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Skeletal muscle expression analysis of fat metabolism genes in pig

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ABSTRACT - Fat content and backfat thickness are important polygenic parameters influencing meat quality and carcass traits in pigs. Up to now, there is a lack of knowledge on the expression level of the genes encoding for enzymes involved in fatty acid metabolism in porcine skeletal muscle. In the present study we analysed, by quantitative real time PCR, the expression of three genes, acetyl-CoA carboxylase (*ACACA*), ATP citrate lyase (*ACL*) and fatty acid synthase (*FASN*) in skeletal muscle tissue samples of Italian Large White and Italian Duroc pigs with divergent breeding values for backfat thickness or visible intramuscular fat. Significant differences of the expression level for *ACACA* gene (P=0.04) and for *ACL* gene (P=0.02) were observed between the two breeds, comparing the samples selected for backfat thickness trait. The expression analysis of *FASN* gene in the samples with different genotype at the SNP c.265*C>T* showed that the TT genotype presented the lowest values in both breeds. The differences observed between breeds should be further considered to investigate the putative involvement of these genes on fat deposition traits.

Key words: Pig, Meat quality, Fat deposition traits, Gene expression.

Introduction - Fat content and backfat thickness are parameters influencing meat and carcass quality traits. In pig, many quantitative trait loci regions are known for fat traits and putative candidate genes for fatty acid metabolism are reported (www.animalgenome.org/QTLdb/pig.html). To our knowledge in literature there are few studies on expression level of fat genes in porcine skeletal muscle tissue. The aim of this study was to analyse, by quantitative real time PCR (qRT-PCR), the transcription level of the genes selected for their involvement in fat metabolism: acetyl-CoA carboxylase (*ACACA*), ATP citrate lyase (*ACL*), and fatty acid synthase (*FASN*). These genes were mapped on porcine chromosome 12: *ACACA* on 12p13-p12 (Calvo *et al*., 2000), *ACL* was localised by comparative mapping according to syntheny conservation proposed by Goureau *et al*., (1996); *FASN* was mapped on 12p1.5 and within the gene a SNP (c.265*C>T*) was reported (Munoz *et al*., 2003). In this study samples of Italian Large White (ILW) and Italian Duroc (ID) pigs with divergent breeding values for backfat thickness (BFT) or visible intramuscular fat (VIF) traits were used. Comparisons of transcription levels were performed between breeds and between divergent groups for each trait.

Material and methods - Skeletal muscle samples were collected from sib tested ILW and ID pigs. We studied the three genes in a group of ILW pigs divergent for BFT and in two groups of ID extreme for BFT and VIF traits. For each trait, 12 pigs were selected from a larger population, 6 with the most positive and 6 with the most negative values. The comparison between different genotypes for the *FASN* c.265*C>T* SNP was performed including 7 additional ILW samples. DNA was obtained from lyophilised blood using a standard protocol. Total RNA was extracted from deep frozen skeletal muscle tissues using Tri-Reagent (SIGMA-Aldrich) and retrotranscribed according to the manufacturer's instructions of Improm-II™ Reverse Transcription System and Oligo-dT primers (Promega Corporation). For all genes

qRT-PCR was carried out using Light Cycler Instrument® (Roche) and PCR reaction mixtures were prepared with SYBR® Premix Ex Taq™ (Takara Bio INC). The calculated copy numbers for each target gene were normalized against the geometric average copy number of cDNA of two stably expressed reference genes, beta-2-microglobulin (*B2M*) and polymerase (RNA) II (DNA directed) polypeptide A, 220kDa (*POLR2A*) according to Vandesompele *et al*. (2002) and GENORM (http://medgen.ugent.be/~jvdesomp/genorm/). The *FASN* SNP c.265*C>T* was analysed as described in Munoz *et al*. (2003). Comparisons were done between divergent pigs for each trait, between the two breeds and, only for *FASN* gene, between different genotypes. T test was calculated by SAS software. Radiation hybrid mapping of *ACL* gene was performed using the INRA-Minnesota 7000 rads radiation hybrid panel (ImpRH), consisting of 118 hamster-porcine hybrid cell lines (Yerle *et al*., 1998).

Results and conclusions - *ACACA* codes for a biotin-dependent pivotal enzyme in the synthesis of fatty acids catalyzing the carboxylation of acetyl-CoA to malonyl-CoA, the rate-limiting step in fatty acid synthesis. The analysis of the expression level of *ACACA* gene showed a significant difference between breeds in the samples analysed for BFT trait (P=0.04; Figure 1A). The comparisons between extreme groups within each breed are not significant.

