

# Characterization of the transcriptome of *Longissimus dorsi* muscle in three closed populations of different Iberian pig strains

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## Summary

The aim of this study is to compare the differences in the loin muscle transcriptome of three different Iberian pig strains. A total of eighteen Iberian male pigs from Lampiño (L; 6), Torbiscal (T; 6) and Retinto (R; 6) were fed with a concentrate-based diet until they reached an average weight of 160 kg and 15 months old. At the slaughterhouse, productive parameters were measured and samples for meat quality analysis were collected. In addition, loin samples for the transcriptome analysis were also collected and immediately frozen in liquid nitrogen, and afterwards stored at -80°C. RNA was extracted from these samples and the pair-end libraries were created with the Novaseq 6000 sequence analyser (Illumina). The RNA-seq technique was performed with the libraries and the obtained data was analyzed with a bioinformatic pipeline (FastQC, TrimGalore, HTseq, Hisat2 and DESeq2). Differential expression analyses were carried out making three comparisons: Lampiño vs Torbiscal (LvsT), Lampiño vs Retinto (LvsR) and Torbiscal vs Retinto (TvsR). Threshold values of  $\log_2FC = |0.58|$ , p-value < 0.05 and q-value < 0.10 were set to consider a gene as differentially expressed (DEG). The largest difference between genotypes was found in the LvsR comparison with 676 DEG identified, 366 of them, upregulated and 310, downregulated in L strain. By contrast, the smallest difference was found in the TvsR comparison with 125 DEG identified, 90 of them, upregulated and 35, downregulated in T strain, and, in the LvsT comparison a total of 272 DEG were found, 188 of them, upregulated, and 88, downregulated in L. There were no DEGs in common in the three comparisons. Functional analysis were also performed with FatiGO and IPA software. As a result, DEGs from TvsR comparison are mainly involved in pathways related with Actine-Myosine function and with cell differentiation pathways. On the other hand, the DEGs observed in LvsR comparison were involved mainly in pathways related with lipid metabolism. Lastly, in LvsT comparison, DEGs were implicated in a wide variety of pathways, such as lipid metabolism, muscular function, cell differentiation and collagen metabolism. It should be noted that pathways related with collagen metabolism were only observed in comparison in which the L strain was present (LvsT and LvsR). Furthermore, significant differences among strains were found for several carcass and meat quality traits, in particular, Retinto had lower ham yield, saturated fatty acid content and higher slaughter weight, carcass weight, and reddish meat colour than the Lampiño, with intermediate values for the Torbiscal. These results are in agreement with the statement that in terms of gene expression, Retinto and Lampiño are the most distant strains, meanwhile Torbiscal is halfway between the other two, probably due to the fact that Torbiscal has common ancestors in Lampiño and Retinto strains.

*Keywords: Iberian Pig Strains, Muscle Transcriptome, Gene Expression,*