Revista Colombiana de Ciencias Pecuarias
ISSN: 0120-0690
rccpecuarias@rccp.udea.edu.co
Universidad de Antioquia
Colombia

Trukhachev, Vladimir; Skripkin, Valentin; Kvochko, Andrey; Kulichenko, Alexander; Kovalev, Dmitry; Pisarenko, Sergey; Volynkina, Anna; Selionova, Marina; Aybazov, Magomet; Krivoruchko, Alexander

Correlation between gene expression profiles in muscle and live weight in Dzhalginsky Merino sheep

Revista Colombiana de Ciencias Pecuarias, vol. 29, núm. 3, julio-septiembre, 2016, pp. 188-198
Universidad de Antioquia
Medellín, Colombia

rccp Revista Colombiana de Ciencias Pecuarias

Correlation between gene expression profiles in muscle and live weight in Dzhalginsky Merino sheep¤

Correlación entre los perfiles de expresión génica en músculo y peso vivo de ovejas Dzhalginsky Merino

Correlação entre perfis de expressão gênica no músculo e peso vivo de ovinos Dzhalginsky Merino

Vladimir Trukhachev[1], MV, DAS; Valentin Skripkin[1], MV, PhD; Andrey Kvochko[1], MV, BD; Alexander Kulichenko[2], MV, MD; Dmitry Kovalev[2], MV, PhD; Sergey Pisarenko[2], AS, PhD; Anna Volynkina[2], AS, PhD; Marina Selionova[3], MV, BD; Magomet Aybazov[3], MV, BD; Alexander Krivoruchko[1*], MV, BD.

[1]*Faculty of Veterinary medicine, Stavropol State Agrarian University, Stavropol, Russian Federation.*

[2]*Stavropol Research Anti-plague Institute, Stavropol, Russian Federation.*

[3]*All-Russian Research Institute of Sheep and Goat Breeding, Stavropol, Russian Federation.*

(Received: August 17, 2015; accepted: April 13, 2016)

doi: 10.17533/udea.rccp.v29n3a04

Summary

Background: marker assisted selection methods of sheep require the identification of genes that positively and negatively affect meat quality. Genes with high expression levels could have the greatest impact on growth and structure of muscle fibers. **Objective:** this study evaluated the expression of genes in the loin muscle of Dzhalginsky Merino sheep. **Methods:** reverse transcription-quantitative real-time PCR (RT-qPCR) was used to investigate the expression of 48 genes in the loin muscle of Dzhalginsky Merino sheep bred in Russia. **Results:** genes *GAPDH, PYGM, CAST, ATP5G1, CAPN3, SOD1, VEGFA, CALM2, YWHAZ, ASIP, MYOD1, CAPN1, GHR, OXTR, BEGAIN, SLC2A3,* and *SS18L2* showed the highest expression. The group of genes with a medium level of expression included *ATOX1, BAMBI, TLR6, IGF2, FOS, FST, GGTA2P, C-MET, FGF5, ACVR2A, CAPN2, GH, DGAT1,* and *IGF1*. Low levels of expression were identified for genes *ABCG2, SPP2, PYGL, PPARG2, TGFB1, CXCR4, MSTN, CYP2J, LEPR, CDKN1A, IGFBP4,* and *SERT*. Trace expression was detected in genes *SST, TSHR, GDF9, FGF7* and *BMP15*. Significant correlation between expression level and live weight was observed for most of the investigated genes. **Conclusion:** our results demonstrate the feasibility of using these newly identified candidate genes as genetic markers in sheep.

Keywords: *growth traits, marker assisted selection (MAS), microarray analysis, transcription.*

¤ To cite this article: Trukhachev V, Skripkin V, Kvochko A, Kulichenko A, Kovalev D, Pisarenko S, Volynkina A, Selionova M, Aybazov M, Krivoruchko A. Correlation between gene expression profiles in muscle and live weight in Dzhalginsky Merino sheep. Rev Colomb Cienc Pecu 2016; 29:188-198.

* Corresponding author: Alexander Krivoruchko. Faculty of Veterinary medicine, Stavropol State Agrarian University, Stavropol, 355019, Russian Federation. Tel.: + 79188814327, Fax: +78652286738. E-mail: rcvm@yandex.ru

Resumen

Antecedentes: los métodos de selección asistida de ovejas a través de marcadores requieren la identificación de los genes que afectan positiva o negativamente la calidad de la carne. Los genes con niveles más altos de expresión podrían tener mayor impacto en el crecimiento y estructura de las fibras musculares. **Objetivo:** evaluar la expresión de los genes en el músculo del lomo de carneros de la raza Dzhalginsky Merino. **Métodos:** se utilizó RT-PCR cuantitativa en tiempo real (RT-qPCR) para investigar la expresión de 48 genes en el músculo del lomo de ovejos raza Merino Dzhalginsky criados en Rusia. **Resultados:** los genes *GAPDH, PYGM, CAST, ATP5G1, CAPN3, SOD1, VEGFa, CALM2, YWHAZ, ASIP, MYOD1, CAPN1, GHR, OXTR, BEGAIN, SLC2A3* y *SS18L2* mostraron la más alta expresión. El grupo de genes con un nivel medio de expresión incluyó *ATOX1, BAMBI, TLR6, IGF2, FOS, FST, GGTA2P, C-MET, FGF5, ACVR2A, CAPN2, GH, DGAT1* y *IGF1*. Se identificaron bajos niveles de expresión en los genes *ABCG2, SPP2, PYGL, PPARG2, TGFB1, CXCR4, NTCR, CYP2J, LEPR, CDKN1A, IGFBP4* y *SERT*. Expresión traza fue detectada en los genes *SST, TSHR, GDF9, FGF7* y *BMP15*. Para la mayoría de los genes investigados hubo una correlación significativa entre el nivel de expresión y el peso vivo de los carneros. **Conclusión:** los resultados demuestran la factibilidad del uso de estos genes candidatos identificados recientemente como marcadores genéticos en ovejas.

