**Expression of genes involved in adipogenesis and lipid metabolism in subcutaneous adipose tissue and *longissimus muscle* in low-marbled Pirenaica beef cattle**

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***Supplementary Table S1*** *Oligonucleotide sequences and amplicon size of the housekeeping [Beta-actin] and target genes [acetyl Co-A carboxylase alpha (ACACA), CCAAT/enhancer binding protein alpha (CEBPA), fatty acid binding protein 4 (FABP4), fatty acid synthase (FASN), lipoprotein lipase (LPL), peroxisome proliferator activated receptor gamma (PPARG), sterol regulatory element binding transcription factor 1 (SREBF1), and wingless-type MMTV integration site family 10B (WNT10B)].*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Gene ID | Accession # | Gene | Primers1 | bp2 |
| Beta-actin | BC\_142413 | β-actin | F: CGC CAT GGA TGA TGA TAT TGC | 65 |
|  | R: AAG CGG CCT TGC ACA T |  |
| Acetyl-CoA carboxylase alpha | NM\_174224 | ACACA | F: AGG ATG GAG GCC ACG TGT T | 144 |
| R: CTA TTA CAC AGC CCG GGT CAA |  |
| CCAAT/enhancer binding protein alpha | NM\_176784 | CEBPA | F: TGG ACA AGA ACA GCA ACG AG | 130 |
| R: TTG TCA CTG GTC AGC TCC AG |  |
| Adipocyte-type fatty acid binding protein 4 | NM\_174314 | FABP4 | F: CAT CTT GCT GAA AGC TGC AC | 202 |
| R: ACC CCC ATT CAA ACT GAT GA |  |
| Fatty acid synthase | AF\_285607 | FASN | F: TGC AAC TCA ACG GGA ACT TAC A | 128 |
| R: AAG TGT CAC GCA CGC CTT G |  |
| Lipoprotein lipase | NM\_001075120 | LPL | F: TCA TCG TGG TGG ACT GGC T | 187 |
| R: TTC CTG CAA TAC CAG CAG CA |  |
| Peroxisome proliferator activated receptor gamma | NM\_181024 | PPARG | F: GTG AAG TTC AAC GCA CTG GA | 113 |
| R: ATG TCC TCA ATG GGC TTC AC |  |
| Sterol regulatory element binding transcription factor 1 | NM\_001113302 | SREBF1 | F: ACC GCT CTT CCA TCA ATG AC | 190 |
| R: TTC AGC GAT TTG CTT TTG TG |  |
| Wingless-type MMTV integration site family 10B | XM\_005206363 | WNT10B | F: AAT GCA AGT GCC ATG GTA CG | 139 |
| R: GAG TTG CGG TTG TGA GTA TCA ATG |  |

1 Primer direction (F – forward; R – reverse) and hybridization position on the sequence.

2 Amplicon size in base pairs (bp).

***Supplementary Table S2*** *Summary statistics for Cq values included in the analyses for acetyl Co-A carboxylase alpha (ACACA), CCAAT/enhancer binding protein alpha (CEBPA), fatty acid binding protein 4 (FABP4), fatty acid synthase (FASN), lipoprotein lipase (LPL), peroxisome proliferator activated receptor gamma (PPARG), sterol regulatory element binding transcription factor 1 (SREBF1), and wingless-type MMTV integration site family 10B (WNT10B).*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Tissue | n1 | Mean | SD | Min. | Max. |
| β-actin | LM2 | 477 | 22.77 | 4.54 | 13.66 | 34.00 |
|  | SC3 | 383 | 22.32 | 3.04 | 15.23 | 38.18 |
| ACACA | LM | 47 | 19.43 | 3.03 | 16.14 | 31.64 |
|  | SC | 48 | 20.24 | 1.98 | 16.57 | 24.79 |
| CEBPA | LM | 48 | 18.91 | 2.12 | 15.58 | 24.00 |
|  | SC | 48 | 23.41 | 4.13 | 15.64 | 31.14 |
| FABP4 | LM | 46 | 19.25 | 3.43 | 15.77 | 37.53 |
|  | SC | 48 | 16.23 | 1.97 | 14.02 | 23.16 |
| FASN | LM | 47 | 20.03 | 3.20 | 15.96 | 31.80 |
|  | SC | 48 | 22.66 | 2.40 | 19.28 | 29.27 |
| LPL | LM | 46 | 23.93 | 3.11 | 18.87 | 29.49 |
|  | SC | 47 | 21.44 | 2.93 | 18.23 | 34.74 |
| PPARG | LM | 46 | 29.14 | 2.67 | 20.14 | 32.39 |
|  | SC | 47 | 18.35 | 2.00 | 16.15 | 27.46 |
| *SREBF1* | LM | 46 | 19.70 | 3.59 | 15.58 | 30.05 |
|  | SC | 48 | 27.17 | 3.36 | 21.31 | 37.08 |
| WNT10B | LM | 47 | 19.80 | 4.72 | 14.88 | 34.30 |
|  | SC | 47 | 29.17 | 2.37 | 23.49 | 33.84 |

