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Expression of key myogenic, fibrogenic and adipogenic genes in *Longissimus thoracis* and *Masseter* muscles in cattle

Lara Martínez del Pino, Olaia Urrutia, Ana Arana, Leopoldo Alfonso, José Antonio Mendizabal, Beatriz Soret

Supplementary Table S1. Oligonucleotide sequences and amplicon size of the target and reference genes used for the quantification of gene expression in cattle.

Gene ID	Accession number	Gene	Primers ¹	bp ²	Source
<i>Peroxisome proliferator activated receptor γ</i>	NM_181024	<i>PPARG</i>	F: GTGAAGTTCAACGCACTGGA R: ATGTCCTCAATGGGCTTCAC	113	Soret <i>et al.</i> , 2016
<i>CCAAT/enhancer-binding protein α</i>	NM_176784	<i>CEBPA</i>	F: TGGACAAGAACAGCAACGAG R: TTGTCACTGGTCAGCTCCAG	130	Soret <i>et al.</i> , 2016
<i>Fatty acid binding protein 4</i>	NM_174314.2	<i>FABP4</i>	F: CATCTTGCTGAAAGCTGCAC R: ACCCCATTCAAAGTATGATGA	202	Bonnet <i>et al.</i> , 2013
<i>Wingless-type MMTV integration site family, member 10B</i>	XM_005206363	<i>WNT10B</i>	F: AATGCAAGTGCCATGGTACG R: GAGTTGCGGTTGTGAGTATCAATG	139	Soret <i>et al.</i> , 2016
<i>Zinc finger protein 423</i>	NM_001101893.1	<i>ZFP423</i>	F: GGATTCCCTCCGTGACAGCA R: TCGTCCTCATTCTCTCTCTCT	120	Duarte <i>et al.</i> 2013
<i>Fibronectin</i>	NM_001163778.1	<i>FN1</i>	F: GCGTGTCACCTGGGCTCCAC R: CTTGCTCCGGCAAGGTCGGGG	149	Duarte <i>et al.</i> 2013
<i>Fibroblast growth factor receptor 1</i>	NM_001110207.1	<i>FGFR1</i>	F: AGGAGGATCGAGCCCACGGC R: CTTGCTCCGGCAAGGTCGGGG	166	Duarte <i>et al.</i> 2013
<i>Fibroblast growth factor 2</i>	NM_174056.3	<i>FGF2</i>	F: GGAGCATCACCACGCTGCCA R: GTGGGTCGCTCTTCTCGCGG	165	Duarte <i>et al.</i> 2013
<i>Transforming growth factor, β 1</i>	NM_001166068.1	<i>TGFB1</i>	F: AGCCAGGGGGATGTGCCA	147	Duarte <i>et al.</i> 2013

<i>Myogenic factor 5</i>	NM_174116.1	<i>MYF5</i>	R: TAGCACGCGGGTGACCTCCT F: AGACGCCTGAAGAAGGTCAA R: TGCCATCAGAGCAACTTGAG	220	This study
<i>Myogenic differentiation 1</i>	NM_001040478.2	<i>MYOD</i>	F: TTCCGACGGCATGATGGACTAC R: TAAGTGCGGTCTAGCAGTTCC	72	Duarte <i>et al.</i> 2013
<i>Myogenin</i>	NM_001111325.1	<i>MYOG</i>	F: TACAGACGCCCACAATCTGCAC R: AGCGACATCCTCCACTGTGATG	67	Duarte <i>et al.</i> 2013
<i>Leptin</i>	NM_173928.2	<i>LEP</i>	F: GGCTGTCCACAGGAGAAGAG R: AGTGAGAGGGAGCTGGAACA	233	This study
<i>Adiponectin</i>	NM_174742.2	<i>ADIPOQ</i>	F: GTGGCTCTGATTCCACACCT R: TCTCCAGGAGTGCCATCTCT	208	This study
<i>Myostatin</i>	AF320998.1	<i>MSTN</i>	F: GTGTTGCAGAACTGGCTCAA R: AGGTGCCTTTGTCTGGCTTA	238	This study
<i>β-actin</i>	BC_142413	<i>ACTB</i>	F: CGCCATGGATGATGATATTGC R: AAGCGGCCTTGACATGC	65	Waters <i>et al.</i> , 2009
<i>Topoisomerase II-beta</i>	XM_001254709	<i>TOP2B</i>	F: CCGATGATGATGACGACAAT R: TGCTATGGGAGATGCTTTGA	62	Bonnet <i>et al.</i> , 2013
<i>Ceroid-lipofuscinosis neuronal 3</i>	NM_001075174	<i>CNL3</i>	F: TTCTGACTCCTTGGGACACA R: CAACCTGCCACCTATCAGT	62	Bonnet <i>et al.</i> , 2013
<i>Peptidylprolyl isomerase A</i>	XM_001252497	<i>PPIA</i>	F: GATTTATGTGCCAGGGTGGT R: GATGCCAGGACCTGTATGCT	116	This study
<i>Ribosomal protein large PO</i>	NM_001012682	<i>RFLPO</i>	F: CAACCCTGAAGTGCTTGACAT R: AGGCAGATGGATCAGCCA	227	Bonnet <i>et al.</i> , 2013