Palabras clave: *análisis de micromatrices, características de crecimiento, selección asistida por marcadores (MAS), transcripción.*

Resumo

Antecedentes: métodos que utilizam marcadores de seleção assistida em ovelhas exigem a identificação de novos genes que afetam a qualidade da carne. Genes com maiores níveis de expressão podem ter maior impacto sobre o crescimento e a estrutura das fibras músculares. **Objetivo:** avaliar a expressão de genes no músculo lombar de ovinos da raça Merino Dzhalginsky. **Métodos:** foi utilizada a reação em cadeia da polimerase-transcriptase reversa e em tempo real (RT-qPCR) para investigar a expressão de 48 genes em músculo do lombo da raça de ovinos Merino Dzhalginsky, que foram criados na Rússia. **Resultados:** os genes *GAPDH, PYGM, CAST, ATP5G1, CAPN3, SOD1, VEGFA, CALM2, YWHAZ, ASIP, MYOD1, CAPN1, GHR, OXTR, BEGAIN, SLC2A3* e *SS18L2* apresentaram a maior expressão. O grupo de genes com um nível médio de expressão incluíram *ATOX1, BAMBI, TLR6, IGF2, FOS, FST, GGTA2P, C-MET, FGF5, ACVR2A, CAPN2, GH, DGAT1* e *IGF1*. Foram identificados baixos níveis de expressão para os genes ABCG2, SPP2, PYGL, PPARG2, TGFB1, CXCR4, MSTN, CYP2J, LEPR, CDKN1A, IGFBP4 e SERT. Foi detectado rastreamento de expressão nos genes SST, TSHR, GDF9, FGF7 e BMP15. Para a maioria dos genes investigados, houve uma correlação significativa entre o nível de expressão e o peso vivo dos carneiros Dzhalginsky Merino. **Conclusão:** os resultados demonstram a viabilidade do uso desses genes candidatos recentemente identificados como marcadores genéticos no desenvolvimento de novas raças de ovinos.

Palavras chave: *características de crescimento, microarray análise, seleção assistida por marcadores (MAS), transcrição.*

Introduction

Marker-assisted selection (MAS) is based on identifying the most reliable genetic markers to predict the increase of muscle mass in farm animals, as well as to use those markers to select the most productive individuals for breeding. Therefore, new candidate genes whose products function in the development of muscle fibers in sheep should be identified (Moradi *et al.*, 2012; Miao and Luo, 2013).

The mRNA expression profile reflects the activity of the individual genes and characterizes the synthetic processes in muscles, which may differ between sheep breeds. Induction of high gene expression levels may indicate the significant impact of a particular gene on growth and development of muscle tissue in sheep, as previously shown by Hamill *et al.* (2012) in pigs.

Using the Affymetrix Bovine Expression Array technique, Fleming-Waddell *et al.* (2007) identified a particular gene expression profile in skeletal muscle of sheep carrying the callipyge mutation. Studies of gene expression profiles in sheep muscles by Lobo *et al.* (2012) revealed differential expression of *MyoD1* and *IGFBP4* genes associated with breed

and productive qualities. In addition, Zhang *et al.* (2013) studied gene expression in two sheep breeds by RNA sequence analysis and revealed significant differences in more than 1,300 genes. Continued research has allowed these researchers to describe 34 genes with differential expression related to the development and differentiation of muscle cells (Zhang *et al.,* 2014).

A number of sheep breeds are bred by local breeders in the Russian Federation. The "Dzhalginsky Merino" breed is well adapted to the dry conditions of the Stavropol Krai steppes. This breed is specialized in wool and meat production. Live weight of rams is 122.8 ± 2.91 Kg, dams are 55.6 ± 0.89 Kg, yearling rams are 79.5 ± 1.16 Kg, and ewes are 41.3 ± 0.71 Kg, which is significantly higher than the standard requirements for wool-breed sheep (Dunin *et al.,* 2013).

To the best of our knowledge, studies on gene structure related to meat productivity and evaluation of muscle gene expression in Russian sheep breeds have not been carried out. In the present study, reverse transcription-quantitative real-time PCR (RT-qPCR) was used to evaluate gene transcription in the loin muscle of Dzhalginsky Merino sheep.