1 Each qPCR was run in triplicate (maximum n=48). -actin was run in all plates for each gene and replicate, thus yielding more values for this gene.

2 LM = *longissimus* muscle

3 SC = subcutaneous adipose tissue

***Supplementary Table S3*** *qPCR performance by gene: acetyl Co-A carboxylase alpha (ACACA), CCAAT/enhancer binding protein alpha (CEBPA), fatty acid binding protein 4 (FABP4), fatty acid synthase (FASN), lipoprotein lipase (LPL), peroxisome proliferator activated receptor gamma (PPARG), sterol regulatory element binding transcription factor 1 (SREBF1), and wingless-type MMTV integration site family 10B (WNT10B).*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Gene |  | Median Cq1 | Slope2 | (R2)3 | Efficiency4 |
| *β-*actin | LM5 | 22.32 | -3.360 | 0.978 | 1.984 |
|  | SC6 | 22.77 |  |  |  |
| ACACA | LM | 18.35 | -3.326 | 0.978 | 1.998 |
|  | SC | 29.14 |  |  |  |
| CEBPA | LM | 23.41 | -3.493 | 0.983 | 1.933 |
|  | SC | 18.91 |  |  |  |
| FABP4 | LM | 21.44 | -3.558 | 0.992 | 1.910 |
|  | SC | 23.93 |  |  |  |
| FASN | LM | 29.17 | -3.385 | 0.991 | 1.974 |
|  | SC | 19.80 |  |  |  |
| LPL | LM | 27.17 | -3.490 | 0989 | 1.934 |
|  | SC | 19.70 |  |  |  |
| PPARG | LM | 20.24 | -3.405 | 0.994 | 1.967 |
|  | SC | 19.43 |  |  |  |
| SREBF1 | LM | 16.23 | -3.3344 | 0.984 | 1.995 |
|  | SC | 19.25 |  |  |  |
| WNT10B | LM | 22.66 | -3.684 | 0.984 | 1.868 |
|  | SC | 20.03 |  |  |  |

1 Calculated taking all groups and bulls: the median for ∆Cq was calculated as [Cq gene–Cq reference gene] for each group and bull