¹F = Forward; R = Reverse.

²Amplicon size in base pairs (bp).

References for Supplementary Table S1

- Bonnet M, Bernard L, Bes S, Leroux C 2013. Selection of reference genes for quantitative real-time PCR normalisation in adipose tissue, muscle, liver and mammary gland from ruminants. *Animal: an international journal of animal bioscience* 7, 1344. doi: 10.1017/S1751731113000475.
- Soret B, Mendizabal JA, Arana A, Alfonso L 2016. Expression of genes involved in adipogenesis and lipid metabolism in subcutaneous adipose tissue and longissimus muscle in low-marbled Pirenaica beef cattle. *Animal* 10, 2018. <https://doi.org/10.1017/S175173111600118X>.
- Waters SM, Kenny DA, Killeen AP, Spellman SA, Fitzgerald A, Hennessy AA, Hynes AC 2009. Effect of level of eicosapentaenoic acid on the transcriptional regulation of Delta-9 desaturase using a novel in vitro bovine intramuscular adipocyte cell culture model. *Animal: an international journal of animal bioscience* 3, 718. doi: 10.1017/S1751731109004054.

Supplementary Table S2. Slope, R² and Efficiency values for the target and reference genes used for the quantification of gene expression in cattle.

Gene	Slope ¹	(R ²) ²	Efficiency ³
<i>PPARG</i> ⁴	-3.405	0.99	1.97
<i>CEBPA</i>	-3.493	0.98	1.93
<i>FABP4</i>	-3.546	0.98	1.91
<i>WNT10B</i>	-3.684	0.98	1.87
<i>ZFP423</i>	-3.371	0.99	1.98
<i>FN1</i>	-3.074	0.99	2.00
<i>FGFR1</i>	-3.350	0.99	1.99
<i>FGF2</i>	-3.179	0.97	1.97
<i>TGFB1</i>	-3.211	0.99	2.00
<i>MYF5</i>	-3.323	0.99	2.00
<i>MYOD</i>	-3.344	0.99	1.99
<i>MYOG</i>	-3.285	0.99	2.00
<i>LEP</i>	-3.309	0.99	2.00
<i>ADIPOQ</i>	-3.160	0.99	2.00
<i>MSTN</i>	-3.314	0.99	1.99
<i>ACTB</i>	-3.450	0.96	1.95
<i>TOP2B</i>	-3.020	0.99	2.00

¹ Slope of the standard curve.

² R²= coefficient of determination of the standard curve.

³ Calculated as 10^{-1/Slope}.

⁴*PPARG* = Peroxisome proliferator-activated receptor γ ; *CEBPA* = CCAAT/enhancer-binding protein α ; *FABP4* = Fatty acid binding protein 4; *WNT10B* = Wingless-type MMTV integration site family, member 10B; *ZFP423* = Zinc finger protein 423; *FN1* = Fibronectin; *FGFR1*= Fibroblast growth factor receptor 1; *FGF2* = Fibroblast growth factor 2; *TGFB1*= Transforming growth factor β 1; *MYF5* = Myogenic factor 5; *MYOG* = Myogenin; *MYOD* = Myogenic differentiation 1; *LEP* = Leptin; *ADIPOQ* = Adiponectin; *MSTN* = Myostatin; *ACTB* = β -actin; *TOP2B* = Topoisomerase II-beta.