Materials and methods

Sample collection

The study was conducted in the Genetic Laboratory of Science-Diagnostic and Veterinary Care Center (Stavropol State Agrarian University, The Russian Federation). We used 17 one-year-old Dzhalginsky Merino rams from a livestock breeding farm located in Stavropol Krai (The Russian Federation). We selected 12 animals with maximum height and weight, and five animals from the same population with a minimum height and weight to gather information about the maximum differential of gene expression patterns. All animals were healthy and kept in optimal conditions with *ad libitum* feed. After slaughter, samples from the center of the loin muscle (1 x 1 x 1 cm) were transported (for 4 hours at 4 °C in a cooling box) to the laboratory, and RNA was immediately isolated.

mRNA collection and cDNA preparation

RNA was isolated from a 0.1 g sample using phenol-chloroform extraction with TRIzol Reagent (ThermoFisher, Waltham, MA, USA) following the manufacturer's protocol. For normalization, RT-qPCR of all samples was performed with 25 ng/ml RNA.

Reverse transcription was performed with the Reverse Transcription Master Mix Kit (Fluidigm, South San-Francisco, CA, USA) using a set of preamplification PreAmp Master Mix and TaqMan Assays (Fluidigm, South San-Francisco, CA, USA). Amplification was carried out in a T100 Thermal Cycler (BioRad, Hercules, CA, USA).

Quantitation of cDNA in samples was performed with a fluorimeter Qubit 2.0 and reagents Qubit ds DNA HS Assay (Invitrogen, Waltham, MA, USA). A qualitative assessment of cDNA (value equal to 1.8) was performed with a NanoDrop spectrophotometer 2000C (ThermoScientific, Waltham, MA, USA) at A260/A280 wavelength.

Reverse-transcription quantitative real-time PCR (RT-qPCR)

Primers to 48 target genes were developed by Fluidigm company (Fluidigm, South San-Francisco, CA, USA). Real-time PCR was performed using a 96.96 Dynamic Array Gene Expression Integrated Fluidic Circuit (IFC) (Fluidigm, South San-Francisco, CA, USA). The preparation array was performed on the IFC Controller (Fluidigm, South San-Francisco, CA, USA) for real-time PCR and results were quantified using a Biomark™ HD System (Fluidigm, South San-Francisco, CA, USA) with negative controls in accordance with the manufacturer protocols and reagents. PCRs were carried out in duplicate samples. The cycle threshold (C_t) was taken into account if the parameter was at a value of 0.65.

Analysis of gene expression was performed using Real-Time PCR Analysis Software (Fluidigm, South San-Francisco, CA, USA). Efficiencies of real-time PCRs were calculated using BioMark™ analysis software (Fluidigm, South San-Francisco, CA, USA) in a 0.95-0.97 range.

Statistical analysis

The Student's t-test in Excel for Windows statistical plugin was used. Significant differences were set at $p<0.05$.

Results

Results of RT-qPCR in the form of individual gene expression profiles are shown in Figure 1.

The data showed that intensity of the same gene expressed in individual animals may be sufficiently close in value or vary significantly. As shown in Figure 1, highly expressed genes (located on the left side of the image) did not differ significantly between individuals. Genes with average expression levels (middle of the image) showed marked heterogeneity in transcription rates. Genes with low expression levels showed even more pronounced variation in mRNA expression levels between various animals (located on the right side of the image).

Animals were divided into two groups of high (12 individuals) and low (five individuals) weight to assess the expression relation of individual genes as integral indicators of meat productivity (as live weight). The average live weight with high reliability ($p<0.001$) differed between groups at 11.04 Kg (19.08%).

Candidate genes were divided into three groups based on digital analysis of gene expression levels in animals with high live weight: genes with high expression (C_t from 8 to 13), genes with an average expression level (C_t from 13 to 16), and those with low expression (C_t above than 16; Tables 1-3).

The *SST, TSHR, GDF9, FGF7,* and *BMP15* genes showed the lowest trace levels of mRNAs (maximum values $C_t>22$ or absence of luminescence in probes), indicating that they were practically not transcribed.

The *LEPR, CDKN1A, IGFBP4,* and *SERT* genes showed the lowest expression values, in which C_t was higher than 19. Two of these genes, *IGFBP4* and *SERT* are on chromosome 11. Expression of the *CDKN1A* gene in animals with low live weights was significantly higher at 23.21% than in rams with high weights. The *IGFBP4* gene showed a similar expression pattern in which the level of mRNA was greater at 8.95% in animals with low weights.

CYP2J gene expression was significantly higher at 7.20% in the low live weight group. A significant difference in expression levels was also found for *ABCG2, PYGL, PPARG2,* and *TGFB1* genes. All of these genes were more intensely transcribed in animals with low live weights; the differences in expression levels of *ABCG2, PYGL, PPARG2,* and *TGFB1* were 6.21, 9.03, 7.04, and 5.88%, respectively.