2 Slope of the standard curve

3 R2 = coefficient of determination of the standard curve

4 Calculated as [10(-1/Slope)]

5LM = *longissimus* muscle

6SC = subcutaneous adipose tissue

***Supplementary Table S4*** *Estimated differences between tissues [subcutaneous (SC) and and longissimus muscle (LM)] by age [6 (6M), 12 (12M), and 18 months (18M)] and diet [low (18ME-) and high (18ME+) energy density] for acetyl Co-A carboxylase alpha (ACACA), CCAAT/enhancer binding protein alpha (CEBPA), fatty acid binding protein 4 (FABP4), fatty acid synthase (FASN), lipoprotein lipase (LPL), peroxisome proliferator activated receptor gamma (PPARG), sterol regulatory element binding transcription factor 1 (SREBF1), and wingless-type MMTV integration site family 10B (WNT10B).*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Factor | Contrast | Gene | Estimate | SED | *P-*value |
| Tissue | SC – LM | ACACA | -0.578 | 0.746 | 0.439 |
|  |  | CEBPA | -6.489 | 1.392 | <.0001 |
|  |  | FABP4 | 5.490 | 1.280 | <.0001 |
|  |  | FASN | 2.093 | 0.975 | 0.034 |
|  |  | LPL | -0.968 | 1.331 | 0.468 |
|  |  | PPARG | 5.066 | 1.154 | <.0001 |
|  |  | SREBF1 | -8.807 | 1.138 | <.0001 |
|  |  | WNT10B | -4.706 | 0.965 | <.0001 |
| Age | 6M - 12M | ACACA | 0.556 | 1.064 | 0.602 |
|  |  | CEBPA | -3.580 | 1.969 | 0.071 |
|  |  | FABP4 | -0.359 | 1.815 | 0.844 |
|  |  | FASN | 0.453 | 1.378 | 0.743 |
|  |  | LPL | -1.466 | 1.886 | 0.439 |
|  |  | PPARG | 0.555 | 1.638 | 0.735 |
|  |  | SREBF1 | -1.387 | 1.616 | 0.392 |
|  |  | WNT10B | -0.937 | 1.360 | 0.489 |
|  | 12M - 18ME+ | ACACA | -0.243 | 1.064 | 0.820 |
|  |  | CEBPA | 0.264 | 1.969 | 0.894 |
|  |  | FABP4 | 0.863 | 1.815 | 0.635 |
|  |  | FASN | -0.526 | 1.378 | 0.704 |
|  |  | LPL | -0.063 | 1.882 | 0.974 |
|  |  | PPARG | 1.472 | 1.630 | 0.368 |
|  |  | SREBF1 | 0.643 | 1.609 | 0.690 |
|  |  | WNT10B | 0.675 | 1.371 | 0.849 |
|  | 6M - 18ME+ | ACACA | 0.313 | 1.044 | 0.765 |
|  |  | CEBPA | -3.316 | 1.969 | 0.095 |
|  |  | FABP4 | 0.505 | 1.806 | 0.780 |
|  |  | FASN | -0.073 | 1.374 | 0.958 |
|  |  | LPL | -1.528 | 1.882 | 0.974 |
|  |  | PPARG | 2.027 | 1.635 | 0.217 |
|  |  | SREBF1 | -0.744 | 1.609 | 0.645 |
|  |  | WNT10B | -0.262 | 1.372 | 0.849 |
| Diet | 18ME+ - 18ME- | ACACA | -0.322 | 1.044 | 0.758 |
|  |  | CEBPA | 0.442 | 1.969 | 0.823 |
|  |  | FABP4 | -1.325 | 1.806 | 0.465 |
|  |  | FASN | 0.378 | 1.380 | 0.785 |
|  |  | LPL | -0.672 | 1.879 | 0.721 |
|  |  | PPARG | -3.621 | 2.934 | 0.219 |
|  |  | SREBF1 | -0.419 | 1.602 | 0.794 |
|  |  | WNT10B | -0.924 | 1.371 | 0.501 |