Supplementary Table S3. Variance components estimates for inter-animal variation, processing steps Sampling, Reverse Transcription (RT) and quantitative Polymerase Chain Reaction (qPCR) (residual), in *Longissimus thoracis* and *Masseter* muscles of Pirenaica and Holstein bulls.

(a) Adipogenic markers¹

<i>Longissimus</i>	Pirenaica					Holstein				
	<i>PPA</i> <i>RG</i>	<i>CEB</i> <i>PA</i>	<i>FABP</i> <i>4</i>	<i>ZFP</i> <i>423</i>	<i>WNT</i> <i>10B</i>	<i>PPA</i> <i>RG</i>	<i>CEB</i> <i>PA</i>	<i>FABP</i> <i>4</i>	<i>ZFP</i> <i>423</i>	<i>WNT</i> <i>10B</i>
Animal	0.16	0.10	0.00	0.53	0.08	0.23	0.14	0.23	0.08	0.19
Sampling	0.24	0.14	2.28	0.07	0.48	0.32	0.62	1.19	0.49	0.61
RT	0.28	0.25	0.92	0.23	2.69	0.16	0.25	0.11	0.30	0.06
qPCR	0.38	0.23	0.29	0.03	1.17	0.05	0.02	0.02	0.02	0.15
<i>Masseter</i>										
Animal	0.00	0.00	0.18	0.00	0.00	0.00	0.00	0.00	0.22	0.00
Sampling	0.59	1.10	0.32	0.12	0.76	0.34	0.46	2.04	0.38	0.28
RT	0.29	0.40	0.22	0.16	0.29	0.10	0.15	0.14	0.14	0.12
qPCR	0.08	0.23	0.58	0.02	0.31	0.02	0.02	0.05	0.02	0.14

(b) Myogenic markers¹

<i>Longissimus</i>	Pirenaica			Holstein		
	<i>MYF5</i>	<i>MYOD</i>	<i>MYOG</i>	<i>MYF5</i>	<i>MYOD</i>	<i>MYOG</i>
Animal	1.59	0.00	0.00	0.00	0.00	0.10
Sampling	1.21	0.00	0.00	1.30	0.55	0.49
RT	0.13	0.00	0.00	0.24	0.72	0.21
qPCR	0.08	0.00	0.00	0.25	0.06	0.01
<i>Masseter</i>						
Animal	0.05	0.25	0.03	0.00	0.21	0.12
Sampling	0.09	0.06	0.08	0.55	0.17	0.37
RT	0.03	0.07	0.01	0.72	0.66	0.67
qPCR	0.04	0.02	0.01	0.06	0.03	0.01

(c) Fibrogenic markers¹

<i>Longissimus</i>	Pirenaica			Holstein		
	<i>FN1</i>	<i>FGFR1</i>	<i>FGF2</i>	<i>FN1</i>	<i>FGFR1</i>	<i>FGF2</i>
Animal	0.00	0.00	0.00	0.00	0.25	0.07
Sampling	1.25	0.90	0.00	1.88	1.08	0.92
RT	0.14	0.22	1.39	0.22	0.24	0.37
qPCR	0.06	0.04	0.14	0.03	0.02	0.16
<i>Masseter</i>						
Animal	0.00	0.03	0.26	0.53	0.38	0.67
Sampling	0.01	0.02	0.00	0.44	0.34	0.66
RT	0.14	0.04	2.53	0.45	0.37	0.48
qPCR	0.03	0.06	0.18	0.03	0.01	0.06

(d) Cytokines¹

<i>Longissimus</i>	Pirenaica				Holstein			
	<i>LEP</i>	<i>ADIPOQ</i>	<i>MSTN</i>	<i>TGFB1</i>	<i>LEP</i>	<i>ADIPOQ</i>	<i>MSTN</i>	<i>TGFB1</i>
Animal	0.00	0.00	0.00	0.00	0.62	0.00	0.85	0.00
Sampling	4.00	0.12	8.29	0.26	1.99	1.14	0.92	0.86
RT	0.40	0.16	0.32	0.16	0.22	2.19	0.10	0.17
qPCR	0.29	0.02	0.42	0.07	0.38	0.05	0.59	0.06
<i>Masseter</i>								
Animal	0.77	1.42	1.59	0.00	2.72	1.80	0.62	0.20
Sampling	1.06	1.14	1.10	0.09	1.74	0.00	1.46	0.46
RT	0.32	0.06	0.10	0.09	0.06	7.67	0.00	0.24
qPCR	0.40	0.03	0.55	0.06	0.26	0.06	1.19	0.11

¹Gene abbreviations are defined in Table S2.