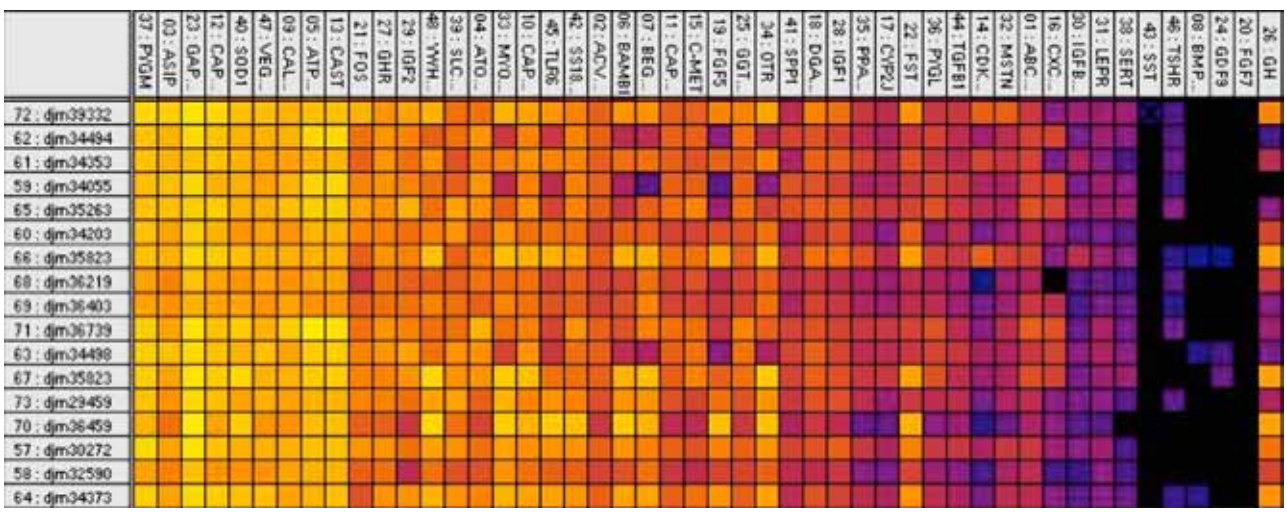

Figure 1. Individual expression profiles of 17 rams (Dzhalginsky Merino breed). Lighter cells indicate higher expression. In columns (genes); in rows (individual rams).

Table 1. Lowly expressed genes in loin muscle of Dzhalginsky Merino sheep in different live weight parameters (M ± m, C_t - cycle threshold).

Gene name	Full name gene	RefSeq ID	Chromosome	Position	C_t, High weight (68.90 ± 0.86 Kg, n = 12)	C_t, Low weight (57.86 ± 1.04 Kg, n = 5)	P-value
ABCG2	ATP-binding cassette, sub-family G (WHITE), member 2	NM_001078657	Chromosome 6	36.514.210-36.556.824	16.73 ± 0.29	15.75 ± 0.26	0.02
SPP2	*Ovis aries* secreted phosphoprotein 2	NM_001009224	Chromosome 1	6.767.995-6.787.884	16.93 ± 0.29	16.33 ± 0.67	0.40
PYGL	Phosphorylase, glycogen, liver	NM_001024861	Chromosome 7	40.814.121-40.860.779	17.29 ± 0.47	15.86 ± 0.27	0.01
PPARG2	Peroxisome proliferator-activated receptor gamma	NM_001100921	Chromosome 19	56.552.358-56.652.679	17.79 ± 0.38	16.62 ± 0.36	0.03
TGFB1	*Ovis aries* transforming growth factor, beta 1	NM_001009400	Chromosome 14	49.659.752-49.674.730	17.81 ± 0.28	16.82 ± 0.15	0.001
CXCR4	C-X-C chemokine receptor type 4	NM_001277168	Chromosome 2	173.602.065-173.605.247	18.04 ± 0.67	17.51 ± 1.16	0.67
MSTN	Myostatin	NM_001009428	Chromosome 2	118.144.443-118.149.433	18.21 ± 0.37	17.09 ± 0.45	0.06
CYP2J	*Ovis aries* cytochrome P450, family 2, subfamily J	NM_001077210	Chromosome 1	34.675.227-34.712.820	18.56 ± 0.44	17.31 ±0.27	0.02
LEPR	Leptin receptor	NM_001009763	Chromosome 1	40.760.256-40.858.312	19.55 ± 0.37	18.84 ± 0.24	0.11
CDKN1A	*Ovis aries* cyclin-dependent kinase inhibitor 1A (p21, Cip1)	NM_001161880	Chromosome 20	10.678.753-10.680.684	20.65 ± 0.67	16.76 ± 0.98	0.01
IGFBP4	Insulin-like growth factor binding protein 4	NM_001134302	Chromosome 11	40.250.021-40.260.382	21.16 ± 0.37	19.42 ± 0.51	0.01
SERT	*Ovis aries* serotonin transporter	NM_001009446	Chromosome 11	20.913.390-20.933.441	21.26 ± 0.39	20.00 ± 0.76	0.15
SST	*Ovis aries* somatostatin	NM_001009196	Chromosome 1	197.885.693-197.888.971	-	-	-
TSHR	Thyrotropin receptor	NM_001009410	Chromosome 7	89.258.424-89.431.877	-	-	-
GDF9	Growth differentiation factor 9	NM_001142888	Chromosome 5	41.841.034-41.843.517	-	-	-
FGF7	*Ovis aries* fibroblast growth factor 7	NM_001009235	Chromosome 7	57.779.972-57.841.735	-	-	-
BMP15	Bone morphogenetic protein 15	NM_001114767	Chromosome X	50.970.938-50.977.454	-	-	-

Table 2. Medium expressed genes in loin muscle of Dzhalginsky Merino sheep in different live weight parameters (M ± m, C_t - cycle threshold).