ACL is the primary enzyme responsible for the synthesis of cytosolic acetyl-CoA in many tissues. In the present study we report the RH mapping of the *ACL* gene on Ssc12 near the marker *SW943* (LOD=11.49) confirming the human-pig comparative mapping of this chromosome (Goureau *et al*., 1996). A significant difference in the expression level between the ILW and ID breeds was found (P=0.02; Figure 1B). Moreover, a difference (P<0.10) was observed in the ID pigs between the divergent groups for VIF trait. The comparison between divergent ILW pigs is not significant.

FASN gene encodes for a protein whose main function is to catalyze the synthesis of palmitate from acetyl-CoA and malonyl-CoA, in the presence of NADPH, into long-chain saturated fatty acids. This gene plays an important role in the energy metabolism. For *FASN* gene a difference (P<0.10) was observed between breeds for BFT trait with the expression level higher in ID breed than in the ILW pigs. For this gene, the transcription level was also studied in samples with different genotypes for the SNP c.265*C>T*. The results showed that for both breeds the expression level varied according to the genotype and the TT samples presented the lowest values. Comparison between genotypes was performed only in the ILW samples, because in the ID breed the *T* allele is rare (0.04). The difference between the expression level of the ILW pigs with CC+CT *vs*. TT genotype was close to significance (P=0.06) (Figure 2).

Figure 1. Average relative expression level for ACACA gene (A) and for ACL gene (B): comparison between 12 ILW and 12 ID pigs extreme for BFT within breed.

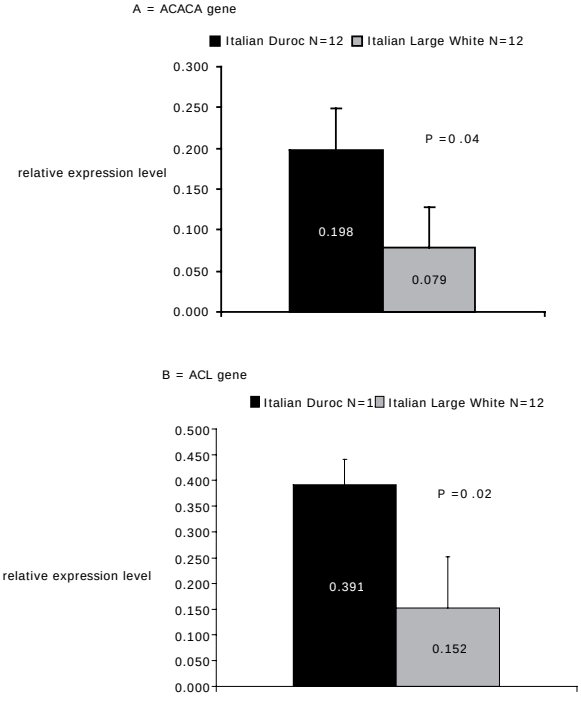

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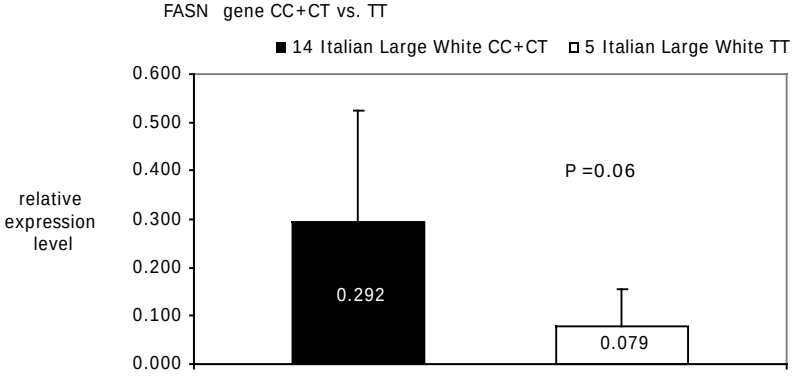
Figure 2. Average relative expression level of FASN gene in 19 Italian Large White: comparison between different genotypes and P value of differences. The only two CC genotyped pigs were grouped to the heterozygous samples for the statistical analysis.



In conclusion, the significant difference of level of *ACACA* and *ACL* transcripts, observed between ID and ILW pigs, suggests a putative role of these genes in the turnover of acetyl-CoA, a rate-limiting molecule of fatty acid metabolism. The obtained results, showing a highest expression level of these two genes in ID than in ILW, may suggest a different modulation among the two breeds of the enzyme activity in the fat-associated metabolic pathways and may reflect the higher capacity to fat deposition in skeletal muscle characteristic of ID compared to ILW.

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