Supplementary Table S4. Differences in normalized expression values of adipogenic, myogenic and fibrogenic genes between *Longissimus thoracis* and *Masseter* in Pirenaica and Holstein bulls.

Gene ¹	Pirenaica				Holstein			
	Contrast	DIF ²	SEM	P-value	Contrast	DIF	SEM	P-value
<i>Adipogenic genes</i>								
<i>PPARG</i>	<i>LT-MS</i> ³	0.53	0.42	0.215	<i>LT-MS</i>	0.19	0.31	0.534
<i>CEBPA</i>	<i>LT-MS</i>	0.42	0.46	0.361	<i>LT-MS</i>	0.25	0.36	0.495
<i>FABP4</i>	<i>LT-MS</i>	1.36	0.57	0.020	<i>LT-MS</i>	0.58	0.52	0.265
<i>ZFP423</i>	<i>LT-MS</i>	0.44	0.28	0.118	<i>LT-MS</i>	0.81	0.39	0.044
<i>WNT10B</i>	<i>LT-MS</i>	-0.32	0.62	0.606	<i>LT-MS</i>	-0.07	0.33	0.841
<i>Myogenic genes</i>								
<i>MYF5</i>	<i>LT-MS</i>	-0.40	0.62	0.516	<i>LT-MS</i>	-0.18	0.47	0.711
<i>MYOG</i>	<i>LT-MS</i>	0.06	0.47	0.902	<i>LT-MS</i>	0.51	0.40	0.198
<i>MYOD</i>	<i>LT-MS</i>	2.45	0.51	0.000	<i>LT-MS</i>	1.98	0.41	0.000
<i>Fibrogenic genes</i>								
<i>FN1</i>	<i>LT-MS</i>	-0.25	0.35	0.481	<i>LT-MS</i>	0.33	0.47	0.492
<i>FGFR1</i>	<i>LT-MS</i>	0.12	0.33	0.725	<i>LT-MS</i>	-0.12	0.44	0.778
<i>FGF2</i>	<i>LT-MS</i>	0.45	0.42	0.281	<i>LT-MS</i>	0.59	0.47	0.213
<i>Cytokines</i>								
<i>LEP</i>	<i>LT-MS</i>	0.63	0.80	0.438	<i>LT-MS</i>	0.26	0.76	0.734
<i>ADIPOQ</i>	<i>LT-MS</i>	0.03	0.50	0.952	<i>LT-MS</i>	-1.18	0.57	0.042
<i>MSTN</i>	<i>LT-MS</i>	1.63	0.94	0.090	<i>LT-MS</i>	2.12	0.59	0.001
<i>TGFB1</i>	<i>LT-MS</i>	-0.27	0.23	0.245	<i>LT-MS</i>	-0.16	0.41	0.697

¹*PPARG* = Peroxisome proliferator activated receptor γ ; *CEBPA* = CCAAT/enhancer binding protein α ; *FABP4* = Fatty acid binding protein 4; *ZFP423* = Zinc finger protein 423; *WNT10B* = wingless-type MMTV integration site family member 10B; *MYF5* = Myogenic factor 5; *MYOG* = Myogenin; *MYOD* = Myogenic differentiation 1; *FN1* = Fibronectin; *FGFR1* = Fibroblast growth factor receptor 1; *FGF2* = Fibroblast growth factor 2; *LEP* = Leptin; *ADIPOQ* = Adiponectin; *MSTN* = Myostatin; *TGFB1* = Transforming growth factor β 1.

²DIF contrasted differences between normalized \log_2 ($E^{-\text{cpq}}$). Values for *PPARG*, *CEBPA*, *FABP4* and *WNT10B* in Pirenaica breed were presented in a previous work [Martínez Del Pino L, Arana A, Alfonso L, Mendizábal JA, Soret B. Adiposity and adipogenic gene expression in four different muscles in beef cattle. PLoS ONE. 2017;12: 1–19. doi:10.1371/journal.pone.01796040].