Gene name	Full name gene	RefSeq ID	Chromosome	Position	C_t, High weight (68.90 ±0.86 Kg, n = 12)	C_t, Low weight (57.86 ± 1.04 Kg, n = 5)	P-value
ATOX1	*Ovis aries* ATX1 antioxidant protein 1 homolog (yeast)	NM_001009429	Chromosome 5	60.474.740-60.479.122	13.29 ± 0.41	11.98 ± 0.22	0.01
BAMBI	*Ovis aries* BMP and activin membrane-bound inhibitor homolog (Xenopus laevis)	NM_001009761	Chromosome 13	35.117.182-35.118.653	13.39 ± 0.73	16.32 ± 0.82	0.01
TLR6	*Ovis aries* toll-like receptor 6	NM_001135927	Chromosome 6	58.034.773-58.037.166	13.44 ± 0.69	15.73 ± 0.86	0.05
IGF2	Insulin-like growth factor 2	NM_001009311	Chromosome 21	48.655.290-48.680.999	13.68 ± 0.51	12.03 ± 0.58	0.04
FOS	FBJ murine osteosarcoma viral oncogene homolog	NM_001166182	Human Chromosome 14	75.278.774-75.282.230	13.85 ± 0.42	12.22 ± 0.56	0.03
FST	Follistatin	NM_001257093	Chromosome 16	25.630.860-25.636.124	13.96 ± 0.70	15.63 ± 0.47	0.05
GGTA2P	Glycoprotein, alpha-galactosyltransferase 2, pseudogene	NM_001009764	Chromosome 3	14.290.904-14.322.530	14.69 ± 0.34	13.69 ± 0.24	0.02
C-MET	*Ovis aries* growth factor receptor c-met	NM_001111071	Chromosome 4	51.540.365-51.625.496	14.93 ± 0.47	14.25 ± 0.18	0.18
FGF5	Fibroblast growth factor 5	NM_001246263	Chromosome 6	94.584.400-94.605.575	14.93 ± 0.68	18.59 ± 1.34	0.03
ACVR2A	Activin receptor IIA	NM_001009293	Chromosome 2	160.457.581-160.548.685	15.08 ± 0.20	14.30 ± 0.11	0.001
CAPN2	Calpain-2 catalytic subunit	NM_001112817	Chromosome 12	25.191.495-25.241.603	15.17 ± 0.34	14.08 ± 0.15	0.01
GH	Growth hormone	NM_001009315	Chromosome 11	47.540.169-47.541.799	15.20 ± 0.95	18.96 ± 1.10	0.02
DGAT1	Diacylglycerol O-acyltransferase 1	NM_001110164	Chromosome 9	13.566.142-13.575.279	15.53 ± 0.30	14.33 ± 0.42	0.03
IGF1	Insulin-like growth factor 1	NM_001009774	Chromosome 3	171.268.400-171.327.752	15.85 ± 0.24	14.88 ± 0.55	0.13

Table 3. Highly expressed genes in loin muscle of Dzhalginsky Merino in different live weight parameters (M ± m, C_t - cycle threshold).

Gene name	Full name gene	RefSeq ID	Chromosome	Position	C_t, High weight (68.90 ± 0.86 Kg, n = 12)	C_t, Low weight (57.86 ± 1.04 Kg, n = 5)	P-value
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	NM_001190390	Chromosome 3	207.818.504-207.822.814	8.48 ± 0.31	8.43 ± 0.44	0.92
PYGM	Phosphorylase, glycogen, muscle	NM_001009192	Chromosome 21	42.295.599-42.307.126	9.74 ± 0.28	9.25 ± 0.41	0.31
CAST	*Ovis aries* calpastatin	NM_001009788	Chromosome 5	93.354.399-93.484.087	9.99 ± 0.36	8.68 ± 0,33	0.01
ATP5G1	ATP synthase F(0) complex subunit C1, mitochondrial	NM_001009396	Chromosome 11	37.173.130-37.175.267	10.00 ± 0.39	8.83 ± 0.33	0.03
CAPN3	*Ovis aries* calpain 3, (p94)	NM_001009212	Chromosome 7	34.747.153-34.805.210	10.36 ± 0.29	9.36 ± 0.35	0.04
SOD1	*Ovis aries* superoxide dismutase 1, soluble	NM_001145185	Chromosome 11	26.456.369-26.456.827	10.52 ± 0.40	10.41 ± 0.14	0.79
VEGFA	*Ovis aries* vascular endothelial growth factor A	NM_001025110	Chromosome 20	17.368.866-17.382.112	10.99 ± 0.28	10.61 ± 0.25	0.30
CALM2	Calmodulin 2 (phosphorylase kinase, delta)	NM_001009759	Chromosome 14	52.810.351-52.818.431	11.07 ± 0.29	10.27 ± 0.22	0.03
YWHAZ	Uncharacterized protein	NM_001267887	Chromosome 13	41.293.013-41.293.750	11.71 ± 0.63	13.35 ± 0.40	0.03
ASIP	Agouti-signaling protein precursor	NM_001134303	Chromosome 13	63.237.431-63.242.627	11.76 ± 0.31	9.94 ± 0.33	0.001
MYOD1	Myoblast determination protein 1	NM_001009390	Chromosome 15	34.370.528-34.371.122	12.10 ± 0.51	14.91 ± 0.98	0.03
CAPN1	*Ovis aries* calpain 1, (mu/I) large subunit (CAPN1)	NM_001127267	Chromosome 21	42.712.976-42.740.799	12.23 ± 0.39	12.14 ± 0.25	0.83
GHR	Growth hormone receptor	NM_001009323	Chromosome 16	31.832.933-32.000.445	12.74 ± 0.25	12.05 ± 0.04	0.01
OXTR	Oxytocin receptor	NM_001009752	Chromosome 19	17.656.099-17.664.613	12.78 ± 0.76	16.47 ± 1.19	0.02
BEGAIN	Brain-enriched guanylate kinase-associated	NM_001009766	Chromosome 18	64.087.503-64.094.914	12.78 ± 0.60	16.82 ± 1.54	0.04
SLC2A3	*Ovis aries* solute carrier family 2	NM_001009770	Chromosome 3	206.193.144-206.206.504	12.79 ± 0.31	13.54 ± 0.64	0.29
SS18L2	*Ovis aries* synovial sarcoma translocation gene on chromosome 18-like 2	NM_001145186	Chromosome 19	14.656.138-14.659.569	12.96 ± 0.40	12.92 ± 0.10	0.92

