.01 $\pm$ 0.01 ^a	0.95 $\pm$ 0.01 ^b

^ASFA: C14:0+C16:0+C18:0+C20:0; MUFA: C16:1n-9+C18:1+C20:1n-9; PUFA: C18:2n-6+C18:3n-3+C20:2n-6+C20:4n-6; C18:1:C18:1n-9+C18:1n-7; ^{a,b,c} Within row, means with different superscripts differ significantly ($P<0.05$).

It has been suggested that the effects of *LEPR* can be an indirect consequence of increased feed intake [4], since the leptin receptor mediates the satiety effect of leptin [1, 12]. To test whether the effect of *LEPR* was mainly due to increased overall fatness, the difference between *LEPR* genotypes for SFA was adjusted for IMF, in LT, and for BT, in SF. Once adjusted, the effect of *LEPR* on SFA decreased in LT ($P<0.05$) and vanished in SF ($P>0.05$; data not shown). In general, the effect of both polymorphisms on FA composition was greater in LT than in SF and for *SCD* than *LEPR*.

Table 2. Least square means ($\pm$ SE) for production traits, intramuscular fat content (IMF) and fatty acid composition in m. *Longissimus thoracis* and subcutaneous fat by *LEPR* genotype during the finishing period (from 160 to 210 days of age)^A

	CC	CT	TT
Body weight, kg	110.8 $\pm$ 1.4	113.5 $\pm$ 1.0	115.1 $\pm$ 1.4
BT, mm	19.4 $\pm$ 0.5 ^b	19.0 $\pm$ 0.3 ^b	20.9 $\pm$ 0.5 ^a
Loin thickness, mm	43.7 $\pm$ 0.5	44.8 $\pm$ 0.3	43.6 $\pm$ 0.5
m. <i>Longissimus thoracis</i>			
IMF, % DM	16.2 $\pm$ 0.7	16.5 $\pm$ 0.5	18.3 $\pm$ 0.7
SFA, mg/g FA	409.8 $\pm$ 3.0 ^b	407.4 $\pm$ 2.1 ^b	423.9 $\pm$ 3.0 ^a
MUFA, mg/g FA	445.4 $\pm$ 2.9 ^a	442.8 $\pm$ 2.0 ^{ab}	435.9 $\pm$ 2.9 ^b
PUFA, mg/g FA	144.9 $\pm$ 3.4 ^{ab}	149.8 $\pm$ 2.3 ^a	139.6 $\pm$ 3.4 ^b
MUFA/SFA	1.09 $\pm$ 0.01 ^a	1.09 $\pm$ 0.01 ^a	1.03 $\pm$ 0.01 ^b
Subcutaneous fat			
SFA, mg/g FA	410.6 $\pm$ 3.5 ^{ab}	407.4 $\pm$ 2.4 ^b	420.6 $\pm$ 3.5 ^a
MUFA, mg/g FA	410.5 $\pm$ 2.6	410.9 $\pm$ 1.8	403.9 $\pm$ 2.6
PUFA, mg/g FA	179.7 $\pm$ 2.9	181.6 $\pm$ 2.0	176.3 $\pm$ 2.9
MUFA/SFA	1.01 $\pm$ 0.01 ^{ab}	1.02 $\pm$ 0.01 ^a	0.96 $\pm$ 0.01 ^b

^ASee footnote in Table 1.; ^{a,b,c} Within row, means with different superscripts differ significantly ($P < 0.05$).

IV. CONCLUSION

Results at hand confirm the positive effect of the T allele at the *SCD* gene on MUFA and provide additional evidence on the positive effect of the T allele at the *LEPR* gene on SFA, both in LT and SF. Contrarily to *SCD*, the analyses indicate that the effect of *LEPR*, particularly in SF, is due to increased overall fatness. Altogether, the results have shown a consistent effect of the *SCD* and *LEPR* polymorphisms throughout the whole finishing period, suggesting that the combined use of both markers can be useful to increase MUFA content regardless of the age at slaughter.

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REFERENCES

- Houseknecht, K. L., Baile, C. A., Matteri, R. L., & Spurlock, M. E. (1998). The biology of leptin: a review. *Journal of Animal Science*, 76(5), 1405–1420.
- Ntambi, J., & Miyazaki, M. (2004). Regulation of stearoyl-CoA desaturases and role in metabolism. *Progress in Lipid Research*, 43(2), 91–104.
- Galve, A., Burgos, C., Silió, L., Varona, L., Rodríguez, C., Ovilo, C., & López-Buesa, P. (2012). The effects of leptin receptor (*LEPR*) and melanocortin-4 receptor (*MC4R*) polymorphisms on fat content, fat distribution and fat composition in a Duroc $\times$ Landrace/Large White cross. *Livestock Science*, 145(1-3), 145–152.
- Óvilo, C., Fernández, A., Noguera, J. L., Barragán, C., Letón, R., Rodríguez, C., Mercadé, A., Alves, E., Folch, J. M., Varona, L., Toro, M. (2005). Fine mapping of porcine chromosome 6 QTL and *LEPR* effects on body composition in multiple generations of an Iberian by Landrace intercross. *Genetical Research*, 85(1), 57–67.
- 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), e86177.
- Henriquez-Rodriguez, 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.
- 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), 358–63.
- Lo Fiego, D. Pietro, Macchioni, P., Minelli, G., & Santoro, P. (2010). Lipid composition of covering and intramuscular fat in pigs at different slaughter age. *Italian Journal of Animal Science*, 9(2), 39.
- Oksbjerg, N., Henckel, P., Andersen, S., Pedersen, B., & Nielsen, B. (2004). Genetic variation of in vivo muscle glycerol, glycogen, and pigment in Danish purebred pigs. *Acta Agriculturae Scandinavica, Section A - Animal Science*, 54(4), 187–192.
- Bosch, L., Tor, M., Villalba, D., Puigvert, X., & Estany, J. (2003). Determinación del contenido y composición de la grasa intramuscular a partir de una biopsia en porcino. *ITEA, Volumen Extra*, 24, 193–195.
- 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), 432–7.

12. Barb, C., Hausman, G., & Houseknecht, K. (2001).
Biology of leptin in the pig. *Domestic Animal
Endocrinology*, 21(4), 297–317.