³*LT*= *Longissimus thoracis*; *MS*= *Masseter*.

Supplementary Table S5. Differences in normalised expression values of adipogenic, myogenic and fibrogenic genes between Pirenaica and Holstein bulls in *Longissimus thoracis* and *Masseter* muscles.

Gene ¹	<i>Longissimus thoracis</i>				<i>Masseter</i>			
	Contrast	DIF ²	SEM	P-value	Contrast	DIF	SEM	P-value
<i>Adipogenic genes</i>								
<i>PPARG</i>	PIR-HOL ³	1.11	0.36	0.003	PIR-HOL	0.81	0.36	0.026
<i>CEBPA</i>	PIR-HOL	-0.64	0.38	0.098	PIR-HOL	-0.77	0.42	0.072
<i>FABP4</i>	PIR-HOL	-1.98	0.57	0.001	PIR-HOL	-2.74	0.51	0.000
<i>ZFP423</i>	PIR-HOL	-0.25	0.46	0.588	PIR-HOL	0.09	0.32	0.772
<i>WNT10B</i>	PIR-HOL	-0.77	0.57	0.180	PIR-HOL	-0.45	0.38	0.241
<i>Myogenic genes</i>								
<i>MYF5</i>	PIR-HOL	-0.92	0.72	0.205	PIR-HOL	-0.69	0.33	0.043
<i>MYOG</i>	PIR-HOL	-0.58	0.55	0.27	PIR-HOL	-0.13	0.31	0.688
<i>MYOD</i>	PIR-HOL	0.05	0.54	0.925	PIR-HOL	-0.42	0.32	0.191
<i>Fibrogenic genes</i>								
<i>FN1</i>	PIR-HOL ³	-0.51	0.56	0.358	PIR-HOL	0.09	0.34	0.801
<i>FGFR1</i>	PIR-HOL	-0.39	0.52	0.454	PIR-HOL	-0.61	0.32	0.066
<i>FGF2</i>	PIR-HOL	0.21	0.48	0.670	PIR-HOL	0.36	0.50	0.479
<i>Cytokines</i>								
<i>LEP</i>	PIR-HOL	-0.12	0.89	0.896	PIR-HOL	-0.46	0.74	0.534
<i>ADIPOQ</i>	PIR-HOL	0.80	0.65	0.222	PIR-HOL	-0.44	0.48	0.368
<i>MSTN</i>	PIR-HOL	2.37	0.92	0.013	PIR-HOL	2.86	0.66	0.000
<i>TGFB1</i>	PIR-HOL	-0.19	0.44	0.667	PIR-HOL	-0.08	0.32	0.813

¹*PPARG* = Peroxisome proliferator activated receptor γ ; *CEBPA* = CCAAT/enhancer binding protein α ; *FABP4* = Fatty acid binding protein 4; *ZFP423* = Zinc finger protein 423; *WNT10B* = wingless-type MMTV integration site family member 10B; *MYF5* = Myogenic factor 5; *MYOG* = Myogenin; *MYOD* = Myogenic differentiation 1; *FN1* = Fibronectin; *FGFR1* = Fibroblast growth factor receptor 1; *FGF2* = Fibroblast growth factor 2; *LEP* = Leptin; *ADIPOQ* = Adiponectin; *MSTN* = Myostatin; *TGFB1* = Transforming growth factor β 1.

²DIF contrasted differences between normalized \log_2 ($E^{-\text{cpq}}$).

³PIR = Pirenaica; HOL = Holstein.

Supplementary Table S6 - Pearson's correlation coefficients (p-values) among gene expression and chemical traits and adipocyte size parameters in cattle.