The *MSTN* gene showed relatively low expression levels that were directly related to the regulation of the intensity of muscle fiber growth, while there was not a significant difference in the amount of mRNA between groups. In addition, the expression of genes with low amounts of mRNA did not show a significant difference, including *SPP2, CXCR4, LEPR,* and *SERT,* in rams with different weights.

Only two of the genes with average mRNA expression levels, *C-MET* and *IGF1*, did not show significant differences in their expression intensities between ram groups. The *ATOX1, IGF2, FOS, GGTA2P, ACVR2A, CAPN2,* and *DGAT1* genes were transcribed with greater intensities in animals with low weight. The significant differences in gene expression intensity between animals with high and low weights for *ATOX1*, *IGF2,* and *FOS* were 10.93, 13.7, and 13.32%, respectively. There were also significant differences in gene expression levels (albeit lower values) between the high and low live weight groups for genes *GGTA2P* (7.28%), *ACVR2A* (5.43%), *CAPN2* (7.68%), and *DGAT1* (8.41%).

Expression levels of *BAMBI, TLR6, FST, FGF5,* and *GH* for the average transcription intensity group were significantly higher in animals with high live weight. Differences in expression between the two groups for *BAMBI, TLR6, FST,* and *FGF5* were 17.99, 14.55, 10.66, and 19.70%. The *GH* gene showed the highest difference in expression among genes, with 19.84% average expression intensity.

For the group of genes with the highest expression indexes, the number of genes with a transcription intensity that did not correlate with live weight (*GAPDH, PYGM, SOD1, VEGFA, CAPN1, SLC2A3,* and *SS18L2*) was almost equal to the number of genes showing reliable differences between groups of animals.

Among genes with the highest transcription indexes, several genes including *CAST, ATP5G1, CAPN3, CALM2, ASIP,* and *GHR* showed significantly higher expression levels in animals with low live weight. The difference in the expression intensity was the highest for *CAST* (15.09%), *ATP5G1* (13.21%) and *ASIP* (18.23%). The remaining genes *CAPN3, CALM2,* and *GHR* showed 10.70, 7.78, and 5.74% expression level differences, respectively.

The *YWHAZ, MYOD1, OXTR,* and *BEGAIN* genes in animals with high live weights showed a significant increase in expression levels. The significant differences in expression intensity for YWHAZ, *MYOD1,* and *OXTR* were 12.30, 18.81, and 22.38%. The *BEGAIN* gene showed the greatest difference in expression levels of all the studied genes. Its expression in animals with high live weight was 24.01% higher than that of the low weight group.

Discussion

This study showed heterogeneity in gene expression patterns in the loin muscle of Dzhalginsky Merino sheep. This heterogeneity reflected gene expression differences of multiple genes within one individual and of one gene in different examined animals. It should be noted that the focus of this investigation was to use differential gene expression patterns indicating involvement of a particular gene in muscle fiber growth regulation, without determining the underlying mechanisms. Furthermore, sequence analysis of candidate genes will reveal new molecular markers for genomic sheep breeding.

The main task of the study was to evaluate gene expression in loin muscle, to define a number of parameters that characterize sheep meat quality. We hypothesized that genes with high expression levels have the greatest impact on growth and structure of muscle fibers.

Weakly expressed genes may have a different effect on myocytes. For example, the low expression of genes encoding enzymes involved in energy metabolism may be rate limiting to muscle fiber development. At the same time, low expression of regulatory genes or genes encoding different hormones and growth factors may be enough to indicate major impact on muscle size and structure, thus, reflecting meat quality (Braun and Gautel, 2011).

We also based our choice of candidate genes on known data from farm animal studies about their impact on meat quality (Kogelman *et al.*, 2011). Relevant information of the impact on muscle growth in humans and laboratory animals from studies of genes in various pathologies and muscular aging

processes was also considered (Braun and Gautel, 2011; Garatachea and Lucía, 2013).