***Supplementary Table S5*** *Estimated differences by tissues [subcutaneous (SC) and and longissimus muscle (LM)], by age [6 (6M), 12 (12M), and 18 months (18M)], and by diet [low (18ME-) and high (18ME+) energy density] for acetyl Co-A carboxylase alpha (ACACA), CCAAT/enhancer binding protein alpha (CEBPA), fatty acid binding protein 4 (FABP4), fatty acid synthase (FASN), lipoprotein lipase (LPL), peroxisome proliferator activated receptor gamma (PPARG), sterol regulatory element binding transcription factor 1 (SREBF1), and wingless-type MMTV integration site family 10B (WNT10B).*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Tissue | Contrast | Gene | Estimate | SED | *P*-value |
| LM | 6M - 12M | ACACA | 3.012 | 1.965 | 0.127 |
|  |  | CEBPA | -1.845 | 2.611 | 0.481 |
|  |  | FABP4 | 2.114 | 3.256 | 0.517 |
|  |  | FASN | 0.543 | 1.942 | 0.780 |
|  |  | LPL | -3.287 | 2.960 | 0.269 |
|  |  | PPARG | 0.672 | 2.946 | 0.820 |
|  |  | SREBF1 | -3.046 | 2.380 | 0.203 |
|  |  | WNT10B | -1.384 | 2.274 | 0.518 |
|  | 12M - 18ME+ | ACACA | -3.447 | 1.965 | 0.081 |
|  |  | CEBPA | -1.777 | 2.611 | 0.497 |
|  |  | FABP4 | -1.694 | 3.256 | 0.604 |
|  |  | FASN | -1.142 | 1.942 | 0.558 |
|  |  | LPL | 0.664 | 2.959 | 0.823 |
|  |  | PPARG | 2.420 | 2.940 | 0.412 |
|  |  | SREBF1 | 0.145 | 2.361 | 0.951 |
|  |  | WNT10B | 1.686 | 2.303 | 0.466 |
|  | 6M - 18ME+ | ACACA | -0.435 | 1.922 | 0.821 |
|  |  | CEBPA | -3.622 | 2.611 | 0.497 |
|  |  | FABP4 | 0.420 | 3.235 | 0.897 |
|  |  | FASN | -0.599 | 1.929 | 0.757 |
|  |  | LPL | -2.623 | 2.951 | 0.376 |
|  |  | PPARG | 3.093 | 2.940 | 0.295 |
|  |  | SREBF1 | -2.902 | 2.361 | 0.222 |
|  |  | WNT10B | 0.328 | 2.303 | 0.887 |
| SC | 6M - 12M | ACACA | -1.899 | 0.818 | 0.022 |
|  |  | CEBPA | -5.316 | 2.948 | 0.074 |
|  |  | FABP4 | -2.832 | 1.606 | 0.080 |
|  |  | FASN | 0.363 | 1.957 | 0.853 |
|  |  | LPL | 0.356 | 2.337 | 0.879 |
|  |  | PPARG | 0.439 | 1.432 | 0.760 |
|  |  | SREBF1 | 0.273 | 2.187 | 0.901 |
|  |  | WNT10B | -0.516 | 1.493 | 0.760 |
|  | 12M - 18ME+ | ACACA | 2.960 | 0.818 | 0.000 |
|  |  | CEBPA | 2.305 | 2.945 | 0.436 |
|  |  | FABP4 | 3.421 | 1.606 | 0.035 |
|  |  | FASN | 0.091 | 1.957 | 0.963 |
|  |  | LPL | -0.789 | 2.327 | 0.735 |
|  |  | PPARG | 0.523 | 1.408 | 0.711 |
|  |  | SREBF1 | 1.141 | 2.187 | 0.603 |
|  |  | WNT10B | -0.336 | 1.487 | 0.822 |
|  | 6M - 18ME+ | ACACA | 1.061 | 0.818 | 0.197 |
|  |  | CEBPA | -3.011 | 2.948 | 0.309 |
|  |  | FABP4 | 0.589 | 1.606 | 0.714 |
|  |  | FASN | 0.453 | 1.957 | 0.817 |
|  |  | LPL | -0.433 | 2.337 | 0.853 |
|  |  | PPARG | 0.962 | 1.432 | 0.503 |
|  |  | SREBF1 | 1.414 | 2.187 | 0.519 |
|  |  | WNT10B | -0.851 | 1.493 | 0.569 |

***Supplementary Table S6*** *Estimated differences by diet [low (18ME-) and high (18ME+) energy density] by tissue [subcutaneous (SC) and longissimus muscle (LM) fat] for: acetyl Co-A carboxylase alpha (ACACA), CCAAT/enhancer binding protein alpha (CEBPA), fatty acid binding protein 4 (FABP4), fatty acid synthase (FASN), lipoprotein lipase (LPL), peroxisome proliferator activated receptor gamma (PPARG), sterol regulatory element binding transcription factor 1 (SREBF1), and wingless-type MMTV integration site family 10B (WNT10B).*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Tissue | Contrast | Gene | Estimate | SED | *P-*value |
| LM | 18ME+ - 18ME- | ACACA | -0.094 | 1.922 | 0.961 |
|  |  | CEBPA | 1.607 | 2.611 | 0.539 |
|  |  | FABP4 | -1.308 | 3.235 | 0.687 |
|  |  | FASN | -1.200 | 1.948 | 0.539 |
|  |  | LPL | -1.298 | 2.950 | 0.661 |
|  |  | PPARG | -0.627 | 1.408 | 0.657 |
|  |  | SREBF1 | 0.275 | 2.343 | 0.907 |
|  |  | WNT10B | -3.230 | 2.303 | 0.131 |
| SC | 18ME+ - 18ME- | ACACA | -0.550 | 0.818 | 0.503 |
|  |  | CEBPA | -0.722 | 2.948 | 0.807 |
|  |  | FABP4 | -1.341 | 1.606 | 0.405 |
|  |  | FASN | 1.956 | 1.957 | 0.319 |
|  |  | LPL | -0.047 | 2.327 | 0.984 |
|  |  | PPARG | 0.965 | 1.418 | 0.498 |
|  |  | SREBF1 | -1.114 | 2.187 | 0.612 |
|  |  | WNT10B | 1.383 | 1.487 | 0.427 |