Gene ¹	Chemical traits (n=16)					Adipocyte size parameters (n=12)					
	Fat, %	Protein, %	Total Collagen, mg/g	Soluble Collagen, %	Moisture, %	Minimum, μm	Maximum, μm	Median, μm	Mean, μm	Mode, μm	Number $10^6/\text{g tissue}$
<i>CEBPA</i>	0.106	0.150	0.138	0.112	-0.306	0.634	0.418	0.209	0.388	0.061	-0.559
<i>p-value</i>	0.697	0.581	0.610	0.680	0.249	0.027	0.177	0.514	0.212	0.851	0.059
<i>FABP4</i>	0.225	0.003	0.424	0.084	-0.410	0.831	0.578	0.350	0.468	0.144	-0.358
<i>p-value</i>	0.403	0.992	0.101	0.758	0.115	0.001	0.049	0.265	0.125	0.656	0.254
<i>FGF2</i>	-0.541	0.517	-0.023	-0.082	0.417	-0.447	-0.174	-0.510	-0.469	-0.513	-0.334
<i>p-value</i>	0.031	0.040	0.934	0.762	0.108	0.145	0.590	0.090	0.124	0.088	0.289
<i>FGFR1</i>	0.387	-0.036	0.418	-0.020	-0.451	0.790	0.919	0.334	0.492	0.223	-0.292
<i>p-value</i>	0.139	0.895	0.107	0.942	0.080	0.002	<.0001	0.289	0.105	0.487	0.357
<i>FN1</i>	-0.096	0.109	-0.200	-0.343	-0.004	0.596	0.639	0.230	0.314	0.113	-0.509
<i>p-value</i>	0.725	0.688	0.458	0.194	0.987	0.041	0.026	0.473	0.321	0.726	0.091
<i>ADIPOQ</i>	0.312	0.047	0.255	0.699	-0.233	-0.146	-0.083	-0.064	0.003	-0.015	0.287
<i>p-value</i>	0.240	0.863	0.340	0.003	0.386	0.650	0.798	0.844	0.993	0.963	0.365
<i>LEP</i>	-0.196	0.436	-0.028	0.019	0.117	0.235	0.356	0.015	0.239	0.023	-0.701
<i>p-value</i>	0.468	0.091	0.918	0.945	0.667	0.462	0.256	0.963	0.454	0.945	0.011
<i>MSTN</i>	-0.577	0.492	-0.293	0.178	0.453	-0.829	-0.358	-0.703	-0.743	-0.623	0.021
<i>p-value</i>	0.019	0.053	0.271	0.509	0.078	0.001	0.254	0.011	0.006	0.031	0.948
<i>MYF5</i>	0.323	-0.061	0.594	0.424	-0.110	0.519	0.270	0.163	0.171	0.065	0.141
<i>p-value</i>	0.222	0.822	0.015	0.102	0.685	0.084	0.397	0.612	0.595	0.842	0.661
<i>MYOD</i>	-0.344	0.365	0.043	0.066	0.042	-0.550	-0.262	-0.730	-0.762	-0.738	0.136
<i>p-value</i>	0.192	0.165	0.874	0.808	0.877	0.064	0.410	0.007	0.004	0.006	0.674
<i>MYOG</i>	-0.003	0.248	0.324	0.287	0.024	0.180	0.109	-0.211	-0.245	-0.356	0.115
<i>p-value</i>	0.990	0.354	0.221	0.282	0.929	0.576	0.736	0.510	0.443	0.257	0.722
<i>PPARG</i>	-0.602	0.542	-0.517	0.130	0.457	-0.806	-0.556	-0.454	-0.460	-0.339	-0.170
<i>p-value</i>	0.014	0.030	0.040	0.632	0.075	0.002	0.060	0.138	0.133	0.282	0.598
<i>TGFB1</i>	0.051	0.088	0.338	0.304	-0.097	0.729	0.673	0.195	0.340	0.081	-0.355
<i>p-value</i>	0.851	0.747	0.201	0.253	0.721	0.007	0.017	0.543	0.280	0.803	0.257
<i>WNT10B</i>	0.121	-0.105	0.241	-0.058	0.074	0.469	0.170	0.444	0.447	0.432	-0.079
<i>p-value</i>	0.656	0.700	0.369	0.831	0.785	0.124	0.598	0.149	0.145	0.160	0.807
<i>ZFP423</i>	-0.330	0.291	-0.041	-0.135	0.050	-0.265	-0.157	-0.586	-0.648	-0.674	0.090
<i>p-value</i>	0.213	0.274	0.880	0.619	0.855	0.406	0.627	0.045	0.023	0.016	0.780

¹Gene abbreviations are defined in Table S2.