A large number of the candidate genes in this study encoded growth factors, activins, chemokines and their receptors, including *MSTN, VEGFA, TGFB1, FGF5, IGFBP4, FGF7, GDF9, IGF1, IGF2, MYOD1, C-MET, BMP15, PPARG2, BAMBI, CAPN1, CAPN2, CAPN3, CAST, ASIP, CXCR4, CDKN1A*. In addition, we investigated the expression of a number of genes encoding hormones and their receptors, including *FST, TLR6, ACVR2A, GH, GHR, SST, TSHR, SERT, OXTR, CALM2,* and *LEPR*. To evaluate the effect of genes on energy metabolism and the transport of substances, we investigated the expression of *PYGL, SPP2, CYP2J, ATOX1, GGTA2P, DGAT1, ABCG2, BEGAIN, SLC2A3, GAPDH, PYGM, ATP5G1,* and *SOD1*.

Mutations in several of the investigated genes, such as *FOS, YWHAZ,* and *SS18L2*, have been associated with the growth and development of muscles in a number of pathological processes in humans and animals. This suggests that they may be useful candidate genes to assess meat quality.

There are several methods to study gene expression, including sequencing cDNAs obtained by reverse transcription (Wang *et al.,* 2014; Zhang *et al.,* 2014), estimating the number of cDNAs by hybridization on biochips (Lobo *et al.,* 2012), and reverse transcription-quantitative real-time PCR (Sun *et al.,* 2014). The latter method is the most accurate and is used to validate the results obtained with microarray hybridization (Lobo *et al.,* 2012). Therefore, we used reverse transcription-quantitative real-time PCR on a 96.96 Dynamic Array Gene Expression system (Fluidigm, USA). This reaction was run at the same time for all the samples we studied, minimizing the impact of variability in terms of PCR standardization.

During the study of gene expression, we have assumed that the maximum intensity of transcriptional performance will be in genes encoding proteins involved in energy metabolism and transport systems, as previously established by Zhu *et al.* (2015), while investigating the variability of gene expression encoding the enzyme glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) in the *musculus longissimus dorsi* of goats. Our results are consistent with those of Zhu *et al.* (2015) as *GAPDH* showed the maximum expression levels in our study.

The *PYGM* gene, encoding a muscle glycogen phosphorylase, showed expression levels similar to those of *GAPDH* in loin muscle. In addition, the mRNA expression levels of calpain 3 (CAPN3) and calpastatin (CAST), which encode regulatory proteins of muscle fibers, were two units less than that of the *GAPDH* C_t value. Since they belong to the gene regulatory peptides group, we expected lower rates of expression intensities, however, the results were consistent and confirm the importance of the calpain-calpastatin system in the development of muscle fibers in sheep, as shown in several previous studies (Azari *et al*., 2012; Ranjbari *et al.,* 2012).

In general, the group of genes with high expression is paramount in the identification of molecular markers of sheep meat productivity. The magnitude of individual gene expression variation within a muscle may be associated with the presence of allelic variants of genes that have different functional activities. Therefore, future studies should focus on the structure of the *ATP5G1* gene, which encodes the ATP synthase enzyme with a key role in energy metabolism. The level of mRNA expression of *ATP5G1* is identical to that of *CAST*, which is a proven indicator of meat sheep productivity.

Gene expression analysis in muscle tissue of sheep (Jeanplong *et al.,* 2015) revealed higher expression levels of *MSTN* than those of *IGF1*. However, our data showed higher *IGF1* mRNA levels than those of *MSTN*. These differences may be explained by the breed characteristic of the animals or the fact that the authors used the semitendinosus muscle tissue for analysis instead of loin muscle. Nevertheless, the expression of these genes should be continued in other breeds of sheep to gather an overall indication of their use in predicting meat quality.

The expression of the majority of the investigated genes correlated with the live weights of the animal. This is supportive evidence of the involvement of a selected list of genes in the productive qualities of the Dzhalginsky Merino breed. Moreover, at this stage of the investigation, it is not important to

increase or decrease the expression of a gene in the group of animals with greater weights. In any case, it is necessary to characterize the structural features of these genes to select new genetic markers of meat productivity.

The growth hormone (*GH*) gene is of interest in these studies. Despite the fact that the main products of growth hormone in animals under the age of one year takes place in the anterior pituitary, our data of extrapituitary production of growth hormone does not contradict the results of other researchers. The presence of growth hormone gene expression in muscle has already been demonstrated in a number of animals (Moria and Devlinb, 1999), and we have confirmed it in sheep. In the group of genes with average expression intensity, *GH* showed the greatest difference in the magnitude of expression between the different weight groups of animals, which indicates a greater probability of its use as a marker of high meat quality in sheep. In addition, it is worth noting the inverse relationship between the levels of *GH* gene expression and that of the gene encoding its receptor *GHR*. This may be due to a compensatory increase in the synthesis of hormone receptor at a lower production of the hormone itself.

Very low trace expression of genes *SST, TSHR, GDF9, FGF7,* and *BMP15* are identified in the loin muscle of sheep. However, they should not be excluded from the list of gene candidates affecting sheep meat quality. It is possible that they may have a remote action after being produced in other tissues. In addition, growth differentiation factor 9 (*GDF9*) belongs to the same group as myostatin (*MSTN, GDF8*), which together with somatostatin (*SST*) have a direct and proven impact on the growth and development of muscle tissue in mammals.

Analysis of the expression of 48 genes in the loin muscle of Dzhalginsky Merino rams has allowed the initial genetic characterization of this breed. The results are relevant to understanding the key regulatory processes of muscle growth and performance of enzyme systems in the energy metabolism. The results showed some differences in gene expression levels in rams with different live weights. These data reveal general genetic aspects of the development of muscle fibers of sheep and of all mammals. The muscle expression of several genes has been studied for the first time, and the results will be used in future work on the genetic analysis of animals and humans. The main result is the justification of the need for additional investigations on the molecular nature of the investigated genes and to identify mutations associated with superior meat quality of sheep and other animals.

Conflict of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

Acknowledgement

This project was funded by the Ministry of Agriculture of the Russian Federation (agreement on the procedure and conditions for granting subsidies to financial security the state order for the provision of public services by December 30, 2013 N.° 3119/13).

References

Azari MA, Dehnavi E, Yousefi S, Shahmohamadi L. Polymorphism of calpastatin, calpain and myostatin genes in native dalagh sheep in Iran. Slovak J Anim Sci 2012; 45:1-6.

Braun T, Gautel M. Transcriptional mechanisms regulating skeletal muscle differentiation, growth and homeostasis. Nat Rev Mol Cell Biol 2011; 12:349-361.

Dunin IM, Serdukov IG, Pavlov MB. New selection achieving fine-fleeced sheep breed Dzhalginsky Merino. Farm Anim 2013; 3-4:46-48.

Fleming-Waddell JN, Wilson LM, Olbricht GR, Vuocolo T, Byrne K, Craig BA, Tellam RL, Cockett NE, Bidwell CA. Analysis of gene expression during the onset of muscle hypertrophy in callipyge lambs. Anim Genet 2007; 38:28-36.

Garatachea N, Lucía A. Genes and the ageing muscle: a review on genetic association studies. AGE 2013; 35:207-233.

Hamill MR, McBryan J, McGee C, Mullen AM, Sweeney T, Talbot T, Cairns MT, Davey GC. Functional analysis of muscle gene expression profiles associated with tenderness and intramuscular fat content in pork. Meat Sci 2012; 92:440-450.

Jeanplong F, Osepchook CC, Falconer SJ, Smith HK, Bass JJ, McMahon CD, Oldham JM. Undernutrition regulates the expression of a novel splice variant of myostatin and insulin-like growth factor 1 in ovine skeletal muscle. Domest Anim Endocrinol 2015; 52:17-24.

Kogelman LJA, Byrne K, Vuocolo T, Watson-Haigh NS, Kadarmideen HN, Kijas JW, Oddy HV, Gardner GE, Gondro C, Tellam RL. Genetic architecture of gene expression in ovine skeletal muscle. BMC Genomics 2011; 12:607-624.

Lobo AMB, Guimaraes SEF, Paiva SR, Cardoso FF, Silva FF, Fernandes GAJ, Lobo RNB. Differentially transcribed genes in skeletal muscle of lambs. Livest Sci 2012; 150:31-41.

Miao X, Luo Q. Genome-wide transcriptome analysis between small-tail Han sheep and the Surabaya fur sheep using high-throughput RNA sequencing. Reproduction 2013; 6:587-596.

Moradi MH, Nejati-Javaremi A, Moradi-Shahrbabak M, Dodds KG, McEwan JC. Genomic scan of selective sweeps in thin and fat tail sheep breeds for identifying of candidate regions associated with fat deposition. BMC Genet 2012; 13:10-27.

Moria T, Devlinb RH. Transgene and host growth hormone gene expression in pituitary and nonpituitary tissues of normal and growth hormone transgenic salmon. Mol Cell Endocrinol 1999; 149:129-139.

Ranjbari M, Hashemi A, Mardani K, Darvishzadeh R. Allelic polymorphism of Makoei sheep calpastatin gene identified by polymerase chain reaction and single strand conformation polymorphism. J Agr Sci Tech 2012; 14:533-538.

Sun W, Su R, Li D, Musa HH, Kong Y, Ding JT, Ma YH, Chen L, Zhang YF, Wu WZ. Developmental changes in IGF-I and MyoG gene expression and their association with meat traits in sheep. Genet Mol Res 2014; 13(2):2772-2783.

Wang X, Zhou C, Xu X, Geng R, Zhou J, Yang Y, Yang Z, Chen Y. Transcriptome profile analysis of adipose tissues from fat and short-tailed sheep. Genet 2014; 549:252-257.

Zhang C, Wang G, Wang J, Ji Z, Dong F, Chao T. analysis of differential gene expression and novel transcript units of ovine muscle transcriptomes. PLoS ONE 2014; 9:1-7.

Zhang C, Wang G, Wang J, Ji Z, Liu Z, Pi X, Chen C. Characterization and Comparative Analyses of Muscle Transcriptomes in Dorper and Small-Tailed Han Sheep Using RNA-Seq Technique. PLoS ONE 2013; 8:1-13.

Zhu W, Lin Y, Liao H, Wang Y. Expression studies related to intramuscular fat deposition in *Capra hircus* skeletal muscle. PLoS ONE 2015; 10(3):e0121280.